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Tetrachloroethene Transformation to Trichloroethene and *cis*-1,2-Dichloroethene by Sulfate-Reducing Enrichment Cultures

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Tetrachloroethene, also known as perchloroethylene, was reductively dechlorinated to trichloroethene and *cis*-1,2-dichloroethene by laboratory sulfate-reducing enrichment cultures. The causative organism or group was not identified. However, tetrachloroethene was dechlorinated to trichloroethene in 50 mM bromoethanesulfonate-inhibited enrichments and to trichloroethene and *cis*-1,2-dichloroethene in 3 mM fluoroacetate-inhibited enrichments. Overall transformation varied from 92% tetrachloroethene removal in 13 days to 22% removal in 65 days, depending on conditions of the inoculum, inhibitor used, and auxiliary substrate used. Neither lactate, acetate, methanol, isobutyric acid, valeric acid, isovaleric acid, hexanoic acid, succinic acid, nor hydrogen appeared directly to support tetrachloroethene dechlorination, although lactate-fed inocula demonstrated longer-term dechlorinating capability.

Tetrachloroethene, also known as perchloroethylene (PCE), is a major groundwater contaminant in the United States. Over 8% of the groundwater used as drinking water sources in the United States showed measurable levels (>0.2 μg/liter) of PCE (26). Of 29 volatile organic compounds measured, PCE was the third most frequently detected, behind trichloroethene (TCE) and 1,1,1-trichloroethane. Although the nationwide median was low (0.6 μg/liter), PCE levels up to 69 μg/liter were measured. PCE 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 (10).

Reductive dechlorination of PCE to TCE has been observed repeatedly in both natural and laboratory methanogenic environments (3–5, 18–20). The enzymatic pathway involved has not been defined, but the general pathway apparently applies to sequential removal of additional chlorines, as dichloroethene (DCE) isomers, vinyl chloride, and ethene have all been observed as products of PCE dechlorination (8, 9, 11, 25). CO_2 has also been observed as a PCE degradation product (25), indicating a possible biooxidation pathway. Recent work with pure cultures of *Methanosarcina* sp. and *Methanosarcina mazei* has shown that methanogens are capable of dechlorinating PCE to TCE (9). Dechlorination occurs only when the organisms are actively producing methane (8), leading to the hypothesis that electrons are diverted from the methane-producing pathway to PCE.

Many groundwaters contain significant concentrations of sulfate, raising the question of whether PCE degradation can occur in sulfate-reducing environments. Only trace removal (less than 15%) of PCE was observed in a sulfate-reducing, continuous-flow column (5). Pure cultures of *Desulfovibrio desulfuricans* produce neither TCE nor other products from PCE (9). Furthermore, degradation of chlorinated phenols and benzenes was observed to cease in the presence of sulfate, even when methanogenesis was stimulated (12).

However, the dechlorinating, sulfidogenic bacterium DCB-1 (23, 24) has been shown to dechlorinate PCE to TCE (9), and *Desulfobacterium autotrophicum* reduces tetrachloromethane quantitatively to trichloromethane and dichloromethane (6), indicating the existence of reductive dechlorination capabilities in sulfidogenic and sulfate-reducing bacteria. Therefore, it appears reasonable that PCE dechlorination may occur in sulfate-reducing environments, provided the appropriate environmental conditions exist and/or a PCE-dechlorinating organism, e.g., a methanogen, is present.

This work examines PCE degradation in sulfate-reducing mixed cultures. Results indicate that PCE is reductively dechlorinated to TCE and *cis*-1,2-DCE by sulfate-reducing enrichment cultures.

MATERIALS AND METHODS

Inoculum source. The inoculum source was a sealed, stirred 4-liter jar with a 3-liter liquid volume, kept in a room at a constant temperature of 35 to 37°C. On 25 March 1988, it was charged with 300 ml of anaerobic digester sludge from the Ithaca, N.Y., wastewater treatment facility and 2,700 ml of basal medium. The basal medium was modified from that of Pfennig et al. (21) for cultivation of sulfate-reducing bacteria. Specifically, the $FeCl_2 \cdot 4H_2O$ quantity was increased by a factor of 5, sulfide addition to the medium was eliminated (sulfide production in the inoculum source maintained the appropriate reducing conditions), and the vitamin and growth factor solutions were added in the appropriate quantities to the auxiliary organic substrate solution instead of to the medium. To avoid excessive sulfide build-up, the inoculum source was stripped daily for 10 min with a 75% N_2–25% CO_2 gas stream deoxygenated by passage through a $TiCl_3$ solution.

The inoculum source was operated semicontinuously (once-daily removal and recharge) with a 15-day hydraulic retention time for 216 days. After 216 days, approximately 2 liters of the contents was removed and replaced with 2 liters of basal medium. The retention time was shortened to 10 days. After two retention times, this process was repeated. These steps were taken to reduce the methanogenic population and were based upon the assumption that sulfate-reducing bacteria have a kinetic advantage over methanogens at high sulfate concentratons and low residual substrate concentrations (15–17, 22, 27).

An auxiliary substrate solution consisting of sodium lac-

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TABLE 1. Inoculum source characteristics[a]

Days from start-up	Alkalinity (mg of $CaCO_3$/liter)	COD (mg/liter)		Sulfate (mg of SO_4^{2-}/liter)	TSS (mg/liter)	VSS (mg/liter)
		Total	Soluble			
77	3,500 (5)	240 (10)	60 (5)	1,370[b]	170 (10)	140 (10)
319	3,550 (10)	210 (5)	100 (10)	1,200 (50)	140 (5)	60 (10)
424	3,700 (25)	270 (20)	60 (20)	960 (60)	290[c]	190[c]
451	NM	NM	NM	870[c]	NM	NM

[a] All values represent the average of triplicates, except as noted. Values in parentheses represent standard deviations. NM, Not measured. TSS, Total suspended solids; VSS, volatile suspended solids.
[b] No replicates measured.
[c] Duplicates measured.

tate (60% aqueous solution) (Sigma Chemical Co., St. Louis, Mo.), vitamins, and growth factors (solutions 7 and 8 from Pfennig et al. [21]) was added to the inoculum source daily through a septum by using a 2.0-ml gastight syringe. With the 15-day retention time, 2 ml of auxiliary substrate solution provided 221 meeq of lactate (meeq = milli-electron equivalents, on the basis of complete oxidation to CO_2) per liter of feed (1.64 M). With the 10-day retention time, 2 ml of substrate solution provided 148 meeq of lactate per liter of feed (1.10 M).

The pH was measured daily in the inoculum source effluent. The pH averaged 7.2 ± 0.1 (mean ± standard deviation) over the course of the experiments. The following parameters were measured periodically in accordance with *Standard Methods for the Examination of Water and Wastewater*, 16th ed. (1), to characterize the inoculum source (Table 1): alkalinity (section 403, titrated to a pH of 4.0); total suspended solids and volatile suspended solids (section 209); sulfate (section 426 C, turbidimetric method, by using a 1.0-cm light path); and chemical oxygen demand (COD), both total and soluble (section 508 A). Soluble COD was defined as the COD present in filtrate passing through a Whatman GF/C glass fiber filter.

Reagents. Tetrachloroethene, trichloroethene, *cis*-1,2-dichloroethene, *trans*-1,2-dichloroethene (neat liquids, 96 to 99%; Supelco, Inc., Bellefonte, Pa.), methane, and hydrogen sulfide (99.0 and 99.5%; Scott Specialty Gases, Plumsteadville, Pa.) were used for gas chromatograph calibration. Neat liquid tetrachloroethene (99.9%) was used for addition to experimental systems; bromoethanesulfonate (BES) (2-bromoethanesulfonic acid, sodium salt; 98%) and fluoroacetate (fluoroacetic acid, sodium salt; 98%) were used for inhibition studies (all reagents used for experimental systems and inhibition studies were from Aldrich Chemical Co., Inc., Milwaukee, Wis.).

Experimental operation. Experiments were conducted in 160-ml borosilicate serum bottles containing 100 ml of liquid. Inocula were anaerobically delivered to the bottles with a Unispense-II pump (Wheaton Industries, Millville, N.J.), while purging the bottle with 75% N_2–25% CO_2. Teflon-lined, butyl-rubber septa (Wheaton Industries) were crimped into place as the purging needle was withdrawn. N_2-CO_2 (5.0 ml) was added to each bottle to account for headspace pressure loss due to sampling. Bottles were kept above 3.0 ml over pressure in the headspace (between 3.0 and 8.0 ml). Water control (WC) and autoclaved-seed control bottles were used to test for abiotic losses.

PCE was added to the bottles as an aqueous stock solution by using a 0.5-ml gastight syringe. The amount of PCE added was measured after 1 h of incubation in a 35°C water-filled shaker bath. The bottles were incubated quiescently in the dark in an inverted position in a room at a constant temperature of 35°C.

The bottles were operated in a batch-fed mode. A solution (50 μl) containing 2,500 meeq of auxiliary organic substrate per liter and 2,500 meeq of SO_4^{2-} (312.5 mM) per liter was provided to bottles periodically. Vitamins and growth factors were usually not included in the substrate solution. Bottle contents were removed as required to keep the bottle liquid contents within ±0.5 ml of 100 ml.

Two metabolic inhibitors were used: BES, a methanogenic inhibitor (14), and fluoroacetate, an acetate-consumer inhibitor (2). The initial BES concentration in BES-inhibited bottles was 50 mM, while the initial fluoroacetate concentration in fluoroacetate-inhibited bottles was 3 mM. To avoid inhibitor depletion, inhibited bottles received substrate containing the same concentration of inhibitor as was initially added to the bottles. The inhibitor concentrations were chosen from metabolic studies with lactate and $^{14}CH_3COOH$ as the minimum concentrations required to effect 95% or greater inhibition of the desired activity.

When most of an initial dose of PCE had been consumed, 4.0 ml of the bottle contents was removed. Basal medium (with no additional substrate), PCE-saturated solution, and concentrated substrate were added back. Basal medium with no additional substrate was also added to the bottles after contents were removed for H_2S testing or organic acid testing. Basal medium for these operations contained Na_2S (21).

Headspace analysis for volatile compounds. Headspace analysis via a gas chromatograph equipped with a flame ionization detector, as described previously (11, 13), was used for determining quantities of PCE, TCE, *cis*- and *trans*-1,2-DCE, and methane in experimental bottles. Calibration factors for volatile organics were prepared as described by Freedman and Gossett (11).

Measurement of H_2S production. Sulfide is extensively partitioned between gaseous, aqueous, and particulate phases. Different soluble forms exist within the aqueous phase, depending upon pH and solution composition. Consequently, total S(−II) in a serum bottle was approximated by determination of total acid-volatile sulfide, defined as the sum of headspace H_2S plus acid-volatile sulfide, as measured on a sample of the liquid suspension (i.e., the mixed-liquor phase).

Headspace H_2S was determined directly from a 0.5-ml headspace sample removed from a serum bottle at 35°C and injected onto an 8500 gas chromatograph (Perkin Elmer-Cetus Instruments) equipped with a thermal conductivity detector. The sample was fractionated on a 2-mm (inside diameter) glass column (6.4 mm by 2.44 m) packed with Chromosil 310 (Supelco) and kept at 10 to 11°C in an external refrigerator. Injection was made with a 1.0-ml gastight syringe with a push-button valve. The injector temperature was 200°C; the detector temperature was 175°C. The He carrier gas flow rate was 24 to 25 ml/min. The detector

setting was for low sensitivity. Data were gathered for 7.5 min, with H_2S eluting at between 5.5 and 6.0 min (time of maximum peak height). Normally, two other peaks eluted: one at 1.1 to 1.2 min (N_2) and one at 2.2 to 2.3 min (CO_2).

Acid-volatile sulfide in the mixed-liquor phase was measured by placing a 1.0-ml mixed-liquor sample into a sealed (Teflon-lined rubber septum), 14.4-ml serum vial containing 5.0 ml of 6 N H_2SO_4 (with a helium headspace), incubating the solution at 35°C for 1 h, and measuring the H_2S present in 0.5 ml of headspace. The detector sensitivity was set at medium for these measurements.

Calibration curves were prepared every time H_2S was measured. For low-sensitivity detection (direct headspace analysis), 14.4-ml serum vials were purged with approximately 75% N_2–25% CO_2 and capped with Teflon-lined rubber septa. H_2S (99.5%) gas was injected into the vials. Volumes from 50 μl to 0.275 ml were used, corresponding approximately to 1.95 to 10.95 μmol of H_2S added. Gas chromatograph peak areas determined for 0.5-ml injection were related to nanomoles of H_2S.

For medium-sensitivity detection (mixed-liquor sample analysis), 14.4-ml serum vials were filled with 5.0 ml of 6 N H_2SO_4, purged with He gas (He was used to remove interference from large N_2 and CO_2 peak tails) for 30 to 40 s, and capped with Teflon-lined rubber septa. Sulfide-free basal medium (1.0 ml) was injected into the sealed vial, followed by injection of 20 to 100 μl of 99.5% H_2S gas (approximately 0.80 to 3.98 μmol of H_2S). The vials were shaken vigorously and placed inverted in a 35°C shaker bath for at least 1 h. Manually determined peak heights determined for the 0.5-ml injection were proportional to the total quantity of H_2S in the vial.

To examine the precision and accuracy of the method, 160-ml serum bottles were prepared with 99.0 ml of basal medium and a 75% N_2–25% CO_2 headspace and were capped with Teflon-lined rubber septa. One milliliter of a stock Na_2S solution (12.35 g of S^{2-} per liter, as determined by the iodometric sulfide procedure, section 427 D [1]) was added to each bottle to provide 386 μmol of H_2S total. The bottles were incubated overnight at 35°C and analyzed for total acid-volatile sulfide by using the headspace and mixed-liquor samples.

A total of 12 bottles were employed: 4 were prepared with no inhibitor, 4 had various levels of BES (5, 10, 25, and 50 mM), and 4 had different levels of fluoroacetate (0.5, 1, 3, and 5 mM). The inhibitors were weighed and placed in the bottles before adding the basal medium. For 11 of the bottles, sulfide recovery averaged 94% ± 2% (mean ± standard deviation); sulfide recovery in the 50.0 mM BES bottle was only 85%, significantly different from the average recoveries of the other bottles (95% confidence). The reason for the decreased recovery was not determined.

Measurement of soluble organics. Residual lactate and acetate concentrations were measured by high-performance liquid chromatography (7). An HP 1090 liquid chromatograph equipped with a Perkin Elmer LC-25 refractive index detector was used to perform the separation and detection. The response was linear ($r^2 > 0.99$) over the range of 10 to 300 mg/liter (compound concentration). On each day of analysis, calibration points were prepared in the vicinity of the expected concentration.

RESULTS

PCE removal in nonfed cultures. Over the course of this study, experiments were conducted by using cultures inoculated at different times after the inoculum source start-up. Each experiment included cultures that received no additional substrate. These cultures provided an indication of the PCE-dechlorinating capability of the inoculum source without additional electron sources and also provided nonfed controls for comparison to the other conditions employed within a given experiment.

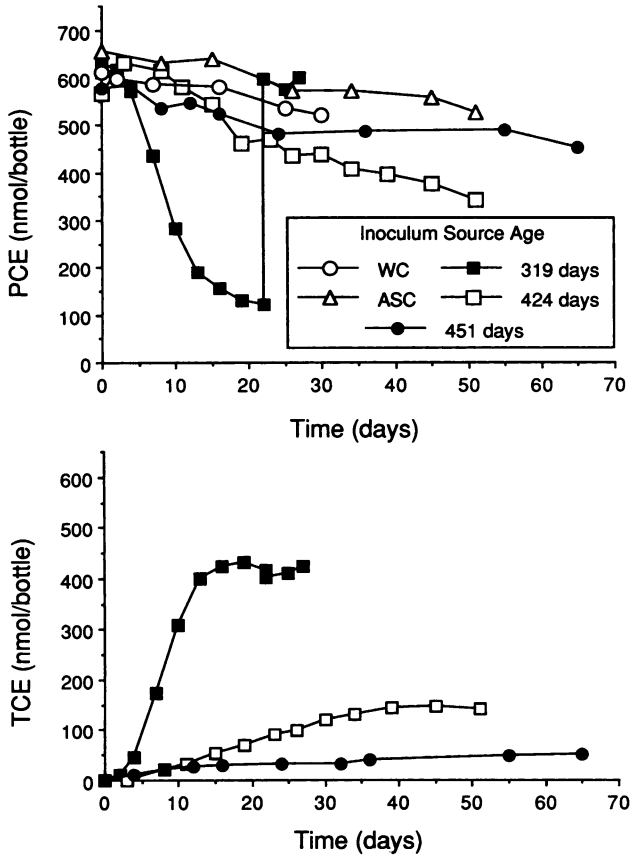

FIG. 1. PCE dechlorination and products in bottles inoculated at different times after inoculum source start-up. Bottles received no auxiliary substrate or inhibitor. ASC, Autoclaved-seed control. The initial PCE aqueous concentrations ranged from 573 to 637 μg/liter. Additional PCE was provided to the 319-day inoculum on day 22.

Dechlorination of PCE in nonfed controls inoculated at different times after the inoculum source start-up is depicted in Fig. 1. The 319-day cultures exhibited 81% PCE removal within 22 days, whereas the 424-day cultures showed 46% PCE removal in 51 days and the 451-day culture showed 22% removal in 65 days. TCE was produced in inoculated bottles, but not in WCs or autoclaved-seed controls. *cis*-1,2-DCE production was observed in the 319-day cultures. Initial PCE addition ranged from 590 to 656 nmol per 100 ml of culture (573 and 637 μg/liter, respectively, equilibrium aqueous concentrations, assuming a Henry's constant of 1.175 mol/m^3 of gas per mol per cubic meter of solution [13]). Although the inoculum source remained vigorously sulfate reducing, its capability to dechlorinate PCE decreased with increased time from start-up.

Impact of inhibitors and substrate addition. Cultures inoculated 319 days after inoculum source start-up were treated with inhibitors and received substrate periodically. One pair of bottles served as nonfed controls. One pair of bottles received 0.125 meeq of lactate every third day (lactate fed).

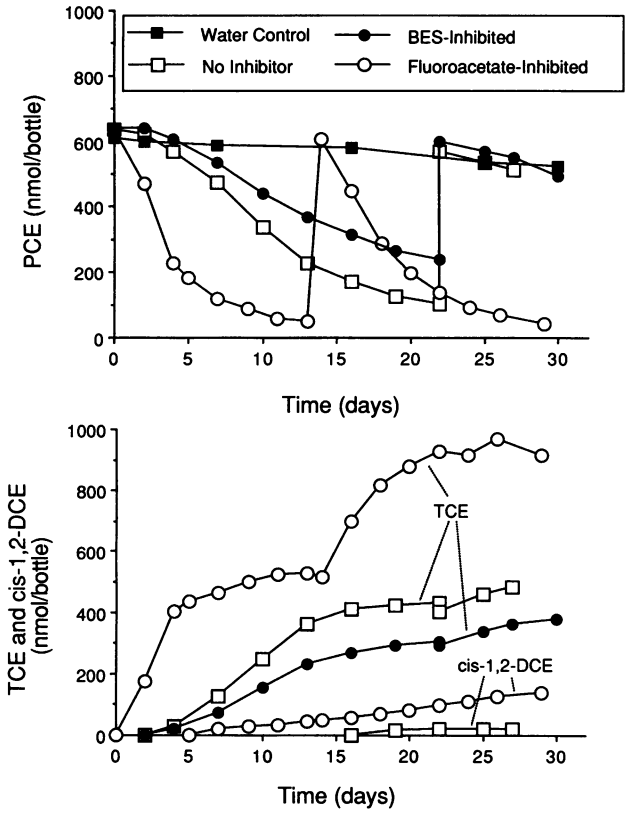

FIG. 2. PCE dechlorination and products in bottles prepared 319 days after inoculum source start-up receiving lactate and either 3 mM fluoroacetate, 50 mM BES, or no inhibitor. Each point is an average of two bottles. The initial PCE aqueous concentration was 617 μg/liter. Additional PCE was provided to fluoroacetate-inhibited bottles on day 14 and to BES-inhibited and noninhibited bottles on day 22.

Two pair of bottles were inhibited with 50 mM BES, with one pair receiving 0.125 meeq of acetate every third day (BES plus acetate) and the other pair receiving 0.125 meeq of lactate every third day (BES plus lactate). A final pair of bottles was inhibited with 3 mM fluoroacetate and received 0.125 meeq of lactate every third day (fluoroacetate plus lactate). WCs were examined in duplicate. The initial PCE addition was 635 nmol per 100 ml of culture (617 μg/liter, equilibrium aqueous concentration).

PCE dechlorination was observed in all pairs except the WCs. Figure 2 shows PCE dechlorination and TCE and *cis*-1,2-DCE productions. The noninhibited, lactate-fed pair degraded PCE similarly (84% removal in 22 days) to the nonfed control pair shown in Fig. 1. The BES plus acetate pair (data not shown) showed PCE degradation similar to the BES plus lactate pair (58 and 63% removal, respectively, in 22 days). PCE dechlorination was most substantial in the fluoroacetate-inhibited bottles (92% removal in 13 days) and least substantial in the BES-inhibited bottles. TCE and *cis*-1,2-DCE productions followed PCE dechlorination essentially stoichiometrically, although BES-inhibited bottles showed no *cis*-1,2-DCE production. Unaccountable PCE losses were of equal magnitude to the WC PCE losses: up to 17% unaccounted PCE in cultures and up to 20% PCE losses from WCs.

H_2S, methane, lactate, and acetate concentrations were measured for all pairs, where possible (Table 2). Essentially stoichiometric sulfide production was observed in the lactate-fed pair after subtracting the sulfide production observed in the nonfed control pair. No residual lactate was detected in any of the bottles receiving lactate. Residual acetate in the lactate-fed pair was low and methane production was very low, accounting for only 2% of the electron equivalents measured.

TABLE 2. Electron balance after 28 days[a]

Substrate and inhibitor	H_2S (meeq)	Acetate (meeq)	CH_4 (meeq)	Total measured meeq	% of added meeq
Lactate	1.61	0.05	0.03	1.69	135
No substrate	0.29	0.00	0.00	0.29	
Lactate + fluA	0.69	0.84	0.00	1.53	122
Lactate + BES	NM	0.05	0.00	0.05	NR
Acetate + BES	NM	0.05	0.00	0.05	NR

[a] Experiment set-up was 319 days after the inoculum source start-up. All 0.00 values mean that the detected quantity was below 0.005 meeq. fluA, Fluoroacetate; NM, not measurable due to BES interference with sulfide measurement; NR, not reported due to inability to measure sulfide concentration.

Sulfide production was also observed in the fluoroacetate plus lactate pair. The number of electron equivalents measured as sulfide was correspondingly less than in the noninhibited lactate-fed pair because of the inhibition of acetate consumption. Methane production in the fluoroacetate-inhibited enrichment was virtually nonexistent (less than 0.2% of the measured electron equivalents).

Sulfide production was not measured in the BES-inhibited cultures. Virtually all added electron equivalents were consumed, however, and less than 0.5% of those equivalents were measured as methane, indicating that sulfate-reducing pathways were consuming the added lactate and acetate.

Bottle pairs inoculated 424 days after the inoculum source start-up were uninhibited and received 0.125 meeq of either lactate or methanol every third day. The initial PCE addition was 625 nmol of PCE per 100 ml of culture (607 μg/liter, equilibrium aqueous concentration). PCE dechlorination was observed, but only to TCE (Table 3). Sulfide and methane productions were measured and indicated that the cultures were sulfate reducing. Methane production was substantial in the methanol-fed pair (40% of the measured electron equivalents), but PCE dechlorination was not significantly different from that in the nonfed control pair.

At day 451 after the inoculum source start-up, a final series of bottles was inoculated. A large matrix of conditions was investigated. Of the 15 bottles inoculated, 5 were inhibited with 3 mM fluoroacetate, 5 were inhibited with 50 mM BES, and 5 received no inhibitor. Within each group of identically

TABLE 3. Electron and PCE balance after 51 days[a]

Substrate	H_2S (meeq)	CH_4 (meeq)	Total measured meeq	% of added meeq	% PCE removed	% TCE produced
Lactate	2.37	0.02	2.39	110	67	44
None	0.30	0.00	0.30		46	23
Methanol	0.45[b]	0.31[b]	0.76	158	49	27

[a] Experiment set-up was 424 days after the inoculum source start-up. All 0.00 values mean that the detected quantity was below 0.005 meeq.

[b] After 11 days (after 51 days, H_2S was measured at 0.40 meeq and the methane quantity was too great to measure with the flame ionization detector at high sensitivity).

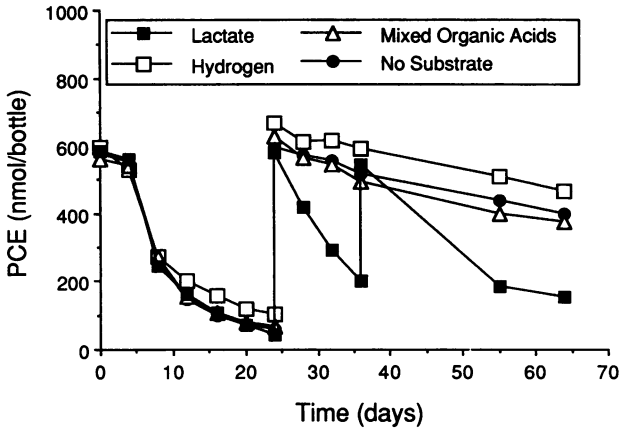

FIG. 3. PCE dechlorination in bottles prepared 451 days after inoculum source start-up inhibited with 3 mM fluoroacetate and receiving lactate, H_2, mixed organic acids, or no substrate. All bottle solutions shown, except that with no substrate, received vitamins. The initial PCE aqueous concentration was 573 μg/liter. Additional PCE was provided to lactate-fed bottles on days 24 and 36 and to other bottles on day 24 only.

inhibited bottles, one received no substrate, one received lactate every third day, one received lactate plus a proportional amount of vitamins every third day, one received hydrogen plus vitamins every third day, and the remaining bottle received a mixture of organic acids plus vitamins every third day. The vitamins were identical to those used to feed the inoculum source. The organic acid mixture consisted of equal electron equivalent additions of isobutyric, valeric, isovaleric, hexanoic, and succinic acids to water to give a concentrated solution of 2,500 meeq (total) per liter, which was then used to feed the appropriate bottles.

All bottles received 590 nmol of PCE per 100-ml culture (573 μg/liter, equilibrium aqueous concentration). Bottles receiving substrate received 0.125 meeq as the designated compound every third day until day 24. Starting on day 24, fed bottles received 0.250 meeq every third day. Neither sulfide production nor residual substrate levels were measured. Stoichiometric PCE dechlorination to TCE was observed in all bottles. 1,2-DCE was observed in fluoroacetate-inhibited bottles, but the quantities produced were too low for determination of the 1,2-DCE isomer. PCE-dechlorinating capability was greatest in the fluoroacetate-inhibited bottle: from 83 to 92% removal in 24 days versus 47 to 63% removal in 65 days for BES-inhibited bottles and 22 to 33% removal in 65 days for bottles receiving no inhibitor.

Fluoroacetate-inhibited bottles receiving lactate showed more dechlorinating capability than those receiving other substrates (Fig. 3). The difference between the lactate-fed bottle with vitamins and that without vitamins was insignificant, so for clarity only the lactate plus vitamin results are shown in Fig. 3. This response to the substrate was also seen for the BES-inhibited bottles and bottles with no inhibitor (data not shown).

Of the added PCE, 2 to 17% could not be accounted for in the chlorinated products. Overall, unaccounted losses appeared to be directly proportional to the length of time required to dechlorinate the first PCE spike. The WCs and autoclaved-seed controls showed PCE losses of 14 to 20%, with no transformation to other chlorinated products. Probably the PCE leaked out of the bottles over time. It is possible, however, that nonchlorinated or soluble products were formed that were not detected.

DISCUSSION

PCE can be dechlorinated to TCE and *cis*-1,2-DCE by laboratory sulfate-reducing enrichment cultures. The stoichiometric production of sulfide in bottles that were observed to dechlorinate PCE supports this conclusion (Tables 2 and 3). Additionally, PCE dechlorination appears to proceed via reductive dechlorination as described previously for methanogenic environments (3, 8, 9, 11, 19, 25). However, the PCE-dechlorinating capability of a sulfate-reducing enrichment is apparently less than that seen in mixed methanogenic enrichments. Vinyl chloride was not detected as a dechlorination end product, and the dechlorination rates were slower than those observed under similar conditions with methanogenic systems (11).

The class of organism responsible for PCE dechlorination was not determined. Recent results with pure cultures have demonstrated that methanogens can dechlorinate PCE, at least to TCE (8, 9). However, when methanogenesis stops, PCE dechlorination by methanogens also stops. This was observed in pure cultures in which methane production ceased due to consumption of added substrate (8, 9) and in mixed cultures with added BES (11).

Methane production in this present study was in all cases less than 2% of the total measured electron equivalents (except in methanol-fed bottles), indicating very little methanogenic activity. Cultures inhibited with 50 mM BES showed virtually no methane production, while demonstrating PCE-dechlorinating capability equal to that of noninhibited bottles. Cultures inhibited with 3 mM fluoroacetate also showed insignificant methane production and enhanced PCE-dechlorinating capability versus the noninhibited bottles. These observations deviate substantially from the pattern of PCE dechlorination observed with methanogenic systems. Therefore, while it is possible that methanogens were responsible for the PCE dechlorination observed in these sulfate-reducing cultures, it appears probable that another class of organisms is responsible for the observed dechlorination.

It is possible, but not conclusive, that sulfate-reducing organisms can dechlorinate PCE. PCE dechlorination was more substantial in cultures receiving lactate as opposed to acetate, hydrogen, methanol, or mixed organic acids. Furthermore, PCE dechlorination was most substantial in cultures inhibited with fluoroacetate. In the fluoroacetate-inhibited enrichment, lactate oxidation to acetate by lactate-consuming sulfate-reducers was the primary metabolic activity observed.

The PCE-dechlorinating capability of the inoculum source decreased over time, both between experiments and during any one experiment (Fig. 1). The reason for this is unknown. Perhaps a growth factor required for dechlorination, but not for growth, was washed out of the inoculum source over time. Alternatively, the organisms responsible for dechlorination may have been washed out.

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