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Reductive Dechlorination of High Concentrations of Tetrachloroethene to Ethene by an Anaerobic Enrichment Culture in the Absence of Methanogenesis

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Tetrachloroethene, also known as perchloroethylene (PCE), is a common groundwater contaminant throughout the United States. The incomplete reductive dechlorination of PCE—resulting in accumulations of trichloroethene, dichloroethene isomers, and/or vinyl chloride—has been observed by many investigators in a wide variety of methanogenic environments. Previous mixed-culture studies have demonstrated that complete dechlorination to ethene is possible, although the final dechlorination step from vinyl chloride to ethene is rate limiting, with significant levels of vinyl chloride typically persisting. In this study, anaerobic methanol-PCE enrichment cultures which proved capable of dechlorinating high concentrations PCE to ethene were developed. Added concentrations of PCE as high as 550 μM (91-mg/liter nominal concentration; approximately 55-mg/liter actual aqueous concentration) were routinely dechlorinated to 80% ethene and 20% vinyl chloride within 2 days at 35°C. The methanol level used was approximately twice that needed for complete dechlorination of PCE to ethene. The observed transformations occurred in the absence of methanogenesis, which was apparently inhibited by the high concentrations of PCE. When incubation was allowed to proceed for as long as 4 days, virtually complete conversion of PCE to ethene resulted, with less than 1% persisting as vinyl chloride. An electron balance demonstrated that methanol consumption was completely accounted for by dechlorination (31%) and acetate production (69%). The high volumetric rates of PCE dechlorination (up to 275 μmol/liter/day) and the relatively large fraction (ca. one-third) of the supplied electron donor used for dechlorination suggest that reductive dechlorination could be exploited for bioremediation of PCE-contaminated sites.

Tetrachloroethene, also known as perchloroethylene (PCE), is a solvent commonly used for the dry cleaning of garments and other purposes. It has been estimated that 6.4×10^9 kg of PCE were synthesized and distributed in the United States between 1945 and 1984, with some significant fraction entering the ground because of improper handling, storage, or disposal (1). PCE has thus become a common constituent at contaminated groundwater sites throughout the United States (28) and is currently listed as a volatile organic contaminant under the Safe Drinking Water Act amendments (1986), with a proposed maximum contaminant level in drinking water of 5 μg/liter (12). Because of its highly oxidized nature, there is little evidence for aerobic breakdown of PCE (13, 19, 26). However, subsurface biological action has been observed to transform PCE by a reductive dechlorination mechanism (22).

The incomplete reductive dechlorination of PCE under anaerobic conditions—resulting in accumulations of trichloroethene (TCE), dichloroethene (DCE) isomers, and/or vinyl chloride (VC)—has been repeatedly observed in laboratory-scale batch and continuous-flow systems (2–4, 14, 15, 27), natural environments (22), and soil microcosms (20, 21). However, these lesser chlorinated ethenes also pose a threat to public health and are regulated under the Safe Drinking Water Act amendments. Complete dechlorination to ethene (ETH) is possible, as demonstrated in previous studies conducted with methanol-PCE methanogenic enrichment cultures (14). However, the final dechlorination step (VC to ETH) appeared to be rate limiting, and significant amounts of VC persisted.

In all of the above-cited studies, the flow of carbon and electrons was primarily to methane, but the role of methanogens in reductive dechlorination is unclear. Fathepure et al. (9–11) reported the transformation of PCE to TCE by pure methanogenic cultures and observed a stoichiometric relationship between methanogenesis and dechlorination. They proposed that electrons transferred during methanogenesis are diverted to PCE by a reduced electron carrier involved in methane production. Reduction beyond TCE was not detected.

In this paper, we report the complete dechlorination of high concentrations (550 μM or 91 mg/liter) of PCE to ETH under anaerobic conditions, with virtually no residual VC. Further, the observed transformations occurred in the absence of methanogenesis. An electron balance is presented as an aid to understanding the important metabolic pathways in this mixed anaerobic culture.

MATERIALS AND METHODS

Chemicals. PCE, TCE, *trans*-1,2-DCE, *cis*-1,2-DCE, and 1,1-DCE were obtained in neat liquid form (1- or 5-g ampoules; Supelco, Inc.) for use as analytical standards. High-pressure liquid chromatography (HPLC)-grade PCE (≥99.9% pure; Aldrich Chemical Co.) and HPLC-grade methanol (99.9% pure; Fisher Scientific) were used as culture substrates. VC, ETH, and methane were obtained as gases (≥99% pure; Scott Specialty Gases). Hydrogen (1% in

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N_2) was purchased from Airco, Inc. Dimethyl sulfide (≥99% pure) was obtained from Aldrich Chemical Co.

Cultures and enrichment procedures. The starting point for our enrichment studies was a methanol-PCE methanogenic culture developed and described previously (14). Maintenance and subculturing operations were performed as described earlier, with a basal medium which contained (among many other constituents) Na_2S and $FeCl_2$ as reductants, bicarbonate as a pH buffer, and 100 mg of yeast extract per liter (14). The cultures were 100-ml suspensions in 160-ml serum bottles, incubated quiescently at 35°C in an inverted position. Teflon-lined natural-rubber septa were kept in place with aluminum crimp caps to maintain anaerobic conditions. The integrity of the septum-seal system with regard to retaining PCE has been demonstrated in previous studies, as has the role of microbial (versus abiotic) activity in mediating the observed reductive dechlorination of PCE (14).

At the outset, duplicate cultures for this investigation were maintained on a semicontinuous basis, with wasting and feeding every 2 days as follows. After headspace analysis was performed, 2 ml of culture was removed and 2 ml of basal medium (containing 100 mg of yeast extract per liter) was added to maintain a liquid volume of 100 ml. The retention time was thus 100 days. Methanol (32 μmol per bottle or 1 mg) was added from an aqueous stock solution. At the beginning of our studies, PCE (0.35 μmol per bottle or 0.06 mg) was added from distilled-deionized water stock solutions which had been saturated with PCE. With each wasting and feeding event, added concentrations were therefore 320 μM methanol, 2 mg of yeast extract per liter, and 3.5 μM PCE (a nominal concentration arrived at by ignoring headspace-liquid partitioning within bottles; in a 160-ml serum bottle containing 100 ml of liquid at 35°C, the PCE concentration expected after phase equilibration would have been approximately 60% of the nominal, added concentration [16]). PCE additions were normally verified by headspace analysis after 20 min of equilibration in a water bath at 35°C. Such verification of initial headspace concentrations also provided evidence that sorption and solution conditions exerted negligible effects on gas-liquid partitioning. The cultures were maintained in this manner for 115 days.

The PCE dose accompanying each wasting and feeding operation was increased incrementally from 0.35 to 55 μmol per bottle (91-mg/liter nominal concentration) over a 42-day period. When dechlorinating capability was demonstrated at a particular dose level, the PCE dose was subsequently increased. Prior to each PCE addition, the bottles were thoroughly purged via a cannula with 70% N_2–30% CO_2. The intent of purging was to prevent potential inhibition of the cultures because of accumulations of VC and/or ETH, which is a known inhibitor of methanogenesis (23). Methanol and yeast extract continued to be added with each PCE addition at each wasting and feeding event. The methanol dose was increased from 32 to 160 μmol after 10 days to ensure an excess of reducing equivalents for reductive dechlorination of ever-increasing PCE additions.

As the PCE dose increased, the use of an aqueous PCE stock solution required a prohibitively large volumetric addition. Neat PCE was dissolved in methanol and used as a stock feed beginning on day 130. Eventually, at even higher PCE doses, PCE was directly added in neat form to the serum bottle cultures. At this point, since volumetric additions became negligible, culture wasting to maintain a constant volume became unnecessary, and the cultures were operated in batch-fed mode, receiving 160 μmol of methanol and 2 mg of yeast extract (from a separate, aqueous stock) with each dose of PCE. (The yeast extract dose was increased to 2 mg at this point to be commensurate with the increases in methanol and PCE doses which had occurred since operations first began.) The quantity of PCE added in neat form to a culture bottle was estimated directly from the delivered syringe volume, rather than from postaddition headspace analysis.

At 177 days, 30 ml of culture was drawn from each duplicate to serve as an inoculum for a set of second-generation enrichments; the volume of each duplicate was restored with basal medium, and routine batch maintenance was resumed. Six second-generation bottles were prepared as follows. Basal medium (90 ml) (containing no yeast extract) was transferred anaerobically to six bottles with a Unispense-II pump (Wheaton Industries, Milleville, N.J.), and 10 ml of inoculum (5 ml from each first-generation culture) was added to each of the six bottles. A gas-tight syringe was used (Dynatech Precision, Inc., Baton Rouge, La.) to transfer the inocula. At start-up, the second-generation cultures received 160 μmol of methanol, 2 mg of yeast extract, and 55 μmol of PCE. Once PCE dechlorination was essentially complete, 10 ml of culture was removed and replaced with basal medium to facilitate enrichment of the dechlorinating culture; bottles were purged with 70% N_2–30% CO_2; and PCE (55 μmol), methanol (160 μmol), and yeast extract (2 mg) were again added. This wasting and feeding protocol continued over a 30-day period of operation. As operations continued, the interval between wasting and feeding operations declined to a minimum of 2 days.

Analysis of organic compounds and hydrogen. The total mass of each volatile organic compound (PCE, TCE, DCE isomers, VC, ETH, methane, and dimethyl sulfide) and hydrogen within a serum bottle culture was determined by a method involving a single 0.5-ml headspace gas injection (16) into a complex network involving the simultaneous use of two gas chromatographs (GCs; Perkin-Elmer model 8500), two analytical columns, two multiport air-actuated switching valves (Valco, Inc.), two flame ionization detectors (FIDs), and one reduction gas detector (Trace Analytical RGD). The GC to which injection was made was equipped with one FID and two columns—a stainless steel column (3.2 mm by 2.44 m) packed with 1% SP-1000 on 60/80 Carbopack-B (Supelco, Inc.) and a stainless steel column (3.2 mm by 3.2 m) packed with 100/120 Carbosieve G (Supelco, Inc.), with the two switching valves situated upstream and downstream of the Carbosieve G column. For 1.5 min after sample injection, the flow was routed from the injector through the Carbopack column to the Carbosieve column. Within that 1.5-min period, hydrogen, methane, and ETH passed through the Carbopack column, relatively unretained, into the Carbosieve column, in which they could be resolved. At 1.5 min, the Carbopack column effluent was redirected via switching valve 1 to the FID for eventual detection of VC, DCE isomers, TCE, PCE, and dimethyl sulfide. For the first 2 min following injection, effluent from the Carbosieve column was routed via switching valve 2 to the RGD for eventual hydrogen detection. At 2 min—just after hydrogen was detected, but before methane was eluted—the effluent from the Carbosieve column was redirected to the FID of the second GC for eventual detection of methane and ETH. Output from each FID was sent to separate calculating integrators and printers (Perkin-Elmer GP-100). Output from the RGD was sent to a third calculating integrator (Perkin-Elmer LCI 100). Helium served as the carrier gas (30 ml/min through each branch of the network at all times) for the

Carbopack and Carbosieve columns. The columns were in a single oven, which was temperature programmed as follows: 100°C for 4 min; increased at 20°C/min to 200°C; kept at 200°C for 8 min.

Initial identification and confirmation of volatile organic compounds were done previously (14). Coefficients of variation (standard deviation/mean × 100) for calibration factors (relating the total mass of each volatile compound in a bottle to the GC peak-area response) ranged from 0.59 to 2.8%.

Methanol and acetate analyses were also completed by a GC technique with a Perkin-Elmer model 8500 GC. A 0.5-μl aqueous injection was made to a Nukol fused-silica capillary column (0.53 mm by 15 m; Supelco, Inc.) connected to the FID. The oven temperature was programmed as follows: 100°C for 3 min; increased at 20°C/min to 160°C. Samples were filtered through a 0.45-μm-pore-size syringe filter (Gelman Sciences) and acidified with 2 N HCl prior to injection. Standard calibration curves were developed for methanol and acetate. Peak-area responses from subsequent samples were compared with the standard curve to obtain methanol and acetate concentrations.

RESULTS

First-generation enrichments. All findings reported here are limited to the period of time during which repetitive PCE additions to bottles containing 100-ml cultures were incrementally increased from 0.35 μmol per bottle and eventually kept at 55 μmol per bottle. Prior to this period, the duplicate 100-ml cultures were routinely given 0.35 μmol of PCE, 32 μmol of methanol, and 0.2 mg of yeast extract; the major product of dechlorination was VC, and a lesser amount of ETH was detected. Near-stoichiometric conversion of methanol to methane was noted, indicating that an insignificant fraction of the reducing equivalents supplied by methanol was being used for reductive dechlorination of such small amounts of PCE. These observations are consistent with those reported previously (14).

Throughout the course of this study, the duplicate cultures performed similarly. Figure 1 depicts the performance of one of the cultures over the 80-day period during which the PCE dose was incrementally increased from 0.35 to 55 μmol. Figure 1a compares cumulative PCE added (essentially equal to cumulative PCE consumed) with cumulative VC and ETH produced. Contributions from TCE and DCE isomers were relatively insignificant and have been omitted in the interest of clarity. From days 115 to 135, each PCE addition was routinely dechlorinated to about two-thirds VC and one-third ETH within the 2-day period allowed between each successive purging and feeding operation. Figure 1b compares cumulative methanol added (essentially equal to cumulative methanol consumed) with methane produced. Theoretical methane yield (based on the stoichiometric conversion of 1 mol of methanol to 0.75 mol of CH_4 and 0.25 mol of CO_2) is also included. From days 115 to 143, near-stoichiometric conversion of methanol to methane occurred. Apparently, little methanol was used for reductive dechlorination. This result was expected, since methanol was added in amounts (initially 32 μmol or 192 μeq; later 160 μmol or 960 μeq) greatly in excess of that required to result in the complete dechlorination of PCE (the dose of which was increased from 0.6 to 12 μmol—i.e., 4.8 to 96 μeq—over this period). Dechlorination performance and methanol conversion therefore did not change appreciably in comparison with initial conditions.

On day 145, the PCE dose was increased to 25 μmol per bottle, and methane production began to decline. On day 150, the PCE dose was increased to 55 μmol per bottle, and methane production essentially ceased. As shown in Fig. 1b, methane production was nil for the remainder of the study (days 150 to 195). Over this same period, PCE transformation was sustained in the absence of methanogenesis; in fact, the degree of VC conversion to ETH increased substantially. Figure 1c shows the distribution of dechlorination products from individual PCE additions over the course of the study. From approximately day 170 on, 80% of the added PCE appeared as ETH within 2 days.

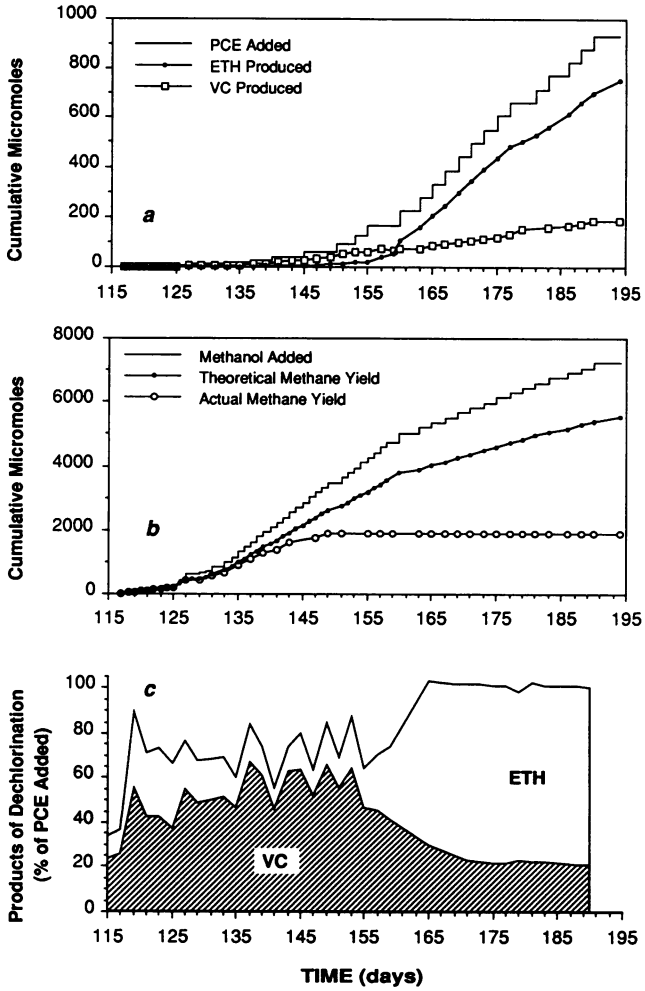

FIG. 1. (a) Cumulative PCE additions and cumulative VC and ETH production. (b) Cumulative methanol additions and cumulative methane production. (c) Trend observed over time in the proportions of VC and ETH produced from PCE within 2 days of PCE additions.

On occasion, periods longer than 2 days were allowed between successive PCE additions. Table 1 presents the distribution of PCE dechlorination products as a function of incubation time between PCE additions. Average values from duplicate bottles are reported. With 3 days of incubation, only 2 μmol of VC remained. Four days of incubation resulted in VC being less than 1% of total products. These observations demonstrate that nearly complete dechlorination of PCE to ETH is possible, with negligible residual VC.

Throughout the study, the major products of dechlorina-

TABLE 1. Proportions of ETH and VC produced from PCE with various incubation times following PCE addition[a]

Interval (days)	Incubation time (days)	PCE degraded (μmol/bottle)	Resulting μmol of the following/ bottle:	
			VC	ETH
165–167	2	55	10	47
183–186	3	55	2	58
190–194	4	55	0.4	56

[a] Values reported are the averages for duplicate bottles.

tion were ETH and VC, with only negligible quantities of TCE and DCE isomers sometimes being observed. The apparent agreement in Fig. 1a between cumulative PCE added and the summation of cumulative VC and ETH formed suggests that the formation of other compounds (such as CO_2) from PCE transformation by this culture must have been insignificant. Previous radiotracer studies performed on the parent culture with [^{14}C]PCE support this suggestion (14).

On day 143, a compound later identified as dimethyl sulfide was eluted from the Carbopack column at about 4.6 min into the headspace analysis. The dimethyl sulfide peak was observed in all subsequent headspace analyses. The production of this compound increased to approximately 1 to 2 μmol per feeding. This became a routine observation, for which no explanation is offered.

Electron balance. Six second-generation bottles were maintained for 25 to 30 days, each bottle receiving repetitive 55-μmol PCE additions, as described above. These second-generation cultures performed in a manner similar to that of the first-generation cultures, in that methane production was not detected. Dimethyl sulfide was observed from the outset, in quantities of up to 20 μmol per bottle within the first 10 days. Thereafter, dimethyl sulfide was routinely detected in quantities of 1 to 4 μmol per bottle. Typically, conversion of PCE to ETH did not occur to as great an extent within 2 days of PCE addition as was observed in first-generation cultures. For example, VC was sometimes present in amounts equal to or greater than those of ETH after 2 days of incubation. Minor amounts of PCE remained at times. The slower dechlorination rate may be attributed to a lower initial population of dechlorinating organisms. A 10% inoculum was used, and the culture dilution rate was up to five times that used in first-generation cultures. Second-generation cultures displayed complete dechlorination to ETH when allowed 4 days of incubation following a 55-μmol PCE addition.

An electron balance was determined for days 17 to 19 for the six bottles. To accomplish this, we performed methanol and acetate analyses, in addition to headspace analyses. Using methanol and PCE as starting compounds, we checked the balance by comparing the microequivalents of methanol consumed (i.e., the presumed, ultimate electron donor) with the sum of the microequivalents of reduced products formed.

The electron balance for one of the six replicate bottles is shown in Table 2. It is apparent that (i) the reducing equivalents available from methanol consumption could be reasonably accounted for and that (ii) about two-thirds of the reducing equivalents from methanol were used to produce acetate, with the remaining one-third being used in reductive dechlorination. Methanogenesis and hydrogen production were insignificant sinks for electrons. The apparent 8.5% discrepancy between reduced-product formation (1,068 μeq) and methanol consumption (984 μeq) is assumed to have resulted from the 2 mg of yeast extract added routinely with methanol.

The other five replicate bottles yielded similar results. The fraction of methanol equivalents used in dechlorination averaged 0.31 among all six bottles (standard deviation, 0.06). The remainder was accounted for via acetogenesis, with insignificant methane and hydrogen production. Among the six bottles, reduced-product formation exceeded methanol consumption by an average of 12%, suggesting a minor electron donor role for yeast extract.

Throughout these studies, bottles were purged thoroughly prior to each PCE addition to preclude potential toxicity from VC or ETH, which would otherwise accumulate. This protocol had been decided upon as strictly a precautionary measure—without any knowledge of the VC or ETH levels which might inhibit dechlorination. To assess the real need for the purging procedure, we undertook a short experiment at the conclusion of the electron balance assays. From 30 to 80 μmol of VC was added to two of the second-generation bottles in addition to the routine additions of PCE, methanol, and yeast extract over two 2-day incubation periods. From 30 to 60 μmol of ETH was added to a third bottle instead of VC. Subsequent headspace analyses indicated that up to 30 μmol of VC did not affect PCE dechlorination; however, PCE reduction was inhibited at VC levels of between 30 and 60 μmol. This observation confirmed the need for purging prior to each PCE addition.

TABLE 2. Electron balance for a single, 100-ml vial fed 160 μmol of methanol, 55 μmol of PCE, and 2 mg of yeast extract

Compound	Amt present (μmol) on day:		Difference (μmol)	Factor (eq/mol)	Methanol consumed (μeq)	Reduced product formed (μeq)[a]
	17	19				
Methanol	164	0	−164	6	984	
PCE	55	2.6	−52.4	0		
TCE	0	0.02	0.02	2		0.04
DCE isomers	0	0.09	0.09	4		0.36
VC	0	34.4	34.4	6		206
ETH	0	18.6	18.6	8		149
Acetate	511	600	89	8		712
H_2	0	<0.01	<0.01	2		<0.02
CH_4	0	0.027	0.027	8		0.22

[a] Total, 1,068 μeq.

ETH had no effect on PCE dechlorination in amounts of up to 60 μmol, the highest ETH level tested. Schink (23) reported that methanogenesis by sewage sludge and anaerobic sediments was markedly inhibited at aqueous ETH concentrations of greater than 36 μM. When partitioning between headspace and liquid is considered in our culture bottles (i.e., with 100 ml of liquid, 60 ml of headspace, and a dimensionless Henry's constant of 9.1 at 35°C, determined by the EPICS method [16]), an aqueous concentration of 36 μM corresponds to a total quantity of 23 μmol of ETH in a culture bottle. Results for first- and second-generation cultures showed that ETH levels reached 45 μmol routinely and occasionally exceeded 50 μmol. Thus, the inhibition of methanogenesis in our cultures could have resulted from high levels of PCE, its chlorinated products, and/or ETH.

DISCUSSION

The results of our experiments indicated that near-millimolar concentrations of PCE could be completely reduced to ETH with relatively little residual VC. This biological transformation was sustained for over 40 days in a first-generation, methanol-enriched culture in the absence of methanogenesis. Successful transfer and enrichment of a second-generation culture further supported the notion of sustenance.

These cultures began as predominantly methanogenic cultures, as evidenced by the initial stoichiometric conversion of methanol to methane. As the PCE dose was increased, methane production ceased and a shift in organism predominance apparently occurred. Presumably, methanogenesis was inhibited by the high concentration of PCE and/or its reduction products. Previous research has implicated methanogens as playing a role in PCE dechlorination (9–11, 14), but the evidence presented here for sustained PCE dechlorination in the absence of methanogenesis suggests a role for other organisms in these cultures. It is conceivable that methanogens were still the mediators of reductive dehalogenation in these cultures and that when methanogenesis was inhibited by high levels of PCE or its reduction products, electron flow in the methanogens was diverted toward reductive dechlorination. However, growth of methanogens in the absence of methanogenesis has never been reported (17). Also, preliminary microbiological studies of the cultures (29) were consistent with a drastic decrease in the number of methanol-utilizing methanogens after the PCE dose was increased.

On the basis of the electron balance, acetogenesis from methanol became the predominant pathway, and the role of acetogens in PCE dehalogenation must be considered. It is possible that an acetogenic organism(s) was directly responsible for the dechlorination. Organisms, such as acetogens, which possess the carbon monoxide-acetyl coenzyme A pathway and its variants (25) have been implicated in the dehalogenation of chlorinated methanes (8), for example. Dehalogenation has been attributed to the high levels of corrinoids involved in this pathway (8). Alternatively, acetogens may have simply arisen in response to the absence of methanogenic competition, while other organisms were responsible for PCE dehalogenation, either using methanol directly as an electron donor or perhaps using hydrogen produced by acetogens during methanol catabolism (5).

It is unknown whether PCE and its lesser chlorinated products serve as electron acceptors for energy conservation in these cultures, as has been demonstrated for anaerobic 3-chlorobenzoate dechlorination (7, 18). The fact that reductive dechlorination consumed one-third of the electrons derived from methanol suggests that dechlorination was more than a minor side reaction in the cultures. With ΔG_f^0 values from standard sources (6, 24), it can be calculated that CO_2 reduction to methane or acetate can provide approximately −34 or −26 kJ per electron pair, considered as H_2, respectively, while reductive dehalogenation of chloroethenes can provide −143 to −171 kJ/H_2. Thus, chloroethenes are potentially more thermodynamically favorable electron acceptors than is CO_2.

The high volumetric rates of PCE dechlorination (up to 275 μmol/liter/day) and the relatively large fraction (ca. one-third) of the supplied electron donor used for dechlorination suggest that reductive dechlorination could be exploited for the bioremediation of PCE-contaminated sites. Clearly, further microbiological studies are necessary to identify the organism(s) causing PCE and/or VC dechlorination in these cultures and to determine factors which favor the complete reductive dechlorination of PCE and other chlorinated ethenes.

REFERENCES

1. **Abelson, P. H.** 1990. Inefficient remediation of ground-water pollution. Science **250**:73.
2. **Bouwer, E. J., and P. L. McCarty.** 1983. Transformations of 1- and 2-carbon halogenated aliphatic organic compounds under methanogenic conditions. Appl. Environ. Microbiol. **45**:1286–1294.
3. **Bouwer, E. J., and P. L. McCarty.** 1985. Utilization rates of trace halogenated organic compounds in acetate-grown biofilms. Biotechnol. Bioeng. **27**:1564–1571.
4. **Bouwer, E. J., and J. P. Wright.** 1988. Transformations of trace halogenated aliphatics in anoxic biofilm columns. J. Contam. Hydrol. **2**:155–169.
5. **Cord-Ruwisch, R., H.-J. Steitz, and R. Conrad.** 1988. The capacity of hydrogenotrophic anaerobic bacteria to compete for traces of hydrogen depends on the redox potential of the terminal electron acceptor. Arch. Microbiol. **149**:350–357.
6. **Dean, J. A. (ed.).** 1985. Lange's handbook of chemistry. McGraw-Hill, New York.
7. **Dolfing, J.** 1990. Reductive dechlorination of 3-chlorobenzoate is coupled to ATP production and growth in an anaerobic bacterium, strain DCB-1. Arch. Microbiol. **153**:264–266.
8. **Egli, C., T. Tschan, R. Scholtz, A. M. Cook, and T. Leisinger.** 1988. Transformation of tetrachloromethane to dichloromethane and carbon dioxide by *Acetobacterium woodii*. Appl. Environ. Microbiol. **54**:2819–2824.
9. **Fathepure, B. Z., and S. A. Boyd.** 1988. Dependence of tetrachloroethylene dechlorination on methanogenic substrate consumption by *Methanosarcina* sp. strain DCM. Appl. Environ. Microbiol. **54**:2976–2980.
10. **Fathepure, B. Z., and S. A. Boyd.** 1988. Reductive dechlorination of perchloroethylene and the role of methanogens. FEMS Microbiol. Lett. **49**:149–156.
11. **Fathepure, B. Z., J. P. Nengu, and S. A. Boyd.** 1987. Anaerobic bacteria that dechlorinate perchloroethene. Appl. Environ. Microbiol. **53**:2671–2674.
12. **Federal Register.** 1989. National primary and secondary drinking water regulations; proposed rule. Fed. Regist. **54**:22062–22160.
13. **Fogel, M. M., A. R. Taddeo, and S. Fogel.** 1986. Biodegradation of chlorinated ethenes by a methane-utilizing mixed culture. Appl. Environ. Microbiol. **51**:720–724.
14. **Freedman, D. L., and J. M. Gossett.** 1989. Biological reductive dechlorination of tetrachloroethylene and trichloroethylene to ethylene under methanogenic conditions. Appl. Environ. Microbiol. **55**:2144–2151.
15. **Gossett, J. M.** 1985. Anaerobic degradation of C_1 and C_2 chlorinated hydrocarbons. NTIS accession no. AD-A165 005/0. Report no. ESL-TR-85-38. Engineering & Services Laboratory, U.S. Air Force Engineering & Services Laboratory, U.S. Air Force Engineering and Services Center, Tyndall Air Force

Base, Fla.
16. **Gossett, J. M.** 1987. Measurement of Henry's law constants for C_1 and C_2 chlorinated hydrocarbons. Environ. Sci. Technol. **21**:202–208.
17. **Jones, W. J., D. P. Nagle, Jr., and W. B. Whitman.** 1987. Methanogens and the diversity of archaebacteria. Microbiol. Rev. **51**:135–177.
18. **Mohn, W. W., and J. M. Tiedje.** 1990. Strain DCB-1 conserves energy for growth from reductive dechlorination coupled to formate oxidation. Arch. Microbiol. **153**:267–271.
19. **Oldenhuis, R., R. L. J. M. Vink, D. B. Janssen, and B. Witholt.** 1989. Degradation of chlorinated aliphatic hydrocarbons by *Methylosinus trichosporium* OB3b expressing soluble methane monooxygenase. Appl. Environ. Microbiol. **55**:2819–2826.
20. **Parsons, F., and G. B. Lage.** 1985. Chlorinated organics in simulated groundwater environments. J. Am. Water Works Assoc. **77**(5):52–59.
21. **Parsons, F., G. B. Lage, and R. Rice.** 1985. Biotransformation of chlorinated organic solvents in static microcosms. Environ. Toxicol. Chem. **4**:739–742.
22. **Parsons, F., P. R. Wood, and J. DeMarco.** 1984. Transformations of tetrachloroethene and trichloroethene in microcosms and groundwater. J. Am. Water Works Assoc. **76**(2):**56–59.**
23. **Schink, B.** 1985. Inhibition of methanogenesis by ethylene and other unsaturated hydrocarbons. FEMS Microbiol. Ecol. **31**:63–68.
24. **Thauer, R. K., K. Jungermann, and K. Decker.** 1977. Energy conservation in chemotrophic anaerobic bacteria. Bacteriol. Rev. **41**:100–180.
25. **Thauer, R. K., D. Möller-Zinkhan, and A. M. Spormann.** 1989. Biochemistry of acetate catabolism in anaerobic bacteria. Annu. Rev. Microbiol. **43**:43–67.
26. **Vogel, T. M., C. S. Criddle, and P. L. McCarty.** 1987. Transformations of halogenated aliphatic compounds. Environ. Sci. Technol. **21**:722–736.
27. **Vogel, T. M., and P. L. McCarty.** 1985. Biotransformation of tetrachloroethylene to trichloroethylene, dichloroethylene, vinyl chloride, and carbon dioxide under methanogenic conditions. Appl. Environ. Microbiol. **49**:1080–1083.
28. **Westrick, J. J., J. W. Mello, and R. F. Thomas.** 1984. The groundwater supply survey. J. Am. Water Works Assoc. **76**(5): 52–59.
29. **Zinder, S. H.** Unpublished results.