Science of the Total Environment 520 (2015) 154–159

Contents lists available at ScienceDirect

Science of the Total Environment

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

Short Communication

Biodegradation of 1,4-dioxane: Effects of enzyme inducers and trichloroethylene

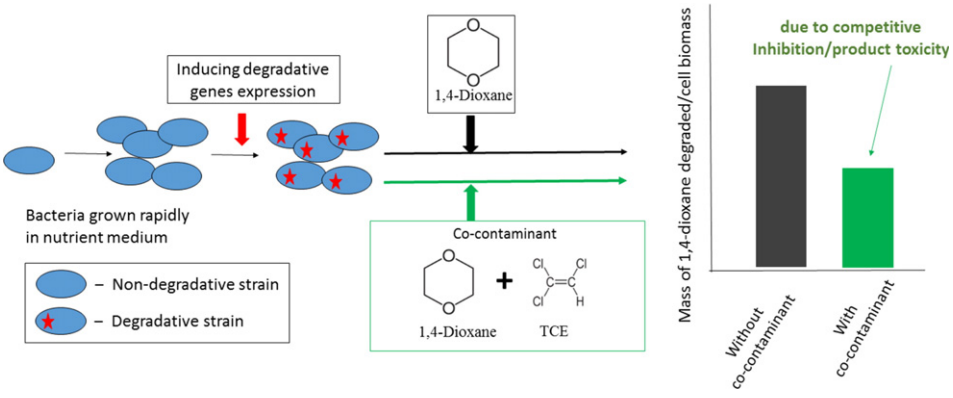
Steven Hand, Baixin Wang, Kung-Hui Chu *

Zachry Department of Civil Engineering, Texas A&M University, College Station, TX 77843-3136, USA

HIGHLIGHTS

- Propane- and 1-butanol-induced *Rhodococcus jostii* RHA1 degraded 1,4-dioxane.
- Propane- and 1-butanol-induced *Mycobacterium vaccae* JOB5 degraded 1,4-dioxane.
- Regardless of inducer, JOB5 and RHA1 degraded 1,4-dioxane, TCE, and mixtures.
- Propane-induced JOB5 showed the highest 1,4-dioxane transformation capacity.
- Product toxicity resulted in inactivation of JOB5 and RHA1.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 21 October 2014
Received in revised form 28 February 2015
Accepted 8 March 2015
Available online 24 March 2015

Editor: Eddy Y. Zeng

Keywords:
1,4-Dioxane
TCE
Oxygenases
Cometabolic degradation
Bioremediation
Product toxicity

ABSTRACT

1,4-Dioxane is a groundwater contaminant and probable human carcinogen. In this study, two well-studied degradative bacteria *Mycobacterium vaccae* JOB5 and *Rhodococcus jostii* RHA1 were examined for their 1,4-dioxane degradation ability in the presence and absence of its co-contaminant, trichloroethylene (TCE), under different oxygenase-expression conditions. These two strains were precultured with R2A broth (complex nutrient medium) before supplementation with propane or 1-butanol to induce the expression of different oxygenases. Both propane- and 1-butanol-induced JOB5 and RHA1 were able to degrade 1,4-dioxane, TCE, and mixtures of 1,4-dioxane/TCE. Complete degradation of 1,4-dioxane/TCE mixture was observed only in propane-induced strain JOB5. Inhibition was observed between 1,4-dioxane and TCE for all cells. Furthermore, product toxicity caused incomplete degradation of 1,4-dioxane by 1-butanol-induced JOB5. In general, the more TCE degraded, the greater extent of product toxicity cells experienced; however, susceptibility to product toxicity was found to be both strain- and inducer-dependent. The findings of this study provide fundamental basis for developing an effective in-situ remediation method for 1,4-dioxane-contaminated ground water and the first known study of 1,4-dioxane degradation by wild-type strain RHA1.

1. Introduction

1,4-Dioxane, a common solvent stabilizer, has been detected along with trichloroethylene (TCE) and other solvents (Abe, 1999; USEPA, 2013) in groundwater and surface water across the United States and

* Corresponding author at: CE/TTI 402A, Civil Engineering Department, Texas A&M University, 3136 TAMU, College Station, TX 77843-3136, USA.
E-mail address: kchu@civil.tamu.edu (K.-H. Chu).

http://dx.doi.org/10.1016/j.scitotenv.2015.03.031
0048-9697/

Japan (Adamson et al., 2014; Anderson et al., 2012; Zenker et al., 2003). Limited 1,4-dioxane exposure can cause respiratory tract irritation, while chronic exposure can cause skin, liver, and kidney damages (USEPA, 2013). The International Agency for Research on Cancer (IARC) has classified 1,4-dioxane as a possible human carcinogen (IARC, 1999). While the EPA has not issued a 1,4-dioxane advisory level, 1,4-dioxane is currently listed on Contaminant Candidate List 3 and the State of Colorado has set a cleanup level of 0.35 μg/L (USEPA, 2013).

Cost-effective in-situ remediation of 1,4-dioxane-contaminated sites is currently limited due to its physical and chemical properties. 1,4-Dioxane is a non-volatile colorless liquid with a low organic carbon partition coefficient ($\log K_{oc} = 1.23$) and a low octanol–water partition coefficient ($\log K_{ow} = -0.27$), making it highly mobile in groundwater, once released into the subsurface. Chemical oxidation like chlorination (Klečka and Gonsior, 1986) and advanced oxidation techniques are impractical for remediating large diluted plumes (Adams et al., 1994; Félix-Navarro et al., 2007; Stefan and Bolton, 1998). Similarly, while natural attenuation is possible for 1,4-dioxane, it is not ubiquitous (Chiang et al., 2012; Li et al., 2013, 2014; Sei et al., 2010). However, 1,4-dioxane is biodegradable. Several pure and mixed cultures are known to degrade 1,4-dioxane via growth-linked (Kim et al., 2009; Nakamiya et al., 2005; Parales et al., 1994; Sei et al., 2013) or non-growth-linked degradation mechanisms (cometabolic reactions) (Burback and Perry, 1993; Kinne et al., 2009; Mahendra and Alvarez-Cohen, 2006; Vainberg et al., 2006; Zenker et al., 2004), suggesting that in-situ bioaugmentation can be an attractive treatment alternative.

Among known degradative cultures, *Mycobacterium vaccae* JOB5 (referred as JOB5 hereafter) (Kim et al., 2009) and *Rhodococcus jostii* RHA1 (referred as RHA1 hereafter) (Seto et al., 1995) are of particular interest because: (i) propane-grown JOB5 is known to degrade 1,4-dioxane (Mahendra and Alvarez-Cohen, 2006) and propane-grown RHA1 is known to degrade nitrosamines (Sharp et al., 2007); (ii) both JOB5 and RHA1 can express oxygenases to co-metabolically degrade TCE and other chlorinated solvents which are also present in 1,4-dioxane-impacted groundwater (Burback and Perry, 1993; Sharp et al., 2007; Vanderberg et al., 1995; Wackett et al., 1989); and (iii) both strains are metabolically diverse, making them capable of growth on multiple substrates easily.

Cometabolic degradation is commonly complicated by inhibition and product toxicity (Alvarez-Cohen and Mccarty, 1991; Bosma and Janssen, 1998; Chu and Alvarez-Cohen, 1998; Ely et al., 1997; Pagan et al., 1998; Travis and Rosenberg, 1997). However, the effects of chlorinated co-contaminants on 1,4-dioxane degradation, particularly related to substrate inhibition and product toxicity, are still poorly understood (Anderson et al., 2012; Mahendra et al., 2013).

Both strains can grow much faster with a liquid nutrient medium than with gaseous substrates like propane and butane. However, cells grown on nutrient rich medium cannot degrade 1,4-dioxane or chlorinated compounds due to lack of inducers (propane and 1-butanol) for degradative enzyme expression. For these strains, propane and/or 1-butanol are known to serve not only as primary substrates but also as inducers for propane monooxygenase or butane monooxygenase expression, respectively. Accordingly, we hypothesized that: (i) wild-type RHA1 can degrade 1,4-dioxane; (ii) bacteria expressing different oxygenases result in different degradation potentials for 1,4-dioxane; and (iii) product toxicity generated from the degradation of 1,4 dioxane and its co-contaminants affect 1,4-dioxane degradation potential of oxygenase-expressing JOB5 and RHA1. Thus, this study was conducted (a) to explore the degradation potential of 1,4-dioxane by wild-type strain RHA1 when expressing different oxygenases; (b) to examine the effects of soluble inducer (1-butanol) and gaseous inducer (propane) on the degradation potential of 1,4-dioxane in the presence of product toxicity and enzymatic completion by these two strains; and (c) to determine the effects of co-contaminants on degradation of 1,4-dioxane by strains JOB5 and RHA1 when expressed with different types of oxygenases. TCE was used as a model chlorinated co-contaminant in this study. The effects of TCE on 1,4-dioxane degradation, particularly related to substrate inhibition and product toxicity, were determined.

2. Materials and methods

2.1. Chemicals

1,4-Dioxane (99%) was purchased from Alfa Aesar (Ward Hill, MA). TCE (99%) was acquired from Sigma-Aldrich (Pittsburgh, PA). Propane (>99.9%) was purchased from MP Biomedicals Inc. (Solon, Ohio) and 1-butanol (≥99.4%) was purchased from Fisher Scientific (Fair Lawn, NJ). Tetrazotized *o*-dianisidine was purchased from Fluka Chemical Corp. (Ronkonkoma, NY), and naphthalene (99.6%) was purchased from Alfa Aesar (Ward Hill, MA). Propidium monoazide (PMA) was purchased from Biotium Inc. (Hayward, CA).

2.2. Strains and culture conditions

Strain JOB5 was kindly provided by Dr. Robert Steffan, CB&I. (Lawrenceville, NJ). Strain RHA1 was kindly provided by Dr. Bill Mohn, University of British Columbia, Canada. Strains RHA1 and JOB5 were cultivated in 50 mL of Reasoner's 2A (R2A) broth medium in a 30 °C incubator while shaken at 150 rpm for approximately 48 h until $OD_{600} = 0.8–1.0$. Cells were harvested by centrifugation at 10,000× *g* for 5 min and then washed and resuspended in ammonium mineral salts (AMS) medium (Chu and Alvarez-Cohen, 1998) to $OD_{600} = 0.8–1.0$. One liter of AMS contained 10.0 g of $(NH_4)_2HPO_4$, 8.66 g of Na_2HPO_4, 1.71 g of K_2SO_4, 0.37 g of $MgSO_4 \cdot 7H_2O$, 0.12 g of $CaSO_4 \cdot 2H_2O$, 0.22 g of $FeSO_4 \cdot 7H_2O$, 0.02 g of KI, 0.06 g of $ZnSO_4 \cdot 7H_2O$, 0.03 g of $MnSO_4$, 0.01 g of H_3BO_3, and 0.11 g of $CoSO_4$. Resuspended cultures were incubated with either 1-butanol (10 mg/L) or propane (40% headspace v/v) for 24 h to induce expression of butane- or propane-monooxygenases, respectively. Cells were then harvested by centrifugation at 10,000× *g* for 5 min and then washed with and resuspended in AMS medium to $OD_{600} = 0.5–1.0$ for experimental use.

2.3. Assay for non-specific oxygenases activity

In order to confirm the expression of non-specific monooxygenases, the activity of non-specific monooxygenases in the model strains was detected using a colorimetric naphthalene oxidation assay (Chu and Alvarez-Cohen, 1998).

2.4. Degradation tests

Biodegradation of 1,4-dioxane was performed in 5 mL liquid samples in a series of 40-mL glass bottles, sealed with Teflon-coated septa and screw caps, containing cells of either propane- or 1-butanol-induced strain JOB5 (or RHA1) and 20 mg/L of 1,4-dioxane. The initial cell concentration was determined as optical density using a spectrophotometer at A_{600} and as volatile suspended solids (VSS). Two additional sets of degradation experiments, one set with TCE (5 mg/L) only and the other set with a mixture of 1,4-dioxane (20 mg/L) and of TCE (5 mg/L), were also performed. All samples include corresponding killed controls. The bottles were then incubated at 30 °C for 72 h at 150 rpm. After 72 h, samples were removed from incubator and liquid and gas phase samples were taken to determine 1,4-dioxane and TCE concentrations.

2.5. Chemical analysis

TCE concentrations were determined by injecting 200 μL headspace samples into an Agilent Technologies 6890N Gas Chromatography (GC)/Flame Ionization Detection (FID) system equipped with a J&W