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Interaction of perchlorate and trichloroethene bioreductions in mixed anaerobic culture

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HIGHLIGHT

- Perchlorate slowed but did not inhibit the complete dechlorination of TCE.
- The inhibition was mainly due to the thermodynamic preference of perchlorate to TCE.
- The generated oxygen was consumed and could not accumulate to inhibit the dechlorinators' activity.

GRAPHICAL ABSTRACT

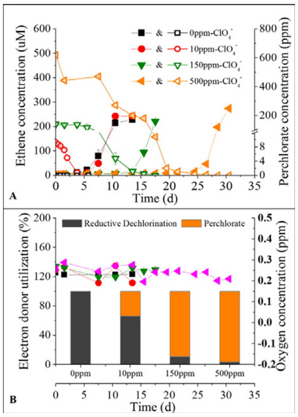

Fig. A plots the interaction of TCE and perchlorate bio-reduction under different concentrations of perchlorate and suggests that initial ethene wasn't formed until the perchlorate was completely reduced. B shows the electron donor utilization and oxygen generated during the experiment and indicates that it is perchlorate reduction over-competed for electron donor rather than oxygen generated that inhibits TCE reductive dechlorination.

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ABSTRACT

This work evaluated the interaction of perchlorate and trichloroethene (TCE), two common co-contaminants in groundwater, during bioreduction in serum bottles containing synthetic mineral salts media and microbial consortia. TCE at concentrations up to 0.3 mM did not significantly affect perchlorate reduction; however, perchlorate concentrations higher than 0.1 mM made the reduction of TCE significantly slower. Perchlorate primarily inhibited the reduction of vinyl chloride (VC, a daughter product of TCE) to ethene. Mechanistic analysis showed that the inhibition was mainly because perchlorate reduction is thermodynamically more favorable than reduction of TCE and its daughter products and not because of toxicity due to accumulation of dissolved oxygen produced during perchlorate reduction. As the initial perchlorate concentration increased from 0 to 600 mg/L in a set of serum bottles, the relative abundance of *Rhodocyclaceae* (a putatively perchlorate-reducing genus) increased from 6.3 to 80.6%, while the relative abundance of *Dehalococcoides*, the only known genus that is able to reduce TCE all the way to ethene, significantly decreased. Similarly, the relative abundance of Proteobacteria (a phylum to which most known perchlorate-reducing bacteria belong) increased from 22% to almost 80%.

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1. Introduction

Trichloroethene (TCE) is a widespread groundwater contaminant due to its use in metal degreasing and other industries (USEPA, 2000). The maximum contaminant level (MCL) of TCE in drinking water is 5 μg/L (USEPA, 2007) because of its strong adverse health effects, such as carcinogenicity and mutagenicity (Lee et al., 2013; USEPA, 2000). Known bacteria able to metabolize TCE can be classified into four phyla: Chloroflexi, Delta-Proteobacteria, Epsilon-Proteobacteria and Firmicutes (Maphosa et al., 2010). *Dehalococcoides*, which belongs to Chloroflexi, is the only known microorganism that can completely reduce TCE via *cis*-dichloroethene (*cis*-DCE) and vinyl chloride (VC) to ethene (Lee et al., 2013), using hydrogen as its obligatory electron (Adrian et al., 2000; Cupples et al., 2003; Lee et al., 2007). Other microbes are only reported to partially metabolize TCE to DCE or VC (Duhamel & Edwards, 2006; Maphosa et al., 2010).

Perchlorate (ClO_4^-) is one of the common co-contaminants of TCE in groundwater (Borden, 2007). Perchlorate was widely used in rocket propellants, munitions, fireworks, and signal flares (Logan, 2001). High levels of perchlorate inhibit thyroid hormone output and ultimately result in hypothyroidism (Coates and Achenbach, 2004). Although the US EPA has not yet established a MCL for ClO_4^-, some states have established clean-up levels ranging from 2 to 18 μg/L for ClO_4^- in drinking water (Gu & Coates, 2006). Recent studies show that concentrations range from 3.1–20 mg/L for ClO_4^- and 0.028–0.21 mg/L for TCE in groundwater (Borden, 2007; Dulaney; Stephenson).

TCE is probably susceptible to microbial transformation under aerobic conditions (Wilson and Wilson, 1985), but various studies showed that chlorinated ethenes are toxic to microorganisms. Kanazawa and Filip (1986) suggested that TCE at a concentration of 100 μg/100 g soil severely inhibited the activities of enzymes in soil. Singh and Olson (2010) indicated that *Pseudomonas putida* F1 close to a TCE-contaminated site was nonviable and used an exponential viability-decay model to test the toxicity of TCE to *P. putida F*1, with a viability-decay constant $k_{TCE} = 0.025\ h^{-4.95}$ ($r^2 = 0.965$). Meanwhile, chlorinated ethenes inhibit the activities of dechlorinators and other members of the microbial community under anaerobic conditions. High concentrations of TCE, *cis*-DCE, and VC (4, 6, and 7 mM, respectively) caused severe inhibition of reductive dechlorination and a decrease in the density of *Dehalococcoides* (Yu and Semprini, 2004; Sabalowsky and Semprini, 2010a, 2010b; Zhao et al., 2010). Yang and McCarty (2000) reported that *cis*-DCE did not inhibit dehalogenation over a range from 0.11 to 0.66 mM, but concentrations of PCE as low as 0.1 mM inhibited methanogenesis and homoacetogenesis. Ziv-El et al. (2012) concluded that 0.56 mM TCE caused stress to *Dehalococcoides* and *Geobacter*, while 0.37 mM TCE stressed *Acetobacterium* and possibly other homoacetogens. García-Solares et al. (2013) found that the tolerance of sulfate reducers to the TCE toxicity depended on the initial activity in sulfate-reducing microcosms. It is thus necessary to consider the toxicity of TCE to perchlorate-reducing bacteria to insure the successful bioreduction of TCE and perchlorate, so the first objective of this work is to evaluate if TCE inhibits perchlorate reduction due to its toxicity, as well as that of its daughter products.

Rapid and complete dechlorination of TCE can be hindered by alternative terminal electron accepting processes competing for hydrogen or acetate as their electron donor and carbon source, respectively. Competing microbial groups include denitrifiers, sulfate reducers, iron-reducing bacteria, methanogens, and hydrogenotrophic acetogens. Cho and Park (2005) reported that the TCE reduction rate decreased by a factor of 5 in the presence of 1.6 mM nitrate because of competition for the electron donor. Heimann et al. (2005) showed that 2.5 mM sulfate limited the rate of TCE dechlorination under steady state hydrogen supply (a few nM H_2) but did not affect dechlorination when rapid fermentation of lactate resulted in transient buildup of higher concentrations of H_2, suggesting that the inhibition was also due to competition for the electron donor. Wei and Finneran (2011) demonstrated that 10 mM Fe(III) reduction did not inhibit complete dechlorination, while the Fe(III) speciation (ferrihydrite versus Fe(III)-NTA) influenced daughter product distribution and the kinetics of dechlorination. Perchlorate is another electron acceptor that may compete for electrons. Therefore, the second objective of this work is to evaluate if perchlorate inhibits the reduction of TCE due to competition for common electron donors.

During perchlorate bioreduction, ClO_4^- is reduced step-wisely to chlorate and chlorite, and chlorite dismutase further catalyzes the transformation of chlorite to chloride and oxygen (Rikken et al., 1996), which is reportedly toxic to *Dehalococcoides*. Several studies reported that brief exposure of *Dehalococcoides* cultures to air or oxygen completely and irreversibly inhibited dechlorination (Adrian et al., 2000; He et al., 2003). Amos et al. (2008) later reported that oxygen inhibited reductive dechlorination, resulting in VC accumulation. Therefore, the authors concluded that *Dehalococcoides* strains were susceptible to oxygen inhibition. However, most perchlorate-reducing bacteria can utilize oxygen, nitrate, and chlorate as electron acceptors (Chaudhuri et al., 2002; Ju et al., 2008; Zhao et al., 2014); therefore, the direct effect of perchlorate on TCE reduction is of great interest. The third objective of this work is to evaluate if the oxygen produced during perchlorate reduction inhibits TCE reduction.

2. Materials and methods

To evaluate the interaction between perchlorate and TCE, we conducted a set of experiments in serum bottles. The serum bottles contained microbial consortia that were capable of reducing perchlorate and/or TCE. The bottles were supplied with lactate as the electron donor and perchlorate and TCE at different initial concentrations. To achieve the three objectives, we measured the concentrations of perchlorate, TCE, and dissolved oxygen during the experiments. We also studied the microbial community structure to further understand the interaction between perchlorate and TCE from the microbial perspective. The experimental details are described below.

2.1. Description of the perchlorate- and TCE-reducing consortia

The perchlorate-reducing consortium was originally enriched in a hydrogen-based membrane biofilm reactor (H_2-MBfR) inoculated with activated sludge obtained from the Qige Wastewater Treatment Plant (WWTP) in Hangzhou (China). The perchlorate-reducing consortium was transferred from the H_2-MBfR to a serum bottle containing 2 mM acetate and 10 mg/L ClO_4^-. The maximum rate of perchlorate removal by the consortium reached 78.2 mg Cl^-/L-g VSS-d. The serum bottle was sealed with a butyl rubber stopper and incubated in the dark at 30 °C at a shake speed of 150 rpm.

The TCE-reducing consortium YH was sub-cultured from the YCQ1 culture (Kranzioch et al., 2013, Wen et al., 2015) grown with lactate as the sole electron donor. The culture was dominated by *Dehalococcoides* spp. and could completely reduce 0.3 mM TCE to non-toxic ethene in 20 days. The culture was maintained under anaerobic conditions and incubated in the dark at 30 °C.

2.2. The effect of perchlorate on TCE-reducing consortia

Reduced anaerobic medium for TCE reduction was prepared according to Wen et al. (2015). The mineral salts medium contained the following reagents (per liter): 3.17 g KH_2PO_4, 14.33 g $Na_2HPO_4 \cdot 12H_2O$, 0.45 g $(NH_4)_2HPO_4$, 0.04 g $MgHPO_4 \cdot 3H_2O$, 1 mL of trace element solution A, and 1 mL of trace element solution B described by Kranzioch et al. (2013). Also, 0.2 mM L-cysteine and 0.2 mM $Na_2S \cdot 9H_2O$ were added as the reducing agents.