

## Anaerobic Oxidation of [1,2-<sup>14</sup>C]Dichloroethene under Mn(IV)-Reducing Conditions

PAUL M. BRADLEY,<sup>1\*</sup> JAMES E. LANDMEYER,<sup>1</sup> AND RICHARD S. DINICOLA<sup>2</sup>

*U.S. Geological Survey, Columbia, South Carolina 29210,<sup>1</sup> and  
U.S. Geological Survey, Tacoma, Washington 98402<sup>2</sup>*

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**Anaerobic oxidation of [1,2-<sup>14</sup>C]dichloroethene to <sup>14</sup>CO<sub>2</sub> under Mn(IV)-reducing conditions was demonstrated. The results indicate that oxidative degradation of partially chlorinated solvents like dichloroethene can be significant even under anoxic conditions and demonstrate the potential importance of Mn(IV) reduction for remediation of chlorinated groundwater contaminants.**

In chlorinated-ethene-contaminated groundwater systems, production of the Environmental Protection Agency priority pollutant vinyl chloride (VC) by anaerobic biodegradation of dichloroethene (DCE) is well documented (1, 2, 5, 7, 8, 10, 15, 17–19) and typically drives environmental concerns (2, 15). Aerobic oxidation of both compounds without accumulation of reduced intermediates is well-known but of limited relevance because DCE and VC are produced in situ under anaerobic conditions (1, 5, 7, 8, 10, 17–19). It is important, therefore, to determine which of the commonly occurring anaerobic terminal electron acceptors may be energetically sufficient to oxidize compounds like DCE and VC. Anaerobic microbial oxidation of VC with CO<sub>2</sub> as the product has been demonstrated in environmental samples under Fe(III)-reducing conditions (3, 4). In contrast, even though low but significant mineralization of DCE under anaerobic conditions has been reported (4, 16), the fact that mineralization involved significant accumulation of VC (4, 16) and was not enhanced by Fe(III) amendment (4) indicates that the initial step was a reduction and that Fe(III) reduction was not sufficient to oxidize DCE directly. These observations suggest that anaerobic oxidation of DCE requires a terminal-electron-accepting process that is more energetically favorable than Fe(III) reduction.

Mn(IV) oxides are common in alluvial and glacial aquifer sediments, and Mn(IV) reduction is more energetically favorable than Fe(III) reduction (9, 11, 14). The natural abundance of Mn(IV) (11, 14), the greater availability for microbial reduction of Mn(IV) oxides [relative to Fe(III) oxides] (14), and the more favorable energetics of Mn(IV) reduction (2, 11, 14) suggest that Mn(IV) reduction may support oxidation of DCE to CO<sub>2</sub> under anaerobic conditions without the accumulation of VC. To test this hypothesis, a microcosm study was initiated by using aquifer (shallow and intermediate) and surface sediments collected from a site at the Naval Undersea Warfare Center, Keyport, Washington. Aquifer material was characterized by fine to medium sands with some gravel and by an Mn(IV) oxide content of 0.05 to 0.1% (dry weight). Dissolved-Mn(II) concentrations at the site ranged from 0.04 to 5.7 mg/liter. Water chemistry analyses, field measurements of dissolved-H<sub>2</sub> concentrations, and laboratory incubations indicated that Mn(IV) reduction was the predominant terminal-electron-accepting process in the shallow and intermediate

aquifers at this site. The surface fill layer was characterized by silt to silty sand and consisted of a mixture of natural material, including glacial till. Water chemistry and H<sub>2</sub> concentration data were not available for the surface fill material. However, based on the shallow sample collection depth, periodic fluctuations in the water table with resultant exposure to air, spatial variability in Mn(IV) oxide availability, and Fe(III) oxide staining on the fill material, the predominant terminal-electron-accepting processes in the zone of the surface fill sample appear to vary among O<sub>2</sub>, Mn(IV), and Fe(III) reduction.

DCE mineralization was evaluated with a neat mixture of [1,2-<sup>14</sup>C]DCE (29% *trans* and 71% *cis* isomers; radiochemical purity  $\geq$  99.9%; Moravek Biochemicals, Inc., Brea, Calif.). Microcosm preparation, incubation conditions, sampling procedures, and methods for verification of <sup>14</sup>CO<sub>2</sub> have been described previously (3, 4, 6). In brief, 20-ml microcosms containing 15 g of saturated material each were created with headspaces of air (aerobic treatments) or 100% helium (anaerobic treatments). Some were amended with 1.0 ml of anoxic, sterile distilled water (aerobic and unamended anaerobic treatments), and the rest were amended with 1.0 ml (approximately 0.05 g) of an anoxic, sterile slurry of poorly crystalline MnO<sub>2</sub> (12, 13) or Fe(OH)<sub>3</sub> (13). Killed controls were autoclaved twice for 1 h each time at 15 lb/in<sup>2</sup> and 121°C. The microcosms were preincubated for 5 days and were then spiked with 40,000 dpm of [1,2-<sup>14</sup>C]DCE (specific activity = 0.6 mCi/mmol). <sup>14</sup>CO<sub>2</sub> was collected in 3 M KOH and quantified by liquid scintillation counting (3, 4, 6). Recovery of <sup>14</sup>CO<sub>2</sub> was confirmed in select microcosms as described previously (3, 4, 6).

The microorganisms indigenous to all three sample materials were capable of significant DCE mineralization to CO<sub>2</sub> under unamended anaerobic conditions (Fig. 1). For aquifer microcosms, mineralization ranged from 14 to 38% over three days. For the surface fill material, approximately 30% mineralization of [1,2-<sup>14</sup>C]DCE was observed after 17 days. In all cases, DCE mineralization was attributable to biological activity because the final recovery of <sup>14</sup>CO<sub>2</sub> in killed control microcosms was less than 4%. Mn(IV) reduction was the predominant terminal-electron-accepting process under unamended, anaerobic conditions, as indicated by the lack of significant dissolved O<sub>2</sub> ([O<sub>2</sub>] < 3 μM), NO<sub>3</sub> ([NO<sub>3</sub>] < 0.2 μM), and SO<sub>4</sub> ([SO<sub>4</sub>] < 20 μM); the lack of significant production of CH<sub>4</sub> (not detected, i.e., [CH<sub>4</sub>] < 1 μmol/liter of headspace), dissolved sulfide (not detected, i.e., [HS] < 0.2 μM), and dissolved Fe(II) [2 ± 2 nmol of Fe(II) produced (Table 1)]; and the significant accumulation of Mn(II) (9 ± 1 nmol produced [Table 1]) within these experimental microcosms.

\* Corresponding author. Mailing address: U.S. Geological Survey, Stephenson Center, Suite 129, Columbia, SC 29210. Phone: (803) 750-6125. Fax: (803) 750-6181. E-mail: pbradley@wrddmail.er.usgs.gov.

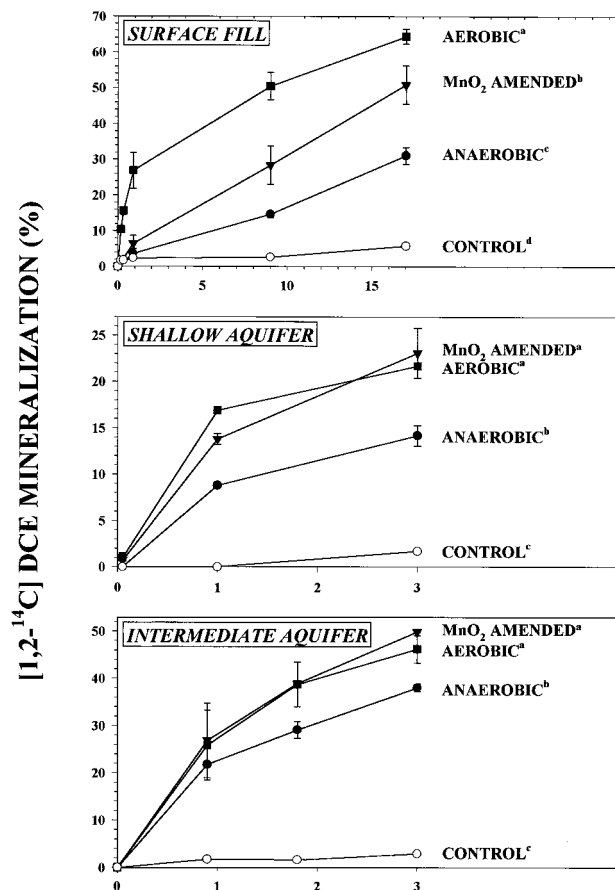


FIG. 1. Percentage mineralization of [1,2-<sup>14</sup>C]DCE to <sup>14</sup>CO<sub>2</sub> in microcosms containing material collected from the surface fill (depth, 1 m), the shallow aquifer (depth, 3 to 5 m) and the intermediate aquifer (depth, 8 to 10 m). Experimental data are means ± standard deviations for duplicate microcosms, and the control data are from a single sterile control microcosm. For each material studied, superscript letters indicate statistically significantly different final mean <sup>14</sup>CO<sub>2</sub> recoveries according to the Kruskal-Wallis one-way analysis of variance on ranks ( $P < 0.05$ ).

The bioavailability of Mn(IV) was a major factor affecting anaerobic DCE mineralization for all three sample materials (Fig. 1). Amendment of anaerobic microcosms with poorly crystalline MnO<sub>2</sub> resulted in a fivefold increase in Mn(IV)

TABLE 1. Mn(IV) and Fe(III) reduction during oxidation of [1,2-<sup>14</sup>C]DCE in intermediate aquifer microcosms<sup>a</sup>

Microcosm	Amt (nmol) of product <sup>b</sup>			
	Mn(II)		Fe(II)	
	Exptl	Control	Exptl	Control
Anaerobic	9 ± 1	1	NS	NS
Fe(OH) <sub>3</sub> amended	NS	NS	35 ± 11	3
MnO <sub>2</sub> amended	47 ± 6	NS	NS	NS
Aerobic	NS	NS	NS	NS

<sup>a</sup> Microcosms were vortex mixed and 1.0 ml of sediment supernatant was removed, filtered (0.2-μm pore size), and analyzed colorimetrically with commercially available test kits (Hach Chemical Co.).

<sup>b</sup> The amount of Mn(II) or Fe(II) produced is presented as a qualitative indicator of Mn(IV) or Fe(III) reduction because the Mn(II) or Fe(II) found in the dissolved phase is a small fraction (less than 10%) (14) of the total Mn(II) or Fe(II) present in the system. Experimental data are means ± standard deviations from duplicate microcosms, and control data are from a single sterile control. NS, no significant change.

TABLE 2. Oxidation of [1,2-<sup>14</sup>C]DCE to <sup>14</sup>CO<sub>2</sub> in anaerobic, intermediate aquifer microcosms<sup>a</sup>

Microcosm	% of DCE lost <sup>b</sup>		% of <sup>14</sup> CO <sub>2</sub> recovered	
	Exptl	Control	Exptl	Control
Anaerobic	37 ± 2	15	38 ± 1	3
MnO <sub>2</sub> amended	42 ± 12	5	50 ± 0	1

<sup>a</sup> Experimental data are means ± standard deviations for duplicate microcosms, and control data are from a single sterile microcosm.

<sup>b</sup> DCE concentrations were determined by flame ionization detection-gas chromatography, and DCE loss was estimated from the final headspace DCE concentration and expressed as a percentage of the initial headspace DCE concentration. All DCE loss was as *cis*-DCE. No significant change in the headspace concentration of *trans*-DCE was observed.

reduction (Table 1). The stimulation of Mn(IV) reduction by MnO<sub>2</sub> amendment was associated with increased DCE mineralization and <sup>14</sup>CO<sub>2</sub> recoveries similar to those under aerobic conditions (Fig. 1). The combined results from the unamended and MnO<sub>2</sub>-amended microcosm studies demonstrate that, under anaerobic conditions, both Mn(IV) reduction and DCE mineralization were limited by the bioavailability of Mn(IV) and indicate that DCE mineralization was coupled to Mn(IV) reduction.

The results further indicate that, under Mn(IV)-reducing conditions, DCE is efficiently oxidized to CO<sub>2</sub> without detectable accumulation of intermediates (Table 2). Following the 3-day incubation period, the headspace of intermediate aquifer microcosms was analyzed for the presence of the daughter products of DCE reductive dechlorination (VC, ethene, and ethane). No volatile organic compounds other than DCE were observed in this study. Moreover, the percent change in headspace DCE concentrations closely corresponded to the percent recoveries of <sup>14</sup>CO<sub>2</sub> in unamended and MnO<sub>2</sub>-amended anaerobic microcosms (Table 2). The stoichiometric conversion of DCE to CO<sub>2</sub>, the lack of accumulation of volatile intermediates, the lack of a detectable lag in the production of <sup>14</sup>CO<sub>2</sub> (Fig. 1), and the similar degrees of mineralization observed in aerobic and MnO<sub>2</sub>-amended microcosms (Fig. 1) are consistent with direct oxidation of DCE with CO<sub>2</sub> as the end product.

The oxidation of DCE observed in this study under anaerobic conditions was not attributable to Fe(III) reduction (Table 3). Because others (12) have shown that Fe(III) reduction can proceed under Mn(IV)-reducing conditions without significant accumulation of dissolved Fe(II), we examined the possibility that anaerobic oxidation of DCE was associated with Fe(III) reduction by quantifying the mineralization of [1,2-<sup>14</sup>C]DCE in anaerobic microcosms amended with poor-

TABLE 3. Effect of Fe(OH)<sub>3</sub> or MnO<sub>2</sub> amendment on the final recovery of <sup>14</sup>CO<sub>2</sub>

Microcosm	% of <sup>14</sup> CO <sub>2</sub> recovered from:			
	Surface fill <sup>a</sup>		Intermediate aquifer <sup>b</sup>	
	Exptl	Control	Exptl	Control
Anaerobic	31 ± 2	1 ± 0	38 ± 1	3
Fe(OH) <sub>3</sub> amended	21 ± 6	5 ± 1	28 ± 1	3
MnO <sub>2</sub> amended	51 ± 5	6 ± 1	50 ± 0	1

<sup>a</sup> 17-day incubation period. Data are means ± standard deviations for triplicate experimental microcosms and duplicate control microcosms.

<sup>b</sup> 3-day incubation period. Experimental data are means ± standard deviations for duplicate microcosms, and control data are from a single sterile control microcosm.

ly crystalline Fe(III) oxide [provided as Fe(OH)<sub>3</sub>]. Addition of Fe(OH)<sub>3</sub> resulted in significant Fe(III) reduction, as indicated by increased accumulation of dissolved Fe(II) (Table 1), but inhibited DCE mineralization (Table 3). The lack of stimulation of DCE mineralization under Fe(III)-amended conditions has been observed previously (4) and indicates that Fe(III)-reducing conditions are not sufficient to support DCE oxidation. Moreover, the apparent inhibitory effect of Fe(III) amendment on DCE oxidation (Table 3) provides additional, compelling evidence that the anaerobic oxidation of DCE observed in the present study was coupled to Mn(IV) reduction.

This investigation is the first report of anaerobic oxidation of DCE under Mn(IV)-reducing conditions and, to our knowledge, the first report of anaerobic mineralization of DCE to CO<sub>2</sub> without the accumulation of reduced intermediates. Mn(IV) oxides are potentially powerful oxidants which are common in aquifer sediments and groundwater systems. Much of the risk associated with DCE contamination in groundwater is due to the potential production of VC. The present results demonstrate that DCE can be oxidized to CO<sub>2</sub> under Mn(IV)-reducing conditions without the environmental risk associated with VC accumulation.

#### REFERENCES

1. **Barcelona, M. J.** 1996. Case study: Wurtsmith Air Force Base, Michigan, p. 98–103. *In* Symposium on natural attenuation of chlorinated organics in ground water. EPA/540/R-96/509. U.S. Environmental Protection Agency, Washington, D.C.
2. **Bouwer, E. J.** 1994. Bioremediation of chlorinated solvents using alternate electron acceptors, p. 149–175. *In* R. D. Norris et al. (ed.), *Handbook of bioremediation*. Lewis Publishers, Boca Raton, Fla.
3. **Bradley, P. M., and F. H. Chapelle.** 1996. Anaerobic mineralization of vinyl chloride in Fe(III)-reducing aquifer sediments. *Environ. Sci. Technol.* **30**: 2084–2086.
4. **Bradley, P. M., and F. H. Chapelle.** 1997. Kinetics of DCE and VC mineralization under methanogenic and Fe(III)-reducing conditions. *Environ. Sci. Technol.* **31**:2692–2696.
5. **Chapelle, F. H.** 1996. Identifying redox conditions that favor the natural attenuation of chlorinated ethenes in contaminated ground-water systems, p. 17–20. *In* Symposium on natural attenuation of chlorinated organics in ground water. EPA/540/R-96/509. U.S. Environmental Protection Agency, Washington, D.C.
6. **Davis, J. W., and C. L. Carpenter.** 1990. Aerobic biodegradation of vinyl chloride in groundwater samples. *Appl. Environ. Microbiol.* **56**:3878–3880.
7. **Dupont, R. R., K. Gorder, D. L. Sorensen, M. W. Kemblowski, and P. Haas.** 1996. Case study: Eielson Air Force Base, Alaska, p. 104–109. *In* Symposium on natural attenuation of chlorinated organics in ground water. EPA/540/R-96/509. U.S. Environmental Protection Agency, Washington, D.C.
8. **Ellis, D. E., E. J. Lutz, G. M. Klecka, D. L. Pardieck, J. J. Salvo, M. A. Heitkamp, D. J. Gannon, C. C. Mikula, C. M. Vogel, G. D. Sayles, D. H. Kampbell, J. T. Wilson, and D. T. Maiers.** 1996. Remediation technology development forum—intrinsic remediation project at Dover Air Force Base, Delaware, p. 93–97. *In* Symposium on natural attenuation of chlorinated organics in ground water. EPA/540/R-96/509. U.S. Environmental Protection Agency, Washington, D.C.
9. **Fairbridge, R. W.** 1972. Manganese: element and characteristics, p. 670–671. *In* *Encyclopedia of geochemistry and environmental sciences*. Van Nostrand Reinhold, New York, N.Y.
10. **Imbrigiotta, T. E., T. A. Ehlike, B. H. Wilson, and J. T. Wilson.** 1996. Case study: natural attenuation of a trichloroethene plume at Picatinny Arsenal, New Jersey, p. 83–89. *In* Symposium on natural attenuation of chlorinated organics in ground water. EPA/540/R-96/509. U.S. Environmental Protection Agency, Washington, D.C.
11. **Langenhoff, A. A. M., D. L. Brouwers-Ceilers, J. H. L. Engelberting, J. J. Quist, J. G. P. N. Wolkenfelt, A. J. B. Zehnder, and G. Schraa.** 1997. Microbial reduction of manganese coupled to toluene oxidation. *Microb. Ecol.* **22**:119–127.
12. **Lovley, D. R., and E. J. P. Phillips.** 1988. Manganese inhibition of microbial iron reduction in anaerobic sediments. *Geomicrobiol. J.* **6**:145–155.
13. **Lovley, D. R., and E. J. P. Phillips.** 1988. Novel mode of microbial energy metabolism: organic carbon oxidation coupled to dissimilatory reduction of iron or manganese. *Appl. Environ. Microbiol.* **54**:1472–1480.
14. **Lovley, D. R.** 1991. Dissimilatory Fe(III) and Mn(IV) reduction. *Microbiol. Rev.* **55**:259–287.
15. **McCarty, P. L., and L. Semprini.** 1994. Ground-water treatment for chlorinated solvents, p. 87–116. *In* R. D. Norris et al. (ed.), *Handbook of bioremediation*. Lewis Publishers, Boca Raton, Fla.
16. **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.
17. **Weaver, J. W., J. T. Wilson, and D. H. Kampbell.** 1996. Extraction of degradation rate constants from the St. Joseph, Michigan, trichloroethene site, p. 69–73. *In* Symposium on natural attenuation of chlorinated organics in ground water. EPA/540/R-96/509. U.S. Environmental Protection Agency, Washington, D.C.
18. **Weidemeier, T. H., J. T. Wilson, and D. H. Kampbell.** 1996. Natural attenuation of chlorinated aliphatic hydrocarbons at Plattsburg Air Force Base, New York, p. 74–82. *In* Symposium on natural attenuation of chlorinated organics in ground water. EPA/540/R-96/509. U.S. Environmental Protection Agency, Washington, D.C.
19. **Wilson, J. T., D. H. Kampbell, and J. W. Weaver.** 1996. Environmental chemistry and the kinetics of biotransformation of chlorinated organic compounds in ground water, p. 124–127. *In* Symposium on natural attenuation of chlorinated organics in ground water. EPA/540/R-96/509. U.S. Environmental Protection Agency, Washington, D.C.