Anaerobic transformation of tetrachloroethane, perchloroethylene, and their mixtures by mixed-cultures enriched from contaminated soils and sediments

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Abstract The focus of this research was to investigate the anaerobic transformation of tetrachloroethane (TeCA), perchloroethylene (PCE), and their mixtures by mixed cultures enriched from contaminated soils or sediments. Batch transformation studies were conducted using TeCA (60 \( \mu \)M), PCE (60 \( \mu \)M), or TeCA + PCE (each added at 60 \( \mu \)M) as electron acceptor(s) and \( \text{H}_2 \) + acetate (each added at 3 mM) or butyrate (3 mM) as electron donor(s). A \textit{Dehalococcoides} spp.-containing, sediment-enrichment dechlorinated PCE rapidly to ethene (ETH) but slowly and incompletely dechlorinated TeCA. Moreover, when present in mixture with PCE, TeCA disrupted the ability of \textit{Dehalococcoides} to dechlorinate vinyl chloride. In contrast, the soil-enrichment culture was able to completely dechlorinate TeCA and PCE to ETH, both when added as single contaminants and when added as a mixture.

Keywords \textit{Dehalococcoides} spp; perchloroethylene; reductive dechlorination; tetrachloroethane

Introduction

Chloroethenes (such as perchloroethylene, PCE, and trichloroethylene, TCE) and chloroethanes (such as 1,1,2,2-tetrachloroethane, TeCA, and 1,1,2-trichloroethane TCA) are often found as co-contaminants in groundwater due to their similar uses as solvents and degreasing agents. These contaminants are of special concern because of their potential for adverse effects following exposure. Anaerobic reductive dechlorination (RD) of highly chlorinated compounds have been well-documented in field and laboratory studies with mixed and pure cultures (Middeldorp \textit{et al.}, 1999). However, different from PCE, and TCE, relatively few studies on TeCA transformation have been conducted (Chen \textit{et al.}, 1996; Lorah and Olsen, 1999; van Eekert \textit{et al.}, 1999) and therefore the fate of TeCA in anaerobic contaminated aquifers is still largely unexplored. One complicating factor in the anaerobic transformation of TeCA is that its degradation can follow biotic and abiotic reaction pathways (i.e. hydrogenolysis, dihaloelimination, dehydrochlorination) producing both chloroethenes and chloroethanes as daughter products (Figure 1).

Elucidation of factors controlling the occurrence of these different reaction pathways is crucial because of differing toxicity, mobility, and persistence of the intermediate daughter products. Despite the number of studies on the biotransformation of individual chlorinated aliphatic hydrocarbons, there is little information concerning mixtures of these compounds. This general lack of knowledge is of particular importance when considering that most of the contaminated sites may contain more than one pollutant. When multiple compounds are present, there are a number of possible factors such as toxicity and inhibition that contribute to the complexity of observed transformation phenomena (Adamson and Parkin, 1999). Without general information on the effects of mixtures of
contaminants, limits are placed on the potential application of bioremediation techniques. The aim of the present paper was to investigate the anaerobic transformation of TeCA, PCE, and their mixtures, in the presence of different electron donors (H₂ + acetate or butyrate), by two mixed-cultures.

**Methods**

**Source enrichment culture.** Batch transformation studies to examine TeCA and PCE transformation were carried out using mixed cultures derived from contaminated sediments and soils, hereafter named sediment-enrichment culture and soil-enrichment culture, respectively. The sediment-enrichment culture was obtained, through sequential transfers, from a microcosm seeded with a brackish sediment from Venice Lagoon (Aulenta et al., 2002). The culture was enriched for over 2 years on PCE and H₂ (or H₂-producing electron donors such as butyrate or methanol) and showed the ability to completely dechlorinate PCE to ethene (ETH). FISH analyses with probes specific to *Dehalococcoides* spp. showed a large abundance (41.5 ± 11.2% of overall VSS) of this microorganism in the culture (Aulenta et al., 2004). The soil-enrichment culture was obtained from microcosms constructed with aquifer material and groundwater from a TeCA and TCE contaminated site located in Northern Italy (Aulenta et al., 2005). The microcosm study indicated the presence in the soil of native dechlorinating populations capable of fully dechlorinate TeCA and TCE into ETH, by using organic compounds (such as yeast extract) as electron donors.

**Anaerobic transformation studies.** The experimental conditions for anaerobic batch transformation studies are reported in **Table 1**. Experiments were conducted in 250-ml serum bottles sealed with Teflon-faced butyl rubber stoppers and aluminium crimps. The bottles were seeded with a fixed volume of source culture (10 ml of suspended biomass from the sediment-enrichment culture or 120 ml of soil suspension from

![Figure 1: Anaerobic transformation pathways of chloroethanes and chloroethenes](https://iwaponline.com/wst/article-pdf/52/1-2/357/433745/357.pdf)

**Table 1** Experimental conditions for anaerobic batch transformation studies. Each treatment was carried out in duplicate on each of the two enrichment cultures.

<table>
<thead>
<tr>
<th>Treatment no.</th>
<th>e⁻-acceptor(s) initial concentration (μM)</th>
<th>e⁻-donor(s) initial concentration (mM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>TeCA 60</td>
<td>Butyrate 3</td>
</tr>
<tr>
<td>2</td>
<td>TeCA 60</td>
<td>Hydrogen 3 + acetate 3</td>
</tr>
<tr>
<td>3</td>
<td>PCE 60</td>
<td>Butyrate 3</td>
</tr>
<tr>
<td>4</td>
<td>PCE 60</td>
<td>Hydrogen 3 + acetate 3</td>
</tr>
<tr>
<td>5</td>
<td>TeCA 60 + PCE 60</td>
<td>Butyrate 3</td>
</tr>
<tr>
<td>6</td>
<td>TeCA 60 + PCE 60</td>
<td>Hydrogen 3 + acetate 3</td>
</tr>
<tr>
<td>Control 1</td>
<td>TeCA 60 (abiotic)</td>
<td>–</td>
</tr>
<tr>
<td>Control 2</td>
<td>PCE 60 (abiotic)</td>
<td>–</td>
</tr>
</tbody>
</table>
the soil-enrichment culture) and reduced anaerobic mineral medium to a final volume of liquid phase of 160 ml. After preparation the bottles were spiked with the selected electron donor(s) (H₂ or acetate or butyrate) and electron acceptor(s) (TeCA, PCE, or their mixture). Duplicate bottles were prepared for each treatment to ensure reproducibility. PCE and TeCA were added in neat form by using gas-tight microsyringes. Butyrate and acetate were added from concentrated anoxic solutions. Hydrogen was added in the headspace of the serum bottles by using gas-tight glass syringes. Bottles were statically incubated at room temperature (18–22°C). Weekly, the serum bottles were analyzed for chlorinated solvents and electron donors. Electron donors were re-added every time analyses indicated that they were completely depleted. Abiotic controls were prepared identically to live treatments with the only exception that bottles were autoclaved at 121°C for 2 hours before adding TeCA or PCE.

**Analytical methods.** Gas chromatography (GC) headspace analysis was employed in the measurement and quantification of volatile compounds (i.e. chloroethanes, chloroethenes, ethane, methane, hydrogen). Headspace samples (100–500 μL) were taken from the reactors using gas-tight syringes and injected manually. GCs, detectors, and column conditions utilized were described elsewhere (Aulenta et al., 2005). Calibration standards for volatile compounds were prepared in 250-ml serum bottles containing 160 ml of liquid volume and known masses of volatile compounds. Calibration curves were then used to determine nominal concentration in the liquid phase (i.e. assuming no partitioning of volatile compounds in the gas phase). Butyrate and fatty acids were determined by direct liquid injections (1 μL) of filtered (0.22 μM) liquid samples into a GC with flame-ionization detectors (FID). Hydrogen formed from butyrate fermentation was analyzed in 2-ml headspace samples by using a GC equipped with reduction gas detector (RGD). All the chemicals used were of analytical grade.

**Results and discussions**

**Anaerobic transformation of PCE**

Sediment-enrichment culture containing *Dehalococcoides* spp. **Figure 2** (left) shows the time course of PCE dechlorination by the *Dehalococcoides* spp.-containing, sediment-enrichment culture, in the presence of butyrate (3 mM) as electron donor. PCE dechlorination commenced without any initial lag and resulted in the dechlorination of added PCE (60 μM) to vinyl chloride (VC) and ETH. Dechlorination proceeded through intermediate formation of TCE, *cis*-dichloroethene (cDCE), and VC. In parallel cultures, in which butyrate was replaced by H₂ acetate mixture, PCE dechlorination to VC proceeded at an almost identical rate whereas the last step from VC to ETH was

![Figure 2](https://iwaponline.com/wst/article-pdf/52/1-2/357/433745/357.pdf)

**Figure 2** Anaerobic transformation of PCE (60 μM) in the presence of butyrate (3 mM) by the sediment-enrichment culture containing *Dehalococcoides* spp. (left) and the soil-enrichment culture (right). Symbols: PCE △; TCE ×; cDCE ●; VC Δ; ETH ■
significantly faster (data not shown), indicating that with butyrate VC dechlorination was probably limited by $\text{H}_2$ availability.

**Soil-enrichment culture.** Figure 2 (right) shows the time course of PCE dechlorination by the soil-enrichment culture in the presence of butyrate (3 mM). The added PCE (60 µM) was completely dechlorinated to ETH in less than 25 days. Different from the sediment-enrichment culture, VC did not accumulate at a high concentration during PCE dechlorination, probably because it was rapidly converted to ETH. In the parallel soil-enrichment cultures fed PCE and $\text{H}_2$ + acetate, the time course of PCE dechlorination (including the last step from VC to ETH) was almost identical to that in the presence of butyrate (data not shown).

**Anaerobic transformation of TeCA**

**Sediment-enrichment culture containing Dehalococcoides spp.** Figure 3 (left) shows the time course of TeCA dechlorination by the *Dehalococcoides* spp.-containing, sediment-enrichment culture, in the presence of butyrate (3 mM) as electron donor. TeCA was degraded very slowly and only little VC accumulated. Similar results were obtained with $\text{H}_2$ + acetate as electron donors (data not shown). Pseudo-first order rate coefficients for TeCA degradation in the presence of butyrate or $\text{H}_2$ + acetate were $0.007 \pm 0.0006$ d$^{-1}$ and $0.009 \pm 0.0002$ d$^{-1}$, respectively. These rates were very similar to those observed in abiotic (killed) controls ($0.009 \pm 0.0005$ d$^{-1}$), with the only difference that TCE (instead of VC) accumulated in killed controls. Since the abiotic dehydrochlorination of TeCA produces TCE (Figure 1), it is likely that, in live cultures, VC resulted from hydrogenolysis of abiotically produced TCE.

**Soil-enrichment culture.** Figure 3 (right) shows the time course of TeCA dechlorination by the soil-enrichment culture, in the presence of butyrate (3 mM) as electron donor.

After an initial lag phase of approximately 20 days, during which only little TCE accumulated (from abiotic dehydrochlorination of TeCA), TeCA was rapidly dechlorinated via dichloroelimination to cDCE and to a minor extent *trans*-dichloroethene (tDCE). Once formed cDCE was then rapidly dechlorinated via hydrogenolysis to ETH (through intermediate formation of VC). In contrast, the *trans*-isomer was more resistant to dechlorination and persisted in the bottles for longer time. Interestingly, in the presence of $\text{H}_2^+$ acetate, TeCA dechlorination proceeded at much slower rate than in the presence of butyrate. This finding might indicate that $\text{H}_2$ is not the ultimate electron donor for TeCA dechlorination for that culture. However, also in the presence of $\text{H}_2$, at the end of the 50-day incubation period ETH (26.3 ± 0.3 µM) and tDCE (3.0 ± 0.1 µM) were
the main dechlorination products present in the bottle, along with remaining TeCA (17.2 ± 0.8 μM).

**Anaerobic transformation of PCE and TeCA when present in mixture**

*Dehalococcoides spp.-containing* sediment-enrichment culture. The presence of TeCA (60 μM) adversely affected the transformation of PCE (60 μM) by the *Dehalococcoides* spp.-containing, sediment-enrichment culture. In the presence of butyrate, PCE was rapidly dechlorinated to TCE (at the same rate as when it was the only compound added) which however, did not degrade further (Figure 4). Differently, in the presence of H₂ acetate, only the last step of PCE dechlorination, i.e. the conversion of VC to ETH, was negatively affected by the presence of TeCA (data not shown). The negative effect of TeCA on VC degradation is consistent with previous studies which have shown that different chlorinated hydrocarbons, such as PCE, TCE, cDCE, carbon tetrachloride, and TCA, inhibit VC degradation in PCE dechlorinating cultures (Maymó-Gatell et al., 2001; Adamson and Parkin, 2000). The more severe inhibitory effect found with butyrate has probably to be attributed to the fact that TeCA also inhibited the formation of H₂ (i.e. the actual electron donor used for the RD of PCE) from butyrate. Indeed, during the course of experiments, the average value of dissolved H₂ concentration in PCE + butyrate-amended cultures (2.11 ± 0.03 nM) was significantly higher, in spite of the intense H₂-consuming dechlorination, than that observed in PCE + TeCA + butyrate-amended cultures (1.57 ± 0.16 nM).

*Soil-enrichment culture.* When present in mixture, TeCA (60 μM) and PCE (60 μM) were completely transformed to ETH (but for some residual tDCE) at similar rates than when added as single substrates. The lack of competitive effects between the two compounds suggests that different bacterial populations (or different enzymes in the same organisms) were responsible for the transformation of the two chlorinated compounds. PCE was initially dechlorinated at a higher rate than TeCA. Also in this case, little VC accumulated during dechlorination. TeCA was initially dechlorinated to cDCE and tDCE via dichloroelimination. It is noteworthy that while the cis-isomer was further dechlorinated to ETH the trans-isomer persisted in the microcosm for longer periods.

**Conclusions**

The anaerobic dechlorination of two common chlorinated groundwater pollutants such as TeCA and PCE by two mixed cultures enriched from contaminated soils and sediments was examined. The *Dehalococcoides* spp.-containing (Aulenta et al., 2004), sediment-enrichment culture was able to completely dechlorinate PCE to ETH but failed to dechlorinate TeCA. Moreover, when present in the mixture, TeCA severely inhibited...
the last step of PCE dechlorination (with H₂+acetate as electron donors), resulting in large accumulation and persistence of the toxic VC. The results of this study indicate that bioaugmentation with *Dehalococcoides* spp. (often considered for remediation of chloroethene-contaminated sites stalling at cDCE or VC) may be ineffective if TeCA is present as a groundwater cocontaminant.

The soil-enrichment culture, derived from a TeCA and TCE contaminated site, showed unique dechlorinating features. TeCA and PCE were fully dechlorinated to ETH with little intermediate accumulation of VC. Moreover, when present in the mixture, TeCA and PCE were transformed at almost identical rates than when added alone. The lack of inhibitory/competitive effects suggests that TeCA and PCE transformations were probably mediated by different microorganisms. TeCA was dechlorinated (at a slower rate than PCE) through initial dichloroelimination to DCEs and further hydrogenolysis to VC and ETH. Different from the *Dehalococcoides*-containing culture, VC dechlorination was not inhibited by TeCA, suggesting that microorganisms, different from *Dehalococcoides*, were responsible for the full dechlorination of PCE to ETH.

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