

Biotransformation of Tetrachloroethylene to Trichloroethylene, Dichloroethylene, Vinyl Chloride, and Carbon Dioxide under Methanogenic Conditions

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Tetrachloroethylene (PCE) and trichloroethylene (TCE), common industrial solvents, are among the most frequent contaminants found in groundwater supplies. Due to the potential toxicity and carcinogenicity of chlorinated ethylenes, knowledge about their transformation potential is important in evaluating their environmental fate. The results of this study confirm that PCE can be transformed by reductive dehalogenation to TCE, dichloroethylene, and vinyl chloride (VC) under anaerobic conditions. In addition, [¹⁴C]PCE was at least partially mineralized to CO₂. Mineralization of 24% of the PCE occurred in a continuous-flow fixed-film methanogenic column with a liquid detention time of 4 days. TCE was the major intermediate formed, but traces of dichloroethylene isomers and VC were also found. In other column studies under a different set of methanogenic conditions, nearly quantitative conversion of PCE to VC was found. These studies clearly demonstrate that TCE and VC are major intermediates in PCE biotransformation under anaerobic conditions and suggest that potential exists for the complete mineralization of PCE to CO₂ in soil and aquifer systems and in biological treatment processes.

Widespread contamination of groundwater by halogenated compounds (12) has led to investigations to determine their fate in the environment. Previous studies have illustrated that a potential exists for their biotransformation under anaerobic conditions that are conducive to methanogenesis. Field studies with reclaimed wastewater injected into an aquifer indicated that trihalomethanes were transformed with half-lives of 30 days and tetrachloroethylene (PCE), trichloroethylene (TCE), and 1,1,1-trichloroethane were transformed with half-lives of 300 days (10). This was confirmed in laboratory studies. The biotransformation of PCE by a mixed methanogenic culture supported by continuous feed of acetate as the primary source of organic carbon was demonstrated by Bouwer and McCarty (4). At elevated PCE levels, partial conversion to TCE was observed. In addition, Parsons et al. (8) demonstrated the formation of traces of dichloroethylene (DCE) isomers and vinyl chloride (VC) after the disappearance of PCE in mucks to which the latter had been added.

Thus, the anaerobic biotransformation of PCE and TCE appears to be the result of reductive dehalogenation. However, research to date has not clearly demonstrated whether conversion of PCE to TCE, DCE, and VC is quantitative or whether this process can lead to the complete mineralization of these compounds. Such information was sought in this study.

MATERIALS AND METHODS

Chemicals and radioisotopes. Chemicals used were reagent-grade PCE, TCE, and 1,1,1-trichloroethane (Matheson, Coleman, and Bell, Norwood, Ohio), sodium acetate and acetone (99.9%; J. T. Baker Chemical Co., Phillipsburg, N.J.), isopropanol (99+% Aldrich Chemical Co., Milwaukee, Wis.), and *o*-xylene (99.5+% Fluka AG, Buchs, Switzer-

land). VC, 0.1 mg per ml of methanol (Supelco Inc., Bellefonte, Pa.), was used. [¹⁴C]PCE (Amersham Corp., Arlington Heights, Ill.) was diluted initially in methanol (absolute; J. T. Baker Chemical Co.) to 5.8×10^4 dpm/ μ l.

Analytical methods. The halogenated aliphatic reactants and products were determined with a lower detection limit of 0.1 μ g/liter by gas chromatography (GC). Samples were sealed in 10- or 60-ml vials and extracted with 1 or 2 ml of pentane (6), respectively, using 1,2-dibromoethane (10 μ g/liter) as an internal standard. A 2- μ l sample of the extract was injected into a packed-column GC (10% squalane on Chromosorb W/AW) equipped with a linearized ⁶³Ni detector. Quantitation was achieved by injecting standards, treated like samples, and comparing relative areas with a Spectra-

TABLE 1. Composition of feed to the large anaerobic column

Compound	Concn (mg/liter)
Inorganics	
NaOH	1,600
NH ₄ Cl	215
MgSO ₄ · 7H ₂ O	150
K ₂ HPO ₄	60
CaCl ₂	25
KCl	25
FeCl ₂	5.0
CoCl ₂	0.5
NiCl ₂	0.25
Organics	
Acetic acid	3,000
Acetone	56
Isopropanol	37
<i>o</i> -Xylene	4.1
PCE	0.76
TCE	0.68
1,1,1-Trichloroethane	0.063

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TABLE 2. Liquid scintillation and GC effluent analysis for the small anaerobic column

Conditions	¹⁴ C activity (% distribution)			GC analysis			
	CO ₂	Non-CO ₂		PCE (nmol/liter)	TCE (nmol/liter)	PCE plus TCE	
		Volatile	Non-volatile			nmol/liter	% of influent PCE
2-Day detention time							
Influent	0			360	0		
Effluent	12	81	7.0	4	234	238	66
4-Day detention time							
Influent	0.6	95	4.2	186	0	186	
Effluent	24	71	4.2	4	134	138	74
4-Day detention time, with VC							
Influent	0.9	95	3.4	196	0	196	
Effluent	12	82	4.8	25	128	153	65
VC, no flow							
Influent	0.9	95	3.4	196	0	196	
Effluent	23	61	16				

Physics 4000 calculating integrator (Spectra-Physics, Sunnyvale, Calif.).

Organic intermediates were determined by GC/mass spectrometry. A 5- or 60-ml amount of sample was purged by closed-loop stripping, trapping the halogenated aliphatics on activated carbon (5a) inside a GC injection liner. The liner was inserted into the GC/mass spectrometer injection port, and the volatile organics were desorbed (approximately 5 min) at 250°C and immediately trapped at the front of a 30-m-thick film capillary column (Durabond 5; J&W, Inc., Rancho Cordova, Calif.) placed in a liquid nitrogen bath (5). Normal GC operating conditions were then used with a Finnigan 4000 GC/mass spectrometer with INCOS data system (Finnigan Corp., Sunnyvale, Calif.).

Samples were mixed with 10 ml of ACS (Amersham Corp.) and assayed for ¹⁴C with a TriCarb model 4530 liquid scintillation spectrometer (Packard Instruments Co., Downers Grove, Ill.). Corrections for counting efficiency were made by the channels ratio method (3), resulting in a minimum detectable activity at the 97.5% confidence level of 3.0 dpm (1).

Small anaerobic continuous-flow column study. Two alumi-

num foil-covered upflow glass columns (2.5-cm inside diameter by 22 cm) filled with 3-mm glass beads were connected in series and operated under anaerobic conditions at 22°C as described previously (4). These columns had been in operation for several years. A sterile medium (7) containing 100 mg of sodium acetate per liter was continuously applied to the lead column with a syringe pump equipped with a 60-ml plastic syringe (4). A second solution containing 110 mg of PCE and 540 mg of acetate per liter was driven from a 10-ml gas-tight syringe and mixed with effluent from the first column at a volumetric ratio of 1:4.4. This resulted in a second-column influent containing 20 mg of PCE and 100 mg of acetate per liter. The feed rate to the second column was 10 ml/day (superficial loading velocity, 2 cm/day), resulting in an actual liquid detention time of 4 days. The tubing, fittings, and sample vials were made of Teflon or glass to minimize sorptive interactions.

After 3 months of operation, [¹⁴C]PCE was added to the second-column feed and the liquid detention time was reduced to 2 days. After an additional 4 months, the second-column influent was reduced to 30 µg of PCE per liter, and the liquid detention time was increased to 4 days.

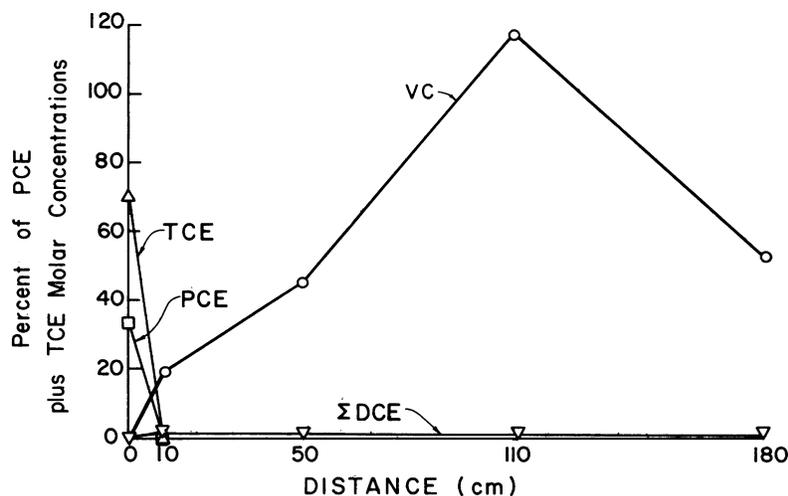


FIG. 1. Profiles of PCE (□), TCE (△), DCE (▽), and VC (○) in the large column after 10 days of operation.

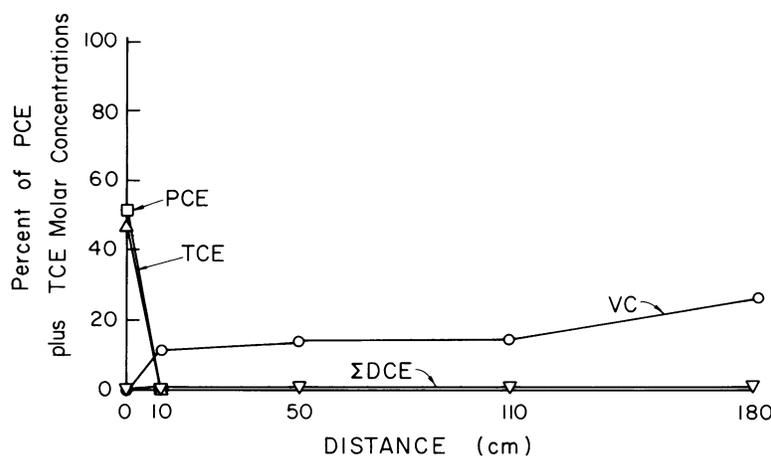


FIG. 2. Profiles of PCE (□), TCE (△), DCE (▽), and VC (○) in the large column after 22 days of operation.

Effluent from the second column was collected in a 20-ml glass syringe barrel with a tight-fitting Teflon float to prevent volatilization losses. Sample extractions were performed on the samples in the syringe barrel. The establishment of steady-state operation was assumed when the concentrations of organics in the effluent no longer varied. This normally required a time period equivalent to at least four detention times.

Large anaerobic continuous-flow column study. An upflow plastic column (20-cm inside diameter by 200 cm) filled with smooth 6-cm-diameter quartzite rocks (13), supporting methanogenic bacteria, and operated at 35°C was fed a non-sterile solution containing acetone, isopropanol, *o*-xylene, 1,1,1-trichloroethane, PCE, TCE, trace nutrients, phosphate, and ammonia (Table 1). This solution, contained in a 20-liter glass bottle stored at 10°C, was delivered to the column by a peristaltic pump at a rate of 2 liters/day (superficial loading velocity, 33 cm/day), resulting in a liquid detention time of 6 days. Active methanogenic conditions in the column had been established over several years through periodic treatment of different organic waste streams. Measurement over time indicated a loss of volatile compounds from the feed storage bottle during each feed cycle (9 days).

Effluent was collected in a 20-liter glass bottle, but analyses were conducted only with samples removed directly from the column through ports at various heights (10, 50, 110, and 180 cm above the influent port). Analyses of samples for halogenated aliphatics were provided by Canonic Engineers, using the purge-and-trap GC method (2). A true steady state of operation was never actually attained due to fluctuations in the feed concentration.

Identification of transformation products by radioactivity. For each ^{14}C measurement, three effluent samples from the small column were counted. One milliliter was injected into a glass counting vial containing 12 drops of 1 N HCl, another 1 ml into a vial containing 12 drops of 1 N NaOH, and a third 1 ml into a vial containing 10 ml of liquid scintillation counting solution (ACS). The first two samples were then purged with nitrogen gas (100 ml/min for 5 min), and 10 ml of liquid scintillation counting fluid was added. Purging provided a presumptive test for [^{14}C]PCE conversion to $^{14}\text{CO}_2$, which would not be stripped at high pH, but would be at low pH. Also, the total amount of volatile ^{14}C -labeled organics, including reactant and intermediates, could be determined, as they would be stripped at any pH.

The production of $^{14}\text{CO}_2$ was confirmed by adding 2.0 g of $\text{Ba}(\text{NO}_3)_2$ to a filtered sample at high pH, mixing for 30 min, and assaying the radioactivity in the solution before and after membrane filtration of the barium carbonate precipitate formed (9).

RESULTS

Small-column study. The small column with a 2-day detention time exhibited efficient removal of PCE from 20.5 mg/liter to 4.4 $\mu\text{g/liter}$, a 99.98% reduction. In the process, 4.1 mg of TCE per liter was formed, representing about 25% of the original PCE on a molar basis (Table 2). Formation of the chlorinated metabolites TCE, DCE, and VC was found by GC/mass spectrometry, but concentrations were not quantified. In addition, traces of ethyl chloride and other unidentified compounds were found. At lower concentrations of PCE, 60 $\mu\text{g/liter}$ (360 nmol/liter) and 31 $\mu\text{g/liter}$ (186 nmol/liter), the small column exhibited reductions of 99 and 98%, respectively (Table 2). Here, the TCE effluent concentrations represented about 66 and 73%, respectively, of the original PCE. With the lower PCE concentrations, [^{14}C]PCE was included in the feed. The effluent $^{14}\text{CO}_2$ activity increased from 12% with a 2-day detention time at 60 μg of PCE per liter to 24% with a 4-day detention time and 31 μg of PCE per liter (Table 2).

To obtain evidence that VC might be a major intermediate in PCE transformation, 100 μg of VC per liter was added to the small-column feed when operated at a 4-day detention time. PCE removal decreased from 98 to 87%, somewhat complicating the interpretation. However, $^{14}\text{CO}_2$ activity decreased from 24 to 12%, suggesting that the large VC pool created may have decreased the extent of [^{14}C]VC conversion. The feed to the small column was then stopped for 48 h, and the $^{14}\text{CO}_2$ activity in the column was monitored. It increased to 23% of the influent, again suggesting that [^{14}C]VC captured in the VC pool may have been oxidized.

Large-column study. The large anaerobic column also exhibited significant biotransformation of PCE. The PCE and TCE influents of about 300 $\mu\text{g/liter}$ were reduced to below 5 $\mu\text{g/liter}$ at the 10-cm port. Although some DCE was detected, concentrations remained below 3 $\mu\text{g/liter}$. VC, on the other hand, increased considerably whereas PCE and TCE disappeared. VC reached 290 $\mu\text{g/liter}$ at the 110-cm port after 10 days of column operation. This represents over 100% of the influent PCE plus TCE molar concentrations.

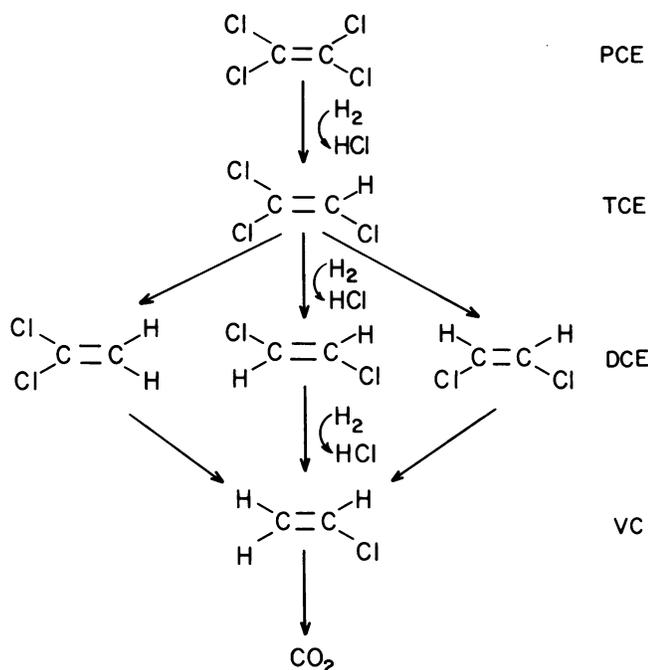


FIG. 3. Schematic for possible pathway for conversion of PCE to CO₂ through reductive dehalogenation.

After 22 days of column operation, the highest concentration of VC found was 57 $\mu\text{g}/\text{liter}$ at the 50-cm port, which represented about 23% of the influent PCE plus TCE molar concentrations. Thus, biotransformation of the VC intermediate appears to have occurred. However, this needs to be confirmed, perhaps with ¹⁴C tracer studies. Profiles of the PCE, TCE, DCE, and VC for days 10 and 22 are shown in Fig. 1 and 2, respectively.

DISCUSSION

Evidence from the small anaerobic continuous-flow fixed-film column study indicates that a major intermediate in the biotransformation of PCE is TCE. Up to 74% of influent PCE (molar basis) was found present as TCE in some effluent samples. This process of reductive dehalogenation was suggested previously (4, 8) and if continued would transform TCE to DCE and then to VC (Fig. 3). The observation of DCE and the essentially quantitative transformation of PCE and TCE to VC in the large anaerobic continuous-flow fixed-film column support this hypothesis.

To date, no data have been available indicating PCE mineralization to carbon dioxide. The production of ¹⁴CO₂ from [¹⁴C]PCE in the small-column study indicates that at least partial mineralization of PCE can occur. At least two different pathways can be suggested for this mineralization. Either the pathway resulting in TCE, DCE, and VC formation is different from that which produces carbon dioxide or carbon dioxide is a result of VC mineralization. Since nonlabeled VC addition reduced the production of ¹⁴CO₂ in the small-column study, circumstantial support is available

for the latter possibility. The hypothesis is that the additional pool of VC created reduced the amount of [¹⁴C]VC transformed to ¹⁴CO₂. The pathway for VC transformation to CO₂ could be similar to that elucidated for the aerobic mineralization of 1,2-dichloroethane (11). The initial step would be the addition of water across the double bond to form 2-chloroethanol, followed by oxidation to form the aldehyde. This may be followed by eventual complete oxidation to CO₂ and perhaps CH₄. The possibility of these reactions occurring anaerobically should not be discounted because molecular oxygen would not be necessary. The conversion of VC to CO₂ under methanogenic conditions is still speculative, however, and a more definitive study is still needed.

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LITERATURE CITED

1. Altshuler, B., and B. Pasternack. 1963. Statistical measurement of the lower limits of detection of a radioactivity counter. *Health Phys.* 9:293-298.
2. American Public Health Association. 1980. Standard methods for examination of water and waste water, 15th ed. American Public Health Association, Washington, D.C.
3. Bell, G. G., and F. N. Hayes. 1958. Liquid scintillation counting. Peragamon Press, Inc., New York.
4. Bouwer, E. J., and P. L. McCarty. 1983. Transformation of 1- and 2-carbon halogenated aliphatic organic compounds under methanogenic conditions. *Environ. Sci. Technol.* 45:1286-1294.
5. Graydon, J. W., K. Grob, F. Zuercher, and W. Giger. 1983. Determination of highly volatile organic water contaminants by the closed-loop gaseous stripping technique followed by thermal desorption of the activated carbon filters. *J. Chromatogr.* 285:307-318.
- 5a. Grob, K., and F. Zuercher. 1976. Stripping of trace organic substances from water—equipment and procedures. *J. Chromatogr.* 117:285-299.
6. Henderson, J. E., G. R. Peyton, and W. H. Glaze. 1976. A convenient liquid-liquid extraction method for the determination of halomethanes in water at the parts-per-billion level, p. 105-112. In L. H. Keith (ed.), *Identification and analysis of organic pollutants in water*. Ann Arbor Science Publishers, Inc., Ann Arbor, Mich.
7. Owen, W. F., D. C. Stuckey, J. B. Healy, Jr., L. Y. Young, and P. L. McCarty. 1979. Bioassay for monitoring biochemical methane potential and anaerobic toxicity. *Water Res.* 13:485-492.
8. Parsons, F., P. R. Wood, and J. DeMarco. 1984. Transformations of tetrachloroethylene and trichloroethylene in microcosms and groundwater. *J. Am. Water Works Assoc.* 76:56-59.
9. Rapkin, E. 1962. Measurement of ¹⁴CO₂ by scintillation techniques. Packard technical bulletin no. 7. Packard Instruments Co., La Grange, Ill.
10. Roberts, P. V., J. E. Schreiner, and G. D. Hopkins. 1982. Field study of organic water quality changes during ground water recharge in the Palo Alto Baylands. *Water Res.* 16:1025-1035.
11. Stucki, G., U. Krebsler, and T. Leisinger. 1983. Bacterial growth on 1,2-dichloroethane. *Experientia* 39:1271-1273.
12. Westerick, J. J., J. W. Mello, and R. F. Thomas. 1984. The groundwater supply survey. *J. Am. Water Works Assoc.* 76:52-59.
13. Young, J. C., and P. L. McCarty. 1969. The anaerobic filter for waste treatment. *J. Water Pollut. Control Fed.* 41:R160-R173.