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Inhibition of aerobic metabolic *cis*-1,2-di-chloroethene biodegradation by other chloroethenes

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ABSTRACT

The presence of other chloroethenes influences aerobic metabolic biodegradation of *cis*-1,2-dichloroethene (cDCE). A new metabolically cDCE degrading enrichment culture was identified as also being capable of degrading vinyl chloride (VC), but not 1,1-dichloroethene (1,1DCE), *trans*-1,2-dichloroethene (tDCE), trichloroethene (TCE), or tetrachloroethene (PCE). The fastest degradation of cDCE was observed in the absence of any other chloroethene. In the presence of a second chloroethene (40–90 μM), the rate of cDCE (60 μM) degradation decreased in the following order: cDCE (+PCE) > cDCE (+tDCE) > cDCE (+VC)> cDCE (+1,1DCE) ≈ cDCE (+TCE). With increasing concentrations of VC, ranging from 10 to 110 μM, the rate of cDCE (60 μM) degradation decreased. This study demonstrates that the inhibiting effects of chloroethene mixtures have to be considered during laboratory studies and bioremediation approaches based on metabolic cDCE degradation.

1. Introduction

The extensive use of chloroethenes as solvents and synthetic feed stocks has resulted in widespread environmental contamination, which is of great concern due to the toxicity and carcinogenicity of such substances (Coleman et al., 2002a). These compounds are a major source of soil and groundwater contamination and, hence, represent a significant threat to human and ecological health. Chlorinated ethenes comprise the higher chlorinated compounds tetrachloroethene (perchloroethene, PCE) and trichloroethene (TCE), the dichloroethenes (*cis*-1,2- (cDCE), *trans*-1,2- (tDCE), and 1,1- (1,1DCE)), and the monochlorinated vinyl chloride (VC) (Abelson, 1990; Bradley, 2003). Microbial metabolism plays a key role in the fate of chloroethenes in the biosphere. Microbial degradation of chlorinated ethenes is known to occur under both anaerobic and aerobic conditions (Beeman and Bleckmann, 2002; Tiehm et al., 2002, 2008; Bradley, 2003).

During complete reductive dechlorination, PCE and TCE are anaerobically dechlorinated via cDCE and VC to the dehalogenated end-products ethene and ethane through halorespiration or co-metabolic degradation (El Fantroussi et al., 1998; Bradley, 2003; Smidt and de Vos, 2004). The microbially driven process of reductive dechlorination can effectively reduce the concentrations of both PCE and TCE in anaerobic groundwater environments (DiStefano, 1999; Major et al., 2002). However, since reductive dechlorination of the more chlorinated ethenes, i.e. PCE and TCE, is often faster than cDCE and VC dechlorination (DiStefano, 1999; Major et al., 2002) those partially dechlorinated products often persist and disperse in groundwater plumes (Semprini, 1997; Tiehm et al., 2008). This is of particular concern due to their toxic (i.e. cDCE and VC) and carcinogenic (VC) properties (Verce et al., 2002).

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Aerobic co-metabolic degradation of chloroethenes (Semprini, 1997) is possible in the presence of auxiliary substrates such as methane (Frascari et al., 2006), toluene (Shim et al., 2001) or ethene (Koziollek et al., 1999). However, in the field the availability of electron donors for reductive dechlorination under anaerobic conditions as well as the availability of auxiliary substrates for co-metabolic degradation under aerobic degradation is limited. Therefore, metabolic degradation pathways, i.e. the use of the target pollutant as growth substrate, are more favorable for efficient bioremediation processes.

It has been shown previously that VC can be degraded metabolically under aerobic conditions (Hartmans and de Bont, 1992; Verce et al., 2001; Coleman et al., 2002b). Metabolic VC degradation can trigger co-metabolic cDCE degradation but the co-metabolic cDCE transformation rates are low as compared to metabolic VC decomposition (Verce et al., 2002; Tiehm et al., 2008).

In case of metabolic biodegradation, cDCE is used as sole carbon source. In recent years the aerobic metabolic degradation of cDCE has been reported (Bradley and Chapelle, 2000; Coleman et al., 2002a; Olaniran et al., 2008; Schmidt et al., 2010). The genome of *Polaromonas* sp. JS 666 – the only known bacterial isolate using cDCE as sole carbon and energy source – has been studied in detail (Mattes et al., 2008). Recently, isotopic carbon fractionation factors have been determined (Abe et al., 2009; Jennings et al., 2009) and two cDCE degradation pathways by *Polaromonas* sp. JS 666 have been proposed (Jennings et al., 2009).

For site remediation, metabolic cDCE degradation has significant advantages since no additional organic substrates are required and the dissolved oxygen is completely available for cDCE degradation. However, in the field, often several chloroethenes are found together in contaminated soil and groundwater (Lendvay et al., 2003; Bennett et al., 2007; Schmidt and Tiehm, 2008) and therefore, the effects of pollutant mixtures on cDCE degradation kinetics have to be considered. The influence of mixtures on the reductive dechlorination of chloroethenes has been reported previously (DiStefano, 1999; Yu et al., 2005); as well as for other classes of pollutants such as polycyclic aromatic hydrocarbons (Tiehm and Fritzsche, 1995).

This study was undertaken with a new enrichment culture capable of aerobic metabolic cDCE degradation, tolerating chloroethene concentrations up to 100 mg/L, a temperature range between 4 °C and 23 °C, and prolonged periods of starvation (Schmidt et al., 2010). It was the objective of the current study (i) to evaluate the ability of the cDCE degrading culture to transform PCE, TCE, tDCE, 1,1DCE, and VC either as single compounds or in binary mixtures with cDCE, and (ii) to study the effects of other chloroethenes on biodegradation kinetics of cDCE.

2. Materials and methods

2.1. Degradation experiments

Experiments were carried out using 2-liter laboratory glass bottles capped with Teflon-coated septa (Butyl/Teflon Pharmafix, gray, 3.0 mm, A-Z Analytikk-Zubehör GmbH, Langen, Germany), held in place with screw-caps. 1800 mL autoclaved mineral salts medium was inoculated with 200 mL FT-culture (1:10 inoculum ratio) in bottles with 2.3 L total volume, resulting in an initial ratio of liquid to head space of approx. 7:1.The FT-culture consists of cDCE aerobic metabolically degrading bacteria, enriched from groundwater from the Frankenthal site in Germany (Schmidt and Tiehm, 2008). The medium was adjusted to pH 7.1 ± 0.2 and contained the following mineral salts (analytical grade or purer, Merck, Darmstadt, Germany) per liter of demineralized water: 1.05 g of $K_2HPO_4 \cdot 3H_2O$, 0.2 g of KH_2PO_4, 0.17 g of $NaNO_3$, 0.04 g of $MgSO_4 \cdot 7H_2O$, 0.023 g of $CaSO_4 \cdot 2H_2O$ and 2 mL of trace element solution (500 mg of $Na_2EDTA \cdot 2H_2O$, 10 mg of $ZnSO_4 \cdot 7H_2O$, 200 mg of $FeSO_4 \cdot 7H_2O$, 3 mg of $MnCl_2 \cdot 4H_2O$, 30 mg of H_3BO_3, 20 mg of $CoCl_2 \cdot 6H_2O$, 10 mg of $CuSO_4 \cdot 2H_2O$, 6 mg of $NiCl_2 \cdot 6H_2O$, 3 mg of $NaMoO_4 \cdot 2H_2O$ per liter).

Bottles were spiked with PCE (99.9%, Fluka, Steinheim, Germany), TCE (99.5%, Fluka), cDCE (≥95%, Fluka), tDCE (≥95%, Fluka), 1,1DCE (≥99.9%, Fluka) and VC (gaseous, 99.97%, Linde, Stuttgart, Germany) (as shown in Fig. 1). Culture bottles were kept at room temperature (22–24 °C). All experiments were performed with two replicates and results are presented as the average values from the two replicates. For each chloroethene a third replicate was spiked with 1 g/L of sodium azide (purum p.a., Fluka) and served as the sterile control. The experimental set-up is illustrated in Fig. 1.

In order to minimize the loss and sorption of chloroethenes during sampling, the Teflon-coated septa were pierced with stainless steel needles, and samples were taken using glass syringes. The septa were then replaced immediately after each sampling. The aerobic degradation of chlorinated ethenes was analyzed by gas chromatography in 10 mL headspace vials containing 5 mL of sample. Chloride formation was monitored by ion chromatography. Samples for gas chromatography were acidified to pH 2 by phosphoric acid in order to stop biological reactions and stored at 4 °C before analysis.

2.2. Analytical methods

Chloroethene concentrations (except 1,1DCE) were determined using an Agilent 6890N gas chromatograph (Waldbronn, Germany) equipped with a capillary column (Varian CP-select 624CB, 60 m, 0.32 mm inside diameter, 94% cross linked methyl siloxane, 6% cyanopropylphenyl, 1.8 μm film thicknesses, Middelburg, The Netherlands) with parallel flame ionization detector (FID) operated at 250 °C and electron capture detector (ECD) operated at 330 °C. Sampling was performed with a Gerstel headspace multipurpose autosampler MPS2 (Mülheim, Germany) and injection of 1 mL headspace sample was performed via a cold-injection system Gerstel KAS4 with an injection temperature of −40 °C. The following oven temperature program was applied: held at 40 °C for 3 min, heated gradually to 90 °C (8 °C/min), held at 90 °C for 4 min, heated gradually to 200 °C (6 °C/min), and held at 200 °C for 10 min.

For the analysis of 1,1DCE 1 mL of headspace samples were injected into a gas chromatograph (series II 5890, Hewlett Packard, Waldbronn, Germany) equipped with headspace sampler, FID and ECD. Separation was accomplished in a capillary column (Hewlett Packard PONA, 50 m, 0.2 mm