Complete Reductive Dechlorination of 1,2-Dichloropropane by Anaerobic Bacteria

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The transformation of 1,2-dichloropropane (1,2-D) was observed in anaerobic microcosms and enrichment cultures derived from Red Cedar Creek sediment. 1-Chloropropane (1-CP) and 2-CP were detected after an incubation period of 4 weeks. After 4 months the initial amount of 1,2-D was stoichiometrically converted to propene, which was not further transformed. Dechlorination of 1,2-D was not inhibited by 2-bromoethanesulfonate. Sequential 5% (vol/vol) transfers from active microcosms yielded a sediment-free, nonmethanogenic culture, which completely dechlorinated 1,2-D to propene at a rate of 5 nmol min⁻¹ mg of protein⁻¹. No intermediate formation of 1-CP or 2-CP was detected in the sediment-free enrichment culture. A variety of electron donors, including hydrogen, supported reductive dechlorination of 1,2-D. The highest dechlorination rates were observed between 20° and 25°C. In the presence of 1,2-D, the hydrogen threshold concentration was below 1 ppm by volume (ppmv). In addition to 1,2-D, the enrichment culture transformed 1,1-D, 2-bromo-1-CP, tetrachloroethene, 1,1,2,2-tetrachloroethane, and 1,2-dichloroethene to less halogenated compounds. These findings extend our knowledge of the reductive dechlorination process and show that halogenated propanes can be completely dechlorinated by anaerobic bacteria.

Halogenated propanes, including 1,2-dichloropropane (1,2-D), have been extensively used as fumigants to control root parasitic nematodes for a variety of crops (2, 4, 21). Due to their toxicity (23, 30) and recalcitrant nature, halogenated propanes have been replaced by other volatile compounds and are thus no longer employed in agriculture in the United States. However, 1,2-D is still used in the United States by industry and for research, where it has application as a solvent, oil and paraffin extractant, metal-degreasing agent, paint and furniture finish remover, lead scavenger in antiknock fluids, and textile stain remover (1, 11, 12, 31). Furthermore, 1,2-D is a chemical intermediate used in the production of tetrachloroethene (PCE), carbon tetrachloride, and other chlorinated compounds (11, 12, 31). In addition to these intentional uses, 1,2-D is formed as an undesired by-product in the chemical production of propylene oxide by the chlorohydrin process (35). In 1991, this process yielded about 165,000 metric tons of 1,2-D worldwide, of which 70,000 tons was produced in the United States. This extensive output of 1,2-D creates a substantial waste management problem. Because 1,2-D is moderately soluble in aqueous systems and recalcitrant to microbial degradation, this compound is found as a significant pollutant of groundwater systems (5, 8, 16, 25). Concentrations of 9 mg m⁻³ in groundwater samples from areas in The Netherlands have been reported (5), and concentrations as high as 1.2 g m⁻³ have been detected in California (16). It has been estimated that the half-life of 1,2-D in groundwater ranges from 6 months to 2 years (1). However, since 1,2-D volatilizes readily at ambient temperatures, the apparent half-life may not represent the actual degradation rates in soils, sediments, or aquifers.

The fate of 1,2-D in natural environments is poorly understood. Under laboratory conditions 1,2-D can be cometabolically oxidized by some methanotrophic (22) and nitrifying (24) bacteria under aerobic conditions. These conversions are due to the fortuitous action of methane monooxygenase and ammonia monooxygenase. Both types of enzymes exhibit a broad range of substrate specificities. For example, whole-cell suspensions of Methylosinus trichosporium OB3b expressing soluble methane monooxygenase partially dechlorinated 1,2-D to 2,3-dichloro-1-propanol (22). In another study, the ammonia-dependent disappearance of 1,2-D was shown in cell suspensions of two soil nitrifiers, but no conversion products could be identified (24). In addition to cometabolic processes, several aerobic bacteria available in pure culture are capable of using halogenated aliphatic compounds as a sole source of carbon and energy (13, 26). Hydrolytic and oxygenolytic dehalogenases have been characterized from these organisms, but none of these enzymes transformed 1,2-D at significant rates, if at all (3, 13, 26, 28, 32). The utilization of 1,2-D as a carbon source under aerobic conditions has been claimed in a single case. Pseudomonas fluorescens PFL12 was reported to degrade 1,2-D under aerobic conditions in the presence of 0.5% glucose and 0.005% yeast extract at a rate of 0.018 nmol min⁻¹ ml of culture fluid⁻¹ (34). Although the authors claim that P. fluorescens PFL12 can grow with 1,2-D as the only available carbon and energy source, this study (34) showed only the disappearance of 1,2-D. No degradation products were detected, and 1,2-D-dependent growth was not shown. In another study, several hundred different environmental samples from pristine areas and contaminated sites were incubated aerobically under different conditions and evaluated for their potential for chloride release from 1,2-D. Chloride release was never observed, nor was a transformation product of 1,2-D or the utilization of 1,2-D as a growth substrate detected (17). Other studies also failed to show the degradation of 1,2-D under aerobic conditions (25, 33).

Very limited information describing the degradation of 1,2-D under anaerobic conditions is available. Boesten et al.

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studied the fate of 1,2-D in three anaerobic sandy subsoil materials. In one of these materials a 96% loss of 1,2-D was observed after 2 years of incubation, with propene and propane suggested as possible transformation products. This paper describes the detection of 1,2-D-dechlorinating activity in anaerobic microcosms derived from a freshwater river sediment and the characterization of a sediment-free, nonmethylene enrichmet culture that completely dechlorinated 1,2-D to propene.

MATERIALS AND METHODS

Chemicals. 1,2-D, 1-chloropropane (1-CP), 2-CP, 2-bromo-1-CP, 2,3-dichloro-1-propene, 1,1-D, and 2,2-D (all from Aldrich, Milwaukee, Wis.). 1,3-D, and 1,2,3-trichloropropane (1,2,3-TCP) (Supelco, Bellefonte, Pa.), and ethane, ethene, propane, and propene (AGA Gas Inc, Cleveland, Ohio) were used in this study. Synonyms for 1,2-D are propylene dichloride, propylene chloride, and 2,3-. Anaerobic aqueous stock solutions were prepared by adding 20 μl of the halogenated aliphatic compound to 10 ml of anoxic water in a 24-ml vial. The vials were closed with Teflon-lined rubber stoppers and autoclaved. Before use the vials were shaken thoroughly to prepare homogeneous suspensions of the incompletely soluble compounds.

Preparation of anaerobic microcosms. Reduced anaerobic basal salts medium was prepared as described by Löfler et al. (19). The medium contained 10 mM acetate as a carbon source when indicated, 1,2-D (2 μmol/24-ml vial and 15 μmol/160-ml serum bottle, unless indicated otherwise), and an electron donor(s) as indicated in the text. The medium was autoclaved before vitamins, electron donor(s), and halogenated aliphatic compounds were added from sterile anoxic stock solutions. Cultures (100 ml) were fed with undiluted halogenated compounds by using 5-μl Hamilton syringes equipped with reproducibility (Chuney) adapters.

Microcosms. Two sediment samples from the Red Cedar Creek were analyzed. One sample, obtained in June 1995, was kept anaerobic at 4°C for 1 week. The other sample, taken from the same location in November 1994, was dried at room temperature and sieved aerobically. A pipetteful slurry (1 g [dry weight] per 5 ml) was prepared by mixing sediment with phosphate-buffered saline (5 mM potassium phosphate, 0.85% NaCl [pH 7.2]). Five milliliters (independently) of the two slurries were transferred to 24-ml vials of four different treatments containing 5 ml of double-strength medium as follows: (i) 2.5 mM pyruvate–5 mM glycerol, (ii) 1 mM acetate–1 mM formate, (iii) no additions, and (iv) 5 ml of phosphate-buffered saline without additional electron donors. All vials were amended with 2 μmol of 1,2-D and sealed with Teflon-lined rubber stoppers. Autoclave-killed controls and live controls without 1,2-D were prepared. Microcosms that depleted the initial dose of 1,2-D were respiked with another 2 μmol of the chlorinated propene.

Incubation conditions. Cultures were incubated in 24-ml (nominal capacity) vials containing 10 ml of medium or in 160-ml serum bottles (nominal capacity) containing 160 ml of medium (referred to hereafter as the 100-ml cultures). When H2 was supplied as an electron donor, 3 ml of the gas was added to the 24-ml vials and 8 ml was added to the 160-ml serum bottles, unless indicated otherwise. All cultures were incubated in inverted containers without shaking (except for the hydrogen threshold experiments) at 25°C in the dark.

Sediment-free enrichment cultures. From microcosms showing propene formation, 0.5 ml of the slurry or supernatant was transferred to fresh medium ([i] 2.5 mM pyruvate–5 mM glycerol and [ii] 10 mM acetate–20% [by volume] H2). All vials were amended with 2 μmol of 1,2-D, and cultures showing dechlorination activity were sequentially transferred (5% [vol/vol]) until sediment-free cultures were obtained.

Countercurrent against methanogens. A 1,2-D-dechlorinating sediment-free culture was transferred in duplicate to medium amended with either 2.5 mM pyruvate–5 mM glycerol or 10 mM acetate–H2. To one of each duplicate vial, 2 mM 2-bromomethanesulfonate (BES) was added to inhibit methanogenesis. Four sequential transfers (2% [vol/vol]) were done for each treatment.

Identification of electron donors supporting reductive dechlorination. The 100-ml cultures were pregrown in standard medium with 10 μM acetate–H2 and two spikes of 15 μmol of 1,2-D. The headspaces were purged with sterile N2-CO2 before incubation at different temperatures. The influence of the different halogenated aliphatic compounds used in this study were identified by GC-MS.

Dechlorination of different halogenated aliphatic compounds. The headspaces of two cultures grown with 10 mM acetate–H2 that converted 30 μmol of 1,2-D to propene were flushed with argon. In a glove box, 10-ml aliquots were dispensed into sterile 24-ml vials. Different chlorinated aliphatic compounds were added (3 ml of 1,2-D was added to each vial. Vials were sealed without flushing at 25°C, and every third day the headspace was analyzed by GC. H2 was replenished as depleted.

Influence of temperature, 1,2-D concentration, Na2S, sulfite, and nitrate on dechlorination. The temperature range was determined as follows. For reconstituting the two heads of active 100-ml cultures that converted 30 μmol of 1,2-D to propene were flushed with argon, and 10 ml was dispersed into each of 24-ml vials in a glove box. The vials were amended with 2 μmol of 1,2-D and 3 ml of H2, and incubated at temperatures of 4, 16, 20, 25, 30, 37, and 45°C. (ii) For growth-dependent determinations, two 100-ml cultures (1 mM acetate–4 mM glycerol) were inoculated with 1 ml of a dechlorinating culture. Samples (10 ml) were aliquoted into 24-ml vials in a glove box. 1,2-D (2 μmol) was added before incubation at different temperatures. The influence of the initial 1,2-D concentration on dechlorination was determined in freshly inoculated cultures (1% inoculum). 1,2-D was added at concentrations ranging from 0.4 to 30 μmol per ml, and propene formation was monitored for 4 months. The influence of the following different amendments on 1,2-D dechlorination was determined as above: sulfite, sulfite, and nitrate (at concentrations of 1, 2, and 10 mM each) and NaCl (to final chloride concentrations of 83, 130, 220, 306, 390, 471, 661, and 836 mM).

Analytical methods. All volatiles were measured in headspace samples at 25°C by GC and direct (splitless) injection. For chlorinated volatiles and propene, samples (0.2 ml) were analyzed on a Varian GC (model 3700) equipped with a Megabore model DB-624 column (45 m by 0.54 mm; J & W Scientific) and a flame ionization detector. Helium was used as the carrier gas. The temperature was held isocratic at 50°C for 4 min, increased at 50°C/min to 200°C, and held at that temperature for 2 min. For propene (in presence of methane) and methane, 0.2-ml headspace samples were injected onto a stainless steel Porapak Q column and detected with a flame ionization detector. The elution was isocratic at oven temperatures of 130°C for propene and of 60°C for ethene. N2 was used as the carrier gas. H2 was measured on a Carle GC equipped with a stainless steel Porapak Q column (1.5 m by 0.32 cm) and a thermal conductivity detector with argon as the carrier gas. In the presence of N2 the detection limit for H2 was 500 ppmv. For more sensitive measurements of H2, a GC equipped with reduction gas detector (Trace Analytical, Menlo Park, Calif.) was used. N2 was used as the carrier gas at a flow rate of approximately 30 ml min−1. The detection limit for H2 under these conditions was 0.5 ppmv. For all headspace measurements, gas-tight 250-μl glass syringes (Hamilton, Reno, Nev.) with gas-tight Teflon valves and Luer Lock adapters were used.

Standards were prepared as described by Gossett (15). A known amount of the compound was added to bottles or vials with the same liquid-to-headspace ratio as that for the cultures being analyzed. Chlorinated hydrocarbons were added from methanol stock solutions. Linear standard curves were obtained for the following concentration ranges: 10 to 18.5 μmol for 1,2-D and 0.7 to 210 μmol for propene (in 160-ml bottles); 0.06 to 18.5 μmol for 1,2-D and 0.09 to 2.4 μmol for 1-CP; 0.07 to 1.2 μmol for 2-CP; and 0.04 to 20 μmol for propene (in 24-ml vials).

Analysis by GC-mass spectrometry (MS) was carried out on a JEOL AX-505H double focusing mass instrument coupled to a Hewlett-Packard 5890 GC via a heated interface. GC separation employed a Porapak U fused-silica capillary column (25 m by 0.32 mm with a 10-μm-thickness film coating) from Chrompack Inc. (Routon, N.J.). Helium gas flow was approximately 1 ml min−1. The GC temperature program was initiated at 50°C, held for 10 min, and increased at 1°C/min to 190°C. MS conditions were as follows: interface temperature, 190°C; ion source temperature, ca. 190°C; electron energy, 3 kV; scan rate of the MS, 1 to 10 m/s against an m/z range of 0 to 200; and ionization current, 100 μA. Protein concentrations were determined according to a modification of the Lowry assay (27) after alkaline cell lysis (14). Sterile medium and ovalbumin were used as the blank and the standard, respectively.

Identification of dechlorination products. Transformation products of the different dechlorinated aliphatic compounds used in this study were identified by comparing their retention times with those of authentic standards by GC. In addition, 1-CP, 2-CP, propene, and 2-chloropropene were identified by GC-MS.

RESULTS

Dechlorination of 1,2-D in microcosms. In all microcosms derived from freshly sampled Red Cedar Creek sediment, the formation of monochlorinated propanes and propene was detected. After 4 weeks, about 0.2 μmol of 1-CP and small amounts of 2-CP (about 1/8 of the amount of 1-CP) were detected. After 6 weeks, the formation of propene was detected, and after 4 months the initial amount of 1,2-D was...
completely recovered as propene. Respiking of microcosms thrice with 2 \( \mu \)mol of 1,2-D after its depletion resulted in the intermediate accumulation of 2.1 \( \mu \)mol of 1-CP and 0.25 \( \mu \)mol of 2-CP before complete dechlorination to propene was observed. Both monochlorinated propanes were identified by GC-MS (data not shown). When microcosms that had completely dechlorinated 2 \( \mu \)mol of 1,2-D to propene were respiked with 2 \( \mu \)mol of 1-CP or 2-CP, the monochlorinated propanes were converted to propene. Propene was the final degradation product in all active microcosms, and propane was never detected. We were led to the initial assumption that propane might be the end product of 1,2-D dechlorination (6) because propene and propane could not be separated on the DB 624 capillary column. By using the Porapak Q column, the retention time of propane was only 1.035 times longer than the retention time of propene. Subsequent gas chromatography-mass spectrometry (GC-MS) analyses of the active microcosms and two sediment-free cultures after 18 transfers have clearly proven that propene is the end product of 1,2-D dechlorination (data not shown). A 30- to 70\% reduction in the initial amount of 1,2-D, 1-CP, or 2-CP over a period of 3 months was observed in killed control microcosms. This reduction was most likely due to volatilization and adsorption processes, since the formation of possible breakdown products was never observed in killed controls. Similarly, no breakdown products of 1,2-D were observed in any of the microcosms derived from the dried Red Cedar Creek sediment.

**Sediment-free, nonmethanogenic enrichment cultures.** A sediment-free culture was obtained from the active microcosms after three 5\% (vol/vol) transfers of the supernatant to fresh basal salts medium amended with glycerol-pyruvate or acetate-H\(_2\). Sediment-free cultures dechlorinated 1,2-D to propene. Hydrogenolysis, which would explain the formation of 1-CP and 2-CP, was not observed in the sediment-free cultures (Fig. 1). Obviously, other organisms in the original microcosm, which were either not present or not active in the sediment-free cultures, were responsible for the formation of monochlorinated propanes.

BES, an inhibitor of methanogenesis, did not affect dechlorination in the sediment-free enrichment cultures containing glycerol-pyruvate or acetate-H\(_2\), although methane production was completely inhibited. After four serial transfers in medium containing 2 mM BES, methanogenic archaea were removed by dilution. None of the derived BES-free cultures showed any methane production with methanol, pyruvate, glycerol, glucose, or acetate-H\(_2\) supplied as the electron donor to the bicarbonate-buffered medium. Small-subunit (16S) rDNA PCR performed with archaeon-specific primers did not result in any amplification product after the BES treatment. In contrast, amplification products were obtained from the methanogenic enrichment cultures (data not shown). Therefore, methanogens were not present after the BES treatment and were not involved in the complete reductive dechlorination of 1,2-D to propene.

The dechlorination of 1,2-D to propene was further characterized for a sediment-free, nonmethanogenic enrichment culture that was obtained from an acetate-H\(_2\) fed culture. The conversion of 1,2-D to propene was monitored in duplicate 100-ml cultures for 45 days (Fig. 2). The inoculum (1\% [vol/vol]) was from a culture transferred twice after the BES treatment (sixth transfer). 1,2-D (15 \( \mu \)mol, undiluted) and H\(_2\) (10 ml of H\(_2\)-CO\(_2\) [80/20]) were both replenished as they were depleted. The lag time prior to propene formation was 12 days in cultures containing acetate-H\(_2\) and 10 days in cultures containing pyruvate-glycerol. The same experiment was repeated with a culture which had undergone 18 successive transfers. With a 1\% (vol/vol) inoculum, the lag time before propene formation was reduced to 4 days.

Dechlorination of 1,2-D by sediment-free enrichment cultures was dependent on the protein content of the cultures (Fig. 3). 1,2-D was stoichiometrically dechlorinated to propene.
TABLE 1. Conversion of halogenated compounds by 1,2-D-dechlorinating enrichment culture

<table>
<thead>
<tr>
<th>Halogenated compound</th>
<th>Dehalogenation product(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1,2-D</td>
<td>Propene</td>
</tr>
<tr>
<td>1,1-D</td>
<td>1-CP</td>
</tr>
<tr>
<td>2,2-D</td>
<td>2-Chloroprene</td>
</tr>
<tr>
<td>1,3-D</td>
<td>—</td>
</tr>
<tr>
<td>1,2,3-TCP</td>
<td>1,2-D, propene</td>
</tr>
<tr>
<td>1-CP</td>
<td>—</td>
</tr>
<tr>
<td>2-CP</td>
<td>—</td>
</tr>
<tr>
<td>2-Bromo-1-CP</td>
<td>1-CP, propene</td>
</tr>
<tr>
<td>2,3-Dichloro-1-propene</td>
<td>—</td>
</tr>
<tr>
<td>PCE</td>
<td>TCE, cis-DCE, trans-DCE</td>
</tr>
<tr>
<td>cis-DCE</td>
<td>—</td>
</tr>
<tr>
<td>trans-DCE</td>
<td>—</td>
</tr>
<tr>
<td>TeCA</td>
<td>TCE, cis-DCE, trans-DCE</td>
</tr>
<tr>
<td>1,2-Dichloroethane</td>
<td>Ethene</td>
</tr>
</tbody>
</table>

a The cultures contained 10 mM acetate and H2 (3 ml) as potential electron donors.
b Complete transformation of the initial amount (2 µmol) of the halogenated compound was observed within 2 weeks, unless indicated otherwise.
c The dechlorination product had the same retention time as did the authentic 1-CP.
d Dechlorination of 2,2-D to 2-chloropropene was also observed in killed controls (see the text).
e —, no transformation products were detected within 3 months.
f Trace amounts of 1,2-D and propene were detected.
g Dechlorination of TeCA to TCE was also observed in killed controls.

at a rate of 5 nmol min⁻¹ mg of protein⁻¹. A paired-sample t test reveals that the observed amount of propene formed was not significantly different from that expected [t(2), 0.05 = 0.5] based on the hypothesis that each molecule of 1,2-D was being dissimilated to propene. The null hypothesis, H₀, was that the mean population difference, μ₀, between paired expected and observed values would have been equal to zero. The ratio, t, of the average differences between observed and expected values and the standard error is smaller than the critical t(2) value of 1.94 for α = 0.05.

The dechlorinating culture was enriched for its ability to reductively dechlorinate 1,2-D to propene and was not exposed to any other halogenated aliphatic compound during the enrichment procedure. A culture that had undergone 18 transfers was examined for its potential to transform halogenated aliphatic compounds other than 1,2-D (Table 1). In contrast to the microcosm studies, sediment-free cultures did not transform monochlorinated propanes. PCE was dechlorinated to cis- and trans-dichloroethene (DCE) as end products, with a trans-DCE/cis-DCE ratio of 2.45. The intermediate formation of small amounts of trichloroethene (TCE) was detected. Also, 1,1,2,2-tetrachloroethane (TeCA) was transformed to TCE and both DCE isomers, with a trans-DCE/cis-DCE ratio of 0.15. The conversion of TeCA to TCE was also detected in killed controls (7), but the formation of DCEs was dependent on live cultures. Dehydrochlorination of 2,2-D to 2-chloropropene was observed in live and killed cultures and in both sterile basal salts medium and oxic water when exposed to light. Small amounts of propene and traces of 1,2-D were formed in live cultures that were spiked with 1,2,3-TCP after an incubation period of 3 months. Live cultures dechlorinated 1,2-dichloroethene to ethene without the intermediate formation of monochlorinated ethanes.

Factors affecting dechlorination of 1,2-D. Dechlorination could be observed at temperatures between 16 and 30°C; however, no dechlorination occurred at 4°C and above 37°C during a 3-month period. Maximal dechlorination rates were observed between 20 and 25°C. Bacterial growth was measured by an increase in turbidity and occurred between 16 and 37°C but not at 4 or 45°C.

The dechlorination of 1,2-D to propene was influenced by the initial concentration of the chlorinated compound. Although growth was observed in 24-ml vials containing up to 30 µmol of 1,2-D, dechlorination was completely inhibited at concentrations above 9 µmol of 1,2-D. Furthermore, the dechlorination rates decreased at concentrations above 2.1 µmol of 1,2-D.

Dechlorination of 1,2-D was not inhibited by NaCl concentrations up to 220 mM, but no dechlorination occurred at 306 mM NaCl. Growth was observed at concentrations up to 661 mM, with complete inhibition at 836 mM NaCl.

Growth of the mixed culture in basal salts medium containing glycerol and acetate was not inhibited by sulfate, sulfite, or nitrate concentrations up to 10 mM. However, all these compounds inhibited dechlorination to different extents. Nitrate showed the most pronounced effect, and no propene formation was observed in the presence of 1 mM nitrate within 3 months. Dechlorination was also completely inhibited at concentrations of 2 mM sulfite or 10 mM sulfate. The time required for complete dechlorination of 1,2-D to propene relative to the time needed by cultures without additions increased by a factor of 1.38 (1 mM sulfate), 2.57 (2 mM sulfate), and 2.0 (1 mM sulfite). The observed inhibition of 1,2-D dechlorination was not due to the lack of a suitable electron donor, since the repeated addition of glycerol or H2 did not stimulate dechlorination. 1,2,3-TCP also inhibited 1,2-D dechlorination. After the addition of 9.4 µmol of 1,2,3-TCP to an actively 1,2-D-dechlorinating 100-ml culture, the reduction of 1,2-D to propene stopped immediately. After 2 months a slight decrease in the 1,2,3-TCP concentration, associated with an increase in propene, was observed. Meanwhile, the concentration of 1,2-D remained constant. This finding indicated that 1,2,3-TCP was dechlorinated by this enrichment culture at low rates and that 1,2-D might be an intermediate (Table 1).

Electron donors for 1,2-D reduction and H2 threshold. A variety of electron donors were tested for their potential to support reductive dechlorination of 1,2-D to propene. Complete dechlorination of 1,2-D to propene was achieved with H2 (in presence of acetate), lactate, fumarate, pyruvate, glycerol, methanol, ethanol, mannitol, sorbitol, glucose, fructose, or yeast extract supplied as the electron donor. Acetate or formate supported dechlorination to some extent, but never more than 30% of the initial amount of 1,2-D was converted to propene. Respiring these cultures with an additional 5 mM acetate or 5 mM formate did not result in increased propene formation, indicating that H2 was the essential electron donor. The addition of H2 or propionate to the bicarbonate-buffered medium that did not contain acetate did not support dechlorination. An increase in turbidity, indicating growth, was observed with all electron donors tested except acetate and propionate.

The H2 threshold concentrations in cultures after 14 and 19 transfers were measured in the presence and absence of 1,2-D. In cultures containing the chlorinated compound, H2 was consumed to concentrations less than 1 ppmv. In contrast, cultures that were not fed with 1,2-D had H2 threshold concentrations of about 450 ppmv. No H2 was consumed in killed control cultures containing 1,2-D.

DISCUSSION

The results presented show the potential for anaerobic bacteria to completely dechlorinate halogenated propanes. These findings are especially important, since the environmental pol-
lutant 1,2-D is resistant to degradation under aerobic conditions (17).

Possible reactions that could contribute to the degradation of 1,2-D under anaerobic conditions are (i) stepwise reductive dechlorination to propane (with the intermediate formation of monochlorinated propanes), (ii) dichloroelimination with the concomitant formation of propene, and (iii) dehydrochlorination resulting in the formation of monochlorinated propenes or propene from 1,2-D or monochlorinated propanes, respectively. Our results indicate that all three dechlorination mechanisms might be involved in the breakdown of 1,2-D in anaerobic environments (Fig. 1). The formation of monochlorinated propanes, as observed in the microcosm studies, can be explained by a simple reduction (hydrogenolysis) step. A second reductive dechlorination step, however, did not occur, and consequently, propane was never detected. The disappearance of 1-CP and 2-CP, which was associated with the formation of propene, is explained by a dehydrochlorination step. Dehydrochlorination is a common abiotic reaction. It is not a redox reaction, and no electron donor (reductant) is required. In killed control microcosms spiked with monochlorinated propanes, the formation of propene was never detected, indicating that the observed dehydrochlorination had been biologically catalyzed. Furthermore, dehydrochlorination of 1,2-D to monochlorinated propanes did not occur in either live or killed microcosms. Because 1,2-D carries two chlorine substituents on adjacent carbon atoms, dichloroelimination (vicinal reduction) can occur. The simultaneous removal of the chlorine substituents coupled with the formation of a double bond between the two carbon atoms (dichloroelimination) explains the conversion of 1,2-D to propene without the intermediate formation of monochlorinated propanes (Fig. 1). In sediment-free enrichment cultures only dichloroelimination was observed. Obviously, the organisms that carried out the single hydrogenolysis step of 1,2-D to monochlorinated propanes in the microcosms were either not present or not active in sediment-free cultures. Similarly, dehydrochlorination of monochlorinated propanes was observed only in the microcosms and not in sediment-free cultures.

In addition to 1,2-D, other halogenated aliphatic compounds were dehalogenated by the enrichment culture. It is unclear whether the 1,2-D-dechlorinating organism(s) was responsible for all dehalogenation reactions or whether other dechlorinating populations were present in the community. Since the 1,2-D-dechlorinating culture was transferred 19 times in basal media and never exposed to other chlorinated compounds, the presence of several different dechlorinating populations in the 1,2-D-enriched culture seems unlikely. This deduction is also reflected in the following observations. A culture which reduced PCE to ethene was enriched (20 serial transfers) from the same Red Cedar sediment. This PCE-dechlorinating culture exhibited specificity for chlorothene and did not dechlorinate 1,2-D (18).

The H2 threshold value of <1 ppmv in the presence of 1,2-D indicates that 1,2-D can be used as electron acceptor by at least one member of the community. In cultures lacking 1,2-D, CO2 was the only available electron acceptor. Therefore, acetogenesis was the only possible terminal-electron-accepting process in the nonmethanogenic cultures, and the resulting H2 threshold values of 400 to 500 ppmv are indicative of acetogenesis as the predominant terminal-electron-accepting process (9). Cord-Ruwisch et al. (9) pointed out that H2 threshold concentrations decrease with increasing Gibbs free energy changes of the H2-consuming reaction. The Gibbs free energy change associated with the formation of acetate from CO2 with H2 as the electron donor is −104.6 kJ/mol (29). This value is considerably less negative compared to the standard free energy change of −183 kJ/mol associated with reductive dechlorination of 1,2-D to propene (10, 20). Since the 1,2-D-dechlorinating organism(s) outcompetes the acetogens for H2, this finding indicates that the dechlorinator(s) is able to benefit from the change in Gibbs free energy associated with reductive dechlorination by generating ATP. To further investigate chlororespiration, our current efforts focus on isolating the dechlorinating population in pure culture.

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REFERENCES

31. TR-263. 1986. Toxicology and carcinogenesis studies of 1,2-dichloropropane (propylene dichloride) (CAS no. 78-87-5) in F344/N rats and B6C3F1 mice (gavage studies). http://ntp-server.niehs.nih.gov/htdocs/LT-studies/tr263.html