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Aerobic biodegradation of trichloroethene without auxiliary substrates

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ABSTRACT

Trichloroethene (TCE) represents a priority pollutant and is among the most frequently detected contaminants in groundwater. The current bioremediation measures have certain drawbacks like e.g. the need for auxiliary substrates. Here, the aerobic biodegradation of TCE as the sole growth substrate is demonstrated. This new process of metabolic TCE degradation was first detected in groundwater samples. TCE degradation was stable in an enriched mixed bacterial culture in mineral salts medium for over five years and repeated transfers of the culture resulting in a 10^{10} times dilution of the original groundwater. Aerobic TCE degradation resulted in stoichiometric chloride formation. Stable carbon isotope fractionation was observed providing a reliable analytical tool to assess this new biodegradation process at field sites. The results suggest that aerobic biodegradation of TCE without auxiliary substrate could be considered as an option for natural attenuation or engineered bioremediation of contaminated sites.

1. Introduction

Due to their extensive industrial use, chloroethenes are among the most frequently detected groundwater contaminants (Chu et al., 2004; Chen et al., 2008; Imfeld et al., 2008; Conrad et al., 2010; Mattes et al., 2010). The microbiological elimination of these pollutants has been demonstrated to be a suitable method for the remediation of contaminated sites. Furthermore, compound-specific stable carbon isotope analysis is a useful tool to provide qualitative proof and a quantitative assessment of the microbiological degradation at field sites (Imfeld et al., 2008; Abe et al., 2009; Pooley et al., 2009).

The biodegradation of chloroethenes can be achieved through the complete anaerobic reductive dechlorination, i.e. halorespiration, of the parent compounds perchloroethene (PCE)/trichloroethene (TCE) to yield ethene via the metabolites *cis*-, *trans*-1,2- and 1,1-dichloroethene (cDCE/tDCE/1,1DCE) and vinyl chloride (VC) (Tiehm and Schmidt, 2011). However, this approach risks the formation of high field concentrations of cDCE or VC (Tiehm and Schmidt, 2011), the latter of which has been detected at more than 40% of the Superfund sites in the United States (US-EPA, 2012a). The parent compounds PCE and TCE and the metabolites cDCE and VC are on the priority list of hazardous substances (ATSDR, 2012). Furthermore they are, except for cDCE, considered priority pollutants by the US-

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EPA (2012b) and are regulated in the European drinking water directive as well as the European water framework directive (EU, 1998; EU, 2008). To stimulate complete anaerobic-reductive dechlorination at field sites, auxiliary substrates often are injected in excess, resulting in the deterioration of the groundwater quality due to competing processes, such as sulphate reduction and methanogenesis (Aulenta et al., 2007; Conrad et al., 2010; Tiehm and Schmidt, 2011).

The aerobic oxidation of TCE, cDCE, and VC is possible co-metabolically in the presence of auxiliary substrates as the electron donors. Auxiliary substrates can be hydrocarbons, such as aromatic pollutants (Barth et al., 2002), ethene (Koziollek et al., 1999), or methane (Chu et al., 2004). In addition, ammonium can serve as an electron donor for co-metabolic chloroethene degradation during nitrification (Sayavedra-Soto et al., 2010). Transformation yields ranging from 0.0056 mg TCE per mg of propane to 0.052 mg TCE per mg of methane demonstrate the need for large amounts of auxiliary substrates (Semprini, 1997). The co-metabolic degradation of TCE, cDCE, and VC has been reported for the remediation of a few contaminated sites (Semprini, 1997; Pooley et al., 2009).

In recent years, aerobic metabolic degradation has been demonstrated for the metabolites of the anaerobic reductive dechlorination, i.e. cDCE and VC (Verce et al., 2000; Coleman et al., 2002; Tiehm et al., 2008b; Schmidt et al., 2010), but not for the primary contaminants, i.e. PCE and TCE (Mattes et al., 2010; Tiehm and Schmidt, 2011). During metabolic degradation, the pollutants are used as growth substrates without the need for auxiliary substrates. Accordingly, these aerobic degradation processes have a strong potential for field application in a sequential anaerobic/aerobic remediation approach (Lohner et al., 2011; Tiehm and Schmidt, 2011).

In this study, aerobic chloroethene degradation was investigated in laboratory microcosms with groundwater samples from a chloroethene-contaminated site. Chemical analysis revealed that none of the known auxiliary substrates for co-metabolic chloroethene degradation was present in the groundwater. After groundwater microcosms results suggested the metabolic degradation of TCE, enrichment cultures in mineral salts medium were performed to confirm TCE degradation as sole growth substrate under defined conditions.

2. Materials and methods

2.1. Groundwater microcosms

The groundwater microcosms were prepared and incubated as previously described (Schmidt et al., 2010). Briefly, gas-tight glass bottles (2.3 L volume) were filled with 2 L of groundwater directly from the wells at a chloroethene-contaminated site in the south of Germany (Fig. S1). The 300 mL headspace contained ambient air. Incubation in the laboratory was done under aerobic conditions at room temperature (23 ± 1 °C). After each sampling event the removed liquid volume was replaced by ambient air. Oxygen concentrations between 4.3 and 8.6 mg/L were measured during the whole incubation period. The abiotic controls were inhibited with 1 g/L sodium azide (purum, Fluka, Steinheim, Germany).

In 2007 groundwater was sampled from the well 819 (Fig. S1). The groundwater contained 1.4 mg/L TCE, 0.15 mg/L cDCE, 0.60 mg/L of dissolved organic carbon (DOC), no oxygen (detection limit: 0.5 mg/L), no ammonium (detection limit: 0.05 mg/L), no methane (detection limit: 0.01 mg/L), no ethene (detection limit: 0.005 mg/L), and no ethane (detection limit: 0.005 mg/L). The alkalinity was 6.3 mM and the pH was 6.95. Initially, this study was conceived to assess the potential for aerobic degradation of cDCE and VC. Therefore, four active microcosms were set up: Two microcosms (+cDCE 1, + cDCE 2) were spiked with 1.0 mg/L cDCE (≥95%, Fluka), one microcosm (+VC) was spiked with 6.7 mg/L VC (99.97%, Linde, Stuttgart, Germany), and one microcosm (+cDCE + VC) was spiked with 1.0 mg/L cDCE plus 4.7 mg/L VC.

In 2010 groundwater samples were again taken from well 819 as well as from seven other wells (Fig. S1, Table S1). Two active microcosms were set up with each groundwater sample (sixteen active microcosms in total): One microcosm of each groundwater sample was incubated without amendments. The second microcosm of each groundwater sample was amended with inorganic nutrients (5.2 mg/L KH_2PO_4, 27 mg/L $K_2HPO_4 \cdot 3H_2O$, 13 mg/L $NaNO_3$ as aseptic stock solution in demineralised water, and 2.0 mL/L trace element solution (2 mL of concentrated phosphoric acid, 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 $Na_2MoO_4 \cdot 2H_2O$ per L of demineralised water)). Mineral salts used were analytical grade or higher (Merck, Darmstadt, Germany). If TCE concentrations in the groundwater were below 0.30 mg/L, microcosms were spiked with 1.0 mg/L TCE (99.5%, Fluka).

2.2. Enrichment cultures in mineral salts medium

Aliquots of two groundwater microcosms (microcosm + cDCE 1 and microcosm + cDCE 2) were transferred into chloride- and ammonium-free mineral salts medium (1.05 g of $K_2HPO_4 \cdot 3H_2O$, 200 mg of KH_2PO_4, 170 mg of $NaNO_3$, 40 mg of $MgSO_4 \cdot 7H_2O$, 23 mg of $CaSO_4 \cdot 2H_2O$, 2 mL of trace element solution as mentioned above). This enriched mixed bacterial culture was maintained active without any auxiliary substrate for five years, with ten transfers into fresh mineral salts medium (10% inoculum) resulting in a 10^{10} times dilution of the original groundwater. The only carbon substrate was TCE, which was added repeatedly after its degradation. Unless otherwise stated all assays were prepared in 1 L or 2 L glass bottles as previously described (Schmidt et al., 2010). Abiotic controls were inhibited with 1 g/L sodium azide.

Bacterial growth was assessed with quantitative polymerase chain reaction (qPCR) targeting bacterial 16S RNA genes. One active assay containing medium plus TCE plus enriched mixed culture (TCE + culture) was accompanied by three controls: Medium plus TCE without enriched mixed culture (TCE only), medium plus enriched mixed culture without TCE (culture only), medium plus TCE plus enriched mixed culture plus 2 g/L copper (II) sulphate (TCE + culture, abiotic).