Simultaneous Anaerobic Transformation of Tetrachloroethene and Carbon Tetrachloride in a Continuous Flow Column

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Abstract

Tetrachloroethene (PCE) and carbon tetrachloride (CT) were simultaneously transformed in a packed column that was bioaugmented with the Evanite culture (EV). The data presented here have been obtained over a period of 1930 days. Initially the column was continuously fed synthetic groundwater with PCE (0.1 mM), sulfate (SO_4^{2-}) (0.2 mM) and formate (2.1 mM) or lactate (1.1 mM), but not CT. In these early stages of the study the effluent H_2 concentrations ranged from 7 to 19 nM, and PCE was transformed to ethene (ETH) (81 to 85%) and vinyl

chloride (VC) (11 to 17%), and SO_4^{2-} was completely reduced when using either lactate or formate as electron donors. SO_4^{2-} reduction occurred concurrently with *cis*-DCE and VC dehalogenation. Formate was a more effective substrate for promoting dehalogenation based on electron donor utilization efficiency. Simultaneous PCE and CT tests found CT (0.015 mM) was completely transformed with 20% observed as chloroform (CF) and trace amounts of chloromethane (CM) and dichloromethane (DCM), but no methane (CH_4) or carbon disulfide (CS_2). PCE transformation to ETH improved with CT addition in response to increases in H_2 concentrations to 160 nM that resulted from acetate formation being inhibited by either CT or CF. Lactate fermentation was negatively impacted after CT transformation tests, with propionate accumulating, H_2 concentrations being reduced to below 1 nM. Under these conditions both SO_4^{2-} reduction and dehalogenation were negatively impacted, with sulfate reduction not occurring and PCE being transformed to *cis*-dichloroethene (*c*-DCE) (52%) and VC (41%). Upon switching to formate, H_2 concentrations increasing to 40 nM, and complete SO_4^{2-} reduction was achieved, while PCE was transformed to ETH (98%) and VC (1%), with no acetate detected. Throughout the study PCE dehalogenation to ethene was positively correlated with the effluent H_2 concentrations.

Keywords

Tertachloroethene, carbon tetrachloride, bioaugmentation, fermentation, lactate, formate, hydrogen

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1. Introduction

Release of chlorinated aliphatic hydrocarbons (CAHs) into groundwater is a widespread environmental problem since they are known or suspected carcinogens. Their movement, transformation, and subsurface remediation is therefore of interest (Löffler et al., 2000). Among the chlorinated solvents, tetrachloroethene (PCE), trichloroethene (TCE), carbon tetrachloride (CT), and trichlormethane (CF) are common subsurface contaminants (ATSDA 2005), therefore, in-situ treatment technologies that simultaneously detoxifies them is highly desirable. CT is of interest since a broad range of transformation products can be formed under anaerobic conditions by biotic and abiotic mechanisms (Vogel et al., 1987; Kriegman-King et al., 1992; Hashsham et al., 1995). Many groundwater sites are contaminated with mixtures of CAHs, including PCE and CT that were both used in dry cleaning (Bagley et al., 2000). These mixtures are problematic for subsurface bioremediation, since they may directly inhibit the other dehalogenation processes or required fermentation processes that supply hydrogen (H_2) required as an electron donor for the organohalogen-respiring bacteria (OHRB) *Dehalococcoides mccartyi* (Löffle and Edwards 2006). CT and especially CF have been observed to inhibit and potentially exert toxicity on reductive dehalogenation of PCE and TCE (Bagley et al., 2000; Maymó-Gatell et al., 2001; He et al., 2005; Weathers et al., 2000; Duhamel et al., 2002) including *Desulfitobacterium* strains (Futagami et al., 2006 and Futagami et al., 2013). CF has also been shown to be a strong inhibitor of anaerobic fermentation processes (Parkin and Owen 1986).

Abiotic transformation of CT has also been observed (Kriegman-King et al., 1992; Criddle and McCarty 1991; Ammonette et al., 2000; Butler and Hayes 2000). CT either directly hydrolyzes to CO_2 or under reducing conditions with electron donors such as sulfide ferrous iron or iron sulfide CT is transformed to CS_2 and CF.

Biotransformation of CT and CF by mixed and pure cultures under methanogenic conditions resulted in the formation of dichloromethane (DCM) and CO₂ (Bagely and Gossett 1995; Gupta et al., 1996a) while under sulfate (SO₄²⁻) reducing and acetogenic conditions higher rates of CT and CF were transformed to DCM and CO₂ (Egli et al., 1988; Egli et al., 1990; Freedman et al., 1995; Gupta et al., 1996b). The anaerobic biotransformation of high concentrations of CT (~ 0.5 mM) with hydroxocobalamin to end products of CO, CO₂ and organic acids has also been reported (Hashsham and Freedman 1999). In the presence of vitamin B₁₂ high concentrations of CF (4.0 mM) were transformed under anaerobic conditions to CO, CO₂ and organic acids (Shan et al., 2010a; Shan et al., 2010b).

Chung and Rittman (2007) and Chung and Rittman (2008) showed the simultaneous biotransformation of a low level mixture of TCE (7-18 µM) CF (8-20 µM) and TCA (7.5 µM) using a hydrogen-base membrane biofilm reactor under SO₄²⁻ and nitrate reducing conditions. DCM and CM were the final CF transformation products with a possibility of the CM hydrolyzing to methanol. In batch experiments with a culture of *Desulfovibrio vulgaris* the sequential removal of PCE (14 µM) and CT (10 µM) showed CS₂ was the major CT transformation possibly due to sulfide production (Koenig and Lee 2012). Previous column studies of PCE and CT transformation indicated the simultaneous transformation could be achieved, however inhibition of PCE transformation to ethene and propionate fermentation occurred (Bagley et al., 2000; Kaseros et al., 2000).

The goal of this study was to achieve the simultaneous anaerobic transformation of PCE and CT and to study the effects of different electron donors lactate or formate on the transformations. The study was conducted in a continuous flow column packed with quartz Ottawa sand. The column was bioaugmented with Evanite culture (EV) that contains strains of

Dehalococcoides mccartyi (*Dhc*) (Behrens et al., 2008; Marshall et al., 2013) that is capable of transforming PCE to ethene (ETH) (Yu et al., 2005). The reductive dehalogenase (*rdh*) genes in the Evenite culture were identified as being closely related to the population of *Dhc* previously found under chemostat growth conditions (Marshall et al., 2013; Mayer-Blackwell et al., 2014), where *Dhc*, *Geobacter*, and *Desulfitobacterium* strains were also present. *Dhc* require for grow on reduced organic compounds as carbon source (e.g. acetate or lactate) (He et al., 2003; Müller et al., 2004). The *16S rRNA* gene abundance for *Dehalococcoides* and *Geobacter* compared with gene counts of hypothesized vinyl-chloride (VC) respiring strains (*vcrA*) and putative vinyl-chloride reductase (*bvcA*) were similar (Mayer-Blackwell et. al., 2014). *vcrA* and *bvcA* strains are known to reduce 1,2-dichloroethene (DCE) and VC to ETH (Müller et al., 2004; Krajmalnik-Brown et al., 2004). The culture was not previously acclimated to CT transformation. The concentration effect of H₂ on PCE dehalogenation was also investigated as well as SO₄²⁻ as a competitive electron acceptor. The studies were conducted as a series of long-term transient tests that permitted pseudo-steady-state conditions to be achieved and electron balances of the donor and acceptor processes. The transient production of intermediate products from CT transformation was also monitored. Studies of PCE transformation and SO₄²⁻ reduction performed after the CT transformation tests evaluated whether the lactate fermentation or the production of H₂ through formate addition was negatively impacted.

2. Materials and methods

2.1. Chemicals

Analytical grade CAHs and ETH for the column influent feed or for analytical standards were used: (PCE, 99.9%; CF, 99.5%; and c-DCE, 97%; Acros Organics) (VC, 99.5%; CT, 99.9%;

CM, 99.5%; and ETH 99.5% Aldrich Chemical) (formate, 99.0%; lactate, 60%; syrup, J.T. Baker) and (H₂, 99.999%; Airgas Inc.).

2.2. Column Construction and Operation

The studies were conducted in a fabricated stainless steel column (standard molybdenum-bearing grade 316, 30 cm L x 7.5 cm ID) packed with quartz Ottawa sand (20/30 mesh; Langston Company Inc). Prior to column packing the Ottawa sand was autoclaved. The column was packed wet as described by Azizian et al., (2008) resulting in a porosity of 0.36 and a pore volume of 476 mL. PEEK tubing (1/8" OD) (Upchurch Scientific) with a low O₂ permeability was used for the pumping of the influent reservoirs and sampling valves and fittings that were connected to the column (Azizian et al., 2008).

The column was amended with synthetic groundwater containing a biocarbonate buffer (3.5 mM) supplemented with 1/10 dilution of anaerobic growth media described by Yu et al. (2005) to which was added PCE (0.1 mM) lactate (0.67 – 1.1 mM) or formate (1.5 – 2.1 mM) and SO₄²⁻ (0 – 0.2 mM) (Table 1). The influent feed (100 ml/day) was added to the column using a syringe pump (M361, Thermo Scientific Orion) via a 100 ml gas tight syringe. This flow rate resulted in a fluid hydraulic residence time in the column of 4.8 days.

2.3. Bioaugmentation Culture

The column was bioaugmented with the EV mixed dehalogenating culture that contained at least two strains of *Dehalococcoides mccartyi* (Marshall et al., 2013). Molecular characterization of the EV culture in a previous column study where the EV was bioaugmented showed 73% of bacteria in the EV culture were strains of *Dhc* (Azizian et al., 2008). In that study after 170 days of column operation all four *RDase* genes (*pceA*, *tceA*, *bvcA*, and *vcrA*) were found (Behrens et al., 2008). Prior to bioaugmentation the EV culture grown in sequential batch mode and fed PCE

and butanol as a fermenting substrate with no exposure to CT. Bioaugmentation was accomplished by adding 50-ml of liquid culture (75 mg/L on a protein basis) to the column influent as a slug input.

2.4. Analytical Methods

Aqueous samples of the column's influent and effluent were analysed for the CAHs, ETH, CS₂, CO, H₂, and CH₄ using a headspace method as described by Azizian et al., 2008. The chlorinated ethenes and ETH were quantified with a HP-6890 gas chromatograph equipped with a flame ionization detector (FID). CT, CF, and DCM were quantified with a HP-6890 GC equipped with an electron capture detector (ECD) while CM was quantified with a HP-5890 GC equipped with an electrolyte conductivity detector (ELCD). The detection limits for PCE, TCE c-DCE, and ETH were 50, 8, 10, and 5 nM respectively. The detection limits for CT, CF, and DCM were approximately 10 80 and 100 nM respectively and 5 nM CM.

H₂ concentrations in the column effluent were analyzed using a reduction gas detector (RGD) (Trace Analytical, ta3000R) (Azizian et al., 2010) with an aqueous concentration detection limit of 0.05 nM. CO was determined by HP-5890 GC with a thermal conductivity detector with packed column (Supelco 15' x 1/8" SS support 60/80 Carboxen 1000) with helium (20 ml/min) as the carrier gas with a detection limit of 20 nM. CH₄ and CS₂ were measured by GC with FID and ECD detectors respectively. The detection limit for CH₄ was 60 nM and for CS₂ 20 nM.

Formate lactate propionate and acetate concentrations were determined using high performance liquid chromatography (HPLC) on a Dionex DX500 HPLC (Azizian et al., 2008). Liquid samples (0.25 mL) of the column influent and effluent were centrifuged (2 min at 14,000 RPM) and diluted (1/10) with ultrapure water prior to analysis. Sulfate concentrations were

determined using a Dionex DX-500 ion chromatograph equipped with an electrical conductivity detector and a Dionex AS14 column (Azizian et al., 2008). Total biomass in the column effluent was determined by filtering 100 mL of solution through 0.2 μ M Millipore filter drying the filter for 1 h at 105° C and weighing.

2.5. Stages of the Column Study

The column study was conducted over a period of 1950 days in ten Stages based influent solution chemical composition (Table 1). Each Stage was conducted for an extended time period so that pseudo-steady-state conditions were achieved with respect to effluent concentrations of CAHs ethene organic acids and H₂. PCE reductive dehalogenation in the absence of CT was conducted in Stages I to VI over 1640 days. Dehalogenation performance was evaluated using different influent concentrations of lactate or formate as electron donors and in the presence and absence of SO₄²⁻ as a competing electron acceptor. The simultaneous transformation of PCE (0.1 mM) and CT (0.015 mM) was studied in Stage VII. A stop-flow experiment was performed in Stage VIII to determine if CF was completely transformed with a longer reaction time. Stages IX and X studied the effect that CT and CF exposure had on lactate fermentation homoacetogenesis and PCE transformation. Electron donor and accept balances were performed when pseudo-steady-state was obtained at the end of each Stage using methods described by (Azizian et al., 2010).

3. Results

3.1. Stages I to III – Bioaugmentation and PCE transformation with lactate addition

Stage I prior to lactate addition and bioaugmentation shows the rapid PCE breakthrough towards the injected concentrations (0.1 mM) with no transformation occurring and little

retardation due to sorption (Fig. 1a). In Stage II lactate addition was initiated on day 13 and bioaugmentation with the EV culture on day 15. A transient build-up TCE concentration occurred and after 50 days PCE is mainly transformed to c-DCE (Fig. 1a). In previous column studies with Hanford aquifer material the transient c-DCE concentrations increased above the injection concentration of PCE which indicated transformation of sorbed PCE (Azizian et al., 2008; Azizian et al., 2010). This did not occur in Stage II which is consistent with minimal retardation of PCE observed in Stage 1. The minimal sorption of PCE is consistent with the very low carbon content of Ottawa sand (Zhuang et al., 2003).

Lactate was fermented to acetate and propionate however SO_4^{2-} reduction was initially not observed (Fig. 1b). Electron donor and acceptor balances at the end of Stage II (Table 2) show 7.3% was associated with CAHs transformation 30.8% with acetate and propionate production while a large fraction (55 %) was missing. Some of the missing balance was likely associated with biomass growth in the column and iron reduction in this early stage.

In Stage III the lactate influent concentration was increased to 1.1 mM to promote more effective dehalogenation and potentially SO_4^{2-} reduction. Acetate and propionate concentrations increased to maximum concentrations of approximately 0.3 mM and SO_4^{2-} was rapidly reduced (Fig. 1b). The accumulated c-DCE was transformed to ethene and VC, with a transient increase in VC concentration observed. Pseudo-steady-state effluent concentrations were achieved showing PCE was transformed to ethene (88.3 %) and VC (11.2 %) (Table 1). Electron balances (Table 2) show about 7.1% of electron donor equivalents were associated with CAHs transformation while 24.9% were associated with SO_4^{2-} reduction. The increase addition of lactate and the observed increase in propionate concentration likely resulted in propionate fermentation and likely an increase in the H_2 concentration. The measurement of aqueous H_2

concentrations commenced at the end of Stage III (Figure 2b), where approximately 20 nM H_2 was observed. This H_2 level is consistent with past column studies where propionate fermentation resulted in effective transformation of TCE to ethene (Azizian et al. 2010).

3.2. Stages IV-VI – Evaluation of formate addition as an electron donor

In Stage IV lactate was replaced by formate as an alternative electron donor. The formate influent concentration of 2.1 mM represented 64% of electron donor potential as 1.1 mM lactate. In response the effluent ethene concentration decreased slightly and VC concentration increased (Table 1) while complete reduction of SO_4^{2-} was maintained (Fig. 2). Since lactate was not being fermented the propionate concentration decreased while acetate concentrations remained fairly constant likely due to homoacetogenesis. The measurement of H_2 concentrations commenced at the end of Stage III. Formate addition maintained fairly high effluent H_2 concentration of 19 nM (Fig. 2b). The percentage of electron equivalents utilized for chloroethene (CE) dechlorination increased with formate addition compared to lactate (Table 2) which is consistent with past column studies (Azizian et al., 2010). Acetate production represented about 34% of the electron equivalents which is also consistent with previous column results (Azizian et al. 2010).

The formate influent concentration was lowered to 1.5 mM in Stage V in order to study the dependence of PCE transformation and SO_4^{2-} reduction on formate concentration. An immediate decrease in ethene concentration and increase c-DCE and VC concentrations occurred that was associated with H_2 concentrations decreasing from 20 to 5 nM. (Fig. 2). The acetate concentration decreased while SO_4^{2-} reduction below the detection limit of 0.01 mM continued. The results are consistent with past columns studies where SO_4^{2-} reduction effectively competed with c-DCE and VC dehalogenation under electron donor limited conditions (Azizian et al., 2010).

A gradual improvement in dehalogenation performance occurred over the period of 640 to 1400 days. By the end of Stage V PCE was transformed to ETH (55%) VC (33%) and c-DCE (11%) (Table 1). H_2 and acetate concentrations remained relatively constant during this period. An interesting observation is that formate increased in the column. One possible explanation for the improvement in dehalogenation performance with time is a population shift over time to strains of *Dehalococcoides mccartyi* that more effectively competed for the lower H_2 concentrations. At the end of Stage V the percentage of electron donor associated with dehalogenation reactions increased to 26% while SO_4^{2-} reduction represented 55% and acetate 7%. Thus under electron donor limited conditions effective electron transfer to dehalogenation reactions was achieved using formate.

In order to further evaluate SO_4^{2-} competition as a competing electron acceptor SO_4^{2-} addition was stopped after 1400 days (Stage VI) while maintaining the influent formate (1.5 mM). In response ethene concentrations increased and c-DCE and VC decreased (Fig. 2a). The termination of SO_4^{2-} addition resulted in an increase in formate effluent to 0.40 mM and increase in H_2 concentrations from 5 nM to 19 nM. Very effective electron donor efficiency was achieved during Stage VI with 48% associated with dehalogenation reactions and 12% to acetate formation.

3.3. Stages VII – Simultaneous PCE and CT Transformation

CT (0.015 mM) was continuously added in Stage VII while maintaining PCE (0.1 mM) and formate (1.5 mM) addition, however SO_4^{2-} was not added (Table 1). After 25 days of CT addition CT reached a maximum concentration of 0.009 mM and then concentrations decreased to below detection (10 nM) (Fig. 3b). CF was produced as an intermediate product and reached

a maximum concentration of 0.006 mM followed by a decrease to a pseudo-steady-state effluent concentration of 0.003 mM (Fig. 2b), which represented 20% of the CT transformed. A trace amount of CM (~10 nM) and DM (data not shown) were detected (Fig. 2b), however CH₄, CO, or CS₂ were not detected.

In response to CT addition and CF production acetate concentrations decreased to below the detection limit (0.01 mM) (Fig. 2c) and the formate effluent concentration increased to about 1.0 mM which is approximately 66% of the influent formate. The increase in formate corresponded to dramatic increase in H₂ concentrations to 160 nM. The increase in formate and H₂ and decrease in acetate supports the inhibition of homoacetogenesis that coincided with CT addition and CF production. The high H₂ concentrations (160 nM) did not result in complete transformation of VC to ethene. The presence of CF may inhibit the rate of VC transformation to ethene. Acetate production was also inhibited, and past studies have shown that acetate is needed as a carbon source by *Dehalococcoides mccartyi* (He et al., 2003). Thus inhibited acetate production may also have been a limiting factor.

A slight increase in ethene concentration and decrease in c-DCE and VC occurred (Fig. 2a) in response to the CT addition. This enhanced dehalogenation performance was likely associated with the increase in H₂ concentrations. At pseudo-steady state electron balances showed that about 71% of the electron equivalents were directed towards chlorinated ethene reduction and 1.0% with CF formation (Table 2). The inhibition of acetogenesis and incomplete formate utilization resulted in a very high percentage of the electron donor being associated with dehalogenation reactions.

3.4. Stage VIII – Stop Flow Test

Flow to the column was stopped after 140 days of CT addition (Stage VIII) while the CAHs, CT, CF, H₂ and formate concentrations of samples from the effluent port were periodically measured. The influent solution was added only to displace the fluid sampled as described by Niemit and Semprini (2005). During the stop-flow Stage CF, CM, and VC concentrations gradually decreased while ETH increased (Fig. 3a and 3b) and no additional transformation products were detected. The formate concentration also decreased along with the H₂ concentration. The stop-flow test showed with longer reaction time CF and VC continued to be transformed with only ETH from PCE transformation observed. CT was completely transformed to unknown product(s). It is possible that the *Ehc* population had not completely developed during the early stage of the column study and incomplete transformation to ethene was limited by the residence time in the column.

3.5. Stage IX – Evaluation of the inhibition of lactate fermentation

Stage IX evaluated whether lactate fermentation was inhibited in response to the prior addition of CT and CF production. Lactate (1.1 mM) and PCE (0.1 mM) were continuously added in the absence of both SO₄²⁻ and CT addition. Lactate was completely transformed however its fermentation was incomplete as indicated by the build-up of acetate (0.36 mM) and propionate (0.48 mM) (Fig. 3c) and decrease in H₂ concentration below detection. While PCE continued to be completely transformed ETH concentrations decreased and VC and c-DCE were the major transformation products. In order to determine if SO₄²⁻ reduction was also inhibited SO₄²⁻ addition (0.2mM) was initiated after 1850 days. No detectable SO₄²⁻ reduction was observed (Fig. 3c) as sulfate increased to the injected concentration.

The influent chemical composition at the end of Stage IX matched those of Stage III (Fig. 1). Transformation to VC and ethene was greatly reduced in Stage IX compared to that achieved in Stage III (Table 1). The amount of propionate and acetate produced was also much greater in Stage IX (Fig. 3c) compared to III (Fig. 1c). Electron balances for Stage IX showed that 19.4 % and 44.4% of the electron equivalents were associated with acetate and propionate production respectively while CAHs consumed only 2% compared to 7% in Stage III (Table 2). The results illustrate that the addition of CT and the production of CF had a negative impact on propionate fermentation which negatively impacted dehalogenation and SO_4^{2-} reduction by limiting H_2 production. A shift in the microbial community likely occurred in response to CF exposure.

3.6. Stage X – Evaluation of the inhibition of homoacetogenesis through formate addition

In Stage X lactate (1.1 mM) was replaced by formate (1.5 mM) to determine whether acetate production was still inhibited and whether effective dehalogenation and SO_4^{2-} reduction could be restored. Acetate concentrations decreased below detection indicating acetate production was still not occurring. The inhibition of acetate production resulted in high H_2 concentrations (44 nM) and formate not being completely utilized (Fig. 3c). However very effective transformation of PCE to ethene (98%) was achieved with only a minor amount of VC (1.0%) observed in the column effluent (Fig. 3a).

The results of Stage X were conducted under the same influent conditions as Stage V (Fig. 2) which was prior to CT addition. In comparison dehalogenation was more effective in Stage X with H_2 concentration of 44 nM compared to 6 - 8 nM in Stage V when acetate was formed. Electron balances showed 33% of electrons were utilized toward CAH reduction compared to 25% in Stage V. The inhibition of homoacetogenesis that resulted from CT addition

and likely CF exposure continued in Stage X 105 days after the CT addition was terminated and corresponded to an increased PCE dehalogenation to ETH.

4. Discussion

4.1. Effective long-term transformation of PCE to ethene

Transformation of PCE to ETH was achieved over 1930 days in a continuous flow column with a single bioaugmentation of the EV culture. The most effective conversion of PCE to ETH (98%) occurred in the Stage X (1885-1950 days) with formate following the simultaneous transformation of PCE and CT and the production of CF (Stages VII and VIII). The EV culture containing strains of *Dehalococcoides mccartyi* which was not pre acclimated to CT or CF was able to tolerate influent CT concentration of 0.015 mM (2.3 mg/L) and CF effluent concentration of 0.003 to 0.006 mM (CF) (0.35 to 0.72 mg/L). Effective fermentation of propionate and the production of acetate as a result of homoacetogenesis however were negatively impacted by CT addition and CF production.

4.2. Comparison with previous column and microcosm studies

Consistent with our past column studies (Azizian, et al., 2010) both lactate and formate were both effective substrates to promote dehalogenation of PCE to ethene. A higher percentage of electron transfer to dehalogenation reactions was achieved using formate than lactate (Table 2) which is also consistent with the results of Azizian, et al., (2010). In both studies when lactate was fermented significant amounts of the electron donor potential were lost in the production of acetate. In both studies sulfate reduction successfully competed with the dehalogenation reaction for electron donor. The aquifer material used in the study of Azizian, et al., (2010) had much higher iron content than the material used in this study and a significant amount of the electron donor was used for iron reduction and sulfate reduction. Therefore the efficiency of the electron

donor directed to dehalogenation in that study 7 to 16% was generally lower than achieved in the present study prior to the addition of CT 7 to 26% (stages II to V). The highest efficiency directed to dehalogenation of 71% was achieved during the CT transformation test (stage VII) when acetate production was inhibited and formate was not completely utilized.

Column and microcosm studies of Bagley et al. (2000) showed CF produced through CT transformation inhibited PCE dehalogenation. In their column studies PCE dehalogenation could not be stimulated in the presence of 0.043 mM CT while feeding ethanol as a fermenting substrate. CT was transformed producing CF (0.022 mM) and DM (0.006mM) and acetate and propionic acid (propionate) accumulated. Microcosm tests confirmed the results of the column studies and supported earlier observations of CF inhibition of propionate-consuming anaerobic systems (Rhee and Speece 1992). They argue that the inhibition of dehalogenation may have resulted from the inhibition of propionate fermentation to produce H_2 which is supported by our observations in Stage IX.

In the column study of Kaseros et al., (2000) PCE transformation to VC and ETH could acclimate to CT exposure and CF production with CT concentrations increasing from 3.3 to 13 μ M while feeding ethanol as an electron donor. Ethanol degradation was not inhibited at the inlet CT concentrations and CF concentration of 3.0 μ M which is in the same range as our study however the further fermentation of propionate and utilization of acetate was completely inhibited. They indicated that PCE transformation was not likely inhibited since ethanol (2.2 mM) and the yeast extract (100 mg/L) were added to produce enough reducing equivalents to reduce PCE (0.03 mM). These concentrations represented higher excess donor conditions compared to our tests. They indicated the inhibition propionate fermentation would likely negatively impact PCE dehalogenation if it was needed as donor. This condition was confirmed

in Stage IX of our study where lactate was completely degraded but acetate and propionate accumulated and the H_2 concentration was reduced below 1 nM due to the inhibition of propionate fermentation. Under these conditions PCE was transformed mainly to c-DCE and VC.

4.3. Relationship of PCE dehalogenation to aqueous H_2 concentrations

The ability to measure low H_2 concentrations was a key aspect of our column study. Fig. 4 shows the relationship of the extent of transformation of PCE to ETH versus the observed effluent H_2 concentrations for different Stages. When the H_2 concentrations were maintained above 30 nM greater than 95% conversion of PCE to ETH was achieved. When the H_2 concentration decreased below 10 nM the conversion to ETH was greatly reduced. The resulting empirical relationship is for the conditions of this study and depends on many factors including donor supply competing acceptor reactions (SO_4^{2-} reduction) and inhibition of the propionate fermentation process. The fit shown in Fig. 4 is a Monod type of plot however the rate that should appear on the y-axis was replaced by the percent conversion to ETH for illustrative purposes. The transformation rates of c-DCE to VC and VC to ETH are dependent on H_2 concentration (Cupples et al., 2004). Cupples et al., 2004 report a half-substrate coefficient (K_s) for H_2 of around 7 nM for c-DCE and VC for the VS dehalogenating culture that contains *D. mccartyi*. If the EV culture had a similar K_s value as the VS culture then the c-DCE and VC dehalogenation rates would be near maximum rates with respect to H_2 when H_2 concentrations were above 30 nM but would be very significantly reduced below the K_s value consistent with the trends shown in Fig. 4. The fitted concentration in Fig. 4 when 50% of the PCE was transformed to ETH was 3.5 nM H_2 which is half of the reported K_s value (Cupples et al., 2004).

4.4. Inhibition of fermentation processes after the transformation of CF to CT

Homoacetogenesis was inhibited during CT addition and CF production (Stage VII) and continued after the addition of CT was discontinued (Stage X) as indicated by the lack of acetate production and increased formate and H₂ concentrations. CF inhibited homoacetogens in studies with microbes associated with excised roots of rice (Conard and Klose 2000). Studies with pure cultures of methanogens homoacetogens and SO₄²⁻ reducing bacteria by Scholten et al. (2000) found CF inhibition was associated with microorganisms that operate the acetyl-CoA cleavage pathway. They found CF inhibited acetate production from H₂ of the homoacetogen *Acetobacterium woodi* which was shown by Egli et al. (1988) to transform CT with CO₂ being a major transformation product.

Microcosm studies with the EV culture (Yu and Semprini 2009) and column studies (Azizian et al., 2008) found methane was not produced likely as a result of exposure high PCE concentrations during long term culture maintenance consistent with observations of Yang and McCarty (1998). Thus H₂ consuming methanogens did not out compete homoacetogens for H₂ in the present study. While feeding formate SO₄²⁻ reducers and homoacetogens were the main competitors for H₂ with the dehalogenating population. When the formate concentration was decreased in Stage V, H₂ concentrations decreased from 20 nM to 8 nM and dehalogenation efficiency decreased while complete SO₄²⁻ reduction was maintained. Sulfate reducing bacteria effectively competed for H₂ with c-DCE and VC dehalogenators. When propionate fermentation was inhibited in Stage IX SO₄²⁻ reduction was also inhibited indicating H₂ was the preferred donor and not acetate which was present.

CF has been applied in activated sludge to increase H₂ production by inhibiting methane production and acetate formation via homoacetogenesis (Xu and Chen 2010). We also found in Stage VII that H₂ concentrations were greatly increased (160 nM) in the presence of CF and

more effective dehalogenation was achieved (Table 2). Thus an important choice for bioremediation when a mixture of PCE and CT or chloroform is present is the donor added. Our study indicates that formate would be a better choice than lactate to achieve effective treatment.

CT essentially completely transformed with a residence time of 4.5 days with 20% observed as CF. It is not known the contribution of biotic and abiotic processes to CT transformation in the column. It is possible that biotic cometabolic processes contributed to the CT transformation. The results show CT addition and CF accumulation inhibited acetate formation by homoacetogenes. Homoacetogenes have been observed to transform CT to CF DCM and soluble non-chlorinated products (Egli et al., 1988; Egli et al., 1990; Syed and Freedman 1999; Stromeyer et al., 1992). Egli et al. (1988) found *Acetobacterium woodi* and *Clostridium thermoaceticum* ability to transform CT was associated with microorganisms that contained the acetyl-coenzyme A pathway, with CO₂ being a major product formed and minor amounts through a reductive pathway of CF and DM. Hashsham et al. (1999) found enhanced biotransformation of CT was achieved by supplemented *Acetobacterium woodi* with hydroxocobalamin that also resulted in a shift from a reductive pathway of CF and DM formation to a pathway of CO formation and CO₂ production. In our study CO was not observed as an intermediated product. Other studies shown that *Dehalobacter* that can gain energy from the respiration of CF to DM has been observed (Grostern et al., 2010) and the reduction of CF to DM and its subsequent fermentation of DM to acetate (Lee et al., 2012) indicates the potential for other biotic pathways.

CT can also be transformed by abiotic processes involving sulfide minerals biotite and vermiculite (Kriegman-King and Reinhard 1992) and pyrite (FeS) (Kriegman-King and Reinhard 1992; Devlin and Müller 1999) producing CF and CS₂. In the column study and batch studies

with sulfate reduction (Devlin and Müller 1999) observed a CF/CS₂ production ratio of around 2:1. We did not observe CS₂ in our study. Active sulfide reduction was not occurring during the Stage VII of CT transformation test; however it is possible that FeS was present in the column due to the production of sulfide through SO₄²⁻ reduction. The Ottawa sand used to pack the column has a low iron content of 28.7 mg/kg solids (Zhuang et al., 2003) thus there was limited potential FeS production which is consistent with our not detecting CS₂. The abiotic transformation of CT by bio-reduced ferric iron produced iron-reducing bacteria has been observed to be a dominant mechanism (McCormick et al., 2002; Maithreepala and Doong 2009) and may have occurred even at the low iron contents of the solids used. A strain of *Geobacter* has recently been shown to represent a significant fraction of the microbial population in a chemostat inoculated with the EV culture that is dehalogenating TCE to ethene with formate being fed as an electron donor (Mayer-Blackwell et al., 2014). Thus *Geobacter* was likely present in the EV culture used to bioaugment the column. One strain of *Geobacter* has been shown to carry *rdh* genes and the capacity for growth linked to PCE-reduction (Sung et al. 2006).

CT was completely transformed and 20% was observed as CF. The other 80% was converted to an unknown product likely CO₂. In a follow-up study we confirmed that CO₂ was the main product formed through the addition of ¹³C labeled CT (Semprini and Azizian 2014).

5. Conclusions

The following conclusions can be drawn from this study:

- PCE was effectively dehalogenated to ethene over a period of 1930 days in a continuous flow column fed either lactate or formate with a single bioaugmentation of the EV culture.

- When formate was fed as the electron donor PCE continued to be effectively dehalogenated to ethene upon the addition of CT with the production of CF and other products.
- CT transformation to CF inhibited homoacetogenesis and the production of acetate when formate was fed as a substrate resulting in incomplete formate utilization high aqueous H₂ concentrations and vary efficient utilization of donor for CE dehalogenation.
- Fermentation processes especially propionate fermentation was greatly inhibited after the exposure to CT and CF which resulted in low aqueous H₂ concentrations and dehalogenation of PCE mainly to c-DCE and VC with the lack of sulfate reduction.
- However the most effective dehalogenation of PCE to ethene was achieved after the exposure to CT and CF when formate was used as an electron donor, and CT was no longer being added that produced CF.

The results of this research indicate the careful consideration should be made as to the substrate used to drive reductive dehalogenation when mixtures of PCE and CT are present. Formate appears to be a better substrate than lactate since fermentation processed and microorganisms required to produce H₂ needed for dehalogenation are greatly inhibited when CF is formed.

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Table 1: Summary of column operation conditions at different Stages electron donors SO_4^{2-} and percentage of PCE transformed to the different products during pseudo-steady-state operation.

Stage	Start (day)	Steady- state (day)	Electron donor	Electron donor conc.		Sulfate (mM)	H ₂ (nM)	CAHs			
				(mM)	(meq)			ETH	VC	c-DCE	TCE
								%	%	%	%
I	0	12		Tracer Test							
II	12	50	Lactate	0.67	8.0	0.2	ND ^a	0.0	0.0	100	0.0
III	50	200	Lactate	1.1	13.2	0.2	ND	88.3	11.2	BDL	BDL
IV	250	500	Formate	2.1	4.2	0.2	18.7	81.3	17.1	BDL	BDL
V	500	1400	Formate	1.5	3.0	0.2	6.8	54.7	32.6	10.5	BDL
VI	1400	1640	Formate	1.5	3.0	0.0	6.8	82.3	16.0	1.0	BDL
VII	1640	1780	Formate	1.5	3.0	0.0	160	85.7	14.3	BDL	BDL
VIII	1780	1850	Stop Flow								
IX	1850	1885	Lactate	1.1	13.2	0.2	0.2	7.7	40.6	51.6	BDL
X	1885	1950	Formate	1.5	3.0	0.2	43.0	97.8	1.0	BDL	BDL

ND^a: not determined BDL^a: below detection limit

Table 2 Electron donor and electron acceptor balances with the percentage representing the donor associated with organic acid or biomass production or sulfate, chlorinated ethene (CE), and CT reduction.

Stage	Electron donor	Acetate	Propionate	Sulfate	CE	CF	Biomass	Electron donor Accountable
<i>Electrons (%)</i>								
<i>II</i>	Lactate	16.0	14.8	11.4	7.3	NA	5.6	55.1
<i>III</i>	Lactate	22.8	21.8	24.9	7.1	NA	2.10	78.7
<i>IV</i>	Formate	33.8	0.0	39.1	17.0	NA	1.64	91.5
<i>V</i>	Formate	6.7	0.0	55.0	25.8	NA	1.77	89.3
<i>VI</i>	Formate	12.2	0.0	0.0	48.4	NA	3.0	63.6
<i>VII</i>	Formate	0.0	0.0	0.0	71.3	1.0	9.8	82.1
<i>VIII</i>	Stop flow							
<i>IX</i>	Lactate	19.4	44.4	8.3	2.2	NA	2.41	76.7
<i>X</i>	Formate	0.0	0.0	53.6	33.4	NA	7.87	94.9

Dehalogenation/reduction reactions w/lactate as electron donor dehalogenation and fermentation products

1. C_2Cl_4 (PCE) + 0.17 $C_3H_5O_3^-$ (Lactate) + 0.67 H_2O $\implies$ C_2HCl_3 (TCE) + HCl + 0.33 CO_2 + 0.17 HCO_3^-
2. C_2Cl_4 (PCE) + 0.33 $C_3H_5O_3^-$ (Lactate) + 1.33 H_2O $\implies$ $C_2H_2Cl_2$ (c-DCE) + 2 HCl + 0.67 CO_2 + 0.33 HCO_3^-
3. C_2Cl_4 (PCE) + 0.51 $C_3H_5O_3^-$ (Lactate) + 2.01 H_2O $\implies$ C_2H_3Cl (VC) + 3 HCl + 1.00 CO_2 + 0.51 HCO_3^-
4. C_2Cl_4 (PCE) + 0.67 $C_3H_5O_3^-$ (Lactate) + 2.68 H_2O $\implies$ C_2H_4 (ETH) + 4 HCl + 1.33 CO_2 + 0.67 HCO_3^-
5. $C_3H_5O_3^-$ (Lactate) + 4.0 H_2O $\implies$ HCO_3^- + 2 CO_2 + 6 H_2
6. $C_2H_3O_2^-$ (Acetate) + 3.0 H_2O $\implies$ HCO_3^- + CO_2 + 4 H_2
7. $C_3H_5O_2^-$ (Propionate) + 6.0 H_2O $\implies$ 2 HCO_3^- + CO_2 + 7 H_2 + H^+
8. $C_3H_5O_3^-$ (Lactate) + 1.5 SO_4^{2-} (Sulfate) $\implies$ 1.5 S^{2-} + 3 CO_2 + 2.5 H_2O
10. SO_4^{2-} (Sulfate) + 0.73 $C_3H_5O_3^-$ (Lactate) $\implies$ S^{2-} + 2.2 CO_2 + 1.8 H_2O + 0.73 e^-

^aAssumptions for computation of equivalents (mequiv/mmol): PCE 8 TCE 6 c-DCE 4 VC 2 H_2 2

formate 2 lactate 12 propionate 14 acetate 8 sulfate 8.

Fig. 1. Column effluent concentrations of (a) PCE, TCE, c-DCE, VC, and ETH and (b) organic acids and SO_4^{2-} during Stages I to III. The dashed line denotes the start of the next Stage of the column study.

Figure#2

Fig. 2. Column effluent concentrations of (a) PCE, TCE, c-DCE, VC, and ETH and (b) organic acids SO_4^{2-} and H_2 during Stages IV to VI. The dashed line denotes the start of the next Stage of the column study.

Figure#3

Fig. 3. Column effluent concentrations of (a) PCE, TCE, c-DCE, VC, and ETH (b) CT, CF, and CM and (c) organic acids SO₄²⁻ and H₂ during Stages VII to X. The dashed line denotes the start of the next Stage of the column study.

Figure#4

Fig. 4. Extent of transformation of PCE to ethene versus the column effluent H₂ concentrations. The solid line represents a Monod type fit where $ETH\% = ETH\ max\% * [H_2] / (K_s + [H_2])$. The fit was achieved with an ETH max of 103.1% and a K_s of 3.3 nM H₂ which represents the H₂ concentration when PCE transformation products are 50% ETH.

Abstract Art