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Electrokinetic-enhanced bioremediation of tetrachloroethylene

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ABSTRACT

This study investigated the feasibility of using electrolysis of water to produce hydrogen and oxygen that were used as the electron donor and the electron acceptor for the anaerobic reductive dechlorination and the aerobic cometabolism of chlorinated ethylene contaminants, respectively. By employing the anaerobic and aerobic conditions in different sections of a column test, intermediates formed in the anaerobic dechlorination of PCE in the up-gradient section were further biodegraded through aerobic cometabolism in the down-gradient section, which used methane as the primary substrate. The results indicated that, the effluent PCE concentration was 0.88 μmol l^{-1} when the influent PCE concentration was 60 μmol l^{-1}, up to 98.5% PCE was degraded in 160 days with an additional current density up to 0.099 mA cm^{-2}. The First-order reaction rate was increased by 1.9–17.3 times with biostimulation by electrolysis method, showing that the benefits of an integrated electrochemistry and biotransformation system to complete mineralization of tetrachloroethylene. In addition, the specific microbial populations of *Dehalococcoides sp.* and methane-oxidizing bacteria were analyzed using the ethidium monoazide-PCR procedure, indicating the presence of viable bacteria in the integrated system.

1. Introduction

Tetrachloroethylene (PCE) is among the most common chlorinated ethylene contaminants in groundwater. PCE has four chlorine atoms with the formula $Cl_2C=CCl_2$. Due to its highly oxidized nature, PCE is difficult to be converted to ethylene under natural conditions (Adamson et al., 2003). Under anaerobic conditions, PCE can be sequentially dechlorinated by certain bacteria to less-chlorinated hydrocarbons, trichloroethylene (TCE), dichloroethylene (DCE), vinyl chloride (VC), and ethylene as the end products (Magnuson et al., 1998). Unfortunately, biodegradation of the metabolites, DCE and VC, is even slower than that of PCE and TCE, creating new long-term pollution challenges (Amos et al., 2009).

Chlorinated ethylene contaminants in soil and groundwater do not easily decompose, nor are they used as carbon sources or energy by microbes. The decomposition of chlorinated hydrocarbons relies on the metabolism of microbes that involves a primary substrate to convert chlorinated ethylene contaminants into vinyl chloride in the environment (Futagami et al., 2008). A common conversion pattern of chlorinated ethylene contaminants is the reductive dechlorination (Nijenhuis and Kuntze, 2016), for which, electron donors, intermediate medium, and electron acceptors are necessary. Hydrogen is typically the electron donor for the anaerobic reductive dechlorination, and is a controlling factor to the biodegradation rate. Alcohols (Kwon et al., 2016), carbohydrates (Révész et al., 2006), organic acids (Hu et al., 2013; Mészáros et al., 2013), H_2 gas (Lee et al., 2007), and metabolic intermediates (Miura et al., 2015) have been used to provide hydrogen to the dechlorination process. Because removing each chlorine atom from PCE requires two electrons, providing electron donors for the microbes would accelerate dechlorination of PCE (Flynn et al., 2000; Smidt and de Vos, 2004).

Previous studies have found that most reductive dechlorination of chlorinated ethylene contaminants is incomplete, causing the accumulation of less-chlorinated but toxic contaminants (Abe et al., 2009). To overcome this shortfall, aerobic microbial cometabolism has been discovered to fully mineralize and convert TCE, DCE, and VC, in addition to PCE, to end products, such as ethylene, carbon dioxide and water (Tiehm and Schmidt, 2011; Mattes et al., 2010). Chan et al. (2009) have also observed that a combination of anaerobic and aerobic biodegradation systems would enhance total degradation efficiency, and

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thereby overcoming the challenges of intermediate products due to incomplete reductive dechlorination (Ponza et al., 2010; Frascari et al., 2013; Yoshikawa et al., 2017).

Because under natural conditional in subsurface, population of appropriate microbes is typically limited, elector donors and acceptor which could be another constraining factor to the dechlorination. Because the substrate affinity (Ks) of *cis*-DCE was greater than that of PCE and TCE, the *cis*-DCE is more difficult to be degraded than PCE and TCE, causing incomplete dechlorination (Haston and McCarty, 1999). This study was based on cometabolic theory, used an electrolysis method to generate elector donors and acceptor to enhance bioremediation (Gill et al., 2014; Wang et al., 2015; Hassan et al., 2016). The electrolysis method was to produce hydrogen at the cathode ($2H_2O$ (l) + $2e^-$ → H_2 (g) + $2OH^-$ (aq)), effectively enhancing the rate of reductive dechlorination by providing additional electron donors (hydrogen) to the dechlorination process. In the meantime, oxygen gas is produced at the anode ($2H_2O$ (l) → O_2 (g) + $4H^+$ (aq) + $4e^-$) to enhance cometabolism of aerobic microbes (such as methane-oxidizing bacteria). These mineralizes which *cis*-DCE, VC and/or ethylene would biodegradation to less chlorine substituents (Frascari et al., 2015; Findlay et al., 2016).

The objective of this study was to the determine effect of water electrolysis for enhanced bioremediation of tetrachloroethylene. Lab experiments demonstrated the survival of microorganism under certain strength of currents and the effectiveness of biostimulation to achieve complete dechlorination of tetrachloroethylene. The results of this study would provide guidance to future application of the electrolysis method in bioremediation sites.

2. Materials and methods

2.1. Microorganisms

The microorganisms used in the study were initially collected from a rice-growing wetland and the effluent of a petroleum wastewater treatment plant. These two microorganisms were isolated in previous study (Chang et al., 2017), and mixed into a chemostat, which was continuously fed with PCE at a concentration of 60 μmol l^{-1} (7.88 mg l^{-1}). The PCE was used as the substrate to grow PCE, DCE, VC and ethylene utilizing cells. In addition to PCE, the nutrients of the chemostat also included: NH_4Cl, 270 mg l^{-1}; $FeCl_3$, 0.5 mg l^{-1}; $CaCl_2$, 6.6 mg l^{-1}; $MgSO_4$, 3.7 mg l^{-1}; KH_2PO_4, 4.5 mg l^{-1}; and K_2HPO_4, 178 mg l^{-1}. The chemostat was operated at a hydraulic retention time for 6 days before the acclimated anaerobic microorganisms, shown in Table 1, were used as the cell source in the experiment. The mixture of aerobic microorganisms which can degrade naphthalene, naphthalene, pyrene, chlorophenol and nitrophenol by long-term acclimation of chemostat. These aerobic cometabolism mixed bacteria, shown in Table 2, were used as the source of dichloroethylene biodegradation.

2.2. Electrolysis method enhanced bioremediation column test

A column test was used in this study to investigate the electrolysis-enhanced bioremediation. The length of the glass column was 60 cm, and the inner diameter is 3 cm. Quartz sand with a particle size ranging from 0.25 to 0.59 mm was used as the porous media. The operating system used DC power to decompose groundwater into hydrogen and oxygen. As shown in Fig. 1, the cathode (graphite electrode) was attached to the entrance of the column and the anode is attached to the mid-point of the column. In the up-gradient section of the column, the hydrogen generated at the cathode created an anaerobic condition and served as the electron donor for the anaerobic reductive dechlorination of PCE and TCE; in the down-gradient section of the column, the oxygen generated at the anode created an aerobic condition and served as the electron acceptor for the aerobic cometabolism of DCE and VC. Water samples were collected at 20 cm down-gradient from each electrode. A

Table 1
Microorganism community structure of the anaerobic mixed bacteria s in the up-gradient section column.

No.	Genus and species	Phylogenetic group	Similarity [%]
A	no gene sequence		
B	Smithella propionica	Bacteria; Proteobacteria; Deltaproteobacteria; Syntrophobacterales; Syntrophaceae; Smithella	99
C	*Curvibacter gracilis*	Bacteria; Proteobacteria; Betaproteobacteria; Burkholderiales; Comamonadaceae; Curvibacter	91
D	*Aquitalea magnusonii*	Bacteria; Proteobacteria; Betaproteobacteria; Neisseriales; Neisseriaceae; Aquitalea	99
E	*Desulfovibrio sp.* BL-157	Bacteria; Proteobacteria; Deltaproteobacteria; Desulfovibrionales; Desulfovibrionaceae; Desulfovibrio	92
F	*Dechloromonas sp.* EMB 50	Bacteria; Proteobacteria; Betaproteobacteria; Rhodocyclales; Rhodocyclaceae; Dechloromonas	98
G	*Desulfovibrio butyratiphilus*	Bacteria; Proteobacteria; Deltaproteobacteria; Desulfovibrionales; Desulfovibrionaceae; Desulfovibrio	99
H	Uncultured bacterium clone A1-C9_M13R	Bacteria; environmental samples	99%

Table 2
Microorganism community structure of the aerobic cometabolism mixed bacteria in the down-gradient section column.

No.	Genus and species	Phylogenetic group	Similarity [%]
S-1	no gene sequence		
S-2	*Candidatus Rhabdochlamydia* sp. cvE88	Bacteria; Chlamydiae; Chlamydiales; Rhabdochlamydiaceae; Candidatus Rhabdochlamydia	91
S-3	*Polaromonas* sp. JS666	Bacteria; Proteobacteria; Betaproteobacteria; Burkholderiales; Comamonadaceae; Polaromonas	93
S-4	Uncultured bacterium clone NK-M27	Bacteria; environmental samples	82
S-5	*Pseudomonas putida* strain BJ-38	Bacteria; Proteobacteria;	98
S-6	*Pseudomonas* sp. Ps1	Gammaproteobacteria; Pseudomonadales; Pseudomonadaceae; Pseudomonas	98
S-7	*Staphylococcus pseudintermedius* ED99	Bacteria; Firmicutes; Bacillales; Staphylococcus	93
S-8	*Comamonadaceae* bacterium OTSz_A_293	Bacteria; Proteobacteria; Betaproteobacteria; Burkholderiales; Comamonadaceae	99