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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-dichloroethane 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 Due to their toxicity (23, 30) and recalcitrant nature, halogenated propanes have been replaced by other volatile compounds and are thus no longer employed in agriculture in the United States.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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(5) 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, nonmethanogenic enrichment 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-D.

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.

Medium preparation. Reduced anaerobic basal salts medium was prepared as described by Löffler 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 donors, 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 (Chaney) 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 pipettable slurry (1 g [dry weight] per 5 ml) was prepared by mixing each 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 propane.

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 100 ml of medium (referred to hereafter as the 100-ml culture). When H_2 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] H_2). 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.

Counterselection 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–H_2. To one of each duplicate vial, 2 mM 2-bromoethanesulfonate (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–H_2 and two spikes of 15 μmol of 1,2-D. The headspaces were purged with sterile N_2-CO_2 (80/20) until no residual propene or H_2 could be detected by gas chromatography (GC). Samples (10 ml) were combined with the different electron donors (1 mM methanol or ethanol and a 5-mM concentration of other donors) in 24-ml vials. Yeast extract was also tested at a final concentration of 0.1 mg/ml. When H_2 was tested as an electron donor, the gas phase contained 20% (by volume) H_2. 1,2-D and propene concentrations were measured every second day, and when depleted, 1,2-D (2 μmol) was added.

Hydrogen threshold experiments. The headspaces of three dechlorinating cultures grown in 10 mM acetate–H_2 medium were flushed with argon until the H_2 concentration in the culture bottles was below 500 ppmv. Serum bottles were amended with 0.2 ml of H_2 (14,000 ppmv) and incubated on a rotary shaker (250 rpm) at 25°C. 1,2-D and H_2 concentrations were measured weekly, and 1,2-D was added (15 μmol) when depleted. When a constant H_2 concentration was observed for 2 months, the cultures were respiked with H_2 (14,000 ppmv), and the H_2 concentration was monitored until a stable plateau was observed. Two autoclaved cultures containing 1,2-D and H_2 and two live cultures that received H_2 but no 1,2-D served as controls. H_2 was measured with a GC equipped with a reduction gas detector (see below).

Dechlorination of different halogenated aliphatic compounds. The headspaces of two cultures grown with 10 mM acetate–H_2 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, and 3 ml of H_2 was added to each vial. Vials were incubated upside down without shaking at 25°C, and every third day the headspace was analyzed by GC. H_2 was replenished as depleted.

Influence of temperature, 1,2-D concentration, NaCl, sulfate, sulfite, and nitrate on dechlorination. The temperature range was determined as follows. (i) For resting cells, the headspaces of two active 100-ml cultures that converted 30 μmol of 1,2-D to propene were flushed with argon, and 10 ml was dispensed 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 H_2 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 24-ml vial, and propene formation was monitored for 4 months. The influence of the following different amendments on 1,2-D dechlorination was determined as described above: sulfate, 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 0.543 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. N_2 was used as the carrier gas. H_2 was measured on a Carle GC equipped with a stainless steel Porapak Q column (1.83 m by 0.32 cm) and a thermal conductivity detector with argon as the carrier gas. In the presence of N_2 the detection limit for H_2 was 500 ppmv. For more sensitive measurements of H_2, a GC equipped with reduction gas detector (Trace Analytical, Menlo Park, Calif.) was used. N_2 was used as the carrier gas at a flow rate of approximately 30 ml min^{-1}. The detection limit for H_2 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: 0.15 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; 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 instrument coupled to a Hewlett-Packard 5890J GC via a heated interface. GC separation employed a Poraplat 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 10°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 s/scan over an *m/z* range of 0 to 200; and ionization current, 100 mA.

Protein concentrations were estimated 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 halogenated 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

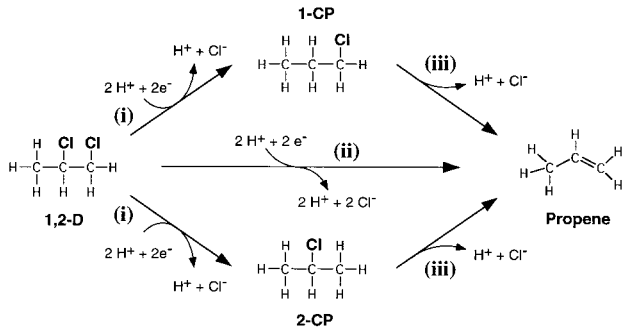

FIG. 1. Anaerobic transformation of 1,2-D detected in microcosms by (i) hydrogenolysis of 1,2-D resulting in the formation of monochlorinated propanes (1-CP and 2-CP), (ii) dichloroelimination (vicinal reduction) of 1,2-D resulting in the formation of propene, the only reaction observed in sediment-free cultures, and (iii) dehydrochlorination of monochlorinated propanes resulting in the formation of propene. Hydrogenolysis of monochlorinated propanes and dehydrochlorination of 1,2-D, a reaction that would result in the formation of monochlorinated propenes, were not observed.

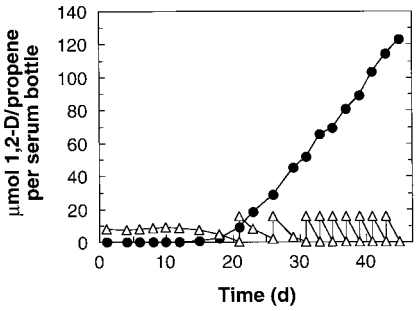

FIG. 2. Reductive dechlorination of 1,2-D (triangles) to propene (circles) in sediment-free, nonmethanogenic cultures. Acetate (10 mM) and H_2 (8 ml) were supplied as potential electron donors. 1,2-D was respiked when depleted, and H_2 was fed when the concentration was below 500 ppmv. Data are the mean values for duplicate cultures.

completely recovered as propene. Respiking of microcosms thrice with 2 μmol of 1,2-D after its depletion resulted in the intermediate accumulation of 2.1 μmol of 1-CP and 0.25 μmol 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 μmol of 1,2-D to propene were respiked with 2 μmol 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 propane and propene 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 headspace 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 time 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 μmol, 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

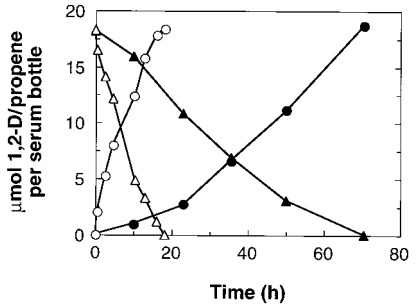

FIG. 3. Dechlorination of 1,2-D to propene: dependence on protein concentration. Two 100-ml cultures that contained 1.0 and 1.1 mg of protein (closed symbols) and two other 100-ml cultures that contained 3.85 and 4 mg of protein (open symbols) were used in this experiment. All cultures were spiked with 18 μmol of 1,2-D and 8 ml of H_2, and the conversion of 1,2-D (triangles) to propene (circles) was monitored for the times indicated. Data are the means for the duplicate cultures.

TABLE 1. Conversion of halogenated compounds by 1,2-D-dechlorinating enrichment culture[a]

Halogenated compound	Dehalogenation product(s)[b]
1,2-D	Propene
1,1-D	1-CP[c]
2,2-D	2-Chloropropene[d]
1,3-D	—[e]
1,2,3-TCP	1,2-D, propene[f]
1-CP	—
2-CP	—
2-Bromo-1-CP	1-CP[c], propene
2,3-Dichloro-1-propene	—
PCE	TCE, *cis*-DCE, *trans*-DCE
cis-DCE	—
trans-DCE	—
TeCA	TCE[g], *cis*-DCE, *trans*-DCE
1,2-Dichloroethane	Ethene

[a] The cultures contained 10 mM acetate and H_2 (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 [$t_{(2),6}$ of 0.45] based on the hypothesis that each molecule of 1,2-D was being dissimilated to propene. The null hypothesis, H_0, was that the mean population difference, μ_d, 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 $\alpha = 0.05$.

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-dichloroethane 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 H_2 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 H_2 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 H_2 (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 50% of the initial amount of 1,2-D was converted to propene. Respiking these cultures with an additional 5 mM acetate or 5 mM formate did not result in increased propene formation, indicating that H_2 was the essential electron donor. The addition of H_2 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 H_2 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, H_2 was consumed to concentrations less than 1 ppmv. In contrast, cultures that were not fed with 1,2-D had H_2 threshold concentrations of about 450 ppmv. No H_2 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 single 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 propenes 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 salts medium containing 1,2-D and was 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 observation. 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 chloroethenes and did not dechlorinate 1,2-D (18).

The H_2 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, CO_2 was the only available electron acceptor. Therefore, acetogenesis was the only possible terminal-electron-accepting process in the nonmethanogenic cultures, and the resulting H_2 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 H_2 threshold concentrations decrease with increasing Gibbs free energy changes of the H_2-consuming reaction. The Gibbs free energy change associated with the formation of acetate from CO_2 with H_2 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 H_2, 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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