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Methane As a Product of Chloroethene Biodegradation under Methanogenic Conditions

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Radiometric detection headspace analyses of microcosms containing bed sediments from two geographically distinct sites indicated that 10−39% of the radiolabeled carbon transformed during anaerobic biodegradation of [1,2-^{14}C]trichloroethene (TCE) or [1,2-^{14}C]vinyl chloride (VC) under methanogenic conditions was ultimately incorporated into $^{14}CH_4$. The results demonstrate that, in addition to ethene, ethane, and CO_2, CH_4 can be a significant product of chloroethene biodegradation in some methanogenic sediments.

Introduction

Under strongly reducing, methanogenic conditions, polychlorinated compounds such as PCE and TCE can be degraded via reductive dechlorination through the less chlorinated intermediates, *c*-DCE and VC, to the nonchlorinated compounds, ethene and ethane (*1*−*7*). A number of investigations conducted in the laboratory using relatively well-defined microbial cultures have reported stoichiometric conversion of chlorinated contaminants to ethene and ethane (*2*−*4*). In groundwater systems, however, where microbial communities and environmental conditions are complex, ethene and ethane accumulation rarely accounts for the total observed loss of chloroethene contaminants (*8*−*10*, authors' unpublished results). This observation, in turn, suggests the possibility that in situ biodegradation of chloroethene contaminants under methanogenic conditions may yield other products such as CO_2 and CH_4 which are not unique to and, therefore, not diagnostic of chloroethene biodegradation. In fact, a number of studies have demonstrated that CO_2, theoretically the ultimate endproduct of oxidative biodegradation, can be a significant product of chloroethene biodegradation even under methanogenic conditions (*11*−*14*). In contrast, significant reduction of chloroethene contaminants to CH_4, theoretically the ultimate reductive endproduct of biodegradation, has not been reported previously (*2*−*4*). The purpose of this report is to provide evidence that CH_4 can be a significant product of chloroethene biodegradation under methanogenic conditions.

Methods

Chemicals. Biodegradation of TCE and VC under methanogenic conditions was investigated using [1,2-^{14}C]TCE (Sigma Chemical Co., St. Louis) and [1,2-^{14}C]VC (NEN Dupont, Boston). Radiometric detection gas chromatography (GC/GRD) and liquid scintillation counting analyses demonstrated that greater than 99% and 98% of the total radioactivity present in the TCE and VC stocks used in this study was, in fact, [1,2-^{14}C] TCE and [1,2-^{14}C] VC, respectively. The chemical purity (>99%) of the [1,2-^{14}C] TCE and [1,2-^{14}C] VC was confirmed in our lab by GC/FID and mass spectrometry gas chromatography (GC/MS) analyses.

Study Sites. Microcosm studies were conducted using bed sediments from two geographically distinct sites: the Naval Weapons Industrial Reserve Plant (NWIRP) Dallas, TX and the Naval Air Station (NAS) Cecil Field, Jacksonville, FL. The NWIRP Dallas bed sediments were collected from a shallow, freshwater lake which continuously receives groundwater contaminated with low concentrations (≤20 ppb) of TCE, *c*-DCE, and VC. The bed sediment was a highly reduced, soft mud composed of clay and fine silt and characterized by vigorous methanogenesis. The NAS Cecil Field bed sediments, which were described in detail previously (*12*), were collected from a shallow, freshwater stream which receives groundwater contaminated with low concentrations (≤20 ppb) of *c*-DCE. The NAS Cecil Field bed sediment samples, a coarse grained sand with a 2−5% organic content, were collected from a location where continuous methane outgassing was observed.

Microcosm Studies. Anaerobic microcosms were prepared as described previously (*12*). In brief, 20 mL serum vials were amended with 15 g of saturated, methanogenic bed sediment under a helium atmosphere, sealed with Teflon-lined butyl rubber stoppers, and flushed with an excess (1000 mL) of high purity helium. Experimental treatments were prepared in triplicate. Duplicate killed control microcosms were prepared as described and autoclaved twice for 1 h at 15 PSI and 121 °C. Sediment microcosms were preincubated for 5 days to reestablish active methanogenesis and then amended with approximately 0.5 μCi of [1,2-^{14}C] TCE or [1,2-^{14}C] VC. For the NWIRP Dallas microcosms, initial dissolved concentrations in equilibrium with the headspace were estimated based on experimentally determined adsorption and Henry's law coefficients to be 550 and 370 μg/L for TCE and VC, respectively. For the NAS Cecil Field microcosms, the initial dissolved concentration was estimated to be 630 μg/L for VC.

For the [1,2-^{14}C] TCE biodegradation study (NWIRP Dallas sediments only), headspace concentrations of TCE and its chlorinated daughter products (*c*-DCE and VC) were monitored periodically by removing 0.5 mL of headspace and analyzing by GC/FID. Headspace concentrations of CH_4, CO_2, ethene, and ethane and the absence of O_2 were monitored in the same manner using thermal conductivity detection gas chromatography (GC/TCD). The headspace sample volumes were replaced with helium. At the end of the study, the final distribution of radioactivity in nonchlorinated products was quantified by GC/TCD coupled to GC/GRD. The GC/GRD output was calibrated by liquid scintillation counting using $H^{14}CO_3$. Because the radioactivity of the nonchlorinated products formed in the experimental treatments was not monitored throughout the study, the results of the [1,2-^{14}C] TCE biodegradation study presented in Figure 1 and Table 1 were not corrected for the loss of constituents due to headspace sample collection. Calculations based on interim TCE concentrations observed in killed control microcosms indicated that the low recovery (66%) in this treatment was entirely attributable to sample collection and headspace replacement. For the [1,2-^{14}C] VC biodegradation studies (NWIRP Dallas and NAS Cecil Field sediments), headspace concentrations of VC and its nonchlorinated daughter products were monitored as described above with the exception that the formation of radiolabeled daughter

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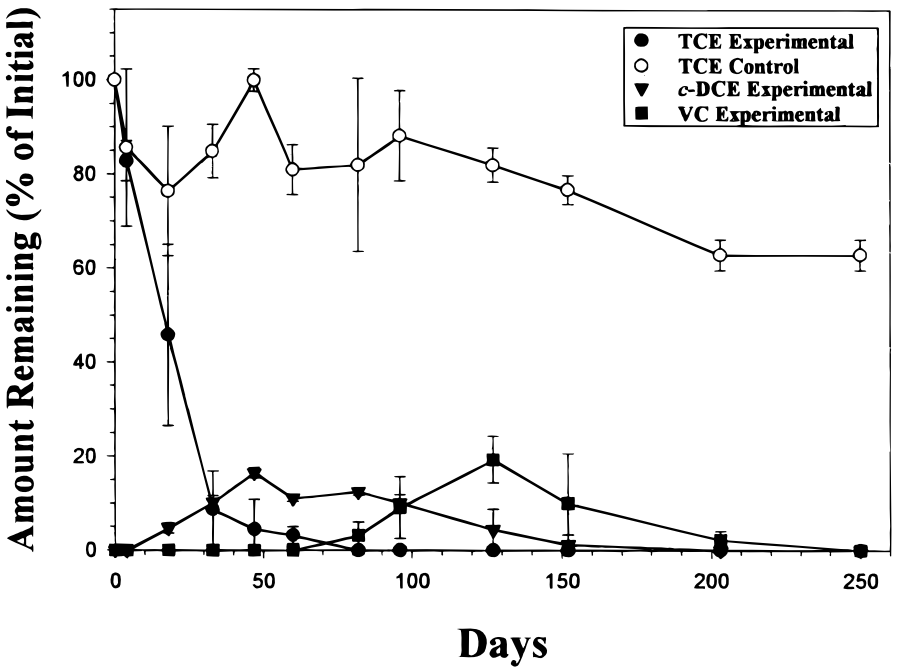

FIGURE 1. Total amount of TCE and its chlorinated daughter products remaining in methanogenic microcosms containing lake bed sediments from NWIRP Dallas. Data are means ± SD for triplicate experimental and duplicate killed control microcosms. Data are not corrected for constituent losses due to headspace sample collection.

TABLE 1. Final Distribution of ^{14}C Radiolabel, Detected as the Original Substrate (TCE) or Its Degradation Daughter Products, in Methanogenic Microcosms Containing Sediment from Naval Weapons Industrial Reserve Plant Dallas, TX[a]

compound	exptl (%)	control (%)
TCE	ND[b]	63 ± 3
c-DCE	ND[b]	ND[b]
VC	ND[b]	ND[b]
ethene	46 ± 8	ND[b]
ethane	ND[b]	ND[b]
CH_4	9 ± 2	ND[b]
CO_2	12 ± 2	3 ± 1
total recovery	67 ± 8	66 ± 3

[a] Data are final recoveries expressed as a percentage of the radioactivity added as TCE. Data are means ± SD for triplicate experimental microcosms and duplicate killed controls. Data are not corrected for constituent losses due to headspace sample collection. [b] Not detected during analysis. Minimum detection limit for radiometric detection was 100 DPM/injection of headspace (equivalent to a final recovery of 2%).

products was monitored continuously throughout the incubation using GC/GRD. The results of the [1,2-^{14}C] VC biodegradation study presented in Figure 2 and Table 2 were corrected for the loss of constituents due to headspace sample collection.

Results and Discussion

As part of a remedial investigation for NWIRP Dallas, the potential for reductive dechlorination of TCE was examined in bed sediment microcosms under anaerobic conditions (Figure 1). Under these conditions microbial activity resulted in vigorous methanogenesis (about 35 μmol/L headspace or 2.1 mg/L microcosm water produced per day, data not shown) and complete disappearance of TCE within 80 days. TCE degradation was followed in turn by accumulation and subsequent disappearance of *c*-DCE and VC. By 250 days, no chloroethene compounds were detected in the experimental microcosms, and the study was terminated. In contrast, TCE

TABLE 2. Final Distribution of ^{14}C Radiolabel, Detected as the Original Substrate (Vinyl Chloride, VC) or its Degradation Daughter Products, in Methanogenic Microcosms Containing Sediment from Naval Weapons Industrial Reserve Plant (NWIRP) Dallas, TX or Naval Air Station (NAS) Cecil Field, Jacksonville, FL[a]

site	compound	exptl (%)	control (%)
NWIRP Dallas	VC	79 ± 5	94 ± 4
	ethene	3 ± 1	ND[b]
	ethane	ND[b]	ND[b]
	CH_4	9 ± 2	ND[b]
	CO_2	11 ± 2	ND[b]
	total recovery	102 ± 5	94 ± 4
NAS Cecil Field	VC	2 ± 3	87[c]
	ethene	10 ± 1	ND[b]
	ethane	39 ± 9	ND[b]
	CH_4	22 ± 1	ND[b]
	CO_2	22 ± 2	ND[b]
	total recovery	95 ± 8	87[c]

[a] Data are final recoveries expressed as a percentage of the radioactivity added as VC. Data are means ± SD for triplicate experimental microcosms and duplicate killed controls. Data are corrected for constituent losses due to headspace sample collection. [b] Not detected during analysis. Minimum detection limit for radiometric detection was 100 DPM/injection of headspace (equivalent to a final recovery of 2%). [c] Datum for single control microcosm.

disappearance in the control microcosms was slow with 63 ± 3% of the TCE remaining after 250 days (Figure 1). No production of methane (data not shown) or chlorinated daughter products was observed in the control microcosms.

Headspace analyses conducted at day 250 using GC/GRD indicated that TCE had been degraded to nonchlorinated compounds (Table 1). The close agreement in the recovery of radioactivity between experimental and control microcosms indicates that degradation to endproducts other than those reported here was not significant. Approximately half of the radioactivity initially added as [1,2-^{14}C] TCE was recovered as ^{14}C-ethene. This result is consistent with numerous reports that chloroethene contaminants can be

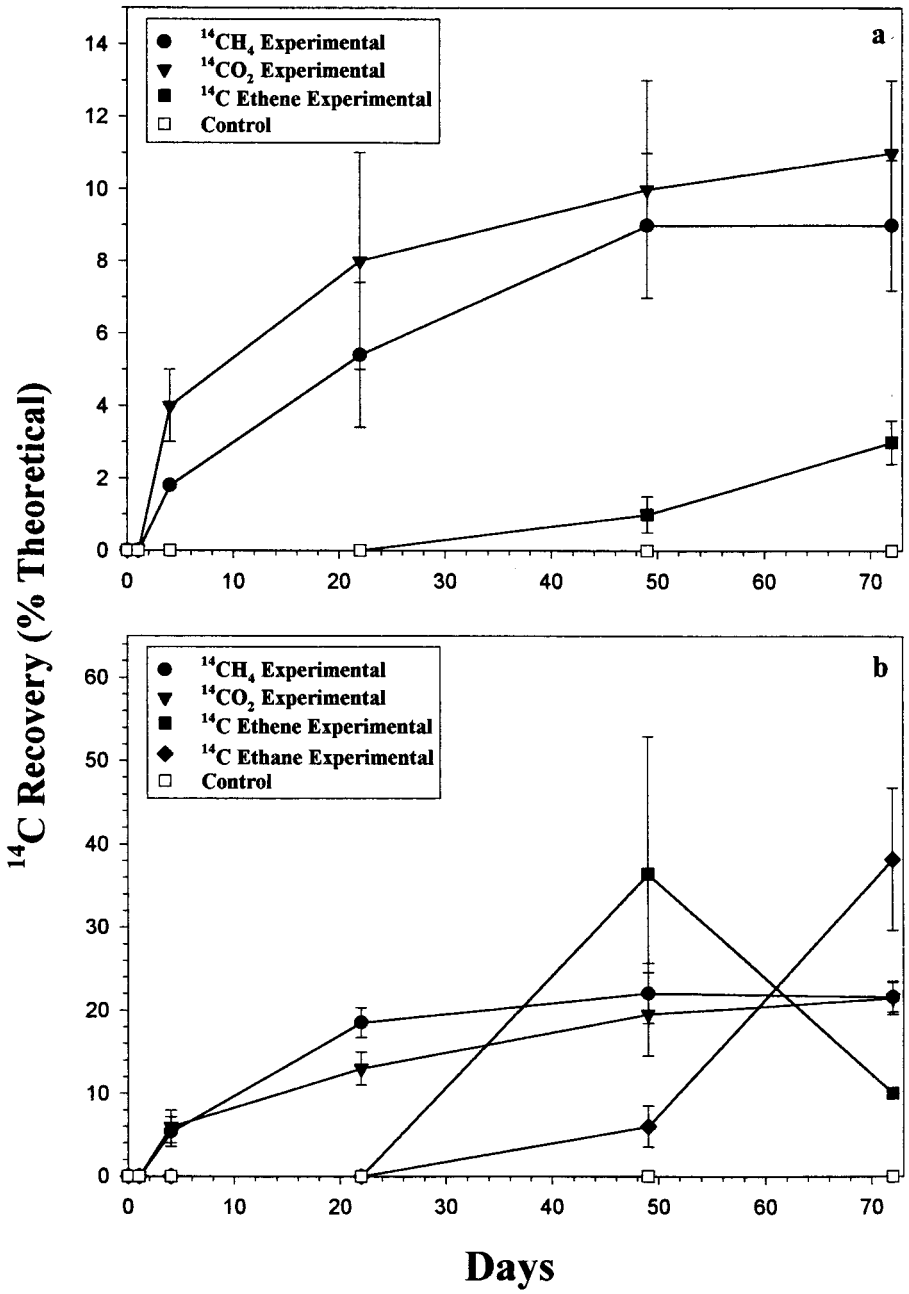

FIGURE 2. Percentage recovery of [1,2-^{14}C] VC radioactivity as nonchlorinated products in methanogenic microcosms containing bed sediments from NWIRP Dallas (a) and NAS Cecil Field (b). Data are means ± SD for triplicate experimental and duplicate killed control microcosms. Data are corrected for constituent losses due to headspace sample collection.

reductively dechlorinated to ethene under methanogenic conditions (*1*−*10*). In addition, 12% of the radioactivity was recovered as $^{14}CO_2$ and 9% as $^{14}CH_4$, respectively. The observed recovery of radioactivity as $^{14}CO_2$ is consistent with previous reports demonstrating significant oxidation of chloroethenes to CO_2 even under methanogenic conditions (*11*−*14*). To our knowledge, however, this is the first evidence of significant degradation of a chloroethene to CH_4.

The biodegradation of chloroethene compounds to CH_4 under methanogenesis was confirmed in NWIRP Dallas sediment microcosms amended with [1,2-^{14}C] VC (Figure 2a). Consistent with the results of the TCE experiment, biodegradation under these conditions was slow and resulted in only a 21% decline in VC concentrations in the experimental treatments after 70 days compared with a 6% decline in the control microcosms (Table 2). In this study [1,2-^{14}C] VC was degraded to ^{14}C-ethene, $^{14}CO_2$, and $^{14}CH_4$ (Figure 2a, Table 2). Significant recovery of $^{14}CO_2$ and $^{14}CH_4$ was observed within 5 days of incubation, but significant production of ^{14}C-ethene was not detected until day 50. The fact that $^{14}CH_4$ represented 39% of the radiolabeled daughter products demonstrated the significance of CH_4 as a product of chloroethene biodegradation in these methanogenic sediments.

A similar production of $^{14}CH_4$ during biodegradation of [1,2-^{14}C] VC was observed in methanogenic microcosms containing stream bed sediments from NAS Cecil Field (Figure 2b). For these sediments, VC loss from experimental treatments was complete after 70 days compared to a 13% loss observed in control microcosms (Table 2). Significant recovery of $^{14}CO_2$ and $^{14}CH_4$ was observed immediately and reached maximum values of 22 ± 2% and 22 ± 1%,

respectively. Significant ^{14}C-ethene accumulation was not observed until day 50. In contrast to the NWIRP Dallas study, ^{14}C-ethene concentrations subsequently declined as ^{14}C-ethene was further reduced to ^{14}C-ethane. By the end of the study, ^{14}C-ethene and ^{14}C-ethane represented about 50% of the recovered radioactivity. The significant degradation of [1,2-^{14}C] VC to $^{14}CH_4$ observed in this study is consistent with that observed in NWIRP Dallas microcosms. The fact that chloroethene biodegradation to CH_4 was observed in sediments from geographically distinct sites suggests that this process may be widespread.

The results of this study have important implications for the use of natural attenuation as a component of contaminant remediation at chloroethene contaminated sites. The results demonstrate that, in addition to ethene, ethane, and CO_2, CH_4 can be a significant product of chloroethene biodegradation in some methanogenic sediments. Because regulatory approval of natural attenuation as a remedial strategy at chloroethene contaminated sites typically depends on the demonstration of efficient degradation to nonchlorinated products, the potential transformation of chloroethenes to nondiagnostic, natural products is of significant interest to the field of environmental restoration. Thus, the mechanism of this process and its significance at other chloroethene contaminated sites merit further investigation.

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