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Reductive Dechlorination of Trichloroethylene and Tetrachloroethylene under Aerobic Conditions in a Sediment Column

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Biodegradation of trichloroethylene and tetrachloroethylene under aerobic conditions was studied in a sediment column. Cumulative mass balances indicated 87 and 90% removal for trichloroethylene and tetrachloroethylene, respectively. These studies suggest the potential for simultaneous aerobic and anaerobic biotransformation processes under bulk aerobic conditions.

Biodegradation of trichloroethylene (TCE) and tetrachloroethylene (PCE) has been demonstrated in pure cultures (11, 12, 16, 22, 25, 27, 28), mixed cultures (1, 2, 3, 14, 15, 17), microcosms (18, 21), and soil columns (29, 30). Field demonstrations of in situ bioremediation of chlorinated solvents have included biostimulation of indigenous methane-oxidizing bacteria (methanotrophs) (24) and bioaugmentation with a metabolic, nutrient inducer (23). Both demonstrations were aerobic systems and focused on biodegradation of vinyl chloride (VC), dichloroethylene (DCE), and TCE.

Aerobic stimulation of methanotrophs may encourage the in situ cometabolic biodegradation of TCE but not PCE. Although TCE is degraded under both aerobic and anaerobic conditions (3, 15, 17), PCE transformation has been demonstrated only under anaerobic conditions (3, 12, 17). Laboratory studies have shown that anaerobic dechlorination of chlorinated ethylenes can proceed to nontoxic, biodegradable products such as ethylene and ethane (7, 8, 17); however, there is the tendency for significant amounts of VC and *cis*-1,2-dichloroethylene (cDCE) to accumulate under anaerobic conditions (5, 26). Although stimulation of reductive dechlorination of PCE and TCE may be a viable alternative at sites where aquifers are already anaerobic, it may be unacceptable to create anaerobic conditions in an aerobic aquifer. It would be desirable, therefore, if both (i) anaerobic PCE or TCE dechlorination and (ii) aerobic TCE, DCE, and VC degradation could occur in sediments maintained under "bulk" aerobic conditions. Phelps et al. demonstrated that this phenomenon does occur in methanotrophic expanded-bed bioreactors (23a). Anaerobic dechlorination of PCE or TCE would produce products, e.g., VC or cDCE, more amenable to subsequent aerobic transformation.

In collaboration with the U.S. Department of Energy (DOE) Office of Technology Development Integrated Technology Demonstration at the Savannah River Site for the in situ bioremediation of chlorinated solvents (19), a sediment column study was conducted to investigate the biodegradation potentials of TCE and PCE during aerobic methanotrophic biostimulation.

Soil column design and operation. A 122-cm sediment column was assembled with composite sediments from three sediment horizons collected during site characterization at the Savannah River Site. Composite A consisted of sediments from the saturated zone at depths of 53.3 to 59.4 m. Composites B and C consisted of sediments from the unsaturated zone at depths of 22.9 to 30.5 and 9.1 to 13.7 m, respectively. Table 1 lists composite sediment characteristics. The column was separated into three sections corresponding to composite types A to C (Fig. 1). Ports for obtaining liquid samples were placed in each section and in influent and effluent lines. Eight side ports were installed in each section for sediment sampling. Sediment samples were taken with a sterile 10-ml syringe barrel and replaced by extruding similar composite sediments from a 10-ml syringe back into the side port. A 5-liter Tedlar gas sampling bag was connected to the column carboy feed water with Viton tubing. The gas bag served two functions: (i) to replace volume lost in the carboy as water levels dropped and (ii) to maintain stable concentrations of nutrients (air, oxygen, and CH_4 in the gas phase) which were in equilibrium with column feed water.

Groundwater from an uncontaminated well was pumped through the column in an upflow direction with a peristaltic pump at an average flow rate of 1.2 ml/min. Column detention time was ≈30 h. Operating conditions with respect to nutrient and TCE and PCE additions to column feed water are listed in Table 2. CH_4 and O_2 were added to column feed water by sparging separate aliquots of well water with either gas and then mixing methane- or oxygen-saturated aliquots in appropriate ratios (Table 2). TCE and PCE (Aldrich, Milwaukee, Wis.) were added to a final concentration of 500 μg/liter to the column feed by using a methanol-based stock solution. The resultant methanol concentration in the feed water was 2.5 mM. Volatile organic carbon (VOC) concentrations in influent and effluent samples were measured twice daily. The column was maintained at room temperature, 18 to 25°C, during the entire experiment. Cumulative masses of TCE and PCE were calculated by Euler integration (6). This stepwise integration was needed because of variations in measured influent concentrations.

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TABLE 1. Characteristics of composite sediments

Composite	Zone	Clay/sand/ gravel ratio	Porosity	TOC[a] (ppm)	Moisture content (%)
A	Saturated	1.7/98.2/0.1	0.33	74.0	18.7
B	Lower vadose	1.6/98.2/0.2	0.35	43.0	1.0
C	Upper vadose	5.2/92.2/2.6	0.32	46.0	2.7

[a] TOC, total organic carbon.

Analytical methods. TCE, PCE, cDCE, and VC concentrations in pore fluids were measured on a Hewlett-Packard 5890A gas chromatograph equipped with a Hewlett-Packard 19395A automated gas headspace analyzer, an electron capture detector, and a 60-m Vocol (Supelco, Bellefonte, Pa.) column. Column temperature was held at 35°C for 8 min and then was increased (5°/min) to 80°C. Helium was used as the carrier gas at a flow rate of 12 ml/min. Samples (1 ml each) were dispensed into headspace vials containing 9 ml of deionized H_2O, which were immediately crimped, and then the samples were equilibrated at 75°C for 1 h prior to analysis. Prior time course analyses indicated 1-h equilibration to be sufficient for VOC partitioning into headspace. Standards containing 10 ml were made with each run, eliminating the need to use Henry's constant for calculations. Detection limits for TCE, PCE, VC, and DCE were, 1.0, 1.0, 150, and 50.0 μg/liter, respectively. Dissolved oxygen and pH were measured with microelectrodes (Microelectrodes Inc., Londonderry, N.H.) following a two-point calibration. Dissolved oxygen

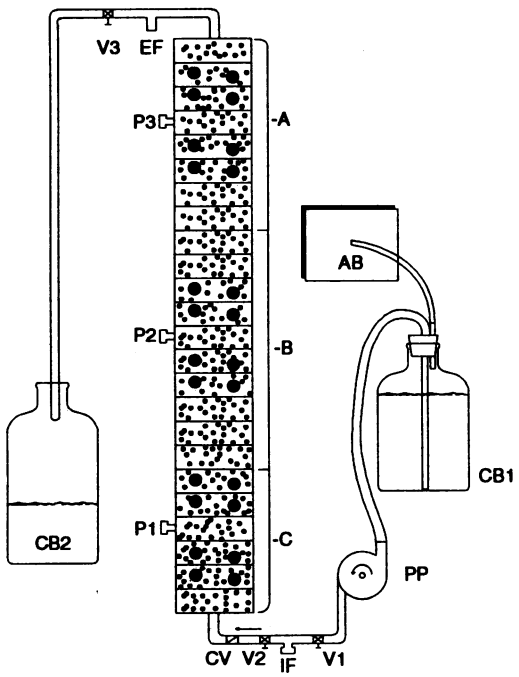

FIG. 1. Diagram of the column. Section A contained composite sediments from the saturated zone; section B and C sediments were from unsaturated zones. Side ports were for sampling sediments and pore waters. V1 to -3, valves; CB1, column feed water carboy; CB2, collection carboy; AB, Teflon gas bag; CV, check valve; P1 to -3, pore water sampling ports; IF and EF, influent and effluent sampling ports, respectively; PP, peristaltic pump. Large solid circles, sediment sampling ports.

TABLE 2. Experimental conditions of column feed water

Days	Gas[a]
0–178	Air
178–262	CH_4-O_2
262–315[b]	CH_4-O_2
315–402	CH_4-O_2
402–436	O_2

[a] CH_4 and O_2 concentrations were used in various ratios of percent saturation from 80:20 to 20:80 for CH_4/O_2. Air and O_2 alone were used at 100% saturation. PCE and TCE (500 μg/liter each) were added beginning at day 140. The column was maintained at room temperature, 18 to 25°C, during the experiment.
[b] Nitrate (940 μM) was added.

measurements of oxygen-free water, sampled by the same technique as pore waters, confirmed that oxygen was not introduced into pore water samples during sampling.

Microbial characterization. Aerobic heterotrophic bacteria were enumerated by the most probable number (MPN) technique on 1% PTYG medium (3). Tenfold serial dilutions were not used, since calculations of MPN were performed by using a computer program which allowed for more flexible dilution schemes (20). Positive aerobic MPN tubes were scored on the basis of turbidity after 3 to 5 days. MPN enumerations of anaerobic bacteria were done in anaerobic culture tubes equipped with butyl rubber stoppers and aluminum crimp seals (Bellco, Vineland, N.J.). The medium used for enumeration of anaerobes contained (per liter) 2.0 g of KH_2PO_4, 0.3 g of NH_4Cl, 0.5 g of NaCl, 0.7 g of Na_2SO_4, 0.4 g of $MgCl_2 \cdot 2H_2O$, 0.5 g of KCl, 0.2 g of $CaCl_2 \cdot 2H_2O$, 0.5 g of Na acetate, 0.4 g of Na formate, 0.5 g of tryptone, 1.0 g of yeast extract, 2.5 g of $NaHCO_3$, 0.5 g of cysteine, 0.5 g of $Na_2S \cdot 9H_2O$, 1.0 mg of resazurin, 2.0 mg of $FeNH_4(SO_4)_2$, 5.0 mg of $NiCl_2$, and 10 ml of trace metal solution. The pH was adjusted to 7.2. The trace metal solution contained (per liter) 1.5 g of nitrilotriacetic acid, 2.0 g of $MgSO_4 \cdot 7H_2O$, 0.5 g of $MnSO_4 \cdot H_2O$, 1.0 g of NaCl, 0.1 g of $FeSO_4 \cdot 7H_2O$, 0.18 g of $CoCl_2 \cdot 6H_2O$, 0.18 g of $ZnSO_2 \cdot 7H_2O$, 14.0 mg of $CuSO_4 \cdot 5H_2O$, 10.0 mg of H_3BO_3, and 10.0 mg of $NaMoO_4 \cdot 2H_2O$. Tubes were pressurized (10 lb/in^2) with oxygen-free 80:20% H_2-CO_2. This medium was not selective for any specific anaerobic population and was meant to support both facultative and strict anaerobes. Tubes for anaerobic enumerations were incubated horizontally at 25°C and scored on the basis of turbidity after 30 days. For all MPN enumerations, a 1:10 sediment-medium slurry served as the initial sample for subsequent dilutions.

Column experiment. Aerobic conditions were maintained in

TABLE 3. Oxygen trends

Port	Oxygen (mg/liter)		
	Mean	Maximum	Minimum
Days 0–337			
Influent	11.0	26.3	3.2
Effluent	4.8	10.4	2.4
A	4.8	9.8	2.4
B	4.3	8.8	1.9
C	5.2	11.1	1.6
Days 338–436			
Influent	15.1	24.7	6.3
Effluent	5.1	7.4	3.5
A	3.8	5.3	2.7
B	4.5	6.5	2.7
C	4.2	6.1	3.1

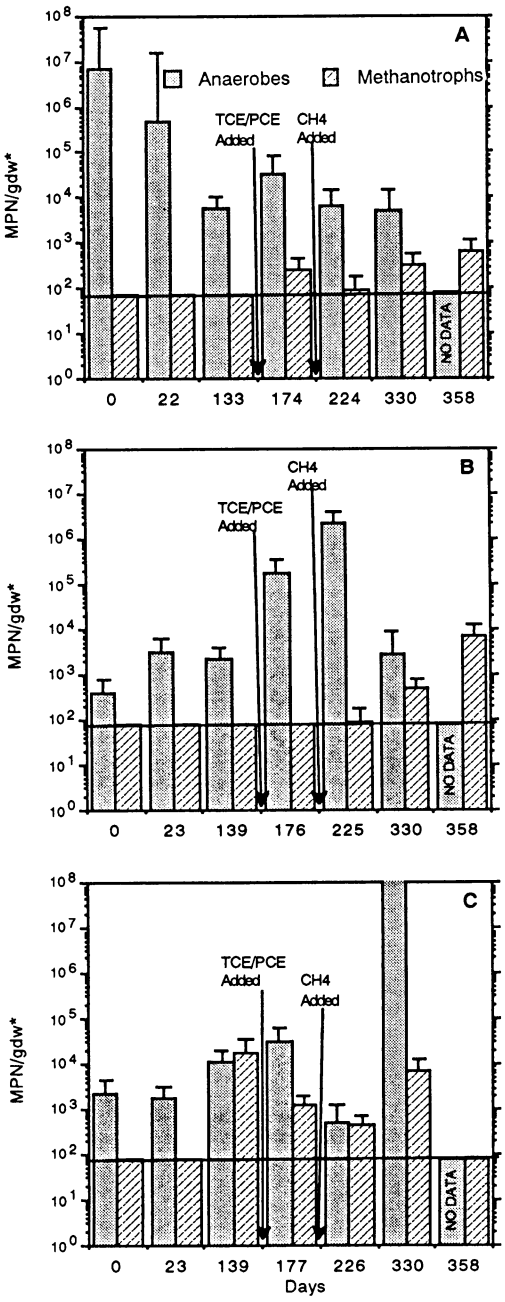

FIG. 2. MPN enumeration of aerobic heterotrophic bacteria in 1% PTYG medium compared with anaerobic bacteria. gdw, grams (dry weight).

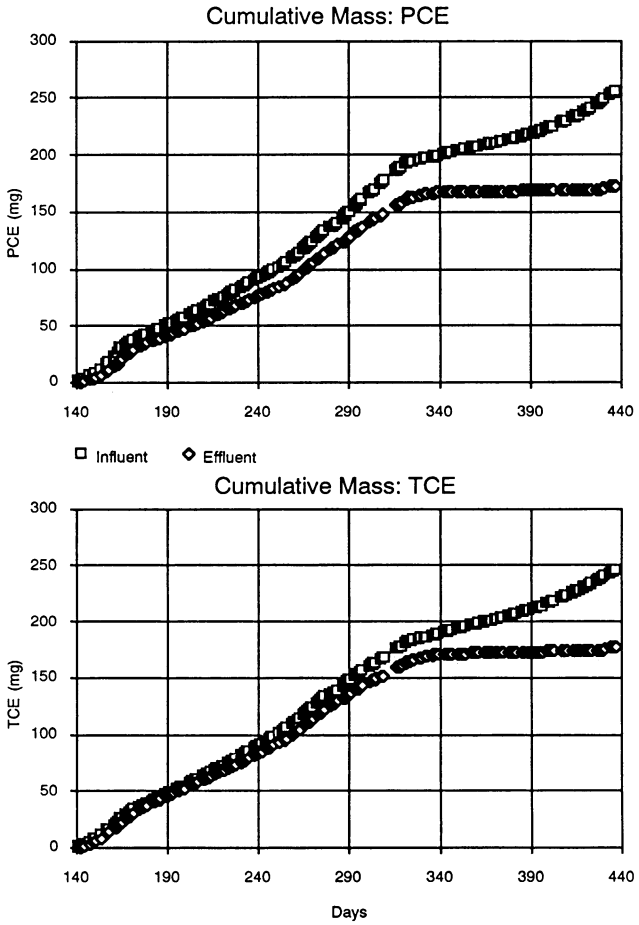

FIG. 3. Cumulative masses for TCE and PCE were derived by Euler integration from concentrations between sampling periods. The influent curve represents total mass loading of TCE and PCE. The difference between the influent and effluent curves represents the amount removed.

the column throughout the experiment (Table 3). In no case were concentrations less than 1.6 mg/liter, i.e., approximately 20% of saturation in air, detected. This low concentration of dissolved oxygen at port C may have resulted from microsite conditions at the sampling port; concentrations in the bulk pore fluids were probably even higher, as indicated by higher concentrations at downstream ports. During the period of greatest TCE and PCE removal, days 338 to 436, the lowest dissolved oxygen concentration was only 2.7 mg/liter (Table 3).

Results of MPN enumerations of aerobic heterotrophs and anaerobes are illustrated in Fig. 2. It is evident that abundant populations of both aerobic and anaerobic microorganisms were present throughout the experiment. A significant portion of the anaerobic enumerations may actually represent facultative anaerobes capable of growing under strictly anaerobic conditions. Methane was measured in pore waters of all sampling ports and in MPN enumeration tubes, suggesting that methanogens were present throughout the column. This suggests that microsites which were capable of supporting strict anaerobes existed in the soil column. Methanotrophs were detected in all three sections of the column (data not shown) at low densities. Even after 6 months of CH_4 exposure, the maximum number of methanotrophs detected was 100 MPN/g (dry weight). However, both methane and dissolved oxygen concentrations were adequate to support methanotroph populations.

TCE and PCE transformation. Significant differences ($P < 0.0001$) in influent and effluent concentrations for TCE and PCE were observed during the first 6.5 months (period 1, days 140 to 337) and the last 3.5 months (period 2, days 338 to 436). Transformation of TCE and PCE was much greater, however, during period 2. Cumulative mass balances indicated 87 and 90% removal for TCE and PCE, respectively, during period 2 compared with 9 and 16% during period 1 (Fig. 3). TCE and

PCE disappearance during period 1 could be due entirely to abiotic losses, i.e., adsorption, volatilization, or abiotic transformation. Losses during period 1 can be subtracted from losses in period 2 to conservatively estimate removal by biotransformation during the latter period. In this manner, conservative TCE and PCE biotransformation rates during period 2 were 76 and 74%, respectively. During period 2, cDCE was observed as the major product of both TCE and PCE transformation. No VC or other chlorinated products were detected.

Considering the low biomass of methanotrophs and the presence of cDCE, cometabolic biodegradation of TCE by methanotrophs was probably insignificant compared with anaerobic dechlorination. Anaerobic conditions apparently developed in microsites since column pore waters remained aerobic. Reductive dechlorination of TCE and PCE under methanogenic conditions can proceed to VC (8, 17, 29), whereas cDCE has tended to accumulate under sulfate-reducing conditions (3, 21). Accumulation of VC and cDCE may occur when there is an insufficient supply of electron donors (8, 9, 17). The addition of 2.5 mM methanol in these studies provided sufficient reducing equivalents to completely reduce the added TCE and PCE to ethylene. Recent studies of anaerobic dechlorination of PCE have shown that the form of carbon substrate determines the dechlorination potential of a selected microbial community (18). In our study methanol may have been effective in stimulating methanogenesis but not in promoting complete reductive dechlorination. The apparent accumulation of cDCE, therefore, suggests that (i) methanogens may not have been solely responsible for the dechlorination of TCE and PCE or (ii) dechlorination activity may have been partially inhibited by oxygen.

Kastner (21) also observed cDCE accumulation in microcosms under sulfate-reducing conditions and suggested that facultative anaerobes may have been responsible for reductive dechlorination on the basis of the dependency of aerobic consortia in microcosms. Facultative anaerobes may also have been, at least partially, responsible for reductive dechlorination activity in our studies. Enumerations of aerotolerant and facultative anaerobic bacteria showed that such populations were comparable in size to aerobic populations (data not shown).

The results from this study clearly show that anaerobic dechlorination of TCE and PCE can be observed in a column maintained under bulk aerobic conditions. Previous work with fluidized expanded-bed bioreactors with Savannah River site consortia from the same site had the same results (23a). Methanogenesis in the column strongly suggests that anaerobic zones or microsites existed, allowing the simultaneous presence of both aerobic and anaerobic microorganisms. These results have important implications for both in situ and on-site PCE and TCE bioremediation projects in which complete anaerobic conditions are either environmentally undesirable or unacceptable by regulatory standards. Sequential anaerobic and aerobic treatments have been suggested to anaerobically dehalogenate fully halogenated compounds and, subsequently, aerobically transform less-halogenated analogs (10, 13). The studies described here suggest that both anaerobic and aerobic populations may be stimulated simultaneously while maintaining an aquifer under bulk aerobic conditions. Data from the Savannah River Site methane injection demonstration also suggest that this is true, since PCE decreased in sediments at some sites in the absence of soil vapor extraction. Pilot and field demonstrations of both strategies, i.e., stimulation of anaerobic microsites in an aerobic aquifer and sequential anaerobic and aerobic treatments, are needed in order to determine the applicability of these remediation designs.

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REFERENCES

1. **Alvarez-Cohen, L., P. L. McCarty, E. Boulygina, R. S. Hanson, G. A. Brusseau, and H. C. Tsien.** 1992. Characterization of a methane-utilizing bacterium from a bacterial consortium that rapidly degrades trichloroethylene and chloroform. Appl. Environ. Microbiol. **58:**1886–1893.
2. **Baek, N. H., and P. R. Jaffe.** 1989. The degradation of trichloroethylene in mixed methanogenic cultures. J. Environ. Qual. **18:** 515–518.
3. **Bagley, D. M., and J. M. Gossett.** 1990. Tetrachloroethene transformation to trichloroethene and *cis*-1,2-dichloroethene by sulfate-reducing enrichment cultures. Appl. Environ. Microbiol. **56:** 2511–2516.
4. **Balkwill, D. L.** 1989. Numbers, diversity, and morphological characteristics of aerobic, chemoheterotrophic bacteria in deep subsurface sediments from a site in South Carolina. Geomicrobiol. J. **7:**33–52.
5. **Beeman, R., S. Shoemaker, J. Howell, E. Salazar, and J. Buttram.** 1993. A field evaluation of in situ microbial reductive dehalogenation by the biotransformation of chlorinated ethylenes, abstr. A-2. *In* In situ and on-site bioreclamation: 2nd International Symposium, San Diego, Calif. Battelle, Columbus, Ohio.
6. **Carnahan, B., H. A. Luther, and J. O. Wilkes.** 1969. Applied numerical methods, p. 344–346. John Wiley & Sons, New York.
7. **Debruin, W. P., M. Kotterman, M. A. Posthumus, G. Schraa, and A. Zehnder.** 1992. Complete biological reductive transformation of tetrachloroethylene to ethane. Appl. Environ. Microbiol. **58:**1996–2000.
8. **DiStefano, T. D., J. M. Gossett, and S. H. Zinder.** 1991. Reductive dechlorination of high concentrations of tetrachloroethylene to ethene by an anaerobic enrichment culture in the absence of methanogenesis. Appl. Environ. Microbiol. **57:**2287–2292.
9. **DiStefano, T. D., J. M. Gossett, and S. H. Zinder.** 1992. Hydrogen as an electron-donor for dechlorination of tetrachloroethylene by an anaerobic mixed culture. Appl. Environ. Microbiol. **58:**3622–3629.
10. **Dooley-Dana, M., S. Fogel, and M. Findlay.** 1989. The sequential anaerobic/aerobic biodegradation of chlorinated ethenes in an aquifer simulator, abstr. Q32, p. 335. Abstr. 89th Annu. Meet. Am. Soc. Microbiol. 1989. American Society for Microbiology, Washington, D.C.
11. **Ensign, S. A., M. R. Hyman, and D. J. Arp.** 1992. Cometabolic degradation of chlorinated alkenes by alkene monooxygenase in a propylene-grown *Xanthobacter* strain. Appl. Environ. Microbiol. **58:**3038–3046.
12. **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.
13. **Fathepure, B. Z., and T. M. Vogel.** 1991. Complete degradation of polychlorinated hydrocarbons by a two-stage biofilm reactor. Appl. Environ. Microbiol. **57:**3418–3422.
14. **Fliermans, C. B., T. J. Phelps, D. Ringelberg, A. T. Mikell, and D. C. White.** 1988. Mineralization of trichloroethylene by heterotrophic enrichment cultures. Appl. Environ. Microbiol. **54:**1709–1714.
15. **Fogel, M. M., A. R. Taddeo, and S. Fogel.** 1986. Biodegradation of chlorinated ethylenes by a methane-utilizing mixed culture. Appl. Environ. Microbiol. **51:**720–724.
16. **Folsom, B. R., P. J. Chapman, and P. H. Pritchard.** 1990. Phenol

and trichloroethylene degradation by *Pseudomonas cepacia* g4: kinetics and interactions between substrates. Appl. Environ. Microbiol. **56:**1279–1285.

17. **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.
18. **Gibson, S. A., and G. W. Sewell.** 1992. Stimulation of reductive dechlorination of tetrachloroethylene in anaerobic aquifer microcosms by addition of short-chain organic acids or alcohols. Appl. Environ. Microbiol. **58:**1392–1393.
19. **Hazen, T. C.** 1992. Test plan for in situ bioremediation demonstration of the Savannah River Integrated Demonstration, WSRC-RD-91-23. Westinghouse Savannah River Co., Aiken, S.C.
20. **Hurley, M. A., and M. E. Roscoe.** 1983. Automated statistical analysis of microbial enumeration by dilution series. J. Appl. Bacteriol. **55:**159–164.
21. **Kastner, M.** 1991. Reductive dechlorination of trichloroethylenes and tetrachloroethylenes depends on transition from aerobic to anaerobic conditions. Appl. Environ. Microbiol. **57:**2039–2046.
22. **Little, C. D., A. V. Palumbo, S. E. Herbes, M. E. Lidstrom, R. L. Tyndall, and P. J. Gilmer.** 1988. Trichloroethylene biodegradation by a methane-oxidizing bacterium. Appl. Environ. Microbiol. **54:**951–956.
23. **Nelson, M. J., J. V. Kinsella, and T. Montoya.** 1990. In situ biodegradation of TCE contaminated groundwater. Environ. Prog. **9:**190–196.

23a. **Phelps, T. J., J. J. Niedzielski, K. J. Malachowsky, R. M. Schram, S. E. Herbes, and D. C. White.** 1991. Biodegradation of mixed-organic wastes by microbial consortia in continuous-recycle expanded-bed bioreactors. Environ. Sci. Technol. **25:**1461–1465.

24. **Semprini, L., P. V. Roberts, G. D. Hopkins, and P. L. McCarty.** 1990. A field-evaluation of in situ biodegradation of chlorinated ethylenes. Results of biostimulation and biotransformation experiments. Ground Water **28:**715–727.
25. **Shields, M. S., S. O. Montgomery, P. J. Chapman, S. M. Cuskey, and P. H. Pritchard.** 1989. Novel pathway of toluene catabolism in the trichloroethylene-degrading bacterium G4. Appl. Environ. Microbiol. **55:**1624–1629.
26. **Smith, G., and G. Ferguson.** 1993. In situ remediation of groundwater contaminated with chlorinated solvents using anaerobic biotransformation, abstr. C-2. In Situ and On-Site Bioreclamation: 2nd International Symposium, San Diego, Calif. Battelle, Columbus, Ohio.
27. **Tsien, H.-C., G. A. Brusseau, R. S. Hanson, and L. P. Wackett.** 1989. Biodegradation of trichloroethylene by *Methylosinus trichosporium* OB3b. Appl. Environ. Microbiol. **55:**3155–3161.
28. **Uchiyama, H., T. Nakajima, O. Yagi, and T. Nakahara.** 1992. Role of heterotrophic bacteria in complete mineralization of trichloroethylene by *Methylocystis* sp. strain M. Appl. Environ. Microbiol. **58:**3067–3071.
29. **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.
30. **Wilson, J. T., and B. H. Wilson.** 1985. Biotransformation of trichloroethylene in soil. Appl. Environ. Microbiol. **49:**242–243.