Journal of Hazardous Materials 275 (2014) 99–106

Contents lists available at ScienceDirect

Journal of Hazardous Materials

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

Enhancing trichloroethylene degradation using non-aromatic compounds as growth substrates

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HIGHLIGHTS

- Microbial growth and toluene degradation rate decreased as toluene increased.
- Co-metabolic TCE degradation was enhanced when non-aromatic compound was supplied.
- Specific TCE degradation rate increased as non-aromatic compound concentration increased.
- TCE degradation rate using ethanol as growth substrate was the highest than others.

ARTICLE INFO

Article history:
Received 1 February 2014
Received in revised form 21 April 2014
Accepted 22 April 2014
Available online 30 April 2014

Keywords:
Cometabolism
Trichloroethylene
Toluene
Burkholderia cepacia G4
Pseudomonas putida

ABSTRACT

The effect of non-aromatic compounds on the trichloroethylene (TCE) degradation of toluene-oxidizing bacteria were evaluated using *Burkholderia cepacia* G4 that expresses toluene 2-monooxygenase and *Pseudomonas putida* that expresses toluene dioxygenase. TCE degradation rates for *B. cepacia* G4 and *P. putida* with toluene alone as growth substrate were 0.144 and 0.123 μg-TCE/mg-protein h, respectively. When glucose, acetate and ethanol were fed as additional growth substrates, those values increased up to 0.196, 0.418 and 0.530 μg-TCE/mg-protein h, respectively for *B. cepacia* G4 and 0.319, 0.219 and 0.373 μg-TCE/mg-protein h, respectively for *P. putida*. In particular, the addition of ethanol resulted in a high TCE degradation rate regardless of the initial concentration. The use of a non-aromatic compound as an additional substrate probably enhanced the TCE degradation because of the additional supply of NADH that is consumed in co-metabolic degradation of TCE. Also, it is expected that the addition of a non-aromatic substrate can reduce the necessary dose of toluene and, subsequently, minimize the potential competitive inhibition upon TCE co-metabolism by toluene.

1. Introduction

Trichloroethylene (TCE) is a chlorinated aliphatic hydrocarbon (CAH) that has been widely used as an ingredient in industrial cleaning. It is one of the most frequently detected contaminants in groundwater. The U.S. National Toxicology Program (NTP) and the U.S. International Agency for Research (IARC) have determined that trichloroethylene is "reasonably anticipated to be a human carcinogen" and "probably carcinogenic to humans," respectively [1]. Because its density is higher than that of water, high vapor pressure and low sorption to soil and sediments, when it is spilled, TCE is likely to move downward through the subsurface soil until its lower permeability features impede its flow into groundwater [2,3].

TCE can be degraded under both anaerobic and aerobic conditions. Under anaerobic conditions, it can be transformed to ethane. However, anaerobic dechlorination has the following limitations: (1) slow degradation rate, (2) incomplete dechlorination and (3) production of highly toxic compounds such as vinyl chloride (VC), 1,1-dichloroethylene (DCE) and *cis*-1,2-DCE to human [4]. On the other hand, under aerobic conditions, TCE can be mineralized via cometabolic transformation reaction to CO_2, without harmful byproducts. Cometabolic transformations are reactions catalyzed by existing microbial enzymes and yield no carbon and energy benefits to the transforming cells. Therefore, a growth substrate must be available at least periodically to grow new cells, provide an energy source, and induce production of the cometabolic enzymes [5,6].

Methane, phenol, toluene, propane, *ortho*-xylene and ammonia are generally used as growth substrates for cometabolic TCE degradation. Some researchers have suggested that the following factors may adversely affect cometabolic TCE degradation: (1) enzyme

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http://dx.doi.org/10.1016/j.jhazmat.2014.04.052
0304-3894/

competition between the aromatic growth substrates and cosubstrate, (2) depletion of NAD(P)H due to its non-regeneration during TCE degradation and (3) toxicity of TCE and transient intermediates of cometabolic substrate oxidation [6–9].

The toluene-to-TCE ratio is a significant determining factor of the extent of TCE degradation. Because toluene oxygenase-expressing is influenced by the toluene concentration, TCE is not fully degraded if the enzyme-expressing is insufficient. Lu et al. [10] reported that the optimal toluene-to-TCE concentration ratio is between 14 and 24 mg toluene per mg TCE. For toluene-to-TCE initial concentration ratios ranging from 6.8 to 1022 mg toluene per mg TCE, TCE degradation ranged from 60 to 97% with 100% removal of toluene [10–12]. Lee and Liu [13] reported that TCE degradation was inhibited by toluene at an initial toluene concentration of 30 mg/L in the mixed culture HHTO4. Moreover, Mirpuri et al. [14] reported that a lag phase at toluene concentration of 20 mg/L and below were shorter than that at higher 20 mg/L, suggesting that higher toluene concentrations could inhibit growth of *Pseudomonas putida* 54G.

On the other hand, *P. putida* F1, *Burkholderia cepacia* G4, *Ralstonia pickettii* PKO1 and *Pseudomonas mendocina* KR1 have demonstrated that TCE degradation can be supported by alternative and non-aromatic growth substrates [15–18].

Therefore, the objective of this study is to investigate the enhancement potentials of cometabolic TCE degradation by adding different non-aromatic compounds such as ethanol, glucose, and acetate as growth substrate. These non-aromatic compounds were supplied to minimize the oxygenase competition between toluene and TCE and to overcome incomplete TCE degradation due to a low toluene-to-TCE ratio.

2. Materials and methods

2.1. Chemicals

Toluene and TCE were purchased from Sigma–Aldrich. Glucose, sodium acetate, ethanol, methanol, sodium formate and other chemicals and reagents were the highest-purity compounds available and were sterilized prior to use.

2.2. Microorganism cultures

P. putida (KCTC 1644) and *B. cepacia* G4 (ATCC 53617) were obtained from the Korean Collection for Type Cultures (KCTC) and the American Type Culture Collection (ATCC), respectively. *P. putida* expresses toluene dioxygenase, and *B. cepacia* G4 expresses toluene monooxygenase. Stock cultures were stored in 15% glycerol at −70 °C.

Cells were grown in an overnight nutrient broth (NB) at 28 °C for each experiment. When the cultures attained an optical density (OD) of 2 at 540 nm, we collected cells by centrifuging the cultures and rinsing them with phosphate-buffered saline (PBS) at a pH of 7.2. Then, 1 mL of the harvested culture was used as the inoculum for the experiments. A phosphate-buffered mineral medium was used to culture microorganisms, and the pH was adjusted to 7.2. The cultures were shaken at 150 rpm and 28 °C. Toluene was fed as the sole carbon source at 10 mg/L every 2 days.

2.3. Batch experiments

The TCE degradation tests in this study were performed as batch tests. The shaking incubator was used at a 150 rpm agitation speed for all of the test bottles, and the temperature was kept constant at 28 °C. To prevent microbial contamination, all of the tests were prepared on a clean bench. Teflon-treated septa and glass bottles were used to prevent the adsorption of TCE onto silicon and amber serum bottles were used to prevent the photolysis of toluene. All serum bottles including medium solution for batch experiments were autoclaved at 120 °C and 1.5 kgf/cm^2 for 20 min. The amber serum bottle containing medium was sealed with a Teflon-treated silicone septa and an aluminum cap. After autoclave treatment, the bottle was cooled at 4 °C. To provide an aerobic condition, pure oxygen was supplied until saturated. The saturated oxygen concentration was approximately 40 mg/L, and the headspace of the bottle was also filled with pure oxygen. TCE (1100 mg/L) and toluene (550 mg/L) were used as stock solutions for this test. Ethanol, glucose, acetate, formate and methanol were also used as 10,000-mg/L stock solutions. The stock solutions of TCE, toluene and non-aromatic compounds were fed at initial concentrations of 0.5 mg/L, 10 mg/L and 0–200 mg/L, respectively. The process was conducted so that the total liquid phase volume was 50 mL, and a gas phase volume equal to the added volume was discharged to maintain a constant pressure in the bottle.

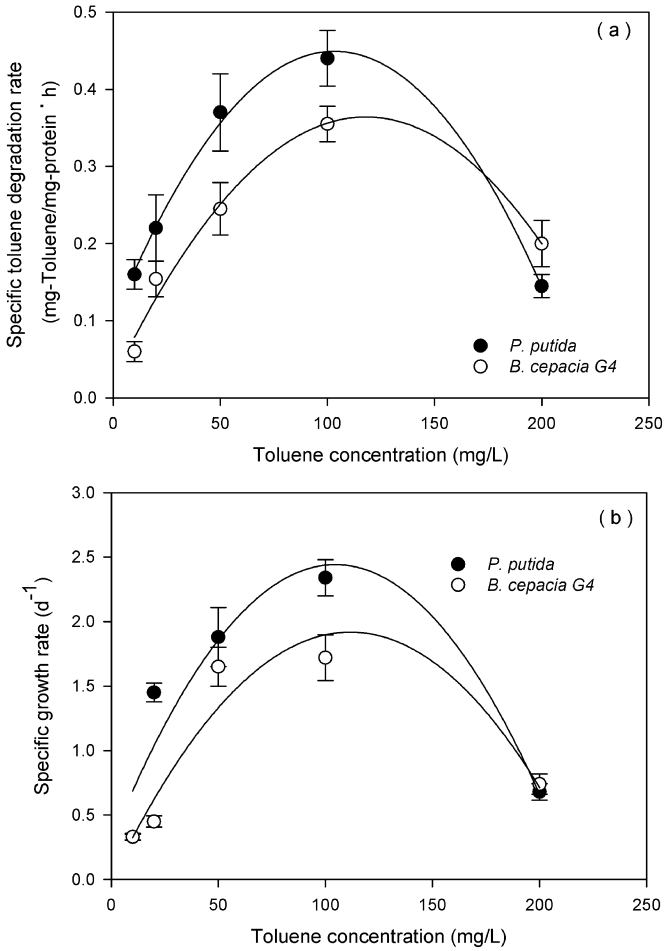

Fig. 1. Specific toluene degradation and growth rates of (a) *B. cepacia* G4 and (b) *P. putida* according to toluene concentration.

2.3.1. Toluene degradation and growth rates of toluene-oxidizing bacteria according to initial toluene concentration

P. putida and *B. cepacia* G4 grown on NB sampled and centrifuged at 3000 rpm for 20 min to recover cells, which were then washed with PBS. The solution was then diluted in an amber serum bottle with a mineral medium to an OD_{540nm} of 0.1. Initial toluene concentrations were ranged from 10 to 200 mg/L. Toluene concentrations and ODs were measured to determine growth rates.