Chemosphere 182 (2017) 65–75

Contents lists available at ScienceDirect

Chemosphere

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

Transformation of carbon tetrachloride and chloroform by trichloroethene respiring anaerobic mixed cultures and supernatant

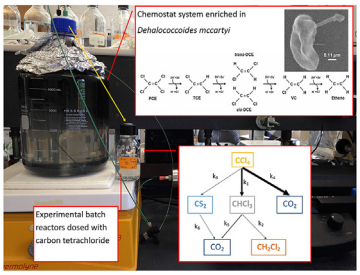
Kyle E. Vickstrom, Mohammad F. Azizian, Lewis Semprini*

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

HIGHLIGHTS

- Anaerobic dechlorinating cultures and supernatant transform inhibitory compounds.
- Transformation of carbon tetrachloride (CT) is predominantly abiotic.
- Transformation of chloroform to dichloromethane was biotic.
- Simultaneous transformation of TCE and CT was observed in the anaerobic batch reactors.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 30 December 2016
Received in revised form
26 April 2017
Accepted 27 April 2017
Available online 29 April 2017

Handling Editor: T Cutright

Keywords:
Carbon tetrachloride
Chloroform
Dehalococcoides mccartyi (*Dhc*)
Reductive dechlorination
Inhibition
Trichloroethene

ABSTRACT

Carbon tetrachloride (CT) and chloroform (CF) were transformed in batch reactor experiments conducted with anaerobic dechlorinating cultures and supernatant (ADC + S) harvested from continuous flow reactors. The Evanite (EV) and Victoria/Stanford (VS) cultures, capable of respiring trichloroethene (TCE), 1,2-*cis*-dichloroethene (cDCE), and vinyl chloride (VC) to ethene (ETH), were grown in continuous flow reactors receiving an influent feed of saturated TCE (10 mM; 60 mEq) and formate (45 mM; 90 mEq) but no CT or CF. Cells and supernatant were harvested from the chemostats and inoculated into batch reactors at the onset of each experiment. CT transformation was complete following first order kinetics with CF, DCM and CS_2 as the measurable transformation products, representing 20–40% of the original mass of CT, with CO_2 likely the unknown transformation product. CF was transformed to DCM and likely CO_2 at an order of magnitude rate lower than CT, while DCM was not further transformed. An analytical first order model including multiple key reactions effectively simulated CT transformation, product formation and transformation, and provided reasonable estimates of transformation rate coefficients. Biotic and abiotic treatments indicated that CT was mainly transformed via abiotic processes. However, the presence of live cells was associated with the transformation of CF to DCM. In biotic tests both TCE and CT were simultaneously transformed, with TCE transformed to ETH and approximately 15–53% less CF formed via CT transformation. A 14-day exposure to CF (CF_{max} = 1.4 μM) reduced all rates of chlorinated ethene respiration by a factor of 10 or greater.

1. Introduction

Carbon tetrachloride (CT) and chloroform (CF) are toxic and recalcitrant groundwater pollutants often found in mixtures with tetrachloroethene (PCE) and trichloroethene (TCE) (Doherty, 2000;

* Corresponding author.
E-mail address: lewis.semprini@oregonstate.edu (L. Semprini).

http://dx.doi.org/10.1016/j.chemosphere.2017.04.139
0045-6535/

Knox and Canter, 1996). Mixtures of chlorinated aliphatic hydrocarbons (CAHs) complicate bioremediation strategies with *Dehalococcoides mccartyi* (Dhc) due to inhibition of reductive dehalogenation exerted by low concentrations of CT and CF, leading to vinyl chloride (VC) accumulation (Bagley et al., 2000; He et al., 2005; Maymó-Gatell et al., 2001). For the bioremediation of mixtures of CT and TCE to be effective, further understanding of the dynamics of CT and CF transformation by *Dhc* needs to be developed.

Transformation of CT and CF in anaerobic environments involves parallel pathways catalyzed by biotic and abiotic mechanisms leading to the formation of dichloromethane (DCM), chloromethane (CM), methane (CH_4), carbon disulfide (CS_2), carbon monoxide (CO), carbon dioxide (CO_2), and formate (Figure SM-1) (Cappelletti et al., 2012; Criddle and McCarty, 1991; de Best et al., 1998; Hashsham and Freedman, 1999). Reduced iron sulfides (Butler and Hayes, 2000; Kriegman-King and Reinhard, 1994, 1992), biogenic iron minerals (McCormick et al., 2002; McCormick and Adriaens, 2004), and metallo-coenzymes (Chiu and Reinhard, 1995; Gantzer and Wackett, 1991; Krone et al., 1989a, 1989b) can catalyze abiotic transformation, while cometabolic CT and CF transformation has been found to occur in methanogenic (Bouwer and McCarty, 1983; Novak et al., 1998), acetogenic (Egli et al., 1988; Hashsham and Freedman, 1999), sulfate reducing (de Best et al., 1998; Egli et al., 1987), iron reducing (Maithreepala and Doong, 2008; McCormick et al., 2002), and fermenting (Criddle et al., 1990; Hashsham et al., 1995) environments. The addition of cofactors such as cobalamins (vitamin B_{12}) greatly enhances degradation rates and shifts product formation away from chlorinated metabolites (Hashsham et al., 1995; Workman et al., 1997).

The ability to couple detoxification of TCE and CT would be advantageous for the *in-situ* bioremediation of co-contaminated sites. A recent study examined the transformation of CT and PCE in a continuous flow column bioaugmented with the Evanite (EV) culture, an anaerobic dechlorinating mixed culture enriched in *Dhc* strains (Marshall et al., 2014) that can transform PCE to ethene (ETH) (Yu et al., 2005). CT (0.015 mM) and PCE (0.1 mM) were transformed simultaneously, but the process was highly dependent on the electron donor (Azizian and Semprini, 2016). When formate (1.5 mM) was provided as the hydrogen (H_2) source, PCE was transformed to VC (20%) and ETH (80%) along with complete CT transformation to CF (20%) and an unknown product (80%). CT addition was stopped and the electron donor was then switched to lactate as a fermenting substrate (1.1 mM). PCE dehalogenation decreased with the formation of cDCE (48%), VC (36%), and ETH (7%). Long-term exposure to CF impacted propionate fermentation, thus reducing the amount of available H_2 (Azizian and Semprini, 2016).

Presented here are the results from a study where CT and CF transformation was investigated by a TCE dehalogenating culture and supernatant produced under chemostat growth conditions. The goals of this study were to (1) determine the extent of CT and CF transformation and products formed by cells and reduced media (supernatant) obtained from chemostats containing TCE respiring anaerobic mixed cultures not previously acclimated to these compounds; (2) develop a kinetic model for CT degradation, product formation, and product transformation; and (3) explore the dynamics of CT, CF, and TCE simultaneous transformation in batch reactor systems.

2. Materials and methods

2.1. Chemicals

All chemicals used in the transformation studies or for external standards were analytical grade: CT, 99.9% (Sigma-Aldrich); CF, 99.9% (OmniSolv); DCM, 99.9% (Fisher Chemical); CS_2, 99.9% (Alfa Aesar); CM, 99.5% (Sigma-Aldrich); CH_4, 99.9% (Air Liquide); CO, 99.0% (Sigma-Aldrich); TCE, 99.9% (Macron Fine Chemicals); cDCE, 99.0% (TCI America); VC, 99.5% (Sigma-Aldrich); ETH, 99.5% (Airgas); formate, 99.0% (Alfa Aesar).

2.2. Anaerobic dechlorinating cultures

Experiments were conducted with the Evanite (EV) and Victoria/Stanford (VS) mixed anaerobic dechlorinating cultures capable of respiring TCE to ETH. The two cultures were grown in 5 L chemostats (Marshall et al., 2014) in a basal anaerobic mineral media (Yang and McCarty, 1998) modified to double the buffering capacity of the system (1 g/L K_2HPO_4 and 3 g/L Na_2CO_3). The EV and VS 5 L chemostats (designated EV-5L and VS-5L) have been operated since July 2007 and July 2009 respectively, with a hydraulic and mean cell retention time of approximately 50 days (Marshall et al., 2014). They receive an influent feed of saturated TCE (10 mM; 60 mEq) as electron acceptor and formate (45 mM; 90 mEq) as electron donor. Molecular characterization of EV-5L and VS-5L concurrent with this experiment found that operational transcription units (OTUs) for *tceA*-containing and *vcrA*-containing *D. mccartyi* populations account for 90–99% of the entire bacterial community (Mayer-Blackwell et al., 2017). The two populations encompass approximately equal fractions of the *D. mccartyi* consortium in EV-5L and VS-5L (Mayer-Blackwell et al., 2017), with the *tceA* strain responsible for the respiration of TCE/DCE (Magnuson et al., 2000) and the *vcrA* strain respiring DCE/VC (Müller et al., 2004). The molecular characterization of the two cultures also revealed that *Geobacter* and *Desulfitobacterium* OTUs accounted for 1–10% of the overall community structure (Mayer-Blackwell et al., 2017). Neither culture had been previously acclimated to CT, CF, or their transformation products.

2.3. Batch transformation studies

Saturated solutions of CT, CF, DCM, CS_2, and cDCE were prepared in anaerobic mineral media (Yu et al., 2005) and used in the batch transformation studies or for analytical standards. Saturated TCE was anaerobically transferred from the chemostat influent media for the same purposes (Marshall et al., 2014). Cells and supernatant (50 mL) from the EV-5L or VS-5L chemostat were transferred to anaerobically-prepared (95:5 N_2/H_2) clear borosilicate glass media bottles (125 mL; Wheaton, Millville, NJ) sealed with chlorobutyl rubber septa screen caps (Wheaton, Millville, NJ). The batch reactor headspace was sparged of residual ETH and chlorinated ethenes with a furnace treated anoxic gas (75:25 N_2/CO_2) prior to the onset of an experiment. Initial slug inputs of CT (0.86, 2.6, or 8.6 μM), CF (2.1 μM) or TCE (50 μM) were added to the batch reactors. Formate electron donor solution (2 mM) produces H_2 and was provided in excess for all experiments. Batch reactors were incubated in the dark at 20 °C and continuously shaken at 200 rpm. Dimensionless Henry's Law coefficients were used to perform mass balances required to estimate transformation rates (Sander, 2015; Staudinger and Roberts, 2001). Controls were conducted in sterile anaerobic mineral media (chemostat feed solution), and showed minimal degradation compared to the ADC + S (Figure SM-2).

Five treatments were investigated in triplicate batch reactors with ADC + S: CT alone (CT), CT and anaerobically-prepared sodium azide (NaN_3) biocide (Lichstein and Soule, 1944) (CT + NaN_3), CT and TCE simultaneous transformation (CT + TCE), TCE without CT or CF (TCE Control) and CF alone (CF). The cell culture density upon harvesting from the chemostat was ~15 mg/L on a cell protein basis (~0.75 mg of cell protein added to each reactor). The appropriate