Anaerobic Microbial Reductive Dechlorination of Tetrachloroethene to Predominately trans-1,2-Dichloroethene

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While most sites and all characterized PCE and TCE dechlorinating anaerobic bacteria produce cis-DCE as the major DCE isomer, significant amounts of trans-DCE are found in the environment. We have obtained microcosms from some sites and enrichment cultures that produce more trans-DCE than cis-DCE. These cultures reductively dechlorinated PCE and TCE to trans-DCE and cis-DCE simultaneously and in a ratio of 3:1 that was stable through serial transfers with a variety of electron donors and occurred in both methanogenic and nonmethanogenic enrichments. Two sediment-free, nonmethanogenic enrichment cultures produced trans-DCE at rates of up to 2.5 mmol L⁻¹·day⁻¹. Dehalococcoides populations were detected in both trans-DCE producing cultures by their 16S rRNA gene sequences, and trans-DCE was produced in the presence of ampicillin. Because trans-DCE can be the major product from PCE and TCE microbial dechlorination, high fractions of trans-DCE at chloroethene-contaminated sites are not necessarily from source contamination.

Introduction

Chlorinated ethenes are significant groundwater contaminants and are present in aquifers as parent compounds (PCE and TCE) and daughter products (DCEs and VC). Of the current or former U.S. Environmental Protection Agency National Priority List Sites, the Agency for Toxic Substances and Disease Registry reports tetrachloroethene (perchloro-ethylene, PCE) at 54% of the sites; trichloroethene (TCE) at 60%; 1,1-dichloroethene (1,1-DCE) at 36%; trans-1,2-dichloroethene (trans-DCE) at 39%; cis-1,2-dichloroethene (cis-DCE) at 10%; and vinyl chloride (VC) at 37% (1). Under anaerobic conditions, chloroethenes are subject to reductive dechlorination (hydrogenolysis) resulting in the stepwise conversion of PCE to TCE, DCE isomers, VC, and ethene. Several anaerobic bacterial populations have been isolated that use PCE or TCE as respiratory electron acceptors for growth through a process termed (de)chlororespiration (2–5). These isolates belong to several genera including Dehalobacter (6, 7), Desulfuromonas (8, 9), Desulfotobacterium (10–13), Dehalococcoides (4, 14), Clostridium (15), Enterobacter (16), and Sulfurospirillum (formerly Dehalospirillum) (17–18). Except for a few Dehalotetrafluorobacterium strains that only convert PCE to TCE and a few Dehalococcoides isolates that dechlorinate PCE or TCE to VC and ethene (4, 14, 19, 20), all other isolates dechlorinate PCE to predominately cis-DCE as the end product. Chloroethene reductive dehalogenases have been characterized from several organisms including Sulforhodospira multivorans (21), Desulfotetrafluorobacterium sp. strain PCE-S (22), Desulfitobacterium sp. strain YS1 (23), Dehalococcoides ethenogenes (24), and Dehalobacter restrictus (25). The reductively dechlorinating enzyme systems contain corrinoid cofactors in addition to iron–sulfur clusters, and in Dehalobacter restrictus, dechlorination is suggested to occur through a radical mechanism (25). A recent computational study examining the stability and transformation rates of radical intermediates explains the favored cis-DCE production by B12 catalyzed reactions (26).

Interestingly, trans-DCE is often found at chloroethene-contaminated sites (1), and its presence has been explained by source contamination or generation through abiotic mechanisms acting on polychlorinated ethenes. No biotic mechanisms that produce predominantly trans-DCE from PCE or TCE are known. A comprehensive understanding of the mechanisms that contribute to trans-DCE occurrence is relevant for natural attenuation monitoring, point-source tracking, and the choice of the most promising remediation strategies. This study describes microcosms and enrichment cultures that produce trans-DCE and cis-DCE in a ratio of 3:1 from PCE and TCE.

Materials and Methods

Chemicals. The following analytical grade chlorinated compounds were used in this study: PCE, TCE, trans-DCE, cis-DCE, all of which were obtained from Supelco (Belleville, PA), and VC, which was obtained from Fluka Chemical Corp. (Ronkonkoma, NY). Other chemicals used in the study were obtained from Sigma-Aldrich (Milwaukee, WI).

Inoculum Sources, Microcosm Preparation, and Growth Conditions. Microcosms that produced a mixture of trans-DCE and cis-DCE were derived from sediment and soil materials collected from the Tahquamenon River and the Pine River, both located in Michigan, the Perfume River near Huei in Vietnam, and a swampy area in Chitwan National Park, Nepal, as described (27, 28). In addition, PCE dechlorinating microcosms were established from a soil and sediment slurry consisting of over 15 agricultural soils, forest soils, and river sediment samples collected in Michigan. The slurry was maintained under nitrate reducing conditions to reduce readily bioavailable electron donors by 10 repeated additions of 0.5 mM nitrate. Following complete removal of nitrate and nitrite, approximately 10 g of slurry was added to 90 mL of basal salts medium reduced with cysteine and sulfide (0.2 mM each) (3) in 160 mL serum bottles. All microcosms were amended with lactate (5 mM) as a source of reducing equivalents and PCE (20 μmol). The microcosms from each site were autoclaved for 60 min at 121 °C on three consecutive days and served as sterile controls. Chloroethenes were analyzed in headspace samples performed following inoculation and periodically thereafter. All cultures were monitored for chloroethene transformation (by gas chromatography) and electron donor consumption (by high performance liquid chromatography) as described previously, and substrates were replenished as needed.
Physiological Studies. To test for spore forming ability of the dechlorinating populations, cultures were pasteurized immediately following inoculation. The temperature was adjusted to a pH of 6.8, 7.5, or 8.2. To examine the effect of temperature on reductive dechlorination, TCE and lactate fed cultures were transferred to fresh medium and incubated at 15, 25, or 30 °C. To examine the effect of cell wall synthesis inhibitors on dechlorination, ampicillin was added from a sterile anoxic aqueous stock solution to a final concentration of 400 mg/L to freshly inoculated TCE and lactate fed cultures. The effect of pH on dechlorination was determined in basal salts medium amended with 30 mM TES (N-tris(hydroxymethyl)methyl-2-aminoethane sulfonic acid) as an additional buffer and adjusted to a pH of 6.8, 7.5, or 8.2.

Nucleic Acid-Based Characterization of trans-DCE Producing Cultures. DNA was extracted from the lactate-amended Tahquamenon and Perfume River cultures after they had consumed the initial dose of TCE (20 μmol). The culture fluids (100 mL) were forced through sterile 0.22 μm polycarbonate membrane filters. The membranes with the cake of biomass were cut with sterile razor blades to fit into the tubes provided in the MoBio UltraClean Soil Kit (Solana Beach, CA). DNA was extracted following the manufacturer’s instructions.

To screen for the presence of known chloroethene-dechlorinating populations, PCR amplification was performed with specific primers targeting the 16S rRNA genes of the following genera: Dehalobacter (31), Desulfuromonas (32), Desulfitobacterium (2 sets (33)), and Dehalococcoides (primer set 3 described in ref 34). PCR reactions were 20 or 50 μL (total volume), and amplification was performed using the conditions described in each of the previous references. Genomic DNA from pure cultures of Dehalobacter restrictus, Desulfuromonas michiganensis, Desulfitobacterium hafniense, and a Dehalococcoides containing mixed culture were used as positive controls for PCR amplification. Whenever amplification with the specific primers occurred, the PCR products were cloned in Escherichia coli using the TOPO TA cloning kit (Invitrogen, Carlsbad, CA). Inserts from at least three positive clones from each culture were sequenced by Michigan State University’s Genomic Technology Support Facility.

Results

Microcosms that reduced PCE to primarily trans-DCE as dechlorination end product were obtained with sediment and soil materials collected from geographically diverse locations. PCE-fed microcosms transiently accumulated TCE before complete conversion to trans-DCE and cis-DCE occurred in a consistent ratio of 3(±0.5):1 (Table 1). No dechlorination and production of DCEs occurred in killed control cultures. This dechlorination profile was independent of the level of enrichment and was observed in methanogenic sediment microcosms, sediment-free cultures, and non-methanogenic cultures transferred over 25 times. The 3:1 ratio of DCE isomers was stable through all transfers, and no further reduction of DCEs to VC was observed even upon extended incubation for several months with ample electron donors.

Physiological Studies. trans-DCE formation was studied in more detail in cultures from the Tahquamenon and Perfume Rivers because these cultures were obtained first, were non-methanogenic, and exhibited robust dechlorination activity after more than 30 and 25 transfers, respectively. Time courses for the dechlorination of TCE showed the simultaneous production of both trans- and cis-DCE (Figure 1). The formation of 1,1-DCE was never observed. The lag time before the onset of dechlorination varied from culture to culture but was always greater than 7 days and could be up to several weeks. Lactate-fed cultures reduced TCE at rates of 2.5 μmol L⁻¹ day⁻¹. The ratio of trans-DCE to cis-DCE was constant over the period of active dechlorination, and trans-DCE accounted for up to 75% of the total chloroethenes in the culture.

The effects of a variety of electron donors on the production of trans-DCE were explored in more detail in the Tahquamenon culture. The trans- to cis-DCE ratio (mean ratio, ±SD, n measurements) with different electron donors was as follows: hydrogen plus acetate (3.33, 0.05, 4), formate (3.14, 0.21, 4), acetate (3.21, −, 1), succinate (2.93, 0.06, 3), and 2-aminoethane sulfonic acid (2.36, 0.05, 4).

### Table 1. Summary of Microcosms and Enrichment Cultures that Produced Mixtures of trans-DCE and cis-DCE

<table>
<thead>
<tr>
<th>Source</th>
<th>Enriched With</th>
<th>Transfers*</th>
<th>Sediment-free</th>
<th>Methane Production</th>
<th>trans/cis DCE†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tahquamenon River, MI</td>
<td>PCE</td>
<td>&gt; 30</td>
<td>yes</td>
<td>no</td>
<td>3.1</td>
</tr>
<tr>
<td>Perfume River, Vietnam</td>
<td>PCE</td>
<td>&gt; 25</td>
<td>yes</td>
<td>no</td>
<td>2.9</td>
</tr>
<tr>
<td>Red Cedar River, MI</td>
<td>1,2-Dc</td>
<td>12</td>
<td>yes</td>
<td>no</td>
<td>2.5</td>
</tr>
<tr>
<td>Pine River, MI</td>
<td>PCE</td>
<td>6</td>
<td>yes</td>
<td>yes</td>
<td>3.4</td>
</tr>
<tr>
<td>Chitwan National Park, Nepal</td>
<td>PCE</td>
<td>1</td>
<td>no</td>
<td>yes</td>
<td>3</td>
</tr>
<tr>
<td>mixed MI soil and sediment inocula</td>
<td>PCE</td>
<td>1</td>
<td>no</td>
<td>yes</td>
<td>3.5</td>
</tr>
</tbody>
</table>

* Transfers were 1% (vol/vol) into fresh minimal medium. † Ratio is the average of at least five measurements (SD < 0.5), except for the Chitwan sample, which is from a single time point and thus only has one significant figure in the ratio. ‡ 1,2-Dichloropropane.
pyruvate (2.92, 0.41, 2), lactate (3.16, 0.31, 5), and glycerol (3.25, 0.28, 3). No dechlorination occurred with propionate or benzoate as electron donors. The average trans- to cis-DCE ratio was 3.13, and trans-DCE was the dominant dechlorination end product under all electron donor conditions tested.

No dechlorination was observed in the Tahquamenon or Perfume River cultures at 12 or 30°C after more than 4 months of incubation. Pasteurized inocula from stationary cultures were no longer able to dechlorinate with all electron donors tested. All cultures produced trans-DCE around pH 7.5, but the Tahquamenon River culture did not dechlorinate at pH 8.2, and only one replicate exhibited dechlorination activity at pH 6.8. The ratios of trans- to cis-DCE isomers produced from TCE by the Perfume River culture were 2.68 (SD 0.05) at pH 6.8, 2.57 (SD 0.06) at pH 7.5, and 2.46 (SD 0.11) at pH 8.2. This slight, but significant (p = 0.027, t-test), decreasing trend in the trans- to cis-DCE ratio with increasing pH was linear (R² = 0.9994) in the pH range tested. Dechlorination of TCE to trans-DCE and cis-DCE occurred in lactate-fed Tahquamenon and Perfume River cultures treated with ampicillin indicating that the organisms involved in the production of trans-DCE were not inhibited by the antibiotic.  

**Nucleic Acid-Based Characterization of trans-DCE Producing Cultures.** Genomic DNA from the Tahquamenon River and Perfume River enrichment cultures was screened using PCR primers targeting 16S rRNA genes of genera known to contain reductively dechlorinating populations. Amplicons from primers targeting the Desulfitobacterium or Desulfuromonas groups were not detected. In both cultures, however, amplicons of the expected size were produced using the Dehalococcoides-targeted primers pairs. The sequences from the 1377 base pair PCR products from both cultures were 99% identical to Dehalococcoides sp. strain FL2 and Dehalococcoides sp. strain CBDB1, which both belong to the Pinellas subgroup of the Dehalococcoides cluster (33). Additionally, Dehalobacter targeted primers produced a band of the expected size from the Perfume River culture DNA. The sequence from the 800 base pair amplicon was 98% identical to the 16S rRNA gene sequence of Dehalobacter restrictus.

**Discussion**

Chlorinated ethenes are frequent groundwater contaminants that are proving amenable to remediation by microbial reductive dechlorination (35, 36). Problems arise, however, if toxic and persistent intermediates such as 1,1-DCE, trans-DCE, cis-DCE, or VC accumulate. Chloroethene dechlorination research has focused on cis-DCE and VC degradation because these compounds are detected at many PCE/TCE contaminated sites and are frequently produced as dechlorination end products in laboratory-based microcosm studies. In addition, all TCE dechlorinating bacteria described to date produce the cis-DCE isomer as the major intermediate or end product. Surprisingly, significant amounts of trans-DCE were detected at some contaminated sites (1). An example is the Key West Navel Air Facility in Florida, where the original spill consisted of TCE, but trans-DCE constitutes the major DCE contaminant detected today (37). Apparent accumulation of one DCE isomer could be due to its preferential production or to isomer-specific degradation. Dehalococcoides populations that only metabolically dechlorinate the cis-isomer were described (4, 14), but recent findings suggest that trans-DCE also serves a growth-supporting electron acceptor in chlororespiration (35).

We evaluated PCE and TCE dechlorination endpoints in microcosms established with soil, aquifer, and sediment materials collected from numerous sites (refs 27, 29, 32, 36, and unpublished results). Albeit rare, the formation of trans-DCE as the major dechlorination end product was observed in microcosms from diverse source materials on several occasions (Table I). These observations are consistent with the more infrequent accumulation of trans-DCE at PCE/TCE contaminated sites undergoing reductive dechlorination. Our findings demonstrated that trans-DCE accumulation in these cultures is in fact due to a microbial process favoring trans-isomer over cis-isomer formation and not because of the preferential consumption of the cis-isomer, as no DCE consumption occurred.

The ratio of DCE isomers was remarkably stable, with the trans-DCE and cis-DCE ratio remaining 3:1 during serial transfers and with different electron donors. Only a slight decrease in the ratio was observed with increasing medium pH in the culture studied. The ratio of 3:1 trans to cis isomers is interesting because there is no precedence in microbial systems for this product distribution, and it is not explained by current understanding of dehalogenase catalyzed reduction. The formation of trans-DCE and the consistency of the ratio produced suggest a yet unknown underlying biochemical mechanism. Abiotic Zn(0) catalyzed hydrogenolysis of TCE produces trans-DCE and cis-DCE in a 2.5:1 ratio (38). There was no significant source of zinc or other zerovalent metals in the medium used in the biological systems described in this study, and formation of dechlorination products never occurred in killed control cultures and bottles not seeded with an inoculum.

The production of trans-DCE in a 3:1 ratio with cis-DCE as described in this study is distinct from the accumulation of trans-DCE by Dehalococcoides ethenogenes strain 195. Strain 195 produced cis-DCE (and 1,1-DCE) prior to and faster than trans-DCE and subsequently consumed cis-DCE at a faster rate (39). trans-DCE only accumulated to a low percentage of the total chloroethene mass. In all cultures investigated in this study, trans- and cis-DCE were produced simultaneously and in a constant 3:1 ratio with no further reduction to VC. Hence, in addition to possible source contamination with trans-DCE, there are at least two biological TCE reduction mechanisms that may lead to the accumulation of trans-DCE in PCE/TCE contaminated subsurface environments.

Dehalococcoides populations may be involved in trans-DCE production in the Tahquamenon and Perfume River cultures because their 16S rRNA gene sequences were readily recovered from these enrichment cultures. Cultures that were transferred twice in the absence of TCE lost the ability to dechlorinate, and Dehalococcoides populations were no longer detectable. This observation is consistent with the known physiology of Dehalococcoides populations, which require a chlorinated compound as growth-supporting electron acceptor (strictly chlororespiratory metabolism). The lag periods before the onset of dechlorination were long, ranging from 1 to several weeks, a phenomenon that has also been observed in Dehalococcoides cultures that grew with VC as terminal electron acceptor (19, 20). Further, the involvement of Dehalococcoides populations is supported by the fact that dechlorination of TCE to trans- and cis-DCE also occurred in the presence of ampicillin. Resistance to cell wall biosynthesis inhibitors such as ampicillin is a characteristic trait of the known Dehalococcoides isolates (4, 14, 20, 40).

Both the Tahquamenon and Perfume River cultures contained 16S rRNA gene sequences most closely related to Dehalococcoides populations of the Pinellas group. The known and characterized Dehalococcoides isolates of the Pinellas group are closely related by 16S rRNA sequence identity but exhibit distinct physiologies (e.g., the range of chloroorganic compounds used as growth-supporting electron acceptors (4, 20, 41)). None of the known isolates, however, produce trans-DCE as a major intermediate in the dechlorination of PCE and TCE, and if Dehalococcoides...
populations are indeed involved, our findings would further imply that similar mechanisms exist among Dehalococcoides populations to act on chlorinated ethenes.

The role of other bacteria, including Dehalobacter, in trans-DCE formation by the mixed cultures at various stages of enrichment cannot be completely ruled out. Pure cultures of the dechlorinating populations are needed to definitively identify the populations responsible for trans-DCE formation. The implication of this study for bioremediation is that trans-DCE can be the major end product from PCE and TCE microbial dechlorination; thus, a high fraction of trans-DCE at a site should not automatically be attributed to source contamination.

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