Bioresource Technology 240 (2017) 98–105

Contents lists available at ScienceDirect

Bioresource Technology

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

Oxygen exposure effects on the dechlorinating activities of a trichloroethene-dechlorination microbial consortium

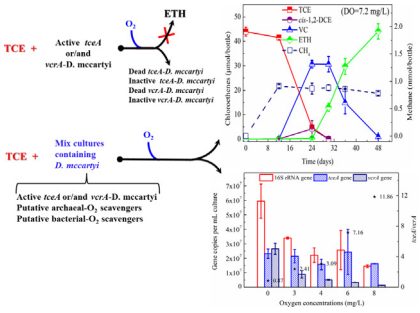
Na Liu [a], Haijun Li [a], Mengyan Li [b], Longzhen Ding [a], Chih-Huang Weng [c,*], Cheng-Di Dong [d]

[a] Key Laboratory of Groundwater Resources and Environment, Ministry of Education, Jilin University, Changchun 130021, China
[b] Department of Chemistry and Environment Science, New Jersey Institute of Technology, Newark, NJ 07102, USA
[c] Department of Civil and Ecological Engineering, I-Shou University, Kaohsiung City 84008, Taiwan
[d] Department of Marine Environment Engineering, National Kaohsiung Marine University, Kaohsiung 81157, Taiwan

HIGHLIGHTS

- *Dehalococcoides mccartyi* (Dhc) exposed to O_2 could degrade TCE to ETH.
- *tceA* gene-Dhc was less sensitive to O_2 exposure than *vcrA* gene-Dhc.
- Non-dechlorinators were crucial to scavenge O_2 to protect Dhc from being damaged.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 5 December 2016
Received in revised form 23 February 2017
Accepted 24 February 2017
Available online 28 February 2017

Keywords:
Trichloroethylene
Oxygen
Dehalococcoides mccartyi
Non-dechlorinators

ABSTRACT

The aim of this work was to study the effects of the presence of oxygen on the dechlorination of trichloroethene by a microbial consortium containing *D. mccartyi*. The 16S rRNA and reductive dechlorination genes of the functional bacteria and the non-dechlorinators were monitored. Exposing the consortium to oxygen altered the overall biotransformation rate of the dechlorination process, biotransformation processes prolonged with oxygen concentrations changing from 0 to 7.2 mg/L, however, trichloroethylene was eventually dechlorinated to ethene. The qPCR analyses revealed that the *D. mccartyi* strains containing the *tceA* gene were less sensitive to exposure to oxygen than were the *D. mccartyi* strains containing the *vcrA* gene. High-throughput sequencing by Illumina MiSeq indicated that the non-dechlorinating organisms were probably crucial to scavenge the oxygen to protect *D. mccartyi* from being damaged.

1. Introduction

Trichloroethene (TCE) is one of the most pervasive groundwater pollutants around the world because it has been widely used in dry cleaning and degreasing applications owing to its excellent solvent properties (Guerrero-Barajas et al., 2014; Kao et al., 2016; Mondal et al., 2016). Microbial anaerobic dechlororespiration is a natural metabolic process that plays a role in the biodegradation of chlorinated compounds (Aulenta et al., 2006). Large numbers of microorganisms commonly found in the subsurface environment have been found to reductively dechlorinate TCE to less-chlorinated ethenes under anaerobic conditions (Duhamel and Edwards, 2006; Frascari et al., 2015; Shukla et al., 2014). However, no strain had been found to completely biodegrade TCE until *Dehalococcoides mccartyi* 195 was discovered (Maymo-Gatell, 1997). This organism completely dechlorinates TCE through a stepwise

* Corresponding author at: Department of Civil and Ecological Engineering, I-Shou University, Da-Shu District, Kaohsiung, 84001, Taiwan.
E-mail address: chweng@isu.edu.tw (C.-H. Weng).

http://dx.doi.org/10.1016/j.biortech.2017.02.112
0960-8524/

dechlorination process, producing ethene (ETH). Microbial anaerobic dechlororespiration is now an important alternative remediation strategy. Several other novel *D. mccartyi* strains have since been cultured and identified (He et al., 2005; Low et al., 2015). Biostimulation has been performed once *D. mccartyi* has been detected, otherwise bioaugmentation has been performed. The anaerobic biodegradation of TCE is catalyzed by reductive dehalogenases (RDases) coded in the *D. mccartyi* genes. Three RDase genes are responsible for the degradation of TCE, the *tceA* gene was found in an anaerobic enrichment culture containing *D. mccartyi*, and the other two genes, *vcrA* and *bvcA* were found later (Krajmalnik-Brown et al., 2004; Magnuson et al., 2000; Muller et al., 2004).

Some studies revealed that *D. mccartyi* strains, especially single-strain cultures, are very sensitive to unfavorable conditions, and this has sometimes caused the biodegradation of TCE to be incomplete. For instance, *D. mccartyi* are strictly anaerobic, so their activities can be seriously decreased in the presence of dissolved oxygen, which can enter a system through the infiltration of oxygenated surface water (e.g., during rain events) or the migration of oxygenated groundwater (Amos et al., 2008). The performances of *D. mccartyi* have been determined in the presence of oxygen (Adrian et al., 2007; Amos et al., 2008; Richardson et al., 2002). The dechlorination activities of all the *D. mccartyi* strain were affected to some extent by oxygen. Adrian et al. (2007) showed that the dechlorination abilities of pure cultures of *D. mccartyi* 195 and CBDB1 were irreversibly inhibited by exposure to oxic conditions for as little as 5 s. In another study, exposing a consortium that could dechlorinate TCE to ethene at an initial dissolved oxygen (DO) concentration of 4 mg/L led to TCE being incompletely dechlorinated to the end product, vinyl chloride (VC), a more toxic product than the parent reactant (Amos et al., 2008). However, all the experiments conducted above focused on the performances of *D. mccartyi*, the non-dechlorinators within a consortium participated in this process were seldom elucidated. Moreover, little is known about the dechlorination activities when the initial concentration of oxygen is different and a different consortium exists.

High-throughput sequencing technologies have brought tremendous improvements in automated sequencing and analysis of genome features (Choi and Liu, 2014; Clark et al., 2016; Lien et al., 2016; Liu et al., 2016; Okonechnikov et al., 2016; Watson et al., 2016). This technique provides an opportunity to generate large amounts of sequence data within a short time at low cost. Its ability to identify large numbers of species from complex communities enabling the analysis of the total microbial communities present within a microbial consortium (Guo et al., 2014; Porazinska et al., 2009; Wu et al., 2015). Apart from identification functional organisms (*D. mccartyi*) directly involved in reductive dechlorination, one advantage of this revolutionized molecular tool applies to characterize a *D. mccartyi*-containing mixed community performing reductive dechlorination is the ability to simultaneously offer insights into the roles of the non-dechlorinators, which is probably of equal importance to the *D. mccartyi*. The quantitative polymerase chain reaction (qPCR) is another widely used culture-independent molecular approaches, owing to its superiority to specifically quantify particular taxonomic or functional markers (Ahn et al., 2008). Quantitative information is critical for establishing cause-and-effect relationships between specific treatments, changes in *D. mccartyi* abundance, and chloroethene detoxification (Porazinska et al., 2009; Tsai et al., 2014).

The aim of this study was to evaluate the effect of oxygen on the dechlorination of TCE by a methanogenic microbial consortium containing *D. mccartyi*. The qPCR approaches was used to enumerate the amounts and variations of functional organisms (*D. mccartyi*) and the *tceA* and *vcrA* RDase genes presented. Illumina MiSeq sequencing of 16S rRNA gene amplicons was likewise applied to analyze the microbial community structure and composition changes of this consortium before and after oxygen was added. A commonly encountered case is that when shallow groundwater table receives oxygenated surface water infiltration (e.g., during rain events), oxygen cannot be consumed immediately by the reducing agents. Then oxygen would become a factor influence dechlorination by *D. mccartyi*. Such profoundly influence bioremediation using *D. mccartyi*-containing consortium should be appropriately evaluated.

2. Materials and methods

2.1. Chemicals

The chloroethenes TCE, 1,1-dichloroethylene(1,1-DCE), *cis*-1,2-DCE, *trans*-1,2-DCE, and VC were purchased from Sigma-Aldrich (St. Louis, MO, USA) and J&K Scientific (Beijing, China). Ultra-high-purity ethylene, methane, nitrogen, hydrogen, two gas mixtures (80:10:10 nitrogen/carbon dioxide/hydrogen and 80:20 nitrogen/carbon dioxide), and other gases were supplied by Changchun Xinguang Gas Manufacturing (Changchun, China). All other chemicals were of reagent grade, and they were used without further purification unless otherwise specified.

2.2. Maintenance of the culture and preparation of the medium

A methanogenic microbial consortium for dechlorinating chloroethenes that is stable and rapidly dechlorinates TCE was kindly donated by Prof. Tielong Li (Xiu et al., 2010). The consortium was cultured in our laboratory for more than 2 years in a 240-mL reactor with 100 mL of liquid medium, using 4.56 mmol methanol and 45.6 μmol TCE as the electron donor–acceptor pair. The culture was periodically transferred to freshly prepared medium and cultivated without stirring at 30 °C in an incubator. Sterile anaerobic medium was prepared as described previously (Carr and Hughes, 1998), and one milliliter of a concentrated filter-sterilized vitamin solution (containing 20 mg/L biotin, 50 mg/L thiamine, and 50 mg/L cyanocobalamin) was added after the autoclaved medium had cooled to room temperature. The medium was then flushed with ultra-pure nitrogen or argon for 30 min and then transferred to an anaerobic chamber. Resazurin (1 mg/L; the redox indicator), sodium bicarbonate (8 g/L; the buffer), and the reductants $Na_2S{\cdot}9H_2O$ (300 mg/L) and $FeCl_2{\cdot}4H_2O$ (40 mg/L) were added successively to the medium. The medium was then adjusted to pH 7.1 ± 0.1 with 3.6 M HCl and kept in an anaerobic chamber until use.

2.3. Oxygen exposure experiments

All the experiments were performed in 240-mL amber screw-capped bottles. Each cap had a Mininert® valve (Supelco, Bellefonte, PA, USA). A 95-mL aliquot of the autoclaved mineral salt medium was added to each bottle, and the medium was inoculated with 5 mL (5% v/v) of the dechlorinating stock culture that had been maintained using TCE as an electron acceptor and methanol as an electron donor. A stock TCE and methanol solution was then added to each bottle to provide a total of about 45.6 μmol TCE and 4.56 mmol methanol per bottle.

Two batches of experiments were designed. One was conducted to test the effects of oxygen concentrations on this trichloroethene-dechlorination microbial consortium. Filter-sterilized oxygen (nitrogen when the oxygen concentration was 0 mg/L) was added to bottles using a gas-tight syringe, to bring the oxygen concentration in the headspace to 0, 7.5, 10.0, 15.0, and 20.0% (v/v), corresponding to DO concentration of 0, 2.7, 3.6, 5.4, and 7.2 mg/L in