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Bio-reduction of tetrachloroethen using a H_2-based membrane biofilm reactor and community fingerprinting

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ABSTRACT

Chlorinated ethenes in drinking water could be reductively dechlorinated to non-toxic ethene by using a hydrogen based membrane biofilm reactor (H_2-MBfR) under denitrifying conditions as it provides an appropriate environment for dechlorinating bacteria in biofilm communities. This study evaluates the reductive dechlorination of perchloroethene (PCE) to non-toxic ethene (ETH) and comparative community analysis of the biofilm grown on the gas permeable membrane fibers. For these purposes, three H_2-MBfRs receiving three different chlorinated ethenes (PCE, TCE and DCE) were operated under different hydraulic retention times (HRTs) and H_2 pressures. Among these reactors, the H_2-MBfR fed with PCE (H_2-MBfR 1) accomplished a complete dechlorination, whereas cis-DCE accumulated in the TCE receiving H_2-MBfR 2 and no dechlorination was detected in the DCE receiving H_2-MBfR 3. The results showed that 95% of PCE dechlorinated to ETH together with over 99.8% dechlorination efficiency. Nitrate was the preferred electron acceptor as the most of electrons generated from H_2 oxidation used for denitrification and dechlorination started under nitrate deficient conditions at increased H_2 pressures. PCR-DGGE analysis showed that *Dehalococcoides* were present in autotrophic biofilm community dechlorinating PCE to ethene, and RDase genes analysis revealed that *pceA*, *tceA*, *bvcA* and *vcrA*, responsible for complete dechlorination step, were available in *Dehalococcoides* strains.

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1. Introduction

Tetrachloroethen (PCE), which causes usually serious and persistent contamination in ground and surface waters, has been widely used in various industries, agriculture, and cleaning operations (Aulenta et al., 2005, 2008). While PCE, like other chlorinated compounds, is not easily biodegraded under natural conditions, it can be reductively dechlorinated when an appropriate electron donor is supplied under anaerobic conditions. H_2 can be effectively used as an electron donor for the dechlorination of PCE in drinking water as it eliminates the contamination of treated water with residual organics (Fig. 1). In dechlorination mechanism, each chlorinated ethene serve as electron acceptors and the chlorine atoms are sequentially replaced with hydrogen atoms. Among these, dichloroethene (DCE) and vinyl chlorinate (VC) are most toxic and ethene is non-toxic forms. Hence, the basic aim in the dechlorination process is the complete conversion of chlorinated ethenes to ethene. *Dehalococcoides* genus was reported to be responsible for the dechlorination of halogenated ethenes (Chung et al., 2008). Recent studies illustrated *Dehalococcoides* strains to use H_2 as electron source during the complete dechlorination of PCE (Popat and Deshusses, 2011; Popat et al., 2012). Unfortunately, most *Dehalococcoides* are not able to completely dechlorinate PCE to ethane whereas VS and BAV1 are capable of complete reduction to non-toxic ethene (Popat et al., 2012).

In order to overcome low solubility of H_2 gas in water, membrane biofilm reactor could be easily used, which allows delivering of it by diffusion through the wall of a bubbleless gas-transfer membrane to the reductive dechlorination bacteria (RDB) efficiently and safely. A number of studies showed that membrane biofilm reactor could be used for bio-reduction of oxidized contaminants (Lee and Rittmann, 2002; Nerenberg et al., 2002, 2006; Chung et al., 2006, 2007, 2008; Hasar et al., 2008). Among these studies, Chung et al. (2008) studied bio-reduction of TCE. They obtained promising results as under denitrifying conditions dechlorination of TCE started immediately and completed to ethane within 120 days accompanying with the increased portion of TCE-dechlorinating bacteria, *Dehalococcoides*, after TCE addition (Chung et al., 2008).

This study aims at investigating the PCE-dechlorination efficiency of H_2-MBfRs, considering that nitrate can be present in water together with various oxidized contaminants. Also, the rate and stoichiometry of each reduction step and change of microbial community in each step were explored in three H_2-MBfRs operated in parallel. Moreover, microbial community in three H_2-MBfRs was also evaluated in detail by considering dechlorination steps to improve understanding of the microbial interactions between each dechlorination step. The microbial tests with *Dehalococcoides* sp. should be monitored completely via the analysis of RDase markers rather than the microcosm analysis of 16rRNA. Therefore, the methods of 16 rRNA analysis and RDase gene monitoring with a simple PCR method have shed light upon the steps of reductive dehalogenation containing a variety of *Dehalococcoides* sp.

2. Materials and methods

2.1. Experimental setup and operational conditions

Three denitrifying hydrogen based Membrane Biofilm Reactors (H_2-MBfRs) were fed with tetrachloroethylene (PCE), trichloroethylene (TCE) and dichloroethene (cis-DCE), respectively. A schematic of the H_2-MBfR is shown in Fig. 2. In brief, each MBfR system consisted of two glass tubes connected with Norprene tubing and plastic barbed fittings. The glass tube contained a main bundle of 32 hollow-fiber non-pores membranes; each 31 cm long, and the other column contained 5 fibers sacrificed for biofilm samples. Each fiber's outside diameter was 280 μm, which provided a surface area of 87.26 cm^2. Each H_2-MBfR consisted of two membrane columns connected by a recirculation loop; a high recirculation ratio (100–150) caused each H_2-MBfR to behave as a completely mixed biofilm reactor. The total volume of each H_2-MBfR was 30 cm^3, of which 0.7 cm^3 was in the fibers, making the liquid volume 29.3 cm^3. Pure H_2 gas was supplied at a set pressure to the inside of the hollow fibers through the manifold at the base. The H_2 was delivered to the inside of the fibers under pressure of 8, 10 and 12 psi. The reactors were operated in a temperature controlled room at 25–30 °C. Reactors were preserved with aluminum foil to prevent light permeability.

2.2. Synthetic medium

The composition of nitrate medium used in this study contained, per liter: 0.0342 g $NaNO_3$, 0.252 g $NaHCO_3$, 0.005 g $MgSO_4 7H_2O$, 0.128 g KH_2PO_4, 0.434 g Na_2HPO_4 and 2 ml trace mineral solution. The trace mineral solution contained, per liter: 100 mg $ZnSO_4 \cdot 7H_2O$, 30 mg $MnCl_2 \cdot 4H_2O$, 300 mg H_3BO_3, 200 mg $CoCl_2 \cdot 6H_2O$, 10 mg $CuCl_2 \cdot 2H_2O$, 10 mg $NiCl_2 \cdot 6H_2O$, and 30 Na_2SeO_3. The composition above was same for all three reactors and only different chlorinated ethenes were separately fed into the reactors using another line.

2.3. Analysis

The influent and effluent of the reactors were sampled for the measurement of PCE, TCE, DCE, VC, ethene, NO_3−−N, and NO_2−−N to evaluate the performance of the H_2-MBfRs. The measurement of PCE, TCE, DCE, VC, and ethane were conducted

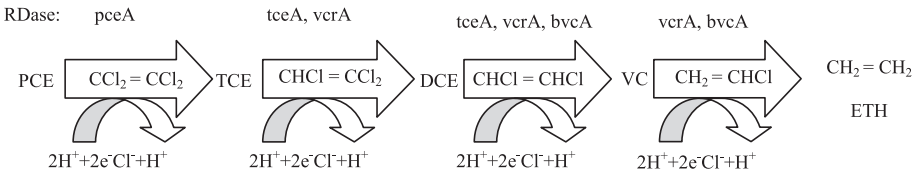

Fig. 1 – Reductive dechlorination pathway of PCE to ethene.