Electron donor availability for microbial reductive processes following thermal treatment

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A B S T R A C T

Thermal treatment is capable of removing significant free-phase chlorinated solvent mass while potentially enhancing bioremediation effectiveness by establishing temperature gradients in the perimeter of the source zone and by increasing electron donor availability. The objectives of this study were to determine the potential for enhanced reductive dechlorination activity at the intermediate temperatures that establish in the perimeter of the heated source zone, and to evaluate the effect of electron donor competition on the performance of the microbial reductive dechlorination process. Microcosms, constructed with tetrachloroethene- (PCE-) and trichloroethene- (TCE-) impacted soils from the Great Lakes, IL, and Ft. Lewis, WA, sites were incubated at temperatures of 24, 35, 50, 70, and 95 °C for 4 months. Reductive dechlorination did not occur in microcosms incubated at temperatures above 24 °C even though mesophilic PCE-to-cis-1,2-dichloroethene dechlorinators were present in Ft. Lewis soil suggesting electron donor limitations. Five days after cooling the microcosms to 24 °C and bioaugmentation with the methanogenic, PCE-to-ethene-dechlorinating consortium OW, at least 85% of the initial PCE and TCE were dechlorinated, but dechlorination ceased prior to complete conversion to ethene. Subsequent biostimulation with hydrogen gas mitigated the dechlorination stall, and conversion to ethene resumed. The results of this study demonstrated that temperatures >35 °C inhibit reductive dechlorination activity at the Great Lakes and Ft. Lewis sites, and that the majority of reducing equivalents released from the soil matrix during heat treatment are consumed in methanogenesis rather than reductive dechlorination. These observations suggest that bioaugmenting thermal treatment sites with cultures that do not contain methanogens may allow practitioners to realize enhanced dechlorination activity, a potential benefit of coupling thermal treatment with bioremediation.

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1. Introduction

Tetrachloroethene (PCE) and trichloroethene (TCE) often exist in the subsurface as dense non-aqueous phase liquids (DNAPLs), which can serve as long-term sources of groundwater contamination (Ramsburg et al., 2004). In situ thermal treatment coupled with soil vapor extraction can rapidly remove significant amounts of chlorinated ethene mass (Heron et al., 1998, 2009; McGuire et al., 2006), but like other source zone remedies, thermal treatment is unlikely to remove 100% of the DNAPL mass (Davis, 1998; Sale and McWhorter, 2001; Christ et al., 2005). Anaerobic bioremediation has been employed successfully for the cleanup of chlorinated ethene plumes (Löffler and Edwards, 2006), and recent studies suggest that bioremediation may be an effective post-treatment polishing step following thermal treatment (Christ et al., 2005; Friis et al., 2006a, 2007). Anaerobic bioremediation of PCE and TCE occurs via stepwise reductive dechlorination reactions, i.e., PCE is transformed to TCE, TCE is transformed to dichloroethenes (mainly cis-1,2-dichloroethene, [cis-DCE]), DCEs are transformed to vinyl chloride (VC), and VC is transformed to non-toxic ethene. While numerous bacteria dechlorinate PCE and TCE to cis-DCE, strictly hydrogenotrophic Dehalococcoides (Dhc) strains are the only known organisms capable of gaining energy from cis-DCE to ethene reductive dechlorination (He et al., 2003).

Dhc have stringent redox, temperature, and nutritional requirements (He et al., 2003; Friis et al., 2007; Amos et al., 2008). While previous studies have suggested that electrical resistance heating (ERH) may have little impact on redox conditions (Friis et al., 2005, 2006b), thermal treatment obviously affects subsurface temperature as source zones are routinely heated to temperatures of 100 °C or greater (Heron et al., 1998). Optimal temperatures for complete microbial reductive dechlorination to ethene are 25–30 °C, higher than those of most aquifers, but much lower than source zone temperatures during heat treatment (Friis et al., 2007; Costanza et al., 2009; Fletcher et al., 2010). Therefore, dechlorination activity is unlikely to occur during active thermal treatment of DNAPL source zones, but accelerated dechlorination and ethene formation may be observed during thermal treatment in the perimeter of the source zone where temperatures are in the range for Dhc activity. To effectively treat residual source zone contamination following thermal treatment, bioaugmentation with Dhc-containing PCE-to-ethene dechlorinating consortia may be necessary (Major et al., 2002; Lendvay et al., 2003; Löffler and Edwards, 2006; Hood et al., 2008; Schuetz et al., 2010).

Previous studies reported the release of organic carbon from the subsurface matrix during thermal treatment and suggested that the increase in bioavailable electron donors could support microbial dechlorination (Friis et al., 2005, 2006a). However, the released organic carbon, suggested to be in the form of long-chain fatty acids (Friis et al., 2005), cannot directly serve as an electron donor for Dhc and must be fermented to produce H₂. Furthermore, Dhc must compete with other organisms, such as methanogens, for available H₂. Thermal treatment has been shown to reduce methanogenesis, even after bioaugmentation with the methanogenic dechlorinating consortium KB-1, suggesting that dechlorinating Dhc may outcompete methanogens for H₂ following thermal treatment (Friis et al., 2006a). Therefore, if thermal treatment both increases H₂ flux and decreases the activity of competing H₂ utilizing microorganisms, dechlorination could be supported by indigenous electron donors, thus alleviating the need for biostimulation (i.e., the introduction of external substrates).

To further explore the utility of combining thermal treatment and bioremediation, (i) the potential for dechlorination activity to occur in the perimeter of the source zone during thermal treatment and (ii) the impact of competing H₂ utilizing (e.g., methanogens) on reductive dechlorination activity were evaluated experimentally. Microcosms, established with soils from two chlorinated ethene-contaminated sites, were incubated at 24, 35, 50, 70, and 95 °C and, following cooling to 24 °C, were bioaugmented with a methanogenic PCE-to-ethene-dechlorinating consortium. Reductive dechlorination and methanogenesis were monitored to determine if the activity of dechlorinators and/or their competitors correlated with previous incubation temperature.

2. Materials and methods

2.1. Site descriptions and soil preparation

Soil and groundwater samples impacted with TCE and cis-DCE (<0.01 μmol/mL of groundwater) were collected from the East Gate Disposal Yard (EGDY) in Fort Lewis, WA, from 28 to 36 feet below ground surface. Flushing with argon gas minimized exposure to oxygen and the analysis of geochemical parameters suggested reducing soil conditions prevailed (Costanza et al., 2009). The soil consisted of well-graded gravel in sand, silt, and clay with a total carbon content (TOC) of 0.69 g TOC/kg. Additional details about the Ft. Lewis site are available (Friis et al., 2007). Ft. Lewis soil samples were collected prior to thermal treatment activities. Additional soil and groundwater samples impacted with PCE and less than 0.02 μmol/mL TCE, cis-DCE, and VC were collected from a former dry cleaner facility at the Naval Training Center in Great Lakes, IL, from between 8 and 10 feet below ground surface (Costanza et al., 2009, 2010). During sampling, the soil was exposed to air before it was broken into fragments with diameters no larger than 10 mm inside a disposable glove bag filled with ultra high purity argon. The solids were combined with an equal weight of site groundwater to create a slurry. Samples from both sites were collected prior to the commencement of thermal treatment activities.

2.2. Microcosm construction, incubation conditions, and sampling

A total of 30 Ft. Lewis microcosms were constructed at room temperature (24 °C) inside an anoxic chamber (Coy Laboratory Products, Ann Arbor, MI) that contained a 96% N₂/4% H₂ (vol/vol) atmosphere. Serum bottles (70 mL, nominal capacity) were filled with 15 mL groundwater and soil was added to achieve a total volume of approximately 37 mL. A total of 20
Great Lakes microcosms were constructed at room temperature inside an argon-filled glove bag. In 70 mL serum bottles, the soil/groundwater slurry was combined with 10–20 mL of sterile mineral salts medium (Amos et al., 2007) or site groundwater to yield a total volume of 40 mL. Mineral salts medium replaced groundwater when insufficient site groundwater was available. All microcosms were capped with sterile black butyl-rubber stoppers prior to removal from the anoxic tents. Preliminary experiments demonstrated that the headspace composition during microcosm setup did not change dechlorination profiles in the Ft. Lewis microcosms (unpublished data; Costanza et al., 2009).

Immediately following microcosm construction, five Great Lakes and 15 Ft. Lewis microcosms were autoclaved at 121 °C for 30 min to serve as killed controls. Triplicate Great Lakes and Ft. Lewis live microcosms and single Great Lakes and triplicate Ft. Lewis autoclaved controls were incubated statically in the dark in water baths adjusted to 24, 35, 50, 70, and 95 °C. The temperature in microcosms incubated at 35 and 50 °C was increased by 1 °C per day whereas all other microcosms were immediately placed at the target temperatures. The 24–95 °C temperature range was selected to include both optimal temperatures for the microbial reductive dechlorination process and temperatures that occur during thermal treatment.

Because of the low chlorinated solvent concentrations in Ft. Lewis soil microcosms (less than 0.5 μmoles/microcosm) after 27 days of incubation, Ft. Lewis microcosms were amended with 5 μmoles of TCE dissolved in 2.25 mL filter-sterilized, anoxic Ft. Lewis site groundwater. Great Lakes microcosms received no additional chlorinated solvent because PCE concentrations were at least 20 μmoles/microcosm. Following 4 months of incubation, the microcosms were cooled at a rate of 5 °C per day to room temperature (24 °C). An incubation period of 4 months was chosen to mimic site conditions where thermal remediation is generally employed for time periods of greater than weeks, but less than years (Heron et al., 2009).

Microcosms were sampled periodically for chlorinated ethenes and gases including acetylene, CH₄, PCE, TCE, VC, cis-DCE, and H₂. Aqueous (1 mL) and gaseous (2 mL) samples were removed from microcosms using sterile plastic syringes with needles. Removed aqueous volumes were replaced with filter-sterilized groundwater or sterile medium, and removed gaseous volumes were replaced with sterile, oxygen-free N₂ gas.

### 2.3 Transfer culture preparation, incubation conditions, and sampling

After 138 days of incubation, 2 mL aqueous volumes were removed from each of the three Ft. Lewis microcosms previously incubated at 35 °C and transferred to individual 160 mL serum bottles containing 100 mL mineral salts medium amended with vitamins (Wolin et al., 1963), 5 mM lactate, and 4 μmoles of TCE. The transfer cultures were initially incubated at 24 °C and 1 mL aqueous samples were removed periodically for chlorinated ethene quantification. Once TCE was completely dechlorinated to cis-DCE during incubation at 24 °C, transfer cultures were amended with another 4 μmoles of TCE and incubated at 35 °C. A second batch of 35 °C transfer cultures was generated by transferring culture suspension (3%, vol/vol) to the same mineral salts medium.

### 2.4 Bioaugmentation and biostimulation

After the microcosms were cooled to 24 °C, 10 mL of culture OW, a methanogenic, PCE-to-ethene-dechlorinating consortium, was added to both live and autoclaved microcosms. Consortium OW contains multiple Dhc strains along with Geobacter, Dehalobacter, and Sulfurospirillum populations implicated in chlorinated ethene reductive dechlorination (Daprato et al., 2007). Prior to inoculation, the OW consortium was removed from a draw-and-fill bioreactor (Daprato et al., 2007) and sparged with N₂ gas for 30 min to remove chlorinated ethenes, ethene, and CH₄. Inoculation (i.e., bioaugmentation) of Ft. Lewis and Great Lakes microcosms occurred on days 139 and 141 of incubation, respectively. The initial biostimulation event in Ft. Lewis and Great Lakes microcosms involved the amendment of microcosms with 5 mL (200 μmoles) of sterile H₂ gas. Secondary biostimulation involved a one-time addition of 5 mL H₂ gas to Ft. Lewis microcosms and involved the biweekly addition of 5 mL of H₂ gas to Great Lakes microcosms. Initial and secondary biostimulation in Ft. Lewis microcosms occurred on days 153 and 258 of incubation or 14 and 119 days following bioaugmentation, respectively. In Great Lakes microcosms, initial and secondary biostimulation occurred on days 164 and 234 of incubation or 23 and 93 days following bioaugmentation, respectively.

### 2.5 Analytical methods and calculations

Aqueous samples (1 mL) were collected for the quantification of chlorinated ethenes and ethene by gas chromatography (GC) following described procedures (Amos et al., 2007). Gaseous samples (2 mL) were collected for analysis of acetylene, CH₄, CO₂, and H₂ using a Hewlett Packard 6890 GC equipped with a heated gas sampling valve, a 250 μL sample loop, and a 30 m length by 0.32 mm OD Carboxen-1010 column (Supelco, Bellefonte, PA) connected to a thermal conductivity detector. Detection limits were determined based on the lowest concentrations that were consistently quantifiable.

To determine the rate of CO₂ production, average CO₂ concentrations measured after 7, 28, 62, 93, and 121 days were plotted versus incubation time. The data were fitted using linear regression analysis and, when concentrations were correlated with incubation time, the rate of CO₂ production was determined from the slope of the linear regression line.

The molar percent of a specific chlorinated ethene was determined according to the formula:

\[
\frac{M_x}{(M_{\text{PCE}} + M_{\text{TCE}} + M_{\text{DCE}} + M_{\text{VC}} + M_{\text{ethene}})} = M_{\text{PFX}}
\]  

(1)

Where \( M_x \) is the number of moles of the compound of interest (e.g., VC), \( M_{\text{PCE}} \) is the number of moles of PCE, \( M_{\text{TCE}} \) is the number of moles of TCE, \( M_{\text{DCE}} \) is the number of moles of cis-DCE, \( M_{\text{VC}} \) is the number or moles of vinyl chloride, \( M_{\text{ethene}} \) is the number of moles of ethene, and \( M_{\text{PFX}} \) is the molar percentage of the compound of interest. To determine how molar percentages change with time, the following formula was applied:
MP_{x,tf} - MP_{x,ti} = \Delta MP_x \tag{2}

Where MP_{x,tf} is the final molar percentage of the compound of interest, MP_{x,ti} is the initial molar percentage of the compound of interest, and \Delta MP_x is the change in the molar percentage of the compound of interest over time, which is also represented as a percentage.

The calculation of the moles of electrons consumed in reductive dechlorination of PCE-to-ethene assumed that each dechlorination step resulted in two electrons (Löffler and Edwards, 2006) and therefore, the moles of electrons consumed in reductive dechlorination were calculated for the Great Lakes microcosms according to the formula:

\[ 2(M_{DCE}) + 4(M_{VC}) + 6(M_{ethylene}) = M_{consumed} \tag{3} \]

Where \( M_{consumed} \) is the number of moles of electrons consumed in reductive dechlorination of PCE to ethene. The moles of electrons used for reductive dechlorination of TCE to ethene were determined according to the formula:

\[ 2(M_{TCE}) + 4(M_{DCE}) + 6(M_{VC}) + 8(M_{ethylene}) = M_{consumed} \tag{4} \]

The calculation of the moles of electrons consumed in methanogenesis assumed that 4 moles of electrons are required per mole of CH\(_4\) formed (Ferry, 1999).

Dechlorination extent in the Great Lakes microcosms was determined according to the formula:

\[ (100)/[1(M_{TCE}) + 2(M_{DCE}) + 3(M_{VC}) + 4(M_{ethylene})]/[4(M_{tot})] = DE \tag{5} \]

Where \( M_{tot} \) is the total number of moles of chlorinated ethenes and ethene, and DE is the dechlorination extent in percent (Friis et al., 2006b).

In the Ft. Lewis microcosms, dechlorination extent was determined according to the formula:

\[ (100)/[1(M_{DCE}) + 2(M_{VC}) + 3(M_{ethylene})]/[3(M_{tot})] = DE \tag{6} \]

Concentrations measured in microcosms were compared using two-tailed student’s t-tests and correlations were identified based on Pearson product moment correlation coefficients. In all cases, \( p \)-values below 0.05 were considered significant. In order to visualize values for multiple variables and correlations between variables in the same space, principal component analysis (PCA) was performed using the ViSta program (Young, 1996). PCA is a statistical approach used with multivariate data to reduce the number of dimensions that account for the observed variance to principal components (PCs). Prior to PCA, the data were normalized so that all arithmetic means had values of 0 and standard deviations of 1 using the ViSta program. The outputs of the PCA were the eigenvalue coordinates for specific microcosm treatments, the eigenvectors, which correspond to measured variables (e.g., the methane concentration, the time required for dechlorination), and the percentages of the total variation explained by each PC (e.g., PC 1 and PC 2). Eigenvectors and eigenvectors were plotted versus the PCs encompassing the highest percentages of the total variation (i.e., PC 1 and PC 2). Eigenvector coordinates can be used to infer how similar the variables included in the PCA were between microcosms. The eigenvalue direction and length provide information as well. For example, eigenvectors pointing in opposite directions indicate that variables have opposite profiles and may be negatively correlated. The longer the eigenvector, the more strongly the variable is related to the eigenvalue coordinates.

3. Results

3.1. Incubation at elevated temperatures

3.1.1. Ft. Lewis microcosms

After 28 days of incubation, \( H_2 \) concentrations in live Ft. Lewis microcosms incubated at 24, 35, and 50 °C were significantly lower \(( p < 0.05)\) compared to those measured in autoclaved microcosms (Fig. 1). In contrast, \( H_2 \) concentrations in live Ft. Lewis microcosms incubated at 70 and 95 °C were not significantly lower than those in autoclaved microcosms (Fig. 1). This pattern persisted throughout the 120 day incubation period. \( CO_2 \) concentrations in all live microcosms, except those previously incubated at 95 °C, were significantly lower \(( p < 0.05)\) after 28 days of incubation than concentrations in autoclaved control microcosms incubated at the same temperatures. \( CO_2 \) concentrations in live Ft. Lewis microcosms ranged from 1830 ± 930 to 10,360 ± 245 ppmv in microcosms incubated at 24 and 95 °C, respectively, and were positively correlated with incubation temperature \(( p < 0.05)\). Between day 7 and day 121, \( CO_2 \) concentrations increased in all live microcosms and were positively correlated with incubation time \(( p < 0.05)\) in microcosms incubated at 24, 35, 50, and 70 °C. In fact, \( CO_2 \) production rates decreased exponentially with increasing temperature (Fig. 2). No positive correlation was observed between \( CO_2 \) concentrations and incubation time in the autoclaved microcosms. After 120 days of incubation, TCE concentrations in all live microcosms, except those incubated at 24 °C, were not significantly lower than concentrations in autoclaved microcosms incubated at the same temperatures (Fig. S1). In live Ft. Lewis microcosms incubated at 24 °C, TCE decreased to below the detection limit.

![Fig. 1 - \( H_2 \) concentrations in live (filled bars) and autoclaved (open bars) Ft. Lewis microcosms after 28 days of incubation. The asterisk indicates that \( H_2 \) was below the detection limit in live microcosms incubated at 24 °C. Error bars represent the standard error.](image-url)
of 0.05 μmoles/microcosm after 120 days of incubation and cis-DCE concentrations increased concomitantly; however, dechlorination beyond cis-DCE did not occur.

3.1.2. Great Lakes microcosms
In Great Lakes microcosms, H₂ concentrations were below the detection limit in all microcosms after 58 days of incubation. After 28 days of incubation, CO₂ concentrations were lower in all live microcosms incubated at 24, 35, 50, and 70 °C (37,330 ± 2,730, 44,560 ± 6,870, 44,830 ± 4,320, and 37,620 ± 1080 ppmv, respectively) than in autoclaved microcosms incubated at the same temperatures (53,450, 73,450, 57,970, and 48,680 ppmv, respectively); however, CO₂ concentrations in live microcosms incubated at 95 °C (48,670 ± 3360 ppmv) were similar to those in the autoclaved microcosm incubated at 95 °C (49,500 ppmv). In live microcosms incubated at 24, 50, and 70 °C, CO₂ concentrations were positively correlated (p < 0.05) with incubation time and increased to 60,680 ± 5810, 64,020 ± 9,480, and 75,060 ± 1740 ppmv, respectively, after 123 days of incubation. CO₂ concentrations were also positively correlated (p < 0.05) with incubation time in autoclaved microcosms incubated at 50 and 70 °C and increased to 97,460 and 70,990 ppmv, respectively, after 123 days of incubation. After 123 days of incubation, PCE persisted in all Great Lakes microcosms, but lower PCE concentrations were measured in the autoclaved microcosms incubated at 24, 35, 50, and 70 °C compared to the live microcosms incubated at the same temperatures (Fig. S1). The PCE concentration in the autoclaved microcosm incubated at 95 °C was within the range of concentrations measured in the live microcosms incubated at 95 °C (11.0 and 11.4 ± 1.2 μmoles/microcosm, respectively). TCE, cis-DCE, and VC concentrations were similar in live and autoclaved microcosms, and the concentrations of possible PCE dechlorination products in all microcosms remained below 0.5 μmoles/microcosm.

3.2. Effects of cooling
Ft. Lewis and Great Lakes microcosms were cooled to 24 °C from elevated temperatures to determine if microbial activity would resume and in preparation for bioaugmentation. The concentration of H₂ decreased significantly (p < 0.05) during cooling in live Ft. Lewis microcosms previously incubated at 70 °C whereas H₂ concentrations changed insignificantly in autoclaved Ft. Lewis microcosms previously incubated at 70 °C. With the exception of the Ft. Lewis microcosms incubated at 24 °C, PCE and TCE persisted in all Great Lakes and Ft. Lewis microcosms, respectively, and no dechlorination products were formed. Higher PCE and TCE concentrations were present in microcosms previously incubated at lower temperatures and PCE and TCE concentrations were negatively correlated with previous incubation temperature (p < 0.05).

3.3. Reductive dechlorination in Ft. Lewis transfer cultures
Since reductive dechlorination occurred in Ft. Lewis microcosms incubated at 24 °C, the lack of dechlorination activity in Ft. Lewis microcosms previously incubated at 35 °C was investigated. In transfer cultures established from microcosms previously incubated at 35 °C and cooled to 24 °C, stoichiometric reductive dechlorination of TCE to cis-DCE occurred in 20 days during incubation at 24 °C, indicating that dechlorinators survived the extended 35 °C incubation period. After the transfer cultures were placed at 35 °C and amended with additional TCE, TCE was completely dechlorinated to cis-DCE within 2 days. When the culture suspension was transferred to fresh medium, TCE to cis-DCE reductive dechlorination activity again occurred during incubation at 35 °C.

3.4. Reductive dechlorination following bioaugmentation
Dechlorination of chlorinated ethenes to ethene did not occur in any of the Ft. Lewis or Great Lakes microcosms and all microcosms were bioaugmented with the PCE-to-ethene-dechlorinating consortium OW. In Ft. Lewis microcosms, at least 95% of the TCE was dechlorinated to VC within 5 days of bioaugmentation (Fig. 3 and Figs. S3, S4). In Great Lakes microcosms, at least 85% of the PCE was dechlorinated to cis-DCE and VC 3 days after bioaugmentation (Fig. 3 and Figs. S2–S4). The only Ft. Lewis microcosms demonstrating a significant increase (p < 0.05) in the molar percentage of ethene 10–13 days following bioaugmentation were live microcosms previously incubated at 24 °C. No significant increase in the molar percentages of VC and ethene occurred in any of the live Great Lakes microcosms between 11 and 23 days following bioaugmentation. In autoclaved Great Lakes microcosms, the molar percentage of VC increased by a maximum of 7.9% between day 11 and day 23 following bioaugmentation (Fig. 3). In all Ft. Lewis and Great Lakes
microcosms, H₂ concentrations were below the detection limit 13 and 23 days after bioaugmentation, respectively.

The electron consumption in reductive dechlorination was calculated to determine how previous incubation temperature affected electron donor utilization (Fig. 4). Over an order of magnitude more electrons were consumed in the reductive dechlorination process in Great Lakes microcosms than in Ft. Lewis microcosms. In microcosms from both sites, the moles of electrons consumed in reductive dechlorination following bioaugmentation (and prior to biostimulation) were negatively correlated with previous incubation temperature \( (p < 0.05) \) (Fig. 4). Apparently, more reducing equivalents were directed toward the reductive dechlorination in microcosms that had been incubated at lower temperatures.

3.5. Reductive dechlorination following biostimulation with H₂

Because dechlorination ceased prior to complete conversion of chlorinated ethenes to ethene, the microcosms were biostimulated with H₂ 14 days after bioaugmentation. The molar percentage of VC in Ft. Lewis microcosms decreased by 47.3 ± 34.2% 17 days after biostimulation (i.e., 31 days following bioaugmentation) and, with the exception of live microcosms previously incubated at 50 and 70 °C, 90% of VC was dechlorinated to ethene within 42 days of biostimulation (Table 1, Fig. 3). In Ft. Lewis live microcosms previously incubated at 50 and 70 °C, at least 70% of the initial TCE existed as VC 92 days after biostimulation in two of three and one of three microcosms, respectively. In all of these microcosms, H₂ concentrations were below the detection limit. Following a second H₂ amendment 105 days after the initial biostimulation, ethene production continued and 90% of the VC was dechlorinated within 29 days (Table 1, Fig. 3). In Great Lakes microcosms, the molar percentage of VC increased by 11.3 ± 7.4% due to the conversion of cis-DCE to VC 2 days after biostimulation (25 days following bioaugmentation). In autoclaved and live Great Lakes microcosms previously incubated at 95 °C, 100% of VC was converted to ethene 29 and 60 days after biostimulation, respectively. The molar percentage of VC did not decrease significantly from 60 to 68 days following biostimulation in live Great Lakes microcosms previously incubated at temperatures below 95 °C. In autoclaved Great Lakes microcosms previously incubated at the same temperatures, the molar percentage of VC decreased by a maximum of 3% during the same period (Fig. 3). No H₂ was detected in any of the Great Lakes microcosms 68 days after biostimulation. The molar percentage of VC decreased by 41.6 ± 24.1% in response to 12 days of biweekly biostimulation. Within 27 days of the beginning of secondary biostimulation, VC was consumed to below the detection limit of 0.05 μmoles per vessel in all microcosms except the live microcosms previously incubated at 24 and 70 °C (Table 1, Fig. 3). In live microcosms previously incubated at 24 and 70 °C, the VC

Fig. 3 – The change in the molar percentage of VC with time after bioaugmentation in Ft. Lewis and Great Lakes microcosms previously incubated at 24 (crosses), 35 (circles), 50 (triangles), 70 (diamonds), and 95 °C (squares) in live (filled symbols) and autoclaved (open symbols) microcosms. Vertical dashed lines indicate the initial biostimulation event and the second biostimulation event (Ft. Lewis microcosms) or the beginning of biweekly secondary biostimulation (Great Lakes microcosms). Error bars have been omitted for clarity. Note that the time scales on the x-axes are shorter prior to the initial biostimulation event.
molar percentages were 0.5 ± 0.1% and 73.6 ± 37.4% 27 days after the beginning of secondary biostimulation. The times required for 90% of VC to be dechlorinated to ethene in Ft. Lewis microcosms and for 100% of VC to be dechlorinated to ethene in Great Lakes microcosms were not correlated with previous incubation temperatures.

3.6. Competition for reducing equivalents

Prior to bioaugmentation, CH₄ was below 600 ppmv in all microcosms. Within 23 days of bioaugmentation, average CH₄ concentrations were at least 21,000 ppmv (Fig. 5). The ratio of the number of electrons consumed in methanogenesis versus reductive dechlorination prior to biostimulation was at least 1,300, indicating that the dechlorinators consumed less than 0.08% of the available reducing equivalents. CH₄ concentrations in live Ft. Lewis microcosms previously incubated at 24 and 35 °C were significantly higher (p < 0.05) than in the autoclaved microcosms incubated at the same temperatures. In live Ft. Lewis microcosms previously incubated at temperatures of 50, 70, and 95 °C, CH₄ concentrations were not significantly different from concentrations in autoclaved microcosms incubated at the same temperatures.

To compare electron donor consumption in reductive dechlorination versus methanogenesis, the CH₄ concentration and dechlorination extent eigenvectors point in a similar direction and CH₄ concentration was significantly positively correlated (p < 0.05) with dechlorination extent. The CH₄ concentration eigenvector is perpendicular to the eigenvector for the moles of electrons consumed in reductive dechlorination, and CH₄ concentrations were significantly negatively correlated (p < 0.05) with the moles of electrons consumed in dechlorination. These results indicate that microcosms that produced more CH₄ consumed fewer electrons in reductive dechlorination, but also exhibited higher dechlorination extents because lower PCE concentrations were present prior to bioaugmentation. The PC1 eigenvalues of microcosms previously incubated at lower temperatures were generally lower than those of microcosms that
experienced higher previous incubation temperatures. The direction of the eigenvectors suggests that microcosms previously incubated at lower temperatures consumed more electrons in the reductive dechlorination process and produced less CH$_4$. In fact, the previous incubation temperature was significantly positively correlated ($p < 0.05$) with CH$_4$ concentration, indicating that microcosms previously incubated at lower temperatures generally produced less CH$_4$ than microcosms incubated at higher temperatures.

In Ft. Lewis microcosms, CH$_4$ concentrations increased by over 30,000 ppmv following the initial H$_2$ biostimulation event and increased by 16,070 ± 9370 ppmv following the second biostimulation event (Fig. 5). At least 19,000 ppmv of CH$_4$ were produced following the initial biostimulation event and prior to the beginning of secondary biostimulation events in all Great Lakes microcosms except for in those microcosms previously incubated at 50 °C (Fig. 5). During the period following the initial biostimulation and prior to secondary biostimulation, no CH$_4$ was produced in the live Great Lakes microcosms previously incubated at 50 °C, and CH$_4$ concentrations increased by less than 3000 ppmv in autoclaved microcosms previously incubated at 50 °C (Fig. 5). In Great Lakes microcosms, CH$_4$ concentrations increased by at least 74,000 ppmv during secondary biostimulation. These concentration increases were not correlated with previous incubation temperature.

CH$_4$ concentration, the time required to achieve 90% VC dechlorination, and the number of electrons consumed in

<table>
<thead>
<tr>
<th>Previous incubation temperature</th>
<th>Ft. Lewis microcosms</th>
<th>Great lakes microcosms</th>
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<tbody>
<tr>
<td></td>
<td>Live</td>
<td>Autoclaved</td>
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<td>95 °C</td>
<td>48 (34)</td>
<td>20 (6)</td>
</tr>
</tbody>
</table>

Fig. 5 – The concentration of CH$_4$ prior to biostimulation (dark gray bars), following the initial biostimulation event (light gray bars), and following secondary biostimulation (white bars). Error bars represent the standard error and negative error bars have been removed for clarity. No error bars are shown for autoclaved Great Lakes microcosms because only one microcosm was incubated at each temperature.
reductive dechlorination in Ft. Lewis microcosms following 90% VC dechlorination were compared using PCA (Fig. 6). None of the eigenvectors point in the same direction and, in fact, none of these variables were significantly positively correlated. Further, as all eigenvalues have similar lengths, each of these variables contributed approximately equally to eigenvalue variation. Similar to the PCA results obtained with the Great Lakes microcoms, the PC1 eigenvalues of microcosms previously incubated at lower temperatures were generally lower than those of microcosms previously incubated at higher temperatures. Based on the directions of eigenvectors, these findings indicate that microcosms previously incubated at lower temperatures were generally lower than those of microcosms previously incubated at higher temperatures. Based on the directions of eigenvectors, these findings indicate that microcosms previously incubated at lower temperatures produced less CH₄ and required more electrons for 90% of VC to be dechlorinated to ethene. In fact, previous incubation temperature was positively correlated \((p < 0.05)\) with CH₄ concentrations and negatively correlated \((p < 0.05)\) with the number of electrons consumed in reductive dechlorination (Fig. 6). Fewer electrons were required for 90% of VC to be dechlorinated to ethene in micorcosms previously incubated at elevated temperatures and TCE concentrations following cooling were negatively correlated with previous incubation temperature.

4. Discussion

4.1. No degradation of chlorinated compounds during incubation at elevated temperatures

PCE and TCE concentrations in autoclaved microcosms were either similar to or less than the concentrations in live microcosms (with the exception of the Ft. Lewis microcosms incubated at 24 °C), indicating that biotic dechlorination did not occur in the Ft. Lewis microcosms incubated at temperatures of 35 °C and above or in any of the Great Lakes microcosms. Previous studies conducted with soils collected from the same sites demonstrated that the half-lives of PCE and TCE were approximately 7000 and 640 days during incubation at 95 °C (Costanza and Pennell, 2007b; Costanza et al., 2009). Therefore, the differences in PCE and TCE concentrations are primarily due to loss (i.e., diffusion through or sorption to the rubber stopper) rather than degradation. This conclusion is consistent with a previous report, which demonstrated that the loss of PCE and TCE from vials sealed with polymer septa and incubated at 50 °C was due to diffusion through and sorption to the septa (Costanza and Pennell, 2007a). The lack of microbial reductive dechlorination activity in Ft. Lewis microcosms previously heated in the laboratory to 100 °C was also observed in an earlier study (Friis et al., 2007). Therefore, for both the Ft. Lewis and Great Lakes sites, transformation of PCE and TCE in the source zone is not likely to occur during thermal treatment.

4.2. Lack of dechlorination activity at non-inhibitory temperatures

Transfer culture experiments demonstrated that microorganisms capable of TCE to cis-DCE dechlorination at 35 °C were present in microcosms incubated at 35 °C, yet no dechlorination occurred. Ft. Lewis soils and microcosms were handled under anoxic conditions, and previous studies have shown that redox conditions do not change due to heating (Friis et al., 2005, 2007). Therefore, unfavorable geochemical/redox conditions cannot explain the lack of dechlorination activity. Increased temperatures affect the activity of both fermenters and consumers of fermentation products, including microbes that compete with the dechlorinators for electron donors (e.g., H₂) (Wiegel, 1990; Rui et al., 2009; Noll...
et al., 2010). Therefore, the lack of dechlorination activity in microcosms incubated at 35 °C may have been due to inadequate electron donor availability. These findings indicate that dechlorination may not occur without electron donor amendment even when native bacteria capable of dechlorination are present and experience non-inhibitory temperatures. Thermal treatment has been shown to release bioavailable electron donors (Friis et al., 2005, 2006a); however, the findings of our study emphasize that careful evaluation of the electron donor availability (e.g., H₂ concentration measurements) should be performed to ensure that the supply of reducing equivalents does not limit reductive dechlorination activity following thermal treatment.

4.3. Biostimulation was required to achieve complete dechlorination to ethene following bioaugmentation

The production of CH₄ coupled to the accumulation of cis-DCE and VC in all Ft. Lewis and Great Lakes microcosms following bioaugmentation demonstrates that the supply of electron donor was inadequate to achieve complete dechlorination to ethene due, at least in part, to consumption of H₂ by competitors. Prior to the stall in dechlorination activity, on average one order of magnitude more electrons were consumed in reductive dechlorination in Great Lakes microcosms than in Ft. Lewis microcosms, but the major dechlorination products in all microcosms were cis-DCE and VC. These results suggest that cis-DCE and VC-dechlorinating Dhc were electron donor (i.e., H₂) limited whereas the PCE and TCE dechlorinators were more competitive for electron donor(s). It is likely that methanogens outcompeted cis-DCE and VC-dechlorinating Dhc, but not PCE and TCE-dechlorinators, because (i) Dhc are strict hydrogenotrophs whereas PCE/TCE dechlorinators are more versatile in terms of electron donor requirement, (ii) reductive dechlorination of cis-DCE and VC generally occurs at slower rates than PCE and TCE dechlorination, and (iii) VC dechlorination is inhibited by polychlorinated ethenes (Hashton and McCarty, 1999; Maymó-Gatell et al., 1999).

After 90% of VC had been dechlorinated in Ft. Lewis microcosms, CH₄ concentrations were positively correlated with previous incubation temperature. That is, more CH₄ was produced in microcosms previously incubated at elevated temperatures, likely by methanogens introduced with the bioaugmentation inoculum. Similarly, following bioaugmentation in Great Lakes microcosms, more CH₄ was generally produced in microcosms previously incubated at higher temperatures. Possible reasons for the higher concentrations of CH₄ in microcosms previously incubated at higher temperatures include the elevated concentrations of CO₂, which serves as an electron acceptor for methanogens (Ferry, 1999) and the release of fermentable carbon substrates as a source of H₂, allowing fast-growing H₂-consumers (e.g., hydrogenotrophic methanogens) to dominate (He et al., 2002).

4.4. Inhibition of reductive dechlorination following thermal treatment

Dechlorination was incomplete in the live Great Lakes microcosms previously incubated at 70 °C even after bioaugmentation and during secondary, biweekly biostimulation, suggesting that inhibitory conditions prevailed. In fact, microcosms constructed with soils collected from both the Ft. Lewis and Great Lakes sites demonstrated limited dechlorination activity following cooling from 70 °C. In a previous bioaugmentation and biostimulation microcosm study, dechlorination of TCE to VC and ethene occurred in unheated microcosms whereas TCE was only dechlorinated to cis-DCE in microcosms that had previously been heated to 100 °C (Friis et al., 2006a). These observations suggest that exposing soils to elevated temperatures can impact dechlorination activity following cooling, possibly due to the release of compounds from the soil matrix that inhibit the microbial reductive dechlorination process. While limited dechlorination was observed in bioaugmented and biostimulated Ft. Lewis and Great Lakes microcosms previously incubated at 70 °C, bioaugmentation and biostimulation supported complete reductive dechlorination to ethene in microcosms previously incubated at 95 °C. These observations suggest that thermal treatment can affect the release and/or availability of compounds inhibitory to the reductive dechlorination process.

4.5. Competition for reducing equivalents

Thermal treatment reduces microbial cell numbers and opens up ecological niches that will be reoccupied following cooling. In a natural setting, microbes surviving the heat treatment (e.g., spore-formers) and microbes transported with the groundwater flow from up-gradient locations will colonize available niches over time. The application of thermal treatment combined with bioaugmentation offers an opportunity to establish microbes with desirable activities, in this case bacteria capable of reductive dechlorination of chlorinated ethenes, in the thermally treated zones. Bioaugmentation can achieve a “founder effect” and establish a microbial community that efficiently ferments the available organic substrates (i.e., electron donors released during heating) to generate H₂. The increased H₂ flux supports chlorinated ethene-respiring bacteria, including Dhc, leading to the enhanced reductive dechlorination of residual contaminants. Since methanogens compete for the same electron donor (i.e., H₂) and generally exhibit higher growth rates than chlorinated ethene-respiring bacteria, methanogens will dominate thermally treated sites following cooling (i.e., methanogens will reoccupy available niches more rapidly and consume H₂). Therefore, consortia without methanogens may offer an advantage for implementing bioaugmentation at thermally treated sites and the application of methanogen-free, PCE-to-ethene-dechlorinating consortia should be explored at thermally treated sites.

5. Conclusions

This study provides new information for the application of the microbial reductive dechlorination process during and following thermal treatment. The findings from this study are relevant for thermal treatment sites; however, the extrapolation of the findings to field sites should be done cautiously because only laboratory experiments were conducted with materials from two field sites. The major conclusions from this study include:
Elevated temperatures above 35 °C inhibit microbial reductive dechlorination of chlorinated ethenes.

Even when temperatures are not inhibitory to dechlorinating populations, dechlorination may not occur due to electron donor limitations (i.e., the electron donors released during soil heating are insufficient or are being consumed in competing microbial processes).

Thermal treatment may generate conditions favorable for competing microbial processes. Specifically, methanogenesis may consume a significant fraction of the reducing equivalents available from electron donor(s) released during thermal treatment, thus negatively impacting dechlorination extent. This observation suggests that bioaugmentation with non-methanogenic consortia can realize higher efficiency in terms of reducing equivalents directed toward the reductive dechlorination process.

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Appendix. Supplementary data

Supplementary data associated with this article can be found in online version at doi:10.1016/j.watres.2011.09.033.

References


