Journal of Contaminant Hydrology 190 (2016) 58–68

Contents lists available at ScienceDirect

Journal of Contaminant Hydrology

journal homepage: www.elsevier.com/locate/jconhyd

Simultaneous anaerobic transformation of tetrachloroethene and carbon tetrachloride in a continuous flow column☆

Mohammad F. Azizian *, Lewis Semprini

School of Chemical, Biological and Environmental Engineering, 102 Gleeson Hall, Oregon State University, Corvallis, OR 97331, United States

ARTICLE INFO

Article history:
Received 4 December 2015
Received in revised form 31 March 2016
Accepted 1 April 2016
Available online 8 April 2016

Keywords:
Tetrachloroethene
Carbon tetrachloride
Bioaugmentation
Fermentation
Lactate
Formate
Hydrogen

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, and 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 increased 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.

Published by Elsevier B.V.

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 and Edwards 2006). Among the chlorinated solvents, tetrachloroethene (PCE), trichloroethene (TCE), carbon tetrachloride (CT), and trichloromethane (CF) are common subsurface contaminants (ATSDR, 2005), therefore, in-situ treatment technologies that simultaneously detoxify them are 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 and Reinhard, 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

☆ Author contributions: The manuscript was written through the contributions of both authors. Both authors have given approval to the final version of the manuscript.

* Corresponding author.
E-mail address: mohammad.azizian@oregonstate.edu (M.F. Azizian).

http://dx.doi.org/10.1016/j.jconhyd.2016.04.002
0169-7722/Published by Elsevier B.V.

fermentation processes that supply hydrogen (H_2) required as an electron donor for the organohalogen-respiring bacteria (OHRB) *Dehalococcoides mccartyi* (Löffler 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 and Parkin, 2000; Duhamel et al., 2002) including *Desulfitobacterium* strains (Futagami et al., 2006, 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 and Reinhard, 1992; Criddle and McCarty, 1991; Ammonette et al., 2000; Butler and Hayes, 2000). CT either directly hydrolizes 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 CO_2 (Bagley and Gossett, 1995; Gupta et al., 1996a) while under sulfate (SO_4^{2-}) reducing and acetogenic conditions higher rates of CT and CF were transformed to DCM and CO_2 (Egli et al., 1988, 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, CO_2 and organic acids has also been reported (Hashsham and Freedman, 1999). In the presence of vitamin $B_{12,}$ high concentrations of CF (4.0 mM) were transformed under anaerobic conditions to CO, $CO_{2,}$ and organic acids (Shan et al., 2010a, 2010b).

Chung and Rittmann (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 SO_4^{2-} 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 CS_2 was the major CT transformation product possibly due to sulfide production (Koenig et al., 2012). Previous column studies of PCE and CT transformation indicated 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 *D. 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 EV 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 present. *Dhc* require reduced organic compounds as a carbon source for growth (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*) (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 H_2 on PCE dehalogenation was also investigated as well as SO_4^{2-} 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 SO_4^{2-} reduction performed after the CT transformation tests evaluated whether the lactate fermentation or the production of H_2 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 ($H_{2,}$ 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 × 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 O_2 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 SO_4^{2-} (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 *D. mccartyi* (Marshall et al., 2013). Molecular characterization of the EV culture in a previous column study in which the EV was bioagumented 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 was grown in sequential batch