Chemosphere xxx (2012) xxx–xxx

Contents lists available at SciVerse ScienceDirect

Chemosphere

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

Technical Note

The impact of chlorinated solvent co-contaminants on the biodegradation kinetics of 1,4-dioxane

Shaily Mahendra [a,*], Ariel Grostern [b], Lisa Alvarez-Cohen [c,d]

[a] Department of Civil and Environmental Engineering, University of California, Los Angeles, CA 90095, United States
[b] Department of Civil and Environmental Engineering, 209 O'Brien Hall, UC Berkeley, CA 94720-1710, United States
[c] Department of Civil and Environmental Engineering, 726 Davis Hall, UC Berkeley, CA 94720-1710, United States
[d] Earth Sciences Division, Lawrence Berkeley National Laboratory, Berkeley, CA, United States

HIGHLIGHTS

- We investigated the effects of chlorinated solvents on dioxane biodegradation.
- Both 1,1,1-trichloroethane and 1,1-dichloroethene inhibited dioxane (co)metabolism.
- Chlorinated solvent non-competitive inhibition was reversible for dioxane metabolism.
- Competitive inhibition was irreversible for dioxane cometabolism only.
- Co-contaminant chlorinated solvents may impair *in situ* dioxane bioremediation.

ARTICLE INFO

Article history:
Received 20 August 2012
Received in revised form 30 October 2012
Accepted 31 October 2012
Available online xxxx

Keywords:
1,4-Dioxane
1,1,1-Trichloroethane
1,1-Dichloroethene
Bioremediation
Competitive
Inhibition

ABSTRACT

1,4-Dioxane (dioxane), a probable human carcinogen, is used as a solvent stabilizer for 1,1,1-trichloroethane (TCA) and other chlorinated solvents. Consequently, TCA and its abiotic breakdown product 1,1-dichloroethene (DCE) are common co-contaminants of dioxane in groundwater. The aerobic degradation of dioxane by microorganisms has been demonstrated in laboratory studies, but the potential effects of environmentally relevant chlorinated solvent co-contaminants on dioxane biodegradation have not yet been investigated. This work evaluated the effects of TCA and DCE on the transformation of dioxane by dioxane-metabolizing strain *Pseudonocardia dioxanivorans* CB1190, dioxane co-metabolizing strain *Pseudonomas mendocina* KR1, as well as *Escherichia coli* expressing the toluene monooxygenase of strain KR1. In all experiments, both TCA and DCE inhibited the degradation of dioxane at the tested concentrations. The inhibition was not competitive and was reversible for strain CB1190, which did not transform the chlorinated solvents. For both strain KR1 and toluene monooxygenase-expressing *E. coli*, inhibition of dioxane degradation by chlorinated solvents was competitive and irreversible, and the chlorinated solvents were degraded concurrently with dioxane. These data suggest that the strategies for biostimulation or bioaugmentation of dioxane will need to consider the presence of chlorinated solvents during site remediation.

1. Introduction

1,4-Dioxane (dioxane) is a groundwater contaminant and potential human carcinogen (Stickney et al., 2003). The predominant use of this cyclic ether has been as a solvent stabilizer for chlorinated solvents such as 1,1,1-trichloroethane (TCA). Dioxane acts as an acid acceptor to protect chlorinated solvents during storage and improves the performance of TCA in vapor degreasing systems and other applications (Mohr et al., 2010). Commercially available TCA contains dioxane in the range of 2–8% by weight (Mohr et al., 2010). Historical practices of production, storage, and disposal of TCA have led to widespread soil and groundwater pollution by both TCA and dioxane, as well as the abiotic breakdown product of TCA, 1,1-dichloroethene (DCE). Recent reports of US Air Force contaminated site data highlighted the co-occurrence of dioxane and chlorinated solvents in the environment (Anderson et al., 2012; Chiang et al., 2012).

While dioxane is resistant to traditional remediation technologies such as air-stripping and activated carbon adsorption (Zenker et al., 2003), microorganisms have been identified that can biode-

* Corresponding author. Address: Department of Civil and Environmental Engineering, 5732 Boetler Hall, University of California, Los Angeles, CA 90095, United States. Tel.: +1 310 794 9850; fax: +1 310 206 2222.
E-mail addresses: mahendra@seas.ucla.edu (S. Mahendra), ariel.grostern@berkeley.edu (A. Grostern), alvarez@ce.berkeley.edu (L. Alvarez-Cohen).

0045-6535/$ - see front matter © 2012 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.chemosphere.2012.10.104

grade dioxane either co-metabolically (e.g., Zenker et al., 2000; Mahendra and Alvarez-Cohen, 2006; Vainberg et al., 2006) or metabolically (e.g., Bernhardt and Diekmann, 1991; Parales et al., 1994; Mahendra and Alvarez-Cohen, 2005), which suggests the feasibility of *in situ* bioremediation for dioxane treatment at contaminated sites. However, the potential impact of co-contaminants on dioxane biodegradation is largely unexplored. In studies where growth-supporting dioxane degradation was observed, dioxane was present as the sole organic compound (e.g., Bernhardt and Diekmann, 1991; Parales et al., 1994; Mahendra and Alvarez-Cohen, 2005, 2006; Mahendra et al., 2007), whereas co-metabolic dioxane degradation requires growth-supporting substrates such as tetrahydrofuran (THF), methane, propane, toluene, or ethanol (Burback and Perry, 1993; Kohlweyer et al., 2000; Zenker et al., 2000; Mahendra and Alvarez-Cohen, 2006). For co-metabolic dioxane transformation, the presence of the growth-inducing substrate can greatly affect dioxane degradation. For example, THF has been shown to inhibit dioxane degradation and limit the dioxane transformation capacity of pure and mixed microbial cultures growing on THF as primary substrate (Kohlweyer et al., 2000; Zenker et al., 2000; Mahendra and Alvarez-Cohen, 2006).

While dioxane in the environment occurs most commonly as a co-contaminant with chlorinated solvents, the effects of these compounds on dioxane biodegradation are unknown. Therefore, this study investigated the effects of two of the most common dioxane co-contaminants, TCA and DCE, on dioxane biodegradation kinetics during metabolic degradation by *Pseudonocardia dioxanivorans* strain CB1190 (CB1190) and co-metabolic degradation by *Pseudomonas mendocina* strain KR1 (KR1) and an *Escherichia coli* recombinant strain (*E. coli* TG1 (T4MO)) that constitutively expresses the toluene-4-monooxygenase of strain KR1.

2. Materials and methods

The metabolic dioxane degrader *P. dioxanivorans* strain CB1190 (Parales et al., 1994; Mahendra and Alvarez-Cohen, 2005) and the co-metabolic dioxane degrader *P. mendocina* strain KR1 (Yen et al., 1991) were grown in nitrate mineral salts medium (NMS) (Chu et al., 2004) with dioxane and toluene, respectively, as energy and carbon sources and monooxygenase-inducing substrates. Dioxane and toluene were added to the medium from aqueous stock solutions to achieve final concentrations of approximately 5 mM. *E. coli* strain TG1/pBS(Kan) (designated *E. coli* TG1 (T4MO)) constitutively expressing strain KR1 toluene-4-monooxygenase (T4MO) (Tao et al., 2004) was grown in Luria–Bertani (LB) broth containing 100 mg L^{-1} kanamycin. All cultures were incubated aerobically at 30 °C with shaking at 150 rpm. Dioxane disappearance was monitored by injecting 5 μL of filtered samples into a Varian 3400 gas chromatograph (GC) equipped with a flame ionization detector (FID) and a GraphPac-GB column (J&W Scientific, Folsom, CA), while toluene disappearance was monitored by 50 μL headspace injections into a Hewlett Packard Series II 5890 GC equipped with a FID and VOCOL capillary column (Supelco, Bellefonte, PA).

Cells were harvested in mid- to late-exponential growth phase. Following centrifugation at 15 000*g* for 5 min, cells were washed twice with fresh NMS medium and finally resuspended in 50 mL NMS. Dioxane biodegradation experiments were conducted at 30 °C in 26 mL vials containing 5 mL cell suspensions. Dioxane was added to the experimental vials in the range of 0.1–5.0 mM for TCA assays and 0.1–1.1 mM for DCE assays and disappearance was measured over time. The effects of chlorinated solvents on dioxane degradation were investigated by spiking vials with concentrations of TCA and DCE ranging from 0.75–75 and 1–100 μM, respectively. TCA and DCE concentrations were monitored by GC of headspace samples, similar to that for toluene. Biological controls without the addition of chlorinated solvents were treated identically. All experiments were performed in duplicate vials.

To test the reversibility of inhibition of chlorinated solvents on dioxane biodegradation, cells exposed to 75 μM TCA or 100 μM DCE for 24 h were centrifuged and resuspended in fresh NMS medium in new vials. Dioxane was then added to 0.6 mM and its degradation was observed over time. Dioxane degradation rates were compared to positive control vials that were washed and resuspended in parallel but were not previously exposed to chlorinated solvents.

Initial degradation rates were calculated by averaging at least two slopes of dioxane disappearance in duplicate vials within the first 4 h of the experiment and were normalized to measured total protein concentrations. The rate equation based on Michaelis–Menten model kinetics was used to characterize dioxane degradation kinetics in no-chlorinated solvent treatments.

$$v_o = \frac{V_{\max}[S]}{K_s + [S]} \quad (1)$$

v_0 represents the initial dioxane degradation rate (μmol of substrate degraded h^{-1} mg^{-1} protein), V_{max} the maximum specific rate of dioxane degradation (μmol of substrate degraded h^{-1} mg^{-1} protein), [S] the substrate (dioxane) concentration (mM), and K_s the half saturation constant (mM). V_{max} and K_s were determined by nonlinear regression using the Enzyme Kinetics module for SigmaPlot 10 (Systat Software, Chicago, IL).

For treatments with chlorinated solvents, the most appropriate substrate inhibition model (competitive, uncompetitive, or non-competitive (mixed) – see Supplementary Material (SM) for model equations) was evaluated with Lineweaver–Burk plots and by fitting the data using SigmaPlot's built-in features for enzyme kinetics, as previously described by Grostern et al. (2009). When inhibition was observed, an inverse inhibition coefficient (K_I – μM) was derived from the nonlinear regression analyses. K_I is an inverse measure of the affinity of the inhibitor for the target enzyme, where a lower value represents a more potent inhibitor.

Cell biomass was quantified from 1 mL samples before and after kinetic experiments as total protein using the Coomassie Plus protein assay kit (Pierce Chemical, Rockford, IL) according to a previously described sample preparation method (Mahendra and Alvarez-Cohen, 2006).

3. Results

3.1. Effects of TCA and DCE on the dioxane metabolizing strain CB1190

Dioxane-grown CB1190 cells were unable to degrade TCA or DCE as either a growth substrate or co-metabolically. The presence of both TCA and DCE decreased initial dioxane degradation rates by CB1190 cells, with the dioxane oxidation rate roughly inversely related to the amount of chlorinated solvent present (Figs. 1 and 2). Analysis of Lineweaver–Burk plots (Figs. 1 and 2) and the kinetic data (Table SM-1) indicates that the mode of inhibition was mixed for both TCA and DCE, with the TCA and DCE having similar inhibitory effects on dioxane degradation, as determined by similar inhibition constants K_I (Table 1). The suppression of dioxane-degrading activity by TCA was found to be reversible in CB1190 cells. That is, dioxane removal rates by TCA-exposed cells rebounded nearly to those of unexposed cells after the TCA was removed and washed cells were re-amended with dioxane. DCE inhibition of dioxane degradation by CB1190 was also reversible, although a 6 h lag occurred prior to resumption of dioxane degradation by washed strain CB1190 cells that had been previously exposed to DCE.

Please cite this article in press as: Mahendra, S., et al. The impact of chlorinated solvent co-contaminants on the biodegradation kinetics of 1,4-dioxane. Chemosphere (2012), http://dx.doi.org/10.1016/j.chemosphere.2012.10.104

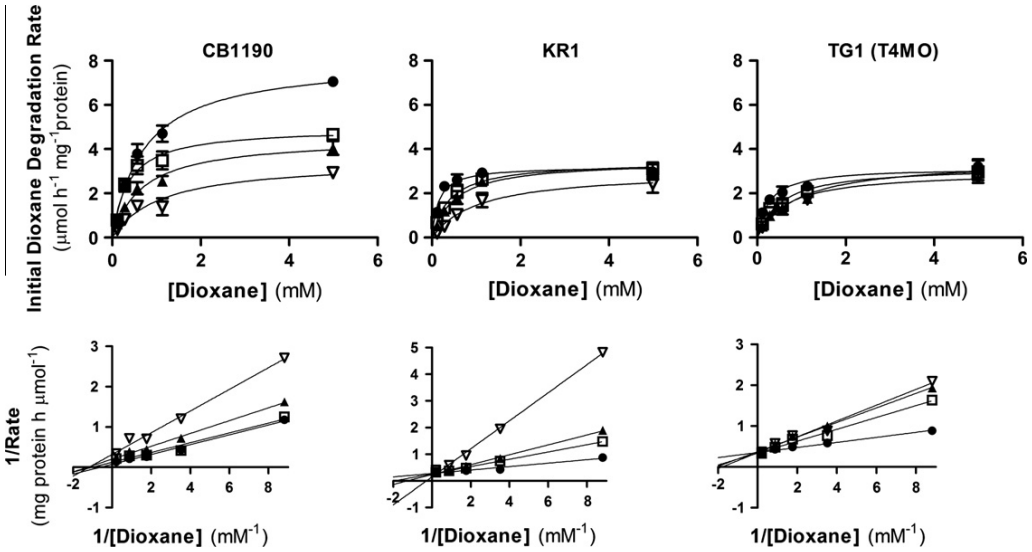

Fig. 1. Dioxane degradation kinetics in the presence of increasing concentrations of the chlorinated solvent TCA. Top: Michaelis–Menten plots of dioxane degradation. Bottom: Lineweaver–Burk transformations of dioxane degradation kinetic data. For each experiment, the individual points represent initial dioxane degradation rates from duplicate assay vials containing: *P. dioxanivorans* CB1190 (CB1190); *P. mendonica* KR1 (KR1); and *E. coli* TG1 (T4MO) (TG1 (T4MO)). Error bars represent the range of duplicate rate measurements. Concentrations of TCA in each series: closed circles – 0 μM; open squares – 0.75 μM; closed triangles – 7.5 μM; open triangles – 75 μM.

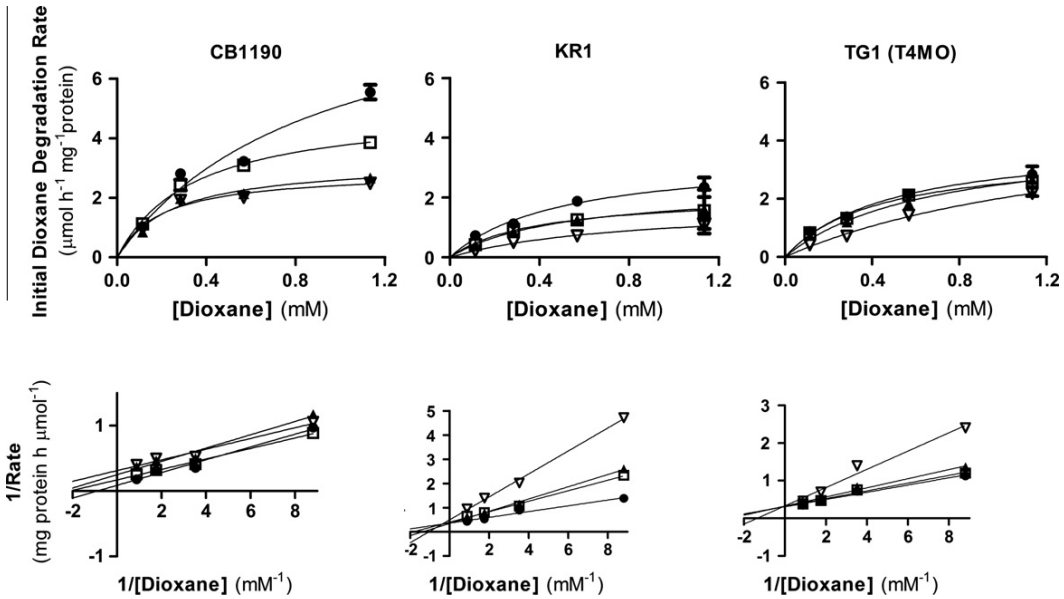

Fig. 2. Dioxane degradation kinetics in the presence of increasing concentrations of the chlorinated solvent DCE. Top: Michaelis–Menten plots of dioxane degradation. Bottom: Lineweaver–Burk transformations of dioxane degradation kinetic data. For each experiment, the individual points represent initial dioxane degradation rates from duplicate assay vials containing: *P. dioxanivorans* CB1190 (CB1190); *P. mendonica* KR1 (KR1); and *E. coli* TG1 (T4MO) (TG1 (T4MO)). Error bars represent the range of duplicate rate measurements. Concentrations of DCE in each series: closed circles – 0 μM; open squares – 0.10 μM; closed triangles – 10 μM; open triangles – 75 μM.

Table 1
Kinetic parameters for dioxane degradation in the presence of TCA or DCE.

Culture	Chlorinated solvent	Best inhibition model	V_{max}[a] (μmol dioxane degraded h^{-1} mg^{-1} protein)	K_s[a] (μM)	K_I[a] (μM)
P. dioxanivorans CB1190	TCA	Noncompetitive (Mixed)	8.0 (±0.9)	0.72 (±0.23)	1.2 (±1.0)
	DCE	Noncompetitive (Mixed)	8.0 (±1.1)	0.65 (±0.25)	3.3 (±2.9)
P. mendocina strain KR-1	TCA	Competitive	3.2 (±0.4)	0.15 (±0.09)	3.8 (±3.7)
	DCE	Competitive	3.4 (±1.2)	0.49 (±0.41)	0.52 (±0.52)
E. coli TG1 (T4MO)	TCA	Competitive	3.2 (±0.5)	0.26 (±0.16)	0.23 (±0.23)
	DCE	Competitive	4.0 (±0.8)	0.49 (±0.23)	22 (±22)

[a] V_{max}, K_s and K_I values are shown with 95% confidence intervals. V_{max} and K_I were obtained from nonlinear regression of kinetics data in the absence of chlorinated solvent.

Please cite this article in press as: Mahendra, S., et al. The impact of chlorinated solvent co-contaminants on the biodegradation kinetics of 1,4-dioxane. Chemosphere (2012), http://dx.doi.org/10.1016/j.chemosphere.2012.10.104

3.2. Effects of TCA and DCE on the dioxane co-metabolizing strain KR1 and E. coli TG1 (T4MO)

When toluene-grown strain KR1 cells were incubated with dioxane and TCA or DCE, both dioxane and the chlorinated solvent were simultaneously degraded. LB-grown *E. coli* TG1 (T4MO) also simultaneously degraded dioxane and TCA or dioxane and DCE. The presence of both TCA and DCE reduced initial dioxane oxidation rates (Figs. 1 and 2). Analysis of kinetic data by Lineweaver–Burk plots (Figs. 1 and 2) and Sigmaplot nonlinear regression indicated a competitive mode of inhibition, where increased chlorinated solvent concentration affected the apparent K_S but not the V_{max} of dioxane degradation. Based on the inhibition constant K_I, DCE was a slightly more potent inhibitor than TCA for strain KR1, while TCA was a much more potent inhibitor (lower K_I) than DCE for *E. coli* TG1 (T4MO) (Table 1). Dioxane degradation rates were not recovered when TCA or DCE exposed cells of either strain KR1 or *E. coli* TG1 (T4MO) were washed and re-amended with dioxane, suggesting irreversible inhibition effects on the cells or enzymes.

4. Discussion

Dioxane degradation is catalyzed by monooxygenase enzymes that exhibit Michaelis–Menten kinetics (Mahendra and Alvarez-Cohen, 2006). The bacterial strains tested in this study all employ monooxygenases to transform dioxane (Mahendra and Alvarez-Cohen, 2006), although the fate of the dioxane metabolites differs between the strains. *P. dioxanivorans* strain CB1190 uses dioxane as a sole carbon and energy source (Parales et al., 1994; Mahendra and Alvarez-Cohen, 2005), and during this growth a plasmid-encoded multi-component monooxygenase is upregulated (Grostern et al., 2012). *P. mendocina* strain KR1 grown with toluene expresses a monooxygenase that degrades dioxane (Mahendra and Alvarez-Cohen, 2006), and while dioxane does not support growth of strain KR1, dioxane is in fact partially mineralized by this strain (Mahendra et al., 2007). Meanwhile, *E. coli* TG1 (T4MO) constitutively expresses the KR1 toluene monooxygenase that is active towards dioxane (Mahendra and Alvarez-Cohen, 2006), and this activity also results in the partial mineralization of dioxane (Mahendra et al., 2007).

The kinetic studies presented here show that the different mechanisms of dioxane transformation and metabolite processing are accompanied by different modes of inhibition by the chlorinated solvents TCA and DCE. The dioxane-degrading monooxygenases of strain CB1190 are sensitive to, but do not transform, TCA or DCE. Both chlorinated solvents decrease the apparent V_{max}, which indicates that the inhibition is not competitive. Increasing chlorinated solvent concentration also increases the apparent K_s, so this type of inhibition is best characterized as mixed. In these interactions, inhibition likely occurs through a complex mechanism, such as the non-covalent and reversible binding of the chlorinated compounds to the enzyme(s) or the enzyme-dioxane complex or to other cellular components (Broholm et al., 1990).

In contrast to the dioxane-induced monooxygenases of strain CB1190, the toluene monooxygenase expressed by strain KR1 and *E. coli* TG1 (T4MO) transformed dioxane, TCA and DCE simultaneously. For these latter strains, the apparent V_{max} of dioxane degradation was unaffected, but the apparent K_s increased, suggesting a decreased enzyme affinity. These patterns are representative of competitive inhibition. Given the simultaneous toluene monooxygenase-catalyzed degradation of dioxane, TCA and DCE, the competitive inhibition model makes sense since both dioxane and the chlorinated solvent are competing for the monooxygenase active site.

The difference in reversibility of TCA and DCE inhibition of dioxane degradation between strains CB1190 and KR1 and *E. coli* TG1 (T4MO) was also notable. Strain CB1190 regained the ability to transform dioxane after removal of the chlorinated solvent inhibitor, whereas strain KR1 and *E. coli* TG1 (T4MO) did not. The reason for the deleterious effect of TCA and DCE on dioxane degradation by the latter strains is not clear. Unlike strain CB1190, strains KR1 and *E. coli* TG1 (T4MO) do not obtain energy from dioxane degradation, so it is possible that internal energy stores are depleted through the oxidation of dioxane, TCA and DCE in these strains. The interactions of the co-metabolic substrates dioxane, TCA and DCE may be better examined using specialized models for multiple substrate effects that account for transformation capacity limitation, enzyme competition, product toxicity, reducing energy deficits, and metabolic flux dilution (e.g., Kim et al., 2002; Stickney et al., 2003; Lovanh and Alvarez, 2004).

While inhibition by co-metabolically-degraded chlorinated organic compounds on primary substrate utilization has been extensively examined (e.g., Speitel et al., 1993; Anderson and McCarty, 1997), the interactions between co-metabolic substrates is less understood. Studies exploring these interactions include a butane-grown enrichment culture (TCA inhibited DCE degradation competitively, and vice versa) (Kim et al., 2002), and a field trial for co-metabolically remediating a mixed chlorinated compound-contaminated plume with phenol and toluene as the primary substrates (DCE was a potent inhibitor of TCE co-metabolism) (Hopkins and McCarty, 1995). It has been proposed that TCA transformation products, such as 2,2,2-trichloroethanol and trichloroacetic acid, are toxic to the enzyme or cells (Newman and Wackett, 1991), and modeling has indicated that DCE transformation toxicity can lead to impaired TCA co-metabolic degradation (Semprini et al., 2007). In lactic acid-grown *Pseudomonas butanovora*, DCE transformation led to the irreversible loss of butane monooxygenase activity (Doughty et al., 2005), and similar DCE transformation product toxicity has been reported for methanotrophic cells (Dolan and McCarty, 1995; Chang and Alvarez-Cohen, 1996). Since concurrent dioxane and chlorinated solvent degradation would be ideal for contaminated site remediation, the interaction of co-metabolic substrates warrants further study.

Historical TCA plumes may be dominated by DCE due to prolonged abiotic degradation, so the relative inhibitory effects of the parent and daughter chlorinated compounds could significantly impact the success of bioremediation efforts aimed at dioxane removal. TCA and DCE both inhibited dioxane degradation by the three tested metabolic and co-metabolic degrading strains. For strains CB1190 and KR1, both TCA- and DCE-induced inhibition of dioxane degradation were similar in extent. This contrasts with the results for *E. coli* TG1 (T4MO), where TCA was a much more potent inhibitor of dioxane degradation than DCE. It is unclear why the heterologous toluene monooxygenase was less sensitive to DCE than the enzyme in the native host.

Regardless, the results for strains CB1190 and KR1 indicate that the presence of either TCA or DCE should be of equal concern for *in situ* dioxane biodegradation. The inhibition constants are an indication of the concentration of the inhibitor at which effects become important, and the low μM (<1 ppm) inhibition constants reported here are well within the range of typical chlorinated solvent contaminated site concentrations (Scheutz et al., 2011).

Acknowledgments

We thank Drs. Jerome Kukor (Rutgers University), Rebecca Parales (UC Davis), and Thomas Wood (Penn State University) for providing bacterial strains. This research was supported by Strategic Environmental Research and Development Program grant ER-1417.

Please cite this article in press as: Mahendra, S., et al. The impact of chlorinated solvent co-contaminants on the biodegradation kinetics of 1,4-dioxane. Chemosphere (2012), http://dx.doi.org/10.1016/j.chemosphere.2012.10.104

Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.chemosphere.2012.10.104.

References

Anderson, J.E., McCarty, P.L., 1997. Effect of chlorinated ethenes on S_{min} for a methanotrophic mixed culture. Environ. Sci. Technol. 31, 2204–2210.

Anderson, R.H., Anderson, J.K., Bower, P.A., 2012. Co-occurrence of 1,4-dioxane with trichloroethylene in chlorinated solvent groundwater plumes at US Air Force installations: Fact or fiction. Integr. Environ. Assess. Manage. 8, 731–737.

Bernhardt, D., Diekmann, H., 1991. Degradation of dioxane, tetrahydrofuran and other cyclic ethers by an environmental *Rhodococcus* strain. Appl. Microbiol. Biotechnol. 36, 120–123.

Broholm, K., Jensen, B.K., Christensen, T.H., Olsen, L., 1990. Toxicity of 1,1,1-trichloroethane and trichloroethene on a mixed culture of methane-oxidizing bacteria. Appl. Environ. Microbiol. 56, 2488–2493.

Burback, B.L., Perry, J.J., 1993. Biodegradation and biotransformation of groundwater pollutant mixtures by *Mycobacterium vaccae*. Appl. Environ. Microbiol. 59, 1025–1029.

Chang, H.L., Alvarez-Cohen, L., 1996. Biodegradation of individual and multiple chlorinated aliphatic hydrocarbons by methane-oxidizing cultures. Appl. Environ. Microbiol. 62, 3371–3377.

Chiang, S.-Y.D., Mora, R., DiGuiseppi, W.H., Davis, G., Sublette, K., Gedalanga, P., Mahendra, S., 2012. Characterizing the intrinsic bioremediation potential of 1,4-dioxane and trichloroethene using innovative environmental diagnostic tools. J. Environ. Monitor. 14, 2317–2326.

Chu, K.H., Mahendra, S., Song, D.L., Conrad, M.E., Alvarez-Cohen, L., 2004. Stable carbon isotope fractionation during aerobic biodegradation of chlorinated ethenes. Environ. Sci. Technol. 38, 3126–3130.

Dolan, M.E., McCarty, P.L., 1995. Methanotrophic chloroethene transformation capacities and 1,1-dichloroethene transformation product toxicity. Environ. Sci. Technol. 29, 2741–2747.

Doughty, D.M., Sayavedra-Soto, L.A., Arp, D.J., Bottomley, P.J., 2005. Effects of dichloroethene isomers on the induction and activity of butane monooxygenase in the alkane-oxidizing bacterium *Pseudomonas butanovora*. Appl. Environ. Microbiol. 71, 6054–6059.

Grostern, A., Chan, W.W., Edwards, E.A., 2009. 1,1,1-Trichloroethane and 1,1-dichloroethane reductive dechlorination kinetics and co-contaminant effects in a *Dehalobacter*-containing mixed culture. Environ. Sci. Technol. 43, 6799–6807.

Grostern, A., Sales, C.M., Zhuang, W.-Q., Erbilgin, O., Alvarez-Cohen, L., 2012. Glyoxylate metabolism is a key feature of the metabolic degradation of 1,4-dioxane by *Pseudonocardia dioxanivorans* strain CB1190. Appl. Environ. Microbiol. 78, 3298–3308.

Hopkins, G.D., McCarty, P.L., 1995. Field evaluation of *in situ* aerobic cometabolism of trichloroethylene and three dichloroethylene isomers using phenol and toluene as the primary substrates. Environ. Sci. Technol. 29, 1628–1637.

Kim, Y., Arp, D.J., Semprini, L., 2002. Kinetic and inhibition studies for the aerobic cometabolism of 1,1,1-trichloroethane, 1,1-dichloroethylene, and 1,1-dichloroethane by a butane-grown mixed culture. Biotechnol. Bioeng. 80, 498–508.

Kohlweyer, U., Thiemer, B., Schrader, T., Andreesen, J.R., 2000. Tetrahydrofuran degradation by a newly isolated culture of *Pseudonocardia* sp. strain K1. FEMS Microbiol. Lett. 186, 301–306.

Lovanh, N., Alvarez, P.J.J., 2004. Effect of ethanol, acetate, and phenol on toluene degradation activity and tod-lux expression in *Pseudomonas putida* TOD102: Evaluation of the metabolic flux dilution model. Biotechnol. Bioeng. 86, 801–808.

Mahendra, S., Alvarez-Cohen, L., 2005. *Pseudonocardia dioxanivorans* sp. nov., a novel actinomycete that grows on 1,4-Dioxane. Int. J. Syst. Evol. Microbiol. 55, 593–598.

Mahendra, S., Alvarez-Cohen, L., 2006. Kinetics of 1,4-dioxane biodegradation by monooxygenase-expressing bacteria. Environ. Sci. Technol. 40, 5435–5442.

Mahendra, S., Petzold, C.J., Baidoo, E.E., Keasling, J.D., Alvarez-Cohen, L., 2007. Identification of the intermediates of *in vivo* oxidation of 1,4-dioxane by monooxygenase-containing bacteria. Environ. Sci. Technol. 41, 7330–7336.

Mohr, T.K.G., Stickney, J.A., DiGuiseppi, W.H., 2010. Environmental Investigation and Remediation: 1,4-Dioxane and Other Solvent Stabilizers. CRC Press, Boca Raton, FL.

Newman, L.M., Wackett, L.P., 1991. Fate of 2,2,2-trichloroacetaldehyde (chloral hydrate) produced during trichloroethylene oxidation by methanotrophs. Appl. Environ. Microbiol. 57, 2399–2402.

Parales, R.E., Adamus, J.E., White, N., May, H.D., 1994. Degradation of 1,4-dioxane by an actinomycete in pure culture. Appl. Environ. Microbiol. 60, 4527–4530.

Scheutz, C., Durant, N.D., Hansen, M.H., Bjerg, P.L., 2011. Natural and enhanced anaerobic degradation of 1,1,1-trichloroethane and its degradation products in the subsurface – A critical review. Water Res. 45, 2701–2723.

Semprini, L., Dolan, M.E., Mathias, M.A., Hopkins, G.D., McCarty, P.L., 2007. Laboratory, field, and modeling studies of bioaugmentation of butane-utilizing microorganisms for the in situ cometabolic treatment of 1,1-dichloroethene, 1,1-dichloroethane, and 1,1,1-trichloroethane. Adv. Water Resources 30, 1528–1546.

Speitel, G.E., Thompson, R.C., Weissman, D., 1993. Biodegradation kinetics of *Methylosinus trichosporium* OB3b at low concentrations of chloroform in the presence and absence of enzyme competition by methane. Water Res. 27, 15–24.

Stickney, J.A., Sager, S.L., Clarkson, J.R., Smith, L.A., Locey, B.J., Bock, M.J., Hartung, R., Olp, S.F., 2003. An updated evaluation of the carcinogenic potential of 1,4-dioxane. Regul. Toxicol. Pharmacol. 38, 183–195.

Tao, Y., Fishman, A., Bentley, W.E., Wood, T.K., 2004. Oxidation of benzene to phenol, catechol, and 1,2,3-trihydroxybenzene by toluene 4-monooxygenase of *Pseudomonas mendocina* KR1 and toluene 3-monooxygenase of *Ralstonia pickettii* PKO1. Appl. Environ. Microbiol. 70, 3814–3820.

Vainberg, S., McClay, K., Masuda, H., Root, D., Condee, C., Zylstra, G.J., Steffan, R.J., 2006. Biodegradation of ether pollutants by *Pseudonocardia* sp. strain ENV478. Appl. Environ. Microbiol. 72, 5218–5224.

Yen, K.M., Karl, M.R., Blatt, L.M., Simon, M.J., Winter, R.B., Fausset, P.R., Lu, H.S., Harcourt, A.A., Chen, K.K., 1991. Cloning and characterization of a *Pseudomonas mendocina* KR1 gene cluster encoding toluene-4-monooxygenase. J. Bacteriol. 173, 5315–5327.

Zenker, M.J., Borden, R.C., Barlaz, M.A., 2000. Mineralization of 1,4-dioxane in the presence of a structural analog. Biodegradation 11, 239–246.

Zenker, M.J., Borden, R.C., Barlaz, M.A., 2003. Occurrence and treatment of 1,4-dioxane in aqueous environments. Environ. Eng. Sci. 20, 423–432.

Please cite this article in press as: Mahendra, S., et al. The impact of chlorinated solvent co-contaminants on the biodegradation kinetics of 1,4-dioxane. Chemosphere (2012), http://dx.doi.org/10.1016/j.chemosphere.2012.10.104