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ORIGINAL PAPER

Microcosm evaluation of bioaugmentation after field-scale thermal treatment of a TCE-contaminated aquifer

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Abstract This paper investigates effects of combining thermal and biological remediation, based on laboratory studies of trichloroethene (TCE) degradation. Aquifer material was collected 6 months after terminating a full-scale Electrical Resistance Heating (ERH), when the site had cooled from approximately 100°C to 40°C. The aquifer material was used to construct bioaugmented microcosms amended with the mixed anaerobic dechlorinating culture, KB-1^{TM}, and an electron donor (5 mM lactate). Microcosms were bioaugmented during cooling at 40, 30, 20, and 10°C, as temperatures continually decreased during laboratory incubation. Redox conditions were generally methanogenic, and electron donors were present to support dechlorination. For microcosms bioaugmented at 10°C and 20°C, dechlorination stalled at *cis*-dichloroethene (*c*DCE) and vinyl chloride (VC) 150 days after bioaugmentation. However, within 300 days of incubation ethene was produced in the majority of these microcosms. In contrast, dechlorination was rapid and complete in microcosms bioaugmented at 30°C. Microcosms bioaugmented at 40°C also showed rapid dechlorination, but stalled at *c*DCE with partial VC and ethene production, even after 150 days of incubation when the temperature had decreased to 10°C. These results suggest that sequential bioremediation of TCE is possible in field-scale thermal treatments after donor addition and bioaugmentation and that the optimal bioaugmentation temperature is approximately 30°C. When biological and thermal remediations are to be applied at the same location, three bioremediation approaches could be considered: (a) treating TCE in perimeter areas outside the source zone at temperatures of approximately 30°C; (b) polishing TCE concentrations in the original source zone during cooling from approximately 30°C to ambient groundwater temperatures; and (c) using bioremediation in downgradient areas taking advantages of the higher temperature and potential release of organic matter.

Keywords Dechlorination · Thermal treatment · Bioaugmentation · Groundwater · TCE

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Introduction

Contamination of groundwater with chlorinated ethenes is challenging to remediate primarily due

to the physical-chemical properties of the contaminants. When released to subsurface environments at high concentrations, they can exist as dense non-aqueous phase liquids (DNAPLs), which are challenging to remediate with conventional in-situ remediation technologies, due to mass transfer limitations. However, heating the subsurface to temperatures around the boiling point of water changes the thermodynamic conditions and makes NAPLs much more mobile (Davis 1998). Thermal treatment is a rapid and efficient technology although it may be cost-prohibitive at many sites. After a thermal treatment, the bulk contaminant is removed (Heron et al. 2005), although residual contamination may still remain dissolved in the water or sorbed to the sediments (Davis 1998).

Temperatures in an Electrical Resistance Heating (ERH) remediated area can remain elevated for months to years after heating (Krauter et al. 1995; Larsen et al. 2004). This suggests a potential for biological remediation after thermal treatment as temperatures remain elevated, because rates for either partial or complete dechlorination of TCE to ethene are increased at temperatures of approximately 30°C for isolates (He et al. 2003) and between 15°C and 30°C for mixed cultures (Friis et al. 2007b).

TCE can be biologically reduced by reductive dechlorination, where it is transformed to *cis*-dichloroethene (*c*DCE), vinyl chloride (VC) and finally to harmless ethene (Holliger et al. 1993). The initial dechlorination step from TCE to *c*DCE can be carried out by a variety of microorganisms (Bradley 2003). However, complete dechlorination to ethene is currently believed to require microorganisms of the genus *Dehalococcoides* (Hendrickson et al. 2002; Maymo-Gatell et al. 2001). At contaminated sites, reductive dechlorination is often limited by a lack of sufficient electron donors and/or specific dechlorinating microorganisms (Hendrickson et al. 2002; Holliger et al. 1993; Major et al. 2002). These limitations can be overcome by injecting electron donors in the subsurface (i.e., biostimulation) or by injecting dechlorinating microorganisms, often following electron donor addition (i.e., bioaugmentation) (Ellis et al. 2000; Major et al. 2002). At field sites undergoing post-thermal bioaugmentation for treatment of chlorinated solvents by anaerobic dechlorination, an electron donor is commonly added initially (Major et al. 2002). When reduced conditions have been obtained, bioaugmentation is applied to stimulate complete dechlorination.

After thermal treatments are performed, elevated temperatures and unfavorable geochemical conditions (e.g., more oxic conditions), (Friis et al. 2005) which have been reached during heating could limit the survival and activity of microorganisms (Friis et al. 2006b). Therefore, there is a need for investigations on the sensitivity of dechlorinating microorganisms in order to establish the optimal time and conditions for bioaugmentation.

Previous work focused on redox processes and anaerobic dechlorination in samples heated under controlled conditions in the laboratory (Friis et al. 2005, 2007a), and field samples heated by ERH (Friis et al. 2006b). In addition, the temperature optima of dechlorination were determined in the diluted mixed culture KB-1^{TM} (Friis et al. 2007b). In the current study the same culture was used, but here we intended to mimic field conditions. We have included the effect of indigenous microorganisms, presence of aquifer material and the field scale temperature development during cooling.

The objectives of this study were to determine favorable temperature conditions for bioaugmentation after a field-scale thermal treatment and the effect of bioaugmentation temperature on dechlorination activity and transformation rates during cooling. The goal was to develop engineering approaches for combining thermal treatment with bioaugmentation for more complete and cost-effective remediation. Aquifer sediments were collected after a full-scale ERH at the Ft. Lewis site in the US when the aquifer had cooled to approximately 40°C. Microcosms were bioaugmented at various temperatures, and the effects on redox activity and anaerobic dechlorination processes were studied during decreasing temperatures. Transformation rates were quantified by a Monod model including inhibition and for simplicity also a zero order degradation rate model.

Materials and methods

Experimental approach

In this study, microcosms were constructed with aquifer sediment and groundwater collected from the ERH heated site after the subsurface temperature had cooled to approximately 40°C, which occurred approximately 6 months after cessation of ERH. This temperature was chosen because it was greater than the expected optimum of approximately 30°C (Friis et al. 2007b). TCE and lactate were added initially and the microcosms were bioaugmented with KB-1™ at various temperatures while cooling (Fig. 1). This was carried out in order to investigate whether the previously determined temperature optima in diluted cultures (Friis et al. 2007b) can be effectively applied in aquifer microcosms collected after a thermal treatment.

In general, post-thermal temperatures are influenced by groundwater flow, properties of the porous media (especially water content), size of the treated area and depth below ground surface. The temperature decrease in this experiment (Fig. 1) was selected from previous field experiences and was intended to mimic the temperature profile in the field. All microcosms were maintained at the same temperatures and controlled in incubators or coolers.

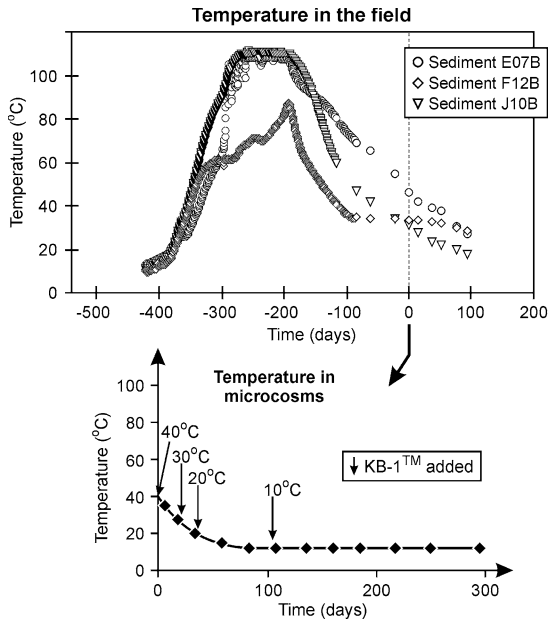

Fig. 1 Temperature history of aquifer materials during Electrical Resistance Heating (ERH). Aquifer samples were collected when the temperatures of the sample locations were: E07B = 46°C, F12B = 34°C, and J10B = 31°C. Microcosms were bioaugmented with KB-1™ at 40°C (0 days of incubation), 30°C (21 days of incubation), 20°C (36 days of incubation), and at 10°C (104 days of incubation)

Site description and sampling

Aquifer and groundwater samples were collected at Ft. Lewis, East Gate Disposal Yard (EGDY), WA, USA in order to establish microcosm experiments. The aquifer was unconfined with a groundwater level approximately 3 m below ground surface (bgs). The average groundwater pore flow velocity was approximately 3 m/day (R. Smith 2005, Personal communication). The subsurface was primarily outwash of cobbles and gravel with till-lenses of sand/gravel with silt. The outwash sediments were situated above a till layer from approximately 9 m (bgs) at the location where samples were collected (U.S. Army Corps of Engineers 2002). The site was primarily contaminated with TCE, *c*DCE and hydrocarbons. Aquifer material was retrieved from three locations after heating (Fig. 1): Sediment E07B (5.8–6.7 mbs, brown/grey sand), sediment F12B (13.3–15 mbs, brown sandy gravel), and sediment J10B (8.3–9.2 mbs, grey sandy gravel).

Sediment cores were collected with a Roto Sonic Drill Rig within a 15 cm polyethylene (PE) sleeve and immediately transferred to a sterilized field-portable glove box with argon (Ar) atmosphere, in order to avoid contact with oxygen (O_2) and the introduction of non-indigenous microorganisms. Sediment was transferred into diffusion-proof aluminum bags coated inside with Teflon (Friis et al. 2005). Groundwater used for all microcosms was sampled at one location (B09) between 6.3 to 8.0 mbs using pre-sterilized and Ar-flushed serum bottles, as previously described (Friis et al. 2006b). Location B09 was approximately 10 m downgradient the thermally treated area.

During transport to the laboratory, samples were kept in an insulated box maintaining temperatures between 30 and 40°C, and temperatures

were recorded to document the temperature history. Samples were set up within 4 days after collection while maintained at 30–40°C. Sediment samples were obtained approximately 6 months after heating. In this time, groundwater could have exchanged the pore volumes 10 times, assuming an average groundwater flow at the site of approximately 3 m/day (R. Smith 2005, Personal communication). With this flow, groundwater could reach all the sediment locations E07B, F12B, and J10B within approximately 10 days. The collected sediment samples were therefore assumed to have been exposed to groundwater which was untreated, but at elevated temperatures, because it had passed through the heated zone.

Microcosm set-up and incubation conditions

Microcosms were established in an anaerobic Coy® glove box by transferring 100 g (wet weight) of well-mixed aquifer material into 500 ml sterile glass bottles and sealed with 1 cm butyl rubber stoppers. In the anaerobic box, aquifer samples and glass bottles were kept at approximately 40°C by using a hot plate. For all microcosms, headspace was flushed for 2–3 min with N_2-CO_2 (80–20% (v/v) to remove excess H_2 from the glove box atmosphere. Groundwater was filtered through 0.2 μm nitrocellulose sterile filters after the filters had been rinsed with 500 ml of milliQ water. Groundwater was purged with N_2-CO_2 during filtering in order to strip off any remaining O_2. The temperature of groundwater was approximately 40°C when 0.2 l was added to each batch while purging. Subsequently, the bottles were sealed with butyl rubber stoppers and then sealed with screw caps. All microcosms were prepared in triplicates. TCE was added to each microcosm at day 0 as an aqueous solution using sterile syringes to a final concentration of 15 mM. Chlorinated ethenes were measured in all triplicates and redox sensitive parameters were measured in individual microcosms.

Lactate was added to all microcosms at day 0 as lactic acid sodium salt solution (Fluka, 50% (w/v) in water) using sterile syringes and preparing a final concentration of 5 mM lactate. KB-1™ is a mixed anaerobic culture which contains dechlorinating microorganisms, methanogens, Fe(III)-reducers, and lesser quantities of putative SO_4^{2-}-reducers and fermenters (Duhamel and Edwards 2006). This culture was provided by SIREM Laboratories, Ontario, Canada. One ml of KB-1™ solution was quickly added to each batch using N_2-rinsed sterile syringes to avoid contact with O_2. KB-1™ was added while the temperature in the microcosms decreased (Fig. 1). In order to avoid fast temperature variations, KB-1™ was kept at room temperature prior to the bioaugmentations at 40, 30, and 20°C and kept at 4°C prior to bioaugmentation at 10°C. Biologically inhibited controls were prepared by forming a solution of 1.8 mM $HgCl_2$ in the microcosms. Pressure in the microcosms was sustained by injecting N_2 in an equal volume to the sampled gas/water ratio. Microcosms were kept inverted in water baths inside dark incubators, while the incubator temperature was controlled and monitored using a Grant 1200 Series Squirrel temperature logger every ten minutes.

Analytical methods

Aqueous samples were obtained from the microcosms using disposable sterile needles (0.60 mm) and syringes, and H_2 and CH_4 gases were sampled from the headspace as previously described (Friis et al. 2005). Dissolved iron (Fe^{2+}) was measured immediately after filtering the samples through sterile 0.45 μm filters, by using the ferrozine method. Dissolved manganese (Mn_{diss}) was analyzed in 0.45 μm filtered samples using an Atomic Absorption Spectrometer, while anions (SO_4^{2-}, NO_3^-) were analyzed using an ion chromatograph. H_2 was analyzed using a Trace Analytical RGD2 Reduction Gas Detector GC, CH_4 using a GC with Flame Ionization Detector, and the pH was analyzed within 2 min of sampling using a microelectrode. Finally, alkalinity was determined by Gran titration as previously described (Friis et al. 2005). Samples for fatty acid analyses were filtered through 0.45 μm nylon filters, acidified with 50 μl 17% (w/v) H_3PO_4 per ml of sample, and kept frozen until analysis by suppressed ion chromatography using a Dionex IonPac Ice AS 1

column, Dionex Amms Ice II suppressor, and Waters 432 conductivity detector.

Chlorinated ethenes, ethene, and ethane were analyzed by gas chromatography using an Agilent 6890N gas chromatograph (GC) equipped with a mass selective detector (MS, Agilent 5973) and a 25 m × 320 μm × 1 μm (nominal) capillary column (J&W GSQ) using helium as the carrier gas. One ml aqueous samples were injected into sealed vials that were heated to 80°C for headspace gas analyses. 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 and added to headspace vials using disposable sterile needles and syringes. VC, ethene, and ethane were added as gases to the headspace vials using a gastight syringe. Ethene and ethane appeared as broad peaks 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. Ethane concentrations remained below detection limit and corresponding data will therefore not be presented. Chloroform served as an internal standard.

Solutions of TCE (>99.5% purity, Merck), *c*DCE (>97%, Acros), 1.1-DCE (>99.5%, Fluka), *t*DCE (>97%, Fluka), and VC was of >99.97% purity (Gerling, Holz & Co.) were used. Finally ethene and ethane were obtained as pure gasses (Mikrolab, Denmark). Standards were prepared as aqueous solutions of chlorinated ethenes for each sampling event, which caused some variation in the concentrations between sampling events. The variation between sampling events was minimized by normalizing concentrations to the TCE concentration in deactivated controls at day 6. The deactivated controls did not show any signs of dechlorination or significant abiotic losses taking the uncertainty in temperature dependency of Henry's law constant into account.

Phase distribution of volatile compounds (chlorinated ethenes, ethene and CH_4) in headspace, water and sediment was calculated in order to quantitatively compare with dissolved electron acceptors (Fe^{2+}, Mn_{diss}, and SO_4^{2-}) on a molar basis. Calculated concentrations were reported as an equivalent aqueous concentration (C^{α}):

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

where m_{total} is the total amount, C is the concentration, V is the volume and m is mass of a given compound in h, headspace; w, water; and s, sediment. Estimated partitioning constants for chloroethenes (Table 1), ethene and CH_4 (Wilhelm et al. 1977) as a function of temperature were applied. However, ethene and CH_4 were assumed not to sorb to the sediment compared to the fraction in water and headspace and distribution between sediment and water was therefore not included for these compounds.

Dechlorination growth rates

Dechlorination growth rates of TCE, *c*DCE and VC were estimated to quantitatively compare rates at various temperatures. A zero order kinetic model (Friis et al. 2007b) and a competitive Monod kinetic model (Cupples et al. 2004; Yu et al. 2005) were applied. Both models assumed that presence of electron donor is not limiting for dechlorination. Zero order dechlorination rates were estimated as increase in concentration of produced compounds divided by the time interval in which dechlorination occurred. It was applied to validate the more complex Monod model. Competitive Monod kinetics was used to estimate maximal growth rates. This model included inhibition of *c*DCE and VC dechlorination by TCE concentrations, and inhibition of TCE and VC dechlorination by *c*DCE concentrations. The Monod model also included biomass growth and the following input parameters were applied: Initial dechlorinating biomass concentrations of 2×10^8 cells/l (Friis et al. 2007b), yields of 5.2×10^8 cells/μmol/Cl^- (Cupples et al. 2004), and decay constant of 0.05 d^{-1} (Cupples et al. 2003). Kinetic parameters which previously have been applied for KB-1™ were used in this model. These were: Half-velocity coefficients for K_{TCE} of 10.0 ± 0.2 μM (Cupples et al. 2004), K_{cDCE} of 3.3 ± 0.2 μM and K_{VC} of 2.6 ± 1.9 μM (Haston and McCarty 1999); inhibition constants for

Table 1 Equations used to estimate temperature dependency of Henry's law constant (H) for chlorinated ethenes and ethene

Compound	Regression equation	Temp interval (°C)	Comment	Reference
TCE	$\ln H = 195.52 - \frac{12540}{T} - 27.11 \ln T$	10–95	Eq given by authors	Heron et al. 1998
*c*DCE	$\log H = 3.36 - \frac{1223.9}{T}$	1.8–70	Eq recalculated[a]	Staudinger and Roberts 2001
VC	$\log H = 4.119 - \frac{1223}{T}$	10–35	Eq given by authors	Shimotori and Arnold 2003
Ethene	$H = (\exp(-151.421 + \frac{7960.55}{T} + 20.5129 \ln T) * T)^{-1}$	–	Estimated from water solubility[b]	Wilhelm et al. 1997

H is dimensionless in all equations

[a] Linear regression made from plotting log H as a function of 1/T for five temperatures with each 10 measuring points ($R^2 = 0.99$). For this estimate it was assumed that the enthalpy of dissolution is constant (Heron et al. 1998).

[b] Calculated from assuming H equals vapour pressure divided by water solubility (Wilhelm et al. 1977). Source Friis (2006)

$K_{I,TCE}$ of 11 ± 0.6 μM, $K_{I,cDCE}$ of 2.2 ± 0.5 μM and no inhibition of $K_{I,VC}$ (Friis et al. 2007b). The model equations are described in Friis et al. (2007b).

Growth rates of TCE, *c*DCE, and VC were estimated at each temperature for each sediment location by including all data in the model.

Results and discussion

Redox processes and fermentation at various temperatures

Redox conditions were interpreted from the change in concentrations of the redox sensitive parameters Mn_{diss}, Fe^{2+}, SO_4^{2-}, and CH_4 (Table 2) over time. In this study, O_2 was eliminated from microcosms during the set-up, and NO_3^- concentrations remained below the detection limit (0.003 mM) at all sampling points. The redox activity in all microcosms was evaluated on the basis of transferred electrons, measured 100 days after bioaugmentation, as described in Fig. 2.

In microcosms bioaugmented at 30°C and 40°C the electron transfer by CH_4-production was orders of magnitude greater than those from Mn/Fe-reduction, SO_4^{2-}-reduction, and reduction of chlorinated ethenes (Fig. 2). In the microcosms bioaugmented later at 10°C and 20°C, CH_4 was produced 100 days after bioaugmentation with sediment from locations E07B and J10B but not with sediment from location F12B (Fig. 2). The presence of methanogenic conditions can positively influence the extent of dechlorination (Bradley and Chapelle 1999), although these conditions are not a prerequisite for ethene production (Duhamel et al. 2004; He et al. 2002). This indicates that the achieved redox conditions were expected to be favorable for complete reductive dechlorination.

Electron donors are also required for reductive dechlorination where chlorinated ethenes are electron acceptors. The electron donors are most often represented as H_2. However, acetate can support cell maintenance in *Dehalococcoides* although it is not an effective donor in complete dechlorination. Acetate has only been shown to directly support dechlorination to *c*DCE (Sung

Table 2 Redox sensitive parameters after bioaugmentation

		40°C		30°C		20°C		10°C	
Days	0	100	200	100	200	100	200	100	200
Sediment E07B									
Mn_{diss}	<0.02	0.06	0.09	0.07	0.09	0.07	0.09	0.06	0.08
Fe^{2+}	<0.02	0.13	0.13	0.15	0.11	0.16	0.35	0.15	0.21
SO_4^{2-}	<0.16	<	<	<	<	<	<	<	<
CH_4	<	4.03	6.98	1.97	6.80	0.77	2.04	1.17	1.65
H_2	<411	5.1	0.5	7.8	0.4	2.4	1.4	2.7	1.2
Lactate	7.25	<	<	<	<	<	<	<	0.02
Acetate	0.18	0.26	<	3.38	<	4.09	3.18	4.39	3.52
Formate	<	<	<	<	<	<	<	<	<
Propionate	<	<	<	<	<	<	<	<	<
pH	5.90	6.47	6.01	6.30	5.91	6.26	5.99	5.80	5.74
Alkalinity	4.18	8.67	9.53	8.24	8.64	8.60	9.59	10.84	8.16
Sediment F12B									
Mn_{diss}	<0.05	0.10	0.11	0.12	0.12	0.11	0.14	0.10	0.11
Fe^{2+}	<	0.07	0.05	0.10	0.10	0.12	0.13	0.09	0.09
SO_4^{2-}	<0.17	<	<	<	<	<	<	<	<
CH_4	<	3.87	5.88	0.33	1.13	0.02	0.18	<	<
H_2	<77	1.7	0.2	1.4	2.2	2.7	0.6	0.7	1.9
Lactate	6.58	<	<	<	<	<	<	<	<
Acetate	<	0.11	0.08	2.31	1.64	5.78	4.25	3.47	2.94
Formate	<	<	<	<	<	<	<	<	<
Propionate	<	<	<	<	<	<	<	<	<
pH	5.94	6.53	6.18	6.61	6.06	6.39	5.98	6.13	6.10
Alkalinity	4.80	10.21	10.40	9.68	10.48	8.94	9.63	11.78	9.5
Sediment J10B									
Mn_{diss}	<0.05	0.11	0.13	0.15	0.15	0.14	0.15	0.14	0.13
Fe^{2+}	<0.09	0.24	0.20	0.15	0.20	0.28	0.29	0.17	0.15
SO_4^{2-}	<0.03	<	<	<	<	<	<	<	<
CH_4	<	0.85	3.89	0.55	4.66	0.14	0.29	0.26	0.47
H_2	<36	0.7	4.3	2.4	1.2	1.2	1.0	1.0	1.4
Lactate	7.25	<	<	<	<	<	<	<	<
Acetate	1.13	4.93	1.49	4.03	0.24	4.47	3.44	4.85	3.43
Formate		<	<	<	<	<	<	<	<
Propionate		<	<	<	<	<	<	<	<
pH	5.85	6.36	6.09	6.29	6.04	6.40	6.02	6.31	6.00
Alkalinity	4.57	7.65	10.08	9.09	10.99	9.05	9.75	9.16	10.05

All parameters are given in mM, except for H_2 which is given in nM

< indicate below detection limit: 0.01 mM Mn_{diss}, 0.015 mM Fe^{2+}, 0.01 mM SO_4^{2-}, 0.03 mM CH_4, and 0.01 mM lactate and acetate. NO_3^- remained below the detection limit of 0.003 mM in all samples and was therefore not included in this table. Parameters detected at day 0 were an average of unamended microcosms with the specific sediment, except for fatty acids, which were only detected in microcosms bioaugmented at 10°C at day 0. CH_4 was estimated as C^{α}, whereas all other constituents were given as aqueous concentrations

et al. 2003). However, acetate can be fermented to H_2 and used for dechlorination in some mixed communities (Duhamel 2005). H_2 is commonly produced by fermentation of various organic substrates, such as lactate (Holliger et al. 1998; Yang and McCarty 1998).

In this experiment fermentative organisms were present in KB-1™ (Duhamel et al. 2004), and therefore in all microcosms. Furthermore, after 100 days of incubation more than 2.3 mM acetate was present in microcosms bioaugmented at 10, 20, and 30°C with sediment from all locations, and microcosms bioaugmented at 40°C with sediment from location J10B (Table 2). In the remaining microcosms (i.e., bioaugmented at 40°C with sediments E07B and

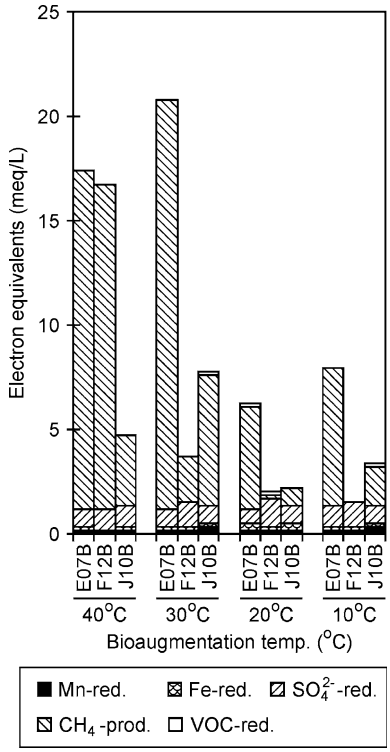

Fig. 2 Magnitude of redox reactions, estimated as transferred electrons 100 days after bioaugmentation. All microcosms were lactate-amended at day 0 and bioaugmented during cooling at 40, 30, 20, and 10°C. Partial redox reactions contributed to the following number of electrons per mole: $Mn_{diss} \times 2$, $Fe^{2+} \times 1$, $SO_4^{2-} \times 8$, $CH_4 \times 4$, TCE × 4, *c*DCE × 4, VC × 2. CH_4 was estimated by including mass in headspace

F12B) the concentrations of the analyzed fatty acids (i.e., lactate, acetate, propionate, and formate) were below 0.3 mM acetate after 100 days of incubation (Table 2). Nevertheless, these microcosms contained 26 nM H_2 in E07B sediments and 30 nM H_2 in F12B sediments, after 48 and 76 days of incubation, respectively (data not shown). At these incubation times, dechlorination stalled at *c*DCE. However, the observed H_2 levels of 26–30 nM were greater than those previously reported in mixed cultures of 0.1–2.5 nM H_2 for *c*DCE reduction and 2–24 nM H_2 for VC reduction at room temperature (Lu et al. 2001; Yang and McCarty 1998). This suggests that sufficient amounts of electron donor were present for reductive dechlorination in these microcosms. This includes microcosms which were bioaugmented at 40°C after 48–76 days of incubation where the temperatures decreased to 13–18°C and no dechlorination occurred.

Overall, the majority of microcosms were anaerobic and showed evidence of methanogenesis and electron donors were present in all microcosms as acetate or H_2. This indicated that redox conditions and electron donor levels, known to be favorable for reductive dechlorination, were achieved. Greatest concentrations of CH_4 were observed in microcosms bioaugmented at 40°C, and in general, the CH_4-production decreased with decreasing bioaugmentation temperature. This demonstrated that methanogens were not inhibited by temperatures of 40°C, corresponding with previous studies (Zehnder 1988).

Dechlorination in KB-1™-amended microcosms

Dechlorination was observed in the majority of microcosms upon bioaugmentation whereas transformation of TCE was not observed in inhibited controls. This indicated that the observed transformation of TCE was biologically mediated. An example of dechlorination over time is demonstrated for sediment J10B in Fig. 3. An overview of dechlorination in all 36 microcosms occurring 150 days after bioaugmentation and after 300 days of incubation is given in Table 3. In microcosms bioaugmented at 40°C (day 0), dechlorination quickly proceeded from TCE to *c*DCE, although VC production was partial and stalled after 20–62 days of incubation in the microcosm demonstrated in Fig. 3. A similar pattern of dechlorination was observed in the remaining microcosms bioaugmented at 40°C, where dechlorination stalled at *c*DCE after 6–62 days of incubation (Table 3). Although microcosms were cooled to 10°C after bioaugmentation at 40°C, dechlorination did not proceed further, demonstrating that temperatures of 30–40°C caused long term irreversible damage to microorganisms dechlorinating *c*DCE to ethene.

Partial dechlorination to *c*DCE was observed at elevated temperatures prior to bioaugmentation. TCE was dechlorinated to *c*DCE within 21 days of incubation at temperatures of 40–30°C

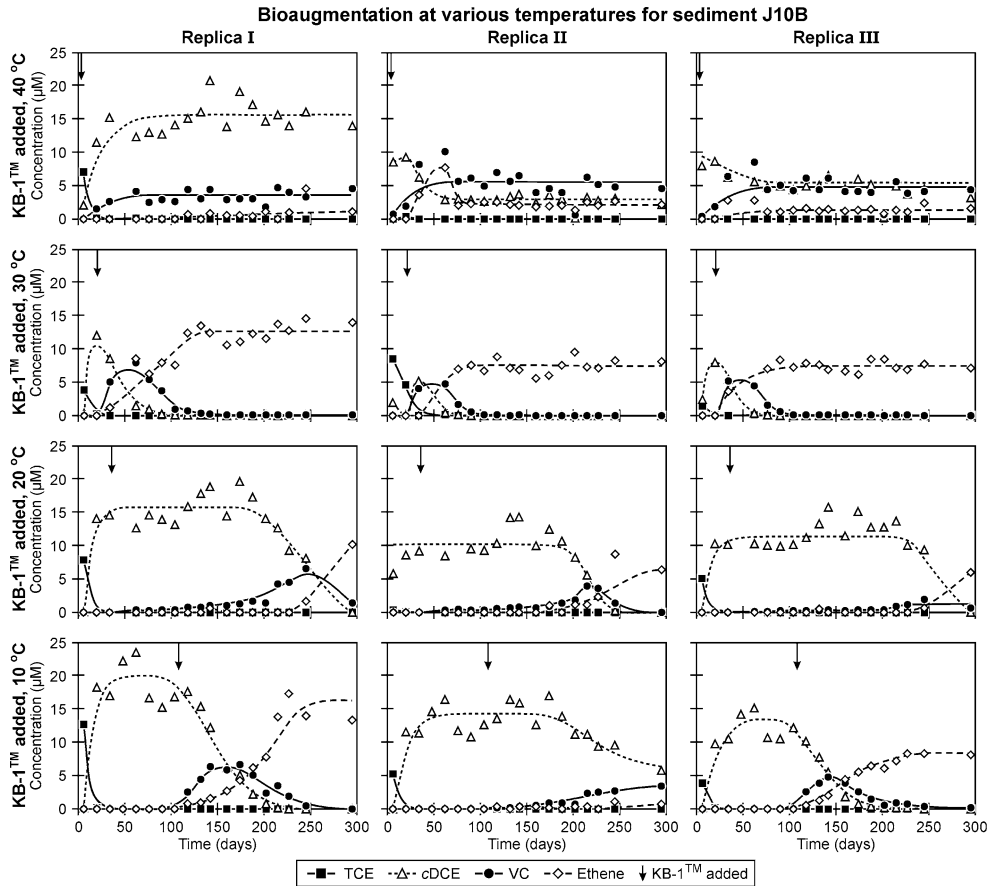

Fig. 3 Dechlorination in individual microcosms with sediment from location J10B. Microcosms bioaugmented at 40°C demonstrated partial dechlorination, and stalled after 62 days of incubation. Microcosms bioaugmented at 30°C demonstated complete dechlorination to ethene within 134 days of incubation. Microcosms bioaugmented at 20°C and 10°C both demonstrated complete dechlorination after 300 days of incubation, although dechlorination was more rapid in microcosms bioaugmented at 10°C. The curves were drawn by hand as visual guidance

in microcosms with sediment from location J10B (Fig. 3). After bioaugmentation at 30°C, dechlorination was complete to ethene within 132 days of incubation (Fig. 3). Complete dechlorination was also observed in microcosms with sediment from locations E07B and F12B, bioaugmented at 30°C, apart from a single microcosm (Table 3). In microcosms bioaugmented at 20°C (day 34), *c*DCE was dominating 150 days after bioaugmentation, although dechlorination proceeded further to ethene in four out of nine microcosms within 300 days of incubation (Table 3). In microcosms bioaugmented at 10°C, *c*DCE was dechlorinated to ethene upon bioaugmentation with sediment from location J10B (Fig. 3). Overall, dechlorination was complete in three out of nine microcosms 150 days after bioaugmentation at 10°C (Table 3).

In general, all microcosms contained sufficient electron donors for reductive dechlorination, and dechlorination was incomplete in the majority of microcosms 150 days after bioaugmentation at 10°C and 20°C. However, out of these 18 microcosms, dechlorination was complete after 300 days of incubation in eight microcosms, ongoing in eight microcosms and stalled in the remaining two microcosms. This demonstrated a potential for subsequent dechlorination with longer incubation periods. In contrast, complete and fast dechlorination was observed upon bio-

Table 3 Overview of dechlorination in triplicate microcosms

Bioaugmentation temperature	Sediment location	Dechlorination 150 days after bioaugmentation		Dechlorination after 300 days of incubation
		End product[a]	Degree of dechl.[b] (%)	
40°C	E07B	*c*DCE (11–24%VC)	40	Stalled since day 6–20
	F12B	*c*DCE (5–16% VC)	39	Stalled since day 34
	J10B	*c*DCE (17–53%VC)	52	Stalled since day 20–62
30°C	E07B	VC (19–61% ethene)	78	Complete to eth, day 202–244
	F12B	Eth	100	Complete to eth, all at day 76
	J10B	Eth	99	Complete to eth, day 104–132
20°C	E07B	*c*DCE	33	Ongoing / stalled since day 6–20
	F12B	*c*DCE (0–34%VC, 0–17%eth)	41	Complete to eth day 62–300 / ongoing
	J10B	*c*DCE (0–11%VC)	36	Complete to eth day 244–300
10°C	E07B	*c*DCE (18–32%VC, 5–8% eth)	45	Ongoing
	F12B	*c*DCE (18–35%VC, 12–100%eth)	67	Ongoing / complete day 230
	J10B	Eth (0–71%*c*DCE, 0–20%VC)	81	Complete to eth, day 202–230 / ongoing

150 days after bioaugmentation and dechlorination after 300 days of incubation. When triplicates behaved differently, the results in two replicas were demonstrated first. Eth indicates ethene

[a] % indicates the fraction of *c*DCE, VC and ethene in moles. Fractions below 5% were not included. TCE concentrations remained below 1% in the calculations and were therefore not included

[b] Estimated for molar masses as: % of *c*DCE × 0.33 + % of VC × 0.66 + % of ethene; and averaged for triplicates. A dechlorination degree of 100% indicated complete conversion to ethene150 days after bioaugmentation and dechlorination after 300 days of incubation. When triplicates behaved differently, the results in two replicas were demonstrated first. Eth indicates ethene

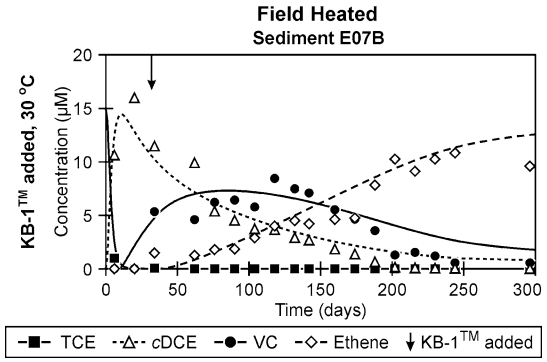

Fig. 4 Example of competitive Monod kinetics model with sediment from location E07B, bioaugmented at 30°C. Curves were simulated from triplicate microcosms at each temperature as described in the text. The model was used to estimate maximal growth rates demonstrated in Fig. 5

augmentation at 30°C. This corresponds to an expected temperature optimum for complete dechlorination at 30°C using KB-1™ (Friis et al. 2007b). Microcosms bioaugmented at 40°C dechlorinated to *c*DCE/VC and stalled even after the temperature decreased. This incomplete dechlorination was most likely caused by the lack or inactivation of dechlorinating microorganisms rather than lack of reducing conditions and/or electron donor availability. This suggests that the dechlorinating capability was destroyed or postponed, when bioaugmenting at temperatures of 40°C with KB-1™.

Dechlorination growth rates

Dechlorination growth rates at various temperatures were compared using a competitive Monod kinetics model in order to quantitatively evaluate the experimental data (Fig. 4). This demonstrated that greatest maximal growth rates of TCE to *c*DCE were observed for bioaugmentation at 40°C (Fig. 5). For the majority of microcosms which were bioaugmented later (i.e., at 30, 20, and 10°C), TCE was rapidly dechlorinated to *c*DCE and prior to bioaugmentation. The higher rates of TCE to *c*DCE dechlorination at 40°C are thus due to a combination of bioaugmentation and elevated temperatures. The efficient transformation of TCE to *c*DCE prior to bioaugmentation shows the robustness of

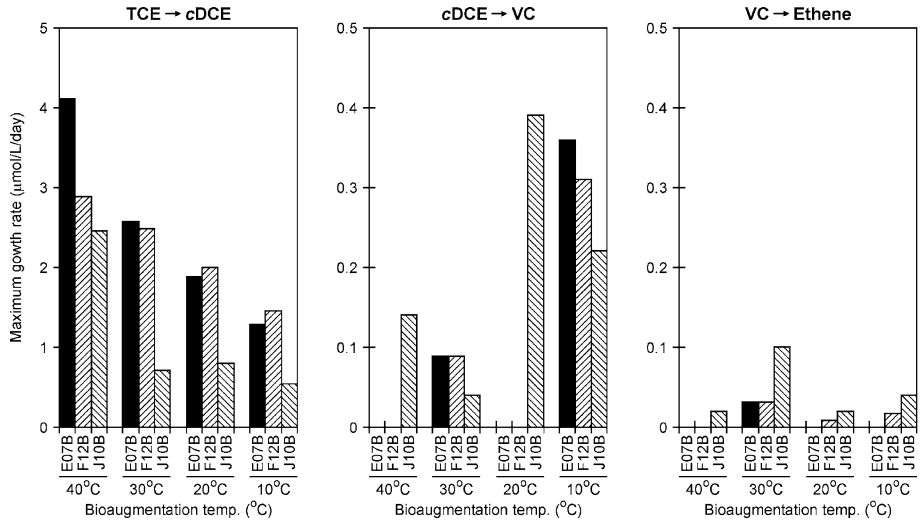

Fig. 5 Maximal growth rates as a function of bioaugmentation temperature estimated using competitive Monod kinetics on data from 0 to 300 days of incubation. Note that the scales for the y-axis vary

TCE to *c*DCE dechlorinators to elevated temperatures. Growth rates of *c*DCE and VC dechlorination were significantly lower (approximately 10 times) than those of TCE, as previously demonstrated (Cupples et al. 2004; Friis et al. 2007b; Yu et al. 2005). In this study, these relative growth rates could, however, also have been influenced by the lower presence of electron donor with time, which is a common issue during sequential dechlorination. Dechlorination of *c*DCE was greatest for microcosms bioaugmented at 10°C and sediment J10B bioaugmented at 20°C. For dechlorination of VC to ethene, the greatest growth rate was observed in microcosms bioaugmented at 30°C in sediment from location J10B (Fig. 5). This can seem in contradiction to the qualitative findings in the previous section, however, the estimated maximum growth rates are influenced by the long lagphase prior to dechlorination. During this period, the decay rate will reduce the biomass concentration to a very low value. When dechlorination initiates, high growth rates are needed in order to simulate the dechlorination.

Table 4 Degradation rates estimated from zero order kinetics

Bioaugmentation temperature	Sediment location	TCE to *c*DCE		*c*DCE to VC		VC to ethene	
		Rate (µmol/l/day)	Lagphase (days)	Rate (µmol/l/day)	Lagphase (days)	Rate (µmol/l/day)	Lagphase (days)
40°C	E07B	2.11 ± 0.77	<6	Below det.	>300	Below det.	>300
	F12B	3.00 ± 0.00	<6	0.13 ± 0.00	34 ± 0	Below det.	>300
	J10B	1.69 ± 0.13	<6	0.07 ± 0.00	4 ± 3	0.03 ± 0.01	20 ± 0
30°C	E07B	2.76 ± 0.42	<6	0.10 ± 0.01	20 ± 0	0.05 ± 0.03	20 ± 14
	F12B	0.44 ± 0.15	2 ± 3	0.70 ± 0.25	20 ± 0	0.70 ± 0.43	20 ± 0
	J10B	2.23 ± 0.60	<6	0.22 ± 0.14	20 ± 0	0.17 ± 0.04	25 ± 8
20°C	E07B	2.28 ± 1.25	<6	Below det.	>300	Below det.	>300
	F12B	0.34 ± 0.07	29 ± 8	0.11 ± 0.03	205 ± 55	0.17 ± 0.12	221 ± 41
	J10B	2.28 ± 0.66	<6	0.04 ± 0.00	137 ± 57	0.17 ± 0.07	214 ± 23
10°C	E07B	2.24 ± 1.31	<6	0.05 ± 0.02	159 ± 16	0.01 ± 0.01	188 ± 0
	F12B	0.80 ± 0.16	85 ± 32	0.07 ± 0.01	173 ± 55	0.09 ± 0.11	160 ± 42
	J10B	1.78 ± 0.80	<6	0.11 ± 0.06	117 ± 22	0.09 ± 0.06	141 ± 64

Rates were estimated as average from triplicate microcosms ± one standard deviation. When concentrations remained below detection limits, rates were not included (indicated as 'below det.'). Lagphases were estimated as the last measuring point prior to detectable concentrations of the transformation product (i.e., *c*DCE, VC, or ethene)

Alternatively a simple zero order degradation kinetic model was applied to compare results from the complex Monods model with a simple alternative by excluding the lagphase prior to dechlorination (Table 4). These results again reveal that the dechlorination rates were highest for TCE to *c*DCE dechlorination, compared to *c*DCE and VC transformation. Furthermore, dechlorination rates of TCE were highest in microcosms bioaugmented at 40°C. However, for dechlorination of *c*DCE and VC, the highest rates and lowest lagphases were observed when the sediments were bioaugmented at 30°C, which is in correspondence with the qualitative findings.

This suggests that growth rates of TCE were increased by bioaugmentation at 40°C, whereas growth rates on *c*DCE and VC generally were higher when bioaugmented at 30, 20, or 10°C and subsequently cooled to 10°C (Fig. 5). Correspondingly, when bioaugmented at 30°C, the dechlorination was initiated much earlier, higher zero order dechlorination for *c*DCE and VC were observed, and complete dechlorination was achieved. This suggests bioaugmentation temperatures at field scale at 30°C or lower. The growth rates, long lagphases, low zero order degradation rates, and incomplete dechlorination underlines the importance of avoiding bioaugmentation at elevated temperatures of 40°C in order to achieve complete dechlorination to ethene.

Implications for bioremediation

Certain aggressive source-zone technologies are suggested to be more promising for the stimulation of sequential reductive dechlorination. Thermal treatment by means of ERH can advantageously be combined with bioaugmentation. For example, increased temperatures after heating can heighten metabolic activity and thereby stimulate biological transformation in the vicinity of thermal treatment zones and inside these zones during cool-down, as demonstrated by this study. Furthermore, redox conditions which will be reached after a thermal treatment may be similar to those observed prior to heating, and organic matter can be released during heating which has been demonstrated in closed microcosms (Friis et al. 2005; Friis et al. 2006b). Although microbial activity and the potential for complete dechlorination to ethene can be decreased or postponed after a thermal treatment (Friis et al. 2007a), bioaugmentation can, as shown in this study, be applied to obtain post-thermal biodegradation. It may actually be beneficial that other microorganisms competing for substrate may be limited after the thermal treatment (Friis et al. 2006a). Lactate, as reducing agent, can as normally done in field scale application be applied prior to bioaugmentation while the subsurface still is at elevated temperatures. This approach provides an excellent opportunity to capitalize on synergies between thermal and biological treatment, using one or more of the following approaches:

- Thermal treatment of hot-spots surrounded by a bioremediation zone and plume treatment. Minimization of the thermally-treated zone reduces overall cost while ensuring compliance.
- Sequential thermal and biological treatment for sites with stringent goals and recalcitrant contaminants.
- Combined solutions, where biological treatment is used both in a perimeter zone and inside the hot-spots during cooling. A subset of the thermal treatment wells are used for electron donor circulation and bioaugmentation.
- Application of bioremediation downgradient a thermally treated area. In these zones, an increased level of dissolved organic carbon (potential electron donor) as well as elevated temperatures can stimulate dechlorination.

Conclusions

This study demonstrates that lactate amendment can be performed at elevated temperatures above 30°C, allowing time for mixing with native groundwater, establishment of anaerobic conditions, and dechlorination of TCE to *c*DCE. Also, bioaugmentation is most favorable once the site has cooled to approximately 30°C to take advantage of short lag times and rapid and complete

dechlorination to ethene, without inhibiting key microorganisms (*Dehalococcoides*) by exposure to high temperatures (i.e., approximately 40°C). These results correspond to the expected temperature optimum for complete dechlorination at 30°C using KB-1^{TM} (Friis et al. 2007b). The results indicate that when these amendments are successfully made, dechlorination of TCE can proceed to ethene, and groundwater cleanup goals can be met.

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