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Dichloroethene and Vinyl Chloride Degradation Potential in Wetland Sediments at Twin Lakes and Pen Branch, Savannah River National Laboratory, South Carolina

By Paul M. Bradley

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Conversion Factors

Temperature in degrees Celsius (°C) may be converted to degrees Fahrenheit (°F) as follows:
°F=(1.8×°C)+32

Abbreviations

ºC	degrees celsius
g	gram(s)
h	hour(s)
M	mole(s) per liter
mg/L	milligram(s) per liter
mL	milliliter(s)
µCi/µmole	microcuries per micromole
µg/L	microgram(s) per liter
µm	micrometer(s)
µM	micromoles per liter
µmole	micromole
nM	nanomoles per liter
nmole	nanomole
DCE	*cis*-dichloroethene
PCE	perchloroethene
PSI	pounds per square inch
SD	standard deviation
TCE	trichloroethene
VC	vinyl chloride

Dichloroethene and Vinyl Chloride Degradation Potential in Wetland Sediments at Twin Lakes and Pen Branch, Savannah River National Laboratory, South Carolina

By Paul M. Bradley

Abstract

A series of ^{14}C-radiotracer-based microcosm experiments was conducted to assess the mechanisms and products of degradation of dichloroethene *(*DCE) and vinyl chloride (VC) in wetland sediments at the Department of Energy (DOE) Savannah River National Laboratory. This project investigated the potential for biotic and abiotic DCE and VC degradation in wetland sediments from the Twin Lakes area of the C-BRP investigative unit and from the portion of Pen Branch located directly down gradient from the CMP investigative unit.

Substantial degradation of [1,2-^{14}C] DCE and [1,2-^{14}C] VC to $^{14}CO_2$ was observed in all viable sediment microcosms prepared under oxic conditions. These results indicate that microbial mineralization processes, involving direct oxidation or cometabolic oxidation, are the primary mechanisms of DCE and VC biodegradation in Twin Lake and Pen Branch sediments under oxic conditions.

Substantial degradation of [1,2-^{14}C] DCE and [1,2-^{14}C] VC was observed in all viable sediment microcosms incubated under anoxic conditions. Production of $^{14}CO_2$ was observed in all sediment microcosms under anoxic conditions. In general, the accumulation of mineralization products ($^{14}CO_2$ and $^{14}CH_4$) was comparable to the accumulation of those reduced daughter products (^{14}C-VC, ^{14}C-ethene or ^{14}C-ethane) traditionally identified with chloroethene reductive dechlorination. These results indicate that microbial mineralization processes can be an important component of DCE and VC degradation in Twin Lake and Pen Branch sediments under anoxic conditions. These results demonstrate that an evaluation of the efficiency of *in situ* DCE and VC biodegradation in Twin Lakes and Pen Branch that is based solely on the observed accumulation of reduced daughter products may underestimate substantially the total extent of contaminant biodegradation and, thus, the contribution of biodegradation to overall contaminant attenuation.

No evidence of abiotic degradation of [1,2-^{14}C] DCE or [1,2-^{14}C] VC was observed in heat-sterilized control treatments in this study under oxic or anoxic conditions. Efforts to enrich and isolate microorganisms involved in the mineralization of [1,2-^{14}C] *cis*-DCE and/or [1,2-^{14}C] VC were unsuccessful.

Introduction

Recent evidence (Bradley and others, variously dated) indicates that biodegradation of chloroethene contaminants in ground-water and surface-water environments can yield two general types of degradation products: reduced daughter products associated with reductive degradation processes or mineralization products. The primary reductive chloroethene degradation mechanism reported in saturated environments is reductive dechlorination. This process involves consecutive hydrogen substitution reactions yielding sequentially less chlorinated daughter products (TCE, DCE and VC) and ultimately ethene. The poly-chlorinated ethenes, PCE and TCE, are highly oxidized compounds that readily undergo reduction reactions under anoxic conditions. Reductive dechlorination is common in anoxic groundwater and surface-water systems and appears to be the primary mechanism for *in situ* biotransformaton of the parent compounds, PCE and TCE. The efficiency of chloroethene reductive dechlorination, however, appears to decrease with decreasing chlorine number. Significant reductive dechlorination of chloroethene contaminants to the non-chlorinated product, ethene, has been reported (Bradley 2003 for review), but is often limited *in situ* due to low electron donor supply, high electron donor competition, the presence of alternative terminal electron acceptors for facultative chlororespiring microorgansims, the potential absence or low activity of DCE and VC dechlorinating microorganisms, and the presence of inhibitory substances including more oxidized chloroethene compounds. In contrast, the potential for chloroethene oxidation to mineralized products increases with decreasing chlorine number and microbial mineralization to CO_2 can be a significant component of *in situ* VC and DCE natural attenuation under oxic and anoxic conditions. The end-product(s) of microbial chloroethene mineralization, primarily CO_2 (or CO_2 and CH_4 under acetotrophic methanogenic conditions), however, are not unique to chloroethene mineralization and, consequently, are not diagnostic of this process. Laboratory microcosm evaluations remain the best approach for assessing the *in situ* potential for microbial mineralization of chloroethene contaminants under oxic or anoxic conditions.

Purpose and Scope

Recent research (Bradley and others, variously dated) indicates that the potential for chloroethene biodegradation is particularly pronounced in wetland environments where spatial and temporal variations in the hydrologic and geochemical character of wetland sediments often lead to highly dynamic microbial communities with a wide range of metabolic capabilities and the demonstrated potential for both reductive dechlorination and mineralization. Because the reduced daughter products, DCE and VC, regularly increase in importance in the down gradient portion of anoxic chloroethene plumes and because wetlands and surface-water bodies are common down gradient receptors, an evaluation of the potentially important biodegradation mechanisms, including oxic and anoxic mineralization mechanisms, is particularly important in potential wetland and surface-water receptors.

This report describes the relative contribution (relative to reductive dechlorination) of oxic and anoxic microbial mineralization processes to the natural

attenuation of DCE and VC in wetland sediments from the Twin Lakes area of the C-BRP investigative unit and from the reach of Pen Branch located directly down gradient from the CMP investigative unit. The information presented in this report is intended to support ongoing efforts to evaluate the contribution of *in situ* microbial degradation to natural attenuation of chloroethene contaminants at the C-BRP and CMP investigative units at the Savannah River National Lab. In addition, this report describes the potential for abiotic degradation of DCE and VC in Twin Lakes and Pen Branch sediments in heat-sterilized, abiotic control microcosms. Finally, this report describes the effort to enrich and/or isolate chloroethene mineralizing microorganisms from viable, sediment treatments that demonstrated significant ^{14}C-VC or ^{14}C-DCE mineralization. Specific objectives of this investigation were:

- Conduct a microcosm investigation using ^{14}C-DCE and ^{14}C-VC to evaluate the mechanisms and products of DCE and VC biodegradation in sediments from Twin Lakes and Pen Branch.
- Determine the contribution of mineralization processes, relative to processes leading to the accumulation of reductive dechlorination daughter products, to the natural attenuation capacity of DCE and VC in these two wetland systems.
- Conduct a microcosm investigation using ^{14}C-DCE and ^{14}C-VC to evaluate the potential for abiotic degradation of DCE and VC in heat-sterilized sediment microcosms from Twin Lakes and Pen Branch.
- Initiate an effort to isolate and enrich defined cultures that are capable of anoxic mineralization of [1,2-^{14}C] DCE and/or [1,2-^{14}C] VC. Isolation and enrichment of microbial communities, however, is resource intensive and offers a low probability of success in the short-term.

Methods

This section presents details of the study sites, sediment collection locations and the experimental approach.

Study Sites

Sediments for conducting microcosm studies were collected from the DOE Savannah River National Laboratory, located at the western border of South Carolina (figure 1).

Wetland sediments were collected in September 2004 from the Twin Lakes area of the C-BRP investigative unit. Twin Lakes sediments were collected from the small stream that drains through the center of the Twin Lakes wetland prior to discharge into Four Mile Branch. Twin Lakes sediments were collected next to and identified by the ground-water monitoring wells; CRP-42, CRP-45, CRP-46 and CRP-49.

Wetland sediments were collected in September 2004 from the reach of Pen Branch that is located directly down gradient from the CMP investigative unit. Sediments were collected from the CMP side of Pen Branch in the area of potential discharge of chloroethene-contaminated ground water. Pen Branch sediments were collected next to and identified by the ground-water monitoring wells; CMP-39, CMP-40 and CMP-41.

Qualitative physical descriptions of the collected sediments are presented in table 1. Sediment organic contents for the collected sediments were estimated based on mass loss on ignition at 550 ºC and are presented in table 1.

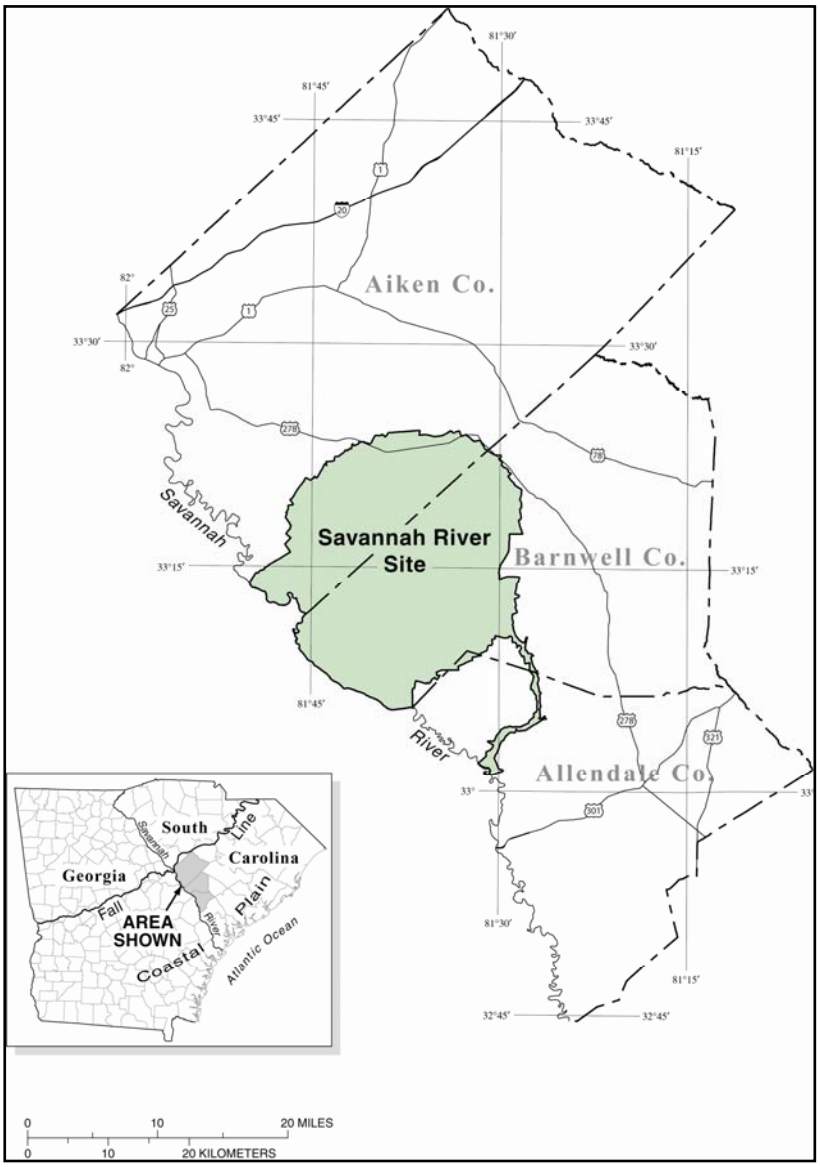

Figure 1. Location of the DOE Savannah River National Laboratory in South Carolina.

Radiochemicals

The potential for chloroethene biodegradation in Twin Lakes and Pen Branch sediments was investigated using uniformly labeled [1,2-^{14}C] DCE (4 µCi/µmole; Moravek Biochemicals, Brea, California) and [1,2-^{14}C] VC (1.6 µCi/µmole; Perkin Elmer Life Sciences, Boston, Massachusetts). The radiochemical purity of the [1,2-^{14}C] chloroethene stocks was evaluated in the U.S. Geological Survey laboratory by direct injection radiometric detection gas chromatography (GC/RD) and found to be greater than 98 percent pure. Authentic $H^{14}CO_3^-$ (Sigma Biochemicals, St. Louis, Missouri) and $^{14}CH_4$ (Perkin Elmer Life Sciences, Boston, Massachusetts) were used as radiolabeled standards for calibration and methods development. Each had radiochemical purities greater than 98 percent.

Site	Sample Location	Description	Organic Content (% dry weight)
C-BRP (Twin Lakes)	**CRP-42**	organic silt/clay	10 0
	CRP-45	organic silt/clay	23 1
	CRP-46	organic silt/clay	21 1
	CRP-49	sand	0.2 0.1
CMP (Pen Branch)	**CMP-39**	sand/gravel	0.1 0.0
	CMP-40	organic sand	2 1
	CMP-41	organic silt/clay	15 1

Table 1. Sample collection locations and sediment descriptions. Organic contents were estimated as loss on ignition. Data are means±SD for duplicate samples. [%, percent]

Microcosm Studies

In general, sediment microcosms were composed of 10 mL serum vials with 10±0.5 g of saturated sediment and an atmosphere of nitrogen (anoxic treatments) or ambient air (oxic treatments). Triplicate experimental (viable) microcosms were prepared for each sediment treatment. Duplicate autoclaved control microcosms and a single sediment-free control microcosm were prepared for each sediment treatment and autoclaved three times for 1 h at 15 PSI and 121 ºC. All anoxic microcosm treatments were pre-incubated in the dark at room temperature for 7 days prior to the addition of ^{14}C-substrates, in order to ensure strictly anoxic conditions. Microcosms were amended with [1,2-^{14}C] DCE or [1,2-^{14}C] VC to yield initial dissolved substrate concentrations of 280 µg/L DCE or 310 µg/L VC, respectively. Following the addition of ^{14}C-substrates, microcosms were incubated in the dark at room temperature (approximately 23 ºC) for 60 days.

Analytical Methods

Headspace concentrations of O_2, CH_4, $^{14}CH_4$, CO_2, $^{14}CO_2$, ethene, ^{14}C-ethene, ethane, ^{14}C-ethane, VC, and ^{14}C-VC were assessed by analyzing 1.0 ml of the microcosm headspace using packed column gas chromatography with sequential radiometric detection and thermal conductivity detection. The headspace sample volumes were replaced with pure oxygen (oxic treatments) or nitrogen (anoxic treatments). Dissolved phase concentrations of ^{14}C-analytes were estimated based on experimentally determined Henry's partition coefficients (Bradley and others, 2000b). Because inorganic carbon is present as dissolved CO_2 and HCO_3^- at the circum-neutral pH values observed in the collected sediments, a dimension less partition coefficient for the distribution of inorganic carbon between the headspace and the dissolved phase was determined by injecting $H^{14}CO_3^-$ into the dissolved phase of triplicate autoclaved sediment microcosms (prepared as described for the degradation study), allowing the microcosms to equilibrate for 24 h, and measuring the $^{14}CO_2$ radioactivity in the headspace using radiometric detection gas chromatography. The radiometric detector was calibrated by liquid scintillation counting using $H^{14}CO_3^-$.

Isolation and Enrichment Methods

Isolation of the microorganisms that are responsible for anoxic chloroethene mineralization is preferred in order to clarify the mechanisms underlying the mineralization process and to provide molecular targets for the identification of the responsible organisms *in situ*. Isolation and enrichment of microbial communities, however, is resource intensive and offers little probability of success in the short-term. The approximate 15 year period between the initial field evidence for chloroethene reductive dechlorination (See for review: Bradley, 2003, McCarty and Semprini, 1994) and the ultimate isolation of a microorganism capable of complete chloroethene reductive dechlorination to ethene (*Dehalococcoides ethenogenes*) (Maymó-Gatell and others, 1997) indicated, from the outset, that identification of anoxic chloroethene mineralizing microorganisms, in all probability, would be a lengthy process that would exceed the DOE project time constraints. Nevertheless, in light of the demonstrated capability of several sediments collected from Twin Lakes and Pen Branch to catalyze substantial mineralization of [1,2-^{14}C] DCE and/or [1,2-^{14}C] VC, an effort to enrich and/or isolate microorganisms capable of chloroethene mineralziation was initiated.

Efforts to isolate and/or identify cultures capable of DCE and/or VC mineralization involved two general approaches: (1) serial dilution of active Twin Lakes and Pen Branch sediment slurries using heat-sterilized water (both filtered and unfiltered treatments were assessed) collected from Twin Lakes and Pen Branch as the growth media and [1,2-^{14}C] DCE or [1,2-^{14}C] VC as the growth substrates, and (2) serial transfer of selected inocula using [1,2-^{14}C] DCE or [1,2-^{14}C] VC as the growth substrate and various artificial growth media. Cultures tested using the second approach included the active sediment slurries as well as a number of fermentative, sulfate-reducing and iron-reducing cultures that had demonstrated capacities for contaminant degradation and that were commercially available from American Type Culture Collection (ATCC; Manassas, Virginia). Artificial media used for the second approach included minimal salt media as well as preparations recommended for and specific to the purchased ATCC cultures. The selection criteria for the isolation effort was the persistent ability to oxidize [1,2-^{14}C] DCE or [1,2-^{14}C] VC in consecutive culture transfers.

Degradation under Oxic and Anoxic Conditions

The following sections present the results of the microcosm degradation studies and the enrichment efforts that were conducted in this investigation.

DCE Degradation under Oxic Conditions

Degradation of [1,2-^{14}C] DCE was observed in all experimental sediment microcosms incubated under oxic conditions (figure 2, table A1). The sole product of [1,2-^{14}C] DCE degradation that was observed under oxic conditions was $^{14}CO_2$. The mean accumulation of $^{14}CO_2$ after 60 days incubation ranged from 35±3 percent of theoretical in microcosms prepared with sediments from CRP-46 to 60±3 percent in microcosms prepared with sediments from CMP-40 treatments. The remaining microcosm ^{14}C-radioactivity was associated with residual [1,2-^{14}C] DCE. The degradation of [1,2-^{14}C] DCE observed in these oxic sediment treatments was attributable to biological activity because no significant ^{14}C-product accumulation was observed in autoclaved control or sediment-free control microcosms. The results of this experiment indicate that microbial mineralization, involving direct oxidation or cometabolic oxidation, is the primary mechanism of DCE degradation in Twin Lake and Pen Branch sediments under oxic conditions. No evidence of reductive

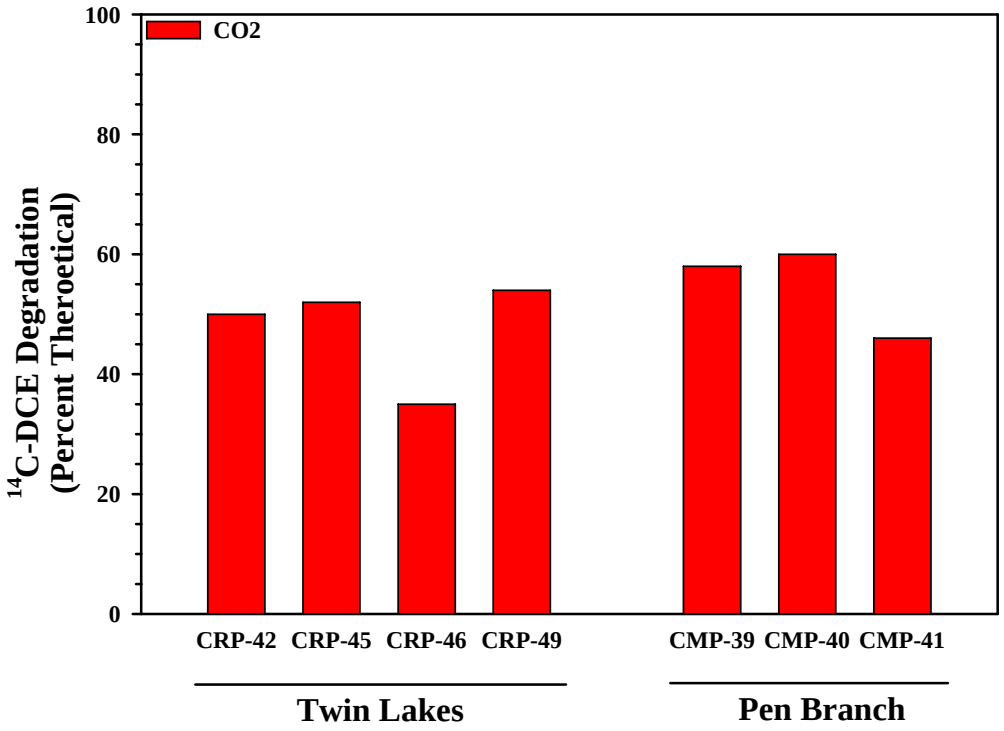

Figure 2. Final percentage recovery of [1,2-^{14}C] DCE radioactivity as $^{14}CO_2$ in viable sediment microcosms incubated under oxic conditions. Data are means of triplicate microcosms. Remaining ^{14}C-radioactivity was recovered as undegraded ^{14}C-DCE.

degradation or reduced daughter products was observed under oxic incubation conditions.

Wetlands and shallow surface-water systems are characterized by considerable temporal and spatial variability in the sediment geochemistry and in the activity of associated microbial redox communities. In such settings, oxic microbial mineralization is expected to be a significant contributor to *in situ* DCE attenuation. Thus, an evaluation of the efficiency of *in situ* DCE biodegradation based solely on the accumulation of reduced daughter products may underestimate substantially the actual extent of contaminant biodegradation and the overall contribution of oxic biodegradation to contaminant attenuation.

DCE Degradation under Anoxic Conditions

Substantial degradation of [1,2-^{14}C] DCE also was observed in all experimental sediment microcosms incubated under anoxic conditions (figure 3, table A1). The degradation of [1,2-^{14}C] DCE observed in this study was attributable to biological activity because no significant ^{14}C-product accumulation was observed in autoclaved control or sediment free control microcosms. The establishment and maintenance of anoxic conditions was confirmed in experimental, autoclaved control and sediment free control microcosms by headspace gas chromatography.

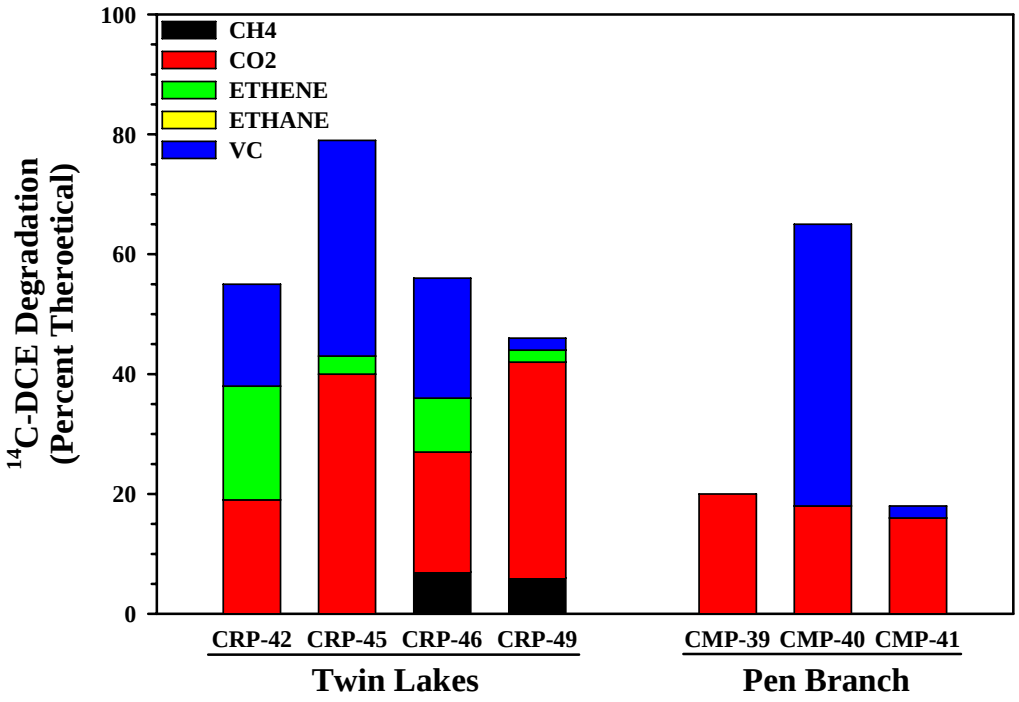

Figure 3. Final percentage recovery of [1,2-^{14}C] DCE radioactivity as $^{14}CH_4$, $^{14}CO_2$, ^{14}C-Ethene, ^{14}C-Ethane, and ^{14}C-VC in viable sediment microcosms incubated under anoxic conditions. Data are means of triplicate microcosms. Remaining ^{14}C-radioactivity was recovered as undegraded ^{14}C-DCE.

Production of $^{14}CO_2$ was observed in all sediment microcosms under anoxic conditions. The mean accumulation of $^{14}CO_2$ after 60 days incubation ranged from 16±2 percent of theoretical in CMP-41 microcosms to 40±8 percent in CRP-45 treatments. The sole product of [1,2-^{14}C] DCE degradation in CMP-39 sediments was $^{14}CO_2$. In contrast, all other sediment treatments contained both reductive dechlorination and mineralization products. In this study, the accumulation of mineralization products ($^{14}CO_2$ and $^{14}CH_4$) was comparable to or greater than the accumulation of those products (^{14}C-VC, ^{14}C-ethene or ^{14}C-ethane) associated with reductive dechlorination.

Previous reports indicate that, with the possible exception of DCE degradation under Mn(IV)-reducing conditions, the net mineralization of DCE involves an initial reduction to VC, which may or may not accumulate to detectable concentrations (Bradley and Chapelle, 1997; 1998b). The detection of ^{14}C-VC in six of the seven sediment treatments in this study is consistent with those reports and indicates that reductive dechlorination is the primary mechanism of biological removal of DCE in Twin Lakes and Pen Branch wetland sediments. The results of this experiment, however, do indicate that mineralization may be an important component of DCE degradation in Twin Lake and Pen Branch sediments under anoxic conditions. Moreover, these results further illustrate that an evaluation of *in situ* DCE biodegradation in Twin Lakes and Pen Branch that is based solely on the accumulation of reduced daughter products may significantly underestimate the extent of anoxic contaminant biodegradation and the contribution of anoxic biodegradation to contaminant attenuation.

VC Degradation under Oxic Conditions

Essentially complete degradation of [1,2-^{14}C] VC was observed in all experimental sediment microcosms incubated under oxic conditions (figure 4, table A2). The sole product of [1,2-^{14}C] VC degradation observed under oxic conditions was $^{14}CO_2$.The degradation of [1,2-^{14}C] VC observed in this study was attributable to biological activity because no significant ^{14}C-product accumulation was observed in autoclaved control or sediment-free control microcosms. The results of this experiment indicate that mineralization, involving direct oxidation or cometabolic oxidation, is the primary mechanism of VC degradation in Twin Lake and Pen Branch sediments under oxic conditions. No evidence of reductive degradation or reduced daughter products was observed under these conditions.

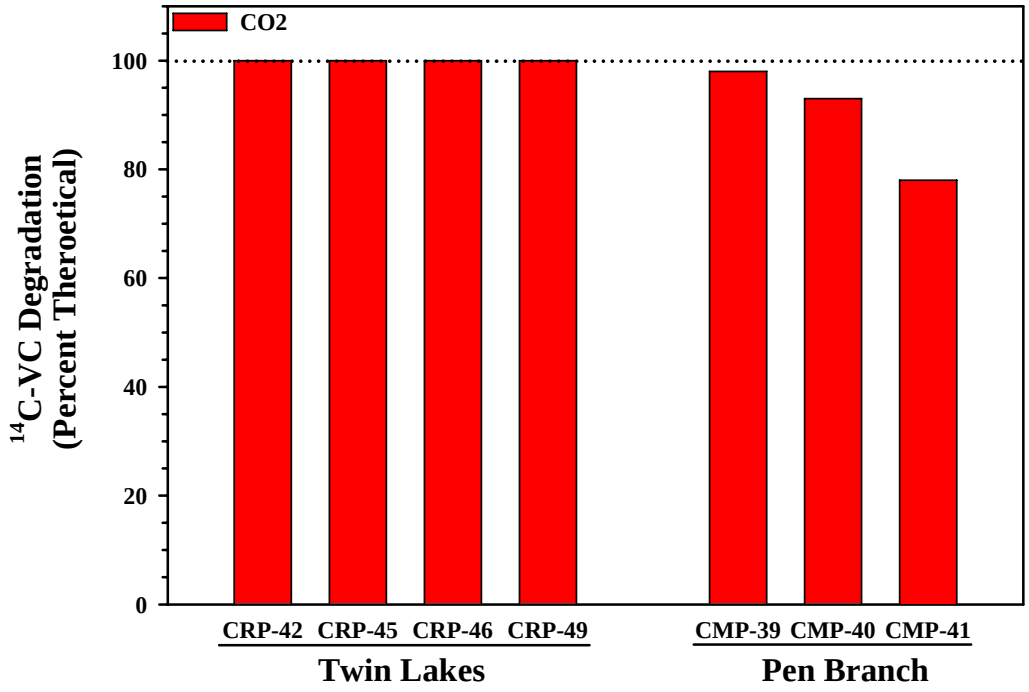

Figure 4. Final percentage recovery of [1,2-^{14}C] VC radioactivity as $^{14}CO_2$ in viable sediment microcosms incubated under oxic conditions. Data are means of triplicate microcosms. Remaining ^{14}C-radioactivity was recovered as undegraded ^{14}C-VC.

As noted earlier for DCE, the spatial and temporal variability in the sediment geochemistry and the activity of associated microbial redox communities in wetland and shallow surface-water systems indicates that oxic, microbial mineralization of VC may contribute significantly to VC attenuation at Twin Lakes and Pen Branch. Thus, an evaluation of the efficiency of *in situ* VC biodegradation based solely on the accumulation of reduced daughter products may significantly underestimate the extent of oxic contaminant biodegradation and the

contribution of oxic biodegradation to contaminant attenuation.

VC Degradation under Anoxic Conditions

Degradation of [1,2-^{14}C] VC also was observed in all experimental sediment microcosms incubated under anoxic conditions (figure 5, table A2). The degradation of [1,2-^{14}C] VC observed in this study was attributed to biological activity because no ^{14}C-product accumulation was evident in autoclaved control or sediment-free control microcosms. The establishment and maintenance of anoxic conditions was confirmed in experimental, autoclaved control and sediment-free control microcosms by headspace gas chromatography.

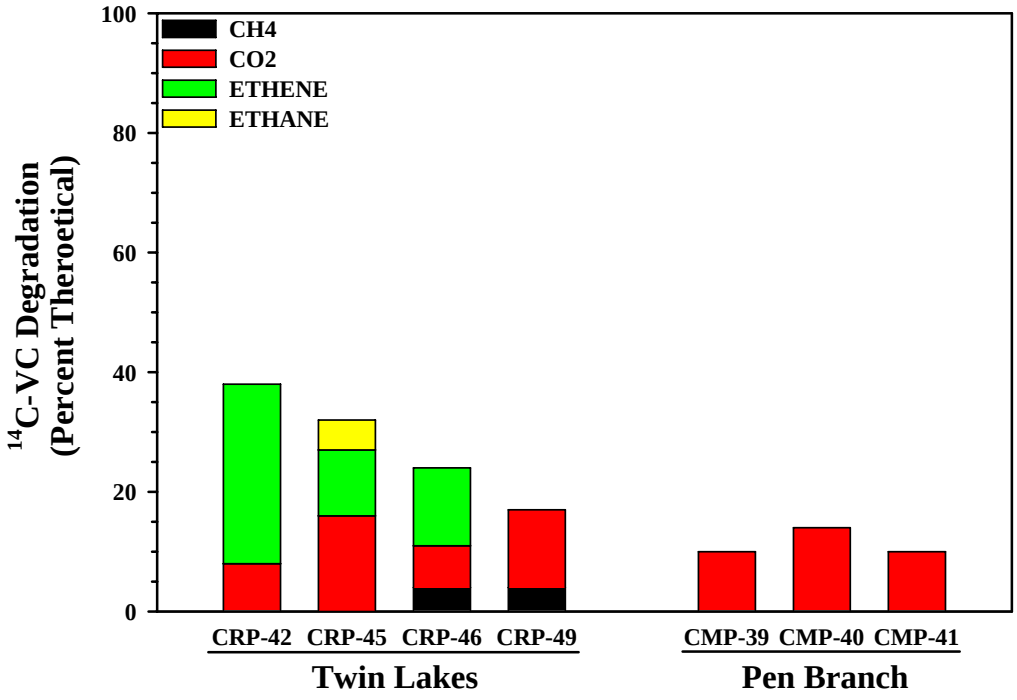

Figure 5. Final percentage recovery of [1,2-^{14}C] VC radioactivity as $^{14}CH_4$, $^{14}CO_2$, ^{14}C-Ethene, and ^{14}C-Ethane, and ^{14}C-VC in viable sediment microcosms incubated under anoxic conditions. Data are means of triplicate microcosms. Remaining ^{14}C-radioactivity was recovered as undegraded ^{14}C-VC.

Production of $^{14}CO_2$ was observed in all sediment microcosms under anoxic conditions. The mean accumulation of $^{14}CO_2$ after 60 days incubation ranged from 7±2 percent of theoretical in CRP-46 microcosms to 16±14 percent in CRP-45 treatments. $^{14}CO_2$ was the sole product of [1,2- ^{14}C] VC degradation observed in anoxic, CMP sediment microcosms, despite the fact that the sediments from CMP-41 were organic-rich. Because oxidation of ^{14}C-ethene to $^{14}CO_2$ under anoxic conditions has been demonstrated previously, these results do not preclude reductive dechlorination of VC in Pen Branch (Bradley and Chapelle, 2002). Rather, these results indicate that microbial degradation of VC to mineralization products may be important in Pen Branch.

In the CRP sediment treatments, the products of [1,2-^{14}C] VC degradation reflected the sediment organic content. CRP-49

sediments were relatively low in organic content, and the sole products of [1,2-^{14}C] VC biodegradation detected in these microcosms were mineralization products ($^{14}CO_2$ and $^{14}CH_4$). In contrast, treatments containing organic-rich CRP sediments accumulated both reductive dechlorination and mineralization products. In these cases, [1,2-^{14}C] VC biodegradation yielded either predominantly reductive dechlorination daughter products (CRP-42) or comparable accumulation of reductive dechlorination and mineralization products (CRP-45 and CRP-46).

Because the accumulation of $^{14}CO_2$ that was observed in Twin Lake and Pen Branch microcosms under anoxic conditions may be the result of a net microbial mineralization of [1,2-^{14}C] VC involving an initial reduction to ^{14}C-ethene followed by oxidation to $^{14}CO_2$ (Bradley and Chapelle, 2002), the results of this study do not necessarily reflect the full contribution of reductive dechlorination processes to the degradation of VC in these sediments. Indeed, the fact that [1,2-^{14}C] VC biodegradation under anoxic conditions (table A2) was less efficient than anoxic [1,2-^{14}C] DCE biodegradation indicates that an initial step, such as reduction to ethene (Bradley and Chapelle, 2002), may limit the net oxidation of VC to CO_2 in these sediments. The results of this experiment, however, do indicate that microbial mineralization may be an important component of VC biodegradation in Twin Lake and Pen Branch sediments under anoxic conditions, and indicate that anoxic mineralization should be considered when evaluating the efficiency of *in situ* VC biodegradation in Twin Lakes and Pen Branch systems.

No Evidence of Degradation in Heat-Sterilized Controls

In this study, no loss of [1,2-^{14}C] DCE or [1,2-^{14}C] VC and no production of ^{14}C-degradation products were observed in heat-sterilized control microcosms containing sediments from Twin Lakes or Pen Branch. Thus, the degradation of [1,2-^{14}C] DCE and [1,2-^{14}C] VC that was observed in this study was consistent with and attributed to biologically driven degradation processes. The possibility remains, however, that abiotic processes may have contributed to the observed degradation of [1,2-^{14}C] DCE and [1,2-^{14}C] VC if the mechanism(s) involved in abiotic degradation were significantly inhibited by the heat-sterilization conditions employed in this study, autoclaving three times for 1 h at 121 ºC and 15 PSI.

Microbial Isolation and Enrichment Efforts

Efforts to isolate or enrich microorganisms capable of mineralizing [1,2-^{14}C] DCE or [1,2-^{14}C] VC have not been successful as of the time of this writing. Sediment-slurry inocula that demonstrated a significant potential to mineralize [1,2-^{14}C] DCE or [1,2-^{14}C] VC failed to retain their mineralization capacity through repeated transfers and/or serial dilutions. In all cases, [1,2-^{14}C] DCE and/or [1,2-^{14}C] VC mineralization activity was extinguished at dilutions greater than 10^5. In all cases, transfers of culture dilutions less than 10^5 failed to grow and/or retain their mineralization activity. As noted earlier, isolation and enrichment of microbial communities is a resource intensive and lengthy endeavor. The approximate 15 year period between the initial field evidence for chloroethene reductive dechlorination and the ultimate isolation of a microorganism capable of complete chloroethene reductive dechlorination to ethene (*Dehalococcoides ethenogenes*) indicated, from the outset, that identification of anoxic chloroethene mineralizing microorganisms, in all probability, would be a lengthy process that would exceed the DOE project time constraints.

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For more information concerning the research in this report, contact:
Paul M. Bradley, U.S. Geological Survey, Stephenson Center, Suite 129, 720 Gracern Road, Columbia, SC 29210.
pbradley@usgs.gov.

Appendix

Table A1. Final percentage accumulation of ^{14}C-products from microbial degradation of [1,2-^{14}C] DCE in viable sediment microcosms after 60 days. Data are means±SD for triplicate microcosms. Remaining ^{14}C-radioactivity was recovered as undegraded ^{14}C-DCE. No accumulation of ^{14}C-products was observed in killed control or sediment free control microcosms. [%, percent]

Site	Sediment	Treatment	Product Accumulation (% Theoretical)				
			$^{14}CH_4$	$^{14}CO_2$	^{14}C-Ethene	^{14}C-Ethane	^{14}C-VC
C-BRP	CRP-42	Oxic	ND[a]	50 2	ND	ND	ND
	CRP-45		ND	52 6	ND	ND	ND
	CRP-46		ND	35 3	ND	ND	ND
	CRP-49		ND	54 12	ND	ND	ND
CMP	CMP-39[b]		ND	58 11	ND	ND	ND
	CMP-40		ND	60 3	ND	ND	ND
	CMP-41		ND	46 4	ND	ND	ND
C-BRP	CRP-42	Anoxic	ND	19 10	19 26	ND	17 5
	CRP-45		ND	40 8	3 0	ND	36 10
	CRP-46		7 7	20 6	9 5	ND	20 8
	CRP-49		6 3	36 6	2 3	ND	2 3
CMP	CMP-39		ND	20 2	ND	ND	ND
	CMP-40		ND	18 2	ND	ND	47 2
	CMP-41		ND	16 2	ND	ND	2 3

Table A2. Final percentage accumulation of ^{14}C-products from microbial degradation of [1,2-^{14}C] VC in viable sediment microcosms after 60 days. Data are means±SD for triplicate microcosms. Remaining ^{14}C-radioactivity was recovered as undegraded ^{14}C-VC. No accumulation of ^{14}C-products was observed in killed control or sediment free control microcosms. [%, percent]

Site	Sediment	Treatment	Product Accumulation (% Theoretical)			
			$^{14}CH_4$	$^{14}CO_2$	^{14}C-Ethene	^{14}C-Ethane
C-BRP	CRP-42	Oxic	ND[a]	100 1	ND	ND
	CRP-45		ND	100 3	ND	ND
	CRP-46		ND	100 2	ND	ND
	CRP-49		ND	100 0	ND	ND
CMP	CMP-39		ND	98 3	ND	ND
	CMP-40		ND	93 12	ND	ND
	CMP-41		ND	78 7	ND	ND
C-BRP	CRP-42	Anoxic	ND	8 0	30 21	ND
	CRP-45		ND	16 14	11 4	5 8
	CRP-46		4 6	7 2	13 7	ND
	CRP-49		4 5	13 0	ND	ND
CMP	CMP-39		ND	10 0	ND	ND
	CMP-40		ND	14 1	ND	ND
	CMP-41		ND	10 1	ND	ND

[a] Not detected. Minimum detection limits were 1%, 2%, 1%, and 1% for $^{14}CH_4$, $^{14}CO_2$, ^{14}C-ethene, and ^{14}C-ethane, respectively.