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Characterization of an H_2-Utilizing Enrichment Culture That Reductively Dechlorinates Tetrachloroethene to Vinyl Chloride and Ethene in the Absence of Methanogenesis and Acetogenesis

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We have been studying an anaerobic enrichment culture which, by using methanol as an electron donor, dechlorinates tetrachloroethene (PCE) to vinyl chloride and ethene. Our previous results indicated that H_2 was the direct electron donor for reductive dechlorination of PCE by the methanol-PCE culture. Most-probable-number counts performed on this culture indicated low numbers ($\leq 10^4$/ml) of methanogens and PCE dechlorinators using methanol and high numbers ($\geq 10^6$/ml) of sulfidogens, methanol-utilizing acetogens, fermentative heterotrophs, and PCE dechlorinators using H_2. An anaerobic H_2-PCE enrichment culture was derived from a 10^{-6} dilution of the methanol-PCE culture. This H_2-PCE culture used PCE at increasing rates over time when transferred to fresh medium and could be transferred indefinitely with H_2 as the electron donor for the PCE dechlorination, indicating that H_2-PCE can serve as an electron donor-acceptor pair for energy conservation and growth. Sustained PCE dechlorination by this culture was supported by supplementation with 0.05 mg of vitamin B_{12} per liter, 25% (vol/vol) anaerobic digestor sludge supernatant, and 2 mM acetate, which presumably served as a carbon source. Neither methanol nor acetate could serve as an electron donor for dechlorination by the H_2-PCE culture, and it did not produce CH_4 or acetate from H_2-CO_2 or methanol, indicating the absence of methanogenic and acetogenic bacteria. Microscopic observations of the purified H_2-PCE culture showed only two major morphotypes: irregular cocci and small rods.

Tetrachloroethene (perchloroethylene or PCE) and trichloroethene (TCE) are commonly used organic solvents which have been released into the environment and have become major groundwater pollutants. PCE appears to be completely resistant to metabolism by aerobes, while TCE can be cooxidized by certain nonspecific oxygenases (9). However, considerable evidence has accrued in studies of anaerobic microcosms and cultures for reductive dechlorination of PCE to TCE (10), dichloroethene (DCE) isomers (1, 14, 16), or vinyl chloride (VC) (12, 24). More importantly, complete dechlorination to ethene (ETH) (7, 12) or ethane (4) has been reported. It is not clear why some anaerobic systems only partially dechlorinate PCE while others effect complete dechlorination. Little is known about the identity of organisms responsible for reductive dechlorination in these systems, although the recent descriptions of strain PER-K23 (16) and *Dehalospirillum multivorans* (20), which are capable of reduction of PCE to *cis*-DCE, provide examples of what is likely to be a diversity of organisms capable of reductive dechlorination of chloroethenes.

We have been studying an anaerobic enrichment culture which uses methanol as the electron donor for reductive dechlorination of PCE to ETH. In initial studies (12), PCE was fed every 2 days at a dose of 3.5 μmol/liter of culture medium. VC was the primary product, and most of the 0.32 mmol of methanol per liter that was added as an electron donor was used for methanogenesis. In a subsequent study (7), the PCE and methanol doses were gradually increased until they reached 0.55 mmol of PCE per liter and 1.6 mmol of methanol per liter. This increase drastically improved the performance of the culture, with nearly complete conversion of PCE to ETH occurring within 4 days. The culture also had essentially ceased producing methane from methanol and, instead, the reducing equivalents from methanol which were not used for PCE reduction were used for acetogenesis.

More recent time course studies (22) have shown that the methanol-PCE culture was capable of reductive dechlorination of all chloroethenes. PCE was initially degraded quantitatively to VC. VC dechlorination to ETH did not usually commence until other chloroethenes were absent and VC dechlorination showed first-order kinetics. The culture also made small amounts of methane, usually after PCE depletion.

While methanol could serve as an electron donor for the culture and appeared to be especially well suited for sustained dechlorination, it was found in early studies that glucose, formate, and H_2 could also serve as electron donors (12). Studies with other cultures have shown that H_2 (16), fatty acids (14), and even toluene (21) could stimulate PCE dechlorination. We suspected that some of these donors were serving as sources of a more universal electron donor, H_2. We found that for the methanol-PCE culture, H_2 could readily replace methanol for several feedings (8), although eventually performance faltered. Good performance was restored by resuspension of the H_2-PCE culture in the supernatant from the methanol-PCE culture, suggesting that the latter contained growth factors required for dechlorination. The eubacterial cell wall synthesis inhibitor vancomycin inhibited PCE dechlorination and acetogenesis from methanol, but PCE dechlorination from H_2 was

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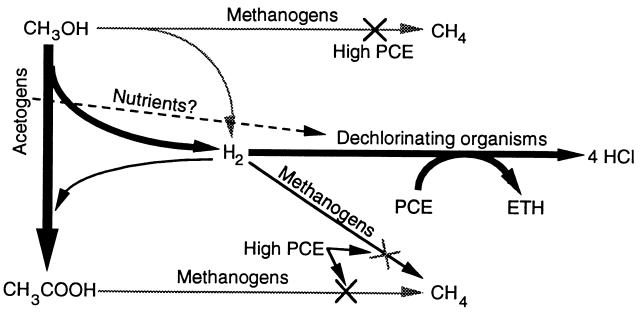

FIG. 1. Model, based on that of DiStefano et al. (8), for carbon and electron flow in a methanol-PCE anaerobic mixed culture.

not affected. This suggested that the PCE dechlorinator was resistant to vancomycin and that it could not use methanol directly but rather required methanol metabolism, most likely to provide H_2 or formate as the electron donor for reductive dechlorination.

Figure 1 shows a model of the metabolism of methanol and PCE by the dechlorinating culture which includes the hypotheses that H_2 is the actual electron donor for dechlorination and that a nutritional contribution is made to PCE dechlorinators by methanol-metabolizing organisms (6, 8). This model suggests that a better-defined culture on H_2 and PCE could be obtained if its nutritional needs were met. In this report, we examine the microbial populations in the methanol-PCE culture and describe the characterization of an H_2-PCE culture derived from a 10^{-6} dilution of the methanol-PCE culture.

MATERIALS AND METHODS

Chemicals. High-performance liquid chromatography-grade PCE (Aldrich Chemical Co., Inc., Milwaukee, Wis.) and methanol (Fisher Scientific, Springfield, N.J.) were used as culture substrates. H_2 was purchased from Empire Airgas, Inc., Elmira, N.Y. VC was obtained as a gas from Matheson Gas Products, Inc., Secaucus, N.J. Other chloroethenes were obtained from Aldrich Chemical Co. Ethane, ethene, and methane were purchased from Supelco, Bellefonte, Pa. All of the other chemicals used were reagent grade or better.

Analyses. For qualitative analysis of ethenes in most-probable-number (MPN) tubes, a model 1400 flame ionization detector-gas chromatograph (GC) (Varian, Walnut Grove, Calif.) was used with a stainless steel column (2 m by 3 mm) packed with 60/80 mesh Carbopak B–1% SP-1000 (Supelco) and operated isothermally at 200°C as described previously (22).

For quantitative analysis of chloroethenes and ETH, samples were analyzed with a model 8610 GC (SRI Instruments, Las Vegas, Nev.) equipped with a flame ionization detector and using the same column and operating conditions as described above. Peak areas were calculated by using the Peaksimple 2 software supplied with the GC and were compared to standard curves for chloroethenes (15). When we added 5 μmol of PCE to a 27-ml crimp-top tube containing 10 ml of growth medium, the nominal concentration was 0.5 mmol/liter, while the estimated aqueous concentration (15) was 0.16 mmol/liter.

When methane and ETH were produced together, they were measured with a Varian 1400 GC using a Poropak R column as described previously (19). The detection limit for methane was ca. 0.1 Pa, or 1 nmol per tube. H_2 was analyzed by gas chromatography with an AGC 110 thermal conductivity GC (Hach Carle, Lehigh Valley, Pa.) using N_2 as the carrier gas as described previously (28), and the detection limit for H_2 was ca. 2 Pa. Headspace samples were 100 μl in all cases. Acetate concentrations were quantified by high-performance liquid chromatography with a fast acid analysis column (Bio-Rad, Richmond, Calif.) and a solvent of 6.5 mM H_2SO_4 as previously described (31). Total organic carbon was measured with a model 5010 organic carbon analyzer (UIC Inc., Joliet, Ill.).

Growth medium and culture conditions. Methanol-PCE cultures were incubated in the growth medium and under conditions described previously (22). Unless stated otherwise, the basal medium contained the following (final concentration in grams per liter): NH_4Cl, 0.5; K_2HPO_4, 0.4; $MgCl_2 \cdot 6H_2O$, 0.1; $CaCl_2 \cdot 2H_2O$, 0.05; resazurin, 0.001; and 10 ml of a trace metal solution (27) per liter modified by addition of 0.01 g of $NiCl_3 \cdot 6H_2O$ per liter. The medium was purged with N_2 and dispensed inside an anaerobic chamber into 27-ml crimp-top tubes (Bellco, Vineland, N.J.). The tubes were then sealed with aluminum-crimp Teflon-coated butyl rubber stoppers (Wheaton, Millville, N.J.) and autoclaved at 121°C for 55 min. The tube headspaces were flushed with sterile 70% N_2–30% CO_2 (Matheson Gas Products, Inc., Secaucus, N.J.) and received the following sterile and anaerobic additions before inoculation (final medium volume, ≈10 ml per tube): $Na_2S \cdot 9H_2O$, 2 mM; $NaHCO_3$, 12 mM; filter-sterilized anaerobic digestor sludge supernatant (SS), 25% (vol/vol); yeast extract (YE), 0.2 g/liter, or sodium acetate, 2 mM; vitamin solution (2) concentrated 10-fold, 0.5% (vol/vol).

The anaerobic sewage digestor sludge for preparation of SS was obtained from the Ithaca Area Wastewater Treatment Facility. Several liters were collected at one time, to ensure uniformity. The sludge was initially clarified by centrifugation for 30 min at 8,000 rpm (10,400 × *g*) with a Sorvall RC-2B centrifuge (DuPont-Sorvall, Wilmington, Del.). Samples were stored frozen at −20°C until use. Thawed samples were then centrifuged for 30 min at 18,000 rpm (39,000 × *g*). The resulting liquid was neutralized to pH 7.0 with 1 M HCl and then prefiltered through a 0.8-μm-pore-size Supor-800 membrane filter (Gelman Sciences, Ann Arbor, Mich.). The SS was then purged with N_2 and filter sterilized in an anaerobic chamber through a double 0.8-0.2-μm-pore-size Acrodisc PF filter (Gelman) into sterile vials. In some instances, the SS was lyophilized in a Labconco LYPH · LOCK 4.5-liter Freeze Dry system, model 77510 (Labconco Corporation, Kansas City, Mo.). Preliminary tests showed that lyophilized SS performed as well as SS. The SS contained 75 mg of organic carbon per liter and ≤0.05 mM acetate or other volatile fatty acids.

Unless otherwise stated, the inoculum size was 2% (vol/vol), all incubations were done in duplicate, and each experiment described was performed at least twice with similar results. Duplicate tubes performed very similarly in the experiments described (less than 5% difference in results for tubes under the same conditions), so the results are presented for individual tubes. Cultures that used H_2 as an electron donor were incubated upside down in the dark in an R76 incubator-shaker (New Brunswick Scientific, Edison, N.J.) operated at 35°C and 150 rpm. H_2 was added to the headspace as overpressure (0.67 atm, 67 kPa, or ca. 47.5 mmol/liter) immediately after inoculation. After several doses of PCE were consumed, $NaHCO_3$ was added to neutralize the HCl produced by the dechlorination process (22) and H_2 was added to replenish the headspace. The quantity of PCE added to a culture tube was estimated directly from the delivered syringe volume (7).

MPN determinations of the methanol-PCE culture. Three-tube MPN determinations were performed for a variety of microbial groups present in the methanol-PCE culture. All tubes contained the basal medium described above.

A master set of dilutions was made in 160-ml serum vials containing 45 ml of basal medium, which was used for all subsequent dilutions. H_2-CO_2-utilizing methanogens and acetogens were enumerated by using medium to which H_2 was added at 130 kPa (ca. 90 mmol/liter) after inoculation. Methanol-utilizing methanogens and acetogens were enumerated by using medium to which 24 mM methanol was added, while 40 mM Na acetate was used to culture acetate-utilizing methanogens. Sulfidogens (e.g., sulfate reducers) were enumerated by using medium which received 5 mM Na acetate, H_2 at 130 kPa, and 10 mM $Na_2S_2O_3$, which is used by more sulfidogens than sulfate (26). Fermentative heterotrophs were enumerated in medium to which an additional 1 g of YE per liter and 0.25 g each of glucose, cellobiose, xylose, and sucrose per liter were added. Tubes were considered positive when significantly greater turbidity was observed than in control dilutions lacking these additions and when the appropriate product (methane or acetate) was detected.

Methanol-utilizing PCE dechlorinators were enumerated in medium to which 12 mM methanol had been added together with the equivalent of 0.2 mmol of PCE per liter. Cultures were considered positive if they produced greater-than-trace quantities of reduction products (generally, VC and ETH were the only products detected). H_2-utilizing PCE dechlorinators were enumerated and assayed in a similar manner, except that H_2 at 130 kPa replaced methanol as the electron donor.

Microscopy. A Zeiss Standard 18 microscope was used for phase-contrast observation of cells and for epifluorescence microscopy to detect organisms with the cofactor F_{420} autofluorescence typical of most methanogens as described previously (29). For photomicroscopy, cells were spread out on an agar-coated slide and stained with 0.01% acridine orange. The cells were observed with a Zeiss LSM-10 laser scanning microscope with either phase-contrast or epifluorescence (488 nm) imaging.

RESULTS

Viable counts of microbial populations in the methanol-PCE culture. We used the MPN technique to estimate the numbers of members of various microbial groups in the methanol-PCE culture (Table 1). Neither methanol- nor acetate-utilizing methanogens were detected in the culture. Relatively few H_2-CO_2-utilizing methanogens were detected, and microscopic examination of the highest positive dilution tubes showed spirals with the distinctive morphology of *Methanospirillum* sp., a methanogen capable of using either H_2-CO_2 or formate (2). High numbers of methanol-utilizing acetogens were found. A preliminary characterization of these chain-forming cocci indicated that they stained gram positive, re-

TABLE 1. MPN determinations for various microbial populations in a methanol-PCE culture

Organism type	MPN (ml^{-1})	Time (wks) until highest dilution was positive	Predominant morphology in highest positive dilution
H_2-CO_2-utilizing methanogens	2.3×10^4	5	Long spirals
Methanol-utilizing methanogens	≤ 2.3		
Acetate-utilizing methanogens	≤ 2.3		
H_2-CO_2-utilizing acetogens	$\leq 2.3 \times 10^4$		
Methanol-utilizing acetogens	9.3×10^6	2	Cocci in chains and doublets
H_2-utilizing thiosulfate reducers	9.3×10^5	2	Small spirals
Fermentative heterotrophs	2.3×10^7	2	Various rods
Methanol-utilizing PCE dechlorinators	2.3×10^3	5	
H_2-utilizing PCE dechlorinators	2.3×10^6	5	Small and large rods, irregular cocci, some small spirals

quired SS for growth, could not use H_2-CO_2 for growth, and did not significantly dechlorinate PCE by using either methanol or H_2 as the electron donor (23). The numbers of H_2-CO_2-utilizing acetogens were lower than those of methanogens. There were relatively high counts of sulfidogens in the culture, and the predominant morphotype in the highest positive dilution tubes resembled desulfovibrios. The highest MPN counts were for fermentative heterotrophs capable of growing in medium containing YE and sugars.

In MPN dilution tubes receiving methanol and PCE, the highest dilution which showed products of reductive dechlorination was 10^{-3}, in which VC was detected within 3 weeks. There was growth in the methanol-PCE tubes to a dilution of 10^{-7}, and chain-forming cocci similar to those seen in tubes receiving methanol without PCE were found in these tubes.

In MPN dilution tubes receiving H_2 and PCE, serial dilutions became positive for VC production sequentially until, after 5 weeks of incubation, two of three 10^{-6} dilution tubes were positive for VC production, with the third tube becoming positive approximately 1 week later. Although analysis of PCE and its products was qualitative, VC was the only daughter product initially detected in positive tubes until PCE was depleted, at which time ETH was detected. Microscopic examination of these cultures revealed small and large rods, individual irregular cocci, and *Desulfovibrio*-like spirals. These 10^{-6} dilution tubes consumed several subsequent doses of PCE, and transfers from these cultures were the source of the purified H_2-PCE cultures described in the rest of this communication.

PCE utilization by the purified H_2-PCE culture. Figure 2 shows that after the H_2-PCE culture was transferred into fresh medium, the rate of PCE utilization increased over time, indicating growth of the PCE-dechlorinating organisms. The major dechlorination products detected were VC and ETH, which were produced nearly stoichiometrically from PCE (there was always some loss of chloroethenes into or through the stoppers). By day 6, the culture was able to consume the equivalent of 1 mmol of added PCE per liter of culture medium in 15 h. Table 2 shows that cultures not provided with H_2 did not dechlorinate PCE and that methanol did not serve as an electron donor for PCE dechlorination. In cultures provided with PCE, there was greater disappearance of H_2 than in cultures lacking PCE or an inoculum, and the net amount consumed, 4.7 mmol/liter, was approximately equal to the amount required to reduce 1.66 mmol of PCE per liter to VC (4.9 mmol/liter). Moreover, cultures provided with H_2 or methanol in the presence or absence of PCE produced neither methane (data not shown) nor acetate (Table 2), indicating the complete absence of methanogens and acetogens, while in dechlorinating cultures the initial acetate concentration decreased as PCE was being consumed.

Nutritional characterization of the H_2-PCE culture. The H_2-PCE culture could be transferred indefinitely in medium containing 0.2 g of YE per liter, 25% (vol/vol) SS, and a vitamin solution as nutritional supplements. It was desirable to replace YE with nutrients which would support less growth of contaminants while still supporting PCE dechlorination.

Our initial studies showed that deleting YE from the growth medium did not allow sustained PCE dechlorination (data not shown). Since it was possible that YE or some product derived from it was serving as a carbon source, we tested whether YE could be replaced with acetate, a common carbon source that is used by many anaerobes but is not utilizable as an energy source by most anaerobes. Figure 3 shows that acetate greatly stimulated dechlorination by the H_2-PCE culture when YE was not present. Formation of dechlorination products (almost completely VC and ETH) essentially stopped after day 5 in cultures grown without acetate, while they continued to increase rapidly when 2 mM Na acetate was present. Acetate concentrations higher than 2 mM were not more stimulatory (data not shown). Cultures could be transferred indefinitely with 2 mM Na acetate (replacing YE), 25% (vol/vol) SS, and vitamins. The MPN of YE-utilizing fermentative heterotrophs in this medium was 2.3×10^6, a 10-fold reduction compared with cultures receiving YE, such as the methanol-PCE culture (Table 1).

TABLE 2. Effects of electron donors on PCE dechlorination (mainly to VC and ETH) and acetogenesis by a purified H_2-PCE culture

Addition	Product concn (mmol/liter)[a] on day 14	H_2 decrease (%)[b] on day 14	Acetate concn (mM)[c]	
			Day 1	Day 14
H_2 + PCE	1.66	10.1	1.97	1.60[d]
Methanol + PCE	0	NA[e]	1.94	2.03
No electron donor + PCE	0	NA	2.04	2.06
No electron donor (no PCE)	NA	NA	1.97	1.98
H_2 (no PCE)	NA	3.3	2.06	1.94
Methanol (no PCE)	NA	NA	1.97	1.97
H_2 (no PCE, no inoculum)	NA	3.2	2.07	2.06

[a] Values obtained from PCE dechlorination to almost completely ETH and VC.

[b] Percentage obtained from the mean of duplicate tubes (sampling headspace). Standard deviations were 1.5 for the H_2-PCE cultures and 1.0 for the H_2-no PCE and H_2-no PCE-no inoculum cultures.

[c] Mean concentration of duplicate tubes (sampling liquid phase). Standard deviations were 0.3 for the H_2-PCE cultures and 0 to 0.1 for the rest of the cultures.

[d] Value measured after 6 days, when 0.23 mmol of the products per liter had been produced.

[e] NA, not applicable.

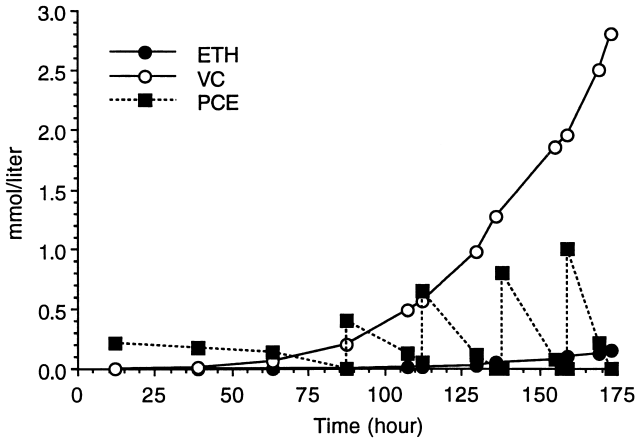

FIG. 2. PCE dechlorination and product formation by a purified H_2-PCE culture to which consecutively increasing PCE doses were added. The basal medium was amended with 25% (vol/vol) SS, a vitamin solution, and 2 mM Na acetate. The inoculum size was 2% (vol/vol).

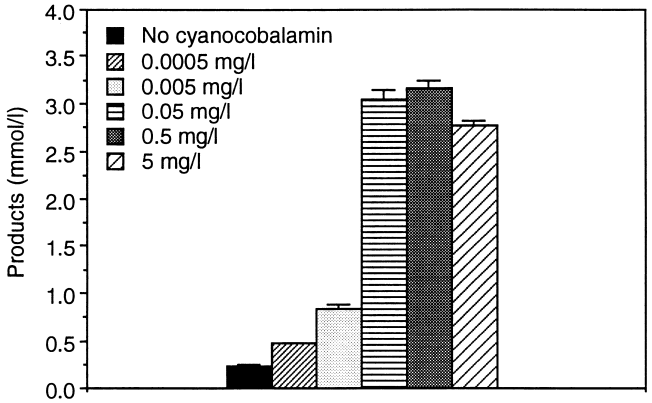

FIG. 4. PCE dechlorination products as measured after 11 days from inoculation (1% [vol/vol]) of the purified H_2-PCE culture in tubes containing different concentrations of vitamin B_{12}. The basal medium was amended with 25% (vol/vol) SS and 2 mM Na acetate.

The potential requirements for vitamins and SS were also examined for the H_2-PCE culture. Previous experiments showed that there was significant limitation of dechlorination in the cultures not receiving vitamins compared with the positive control cultures (data not shown). To identify which vitamins were required, an experiment was performed in which 1 of the 10 vitamins present in the standard solution was deleted from each set of duplicate tubes. It was found that cultures with vitamin B_{12} deleted showed dechlorination as poor as that of cultures receiving no vitamins, while deletion of the other vitamins had negligible effects on product formation (data not shown). The requirement for vitamin B_{12} was then examined more closely. Figure 4 shows the effect of adding various amounts of vitamin B_{12} on product formation (mainly VC and ETH) by the culture after 11 days of incubation (no other vitamins were added). Increasing amounts of vitamin B_{12} led to increasing product formation, although the response was not linear. Saturation occurred near 0.05 mg of vitamin B_{12} per liter, at which concentration the cultures performed as well as those receiving all 10 vitamins. In uninoculated cultures, vitamin B_{12} added to concentrations of up to 5 mg/liter did not catalyze measurable reductive dechlorination of PCE (data not shown).

When SS was not added to the growth medium, the culture did not consume the first dose of PCE within 14 days (Fig. 5), suggesting nutrient limitation. Adding the equivalent of 5% (vol/vol) SS allowed the consumption of four increasing doses of PCE, with little accumulation of ETH and significant accumulation of DCEs and TCE between feedings. Adding the equivalent of 25% (vol/vol) SS, the standard dose used in other experiments, allowed the consumption of multiple and increasing doses of PCE, although there were signs of limitation (residual TCE and PCE) after the last dose. Addition of 50% (vol/vol) SS allowed the best consumption of PCE, with the greatest ETH accumulation, while cultures amended with 100% (vol/vol) SS did not perform as well, showing a decrease in the rate of PCE degradation at the end of the incubation.

Microscopic observations of the methanol-PCE culture and the purified H_2-PCE culture. In the purified H_2-PCE culture grown in medium with acetate, SS, and vitamins, the two major morphotypes were irregular cocci and small rods (Fig. 6). Larger rods were also readily observed but were far less nu-

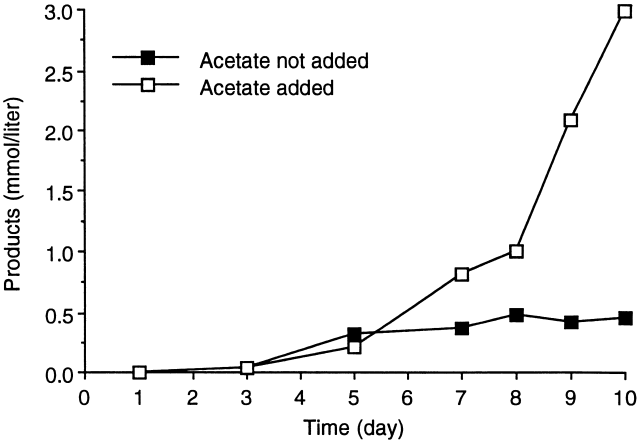

FIG. 3. Product formation (predominantly VC and ETH) from PCE dechlorination by a purified H_2-PCE culture in the presence or absence of 2 mM Na acetate. The basal medium was amended with 25% (vol/vol) SS and vitamins, and the inoculum was 1% (vol/vol).

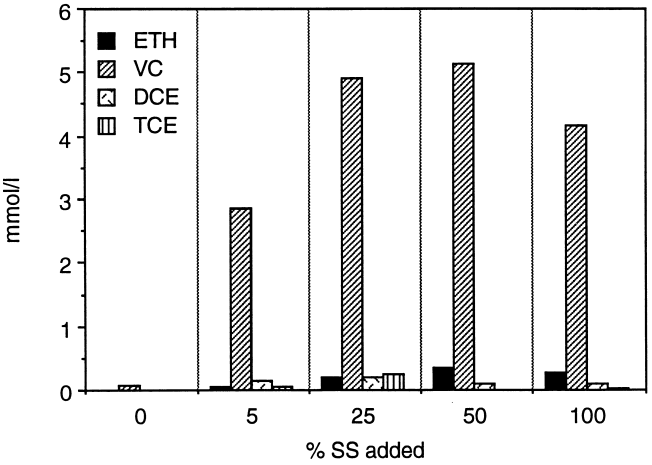

FIG. 5. PCE dechlorination products as measured after 14 days from inoculation (2% [vol/vol]) of the purified H_2-PCE culture. Tubes were amended with different percentages of lyophilized SS and were given new PCE doses as soon as PCE was consumed. The basal medium was amended with vitamins and 2 mM Na acetate.

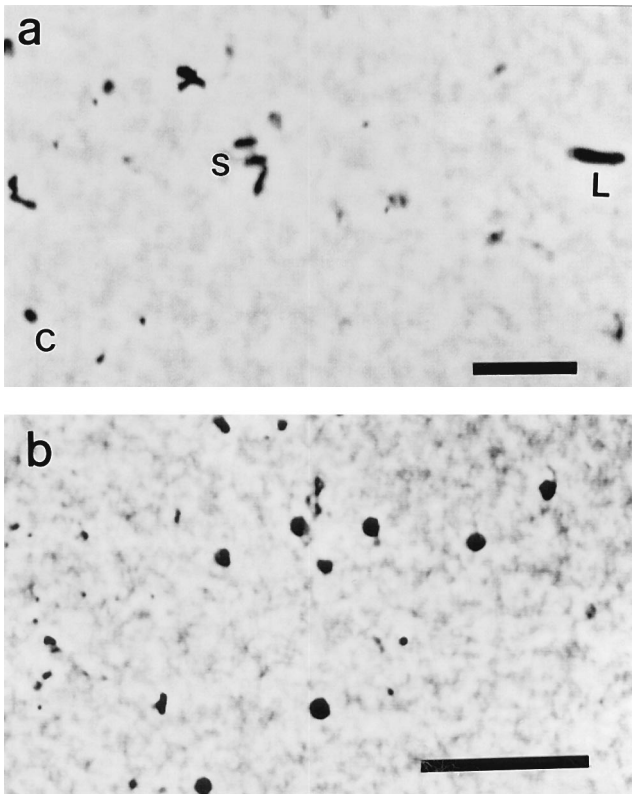

FIG. 6. (a) Photomicrograph of a purified H_2-PCE culture concentrated 25-fold showing short rods (S), a long rod (L), and irregular cocci (C). (b) Higher-magnification photomicrograph of the concentrated H_2-PCE culture showing a microscopic field containing many irregular cocci. The microscopy conditions were adjusted to enhance the contrast between the cocci and the background. Bars, 5 μm.

merous than the previously mentioned morphotypes. The irregular cocci were absent from cultures which did not show PCE dechlorination (data not shown), suggesting their involvement in that process, although more conclusive evidence is needed before the process can be ascribed to any one organism.

DISCUSSION

The results presented here are consistent with the primary prediction of the model of DiStefano et al. (8), in which H_2 (or possibly formate) is the primary electron donor for reductive dechlorination in the methanol-PCE culture (Fig. 1). Specifically, the prediction was borne out that there should be relatively high numbers of methanol-utilizing acetogens and H_2-utilizing PCE dechlorinators in the culture. Indeed, when tested after several transfers, the H_2-PCE culture derived from a 10^{-6} dilution of the methanol-PCE culture was no longer capable of using methanol as an electron donor for dechlorination or for acetogenesis (Table 2). Presumably, the methanol-utilizing acetogens were initially present in the 10^{-6} H_2-PCE dilutions, since they were present in the original methanol-PCE culture at numbers exceeding 10^7/ml, but were lost after several transfers in medium lacking a substrate for their growth. Acetate, which was present in the medium at 2 mM, also could not serve as an electron donor for dechlorination (Table 2).

The model also predicts low numbers of methanogens in the methanol-PCE culture, and only few H_2-CO_2-utilizing methanogens were detected (Table 1). This is in contrast to earlier studies on the methanol-PCE culture, in which the PCE dose was much lower and the culture was actively methanogenic (12) and we found over 10^7 cells of a methanol-utilizing methanogen resembling *Methanosarcina* sp. per ml (30). The lack of evidence of the presence of methanogens and acetogens in the purified H_2-PCE culture indicates that these organisms were not primarily responsible for the high-rate PCE dechlorination in the original methanol-PCE culture.

H_2 was able to serve indefinitely as the electron donor for reductive dechlorination of PCE to VC and ETH in cultures provided with proper nutritional supplements, in agreement with previous results (8). The degradation of PCE by the H_2-PCE culture at an increasing rate, as well as our ability to transfer it indefinitely, is indicative of growth of the culture concurrent with the dechlorination process. H_2 and PCE have been shown to serve as an electron donor-acceptor pair for the growth of strain PER-K23 (16) and *D. multivorans* (20) cultures, but in those cases, reductive dechlorination stopped at *cis*-DCE. No evidence of DCE accumulation in this culture was obtained, and neither of the two dominant morphotypes in the purified H_2-PCE culture, when examined microscopically, resembled strain PER-K23 or *D. multivorans*, so it is likely that a different organism is responsible for PCE dechlorination in this culture.

That a 10^{-6} dilution of the methanol-PCE culture on H_2-PCE required 35 days (Table 1) to accomplish approximately 20 doublings ($2^{20} \approx 10^6$) suggests a doubling time of less than 2 days for the H_2-PCE culture, which is consistent with the increasing rate of metabolism in Fig. 2.

The purified H_2-PCE culture was able to convert PCE to ETH, although the ratio of ETH/VC produced from PCE was much lower than that described for the methanol-PCE culture (7). This is at least partially due to the feeding regimen we used for the H_2-PCE culture in these experiments, in which we provided a dose of PCE as soon as its depletion was detected. Since PCE can inhibit VC dechlorination (22), the cultures had little opportunity to accumulate ETH.

The purified H_2-PCE culture could be transferred indefinitely in growth medium supplemented with acetate, SS, and vitamins. The acetate most likely serves as a carbon source for the culture and, as such, supports considerably less growth of potential contaminants than does YE. The optimal amount of SS required was 25 to 50% (vol/vol). It is not clear whether the SS directly provides nutrients required by PCE dechlorinators or whether products of metabolism of SS components by other organisms are required. The SS contributed less than 20 mg of carbon per liter to the culture when added at 25% (vol/vol), and since some portion of it is not readily catabolizable, it should support only a low number of contaminants. The amount of acetate provided by 25% (vol/vol) SS, <0.05 mM, was apparently too low to support the growth of dechlorinators.

The vitamin requirement for PCE dechlorination could be met by adding vitamin B_{12} alone, but since we also needed to add SS in these experiments, it is possible that the SS was providing sufficient quantities of other required vitamins. The vitamin B_{12} requirement is intriguing because it and other corrinoid compounds have been shown to carry out reductive dechlorination of chloro-organics, including chloroethenes, in vitro (13, 17, 20). We verified that no reductive dechlorination of PCE occurred in uninoculated medium supplemented with vitamin B_{12}. Still, the requirement for vitamin B_{12} suggests a role in dechlorination in this organism, perhaps as a prosthetic group of a dechlorinating enzyme. The amount required, between 0.005 and 0.05 mg/liter, is considerably greater than the amount (0.001 mg/liter) typically supplied to organisms which

use vitamin B_{12} for anabolic reactions (3). The vitamin B_{12} requirement may explain why methanol was the electron donor which led to the best sustained dechlorination in the early studies on this culture (7, 12). Those cultures were amended with YE, which lacks vitamin B_{12} (3). However, methylotrophic methanogens (5) and acetogens (18) growing on methanol are rich in cobamides, which could be cross-fed to the dechlorinating organisms by lysis and possibly excretion. For example, a *Methanosarcina* culture grown on acetate supported the growth of a vitamin B_{12}-requiring contaminant (25).

These results show that like many other anaerobes, the PCE dechlorinators in this culture are dependent on other organisms for several of their nutritional requirements. Knowledge of some of these requirements has allowed us to simplify the culture medium we use for them, and it is hoped that further unraveling of these requirements will lead to isolation of PCE dechlorinators by providing the nutrients they require to grow axenically without supporting significant growth of contaminants. Knowledge of the nutrition of PCE dechlorinators can also have practical significance. Fennell and Gossett (11) have found in bioreactor studies that the methanol-PCE culture could be switched to butyrate as an electron donor, but only when vitamins, including vitamin B_{12}, were added. Thus, addition of a simple vitamin solution has allowed greater flexibility in electron donor use by a PCE-dechlorinating mixed culture.

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