In cooperation with the
U.S. Army Garrison, Aberdeen Proving Ground
Environmental Conservation and Restoration Division
Aberdeen Proving Ground, Maryland

Anaerobic Degradation of 1,1,2,2-Tetrachloroethane and Association with Microbial Communities in a Freshwater Tidal Wetland, Aberdeen Proving Ground, Maryland: Laboratory Experiments and Comparisons to Field Data

Water-Resources Investigations Report 02–4157

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Conversion Factors, Vertical Datum, and Abbreviations

Multiply	By	To obtain
Length		
centimeter (cm)	0.3937	inch
millimeter (mm)	0.03937	inch
meter (m)	3.281	foot
meter (m)	1.094	yard
Volume		
liter (L)	33.82	ounce, fluid
liter (L)	2.113	pint
liter (L)	1.057	quart
liter (L)	0.2642	gallon
liter (L)	61.02	cubic inch
Flow rate		
meter per year (m/yr)	3.281	foot per year
Mass		
gram (g)	0.03527	ounce, avoirdupois
kilogram (kg)	2.205	pound, avoirdupois

Temperatures in degrees Celsius ($^{\circ}$C) may be converted to degrees Fahrenheit ($^{\circ}$F) as follows:

$$^{\circ}F = (1.8 \times {}^{\circ}C) + 32$$

Vertical Datum: In this report, “sea level” refers to the National Geodetic Vertical Datum of 1929—a geodetic datum derived from a general adjustment of the first-order level nets of the United States and Canada, formerly called Sea Level Datum of 1929.

Concentrations of chemical constituents in water are given either in milligrams per liter (mg/L), micrograms per liter (μg/L), millimoles per liter (mmol/L), or micromoles per liter (μmol/L).

Anaerobic Degradation of 1,1,2,2-Tetrachloroethane and Association with Microbial Communities in a Freshwater Tidal Wetland, Aberdeen Proving Ground, Maryland: *Laboratory Experiments and Comparisons to Field Data*

By Michelle M. Lorah, Mary A. Voytek, Julie D. Kirshtein, and Elizabeth J. (Phillips) Jones

Abstract

Defining biodegradation rates and processes is a critical part of assessing the feasibility of monitored natural attenuation as a remediation method for ground water containing organic contaminants. During 1998–2001, the U.S. Geological Survey conducted a microbial study at a freshwater tidal wetland along the West Branch Canal Creek, Aberdeen Proving Ground, Maryland, as part of an investigation of natural attenuation of chlorinated volatile organic compounds (VOCs) in the wetland sediments. Geochemical analyses and molecular biology techniques were used to investigate factors controlling anaerobic degradation of 1,1,2,2-tetrachloroethane (TeCA), and to characterize the microbial communities that potentially are important in its degradation. Rapid TeCA and daughter product degradation observed in laboratory experiments and estimated with field data confirm that natural attenuation is a feasible remediation method at this site. The diverse microbial community that seems to be involved in TeCA degradation in the wetland sediments varies with changing spatial and seasonal conditions, allowing continued effective natural attenuation throughout the year.

Rates of TeCA degradation in anaerobic microcosm experiments conducted with wetland sediment collected from two different sites (WB23 and WB30) and during three different seasons (March–April 1999, July–August 1999, and October–November 2000) showed little spatial variability but high seasonal variability. Initial first-order degradation rate constants for TeCA ranged from 0.10±0.01 to 0.16±0.05 per day (half-lives of 4.3 to 6.9 days) for March–April 1999 and October–November 2000 microcosms incubated at 19 degrees Celsius, whereas lower rate constants of 0 ± 0.03 and 0.06 ± 0.03 per day were obtained in July–August 1999 microcosms incubated at 19 degrees Celsius. Microbial community profiles showed that low microbial biomass and microbial diversity in the summer, possibly due to competition for nutrients by the wetland vegetation, could account for these unexpectedly low degradation rates. In microcosms incubated at 5 degrees Celsius, about 50 percent of the initial TeCA in solution was converted to daughter products within a 35-day incubation period, indicating that biodegradation in the wetland sediments can continue during cold winter temperatures.

Initial pathways of TeCA degradation were the same in the wetland sediment microcosms regardless of the season or sediment collection site, the reduction-oxidation conditions, and the previous exposure of the sediment to contamination. Immediate and simultaneous dichloroelimination and hydrogenolysis, producing 1,2-dichloroethene (12DCE) and 1,1,2-trichloroethane (112TCA), respectively, were the initial TeCA degradation pathways in all live microcosm experiments. The production and degradation of vinyl chloride (VC), which is the most toxic of the TeCA daughter compounds, was affected by spatial and seasonal variability, reduction-oxidation condition, and pre-exposure of the wetland sediment. TeCA-amended microcosms constructed with WB30 sediment showed approximately twice as much VC production as those constructed with WB23 sediment. Results of 112TCA-amended microcosms indicated that the greater production of VC in the WB30 sediment resulted from a greater predominance of the 112TCA dichloro-

elimination pathway in these sediments. VC degradation also was substantially higher in microcosms constructed with WB30 sediment than those constructed with WB23 sediment, resulting in lower VC concentrations at the end of WB30 microcosms. Enrichment experiments in which microcosm slurry was amended with high initial VC concentrations showed that the spatial difference in VC degradation was negligible after prolonged incubation under methanogenic conditions. Inhibition of methanogenic activity in microcosms by addition of sulfate or of 2-bromoethanesulfonic acid inhibited production and degradation of VC. Inhibition of methanogenesis by addition of ferric iron or of 2-bromoethanesulfonic acid also completely inhibited VC degradation in VC-amended enrichment experiments. Pre-exposure to VC substantially increased degradation in VC-amended enrichment experiments.

A microbial consortium, rather than one microbial species or group, likely is involved in the degradation of TeCA, as indicated by the occurrence of multiple degradation pathways and the variability in VC production and degradation. A bacterial peak at 90 base pair (bp) fragment length in terminal-restriction fragment length polymorphism (TRFLP) profiles was associated with TeCA hydrogenolysis to 112TCA, and bacterial species represented by 198 and 170 bp fragment lengths were associated with TeCA dichloroelimination to 12DCE. Dichloroelimination of 112TCA to VC was associated with increasing dominance of the 198 bp bacterial peak in March–April 1999 and October–November 2000 microcosms, whereas an 86 bp or the 170 bp bacterial peak was associated with 112TCA dichloroelimination in the summer experiment. Hydrogenolysis of 12DCE to VC was associated with a carbon dioxide-utilizing methanogen at 307 bp in the March–April 1999 and October–November 2000 microcosm experiments, whereas production of VC occurred despite low methanogen biomass and methane production in the July–August 1999 experiments. Production of VC in the absence of methane production also occurred in 12DCE-amended enrichment cultures. The exponential production of VC in the 12DCE-amended enrichment cultures after an initial lag indicated growth of a microbial species or group, possibly one of the known dehalorespiring bacteria. Molecular analyses using specific primers targeting dehalorespiring bacteria of the *Dehalococcoides* group (*Dehalococcoides ethenogenes* and *Dehalococcoides* sp. strain FL2) and of the acetate-oxidizing *Desulfuromonas* group (*Desulfuromonas* sp. strain BB1 and *Desulfuromonas chloroethenica*) showed the presence of these bacteria in microcosm slurry from site WB30 but not from site WB23. Addition of hydrogen, which is the favored substrate of *Dehalococcoides*, tripled VC production in 12DCE-amended enrichment cultures. VC degradation showed a marked association with an increase in the relative proportion of Methanosarcinaceae, a family of methanogens that includes all those capable of utilizing acetate as a substrate, in the total methanogen community.

Half-lives for TeCA and TCE estimated from field data were in the range of 60 to 100 days, which agrees well with laboratory estimates of degradation rates considering the inherent differences in the laboratory and field systems. Both laboratory microcosm experiments and field data showed that 12DCE and VC are the predominant, persistent daughter compounds from TeCA degradation. In addition, porewater chemistry showed higher accumulation of VC in the wetland sediment at site WB30 than at site WB23, as was observed in the microcosm experiments. Molecular analyses of grab samples of surficial wetland sediment showed that all the microbial species or groups linked to TeCA degradation in the microcosm experiments were present in all sediment samples. Microbial biomass and diversity were lowest in an area of the wetland (transect C-C′) where porewater VOC concentrations are highest, indicating that the higher VOC concentrations could result from lower degradation rates. The lower microbial biomass and diversity in this area could be caused by toxic effects of the contaminants, or possibly from differences in frequency and duration of tidal inundation.

Introduction

Chlorinated solvents such as trichloroethene (TCE) and 1,1,2,2-tetrachloroethane (TeCA) tend to be relatively resistant to biodegradation in aquifers, compared to the biodegradation potential of petroleum hydrocarbons (National Research Council, 2000). This has led to the development of

long chlorinated solvent plumes at a number of Department of Defense (DoD) and private facilities that have reached, or may reach, discharge areas in surface-water bodies or wetlands. About 75 percent of Resource Conservation and Recovery Act (RCRA) and Superfund sites, including DoD sites, are located within a half mile of a surface-water body (Tomassoni, 2000), where wetlands often are part of the landscape. Conventional engineered remediation of ground water contaminated with chlorinated solvents can be extremely costly and could be detrimental to ecologically sensitive wetland environments. Although chlorinated solvents tend to be relatively resistant to degradation within most aquifers, rapid and complete transformations can occur within the organic-rich reducing environment typical of wetland sediments, indicating that monitored natural attenuation may be an effective remediation option for discharges into wetlands (Lorah and others, 1997; Lorah and Olsen, 1999a, b; Lorah and others, 2001). The study of TeCA degradation and associated microbial communities in wetland sediments reported here is part of a larger ongoing study by the U.S. Geological Survey (USGS) of natural attenuation of chlorinated volatile organic compounds (VOCs) in a freshwater tidal wetland along the West Branch Canal Creek, Aberdeen Proving Ground (APG), Maryland (fig. 1) (Lorah and others, 1997). TeCA and TCE, which can be a daughter product of TeCA degradation in addition to being a widely used solvent, are the major contaminants in the shallow aquifer (less than about 15 m deep) and wetland sediments at this site (Lorah and others, 1997; Lorah and Olsen, 1999a, b; Lorah and others, 2001).

Defining biodegradation rates and reactions is a critical part of assessing the feasibility of monitored natural attenuation as a remediation method for ground water containing organic contaminants because biodegradation is commonly the primary destructive process for these contaminants (Wiedemeier and others, 1998; National Research Council, 2000). Laboratory experiments to define biodegradation processes and rates may be warranted during investigation of natural attenuation at a site, depending on the availability of information in the literature and on the expected complexity of biodegradation processes for contaminants at the site (Wiedemeier and others, 1998). Relatively few studies of TeCA degradation in the environment have been conducted compared to the numerous field and laboratory studies of TCE degradation. In addition, degradation of TeCA is expected to be complex because there are three main initial pathways of reaction that can occur, producing both chlorinated ethene and chlorinated ethane daughter products (fig. 2). Each of these intermediate daughter products could then be degraded through several different reaction pathways. Elucidation of factors controlling the occurrence of these diverse degradation pathways for TeCA is critical because of differing toxicity and persistence of the intermediate daughter products. Of the chlorinated ethenes and ethanes, accumulation of vinyl chloride (VC) might be considered of the greatest concern for remediation of a contaminated site because it is the only known carcinogen and has the lowest maximum contaminant level (MCL) for drinking water (2 µg/L, or micrograms per liter) (U.S. Environmental Protection Agency, 2001). Higher MCLs of 5, 70, 100, 5, and 5 µg/L, respectively, have been established for TCE; *cis*-1,2-dichloroethene (cDCE); *trans*-1,2-dichloroethene (tDCE); 1,1,2-trichloroethane (112TCA); and 1,2-dichloroethane (12DCA) (U.S. Environmental Protection Agency, 2001). Although TeCA currently (2002) is not a regulated drinking-water contaminant, the U.S. Environmental Protection Agency (1998) has placed it on the Drinking Water Contaminant Candidate List, where it is listed as a priority contaminant for determination of regulatory needs based on potential to occur in public water systems with a relatively high frequency and at concentrations that could pose a threat to public health.

Site characteristics, in addition to contaminant properties, determine the potential complexity of biodegradation processes. Wetlands can be extremely complex because they typically have a high diversity of reduction-oxidation (redox) zones and of microbial populations. In addition, wetlands are dynamic surficial systems that can have a high degree of spatial and temporal variability in hydrology, microbiology, and water chemistry because of variable surface-water flow, ground-water recharge, air temperature, sediment organic carbon content, vegetation type, density, and activity. Wetlands, therefore, can show greater spatial and seasonal variability in contaminant distribution and biodegradation rates than observed in most deeper ground-water systems. This variability needs to be understood to determine whether natural attenuation is sufficient as a remediation method at all times of the year, and to predict the amount of time needed for complete attenuation of contaminants in the wetland sediment. In addition, this information can improve long-term monitoring plans by identifying possible factors that could cause changes in natural attenuation efficiency. Because hydrogeologic and geochemical data do not always indicate obvious reasons for variations in degradation processes and rates, molecular biology techniques to characterize microbial communities can be useful (Flynn and others, 2000; Fennel and others, 2001).

The purpose of the USGS microbial study at the freshwater tidal wetland at APG (fig. 1) is to use a combination of geochemical analyses and molecular biology techniques to investigate factors controlling the occurrence of different anaerobic TeCA degradation pathways, and to characterize the microbial communities that potentially are important in these degradation reactions (fig. 2). The objectives of this ongoing study are to (1) evaluate spatial and seasonal variations in TeCA degradation pathways and rates in the wetland sediment, (2) identify microbial groups and/or species involved in biodegradation of TeCA in the wetland sediment, (3) determine the spatial and seasonal variations in microbial communities in the wetland sediment, and (4) ultimately develop gene probes for indicator microbial species that are critical for different reaction pathways of TeCA degradation to occur. Gene probes could be used for long-term monitoring of the presence of critical microbial species over time in

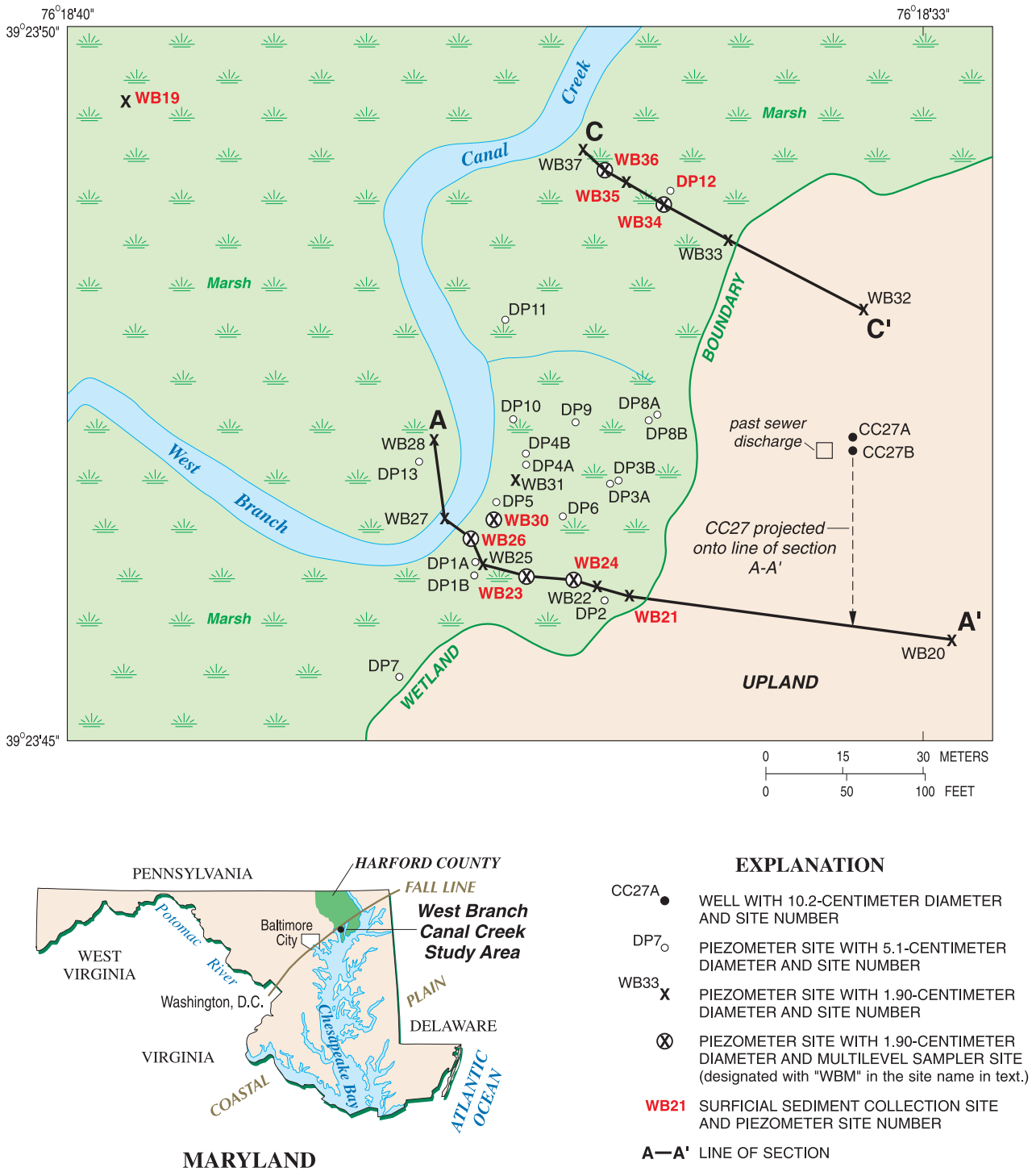

Figure 1. Sampling sites and locations of sections A-A' and C-C' in the wetland study area along the West Branch Canal Creek, Aberdeen Proving Ground, Maryland.

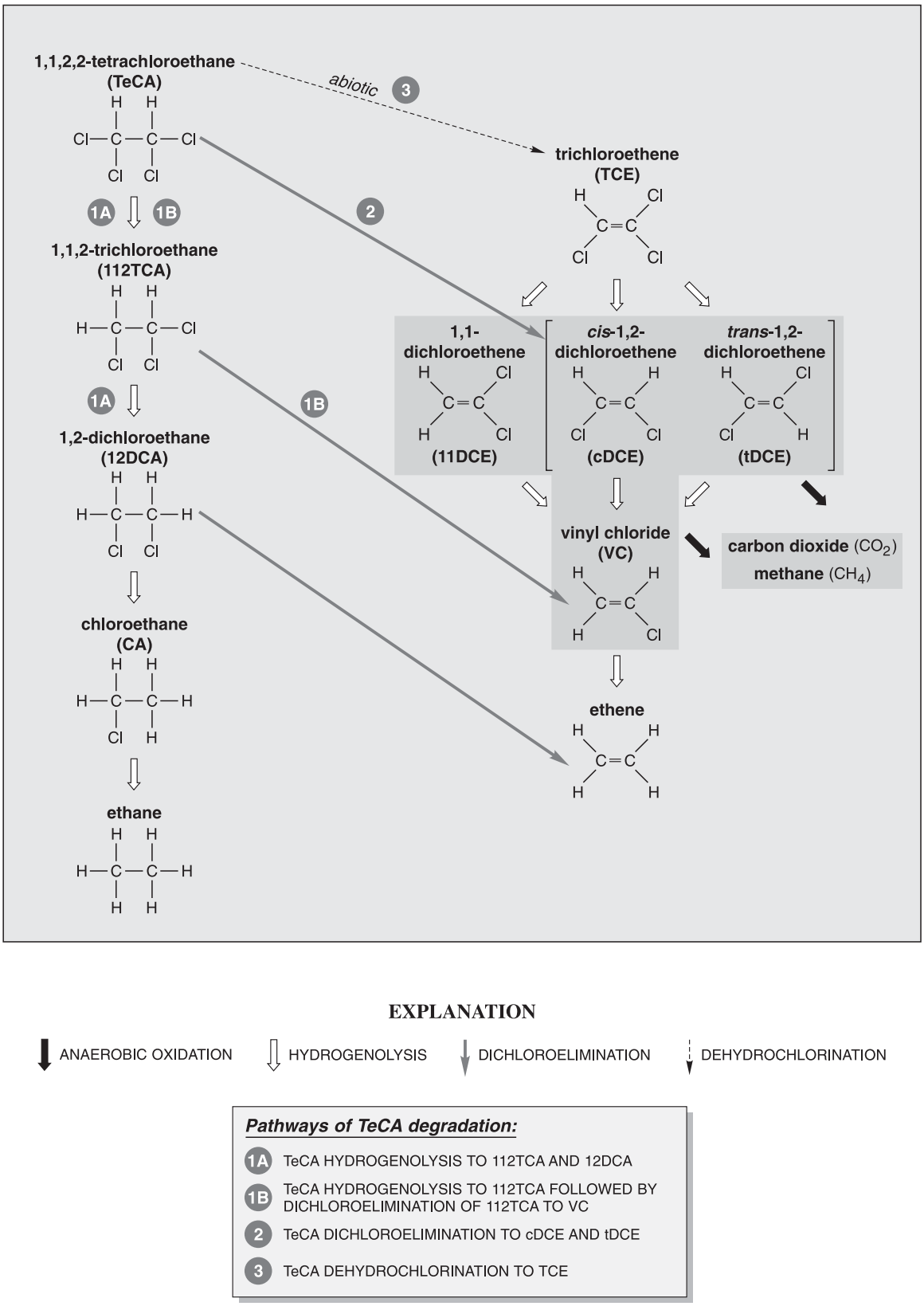

Figure 2. Anaerobic degradation pathways for 1,1,2,2-tetrachloroethane [modified from Chen and others (1996) and Vogel and others (1987)].

the wetland sediment and for evaluating the potential for natural attenuation at other contaminated sites. Because anaerobic TeCA degradation encompasses chlorinated ethane and ethene pathways (fig. 2), results of this study are transferable to other sites at APG and elsewhere that are contaminated with TeCA, TCE, or their anaerobic degradation products.

Purpose and Scope

The purpose of this report is to describe the anaerobic TeCA degradation reactions occurring in wetland sediments in the West Branch Canal Creek study area at APG (fig. 1), and the association between these degradation reactions and the microbial communities in the wetland sediment. Anaerobic laboratory microcosm experiments and enrichment cultures are used to evaluate spatial and seasonal variability in TeCA degradation rates and pathways, and to evaluate other potential factors controlling TeCA degradation, including redox conditions, substrate type, and pre-exposure of the sediment to the contaminants. In addition to microcosms incubated with TeCA, microcosms and/or enrichment cultures amended with the daughter products VC, 112TCA, cDCE, or tDCE (fig. 2) assist in elucidation of TeCA degradation pathways and controlling factors. Enrichment experiments focus primarily on VC production and degradation because of the importance of this toxic TeCA daughter product that potentially can be produced through three reaction pathways (fig. 2). Concomitant geochemical and microbial analyses made during anaerobic microcosm experiments are used to define TeCA degradation pathways and relate these pathways to the microbial communities in the wetland sediment. Geochemical analyses include measurements of the distributions of TeCA, its daughter products, and redox-sensitive constituents over time in the microcosms. Microbial analyses include terminal-restriction fragment length polymorphism (TRFLP) analysis of polymerase chain reaction (PCR)-amplified bacterial 16S rDNA and *mcr*A (methylcoreductase gene) to obtain profiles of the bacterial and methanogen communities, respectively. These genetic analyses commonly are referred to as "DNA fingerprinting." Although the primary objective of this report is to present results of laboratory experiments, selected field data are used to estimate biodegradation rates, evaluate TeCA daughter compound distributions in the wetland porewater, and evaluate distribution of microbial communities in the wetland sediment.

Between 1998 and 2000, four laboratory microcosm experiments were conducted with wetland sediment and porewater collected at different times of the year and from two contaminated sites in the wetland area, sites WB23 and WB30 (fig. 1). During the 35- to 43-day incubation period for each experiment, water in the microcosms was analyzed for TeCA and its potential daughter products and for redox-sensitive constituents, while sediment in the microcosms was analyzed for microbial communities. To allow comparisons of TeCA degradation rates among the experiments, all microcosms were incubated at a temperature of 19 ˚C (degrees Celsius), which was the average summer temperature in the shallow wetland sediment (Lorah and others, 1997). One additional set of microcosms was incubated at a winter temperature of 5 ˚C. The effect of redox conditions on TeCA degradation is evaluated using microcosm and enrichment experiments with added iron or sulfate, or with an inhibitor of methanogenesis. Substrate type effects are examined through the addition of methanol, hydrogen, or acetate in some experiments. The effect of prior exposure, or "pre-exposure," of the wetland sediment to the chlorinated organic contaminants is evaluated using TeCA-amended microcosms constructed with wetland sediment and porewater collected from a background site to compare to those constructed with sediment and porewater from contaminated sites. In addition, pre-exposure effects on VC degradation are examined in VC-amended sediment enrichments constructed using microcosm slurry that was or was not previously incubated with TeCA.

For comparison to laboratory-derived rates, changes in porewater concentrations along flowpaths through the wetland sediment are used to estimate TeCA degradation rates at several multi-level sampling and piezometer sites. Distributions of daughter products in the wetland porewater at sites WB23 and WB30, which were the two main sites used for sediment collection for the microcosm experiments, are compared to distributions observed in laboratory microcosms. To evaluate how the microbial communities change seasonally and spatially in the wetland sediment, surficial sediment samples were collected at 10 piezometer sites (including sites used to obtain sediment for microcosm experiments) in March 1999 and August 1999 (fig. 1). The laboratory- and field-derived data are compared, and the implications of these results to natural attenuation of the contaminants in the wetland are discussed.

Description of Study Area

The study area is located along the West Branch Canal Creek at APG, a U.S. Army base in Maryland near the head of the Chesapeake Bay (fig. 1). Freshwater tidal wetlands surround much of West Branch Canal Creek, which has tidal fluctuations in the range of 0.15 to 0.61 m (meters). Release of waste products by spills, landfilling, and discharge to sewers during chemical manufacturing operations near the creek resulted in ground-water contamination (Lorah and Clark, 1996; Lorah and others, 1997). As part of the study of natural attenuation of VOCs begun by the USGS in 1992, nested drive-point piezometers were installed in the wetland study area primarily along two transects on the eastern side of West Branch Canal Creek (Lorah and others, 1997) (figs. 1 and 3). Transects are aligned perpendicular to the creek, along the general direction of ground-water flow in the aquifer (Lorah and others, 1997). As part of a study under the DoD Environmental Security Technology Certification Program (ESTCP), additional multi-level samplers and piezometers, described in Spencer and others (2002) and Dyer and others (2002), were installed in the wetland and shallow aquifer sediments at six of the pre-existing drive-point piezometer sites (fig. 1).

The contaminated aquifer, known as the Canal Creek aquifer, is about 12 to 14 m thick in the study area and

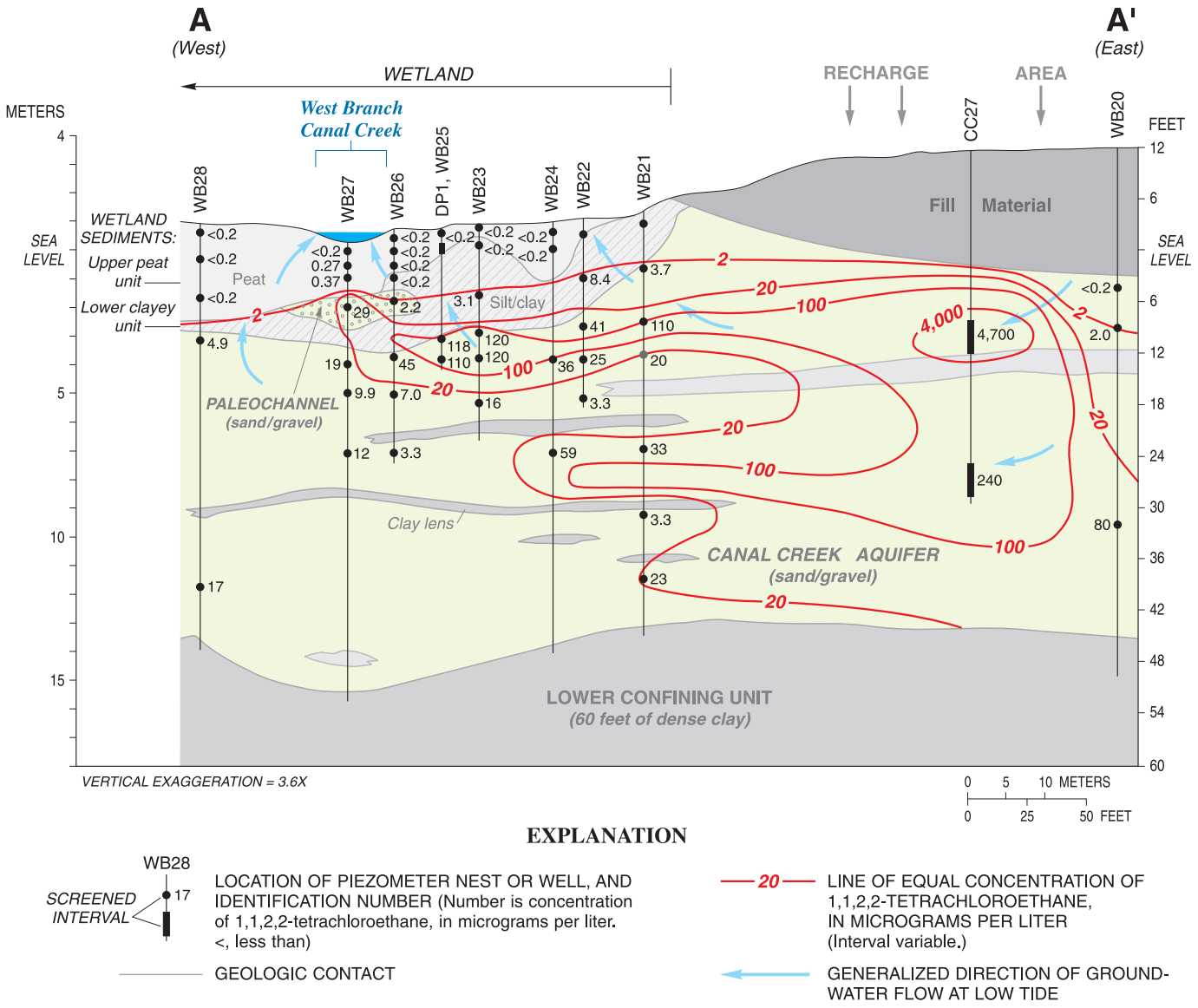

Figure 3. Hydrogeology and 1,1,2,2-tetrachloroethane (TeCA) distribution in ground water along section A-A', June-October 1995 (modified from Lorah and others, 1997, p. 45).

consists mainly of medium- to coarse-grained sand and gravel (fig. 3). Wetland sediments that overlie the aquifer consist of two distinct layers that have a combined thickness of about 1.8 to 3.6 m in the study area--a lower unit of silty to sandy clay or clayey sand, and an upper unit of peat mixed with variable amounts of clay and silt (Lorah and others, 1997). The total organic carbon content in the wetland sediment averaged about 1 percent in the lower clayey unit and 18 percent in the upper peat unit (Lorah and others, 1997; Olsen and others, 1997). Ground-water-flow directions within the wetland area are predominantly upward. At low tide, water from the aquifer discharges through both the wetland and channel sediments. At high tide, however, reversals in ground-water-flow directions can occur at some sites (Lorah and others, 1997). The linear ground-water-flow velocity in the wetland sediments is estimated to be about 0.6 to 0.9 m/yr (meters per year) (Lorah and others, 1997).

The contaminant plume in the aquifer contains both TeCA and TCE from disposal sources, and previous field and laboratory studies have shown that rapid TeCA and TCE degradation occurs as the plume flows upward from the aerobic sand aquifer and through the anaerobic wetland sediments (Lorah and others, 1997, 2001; Lorah and Olsen, 1999a, b). Although carbon tetrachloride and chloroform also are present in the aquifer along the A-A′ and C-C′ transects, their concentrations are below detection levels (0.5 µg/L, or micrograms per liter) in the wetland sediments (Lorah and others, 1997). TeCA and TCE concentrations were highest (about 4,700 and 300 µg/L, respectively)

upgradient from the eastern edge of the wetland at site CC 27 (figs. 1 and 3), which is near a suspected source from a past sewerline discharge point (Lorah and Clark, 1996; Lorah and others, 1997). TCE is a major contaminant in the aquifer along section A-A′, but its concentrations are an order of magnitude lower along section C-C′ (Lorah and others, 1997; Lorah and Olsen, 1999b). Concentrations of VOCs observed in ground water at site CC 27 have remained relatively constant from 1987 through 2001 (Lorah and Clark, 1996; Lorah and others, 1997; Spencer and others, 2002), indicating that there is a continuous source of VOCs to the shallow aquifer and that the contaminant plume discharging to the wetland is at steady state. Moving downgradient into the wetland area, the TeCA and TCE plume remains shallow in the aquifer, reflecting the upward head gradients in the aquifer (fig. 3). Total concentrations of VOCs are in the range of 400 to 500 µg/L in a thin zone that lies directly beneath the wetland sediments (Lorah and others, 1997). Along the upward direction of flow through the overlying wetland and creek-bottom sediments, total concentrations of VOCs decrease substantially until concentrations generally are below detection levels within 0.15 to 0.30 m of land surface (Lorah and others, 1997; Lorah and Olsen, 1999a,b) (fig. 3).

The decrease in VOC concentrations is associated with the natural increase in dissolved organic carbon concentrations and decrease in redox state of the ground water along the upward flow direction in the wetland sediments (Lorah and others, 1997; Lorah and Olsen, 1999b). The aquifer typically is aerobic or hypoxic. Iron-reducing conditions are predominant in the lower clayey unit of the wetland sediments, whereas methanogenic or methanogenic/mixed zones that contain variable concentrations of ferrous iron and sulfide in addition to methane are predominant in the upper peat unit. Production of anaerobic daughter products (fig. 2) coincides with a decrease in TeCA and TCE concentrations and a decrease in redox state of the ground water along upward flowpaths through the wetland sediments at each site (Lorah and others, 1997*;* Lorah and Olsen, 1999b). Laboratory microcosm experiments conducted during 1996–1997 with TeCA and TCE confirmed the rapid degradation of these compounds in the wetland sediments (Lorah and others, 1997, 2001; Lorah and Olsen, 1999a).

Background on Anaerobic Degradation Pathways

TeCA can degrade under anaerobic conditions by three main reaction pathways—a hydrogenolysis pathway that produces 1,1,2-trichloroethane (112TCA) and 1,2-dichloroethane (12DCA) as intermediate daughter compounds; a dichloroelimination pathway that produces 1,2-dichloroethene (12DCE, both *cis*- and *trans*- isomers) and VC as intermediate daughter products; and an abiotic dehydrochlorination reaction that produces TCE (fig. 2) (Vogel and others, 1987; Lorah and others, 1997; Chen and others, 1996). Hydrogenolysis entails the sequential replacement of a single chlorine atom by hydrogen, whereas dichloroelimination entails the simultaneous replacement of two adjacent chlorine atoms by hydrogen to produce a double bond. For both of these types of reductive dechlorination reactions, the contaminant serves as an electron acceptor, resulting in production of more reduced, less-chlorinated daughter compounds. Microorganisms require the presence of suitable electron donors (substrates) for reductive dechlorination to occur. Possible electron donors include natural compounds such as hydrogen, acetate, and methanol, and anthropogenic organic compounds such as benzene and toluene. Low natural organic carbon content may limit the extent of reductive dechlorination in many aquifers (Chapelle, 1993; Natural Research Council, 2000), whereas a source of organic substrates is not expected to be a limiting factor in organic-rich wetland sediments (Lorah and Olsen, 1999b).

Microbially catalyzed reductive dechlorination reactions often are believed to be cometabolic (a fortuitous reaction from which the microorganism does not derive energy), but a few bacteria capable of obtaining energy from reductive dechlorination reactions also have been isolated in the past decade (Löffler and others, 1996). Cometabolic reductive dechlorination can occur through reactive transition metal cofactors, such as vitamin B_{12}, heme, and coenzyme F_{430}, in anaerobic microorganisms that catalyze the replacement of chlorines by hydrogen atoms (Fathepure and Boyd, 1988; Gantzer and Wackett, 1991; Schanke and Wackett, 1992; Yager and others, 1997; Novak and others, 1998). These reactive coenzymes are especially abundant in methanogens (anaerobic microorganisms that produce methane predominantly through metabolism of hydrogen plus carbon dioxide or metabolism of acetate) and acetogens (anaerobic microorganisms that synthesize acetate from carbon dioxide through the acetyl-CoA pathway) (Fathepure and Boyd, 1988; Maymó-Gatell and others, 1997; Yager and others, 1997). The highly chlorinated solvents such as TeCA and TCE have been shown to biodegrade faster and undergo more complete dechlorination under methanogenic conditions than under the less reducing conditions of nitrate or sulfate reduction (McCarty and Semprini, 1994; Lorah and others, 1997). Methanogenic conditions often are predominant in freshwater wetland sediments (Capone and Kiene, 1988). In addition to methanogens and bacteria capable of cometabolic reactions, pure cultures of anaerobic dehalorespiring bacteria that can use chlorinated ethenes, including tetrachloroethene (PCE), TCE, and cDCE, as terminal electron acceptors to derive energy and grow have been isolated (Holliger and others, 1993; Krumholz and others, 1996; Maymó-Gatell and others, 1997, 1999, 2001). This growth-coupled metabolism of halogenated VOCs generally is believed to be faster than cometabolic reductive dechlorination, and thus may be more effective for remediation of contaminated ground water (Maymó-Gatell and others, 1997; Yager and others, 1997). The only chlorinated ethane reported to be degraded by dehalorespiring bacteria is 12DCA (Maymó-Gatell and others, 1997, 1999).

Few previous studies have examined the *in situ* degradation pathways of TeCA in ground water or soil. In previous USGS field and laboratory investigations at the West Branch

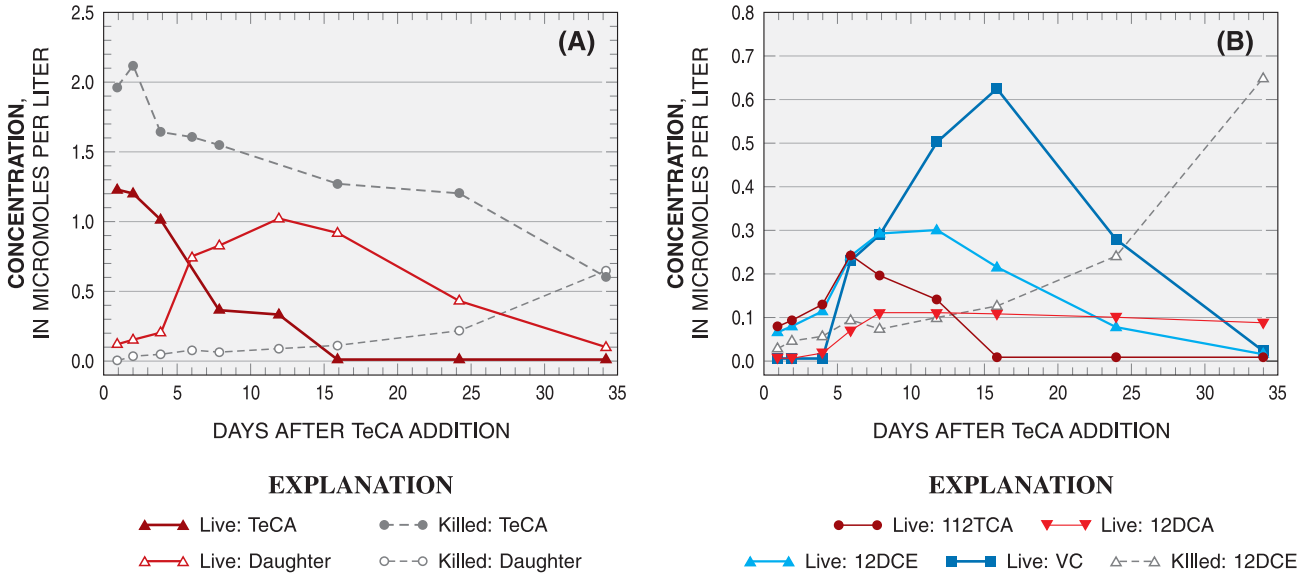

Figure 4. Degradation of 1,1,2,2-tetrachloroethane in previous anaerobic microcosm experiment (30E4) with wetland sediment (site WB30), May-June 1996 (from Lorah and others, 1997; Lorah and Olsen, 1999a): **(A)** 1,1,2,2-tetrachloroethane (TeCA) and the sum of daughter products in the TeCA-amended live microcosms and sterile controls, and **(B)** Daughter products 1,2-dichloroethene (12DCE, total of the *cis* and *trans* isomers); vinyl chloride (VC); 1,1,2-trichloroethane (112TCA); and 1,2-dichloroethane (12DCA) in the TeCA-amended microcosms.

Canal Creek wetland study area at APG, 12DCE (both *cis*- and *trans*- isomers) and VC were the predominant persistent daughter compounds (Lorah and others, 1997; Lorah and Olsen, 1999a, b). These daughter compounds primarily resulted from the combined hydrogenolysis and dichloroelimination pathway 1B and dichloroelimination pathway 2 (fig. 2), whereas abiotic production of TCE was a minor degradation pathway (accounting for less than 2 percent of the initial TeCA) (Lorah and others, 1997; Lorah and Olsen, 1999a, b). Although 12DCE and VC temporarily accumulated, complete anaerobic degradation to non-chlorinated end products was observed within 34 days in microcosms constructed with the wetland sediment and amended with an initial TeCA concentration of about 2.9 µmol/L (micromoles per liter) (Lorah and others, 1997; Lorah and Olsen, 1999a) (fig. 4). In laboratory experiments conducted using anaerobic municipal digester sludge, a substantially higher percentage of TCE and lower percentage of 112TCA and VC was observed from TeCA degradation (Chen and others, 1996) than was observed in the anaerobic wetland sediment (Lorah and Olsen, 1999a). In contrast, TeCA degraded solely to cDCE by pathway 2 (fig. 2) in anaerobic batch experiments conducted with bottom sediment from a slow-moving stream in the Netherlands (Peijnenburg and others, 1998). Dichloroelimination pathway 2 (fig. 2) also was reported to be the major transformation route for TeCA in anaerobic batch experiments conducted with bottom sediment from a pond or a slow-moving stream in Georgia (Jafvert and Wolfe, 1987). Continued degradation of 12DCE to VC was not observed in either the experiments by Jafvert and Wolfe (1987) or by Peijnenburg and others (1998), possibly because they were conducted for less than 10 and 25 days, respectively. Alternatively, the sediments used in these experiments may have been lacking a critical microbial species or group.

Field and laboratory studies of reductive dechlorination of TCE, which are more numerous than studies of TeCA degradation, have shown that dehalorespiring bacteria that can reductively dechlorinate 12DCE to VC and ethene are relatively uncommon, whereas biotic conversion of TCE to 12DCE occurs at many chlorinated solvent sites (Maymó-Gatell and others, 1995; Fennell and others, 2001). Complete dechlorination of TCE and PCE is more commonly observed in the field or in microcosms and reactors that contain mixed cultures than in experiments with pure cultures (Flynn and others, 2000). Pure cultures of dehalorespiring bacteria that can use PCE and TCE as terminal electron acceptors include *Dehalospirillum multivorans*, *Dehalobacter restrictus* strains PER-K23A and TEA, *Desulfuromonas* sp. strain BB1 and *Desulfuromonas chloroethenica*, *Enterobacter* sp. strain MS1, *Desulfitobacterium* sp. strain PCE-S, and *Dehalococcoides ethenogenes* strain 195 (Löffler and others, 2000). *Dehalococcoides ethenogenes* strain 195 is the only known isolate, however, that completely dechlorinates PCE or TCE to the non-chlorinated end product ethene (Maymó-Gatell and others, 1997). Although *Dehalococcoides ethenogenes* strain 195 achieves complete reduction of PCE and TCE, the reduction of VC does not support its growth. Complete dechlorination to ethene could be sustained only by periodic additions of filtered supernatant from the mixed culture of which it was

isolated originally (Maymó-Gatell and others, 1995, 1997, 2001), implying that mixed cultures (or a consortium) are required to achieve complete dechlorination. The accumulation of VC that commonly is observed during anaerobic reduction of chlorinated ethenes in field studies and laboratory experiments may indicate that one or more critical microorganisms are lacking.

In addition to reduction of VC, anaerobic oxidation or mineralization of VC to carbon dioxide (CO_2) or to CO_2 and methane (CH_4) has been reported in laboratory experiments under iron-reducing, sulfate-reducing, humic acid-reducing, and methanogenic conditions (Vogel and McCarty, 1985; Bradley and Chapelle, 1996, 1998, 1999a; Bradley and others, 1998). 12DCE also can undergo anaerobic oxidation, but at substantially slower rates than those observed for VC (Bradley and others, 1998). Recent experiments with organic-rich streambed sediments incubated with ^{14}C-radiolabeled VC showed the transient presence of ^{14}C-acetate as an intermediate of VC degradation under methanogenic conditions, and the production of approximately equal molar concentrations of CO_2 and CH_4 (Bradley and Chapelle, 1999b, 2000). Furthermore, although addition of an inhibitor of methanogenesis (BES, or 2-bromoethanesulfonic acid) caused a decrease in VC degradation rate, VC degradation was not stopped completely. These results led Bradley and Chapelle (2000) to propose that VC mineralization to CO_2 and CH_4 involves two steps: (1) oxidation of VC by acetogens, which produces acetate, (2) and metabolism of acetate to CO_2 and CH_4 by acetotrophic methanogens. This theory proposes that methanogens are indirectly, rather than directly, involved in the anaerobic mineralization of VC. The relative importance of reductive dechlorination reactions (either cometabolic or dehalorespiratory) and of anaerobic oxidation reactions in controlling the fate of VC and other chlorinated daughter products in the environment is unknown.

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Methods and Data Analysis

Anaerobic laboratory microcosm experiments to examine TeCA degradation were conducted between 1998 and 2000 (February–April 1998, October 1998, March–April 1999, July–August 1999, and October–November 2000). A previously reported experiment (Lorah and others, 1997; Lorah and Olsen, 1999a) that was conducted in May–June 1996 is included for comparisons (table 1). Microcosm preparation, incubation, and geochemical analysis techniques generally were the same in the 1998–2000 experiments as those methods reported previously (Lorah and others, 1997; Lorah and Olsen, 1999a). The only differences in microcosm construction methods from the May–June 1996 experiment are that wetland sediment was collected at a different site and during a different season, and that methanol (instead of deionized organic-free water) was used in preparing TeCA and 112TCA stock solutions for the February–April 1998 experiment (table 1). The February–April 1998 experiment was designed to test the effect of different initial concentrations of TeCA, the presence of sulfate as an electron acceptor, and the inhibition of methanogenesis on TeCA degradation rates and pathways. The 1999 and 2000 experiments were designed to evaluate the spatial and seasonal variability in TeCA degradation rates and pathways. In the October 1998 and October–November 2000 experiments, treatments also were prepared with and without addition of methanol (MeOH) to evaluate the effect of the added MeOH (table 1). Enrichment experiments discussed in this report were conducted in January–June 2001.

Anaerobic microcosm experiments and geochemical analyses were performed by USGS personnel in Baltimore, Maryland, whereas enrichment experiments and all micro-

Table 1. *Treatments used in anaerobic microcosm experiments to examine 1,1,2,2-tetrachloroethane or 1,1,2-trichloroethane degradation in wetland sediments from the West Branch Canal Creek study area, Aberdeen Proving Ground, Maryland, May 1996–November 2000*

[Microcosms were amended with 1,1,2,2-tetrachloroethane (TeCA) or 1,1,2-trichloroethane (TCA) at day 0. Treatment names beginning with "23," "30," or "Bck" were constructed with sediment from site WB23, WB30, or background site WB19, respectively. Treatment names containing "LC" indicate live unamended controls. Killed controls were prepared for all treatments, except 23BES-TeCA.2, by adding 1 percent formaldehyde by volume. Initial concentrations (C_i) of TeCA or TCA, which are given in micromolar (μM) followed (in parentheses) by micrograms per liter (μg/L), are the highest concentration detected in solution during days 1 to 3 (T_i). Other amendments included 4 millimolar (mM) methanol (MeOH), used in preparing stock solutions of TeCA or TCA in some experiments; 11 mM sulfate (SO_4); and 30 mM 2-bromoethanesulfonic acid (BES) added on day 5. Dashes indicate no amendment.]

Treatment name	C_i TeCA [μM (μg/L)]	C_i TCA [μM (μg/L)]	T_i (day)	Other amendments
May–June 1996:				
30E4	1.2 (210)	--	1	--
February–April 1998:				
23TeCA.1	1.4 (240)	--	2	MeOH
23TeCA.2	3.6 (610)	--	3	MeOH
23TeCA.3	13 (2200)	--	1	MeOH
Bck-TeCA.2	3.3 (550)	--	2	MeOH
23SO_4-TeCA.2	3.1 (520)	--	2	MeOH; SO_4
23BES-TeCA.2	3.2 (540)	--	1	MeOH; BES
23TCA.1	--	2.4 (320)	1	MeOH
23TCA.2	--	6.3 (840)	1	MeOH
23LC	--	--	--	--
Water control	3.3 (550)	3.9 (520)	0	MeOH
October 1998:				
23TeCA.2	2.8 (480)	--	1	MeOH
23TeCA.2	2.0 (340)	--	1	--
23TCA.1	--	1.6 (220)	1	MeOH
23TCA.1	--	1.9 (250)	1	--
March–April 1999:				
23TeCA.3/99	2.8 (470)	--	1	--
23TeCA5.3/99	3.0 (500)	--	1	--
23TCA.3/99	--	3.2 (430)	1	--
23LC.3/99	--	--	1	--
30TeCA.3/99	2.2 (370)	--	1	--
30TCA.3/99	--	3.1 (410)	1	--
30LC.3/99	--	--	1	--
Water Control	3.2 (540)	3.4 (450)	2	--
July–August 1999:				
23TeCA.7/99	4.3 (720)	--	3	--
23TCA.7/99	--	2.0 (270)	1	--
23LC.7/99	--	--	1	--
30TeCA.7/99	4.0 (670)	--	3	--
Water Control	5.6 (940)	4.6 (620)	0	--
October-November 2000:				
23TeCA.10/00	2.0 (340)	--	1	--
30TeCA.10/00	2.5 (420)	--	3	--
30MeOH.10/00	3.1 (520)	--	1	MeOH
30TCA.10/00	--	2.6 (350)	1	--
Water Control	4.8 (800)	1.6 (330)	0	--

bial community analyses were performed by USGS National Research Program personnel in Reston, Virginia. Ground-water-sampling methods for selected chemical data that are used in this report for comparison to laboratory data were published in Spencer and others (2002). The same analytical techniques were used to determine geochemical constituents in water samples collected from the laboratory microcosms and in ground-water samples collected in the field. The same microbial community analytical techniques also were used for sediment samples collected from the laboratory microcosms and from the field. These analytical techniques, therefore, are described only in the first section on laboratory microcosm experiments.

Laboratory Microcosm Experiments

Sediment and Ground-Water Collection To evaluate spatial and seasonal effects on biodegradation pathways and rates, microcosms were constructed with shallow wetland sediment collected during three seasons (late winter/early spring samples in March 1999, summer samples in July 1999, and fall samples in October 2000) and from two different contaminated sites (near piezometer sites WB23 and WB30 (fig. 1). Sites WB23 and WB30 are separated horizontally by a distance of about 9 m (fig. 1). As part of an effort to evaluate the possible effect of pre-exposure to the contaminants on degradation, sediment also was collected from the upper peat unit at background site WB19 (fig. 1) for one microcosm experiment in February–April 1998 (table 1). Measurements of dissolved oxygen, ferrous and ferric iron, sulfide, and methane indicate that the upper peat unit of the wetland sediment at and near sites WB23, WB30, and WB19 is methanogenic or mixed iron-reducing and methanogenic (Lorah and others, 1997; Olsen and others, 1997). The total organic carbon content in sediment samples collected in the upper 30 cm (centimeters) at or near these two sites was between 6.9 and 10.6 percent (Olsen and others, 1997). Wetland sediment from the upper peat unit at both sites was collected from a depth of 0 to 25 cm below land surface. The sediment was placed in mason jars and packed to leave minimum airspace.

Wetland porewater for the microcosms constructed with WB23 and WB30 sediment was collected from a nearby contaminated piezometer, WB24B (figs. 1 and 3). The chemistry of water from piezometer WB24B is similar to that from shallow piezometers at WB23 and WB30, but piezometer WB24B has a more rapid recovery rate during sampling. For microcosms constructed with sediment from background site WB19, wetland porewater was collected from piezometer WB19B. Porewater was collected using a peristaltic pump with tubing extended to the top of the screened interval. No effort was made to prevent loss of VOCs from the water during sample collection, and the water was purged with nitrogen gas immediately prior to microcosm construction to remove oxygen that potentially was added during sample collection. Sediment and porewater for the microcosms were collected during the week prior to microcosm construction and then were stored at the planned incubation temperature.

Microcosm Preparation and Incubation All microcosms were constructed under a nitrogen atmosphere in 162-mL (milliliter) serum bottles using a 1.5:1 volumetric ratio of ground water to wetland sediment (with no headspace) and incubated upside down in the dark. All microcosms were incubated at the summer temperature of 19 °C (Lorah and others, 1997), although an additional set of microcosms in the March–April 1999 experiment was incubated at the winter temperature of 5 °C.

Duplicate microcosm bottles were prepared for each treatment and sacrificed for chemical and microbiological analyses at each time step. Microcosms generally were incubated for 35 to 42 days and sampled at 9 or 11 time steps during this period. One exception was the October 1998 microcosms, which were designed solely to observe the effect of MeOH addition, and were incubated for only 15 to 20 days and sampled at 5 or 6 time steps (Lorah-Devereux, 1999). Experiments included killed controls that were prepared with 1 percent by volume of formaldehyde for each treatment. Unamended live controls that did not contain added TeCA or 112TCA also were prepared for each sediment (WB23 or WB30) used to construct microcosms. Water controls (microcosms containing only deionized water and amended with TeCA or 112TCA) were constructed to account for any potential losses due to volatilization or to sorption to the serum bottle or Teflon-coated rubber stoppers. Stock TeCA and 112TCA solutions were made from neat chemicals (Supelco, Bellefonte, Pennsylvania) and added to microcosms using gas-tight syringes. Stock solutions that were prepared using only deionized water were placed in the dark on magnetic stir plates for 24 hr before spiking the microcosms. For stock solutions mixed in a flask with 50 percent water and 50 percent MeOH (table 1), the stock solutions were stirred for 4 hr before spiking. Aliquots of the stock solutions were analyzed before addition to the microcosms to estimate the injection volumes needed to obtain the planned initial concentrations at day 1. Because of expected losses due to sorption during the first 24 hr (Lorah and others, 1997; Lorah and Olsen, 1999a), the microcosms were spiked on day 0 with a concentration about 2.4 times greater than the desired initial (day 1) aqueous concentration. Most microcosms were expected to reach the wetland sediments' natural methanogenic conditions within several days of incubation, but high sulfate concentrations (11 mM, or millimolar) were added in one treatment to induce sulfate-reducing conditions (table 1). In addition, BES, a known specific inhibitor of methanogenesis (Oremland and Capone, 1988), was added to one set of TeCA-amended microcosms (table 1).

Geochemical and Microbial Community Analyses At each time step, supernatant from duplicate microcosm bottles was withdrawn for analyses of pH, VOCs, ferrous iron, sulfate, and CH_4 by the same methods as previously reported (Lorah and others, 1997; Lorah and Olsen, 1999a, b). The initial pH in all microcosms ranged from 6.2 to 6.6, which is in the range of pH measured in the wetland porewater in the field (Lorah and others, 1997; Lorah and Olsen, 1999b), and

increased to as high as 7.2 at the end of the experiments. VOCs were analyzed at the on-site laboratory facility at APG using a purge and trap capillary gas chromatography/mass spectrometry (GC/MS) method that is equivalent to U.S. Environmental Protection Agency Method 524.2 (Rose and Schroeder, 1995). Average relative percent differences for analyses of replicate VOC samples ranged from 5 to 13 percent for TeCA and its potential daughter compounds (Spencer and others, 2002). Samples for ferrous iron were filtered through 0.2-µm (micrometer) filters and analyzed on-site by the colorimetric bipyridine technique. Sulfate was determined on 0.45-µm filtered samples by ion chromatography. Methane was determined by collecting unfiltered water in sealed serum bottles and analyzing the headspace by gas chromatography with a flame ionization detector (GC/FID) at the on-site laboratory. Methane concentration in the water then was calculated using the ideal gas law and Henry's Law constant (Baedecker and Cozzarelli, 1992).

After supernatant was removed for chemical analyses at each time interval, the remaining sediment slurry from the microcosms was transferred to plastic bags and immediately frozen for later microbial analyses. For most experiments, sediment samples from the sites used in microcosm construction also were collected in the field and immediately frozen for microbial analyses. The bacterial and methanogen communities from TeCA- or 112TCA-amended microcosm slurries were characterized using a polymerase chain reaction (PCR) procedure of amplifying specific targeted DNA and performing terminal restriction fragment length polymorphism (TRFLP) analysis on the PCR products (Clement and others, 1998). TRFLP provides a molecularly derived microbial "fingerprint" that provides a relative estimate of the total microbial abundance and of the diversity of bacterial and methanogen communities. In order to evaluate bacterial communities, 16S rDNA was targeted for analysis because it codes for a molecule present in all bacteria. For microcosm samples from the March–April 1999 experiment, a newly available nested PCR methodology also was used that targeted specific dehalorespiratory bacteria (Löffler and others, 2000). The two primer pairs are specific to hypervariable regions of the 16S rRNA genes of the *Dehalococcoides* group (*Dehalococcoides ethenogenes* and *Dehalococcoides* sp. strain FL2) and of the acetate-oxidizing *Desulfuromonas* group (*Desulfuromonas* sp. strain BB1 and *Desulfuromonas chloroethenica*) (Löffler and others, 2000).

To characterize the methanogenic community, a key functional gene involved in methanogenesis, *mcr*A, was amplified and analyzed. The gene *mcr*A codes for the enzyme methylcoreductase that is involved in the final reaction in methanogenesis, which is release of CH_4 after reduction of a methyl group bound to a coenzyme (Klein and others, 1988). The size of the PCR *mcr*A products (464 and 481 base pairs, respectively) differs between the Methanosarcinaceae, the only group that contains acetate-utilizing methanogens (acetoclasts), and the other main methanogen groups Methanococcales, Methanobacteriales, and Methanomicrobiales. Because of this fragment length heterogeneity, the proportion of Methanosarcinaceae in the total methanogen community can be determined to evaluate the potential importance of acetoclasts in the microcosm experiments.

To obtain the TRFLP profiles, DNA was extracted from the same amount of sediment for all samples, re-suspended in the same volume of buffer, and the same volume was subsequently used to amplify and digest the samples. Sample TRFLP profiles, therefore, were normalized to equal amounts of sediment. DNA was extracted from microcosm slurries or surficial sediments using the Bio101 FastDNA spin kit for soil according to manufacturer's instructions (Bio101 Inc., Vista, California). PCR was performed to amplify a portion of the bacterial 16S rDNA using the primers 46 forward with a fluorescent tag (FAM) and 519 reverse (Brunk and others, 1996) at a concentration of 0.2 µM. PCR conditions were as follows: one cycle of 94 °C for 5 minutes followed by 30 cycles of 94 °C for 30 seconds, 56 °C for 30 seconds, 72 °C for 90 seconds, and a final extension for 7 minutes at 72 °C. *Mcr*A DNA amplifications were performed using the degenerate *mcr*A oligonucleotide primer pair described by Luton (1996) at 0.2 µM, except the forward primer was labeled with the fluorescent tag FAM. PCR conditions were as follows: one cycle of 94 °C for 5 minutes followed by 38 cycles of 94 °C for 45 seconds, 56 °C for 45 seconds, and 72 °C for 2 minutes, with the first four cycles employing a slow (50 percent) ramp between the 56 °C and 72 °C incubation.

After amplification, bacterial 16S rDNA amplicons were digested with the restriction enzyme *Mnl*I, whereas *mcr*A amplicons were digested with *Rsa*I (New England Biolabs, Beverly, Massachusetts) at 37 °C overnight. Digests were precipitated in ethanol, centrifuged, dried, and resuspended in Tris-EDTA buffer. Samples were electrophoresed on an ABI310 sequencer (Applied Biosystems, Foster City, California), generating a plot called an electropherogram of the size (using internal standards TAMRA 500) and the relative intensity of each fluorescent fragment. Undigested *mcr*A amplicons also were analyzed to determine the proportion of Methanosarcinaceae in the methanogen population on the basis of fragment length heterogeneity. Biomass in the microcosm slurry was estimated from total DNA extracted, the intensity of PCR amplifications, and TRFLP peak intensities.

Calculation of Degradation Rates Biodegradation rates were calculated assuming first-order kinetics, as in earlier experiments (Lorah and others, 1997). The apparent biodegradation rate constant, k, was determined from the slope of a linear regression of the natural logarithm of the aqueous TeCA or 112TCA concentration [ln(TeCA) or ln(112TCA)] remaining in the live microcosms against time of incubation. The rate constants calculated for the killed controls were subtracted from the rate constants in the live microcosm if they were greater than the standard error calculated for the rate constants in the live microcosms. Biotic daughter products generally were not detected in the killed controls during the time periods used to calculate rate constants.

After the rate constant *k* was estimated, biodegradation rates of TeCA or 112TCA (units of μM/day) were calculated by multiplying *k* (units of per day) by the initial TeCA or 112TCA concentration (units of μM) in the experiment.

Laboratory Enrichment Experiments

Enrichment Preparation and Incubation Two types of enrichment experiments were done: (1) enrichment culture experiments that were used to select for members of the microbial community with the ability to produce VC, and (2) VC/sediment enrichments that were used to study the potential of the sediment to degrade VC under various geochemical and physiological conditions (table 2). For the enrichment culture experiments, sediment slurry from the October–November 2000 microcosms that were amended with TeCA (no MeOH added) was used as the inoculum to examine VC production from different precursors (table 2). After sampling the microcosm bottles on day 39, which required removal of about 7 percent of the liquid, the microcosm bottles were resealed with an anaerobic headspace of 95 percent nitrogen and 5 percent carbon dioxide and preincubated for an additional 6 weeks at room temperature (microcosms were methanogenic and depleted of VOCs by this time). After this preincubation, 10 mL of microcosm slurry from WB23 and WB30 treatments was added to 100 mL of a bicarbonate-buffered (pH of 6.8) anaerobic enrichment medium in a 160-mL serum bottle. The remaining 50 mL of headspace in the bottles initially contained 80 percent nitrogen and 20 percent carbon dioxide. The enrichment medium contained nutrients and minerals (500 mg/L of NH4Cl, 600 mg/L of NaH2PO4, and 100 mg/L of KCl), a suite of vitamins and trace minerals, yeast extract (50 mg/L), and cysteine HCl (250 mg/L) as a reductant. Each enrichment culture was amended with one of three potential precursors of VC (112TCA, cDCE, and tDCE) as the electron acceptor (table 2). These compounds were added as neat liquids. The enrichment cultures were given a non-fermentable electron donor of either acetate (5 mM) or hydrogen (H_2) (10mL overpressure in the headspace). Bottles were inverted, incubated at room temperature, and a small subsample of the headspace was removed periodically to monitor VOC concentrations.

Three sets of VC/sediment enrichment experiments were conducted (table 2). The first two VC/sediment enrichments were designed to evaluate the effects of redox condition and spatial variability in the wetland sediment on VC degradation, whereas the third experiment was designed to evaluate the effect of pre-exposure of the sediment to VOCs on VC degradation (table 2). The concentration of VC provided to the enrichments represented a concentration about 7 times higher than that observed during the peak accumulation of VC in the microcosms. The first VC/sediment enrichment was constructed using sediment slurries from the TeCA-amended (no MeOH added) microcosms constructed in October–November 2000. The microcosm slurries were preincubated as described above to deplete sulfate, iron, and VOCs. After preincubation, sediment slurry (10 mL) was placed in a pressure tube (28 mL), and 1 mL of 1 percent VC gas was added. Some treatments also received ferric iron (Fe(III)) in the form of amorphous ferric oxyhydroxide (FeOOH) (1 mL of a suspension resulting in a final addition of 10 mM Fe(III)) to induce iron-reducing conditions or BES (20 or 50 mM) to inhibit methanogenesis. No excess electron donor (such as acetate or H2) was added. Tubes were incubated on their sides in order to maximize the surface area for exchange of VC across the interface between the headspace and the sediment slurry. The enrichments were analyzed for VC, Fe(III), and CH4 concentrations.

For the second VC/sediment enrichment, a test of the potential effect of iron-reducing conditions on VC degradation was repeated, this time using a soluble chelated form of ferric iron (Fe(III)-NTA) that is reported to be more readily available to iron-reducing bacteria than FeOOH (table 2) (Bradley and Chapelle, 1996). The second VC/sediment enrichment was prepared transferring 3 mL of proven VC degrading sediment from site WB23 into three tubes. The tubes were resealed, and the headspace was purged with nitrogen before amending again with VC.

In April 2001, the third VC/sediment enrichment experiment was performed to evaluate the effect of pre-exposure to TeCA on VC degradation (table 2). Fresh sediment was collected from sites WB23 and WB30 to prepare three sets of VC/sediment enrichments for this experiment (table 2). One set of VC/sediment enrichments was amended with VC 5 days after collection, and this sediment was referred to as "fresh." A second set of VC/sediment enrichments, referred to as "pre-exposed," was preincubated for 42 days with TeCA (7.1 μM added at day 0 to give an initial aqueous concentration of about 3.0 μM), using the same methods to prepare and incubate anaerobic microcosms as reported in the preceding section on Laboratory Microcosm Experiments. A third set of VC/sediment enrichments, referred to as "preincubated," also was preincubated for 42 days but was not amended with TeCA or any other compounds. Each set of VC/sediment enrichments was tested for VC degradation (initial VC concentration of 44.6 μM) in triplicate tubes of three treatments: (1) no amendments other than VC; (2) amended with amorphous FeOOH; and (3) amended with BES (table 2). In addition, VC was added to killed controls to monitor abiotic loss of VC. The killed controls for the "fresh" experiment were poisoned with 1 percent formaldehyde. Sediment for the "preincubated" and "pre-exposed" killed controls was autoclaved three times on 3 consecutive days for 1 hr each. Individual tubes were sacrificed over time to obtain geochemical and microbial samples.

Geochemical and Microbial Community Analyses In all the enrichment experiments, VC and methane were analyzed by injecting 0.3 mL subsample of the headspace onto a packed column and measuring with a GC/FID at the USGS in Reston, Virginia. For the April 2001 enrichment experiments, the anaerobic microcosms that were constructed prior to preparation of the preincubated and pre-exposed enrichment cultures were monitored periodically for VOCs by GC/MS analysis at the on-site laboratory at APG as reported in the preceding section for other microcosm experiments.

Table 2. *Treatments used in enrichment experiments with anaerobic daughter products of 1,1,2,2-tetrachloroethane (TeCA) degradation*

[112TCA, 1,1,2-trichloroethane; cDCE, *cis*-1,2-dichloroethene; tDCE, *trans*-1,2-dichloroethene; VC, vinyl chloride; FeOOH, ferric oxyhydroxide; Fe(III)-NTA; BES, 2-bromoethanesulfonic acid. Concentrations given in millimolar (mM) or micromolar (μM)]

Sediment	Daughter compound added	Other amendments
Enrichment Cultures (October–November 2000 Microcosm Slurry)		
WB23 (microcosm 23TeCA.10/00)	112TCA (10.8 mM), cDCE (8 mM), or tDCE (14.9 mM)	acetate (5 mM)
WB23 (microcosm 23TeCA.10/00)	112TCA (10.8 mM), cDCE (8 mM), or tDCE (14.9 mM)	hydrogen
WB30 (microcosm 30TeCA.10/00)	112TCA (10.8 mM), cDCE (8 mM), or tDCE (14.9 mM)	acetate (5 mM)
WB30 (microcosm 30TeCA.10/00)	112TCA (10.8 mM), cDCE (8 mM), or tDCE (14.9 mM)	hydrogen
VC/Sediment Enrichment Experiment 1 (October–November 2000 Microcosm Slurry)		
WB23 (microcosm 23TeCA.10/00)	VC (44.6 μM)	none
WB23 (microcosm 23TeCA.10/00)	VC (44.6 μM)	amorphous FeOOH
WB23 (microcosm 23TeCA.10/00)	VC (44.6 μM)	BES (20 mM)
WB30 (microcosm 30TeCA.10/00)	VC (44.6 μM)	none
WB30 (microcosm 30TeCA.10/00)	VC (44.6 μM)	amorphous FeOOH
WB30 (microcosm 30TeCA.10/00)	VC (44.6 μM)	BES (50 mM)
VC/Sediment Enrichment Experiment 2 (October–November 2000 Microcosm Slurry)		
WB23 from Enrichment 1	VC (44.6 μM)	none
WB23 from Enrichment 1	VC (44.6 μM)	Fe(III)-NTA
WB23 from Enrichment 1	VC (44.6 μM)	BES (20 mM)
VC/Sediment Enrichment Experiment 3 (April 2001 Microcosm Slurry)		
WB23, fresh, preincubated, and pre-exposed	VC (44.6 μM)	none
WB23, fresh, preincubated, and pre-exposed	VC (44.6 μM)	amorphous FeOOH
WB23, fresh, preincubated, and pre-exposed	VC (44.6 μM)	BES (20 mM)
WB30, fresh, preincubated, and pre-exposed	VC (44.6 μM)	none
WB30, fresh, preincubated, and pre-exposed	VC (44.6 μM)	amorphous FeOOH
WB30, fresh, preincubated, and pre-exposed	VC (44.6 μM)	BES (20 mM)

Ferrous and ferric iron concentrations available for microbial reduction in the sediment slurries were monitored in the April 2001 microcosms using the extraction and ferrozine assay technique detailed in Lovley and Phillips (1987). Sediment slurry samples were collected at selected time intervals for all enrichment experiments for molecular analysis of the microbial communities (as detailed in the preceding section on Laboratory Microcosm Experiments); however, these analyses have not been completed yet.

Field Data Collection and Analyses

Surficial Wetland Sediment Samples To evaluate spatial and seasonal variability, grab samples of the surficial wetland sediment were collected from 0 to 7.6 cm below land surface near piezometer sites along the A-A′ and C-C′ transects in March and August 1999. The sediment samples were frozen immediately and analyzed later for microbial communities as detailed previously in the section on Laboratory Microcosm Experiments. Biomass was estimated from the TRFLP procedure and also from direct cell counts on stained slides. Cores of the wetland sediment also were

collected at sites WB23 and WB30 (fig. 1) in August 2000 by hammering a 1.2 m-long, 0.076-m-diameter polyvinyl chloride (PVC) pipe with a clear plastic liner into the sediment. Depths to the top of the sediment core were measured every 30 cm to determine the amount of sediment compaction. The PVC pipes then were capped before manually pulling the PVC pipe from the sediment. Sediment samples from selected depths in these cores were analyzed at the USGS laboratory in Reston, Virginia for bioavailable ferrous and ferric iron concentrations using the extraction and ferrozine assay technique (Lovley and Phillips, 1987).

Ground-Water Samples Ground-water-quality data collected during 1995 and 2000 from selected piezometers and multi-level samplers are used in this report to estimate contaminant degradation rates and to compare daughter product distributions and redox characteristics in the laboratory microcosms to field observations. Sampling and analytical methods for the 1995 data are reported in Lorah and others (1997) and Olsen and others (1997). Sampling and analytical methods for the 2000 data are reported in Spencer and others (2002). The analytical techniques used in 1995 were the same as those used in 2000.

Calculation of Degradation Rates TCE degradation rates that were estimated using field data collected along or near section A-A′ during June–October 1995 were reported previously in Lorah and others (2001). For this report, TeCA degradation rates were calculated for the same sites using the data reported in Lorah and others (1997) and Olsen and others (1997), and the same rate estimation method for TCE reported in Lorah and others (2001). Data from the multi-level sampler at site WB30 that were collected in June–July 2000 (Spencer and others, 2002) also are used to calculate TeCA and TCE degradation rates using the same estimation method. Field rates of TeCA and TCE degradation were estimated using TeCA, TCE, and chloride concentrations along upward flowpaths from the shallow aquifer to the top of the wetland surface. At sites selected for rate calculations, there were sufficient screened intervals and detectable TeCA and TCE concentrations in the wetland porewater to give at least two points (usually at least three points) along the flowpath to estimate rates. Measured TeCA and TCE concentrations were corrected for the effects of advection, dispersion, and dilution by normalization with the conservative tracer chloride, using the correction method outlined by Wiedemeier and others (1998). Ravi and others (1998) found excellent agreement between rate constants estimated with this tracer-corrected method and a method that utilizes a one-dimensional analytical model. Because TCE degradation rates estimated using both chloride and bromide as conservative constituents at the same sites were essentially the same (Lorah and others, 2001), only chloride was used in this study. Because chloride concentrations in the aquifer are in the range of 50 to 100 mg/L and the VOC concentrations are in the µg/L range (Lorah and others, 1997; 2001), the effect of dechlorination reactions on the chloride concentrations would be negligible.

The tracer-corrected rates can be assumed to represent biodegradation rates if volatilization and sorption effects are assumed to be negligible. Volatilization was shown to be a negligible transport mechanism for the volatile contaminants in the wetland study area by using sulfur hexafluoride as a conservative gas in a ground-water tracer test (L.D. Olsen, U.S. Geological Survey, written commun., 2002). Sorption also is assumed to have a negligible effect on the estimated field rates because the plume is believed to be at steady state (Lorah and others, 1997; Chapelle and Bradley, 1998). The first-order biodegradation rate at each site was estimated by linear regression of the natural logarithm of the normalized TCE concentration against traveltime along the flowpath. The traveltime was calculated using the linear ground-water-flow velocity in the wetland sediments and the travel distance measured between the mid-point of each screened interval. The piezometers used for the 1995 calculations have 15-cm-long screens, whereas the multi-level sampler has 7.5-cm-long screens at each sampling point (Spencer and others, 2002). Linear ground-water-flow velocities between 0.6 and 0.9 m/yr were estimated using flow-net analyses along sections A-A′ and C-C′, measured horizontal hydraulic conductivities, and estimated vertical hydraulic conductivities (Lorah and others, 1997). The maximum of this estimated velocity range (0.9 m/yr) was selected for use in the original field-estimated TCE degradation rates (Lorah and others, 2001) and was used in this report for consistent comparison. Variations of ground-water-flow velocities in time and space because of the heterogeneity of the wetland sediments and the seasonal and tidal fluctuations in the hydraulic gradient still are large, undefined sources of uncertainty.

Laboratory Experiments on Anaerobic Degradation of 1,1,2,2-Tetrachloroethane and Association with Microbial Communities

The following sections discuss the anaerobic degradation of TeCA in the laboratory microcosm and enrichment experiments that were conducted during 1998–2001, and the association between degradation pathways and the microbial communities in the microcosm sediment. TeCA degradation rates in all the microcosm experiments that were conducted at this site, including the previously reported experiment (Lorah and others, 1997; Lorah and Olsen, 1999a), are discussed in the first subsection, providing a partial overview of the results. Understanding the degradation pathways of TeCA requires an understanding of the production and degradation of the many possible daughter products (fig. 2). Ultimately, the feasibility of natural attenuation as a remediation method for the chlorinated VOC plumes discharging to the wetland sediment depends on the fate of these daughter products. Discussion of the many laboratory experiments that were conducted (tables 1 and 2), therefore, focuses on the daughter products and the possible factors that could control their distribution and degradation in the wetland

sediments. Laboratory microcosm and enrichment culture experiments that provide information for each factor are discussed together and not in chronological order. Some experiments are discussed in more than one section because the results have a bearing on more than one factor. Results of the microcosm and enrichment experiments are presented as averages of duplicate samples, unless otherwise noted. After the first 1 to 3 days of incubation, reproducibility in the microcosm experiments generally was within or slightly greater than the relative percent difference of the analysis, which ranged from 5 to 13 percent for TeCA and its potential daughter compounds.

Degradation Rate of 1,1,2,2-Tetrachloroethane

Rapid removal of TeCA from solution occurred in all live (no formaldehyde added) microcosm experiments with wetland sediment and ground water, regardless of the site at which the sediment was collected (table 3; figs. 5–10). The TeCA biodegradation rate was affected substantially, however, by the addition of MeOH to microcosms and in some cases by the season during which the sediment was collected (fig. 5a). Apparent first-order biodegradation rate constants, which were corrected for losses in killed (formaldehyde added) controls to account for abiotic losses such as sorption and volatilization, ranged from 0 to 0.50 per day in the WB23 microcosms and from 0.06 to 0.63 per day in the WB30 microcosms (table 3). Figure 5a shows the initial apparent rate of TeCA degradation, which is calculated by multiplying the initial first-order rate constant estimated for each experiment by the initial substrate (TeCA) concentration (table 3) in each of the microcosm experiments. The slope of the best-fit line through the plotted TeCA degradation rates against the initial TeCA concentration (using only those experiments that did not contain amendments other than TeCA) gives the first-order rate constant that best fits the studied range of concentrations (from about 1.0 to 13.0 µmol/L) (fig. 5a). It should be noted that for many experiments, there was a second period of faster TeCA degradation (table 3) after the initial period of degradation that is shown in figure 5a. For the July–August 1999 experiment with sediment from site WB23, for example, the initial TeCA degradation rate (day 3 to 12) was 0. Substantial TeCA degradation did occur after the initial period of day 3 to 12, as evidenced by the production of daughter compounds. A degradation rate was not estimated for this later period, however, because a slight rise in TeCA concentrations (that most probably is due to desorption) occurs between day 12 and 21 (see fig. 8a). For other experiments, a distinct change in slope in plots of the natural log of TeCA concentrations against time was observed and a second rate was calculated (table 3).

Plotting the initial TeCA degradation rate for each microcosm gives two distinct lines, with the first-order rate constant about two times higher in the MeOH-amended microcosms than in those constructed without MeOH (fig. 5a). Both data groups show a linear increase in degradation rate with initial TeCA concentration, but the trend is more evident in the MeOH-amended microcosms because they span a larger concentration range. The 1998 microcosms, which were amended with MeOH in the TeCA stock solution, were designed to include three initial TeCA levels, whereas the other experiments only targeted one initial concentration level (table 1) that was thought to be most representative of the average ground-water TeCA concentrations in the aquifer (Lorah and others, 1997; Lorah and Olsen, 1999b). The first-order rate constant for TeCA biodegradation in the MeOH-amended microcosms was 0.12 per day, corresponding to a half-life of 5.8 days (fig. 5a). Compared to the other microcosms that contained MeOH, the TeCA degradation rate was relatively high in the microcosm constructed with sediment from the background site WB19, and relatively low in the microcosms amended with sulfate and BES (fig. 5a). The first-order rate constant of 0.053 per day (half-life of 13 days) estimated for the microcosms without MeOH (fig. 5a) is more applicable to natural field conditions. It is unknown, however, if this first-order estimate is applicable to initial TeCA concentrations above about 3 µmol/L (500 µg/L) (fig. 5a). The linear increase in biodegradation rate indicates that TeCA is not toxic to the degrading microorganisms up to an initial TeCA concentration of 13 µmol/L, and that a maximum degradation rate has not been reached as described by Monod kinetic theory for utilization of a single rate limiting substrate (fig. 5a). A greater range in initial concentrations, however, should be investigated to fully describe TeCA degradation kinetics.

The higher degradation rates with addition of MeOH may be due to increased bioavailability of the TeCA because MeOH increases the solubility of TeCA and decreases sorption by a cosolvent effect (Schwarzenbach and others, 1993, p. 96; Pankow and Cherry, 1996, p. 255). The higher bioavailability and degradation rates with MeOH are indicated by the high rebound in daughter compound concentrations (especially 12DCA) near the end of experiments 23TeCA.2 and 23TeCA.3 (figs. 6b and 6c). It is not possible to determine if this effect occurred in experiment 23TeCA.1, which also contained added MeOH, because a shorter incubation and sampling period was used (fig. 6a). After daughter compound concentrations decreased to below detection levels by day 40 in experiments 23TeCA.2 and 23TeCA.3, 12DCA concentrations again increased to between 1.5 and 2.0 µmol/L by day 45, without any rebound in TeCA concentrations above the detection level (figs. 6b and 6c). These concentrations indicate desorption of TeCA and subsequent rapid biodegradation. This high of a rebound in concentrations was not evident in the microcosms that were not amended with MeOH (figs. 7–9). In addition, the availability of MeOH as a substrate for microorganisms may have affected biodegradation rates by altering degradation pathways and composition of the microbial community, as discussed in the section on Daughter Compound Production and Degradation.

Seasonally, the lowest initial TeCA biodegradation rate was observed in the one set of microcosms conducted during the summer (July–August 1999) (table 2; fig. 5a). In the first 12 days of the July–August 1999 experiment, the TeCA

Table 3. *First-order rate constants and half-lives for degradation of 1,1,2,2-tetrachloroethane (TeCA) or 1,1,2-trichloroethane (112TCA) in anaerobic microcosm experiments conducted using wetland sediments, May 1996–November 2000*

[Rate constants (± standard error for a 95-percent confidence interval) are presented for the average of duplicate microcosms for each experiment. C_i is the concentration (in micromoles per liter, or µmol/L) of TeCA or 112TCA remaining at the first day of the time period used to calculate the first-order degradation rate. Concentrations were corrected for losses in killed controls before determining rates, except for BES-TeCA.2, which did not have killed controls. Dashes indicate not applicable]

Treatment name	C_i (µmol/L)	Rate constant (day^{-1})	Half-life (days)	Correlation coefficient, r^2	Time period (days)
May–June 1996:					
30E4	1.2	0.25 ± 0.03	2.8	0.99	4-8
February–April 1998:					
23TeCA.1	1.4	0.50 ± 0.14	1.4	.86	2-8
23TeCA.2	3.6	0.28 ± 0.04	2.5	.98	3-9
23TeCA.3	13	0.16 ± 0.04	4.3	.85	3-16
Bck-TeCA.2	3.3	0.39 ± 0.11	1.8	.86	2-11
$23SO_4$-TeCA.2	3.1	0.25 ± 0.04	2.8	.96	2-10
23BES-TeCA.2	3.2	0.15 ± 0.02	4.6	.95	1-12
23TCA.1	2.4	0.38 ± 0.13	1.8	.82	1-9
23TCA.2	6.3	0.46 ± 0.07	1.5	.91	1-16
October 1998:					
23TeCA.2 (methanol)	2.8	0.36 ± 0.03	1.9	.98	2-11
23TeCA.2 (no methanol)	2.0	0.16 ± 0.02	4.3	.96	2-14
23TCA.1 (methanol)	1.6	0.58 ± 0.05	1.2	.99	2-8
23TCA.1 (no methanol)	1.9	0.22 ± 0.03	3.2	.95	2-14
March–April 1999:					
23TeCA.3/99	2.8	0.15 ± 0.02	4.6	.98	1-9
	.73	0.41 ± 0.02	1.7	.99	12-25
30TeCA.3/99	2.2	0.15 ± 0.02	4.6	.92	1-12
	.39	0.63 ± 0.02	1.1	1.0	16-25
23TeCA.3/99	3.2	0.22 ± 0.02	3.2	.93	1-35
30TeCA.3/99	2.8	0.32 ± 0.01	2.2	.99	16-25
July–August 1999:					
23TeCA.7/99	4.3	0 ± 0.03 [A]	--	--	3-12
30TeCA.7/99	4.0	0.06 ± 0.03	12	.96	3-9
	1.9	0.55 ± 0.17	1.3	.91	21-30
23TCA.7/99	2.4	0.13 ± 0.03	5.3	.92	21-35
October–November 2000:					
23TeCA.10/00	2.0	0.16 ± 0.05	4.3	.83	1-19
30TeCA.10/00	2.5	0.10 ± 0.01	6.9	.98	3-10
	1.3	0.24 ± 0.01	2.9	.99	10-21
30MeOH.10/00	3.1	0.10 ± 0.05	6.9	.94	1-6
	1.9	0.39 ± 0.05	1.8	1.0	6-10
30TCA.10/00	2.6	0.07 ± 0.02	9.6	.85	1-10
	1.3	0.27 ± 0.003	2.6	1.0	10-18

[A] Substantial TeCA degradation does occur after the initial period of day 3 to 12, as evidenced by the production of daughter compounds. A degradation rate was not estimated, however, because a slight rise in TeCA concentrations (most likely caused by desorption) occurs between day 12 and 21 (see fig. 8a).

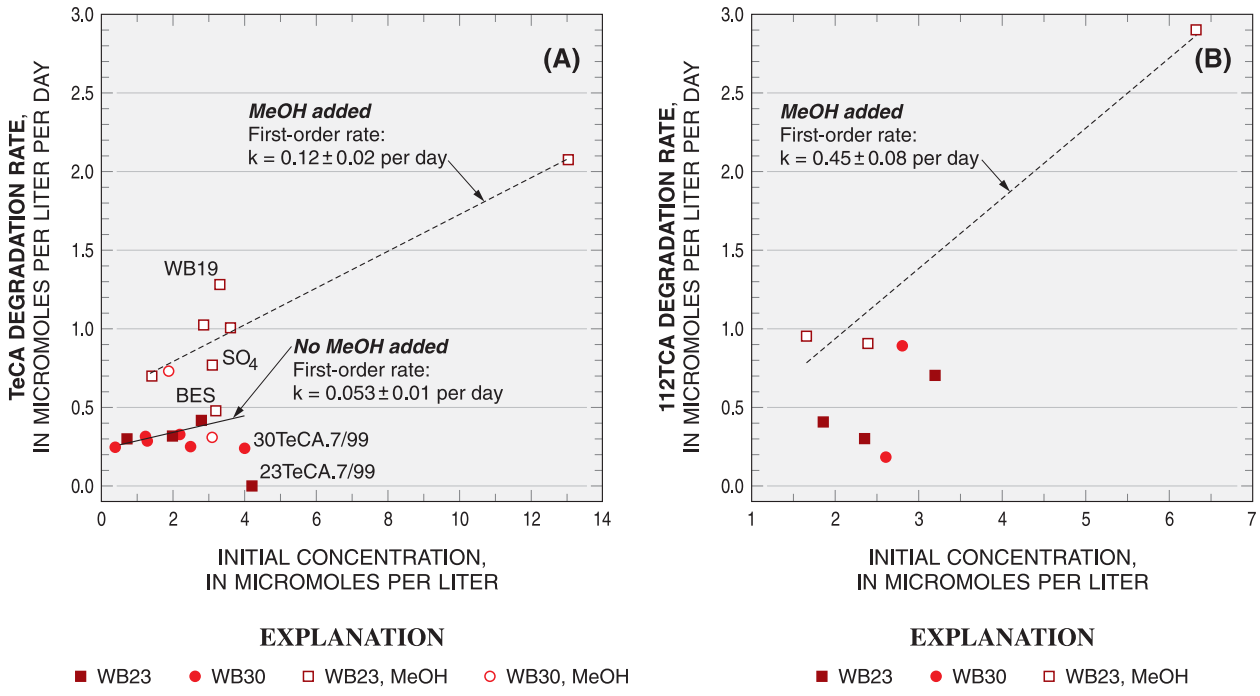

Figure 5. Apparent first-order degradation rates of **(A)** 1,1,2,2-tetrachloroethane (TeCA) and **(B)** 1,1,2-trichloroethane (112TCA) in anaerobic microcosm experiments, 1998-2000. *[Note that the microcosm constructed with sediment from the background site (WB19), and those amended with sulfate (SO_4) and 2-bromoethanesulfonic acid (BES) were not included in the first-order rate constant estimates.]*

biodegradation rate in the WB23 microcosm was insignificant after the rate of TeCA loss in the killed controls was subtracted from that of the live microcosms (the difference was within the standard error for the rate constant of 0.02 per day). The lower concentrations of biotic daughter products (all except TCE) in the first 12 days of the July–August 1999 experiments compared to the March–April 1999 experiments also shows the lower TeCA degradation rate in the July–August 1999 experiments (figs. 7 and 8). The similar loss of TeCA in the killed and live microcosms for the July–August 1999 experiments constructed from WB23 and WB30 sediments is evident in figure 8. For the WB30 microcosms in July–August 1999, however, the initial TeCA biodegradation rate was significant compared to loss in the killed controls, although the rate is relatively low compared to the other microcosms (table 3 and fig. 5a).

Comparison of the decrease in TeCA concentrations in the live microcosms to the killed controls indicates that abiotic processes caused substantial decrease in TeCA concentrations at times (figs. 6–9). During the first 4 to 7 days of incubation, some loss of TeCA often was observed in the killed microcosms without concurrent production of biotic daughter products and without corresponding losses in the water controls. Sorption to the organic-rich wetland sediments is the likely cause of most of this abiotic loss of TeCA in the microcosms. Volatilization from the microcosm bottle or sorption to the microcosm bottle or stopper was determined to be insignificant because losses in water controls, which were constructed with most experiments (table 1), were insignificant (within the relative percent difference of the analyses) (data on file at the USGS office in Baltimore, Maryland). TCE, which is an abiotic daughter product of TeCA, commonly was observed in the killed controls, although concentrations were less than 6 percent of the initial added TeCA (data on file at the USGS office in Baltimore, Maryland). TCE concentrations in live and killed controls were highest in the July–August 1999 microcosms and the microcosms incubated at 5 °C, as discussed in the section on Seasonal Variability. As much as 2 percent of the TCE observed in all microcosms may have resulted from contamination by the neat TeCA used to amend the microcosms. Analyses of the neat TeCA showed that TCE was an impurity, comprising up to 2 percent of the solution. The only other daughter compounds observed in the killed TeCA-amended microcosms were cDCE and tDCE. The total concentration of these compounds remained below 10 percent of the initial TeCA concentration during the time that complete degradation of TeCA was observed in the corresponding live microcosms (generally the first 12 to 25 days of incubation; figs. 6–9). Concentrations of

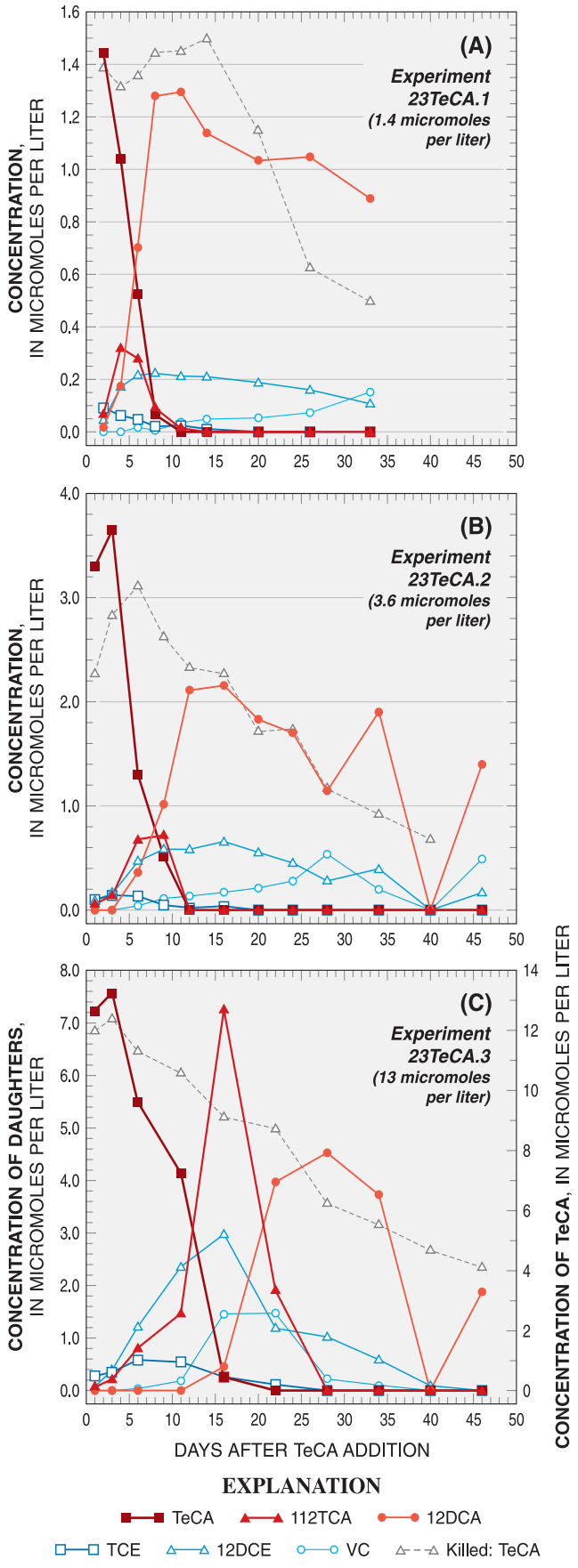

Figure 6. Degradation of 1,1,2,2-tetrachloroethane (TeCA) and production of daughter compounds in anaerobic microcosm experiments **(A)** 23TeCA.1, **(B)** 23TeCA.2, and **(C)** 23TeCA.3 conducted in February-April 1998 using sediment from site WB23, and amended with methanol and three different initial TeCA concentrations (noted on figures).

12DCE sometimes did increase greatly after this time, possibly indicating the recovery of microorganisms from the effect of formaldehyde or the occurrence of abiotic dichloroelimination of TeCA (Lorah and Olsen, 1999a). TRFLP profiles of the microbial communities in the killed controls sometimes did indicate the recovery of microorganisms near the end of incubations, as discussed in the section on Microbial Communities and Associations with Degradation Pathways. Of all the killed microcosm controls conducted, the most rapid decrease in TeCA (figs. 6–9) and earliest increase in 12DCE were observed in the February–April 1998 experiments that were amended with TeCA mixed in a MeOH solution. After day 16 in the February–April 1998 experiments, all the loss of TeCA in the killed controls can be attributed to 12DCE production, and over 70 percent of the initial TeCA was degraded to 12DCE by the end of the experiments (fig. 6a). Daughter compounds were not detected in any 112TCA-amended killed controls, but sorption losses were similar to those in the TeCA-amended killed controls.

Mass balances between the aqueous TeCA concentration and the sum of daughter products produced provide evidence that the decrease in TeCA concentrations over time in the live microcosms was due largely to degradation of the TeCA, rather than to sorption. In all experiments, production of daughter compounds in the live microcosms showed that biodegradation of TeCA by microorganisms indigenous to the wetland sediments was occurring. Figure 10 shows the sum of daughter compounds (maximum sum at any one time step in the experiment) compared to the initial (day 1) TeCA concentration for each live microcosm experiment. Most of these points fall close to the 1:1 line that indicates parent compound concentration equals the sum of daughter compounds (fig. 10). The three points that are well below the 1:1 mass-balance line are the two TeCA-amended microcosm experiments in July–August 1999 (23TeCA.7/99 and 30TeCA.7/99) and the microcosm that was incubated at 5 °C. These three microcosm experiments also had the lowest TeCA degradation rates (table 3), and the lack of mass balance reflects the fact that the TeCA was not completely degraded within the incubation time of the experiments. None of the live controls (microcosms with sediment and water that were not amended with TeCA and were incubated in the same manner as the amended microcosms) had detectable TeCA or daughter compound concentrations throughout the experiments. Desorption and degradation of background contaminants in the wetland sediment, therefore, did not affect the mass-balance calculations.

In the microcosms that were amended with 112TCA rather than TeCA, 112TCA degradation rates were as high or higher than TeCA degradation rates (table 3; fig. 5). The first-order rate constant estimated for the 112TCA-amended microcosms that contained MeOH was 0.45 per day (corresponding to a half-life of 1.5 days), which was about four times faster than TeCA degradation in comparable MeOH-amended microcosms (fig. 5). As observed for the TeCA-amended microcosms, 112TCA degradation rates were about

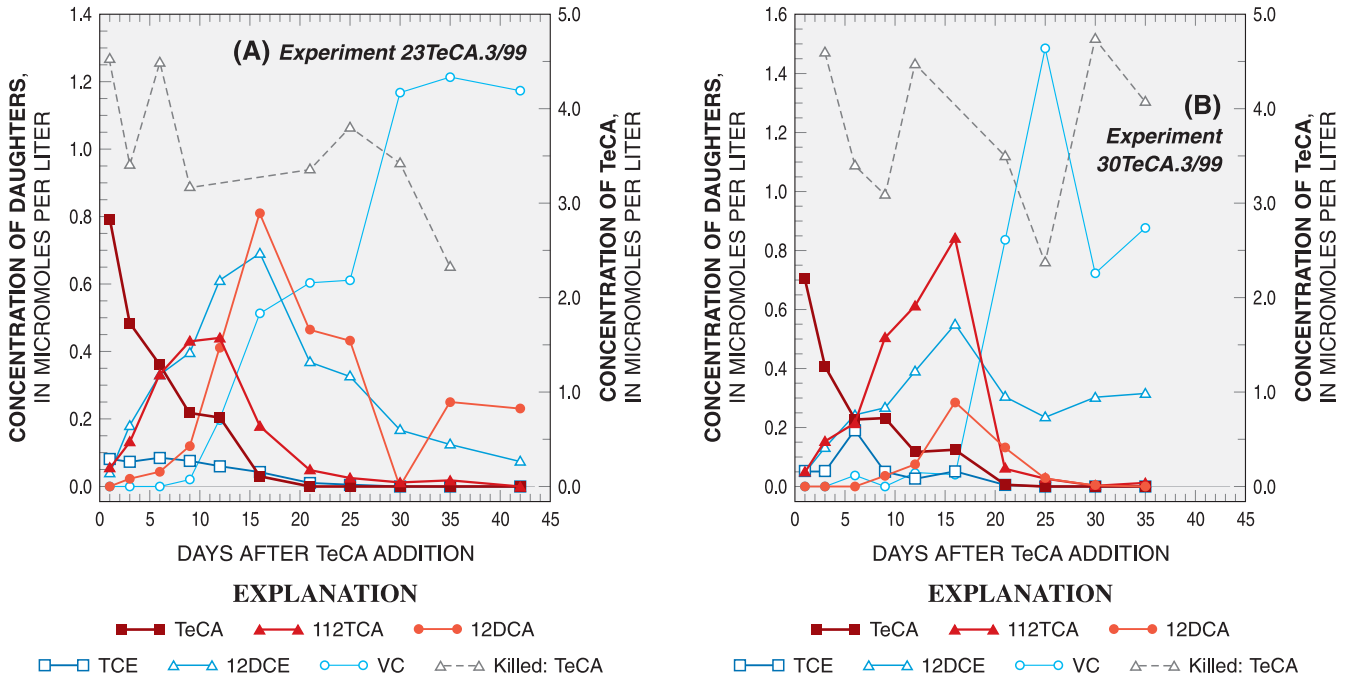

Figure 7. Degradation of 1,1,2,2-tetrachloroethane (TeCA) and production of daughter compounds in anaerobic microcosms, March-April 1999, constructed with wetland sediment from **(A)** site WB23 and **(B)** site WB30.

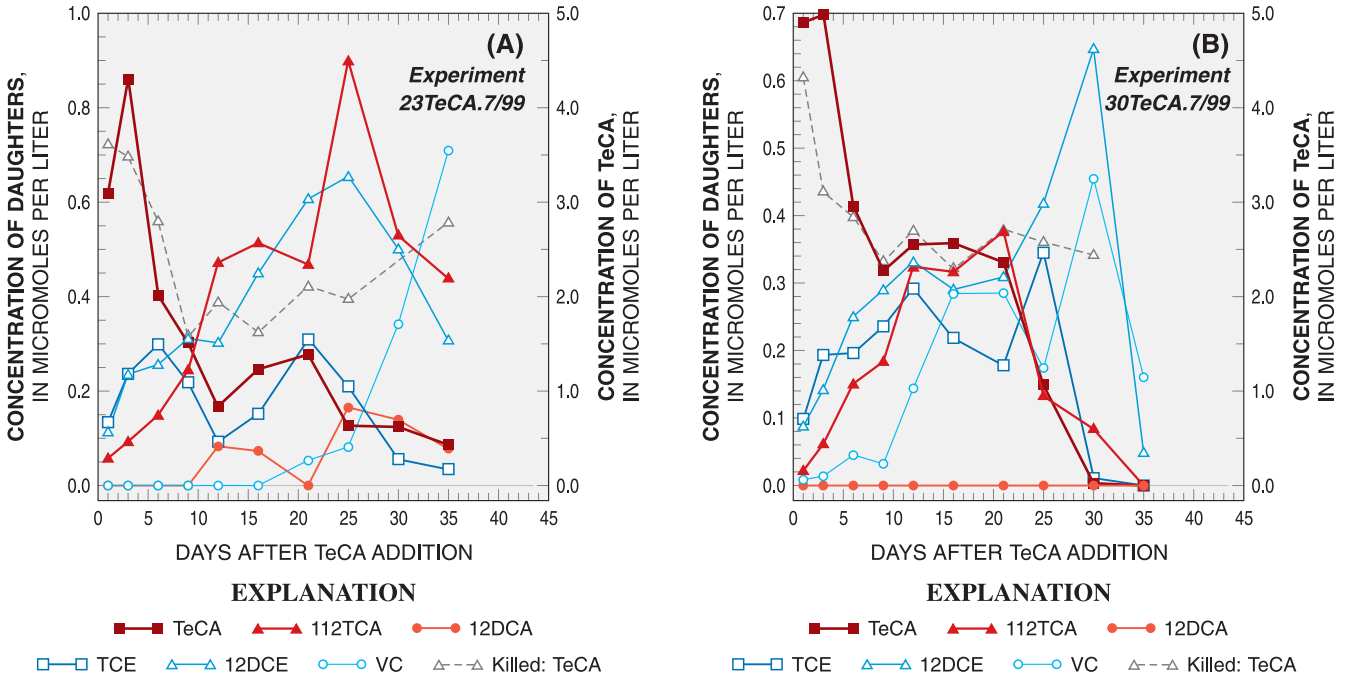

Figure 8. Degradation of 1,1,2,2-tetrachloroethane (TeCA) and production of daughter compounds in anaerobic microcosms, July-August 1999, constructed with wetland sediment from **(A)** site WB23 and **(B)** site WB30.

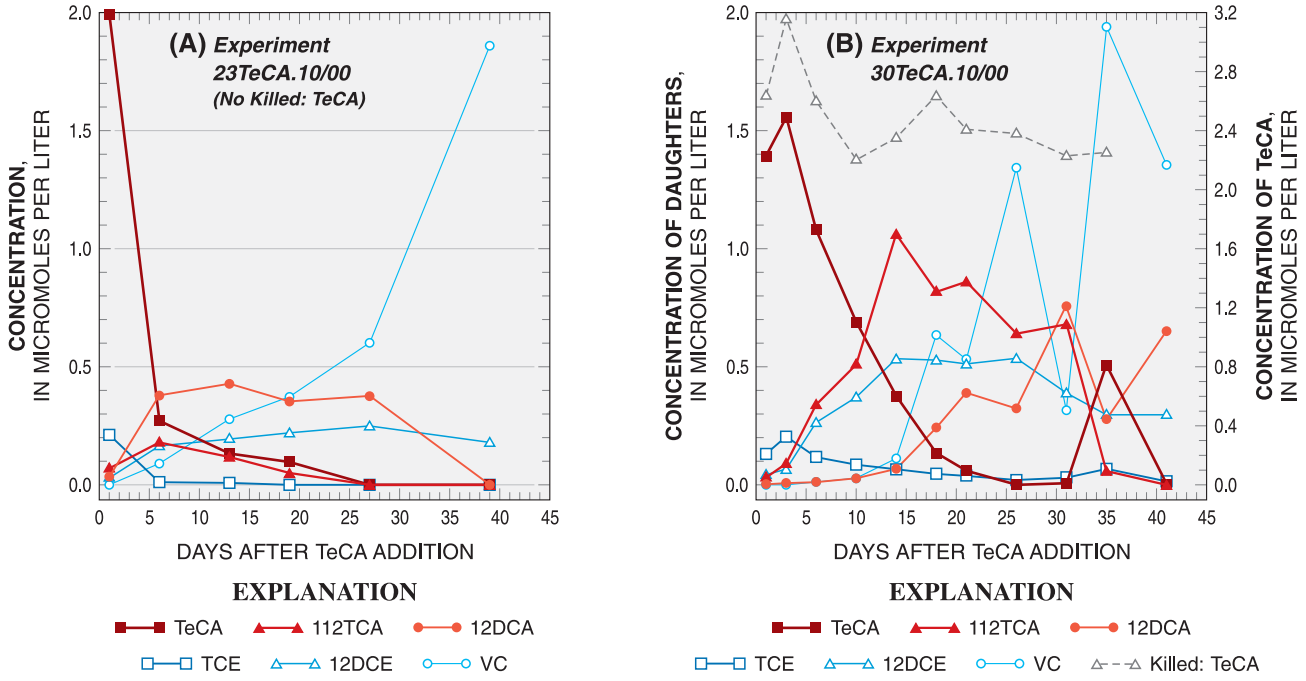

Figure 9. Degradation of 1,1,2,2-tetrachloroethane (TeCA) and production of daughter compounds in anaerobic microcosms, October-November 2000, constructed with wetland sediment from **(A)** site WB23 and **(B)** site WB30.

twice as high in microcosms that contained MeOH compared to those that did not. No significant difference was observed between the 112TCA degradation rates for microcosms constructed with sediment from site WB23 compared to those constructed with sediment from site WB30, but few data points are available for comparison (fig. 5b).

Daughter Compound Production and Degradation

Spatial Variability Although TeCA degradation rates were similar in the microcosms constructed with WB23 and WB30 sediments (fig. 5), the daughter product distributions in the microcosms with the two sediments did show significant differences. In particular, the relative proportions of VC produced and degraded differed with the location from which the sediment was collected (figs. 7–9; fig. 11). Daughter product distributions as molar percentages were calculated by dividing the maximum measured concentration (in molar units) of each daughter product over the incubation period by the initial TeCA concentration in the microcosm (fig. 11). The spatial variability effect on daughter product distributions is most clearly observed by comparing the WB23 and WB30 microcosms in the March–April 1999 and the October–November 2000 (without MeOH added) experiments (fig. 11). Because the February–April 1998 experiments were constructed only with WB23 sediment and with the addition of MeOH (table 1), these experiments will be discussed in the section on Substrate Type. In the July–August 1999 microcosms, the concentrations of some

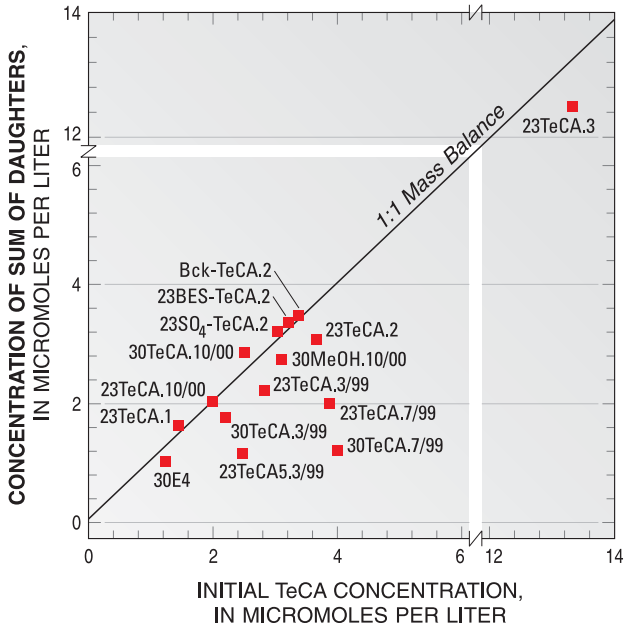

Figure 10. Mass balance between initial (day 1) concentration of the parent compound 1,1,2,2-tetrachloroethane (TeCA) and the sum of daughter compounds produced in the live anaerobic microcosms, 1998-2000.

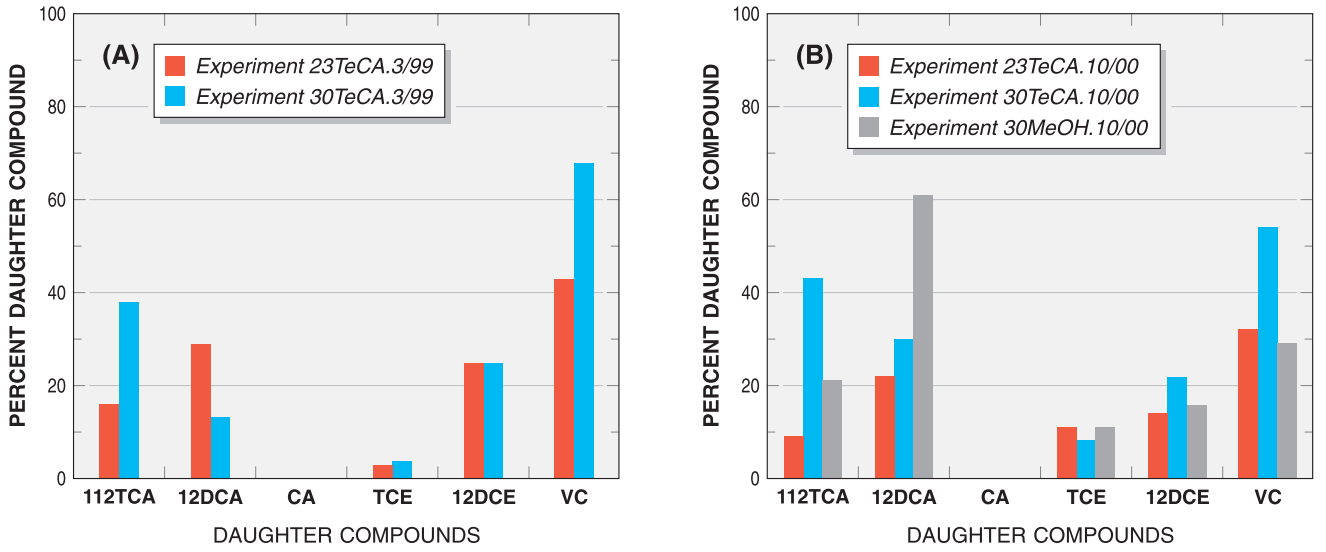

Figure 11. Daughter product distribution in anaerobic microcosms amended with 1,1,2,2-tetrachloroethane (TeCA) and constructed with wetland sediment from sites WB23 and WB30 in microcosm experiments in **(A)** March-April 1999 and **(B)** October-November 2000. *(Daughter compounds are shown as molar percentages of the initial TeCA concentration.)*

daughter compounds still were rising at the end of the incubation period because of the slower TeCA degradation rates (figs. 5 and 10). Therefore, the spatial variability in daughter compound distributions in the July–August 1999 microcosms could not be evaluated by comparing peak concentrations, as was done for the March–April 1999 and the October–November 2000 experiments.

Spatial Variability of Vinyl Chloride Production—The microcosms constructed with WB23 sediment had lower concentrations of VC and 112TCA than those constructed with WB30 sediment (fig. 11). Peak production of VC in the microcosms constructed with WB23 sediment was 43 and 32 percent of the initial TeCA concentrations in the March–April 1999 and the October–November 2000 experiments, respectively. The peak production of VC in the WB30 microcosms was about twice as high as that in the WB23 microcosms (fig. 11). Similarly, peak production of 112TCA was about twice as high in the WB30 microcosms, indicating that the higher VC production resulted from a greater predominance of the 112TCA dichloroelimination pathway in these microcosms (pathway 1B in fig. 2). Because production of TCE and 12DCE was about the same in the microcosms constructed with sediment from WB23 and WB30 (fig. 11), it is unlikely that the higher VC production in the microcosms with WB30 sediment resulted from greater hydrogenolysis of 12DCE, which can be produced through either dichloroelimination or dehydrochlorination of TeCA (pathways 2 or 3 in fig. 2). Further evidence of the pathway through which the higher VC is produced in the microcosms constructed with WB30 sediments is provided by the distribution of daughter products in the microcosms amended with 112TCA, which could produce VC only through dichloroelimination (fig. 12). The 112TCA-amended microcosms constructed with sediment from WB30 also showed twice as much VC production as those constructed with sediment from WB23 (fig. 12).

A possible alternative explanation for the higher peak VC concentrations in the WB30 microcosms compared to the WB23 microcosms is that VC degradation was more rapid in the WB23 microcosms. This explanation was discounted, however, by the higher VC degradation that also was noted in the WB30 microcosms, as discussed in the section on Spatial Variability of VC Degradation.

Although VC production was higher in WB30 microcosms than the WB23 microcosms, it is important to note that VC was the predominant daughter compound produced from all microcosms in 1999 and 2000 that did not contain added MeOH (figs. 7–9; fig. 11). The high production of this compound clearly indicates that VC results from multiple degradation pathways. Dichloroelimination of 112TCA and hydrogenolysis of 12DCE produced through TeCA dichloroelimination appear to be the predominant pathways of VC production because TCE concentrations generally were less than 10 percent of the initial TeCA concentration. In all microcosms, 12DCE and 112TCA were produced simultaneously and immediately from the beginning of the experiment, regardless of the site from which the sediment was collected (figs. 7–9). The spatial variability observed in the daughter product distributions results from the predominant pathway of 112TCA degradation, with the production of VC being predominant in the WB30 sediment (fig. 11). A correspondingly higher production of 12DCA from

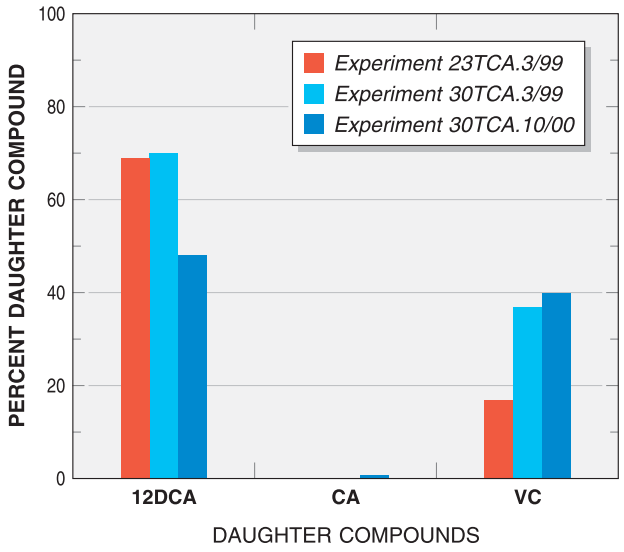

Figure 12. Daughter product distribution in anaerobic microcosms amended with 1,1,2-trichloroethane (112TCA) and constructed with wetland sediment from site WB23 (March-April 1999) and site WB30 (March-April 1999 and October-November 2000). *(Daughter compounds are shown as molar percentages of the initial 112TCA concentrations.)*

112TCA in the WB23 sediment compared to the WB30 sediment sometimes was observed (fig. 11). The higher production of 12DCA in the WB23 sediment was most evident in the March–April 1999 microcosms amended with TeCA (fig. 11). It is possible that the peak concentration of 12DCA was missed in the October–November 2000 microcosm with WB23 sediment because fewer sampling points were obtained during this experiment than during other experiments (figs. 9a–b).

The spatial variability in production of VC in the microcosms shows some association with ferrous iron production (from reduction of ferric iron) and CH_4 production (figs. 7–9; fig. 13). In the March–April 1999 and October–November 2000 microcosms, iron reduction occurred throughout the experiments with WB30 sediment, whereas iron concentrations remained relatively low and constant in the microcosms with WB23 sediment (figs. 13a–b). The early occurrence of iron reduction in the microcosms with WB30 sediment appeared to cause a delay in the onset of methanogenesis in these microcosms (figs. 13b and 13d). Methanogenesis did not begin in the microcosms with WB30 sediment until days 16 and 21 in the March–April 1999 and October–November 2000 microcosms, respectively (fig. 13d). In contrast, methanogenesis was evident from day 1 in the WB23 microcosms in March–April 1999 (fig. 13c). Similarly, the onset of VC production was delayed in the microcosms with WB30 sediment compared to those with WB23 sediment (figs. 7 and 9). The onset of 12DCA production also was delayed in the microcosms with WB30 sediment compared to those with WB23 sediment, although 12DCA production generally began before VC production (figs. 7 and 9). The delay in the onset of methanogenesis in the WB30 microcosms was associated with a greater accumulation of 112TCA. When methanogenic conditions did occur, 112TCA dichloroelimination to VC may have been more rapid than the 112TCA hydrogenolysis to 12DCA, resulting in the relatively higher VC production in these WB30 microcosms than the WB23 microcosms (fig. 11). Alternatively, the WB30 sediment may have a higher prevalence of microorganisms that directly or indirectly mediate 112TCA dichloroelimination to VC than those that can mediate 112TCA hydrogenolysis to 12DCA.

The microcosms that were amended with 112TCA provide further evidence of the relation between VC and 12DCA production and methanogenic conditions (figs. 14 and 15). In the March–April 1999 and October–November 2000 microcosms, onset of VC production from 112TCA dichloroelimination generally coincided with the onset of methanogenesis in microcosms with WB23 and WB30 sediments (figs. 14 and 15). In contrast, 112TCA hydrogenolysis to 12DCA occurred prior to the onset of methanogenesis in all the microcosms (figs. 14 and 15). Comparison of the steepest slopes of the 12DCA and VC concentration lines indicates that 12DCA production is faster or about the same as VC production in these microcosms. Therefore, the doubling of VC production in the 112TCA-amended microcosms constructed with WB30 sediment compared to those constructed with the WB23 sediment (fig. 12) is most likely due to differences in the microbial communities in the two sediments.

Enrichment cultures amended with 112TCA and 12DCE were used to select for members of the microbial community with the ability to produce VC and to examine the apparent differences in VC production in WB30 wetland sediment compared to WB23 wetland sediment (table 2). Two important differences were observed in the biogeochemical reactions in the microcosm experiments compared to those in the enrichment culture experiments. First, although the starting microcosm slurry used to inoculate the enrichment cultures was methanogenic, methane production was not observed during the enrichment culture experiments with either WB23 or WB30 microcosm slurry. Because 112TCA dichloroelimination to VC occurred without production of CH_4 in these enrichment culture experiments, in contrast to the microcosm experiments (figs. 14 and 15), methanogens were not necessarily directly involved in this reaction pathway. Second, 12DCA was not produced in any of the 112TCA-amended enrichment cultures, whereas hydrogenolysis of 112TCA to 12DCA was an important reaction in the microcosms, especially in those constructed with WB23 sediment. These differences between the microcosms and the enrichment cultures are not surprising because the enrichment cultures reflect a selection from the original microbial community. Selectivity results from a combination of effects, including dilution of the microbial community, culturing conditions differentially affecting growth or activity of organisms, and differential sensitivity to VOC toxicity.

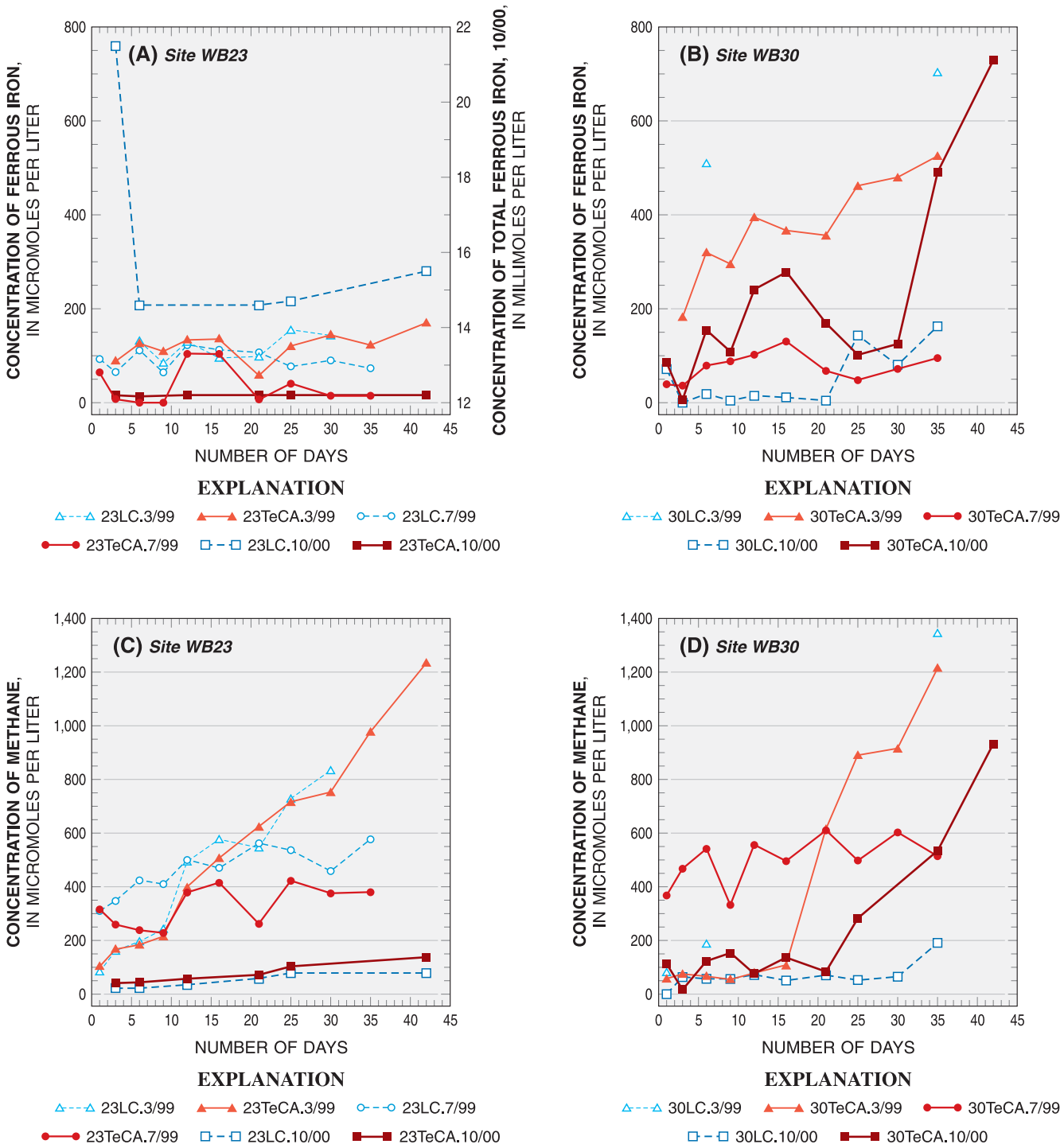

Figure 13. Methane and ferrous iron concentrations in 1999 and 2000 microcosms amended with 1,1,2,2-tetrachloroethane (TeCA) (and live controls, LC): **(A)** Ferrous iron in microcosms constructed with sediment from site WB23 *(Total ferrous iron was determined using the ferrozine method for experiments 23LC.10/00 and 23TeCA.10/00, rather than the dissolved ferrous iron concentrations shown for the other experiments.)*; **(B)** Ferrous iron in microcosms constructed with sediment from site WB30; **(C)** Methane in microcosms constructed with sediment from site WB23 *(Methane concentrations in killed controls were less than 100 micromoles per liter.)*; and **(D)** Methane in microcosms constructed with sediment from site WB30 *(Methane concentrations in killed controls were less than 100 micromoles per liter.)*.

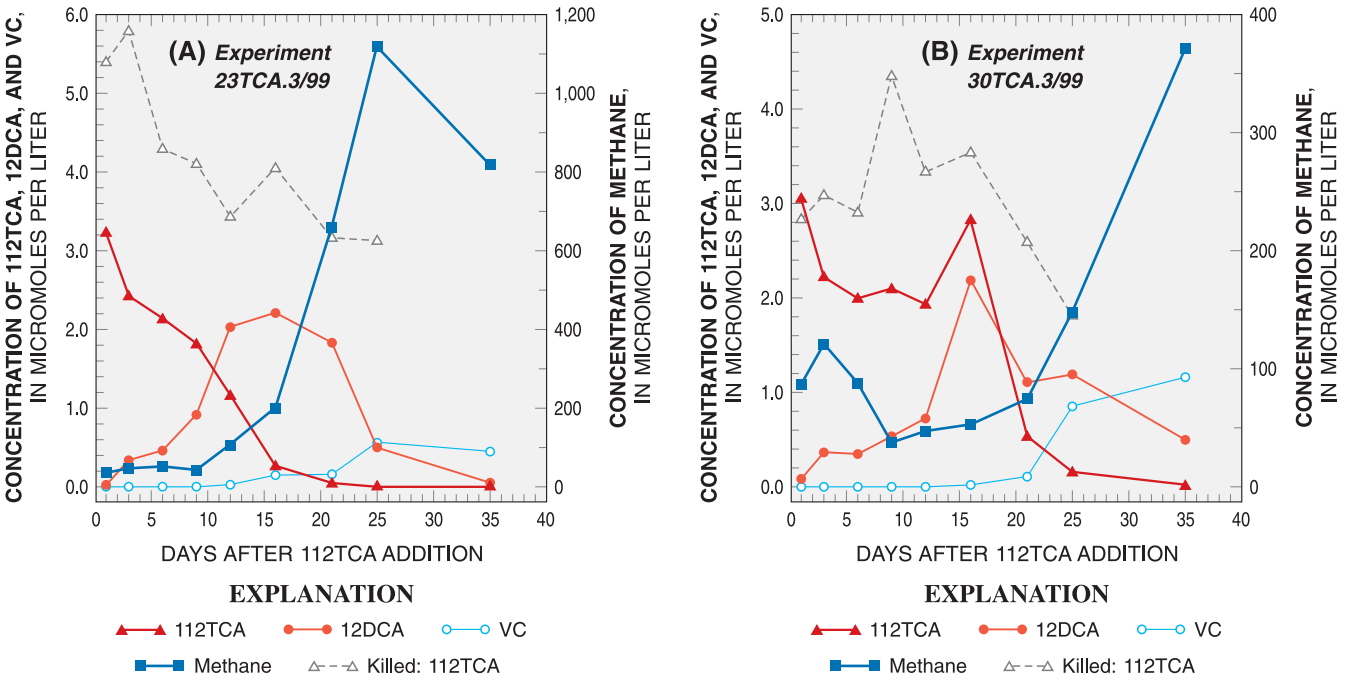

Figure 14. Degradation of 1,1,2-trichloroethane (112TCA), production of daughter compounds 1,2-dichloroethane (12DCA) and vinyl chloride (VC), and production of methane in anaerobic microcosms, March-April 1999, constructed with wetland sediment from **(A)** site WB23 and **(B)** site WB30.

VOC concentrations added to the enrichment cultures were substantially greater than the VOC concentrations added to the microcosms (tables 1 and 2).

In enrichment cultures amended with 112TCA, linear production of VC began without a lag in enrichments inoculated with either WB23 or WB30 sediments and provided with either acetate or H_2 as an electron donor (fig. 16a). This similar behavior in 112TCA metabolism shows that the enrichment cultures reflect selection of a similar sub-component of the microbial community from both the WB23 and WB30 wetland sediments. Enrichment cultures with the WB23 microcosm slurry produced about twice as much VC from 112TCA after 16 days as cultures with the WB30 microcosm slurry (fig. 16a). This contrasts with the higher VC production from 112TCA dichloroelimination observed in the microcosm experiments with WB30 sediment (figs. 11 and 12). Apparently, the wetland sediment at site WB23 has a higher potential than sediment at site WB30 to degrade 112TCA to VC that is not expressed *in situ*.

The cDCE-amended enrichment cultures inoculated with WB30 or WB23 microcosm slurry, respectively, produced VC after an initial lag of 6 or 19 days (fig. 16b). After this lag, the amount of VC production in the cDCE-amended enrichment cultures surpassed that observed in the 112TCA-amended enrichment cultures. Enrichment cultures with the WB23 and WB30 microcosm slurries that were amended with tDCE also produced VC, but the lag periods before

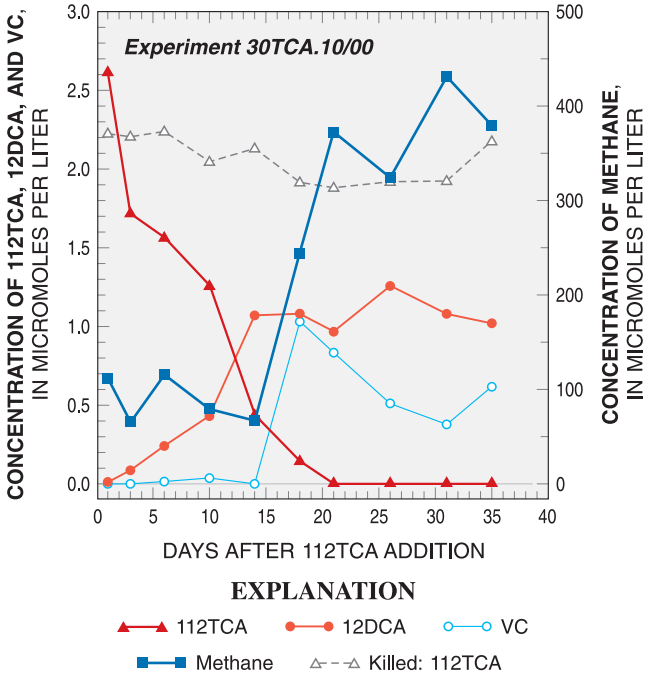

Figure 15. Degradation of 1,1,2-trichloroethane (112TCA), production of daughter compounds 1,2-dichloroethane (12DCA) and vinyl chloride (VC), and production of methane in anaerobic microcosms, October-November 2000, constructed with wetland sediment from site WB30.

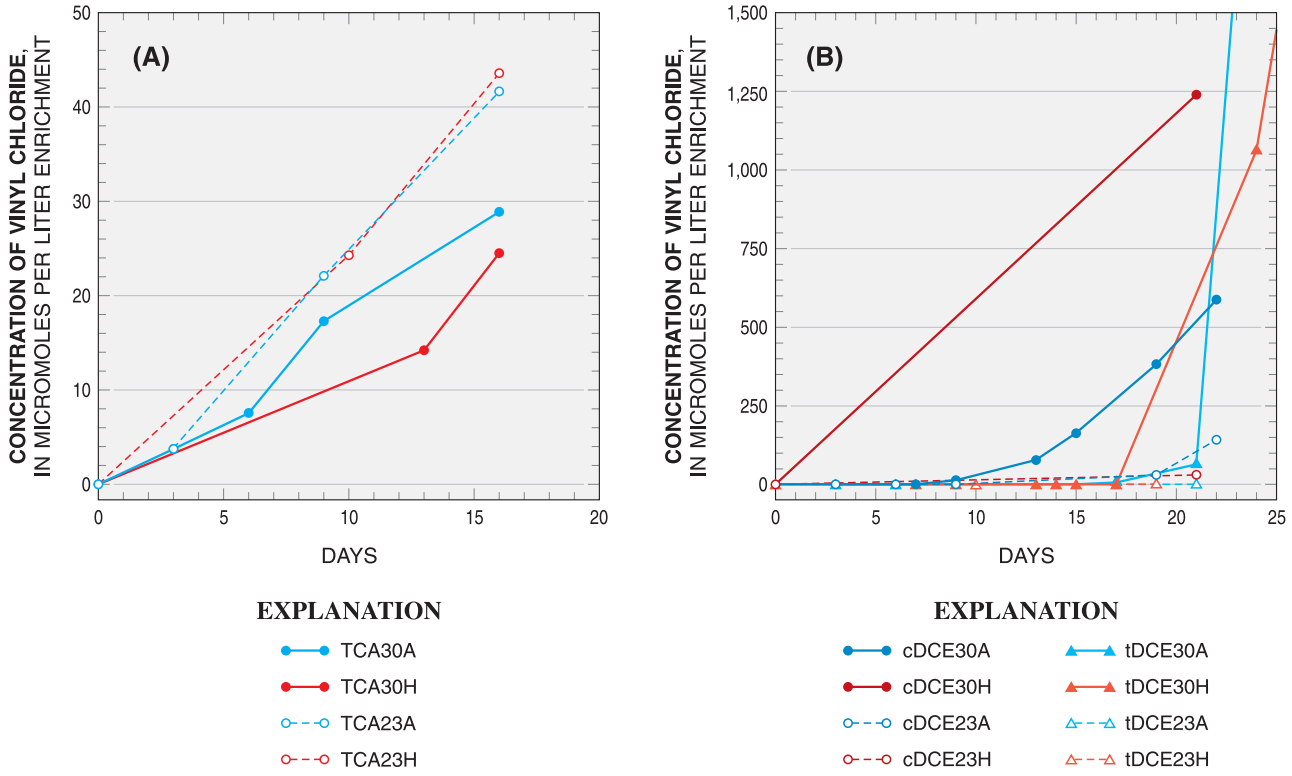

Figure 16. Production of vinyl chloride in enrichment cultures inoculated with WB23 or WB30 microcosm slurry, October-November 2000 and **(A)** amended with 1,1,2-trichloroethane and either acetate (TCA30A and TCA23A) or hydrogen (TCA30H and TCA23H), or **(B)** amended with *cis*-1,2-dichloroethene (cDCE) or *trans*-1,2-dichloroethene (tDCE) and with either acetate (cDCE30A and cDCE23A) or hydrogen (cDCE30H and cDCE23H).

increasing VC concentrations were substantially longer than those in the cDCE-amended cultures (fig. 16b). After the lag period, VC production rates in the tDCE-amended cultures were similar to those in the cDCE-amended cultures with WB30 sediment. VC concentrations increased exponentially in 12DCE-amended enrichment cultures, as opposed to the linear increase observed in the 112TCA-amended enrichment cultures (fig. 16). The exponential increase suggests the growth of microbial species that could utilize cDCE with either acetate or H_2 as the electron donor (fig. 16b). The shorter lag before VC production began in the cDCE-amended cultures inoculated with WB30 microcosm slurry compared to those with WB23 microcosm slurry indicates that the WB30 inoculation provided a higher number of these microorganisms or a more active population.

To summarize, the enrichment culture results indicated that the WB30 sediment has a higher capacity to produce VC from cDCE than the WB23 sediment does, whereas the WB23 sediment has a higher capacity to produce VC from 112TCA than the WB30 sediment does. In contrast, microcosm results indicated that the WB30 sediment has a higher capacity to produce VC from 112TCA than the WB23 sediment does, whereas there was no apparent difference in the capacity of the two sediments to produce VC from 12DCE (at least in the March–April 1999 and October–November 2000 microcosms discussed here). The combined microcosm and enrichment culture results, therefore, indicate that both sediments have the microorganisms necessary to carry out these VC production reactions, but their population size or activity varies with conditions such as redox or toxicity effects. For example, the apparent difference in the capacities of the sediment to produce VC from dichloroelimination of 112TCA in the microcosm compared to the enrichment cultures could be associated with the difference in methane production in the two types of experiments. The immediate and linear increase in VC concentrations from 112TCA degradation suggests that the enrichment cultures did not select for the growth of a microorganism with 112TCA-degrading metabolic capability, unlike the cDCE degradation reaction in the enrichment cultures. The linear increase and implied lack of growth indicates that the organisms may not be deriving energy from the 112TCA dichloroelimination reaction and that the reaction is cometabolic under the non-methanogenic conditions in the enrichment cultures. It is possible that a cometabolic 112TCA dichloroelimination reaction was enhanced in the methanogenic microcosms with WB30 sediment compared to WB23 sediment because a specific methanogen species or group that enhances this dichloro-

elimination reaction was more prominent at site WB30. Alternatively, a microorganism capable of utilizing 112TCA for growth may be active in the WB30 sediment under methanogenic conditions, adding to the cometabolic reaction and causing the approximate doubling of VC production in the 112TCA-amended microcosms constructed with WB30 sediment compared to those constructed with the WB23 sediment (fig. 12).

Spatial Variability of Vinyl Chloride Degradation—The greater production of VC in surficial wetland sediment from site WB30 could be of concern because of the highly toxic nature of this daughter compound. However, all microcosms constructed with WB30 sediment also showed a greater capacity to degrade VC than those constructed with WB23 sediment. For example, in the March–April 1999 microcosms constructed with WB23 sediment, VC concentrations increased beginning at day 9 and then remained approximately constant from day 30 through day 42 (end of the experiment) (fig. 7a). In the March–April 1999 microcosms constructed with WB30 sediment (and without MeOH), VC concentrations increased rapidly between days 16 and 25 and then decreased by approximately 50 percent over the next 5 days (fig. 7b). The greater VC degradation in wetland sediment from WB30 was a consistent characteristic of all microcosms conducted during 1996–2000 (fig. 17). The percent of VC degraded, calculated using the peak VC concentration detected during each experiment and the VC concentration remaining at the end of each experiment, was greater than 30 percent and as high as 96 percent in the microcosms constructed with WB30 sediment. In contrast, the percent of VC degraded was between 0 and 14 percent in the microcosms with WB23 sediment without MeOH added (fig. 17).

The spatial variability in VC degradation that was observed in microcosms constructed with wetland sediment from sites WB23 and WB30 (fig. 17) also was investigated further with VC/sediment enrichment experiments (table 2). The removal rates of VC and the production of methane in enrichments with the two sediment slurries were similar after extended incubation under methanogenic conditions (fig. 18). The WB23 and WB30 wetland sediment, therefore, showed an equally large capacity to degrade high concentrations of VC (44.6 µM, or 2,750 µg/L) after pre-exposure to the contaminants and prolonged incubation under methanogenic conditions (the effects of added BES or iron are discussed in the section on Redox Conditions). VC degradation was rapid under methanogenic conditions, with 90 percent of the initial VC removed in 15 days (fig. 18).

Although VC production and degradation clearly are important processes in the wetland microcosms, the pathways of VC degradation could not be determined definitively from the microcosm or enrichment experiments. Degradation of VC by hydrogenolysis would result in production of ethene and/or ethane. Ethene and ethane, however, were detected infrequently in the microcosm experiments and in trace concentrations. Ethene and ethane also were not detected in the VC/sediment enrichment experiments. Either these compounds were not daughter products

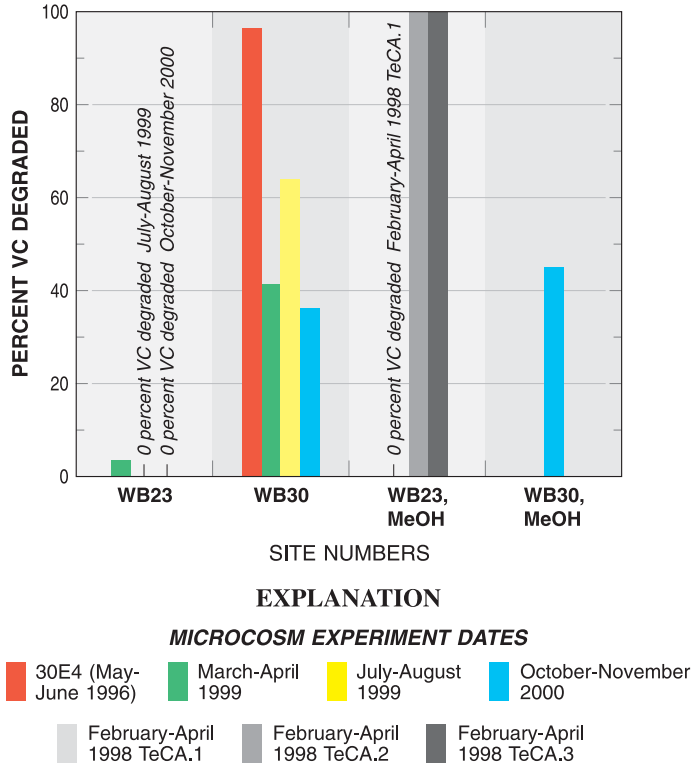

Figure 17. Percent of vinyl chloride (VC) degraded in anaerobic microcosms amended with 1,1,2,2-tetrachloroethane (TeCA) or amended with TeCA and methanol (MeOH), 1996-2000.

of the TeCA and 112TCA degradation, or ethene and/or ethane degradation rates were much faster than the VC degradation rate so that they did not accumulate in the microcosms. In preliminary enrichment experiments, ethene was added to sediment slurries from WB23 and WB30 that had been exposed previously to VC in VC/sediment enrichment experiments. VC degradation was much faster than ethene degradation in these enrichments, and no substantial accumulation of ethene was observed (data on file at USGS, Reston, Virginia). Ethene was degraded to ethane in the VC-adapted, ethene-amended sediment with WB23 sediment but was not degraded (or showed very limited degradation) in the amended WB30 sediment. These results indicate that the primary pathway of VC degradation does not produce ethene in these wetland sediments, but duplicate experiments with sediment spiked with VC and ethene at the same time would need to be conducted in order to directly compare the rates of degradation. Studies by Bradley and Chapelle (1996, 1998, 1999a,b, 2000) indicated that anaerobic oxidation of VC can occur, producing CO_2 or both CH_4 and CO_2 as the end products (fig. 2). The micromolar concentrations of CH_4 and CO_2 that would be produced from this VC oxidation could not be discerned from the ambient CH_4 and CO_2 produced by other natural organic carbon substrates in the wetland sediment, unless experiments with radiolabeled VOCs were conducted.

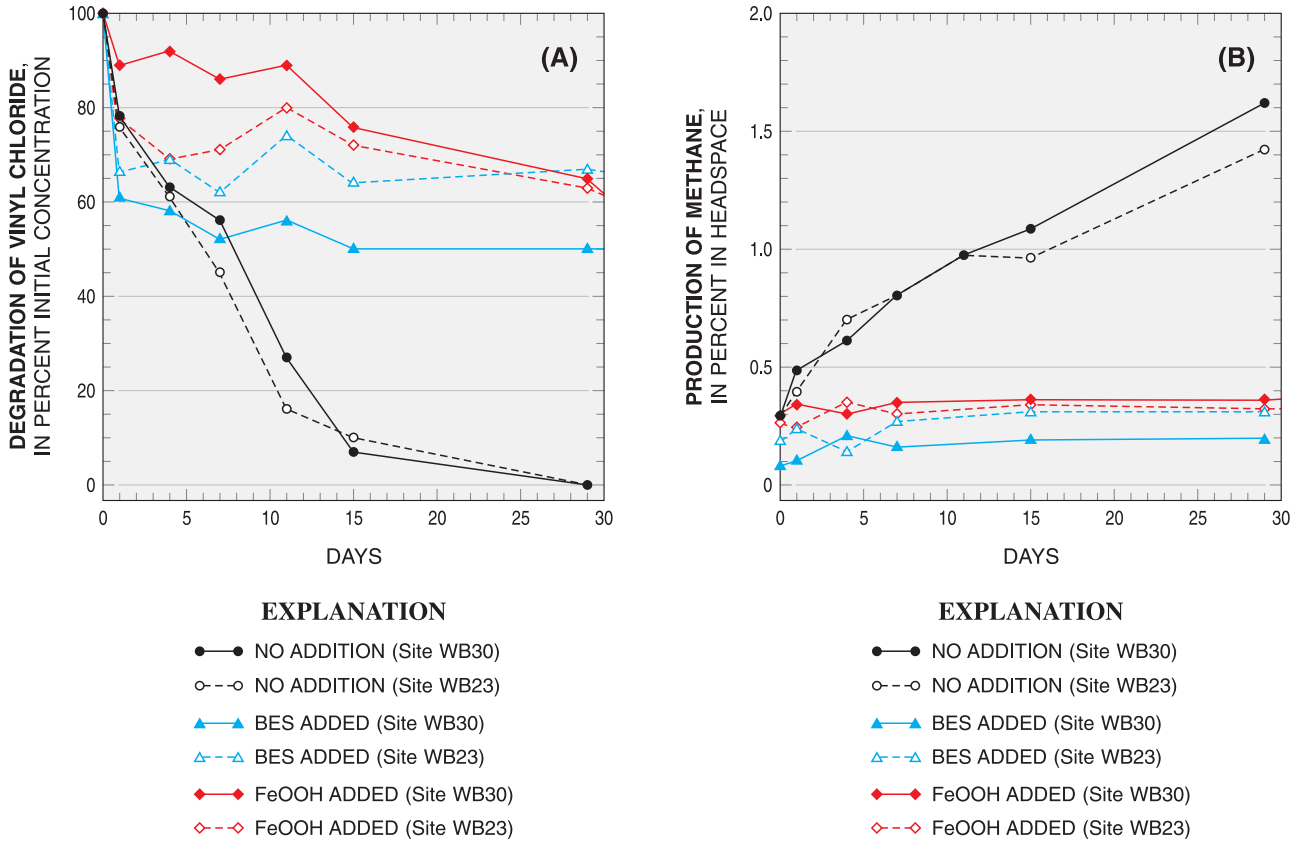

Figure 18. **(A)** Degradation of vinyl chloride and **(B)** production of methane in sediment enrichments inoculated with WB23 or WB30 microcosm slurry, October-November 2000, and amended with only vinyl chloride ("no addition"), with vinyl chloride and 2-bromoethanesulfonic acid (BES), or with vinyl chloride and amorphous ferric oxyhydroxide (FeOOH).

Seasonal Variability The greatest seasonal variability in the microcosm experiments incubated under the same temperature of 19 °C was the substantially lower TeCA degradation rates and lower daughter compound production in microcosms constructed with wetland sediment collected in the summer (July 1999) compared to those constructed with sediment collected in the late winter/early spring (early March 1999) or fall (October 1999) (figs. 5a and 10). Except for the abiotic daughter product TCE, the peak daughter product concentrations in the July–August 1999 microcosms generally were lower and occurred after a longer incubation time than those in the March–April 1999 experiments (figs. 7 and 8). TCE was present between day 1 and 16 in the March–April 1999 experiments, but comprised only about 3 percent of the initial TeCA added (figs. 7 and 11a). In contrast, TCE was present from days 1 through 35 in the July–August 1999 experiment and comprised 7.2 percent of the initial TeCA, which is more than twice the TCE observed in March–April 1999 (fig. 8). TCE also was a more prominent daughter product in the October–November 2000 experiment than in the March–April 1999 experiment (fig. 11). The broader peaks for the daughter products over time indicate that production and degradation of the daughter compounds also was slower for the October–November 2000 experiment, although not as slow as for the July–August 1999 experiment (figs. 7–9). It should be noted that the October–November 2000 microcosm experiment with sediment from WB23 had fewer sampling times than the other experiments and, as a consequence, peak concentrations of some of the daughter products may have been missed.

There were two other differences between the July–August 1999 microcosm experiments and the March–April 1999 and October–November 2000 microcosm experiments. First, the July–August 1999 microcosms amended with TeCA and 112TCA did not show a relation between VC production and methane production. VC production was observed by day 6 in the WB30 microcosms in the July–August 1999 experiment (fig. 8b), although methane production was minimal by day 6 and remained low throughout incubation (figs. 13 c–d). In contrast, VC production in the WB30 microcosms in March–April 1999 coincided with an increase in methane concentrations from day 16 to 21 (figs. 7b and 13d). Second, VC production began earlier in the microcosms constructed with WB30 sediment than those constructed with WB23 sediment in the July–August 1999 experiment, whereas VC production was delayed in the WB30 microcosms compared to WB23 microcosms in the

March–April 1999 and October–November 2000 experiments (figs. 7–9).

To evaluate the effect of seasonal changes in temperature on degradation, one set of microcosms was constructed with sediment from site WB23 in early March 1999, when ice was present on the surface of the wetland, and incubated at 5 °C. Substantial but slower degradation of TeCA was observed at the cold temperature of 5 °C compared to 19 °C. About 50 percent of the initial TeCA in solution was converted to daughter products within the 35-day incubation period at 5 °C, whereas complete loss of the added TeCA occurred within 21 days in duplicate microcosms incubated at 19 °C (table 4; figs. 7a and 10). Of the remaining 50 percent of the initial TeCA in the 5 °C microcosms, 20 percent was in solution at day 35, while the rest most likely was sorbed to the sediment based on comparison to killed controls (data not shown). The daughter product distributions over time (data not shown) and the maximum percentages of each daughter product (table 4) in the 5 °C microcosms were similar to the July–August 1999 experiment that was conducted at 19 °C. The abiotic daughter product TCE comprised 7.4 percent of the initial TeCA in the microcosms that were incubated at 5 °C. Methane production was negligible in the 5 °C microcosms, and also was low in the July–August 1999 experiment that was conducted at 19 °C compared to the March–April 1999 experiment (fig. 13c).

The relatively slow degradation rate in July–August 1999 microcosms incubated at 19 °C compared to other microcosms incubated at this temperature and the similarities between the summer experiment (July–August 1999) and microcosms incubated at 5 °C were unexpected. Because microbial growth and activity generally increases with increasing temperature, higher microbial biomass and higher degradation rates were expected with sediment collected in the summer compared to sediment collected during late winter/early spring. Instead, seasonal variation in TeCA biodegradation in the surficial wetland sediments did not seem to be associated with temperature effects. The trend observed was highest degradation in the winter/early spring and lowest degradation in the summer, followed by increasing degradation in the fall. The degradation of TeCA and the production and degradation of daughter compounds in the microcosms constructed in March 1999 with sediment from site WB30 was similar to that in the May 1996 microcosms (figs. 3 and 7b), indicating some consistency in this seasonal trend from year to year.

Substrate Type Production and degradation of VC varied with the substrate type added to microcosms and enrichment experiments. The effect of MeOH as a substrate during TeCA degradation was tested inadvertently in the February–April 1998 experiments when TeCA and 112TCA stock solutions used to amend the microcosms were mixed in MeOH. The February–April 1998 experiments that were amended with TeCA and prepared with sediment from site WB23 (except the background experiment Bck-TeCA.2), had substantially higher 12DCA and lower VC concentrations than experiments constructed without MeOH (figs. 6, 11, and 19a). Methane production also was substantially higher in these MeOH-amended microcosms. Methane concentrations increased above 1,500 µmol/L after 20 days of incubation in the MeOH-amended experiments 23TeCA.2 and 23TeCA.3 (fig. 20), compared to less than 800 µmol/L in microcosms without MeOH and amended with similar initial TeCA concentrations (fig. 13 c).

Table 4. *Percentage of each daughter product observed in the TeCA-amended microcosms constructed with sediment from site WB23 and incubated at 5 degrees Celsius (experiment 23TeCA5.3/99) compared to those incubated at 19 degrees Celsius in March–April 1999 (experiment 23TeCA.3/99) and July–August 1999 (experiment 23TeCA.7/99)* [A]

[112TCA, 1,1,2-trichloroethane; 12DCA, 1,2-dichloroethane; CA, chloroethane; TCE, trichloroethene; 12DCE, *cis*-1,2-dichloroethene and *trans*-1,2-dichloroethene; VC, vinyl chloride]

Experiment name	112TCA	12DCA	CA	TCE	12DCE	VC	Total [B]
23TeCA.3/99 (19 °C)	16	29	0	3.1	25	43	116
23TeCA.7/99 (19 °C)	21	3.8	0	7.2	16	16	64
23TeCA5.3/99 (5 °C)	20	6.3	0	7.4	14	1.8	50

[A] To calculate percentages, maximum molar concentrations were used for each daughter product observed over the course of the experiment and divided by the initial TeCA molar concentration. Note that as much as 2 percent of the TCE may have resulted from contamination by the neat TeCA used to amend the microcosms.

[B] Sum of the percentages of daughter products observed over the course of the experiment.

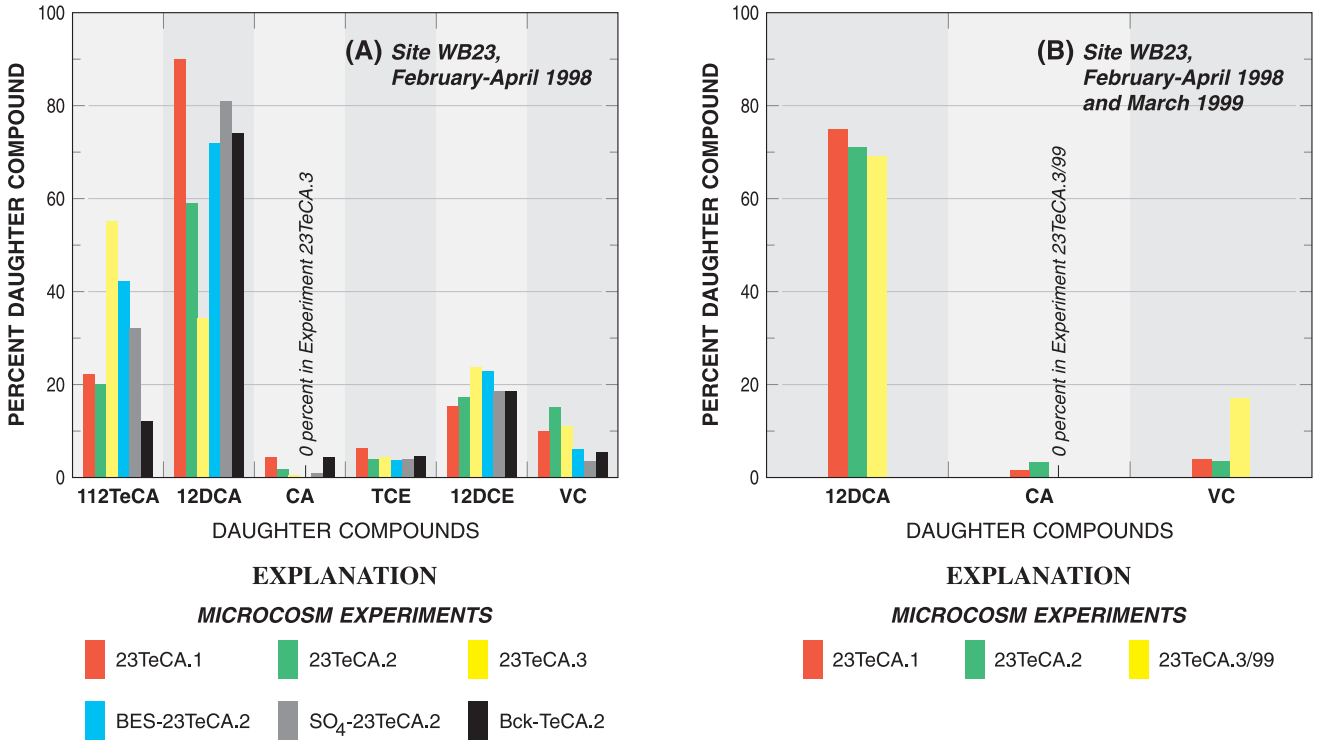

Figure 19. Daughter product distribution in anaerobic microcosms constructed with wetland sediment from site WB23, February-April 1998, that were amended with methanol and with **(A)** 1,1,2,2-tetrachloroethane (TeCA) and **(B)** 1,1,2-trichloroethane (112TCA). *(The 112TCA-amended microcosm experiment that was constructed with wetland sediment from site WB23 in March-April 1999 and that was not amended with methanol (23TeCA.3/99) also is shown for comparison. Daughter compounds are shown as molar percentages of the initial TeCA or 112TCA concentration.)*

The February–April 1998 experiment was designed largely to elucidate whether VC, which was one of the predominant daughter products observed in the first experiment conducted in 1996, was produced mainly from dichloroelimination of 112TCA or from hydrogenolysis of 12DCE. VC, however, was a relatively minor daughter product of TeCA and 112TCA degradation in the February–April 1998 experiment compared to other microcosm experiments that did not contain MeOH (figs. 11, 12, and 19). It appears that the differences between the two experiments resulted primarily from differing pathways of 112TCA degradation, rather than from differences in the initial TeCA degradation pathway. Similar percentages of 12DCE were observed in all TeCA-amended microcosms (figs. 11 and 19a), indicating that the proportion of TeCA degrading by hydrogenolysis and dichloroelimination was similar in all experiments. The 112TCA produced from hydrogenolysis of TeCA subsequently may have degraded predominantly by continued hydrogenolysis to 12DCA in the MeOH-amended microcosms and predominantly by dichloroelimination to VC in microcosms without MeOH (figs. 11 and 19). VC production was lower in 112TCA-amended microcosms with WB23 sediment when MeOH was added (fig. 19b). VC production accounted for about 20 percent of the 112TCA degraded in the March–April 1999 experiment when MeOH was not added, and only 4 percent in the February–April 1998 when MeOH was added. It is possible that the differences in the peak VC concentrations are due partly to differences in VC degradation rates, rather than VC production rates. Faster VC degradation in the MeOH-amended microcosms could result in lower peak VC concentrations than in those without MeOH. In fact, all the VC measured in the TeCA-amended microcosms with MeOH from the February–April 1998 experiment was degraded by the end of the incubation period, and the percent VC degraded was higher in these experiments than in other microcosms that did not contain added MeOH (fig. 17). As discussed earlier for the microcosms without MeOH, ethene and ethane were not detected or were detected only at trace concentrations in the microcosms.

The addition of MeOH to microcosms with sediment from site WB23 enhanced the spatial variability effect that was discussed in a previous section. A short microcosm experiment that was conducted in October 1998 was designed to test the TeCA degradation in microcosms constructed with WB23 sediment with and without added MeOH (table 1). These results indicated that added MeOH alone did not cause the high 12DCA and low VC concentrations observed in the February–April 1998 microcosms with WB23 sediment (Lorah-Devereux, 1999). The 1999 experi-

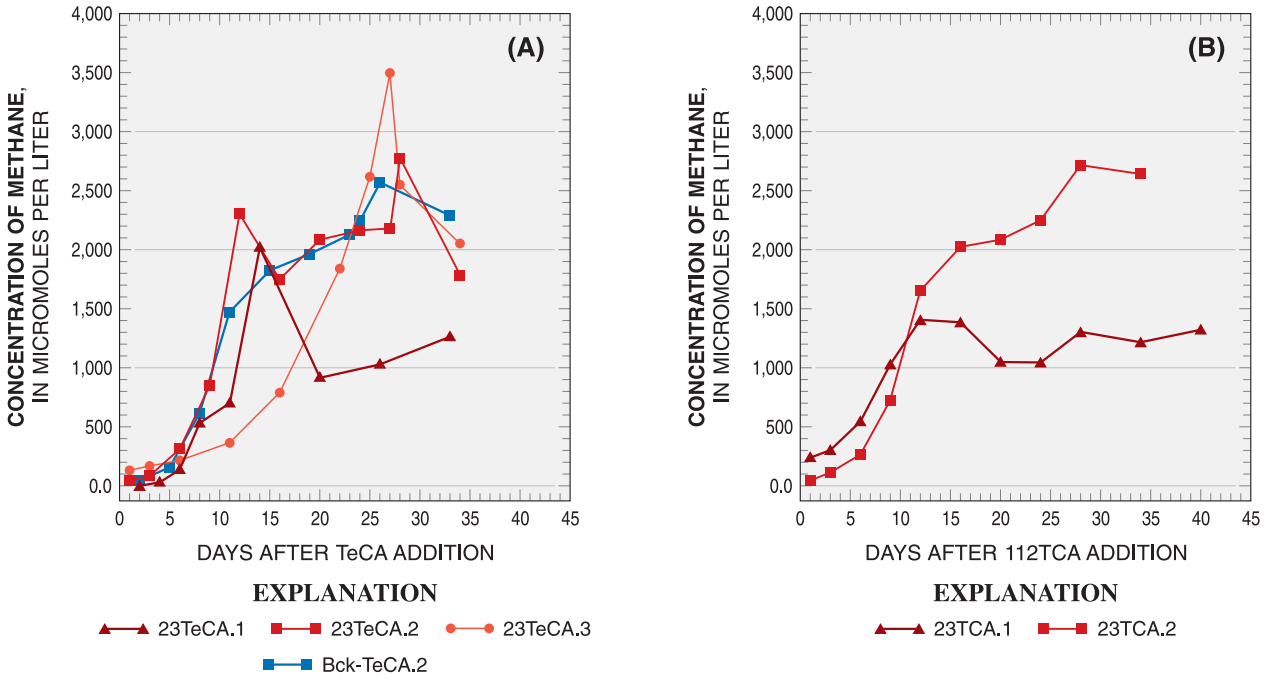

Figure 20. Methane concentrations in February-April 1998 anaerobic microcosms amended with methanol and **(A)** 1,1,2,2-tetrachloroethane (TeCA) and **(B)** 1,1,2-trichloroethane (112TCA).
(Methane concentrations in killed controls were less than 100 micromoles per liter.)

ments comparing degradation in WB23 and WB30 sediments confirmed that WB23 sediments produced more 12DCA and less VC even without the addition of MeOH (figs. 11 and 19). The use of MeOH increased VC degradation, however, whereas the sediment from site WB23 showed a lower ability to degrade VC than sediment from site WB30 did in the microcosms without MeOH (fig. 17). In October–November 2000, microcosms with and without added MeOH also were compared for WB30 sediment (fig. 11b). These results showed that MeOH also increased 12DCA production and decreased observed VC production in WB30 surficial wetland sediment (fig. 11b). Methanol addition increased methane production in microcosms with WB23 (Lorah-Devereux, 1999) and WB30 sediment (fig. 13d).

Concentrations of 12DCA decreased in the 112TCA-amended microcosms after about 15 days of incubation, whether or not MeOH was added (Lorah-Devereux, 1999; fig. 14a). In the 1998 experiments with MeOH, however, the 12DCA hydrogenolysis product chloroethane (fig. 2) was observed whereas it was not detected in the experiments without MeOH (fig. 19b). Possibly, the dichloroelimination degradation pathway for 12DCA (fig. 2) is predominant in the microcosms without MeOH, as it is for 112TCA degradation. Dichloroelimination of 12DCA produces ethene, which also was not detected in any of these microcosms, however.

The effect of hydrogen and acetate as available substrates during VC production was examined in enrichment culture experiments amended with 112TCA, cDCE, or tDCE (table 2). VC production from all three precursors occurred without methane production in the enrichment culture experiments. VC was produced from 112TCA immediately and linearly without significant difference between enrichment cultures incubated with the different substrates (fig. 16a). In contrast, there was a lag in VC production from cDCE and tDCE followed by an exponential increase (fig. 16b). Although VC was produced in 12DCE-enrichment cultures with acetate as a substrate, VC concentrations after 20 days of incubation were more than three times higher when hydrogen was the added substrate. Ethene was produced in high concentrations only in enrichment cultures supplied with hydrogen. These results indicate that the pathway and efficiency of VC production and degradation varied with substrate type.

Redox Conditions The availability of different terminal electron acceptors under different redox conditions causes varying pathways and efficiencies of TeCA and daughter compound production and degradation. The effect of methanogenic and sulfate-reducing conditions on TeCA degradation was investigated in the February–April 1998 microcosm experiments by addition of BES as a methanogenic inhibitor or by addition of sulfate as an alternate electron acceptor (table 1; figs. 21 and 22). Methanol was added to all micro-

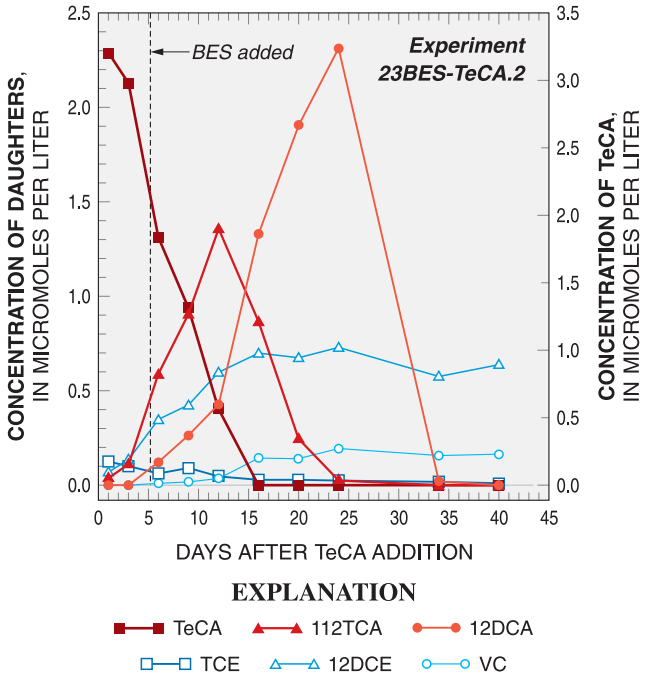

Figure 21. Degradation of 1,1,2,2-tetrachloroethane (TeCA) and production of daughter compounds in anaerobic microcosms amended with 2-bromoethanesulfonic acid (BES), methanol, and TeCA and constructed with wetland sediment from site WB23, February-April 1998.

cosms in the February–April 1998 experiment, and its possible effect on the BES- and sulfate-amended results is unknown. The effect of BES as a methanogenic inhibitor and of iron as a terminal electron acceptor on VC degradation was evaluated in VC/sediment enrichment experiments (table 2).

Addition of BES to February–April 1998 microcosms did inhibit methanogenic activity significantly, although a slight increase in methane concentrations was observed over the 41-day incubation (fig. 22b). Of the MeOH-amended microcosms, TeCA degradation rates were lowest in those amended with BES (fig. 5a). The half-life of 4.6 days for TeCA in the BES-amended microcosms still was low, however, indicating that methanogenic activity was not necessary for TeCA degradation to occur. Substantial production of 112TCA, 12DCA, and 12DCE occurred in the BES-amended microcosms, indicating that methanogens were not directly involved in the production of these compounds (fig. 21). Peak concentrations of 112TCA, 12DCA, and 12DCE in the BES-amended microcosms were about the same or higher than those in the treatment without BES that had a similar initial TeCA concentration (23TeCA.2) (fig. 19a). VC and chloroethane (CA) production, however, were substantially lower in the BES-amended microcosms than in 23TeCA.2 (fig. 19a). The lower VC production in the BES-amended microcosms is consistent with the association noted between onset of methane production and of VC production from 112TCA dichloroelimination in the microcosms (figs. 14 and 15).

Methanogenic activity also was important in degradation of the daughter products 12DCE and VC, whereas 12DCA degradation was unaffected by BES addition (fig. 21). 12DCE and VC were degraded to below detection levels by day 40 in the methanogenic microcosms, but their concentrations remained approximately constant from days 25 through 40 in the BES-amended microcosms (figs. 6b and 21). In the sulfate-reducing microcosms where methane production was not evident after day 16, the approximately constant 12DCE and VC concentrations from day 16 through 46 indicated that degradation of 12DCE and VC also was negligible (fig. 22). In previous TCE-amended microcosms with wetland sediment from site WB30, similar inhibition of 12DCE and VC degradation in BES-amended microcosms and under sulfate-reducing conditions was observed (Lorah and others, 1997; Lorah and others, 2001). Other laboratory and field studies have shown that reductive dechlorination of 12DCE and VC is partially or completely inhibited under sulfate-reducing conditions compared to methanogenic conditions (Bagley and Gossett, 1990; Kästner, 1991; Pavlostathis and Zhuang, 1991; Semprini and others, 1995).

Early experiments by Bradley and Chapelle (1996) indicated that oxidation of VC occurred under iron-reducing conditions. Because the WB30 sediments that showed a greater capacity to degrade VC in the microcosm experiments were initially iron-reducing (figs. 13 and 17), this possible oxidation pathway was investigated in the VC/sediment enrichment experiments by adding FeOOH to induce iron-reducing conditions in the methanogenic sediments. Iron reduction occurred and methanogenesis was inhibited in these FeOOH-amended cultures (fig. 18). VC degradation was inhibited in the iron-reducing enrichments, indicating that anaerobic oxidation of VC by iron-reducing bacteria did not occur (fig. 18). The addition of a more bioavailable, chelated form of iron (Fe(III)-NTA) also did not stimulate degradation under iron-reducing conditions (data on file at USGS, Reston, Virginia). Substantial degradation of VC in the VC/sediment enrichments was observed only under methanogenic conditions. Inhibition of methanogenesis by BES inhibited VC degradation in the enrichment experiments, as observed in the microcosm experiments (figs. 18 and 21). None of these sediment incubation experiments, however, can discern between an oxidation reaction (production of CH_4 and CO_2) and a reduction reaction (production of ethene or ethane) for the VC degradation observed under methanogenic conditions.

Toxicity Although the linear increase in TeCA degradation rates indicates that TeCA concentrations up to 13 µmol/L were not noticeably toxic to the microorganisms responsible for the degradation (fig. 5a), methanogenic activity appeared to be partially inhibited in microcosms with the highest initial TeCA concentration (23TeCA.3), as shown by a slower increase in methane concentrations than observed at lower initial TeCA concentrations (23TeCA.1 and 23TeCA.2) (fig. 20a). Methane concentrations of about

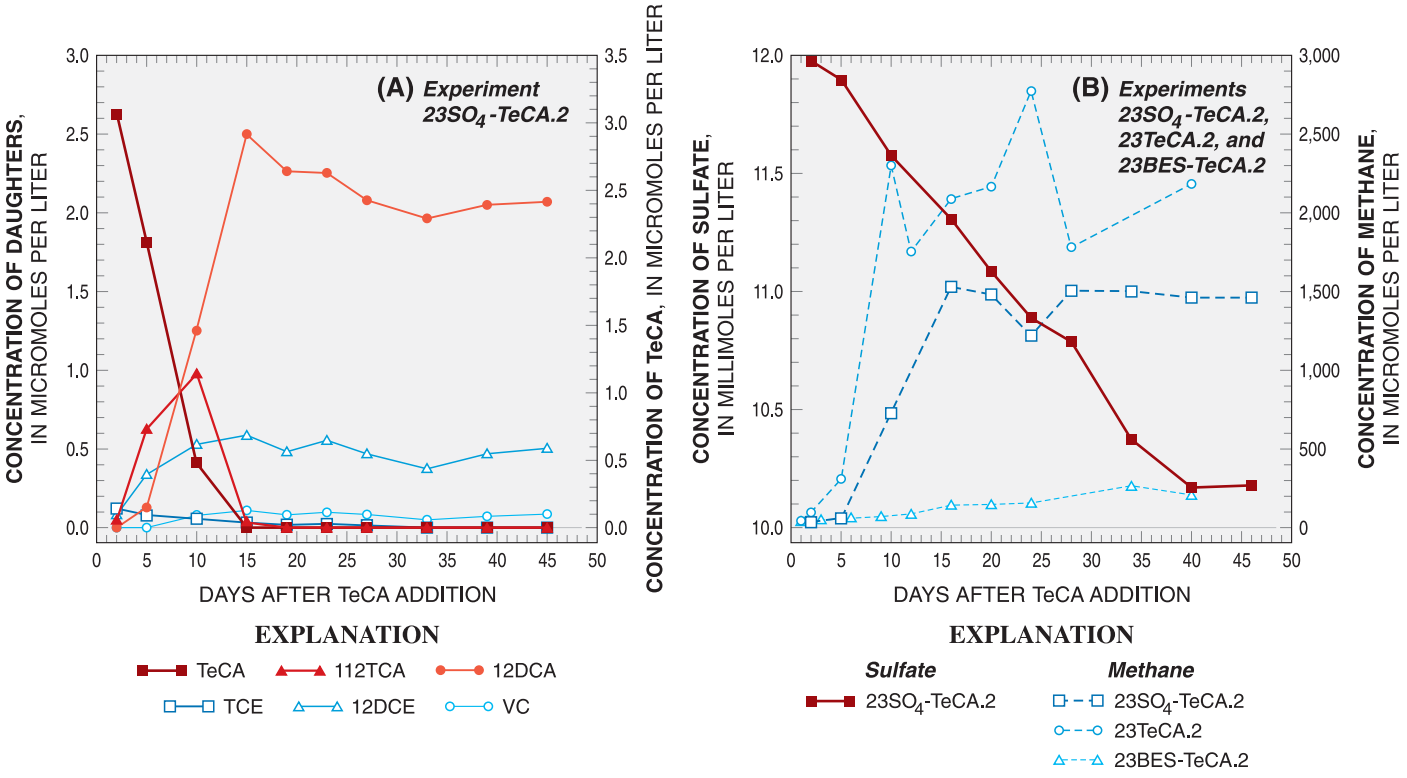

Figure 22. Degradation of **(A)** 1,1,2,2-tetrachloroethane (TeCA) and production of daughter compounds in anaerobic microcosms amended with sulfate (SO_4), methanol, and TeCA and constructed with wetland sediment from site WB23, February-April 1998, and **(B)** effect of SO_4 and 2-bromoethanesulfonic acid (BES) addition on methane and SO_4 concentrations.

2,000 µmol/L were reached by day 15 at the lower initial TeCA concentrations and by later than day 22 at the highest initial TeCA concentration (fig. 20a). In addition, at the highest initial TeCA concentration (23TeCA.3), the greatest time lag was observed between the peak concentrations of 112TCA and 12DCA, and the peak 112TCA concentration was substantially higher than the peak 12DCA concentration (fig. 6). Microcosm experiment 23TeCA.3 with the highest initial TeCA concentration also showed the lowest percentage of CA (fig. 19a). These results indicate that hydrogenolysis of 112TCA to 12DCA and CA was inhibited at the highest initial TeCA concentration, at least in microcosms amended with MeOH.

For the two initial concentrations of 112TCA that were tested in February–April 1998 (2.4 and 6.3 µmol/L), no toxic effect was observed on methanogenic activity, and rapid 112TCA degradation rates occurred (figs. 5b and 20b). In enrichment cultures that were amended with much higher concentrations of 112TCA (10.8 millimolar, see table 2), degradation of 112TCA to VC did occur but without CH_4 production (fig. 16). Thus, the methanogenic community in the original microcosm slurry used for the enrichment cultures may have been inhibited by the 112TCA, although selective effects of dilution and the enrichment medium also could have affected methanogen growth in the enrichment cultures. Microcosms in the October–November 2000 experiment that were constructed using sediment from site WB30 and amended with 3.6 µmol/L of 12DCA showed no degradation or methanogenic activity. Decreases in 12DCA concentrations in the live microcosms were not different from those in the killed controls throughout the 30-day incubation, and production of CA or ethane was not observed (data on file at the USGS office in Baltimore, Maryland). Therefore, 12DCA seemed to have a greater toxic effect than either TeCA or 112TCA. Other studies also have shown that chlorinated alkanes, including 12DCA, chloroform, and carbon tetrachloride, inhibit methanogenic activity at relatively high concentrations (Belay and Daniels, 1987; Liang and Grbic´-Galic´, 1993). It is important to note, however, that apparent toxicity effects in the batch microcosm experiments may not occur in a flowing system.

Pre-Exposure to Contaminants Pre-exposure of the wetland sediment to the presence of TeCA was not required for degradation of TeCA. In microcosms constructed with wetland sediment and ground water from background site WB19, the TeCA degradation rate was slightly higher than the other TeCA-amended microcosms that contained MeOH (fig. 5a). In addition, no lag occurred before degradation of

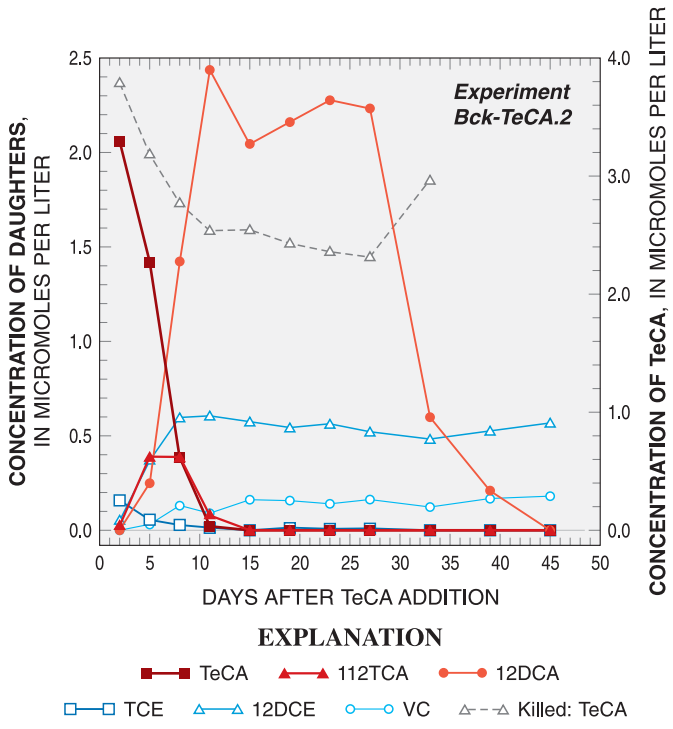

Figure 23. Degradation of 1,1,2,2-tetrachloroethane (TeCA) and production of daughter compounds in anaerobic microcosms conducted in February-April 1998 using sediment from background site WB19 and amended with methanol.

TeCA commenced in the microcosms constructed from the contaminated or the uncontaminated sites (figs. 6 and 23). Thus, acclimation to the presence of TeCA by sediment microorganisms was not needed for degradation to occur by the pathways observed in the MeOH-amended microcosm experiment.

To test the effect of pre-exposure to TeCA on VC degradation in the wetland sediments, replicate VC/sediment enrichment experiments were constructed using three sets of sediments—"fresh" sediment that was amended with VC within 5 days of collection, "pre-exposed" sediment that was incubated with added TeCA for 40 days in the same manner as the microcosm experiments, and "preincubated" sediment that was incubated in the same manner as the "pre-exposed" sediment, but without the addition of TeCA (table 2). VC was not degraded in killed controls for any of these enrichments (data on file at USGS, Reston, Virginia), but all live and killed treatments showed an abiotic loss of VC in the first 2 or 3 days that probably was caused by sorption (fig. 24a). In the live VC/sediment ennrichments, VC degradation was fastest in the pre-exposed enrichments with both WB23 and WB30 sediment, and slowest in the fresh sediment (fig. 24a). VC was degraded completely by day 6 in the pre-exposed enrichments (fig. 24a). VC degradation in the fresh WB23 sediment enrichments lagged for the first 2 weeks, and then VC concentrations decreased at a rate similar to that observed in previous VC/sediment enrichment experiments (fig. 18). Complete removal of the VC was observed by day 28 in the fresh WB23 sediment enrichments. In the fresh WB30 sediment enrichments, degradation lagged for at least 3 weeks, and then VC concentrations decreased at a rate similar to that in fresh WB23 sediment cultures (fig. 24a). Methane production was greater in the three sets of enrichment cultures with WB23 sediment than in those with WB30 sediment (fig. 24b). The longer lag before VC degradation in the cultures with fresh and preincubated WB30 sediment could be associated with the lower methane production in these cultures compared to the pre-exposed WB30 enrichments and to the WB23 VC/sediment enrichments. In the pre-exposed enrichments, VC degradation was equally rapid in WB30 and WB23 sediment, although methane production was lower in the WB30 pre-exposed culture (fig. 24).

Microbial Communities and Associations with Degradation Pathways

A microbial consortium, rather than one microbial species or functional group, apparently is involved in the degradation of TeCA, as indicated by the variability in daughter product distributions in the microcosms with the site or season from which the sediment was collected, and by the variable effect of redox conditions on different steps of the degradation pathways. The microbial community analyses, combined with the geochemical data, assist in evaluation of the reaction pathways of TeCA degradation in the anaerobic microcosms, and lead to identification of the possible microbial species or groups involved, either directly or indirectly, in different steps of the reaction pathways. To identify possible critical microbial species or groups, shifts in the TRFLP profiles (DNA fingerprints) of the microbial communities in the microcosm sediments over time and between different experiments were compared to shifts in the daughter product distributions to note consistent associations. In the discussion below, changes in degradation pathways associated with spatial and seasonal variability are linked to changes in the microbial communities. Other factors affecting degradation pathways and microbial communities, such as substrate type, are discussed where molecular data are available. In this report, the potential critical microbial species or groups are identified primarily by base pair sizes for the specific peaks on the DNA fingerprint. Future analyses of these specific peaks in archived frozen samples should provide definitive identification by using primers for specific microbial functional groups, such as iron-reducing bacteria, sulfate-reducing bacteria, or acetogens. The planned completion of genetic analyses of the enrichment experiments should assist in these identifications because enrichment decreases the number of TRFLP peaks to be identified by selecting for community members involved in the reaction of interest and diluting out others. Enrichment greatly decreases the diversity of microbial species and increases the intensity of peaks associated with the selected members of the microbial community. The following sections discuss the degradation reactions of TeCA for which possible indicator microbial

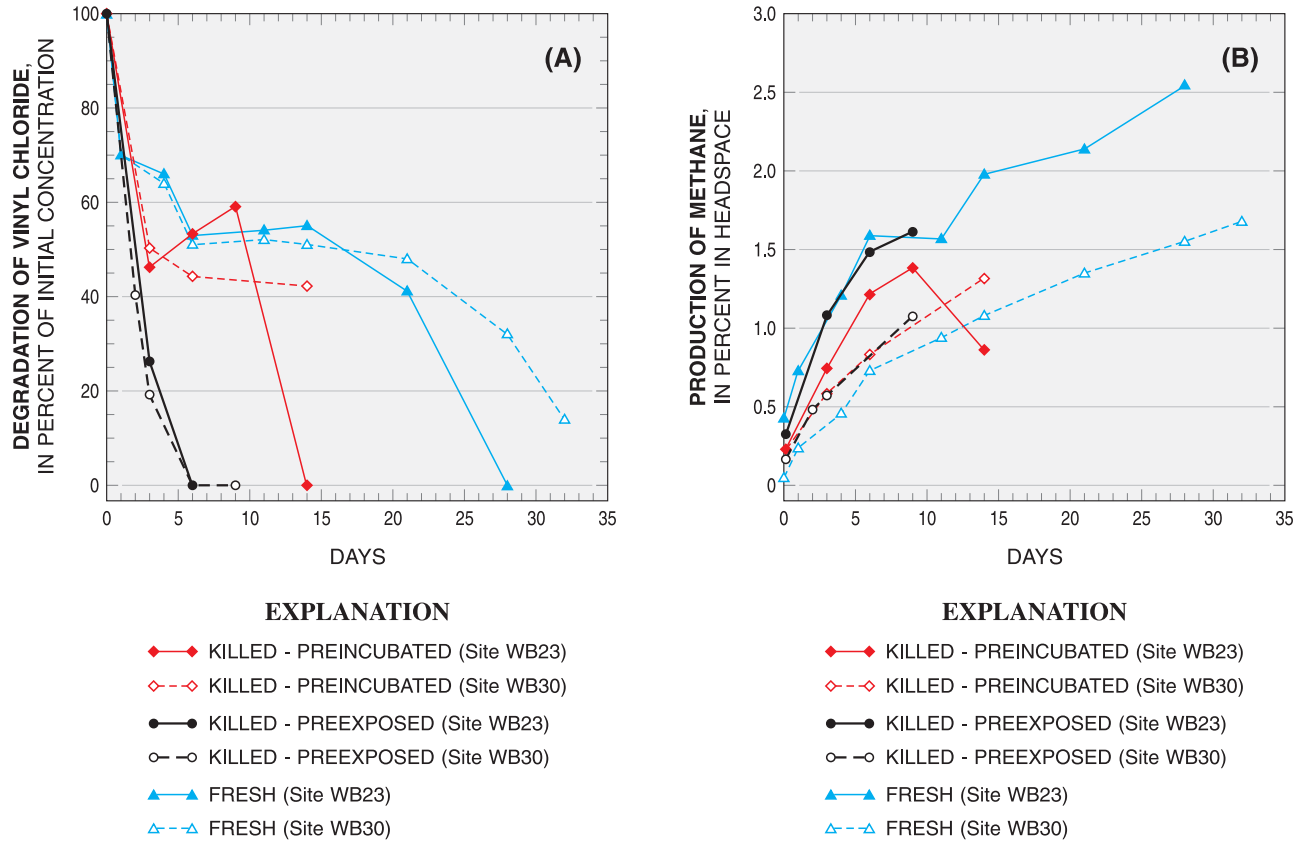

Figure 24. **(A)** Degradation of vinyl chloride and **(B)** production of methane in sediment enrichment experiments amended with vinyl chloride and constructed with fresh, preexposed, and preincubated wetland sediment from sites WB23 and WB30.

species or groups have been identified on the basis of the microcosm experiments.

Degradation of 1,1,2,2-Tetrachloroethane Simultaneous production of 12DCE and 112TCA from dichloroelimination and hydrogenolysis of TeCA, respectively, occurred without a lag in all live microcosm experiments that were conducted (figs. 6–9), including the experiments where low degradation rates of TeCA were observed—the July–August 1999 microcosms incubated at 19 °C and the March–April 1999 microcosms incubated at 5 °C (fig. 5; table 4). Because methane production often did not occur when 12DCE and 112TCA production occurred, bacteria (rather than methanogens) are most likely involved in these TeCA degradation reactions. On a molar percentage basis, the proportion of 12DCE produced was between 16 to 25 percent of the initial TeCA in microcosms constructed with wetland sediment from three different sites and under all observed redox conditions (figs. 11 and 19a; table 4), indicating the involvement of metabolically versatile anaerobic bacteria or a cometabolic process in the dichloroelimination of TeCA. Although the percentage of 112TCA produced was more variable (figs. 11 and 19a; table 4), hydrogenolysis of TeCA to 112TCA also seems to involve metabolically versatile anaerobic bacteria. Identification of the microorganisms that potentially are involved in these early TeCA degradation reactions can be best examined by using the molecular data from microcosms where production of daughter compounds other than 12DCE and 112TCA was minimal.

For dichloroelimination of TeCA to 12DCE, the molecular data for killed controls that showed production of 12DCE provides the best information because 12DCE (both *cis*- and *trans*- isomers) was the only daughter compound observed in these microcosms. Production of 12DCE in killed controls was greatest in the February–April 1998 microcosms that contained MeOH, as discussed in the preceding section on Degradation Rate of 1,1,2,2-Tetrachloroethane. Recovery of metabolic activity in the killed controls was indicated by decreasing TeCA concentrations (fig. 6) and corresponding increasing 12DCE concentrations by the end of the experiments (Lorah-Devereux, 1999; Lorah and Olsen, 1999a). Killed controls for experiments 23TeCA.1 and 23SO_4-TeCA.2 had the highest production of 12DCE of all microcosm experiments; the maximum molar percentage of 12DCE produced was greater than 80 percent. The extensive metabolic activity in the killed controls for the MeOH-amended experiments indicates that microbes involved in

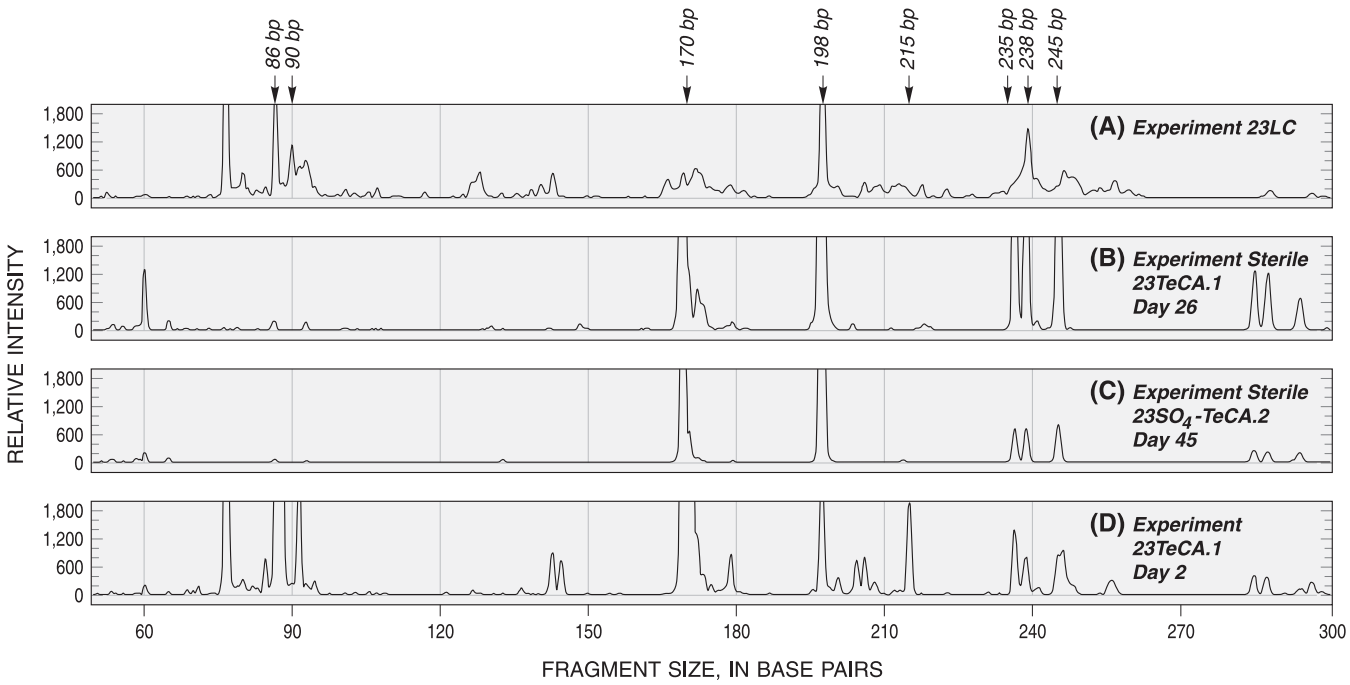

Figure 25. Bacteria terminal-restriction fragment length polymorphism profiles in selected TeCA-amended microcosms constructed with sediment from site WB23, February-April 1998: **(A)** live control [no 1,1,2,2-tetrachloroethane (TeCA) added, experiment 23LC], **(B)** killed control for microcosms with low initial TeCA concentration [experiment killed 23TeCA.1], **(C)** killed control for microcosms with TeCA and sulfate amendment [experiment killed $23SO_4$-TeCA.2], and **(D)** live microcosm with low initial TeCA concentration [experiment 23TeCA.1].

TeCA dichloroelimination can utilize MeOH, and possibly formaldehyde, as substrates. Although DNA was not recovered from most killed microcosm samples from early time steps, DNA was recovered and analyzed by TRFLP from later time points where 12DCE production occurred. Bacterial TRFLP profiles in sediment from killed controls in the 1998 experiment show a substantial increase over the course of the experiment in intensity of several peaks, including peaks at 170, 198, 235, and 238 bp (base pairs) (fig. 25). There were other less prominent peaks, but none that consistently appeared in all microcosms that showed 12DCE production from TeCA dichloroelimination. The bacteria TRFLP profiles for the TeCA-amended microcosms in July–August 1999 are shown for comparison of prominent peaks (fig. 26). Microbial biomass and diversity were substantially lower in the live July–August 1999 microcosms compared to all other microcosms, as discussed in more detail in the following section on Seasonal Variability (figs. 26 to 28). Prominent bacterial peaks, therefore, were fewer and more distinct. At day 3 in the TeCA-amended live microcosms in July–August 1999, the daughter compound present in the highest concentrations was 12DCE, except for the abiotic daughter product TCE (fig. 8). Of the prominent bacterial peaks in the killed controls that produced 12DCE (fig. 25), the 198-bp peak was the most prominent at day 3 in the July–August 1999 microcosms (fig. 26). A role for the bacterial species or groups represented by 170, 235, and 238 in TeCA dichloroelimination, however, can not be discounted as more than one bacterial group may be involved. TeCA dichloroelimination produced both the 12DCE isomers, although production of cDCE commonly was three or more times greater than production of tDCE in these and previously reported microcosms at this site (Lorah and Olsen, 1999a). Different bacteria likely are involved in production of the two 12DCE isomers.

The February–April 1998 microcosms with WB23 sediment also were used to help identify bacteria potentially involved in hydrogenolysis of TeCA to 112TCA because both the use of MeOH and the use of WB23 sediment seemed to enhance this hydrogenolysis pathway, as shown by the predominance of 112TCA and 12DCA compared to VC in these microcosms (discussed previously in the section on Substrate Type). As an example, figure 25 shows the bacterial TRFLP profiles from experiment 23TeCA.1 after 2 days of incubation, compared to bacteria profiles in the WB23 sediment prior to any microcosm preparations and to the live control microcosms that did not contain added TeCA or MeOH. 112TCA and 12DCE were the only biotic daughter products observed at day 2 in 23TeCA.1 (fig. 6a). The bacterial peaks at 90 and about 215 bp were enhanced in 23TeCA.1 compared to the live control microcosm (fig. 25).

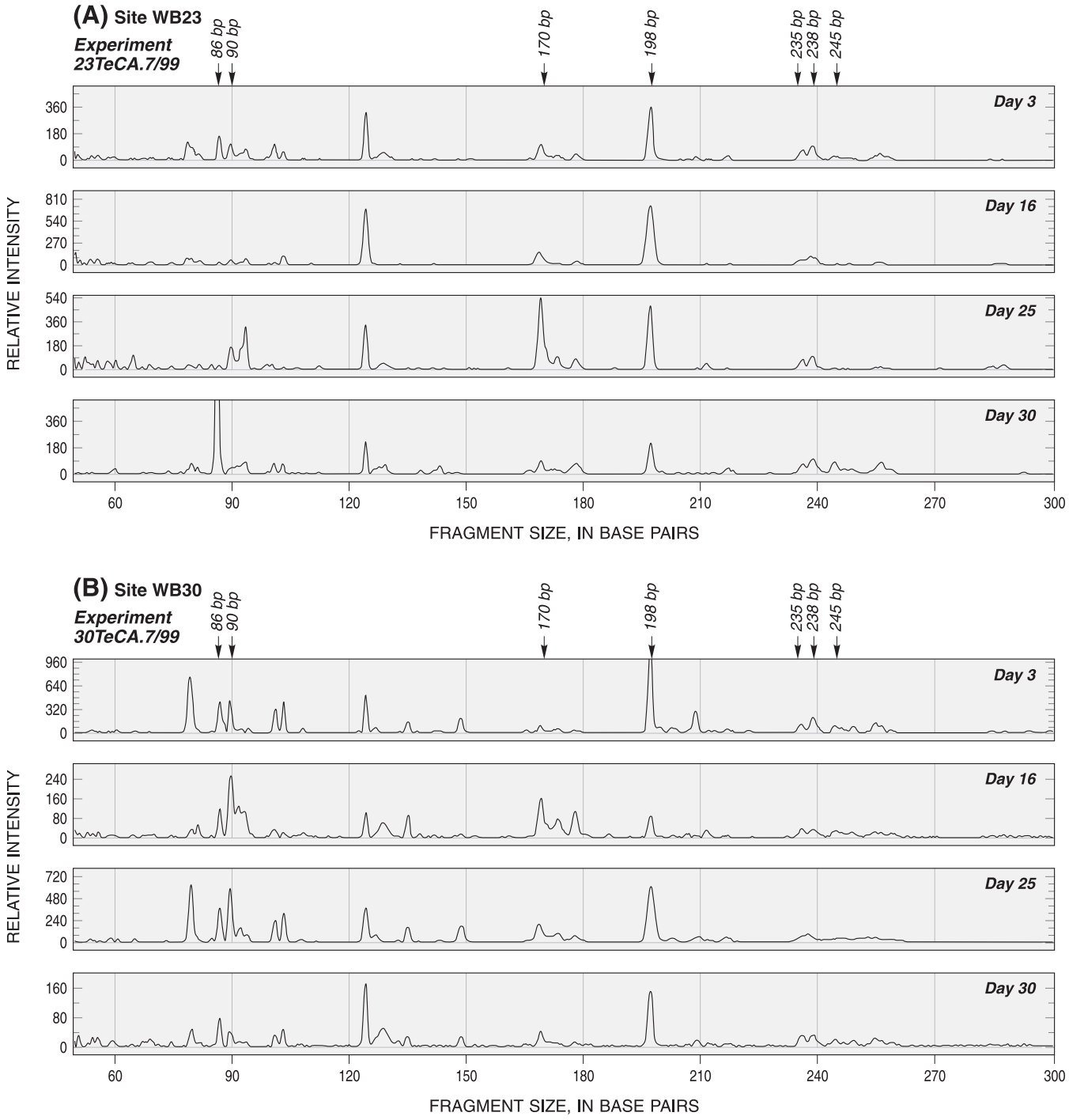

Figure 26. Bacteria terminal-restriction fragment length polymorphism profiles in 1,1,2,2-tetrachloroethane- (TeCA-) amended microcosms constructed with sediment from site WB23 compared to site WB30, July-August 1999: **(A)** Site WB23 [experiment 23TeCA.7/99], and **(B)** site WB30 [experiment 30TeCA.7/99].

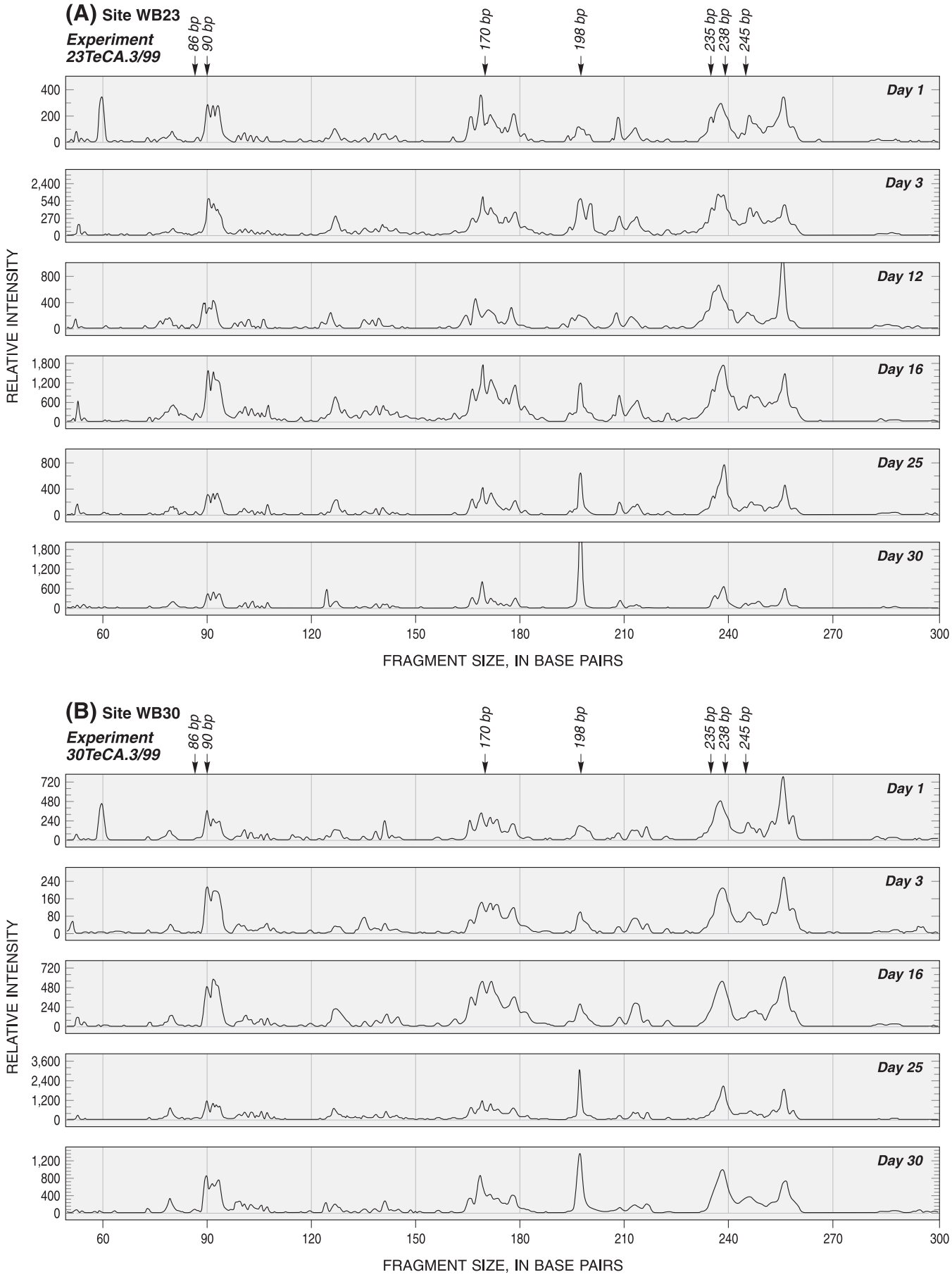

Figure 27. Bacteria terminal-restriction fragment length polymorphism profiles in 1,1,2,2-tetrachloroethane- (TeCA-) amended microcosms constructed with sediment from site WB23 compared to site WB30, March-April 1999: **(A)** Site WB23 [experiment 23TeCA.3/99], and **(B)** site WB30 [experiment 30TeCA.3/99].

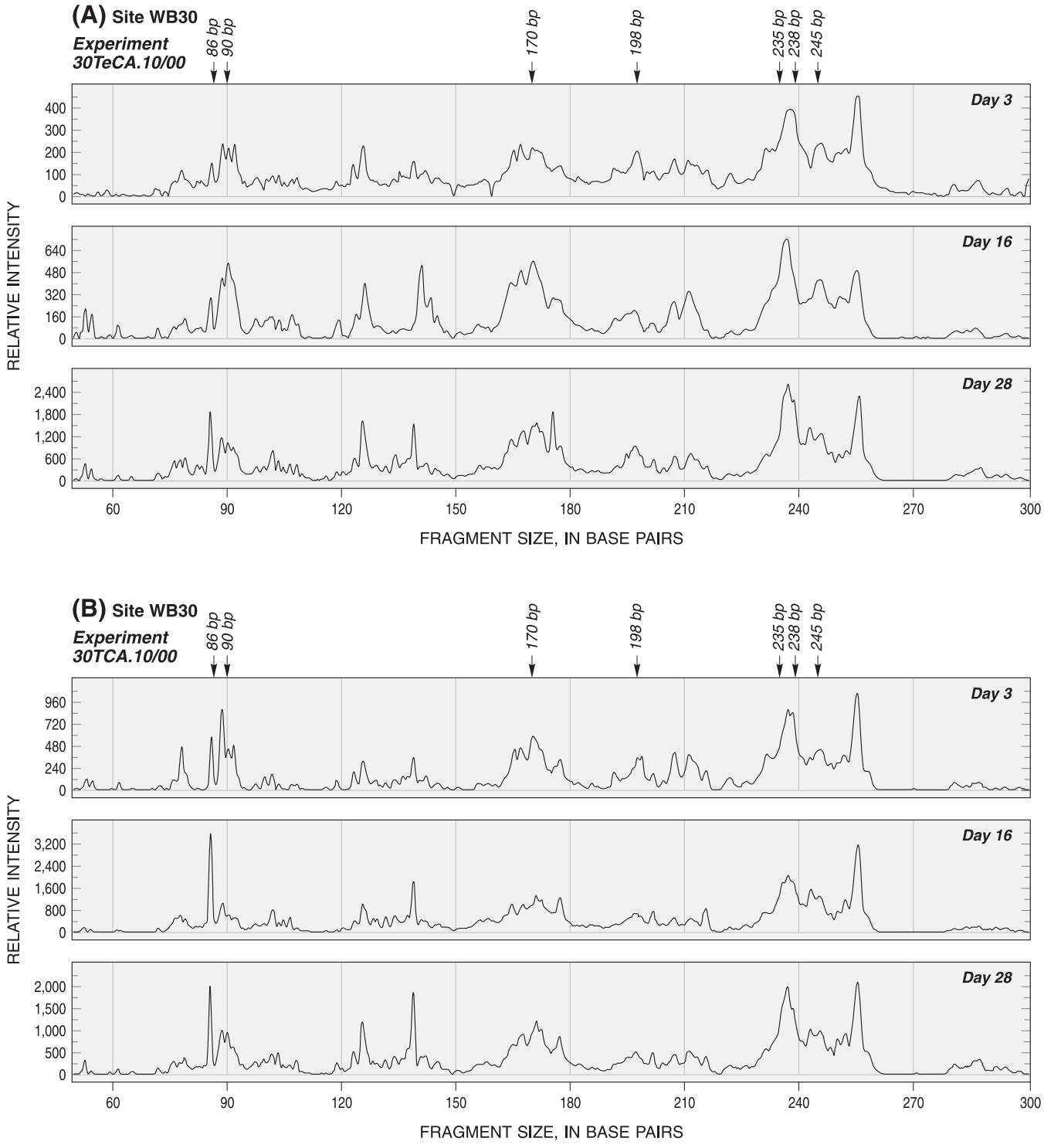

Figure 28. Bacteria terminal-restriction fragment length polymorphism profiles in 1,1,2,2-tetrachloroethane- (TeCA-) and 1,1,2-trichloroethane- (112TCA-) amended microcosms constructed with sediment from site WB30, October-November 2000: **(A)** Site WB30, amended with TeCA [experiment 30TeCA.10/00], and **(B)** site WB30, amended with 112TCA [experiment 30TCA.10/00].

Although other peaks were enhanced in 23TeCA.1, they were discounted as having a role in 112TCA production because they also were present in the killed controls (fig. 25). The killed controls only showed production of 12DCE and not 112TCA. The bacterial group represented by the 90 bp peak is more likely to be involved in TeCA hydrogenolysis to 112TCA than the 215 bp peak because the 90 bp peak was observed in all live TeCA-amended microcosm sediment samples (figs. 26–28). The presence of the 215 bp peak was not associated consistently with 112TCA production, sometimes occurring in extremely low intensity or not at all when substantial 112TCA production was observed (figs. 8 and 26).

Daughter Compound Production and Degradation

Spatial Variability of Vinyl Chloride Production—One of the greatest spatial variability effects observed in the microcosms was in the production of VC. At least three distinct VC production reactions were noted from the microcosm and enrichment culture experiments: (1) 112TCA dichloroelimination (possibly cometabolic) that was enhanced under methanogenic conditions and resulted in approximately twice as much VC production in WB30 microcosms compared to WB23 microcosms amended with either TeCA or 112TCA, (2) cometabolic 112TCA dichloroelimination that occurred without methane production and at a similar rate in cultures inoculated with sediment slurry from WB23 and WB30, and (3) growth-linked hydrogenolysis of 12DCE that occurred without methane production and more rapidly in WB30 enrichments than in WB23 enrichments (see earlier section on Spatial Variability of VC Production). Thus, at least three different microbial species/groups potentially are involved in VC production in these wetland sediments. The combined microcosm and enrichment culture results indicate that both sediments have the microorganisms necessary to carry out these VC production reactions, but that their population size or activity vary with conditions such as redox or toxicity effects. In the discussion below, shifts in the TRFLP profiles that correspond to the variable timing of production of VC observed in the WB30 microcosms compared to the WB23 microcosms are used to identify possible microbial species/groups that are involved in VC production reactions, either directly or indirectly. Thus, spatial variability effects are discussed primarily in the context of clarifying degradation pathways and associated microorganisms, instead of identifying the causes of spatial variability. The underlying causes of spatial variability likely are complex and result from a combination of interconnected factors, including redox conditions, substrate types, and toxicity effects.

In March–April 1999, the onset of VC production was delayed in the TeCA- and 112TCA-amended microcosms constructed with WB30 sediment compared to those constructed with WB23 sediment (figs. 7 and 14), but VC production was greater in the WB30 microcosms (figs. 11 and 12). Conditions in the sediment at WB30 seemed to favor the dichloroelimination pathway of 112TCA degradation over the hydrogenolysis pathway, as discussed in the preceding section. Because methane production enhanced but was not required for 112TCA dichloroelimination to VC, bacteria rather than methanogens seem to be directly involved in this degradation reaction. Comparison of bacterial TRFLP profiles from both WB23 and WB30 microcosms in March–April 1999 reveal development of a specific peak concomitant with the onset of VC production. This peak, a 198 bp fragment, appeared early in the experiments and grew in prominence as VC production increased (figs. 27a–b). VC production began by day 10 in the WB23 TeCA-amended microcosm, and the 198 bp peak was prominent by day 3 (figs. 7a and 27a). In contrast, VC production began at day 16 in the WB30 TeCA-microcosm, and the 198 bp peak also became prominent by day 16 (figs. 7b and 27b). The live control that was not amended with TeCA did not show an increase in intensity in the 198 bp peak, indicating that growth of this bacterial species or group was stimulated in the TeCA-amended microcosms (fig. 29a). This same 198 bp peak possibly is associated with another dichloroelimination reaction--TeCA to 12DCE (fig. 25).

The microbial profiles for the 112TCA-amended microcosms did not show as clear a relation between VC production and the 198 bp peak (fig. 30), but these microcosms may have been affected by toxicity effects of 112TCA (see previous section on Toxicity). The peak intensity and diversity generally was lower in the TRFLP profiles of the 112TCA-amended microcosms compared to the TeCA-amended microcosms, as might be expected from toxicity effects (figs. 27 and 30). The 112TCA-amended microcosm with sediment from site WB23 in March–April 1999, in which VC concentrations first increased between days 21 and 25 (fig. 14a), did show a distinct increase in the 198 bp peak by day 25 (fig. 30a). In the 112TCA-amended microcosm for site WB30, however, the 198 bp peak initially was prominent but then decreased substantially by the end of the experiment. It is possible that another bacterial species or group, such as the 255 and 258 bp peaks in fig. 30b, became involved in 112TCA dichloroelimination under 112TCA toxicity conditions. A change in the bacterial species or group involved in VC production from 112TCA also was observed seasonally, as discussed in the following section on Seasonal Variability.

A change in slope was observed in VC production in the TeCA-amended microcosms in March–April 1999, particularly those constructed with WB23 sediment (fig. 7a). The first increase in VC coincided with decreasing 112TCA concentrations and the second increase in VC coincided with decreasing 12DCE concentrations (fig. 7a). The second increase in VC production in the TeCA-amended microcosms in March–April 1999 began at day 25 (fig. 7a). A corresponding change in the bacterial community was not evident with the onset of this second major pathway for production of VC—hydrogenolysis of 12DCE (fig. 27a). A change in the methanogen community, however, was evident (fig. 31a). The methanogen TRFLP profile showed an increase by day 25 in a 307 bp peak, which is from a methanogen in the Methanococcales or Methanobacteriales family, methanogens that predominantly utilize CO_2 and hydrogen to produce CH_4 (fig. 31a). By day 30, the peak intensity of

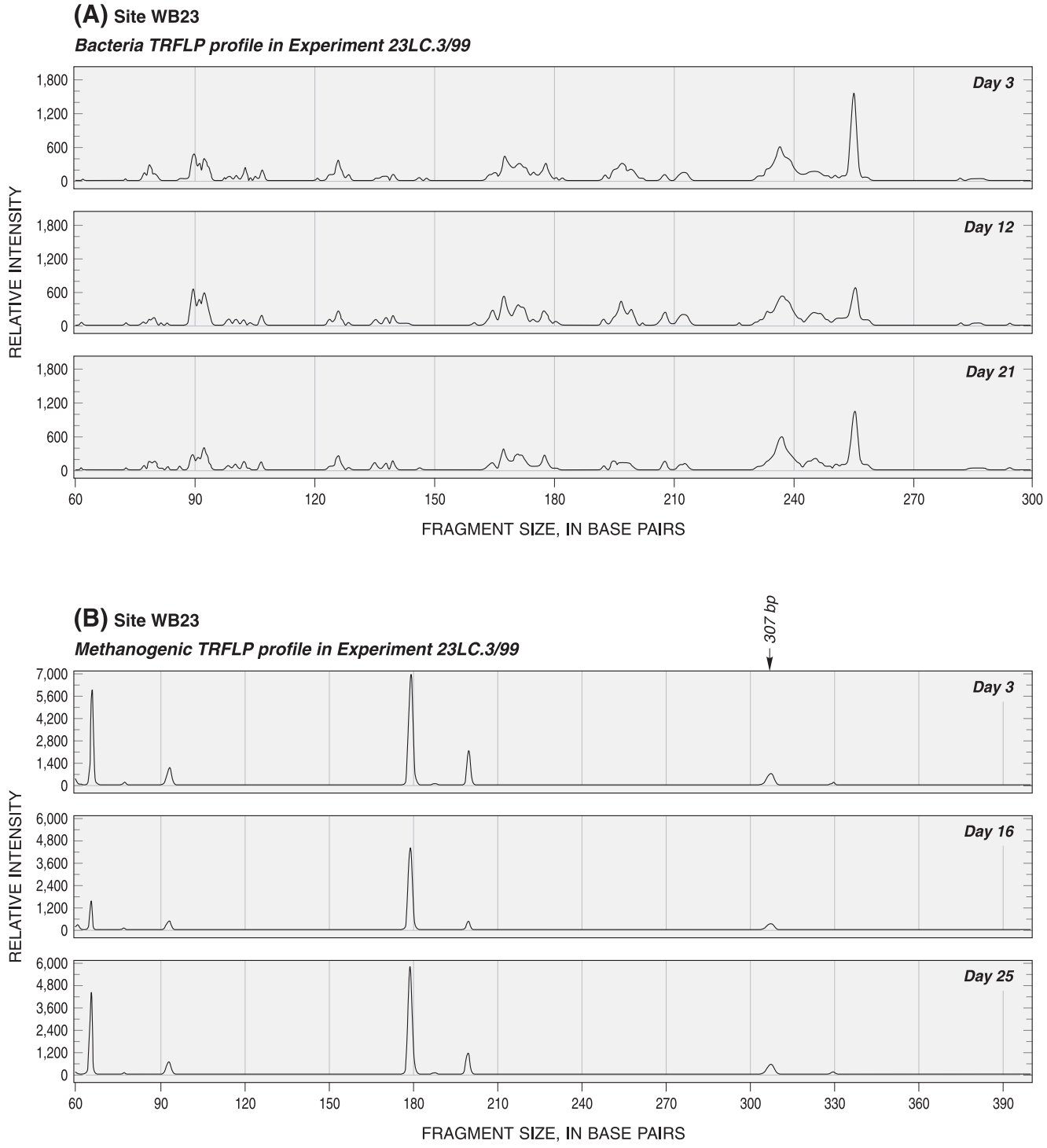

Figure 29. Terminal-restriction fragment length polymorphism (TRFLP) profiles for **(A)** bacteria and **(B)** methanogens in live control microcosms [experiment 23LC.3/99] constructed with sediment from site WB23, March-April 1999.

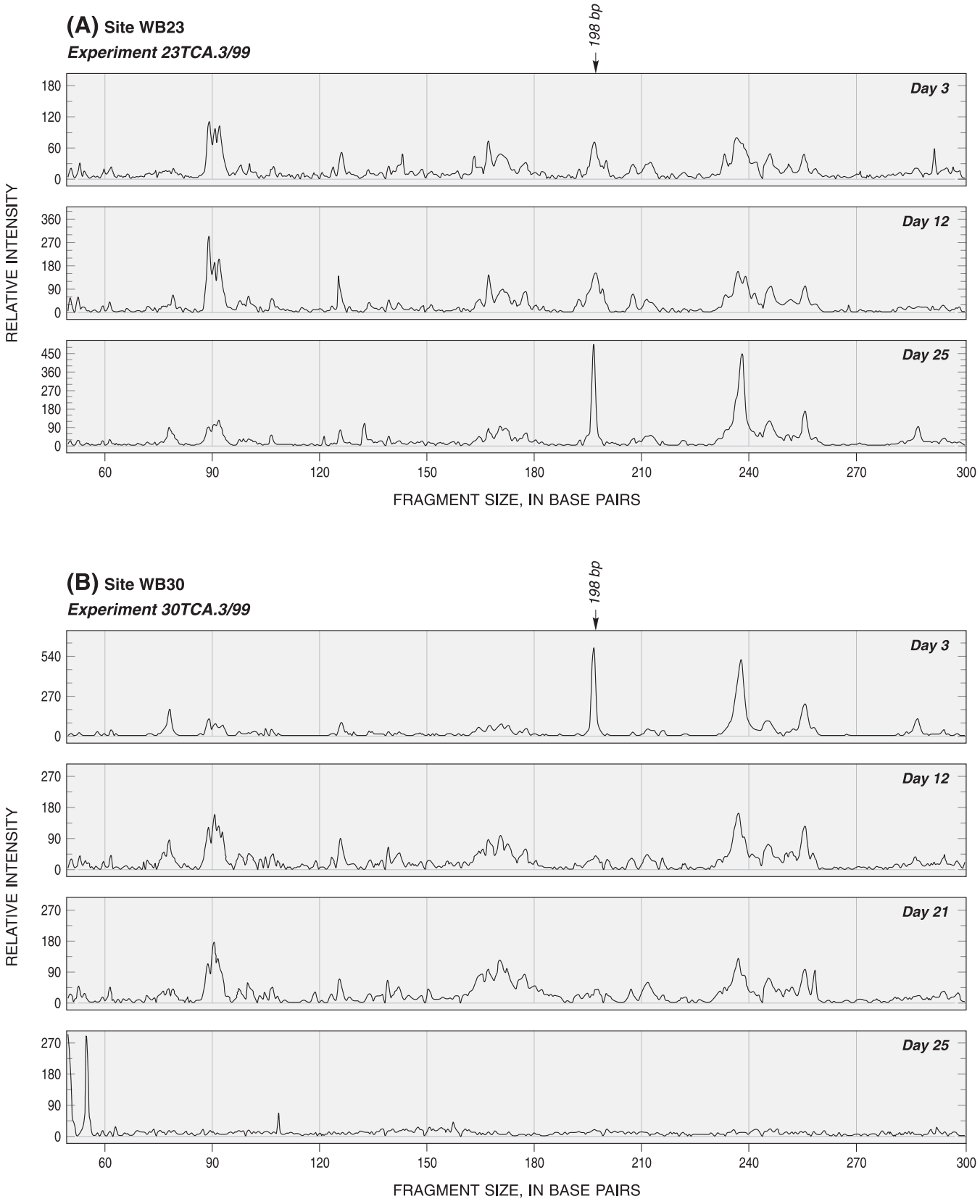

Figure 30. Bacteria terminal-restriction fragment length polymorphism profiles in 1,1,2-trichloroethane- (112TCA-) amended microcosms constructed with sediment from site WB23 compared to site WB30, March-April 1999: **(A)** Site WB23 [experiment 23TCA.3/99], and **(B)** site WB30 [experiment 30TCA.3/99].

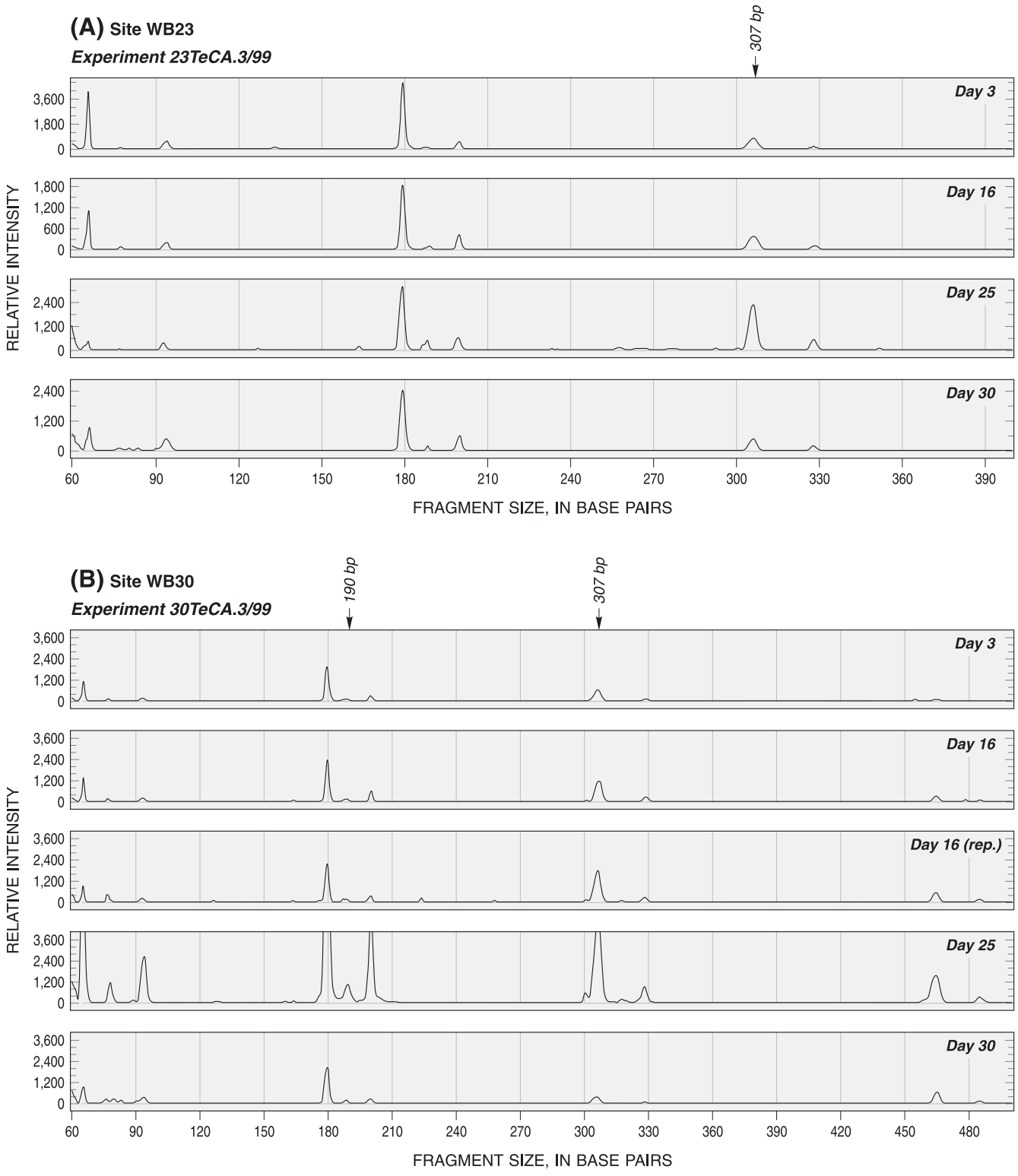

Figure 31. Methanogen terminal-restriction fragment length polymorphism profiles in 1,1,2,2-tetrachloroethane- (TeCA-) amended microcosms constructed with sediment from site WB23 compared to site WB30, March-April 1999: **(A)** Site WB23 [experiment 23TeCA.3/99], and **(B)** site WB30 [experiment 30TeCA.3/99].

this 307 bp methanogen had decreased, and VC concentrations had decreased below 0.15 µmol/L (9.4 µg/L). Similarly, there was an increase in a 190 bp peak between days 16 and 25, which also is believed to be from a methanogen in the Methanococcales or Methanobacteriales family and may be involved in the hydrogenolysis of 12DCE to VC. The intensity of this peak also decreased by day 30 (fig. 31a). The methanogen profiles for the March–April 1999 microcosms with WB30 sediment may indicate that the 307 bp methanogen is involved in 12DCE hydrogenolysis to VC, rather than the 190 bp methanogen (fig. 31b). In the WB30 microcosms, CH_4 production began between days 16 and 21 (fig. 13d). Substantial VC production also began between days 16 and 21, as both 112TCA and 12DCE concentrations decreased (fig. 7b). The methanogen profiles for this experiment show that the 307 bp peak was prominent at day 16, whereas the 190 bp peak did not become prominent until day 25, when VC production already was occurring (fig. 31b). The live control that was not amended with TeCA did not show an increase in intensity in the 307 or 190 bp peaks, indicating that growth of these CO_2-utilizing methanogens was stimulated in the TeCA-amended microcosms (fig. 29b).

In the 12DCE-amended enrichment culture experiments, the exponential increase in VC concentrations with addition of 12DCE indicated growth of a microbial population that can utilize 12DCE in non-methanogenic conditions (fig. 16). Bacteria, rather than methanogens, mediated hydrogenolysis of 12DCE in these cultures, and the WB30-inoculated cultures degraded cDCE more readily than the WB23-inoculated cultures (fig. 16). Molecular analyses of WB30 sediment from the March–April 1999 microcosms using primers specific for dehalorespiring bacteria revealed the presence of *Dehalococcoides ethenogenes* and members of *Desulfuromonas* throughout the experiment. Similar molecular analyses targeting these dehalorespiring bacteria were unsuccessful with the WB23 sediment. It should be noted, however, that the detection limits of the nested PCR methodology for these dehalorespiring bacteria are unknown. Löffler and others (2000) recommend that enrichments be done to prevent false negative results. In addition, there could be unidentified dehalorespiring bacteria at this site that these specific primers would not detect. The eventual production of VC from 12DCE in the WB23-inoculated enrichment cultures after a long incubation (fig. 16) indicated that dehalorespiring bacteria were present at site WB23, but that their population size likely was much lower than at site WB30. Completion of the TRFLP analyses of the enrichment cultures and completion of molecular analyses of the microcosm slurry targeting the dehalorespiring bacteria (these analyses have been completed only for one set of microcosm experiments) could allow more definitive identification of the different bacteria and factors controlling VC production in the wetland sediment.

Spatial Variability of Vinyl Chloride Degradation—The higher VC degradation that consistently occurred in the microcosms constructed with WB30 wetland sediment compared to those with WB23 sediment (fig. 17) was associated with an

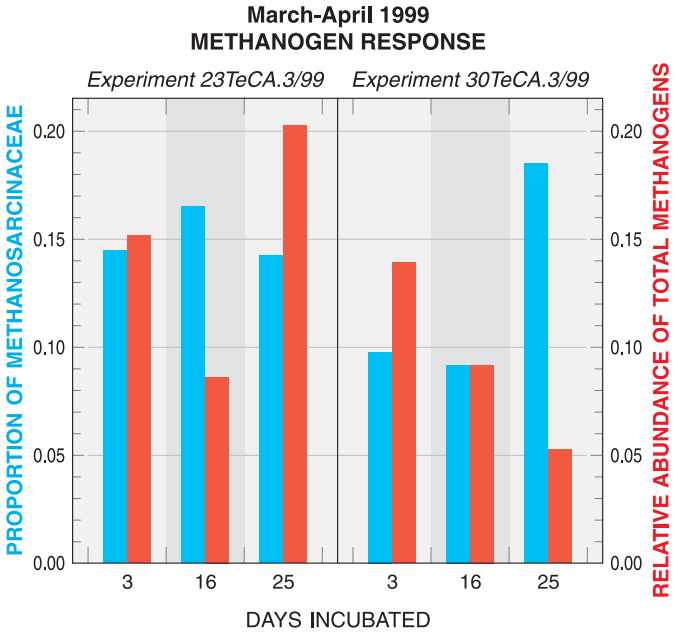

Figure 32. Proportion of Methanosarcinaceae and the relative abundance of total methanogens in 1,1,2,2-tetrachloroethane- (TeCA-) amended microcosms constructed with WB23 and WB30 sediments, March-April 1999 (experiments 23TeCA.3/99 and 30TeCA.3/99).

increase in the proportion of Methanosarcinaceae, a group that includes all methanogens that can utilize acetate to generate CH_4 (acetotrophic methanogens). In the March–April 1999 microcosms with WB30 sediment, the proportion of Methanosarcinaceae in the total methanogen community doubled between days 16 and 25, increasing from about 10 to 20 percent (fig. 32). At day 25, VC concentrations began to decrease in these microcosms (fig. 7b). Similarly, in the October–November 2000 microcosms with WB30 sediment (and no added MeOH; the MeOH effect is discussed later), the proportion of Methanosarcinaceae tripled from 6 to 19 percent between days 18 and 26 (fig. 33), and VC concentrations first began to decrease at day 26 (fig. 9b). These acetotrophic methanogens, therefore, may be linked either directly or indirectly to VC degradation at site WB30. In contrast, the Methanosarcinaceae proportional abundance remained about the same in the WB23 microcosms (fig. 32), and VC concentrations did not decrease substantially within the 42-day experiment (fig. 7a). It also is interesting to note that while the proportion of Methanosarcinaceae increased when CH_4 production began and VC was degrading in the WB30 microcosms (figs. 7b, 13d, and 32), the relative abundance of total methanogens actually decreased in WB30, both between days 3 and 16 as well as days 16 and 25 in the March–April 1999 experiment (fig. 32). Note that the proportion of Methanosarcinaceae shown in figure 32 was calculated as a percentage of the total methanogen peak area on a given sampling day, whereas the relative abundance of total methanogens was calculated by comparing the total

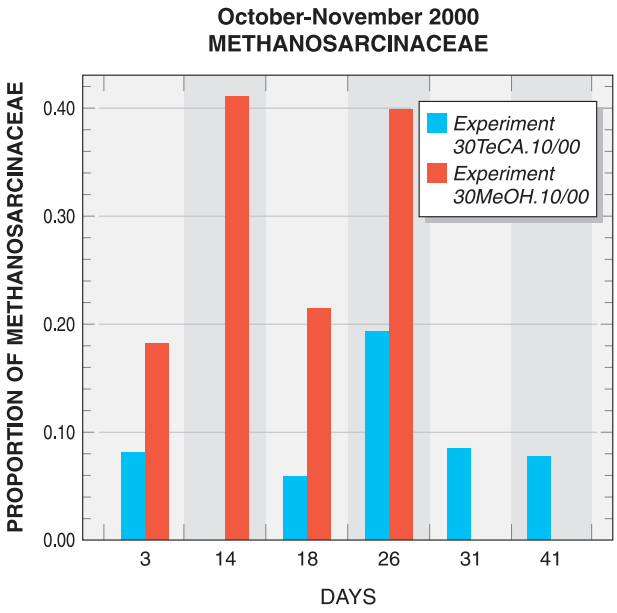

Figure 33. Proportion of Methanosarcinaceae in 1,1,2,2-tetrachloroethane- (TeCA-) amended microcosms constructed with WB30 sediments, with or without addition of methanol (MeOH), October-November 2000.

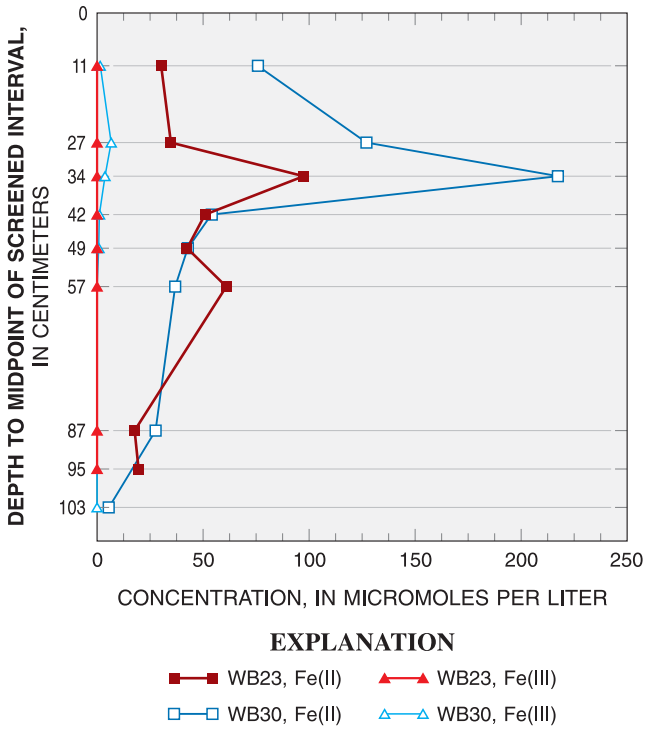

Figure 34. Ferrous [Fe(II)] and ferric [Fe(III)] iron concentrations (in micromoles per gram wet weight) in wetland sediment samples that were collected by coring at sites WB23 and WB30, August 2000.

*mcr*A peak from timepoint to timepoint over the incubation period.

The Methanosarcinaceae could increase in proportion and potential importance by outcompeting the other methanogens in some way, such as the ability to use a substrate for methanogenesis such as acetate that the other methanogen groups cannot use. The initial dominance of iron-reducing conditions in the WB30 microcosms (fig. 13) was the only noticeable geochemical difference between the WB30 and WB23 microcosms. The higher availability of ferric iron in the surficial wetland sediments at site WB30 than at site WB23 accounts for the higher iron reduction observed in the WB30 microcosms (fig. 34). The initial iron reduction may have stimulated the growth of acetate-utilizing methanogens compared to CO_2-utilizing methanogens, although the mechanism by which this enhancement might have occurred is unknown. Possibly, iron reduction released phosphorus from the sediment, and the increased availability of this nutrient in the WB30 microcosms stimulated the acetate-utilizing methanogens. Because phosphorus can be retained in wetland sediments as ferric phosphate compounds and as oxides and hydroxides of iron and aluminum, the iron and aluminum content of wetland sediments affects nutrient availability (Mitsch and Gosselink, 1993, p. 605). Although onset of CH_4 production was delayed in the WB30 microcosms compared to WB23 microcosms, the CH_4 production rate was much higher in the WB30 microcosms once it began (fig. 13d). Higher nutrient availability in the WB30 microcosms could have increased metabolism of the Methanosarcinaceae compared to the CO_2-utilizers that were predominant in the WB23 microcosms by the end of the experiment (fig. 32). Alternatively, other factors such as toxicity or substrate type could have caused the decline in CO_2-utilizing methanogens. Although the cause is unclear, it is clear that the non-acetate utilizing methanogens decreased in proportional abundance in the WB30 microcosms, whereas proportional abundance of acetate-utilizers was correlated with both the CH_4 production and the VC degradation.

The apparent link between VC degradation and acetotrophic methanogens observed in these microcosm experiments may support the theory that VC mineralization under anaerobic conditions begins with oxidation by acetogens and that acetotrophic methanogens enhance degradation, possibly by converting the acetate to CO_2 and CH_4 (Bradley and Chapelle, 2000) or by excretion of some biomolecule that stimulates degradation (Novak and others, 1998). Although the experiments reported here clearly show involvement of acetotrophic methanogens, involvement of acetogens is purely speculative. Evidence for the role of acetogens possibly could be obtained by conducting TRFLP analyses of archived microcosm samples with acetogen primers. Another possibility is that dehalorespiring bacteria (bacteria that can utilize VC as a terminal electron acceptor for

energy) actually degrade VC in the WB30 microcosm and subsequently cause low hydrogen levels compared to the WB23 microcosms. Hydrogen is a favored substrate of some dehalorespiring bacteria identified to date, and Fennel and Gossett (1998) have shown that low hydrogen levels driven by dechlorination reactions in a dehalorespiring bacteria culture favor acetotrophic methanogens over CO_2-utilizing methanogens. Molecular analyses of WB30 sediment from the March–April 1999 microcosms revealed the presence of the dehalorespiring bacteria *Dehalococcoides ethenogenes* and *Desulfuromonas* throughout the experiment, whereas they were not detected in the WB23 microcosm sediment. VC/sediment enrichment experiments, however, showed similar rapid VC degradation rates in WB23 and WB30 sediment after prolonged incubation under methanogenic conditions, indicating that the presence or absence of dehalorespiring bacteria was not a critical factor in the occurrence or rate of VC degradation in these wetland sediments (fig. 18). In addition, ethene is the end product of reductive dechlorination by *Dehalococcoides ethenogenes* (Maymó-Gatell and others, 1997, 1999), and no ethene was detected in these microcosm experiments (although it had been detected in non-methanogenic enrichment cultures, as described in preceding sections). Possibly, ethene was not detected because it was degraded faster than VC, as discussed in the preceding section on Spatial Variability of Vinyl Chloride Degradation.

In any case, the activity of dehalorespiring bacteria would not discount a role of the acetotrophic methanogens in VC degradation. Maymó-Gatell and others (1995) concluded that *Dehalococcoides* requires growth factors such as vitamin B_{12} from other anaerobic bacteria or methanogens to sustain dechlorination of VC to ethene. In other words, VC utilization by *Dehalococcoides ethenogenes* can be considered cometabolic (Maymó-Gatell and others, 2001). It is possible that VC can be degraded by more than one reaction pathway in the wetland sediments, and the dominant pathway could change with changing redox conditions, substrate type, or other factors that affect the microbial community.

Seasonal Variability—The low TeCA degradation rates in the July–August 1999 microcosms (fig. 5) seem to be related to low microbial biomass. The microbial community analyses consistently showed that bacterial biomass was lower in the July–August 1999 microcosm sediment samples than in the March–April 1999 microcosms (figs. 26 and 27). The relative intensity of the peaks in the TRFLP profiles is related to the biomass present for each fragment length. The relative intensities were markedly lower in July–August 1999 microcosms than in March–April 1999, especially in the bp size range of about 160 to 240 (figs. 26 and 27). Sediment samples collected from sites WB23 and WB30 and analyzed for microbial communities prior to microcosm preparation and incubation also show a lower intensity of peaks in the summer, indicating that the low microbial biomass in the July–August 1999 microcosms did not result from sediment manipulation (fig. 35). The methanogen biomass also was lower in the July–August 1999 microcosms, which is consistent with the low methane production in these microcosms (figs. 13c–d). This low biomass was contrary to the expected high microbial biomass and activity with high summertime temperatures. The October–November 2000 microcosm sediment samples, which had removal rates of TeCA and daughter products that were higher than those in July–August 1999 and lower than those in March–April 1999, had biomass levels that similarly were intermediate of those in March–April and July–August 1999. The low microbial biomass observed using the TRFLP profiles, which measures the amount of DNA amplifiable by PCR from 1 g (gram) of sediment, was confirmed on selected sediment samples using direct cell counts (data on file at USGS, Reston, Virginia). Epifluorescent cell counts are difficult in sediment samples because of the natural fluorescence of the soil material. Although biomass estimates based on the concentration of PCR-amplifiable DNA are only semi-quantitative, these estimates probably are more sensitive measures of biomass than cell counts from soil matrices.

Although the low microbial biomass and TeCA degradation rates seem to be a summertime occurrence, it is unknown if this is a recurrent phenomenon, or if the effect occurs at depths greater than the 0 to 25 cm from which surficial wetland sediments were collected for this study. Possibly, the drought conditions that occurred in the summer of 1999 caused unusual stress on the microbial communities in July–August 1999, resulting in unusually low degradation rates in the summer 1999 microcosms. No drought effects, however, were obvious in the wetland. Wetland sediments remained saturated close to or at land surface at sites WB23 and WB30 throughout this study, and diurnal tidal flooding occurred as usual at these sites. A possible recurrent event that could cause low microbial biomass in the summer is the growth of vegetation beginning in May and continuing through the summer in the wetland. *Phragmites* (common reed) shows a marked increase in growth by July, commonly reaching heights of 3 to 5 m in the wetland area. Competition from this macrophyte growth could cause a decreased availability of the nutrients that are required for microbial growth.

One effect of the low microbial biomass in the surficial wetland sediments in the summer was the doubling in percentage of TCE produced by abiotic TeCA degradation in July–August 1999 compared to March–April 1999 microcosms (table 4). The TCE production in the July–August 1999 microcosms was equally high (about 7 percent) in the microcosms that were incubated at 5 °C, where low microbial activity was expected. The October–November 2000 TeCA-amended microcosms that had intermediate biomass conditions also had high TCE production (8 to 11 percent; fig. 11b). During experiments with anaerobic municipal digester sludge, Chen and others (1996) found that concentrations of this abiotic daughter product increased under low biomass conditions. In experiments with digester sludge, TCE comprised about 9 to 16 percent of the initial TeCA added in four tests with sludge-seeded cultures that had high biomass and about 49 percent in diluted cultures with low

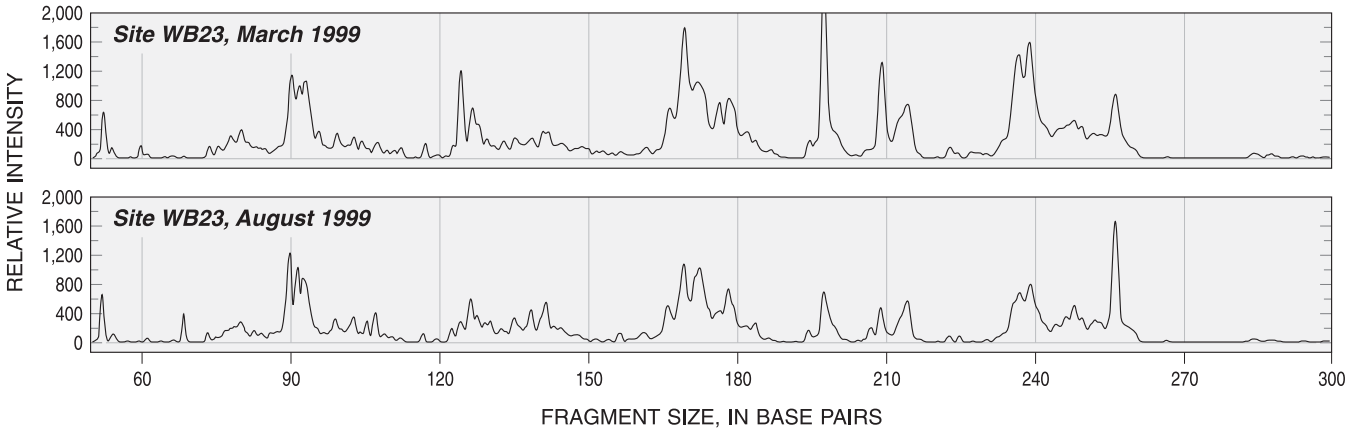

Figure 35. Bacteria terminal-restriction fragment length polymorphism profiles in sediment samples from site WB23 prior to microcosm preparation and incubation, March and August 1999.

biomass (Chen and others, 1996). The higher TCE production observed in these low biomass sludge-seeded cultures than in the low biomass wetland sediment microcosms could be caused partly by the degree of dilution of the biomass and partly by the initial TeCA concentration. The initial TeCA concentrations that were used by Chen and others (1996) ranged from about 60 to 100 µmol/L, compared to the maximum of 13 µmol/L used in the microcosms presented in this report (table 1). It is possible that abiotic dehydrochlorination becomes more significant with extremely high TeCA concentrations, although a substantial difference in the percentage of TCE produced was not observed over the moderate concentration range used in the wetland sediment microcosms (fig. 19a).

Seasonal changes in the predominant microbial species or group involved in VC production were indicated by the lack of association between methane and VC production in all July–August 1999 microcosms. In addition, VC concentrations increased by days 6 and 21, respectively, in the WB30 and WB23 microcosms in July–August 1999 (fig. 8). The largest increase in VC in the WB23 microcosms in July–August 1999 occurred between days 25 and 30, whereas it was the WB30 microcosms that showed this delay in VC production in March–April 1999 (figs. 7–8). The 198 bp bacteria peak that seemed linked to VC production from 112TCA dichloroelimination in the March–April 1999 microcosms was prominent in both WB23 and WB30 microcosms from the beginning of incubation in July–August 1999 (fig. 26). These bacteria, therefore, do not seem to be associated with VC production in the July–August 1999 microcosms. Comparing the times of onset of VC production and the increase in intensity of bacteria peaks for the July–August 1999 experiment, peaks at 86 bp and 170 bp seem to show the best link to VC production for both the WB23 and WB30 microcosms (fig. 26). Increases in intensity of these peaks also coincided with VC production in the TeCA- and 112TCA-amended microcosms in October–November 2000 (fig. 28). Although the 198 bp peak also was evident in the October–November 2000 microcosms, its intensity was lower than the 86 and 170 bp peaks and decreased over the incubation time (fig. 28). Note that the 170 bp peak, along with the 198 bp peak, was present in the killed controls that showed 12DCE production from TeCA dichloroelimination (fig. 25). The same bacterial species or groups, therefore, may be involved in both TeCA and 112TCA dichloroelimination reactions. The CO_2-utilizing methanogens that were associated with VC production from 12DCE hydrogenolysis in the March–April 1999 microcosms also were observed in the July–August 1999 microcosms, indicating little seasonal change in this reaction (figs. 31 and 36).

Although the microorganisms involved in VC production may change seasonally, the wetland sediment from site WB30 consistently showed greater VC production than sediment from site WB23 (figs. 11 and 12). The involvement of Methanosarcinaceae in VC degradation and the greater percentage of VC degradation observed with WB30 sediment also seemed to be consistent seasonally on the basis of the March–April 1999 and October–November 2000 data (figs. 32 and 33). Although data on the proportion of Methanosarcinaceae are not available for the July–August 1999 microcosms, substantial VC degradation was observed in the WB30 microcosms within the 35-day incubation period, whereas none was observed in the WB23 microcosms (fig. 8). The presence of *Dehalococcoides* and *Desulfuromonas* dehalorespiring bacteria in WB30 sediment samples, as discussed in the preceding section on Spatial Variability, could be at least partly responsible for the apparently greater capacity of these sediments to produce and degrade VC. Because methanogens compete for the supply of H_2 as a substrate (Maymó-Gatell and others, 1995; Smatlak and others, 1996), these dehalorespiring bacteria may be more active, or

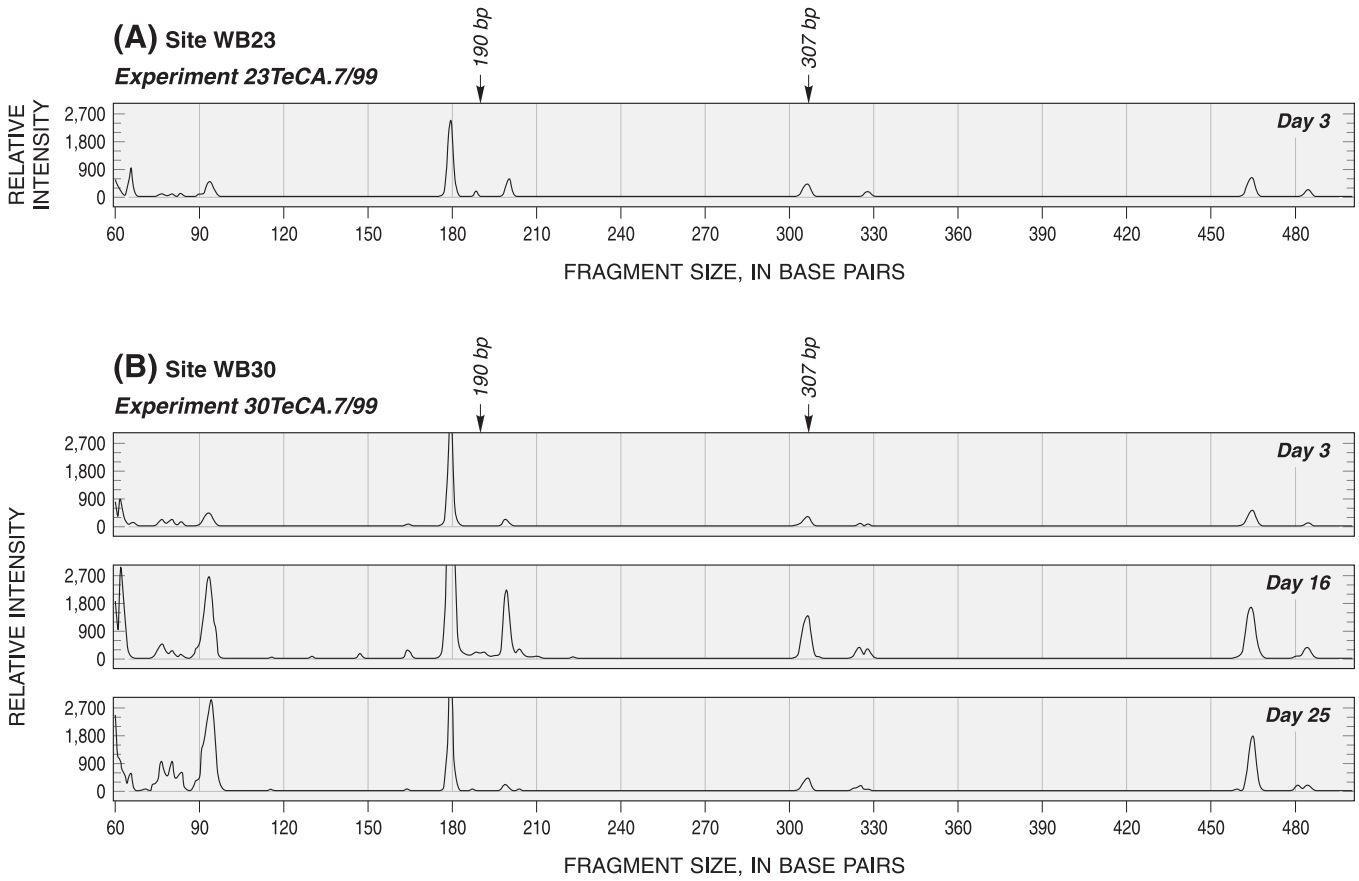

Figure 36. Methanogen terminal-restriction fragment length polymorphism profiles in 1,1,2,2-tetrachloroethane- (TeCA-) amended microcosms constructed with sediment from sites WB23 and WB30, July-August 1999: **(A)** Site WB23 [experiment 23TeCA.7/99], and **(B)** site WB30 [experiment 30TeCA.7/99].

more noticeably active, in the microcosms constructed in the summer when methanogen activity and biomass were lower (figs. 13). The presence of *Dehalococcoides ethenogenes*, which are known to obtain energy from 12DCE hydrogenolysis to VC, may explain why VC production unrelated to methane production was observed early in the WB30 microcosms in July–August 1999. *Dehalococcoides ethenogenes* also is known to utilize 12DCA reductive dechlorination for growth (Maymó-Gatell and others, 1997, 1999). The presence of *Dehalococcoides ethenogenes* and its potentially greater activity in the summer also could explain the undetectable concentrations of 12DCA throughout the July–August 1999 microcosm with WB30 sediment (fig. 8b). 12DCA was observed in WB30 microcosms in February–April 1998, March–April 1999, and October–November 2000 (figs. 11 and 19). Despite the presence of the dehalorespiring bacteria at site WB30, TeCA degradation rates were lowest in the July–August 1999 microcosms, although the July–August 1999 rates were higher in the WB30 microcosms than the WB23 microcosms (fig. 5).

Substrate Type—Both VC production and VC degradation showed some variability with the predominant available substrate in the laboratory experiments. VC production from 12DCE was substantially higher with addition of hydrogen as a substrate than with acetate in non-methanogenic enrichment cultures (fig. 16). In addition, enrichments that were amended with cDCE and hydrogen produced ethene in addition to VC, whereas enrichments that were amended with cDCE and acetate produced only VC (data on file at USGS, Reston, Virginia). These differences with substrate type are consistent with previous studies of two separate groups of dehalorespiring bacteria. One group, *Dehalococcoides*, utilized hydrogen as the favored substrate for reductive dechlorination of cDCE to VC and ethene (Maymó-Gatell and others, 1997, 1999), whereas a second group, *Desulfuromonas*, utilized acetate as a substrate to produce VC as the most reduced end product of reductive dechlorination (Löffler and others, 2000). VC production in the hydrogen-amended enrichments was about three times higher than the acetate-amended enrichments (fig. 16), indicating that dehalorespiring bacteria such as *Dehalococcoides* that prefer

hydrogen as a substrate were dominant in the wetland sediment.

The microcosm experiments with added MeOH showed a substantial increase in VC degradation and an associated increase in the percentage of Methanosarcinaceae compared to microcosms without added MeOH (figs. 17 and 33). Methanol can be utilized directly as a substrate by Methanosarcinaceae, and MeOH also favors growth of acetogens. Because acetogens produce acetate that can be metabolized by Methanosarcinaceae, their growth on MeOH can stimulate growth of Methanosarcinaceae compared to CO_2-utilizers (Maymó-Gatell and others, 1995). This increase occurred for both WB23 and WB30 sediments, indicating that substrate type could be an important factor in determining the methanogen type that is predominant. The enhanced growth of acetotrophic methanogens could be another factor causing the higher TeCA degradation rates in the MeOH-amended microcosms (fig. 5).

Comparisons to Field Data on Anaerobic Degradation of 1,1,2,2-Tetrachloroethane and Association with Microbial Communities

Degradation Rate of 1,1,2,2-Tetrachloroethane

Using normalized concentrations from 1995 piezometer samples, estimated rate constants for TeCA degradation in the wetland porewater ranged from 0.0065 to 0.0085 per day at a flow velocity of 0.91 m/yr (table 5). These field-derived degradation rates correspond to the estimated minimum and maximum flow velocities, respectively, estimated for the wetland sediments using two different methods. Estimated half-lives for TeCA based on the field data range from 82 to 107 days (table 5). These rate estimates were based on summer 1995 data from four piezometer nests where three or more samples from piezometers screened in the wetland sediment had detectable concentrations of TeCA, including sites WB23 and WB30 that were used for sediment collection for the laboratory microcosm experiments (table 5; fig. 1). Late winter/spring sampling periods generally had lower VOC concentrations in the wetland porewater, and insufficient data were available to calculate degradation rates (Lorah and others, 1997; Olsen and others, 1997; Spencer and others, 2002). These field observations of low porewater VOC concentrations in the winter/early spring are consistent with the higher microbial biomass and degradation rates observed in the March–April 1999 microcosms compared to the July–August 1999 microcosms.

The rate constants for TeCA degradation are slightly lower than TCE degradation rates estimated previously using 1995 data from the same sites (Lorah and others, 2001) (table 5). Because TCE can be an abiotic daughter product of TeCA, the field-derived rate constants for TCE degradation could be underestimated. This effect probably is small, however, because TCE accounted for only 3 to 11 percent of the TeCA degraded in laboratory microcosm experiments with the wetland sediment (table 4; figs. 11 and 19). Therefore, TCE concentrations used for calculating field degradation rates are predominantly from TCE present as a result of disposal activities rather than as a result of TeCA degradation.

The rate constants estimated based on the 1995 field data (table 5) agree well with the rate constants estimated from anaerobic laboratory microcosms that were amended with TCE (Lorah and others, 1997, 2001) and TeCA (fig. 5), although laboratory-derived rates were consistently higher. The laboratory-derived TCE rate constants ranged from 0.032 to 0.37 per day (half-life of 2 to 22 days) in the laboratory experiments compared to field estimates of 0.009 to 0.012 per day (half-life of 58 to 77 days). The best-fit line for TeCA degradation in the microcosms that did not contain MeOH gave a rate constant of 0.053 per day (half-life of 13 days) (fig. 5) compared to estimated half-lives of 82 to 107 days from the field data (table 5). The agreement between the laboratory- and field-derived rate constants can be considered good considering the inherent differences in a laboratory batch experiment and an uncontrolled field system. In addition, the low summer degradation rates were excluded from the regression line to calculate the laboratory-derived rate constant (fig. 5), but summer data were used to estimate field-derived degradation rate constants. The laboratory-derived rate constant, therefore, that was used for comparison to the field-derived rates was a maximum rate, whereas the field-derived rate constants in table 5 may represent minimum rates.

The laboratory- and field-derived estimates show similar TeCA degradation rates in the wetland sediments at sites WB23 and WB30 (table 5 and fig. 5). Rate constants for TeCA and TCE degradation also were estimated for site WB30 using porewater samples collected from a multi-level sampler that was added in 1999 as part of the ESTCP project (fig. 1 and table 5). Although a multi-level sampler also was installed at site WB23, chloride data were insufficient to correct TeCA and TCE concentrations and calculate degradation rate constants. Data collected from the multi-level sampler at WB30 in June–July 2000 allowed rate constants to be estimated using concentrations from twice as many depths in the wetland sediment as the two to three depths available from the drive-point piezometers in 1995 (table 5; figs. 3 and 37). For site WB30, the TeCA and TCE rate constants estimated from the piezometer and multi-level samplers generally are consistent, especially for TeCA with half-lives of 107 and 91 days using a flow velocity of 0.9 m/yr (table 5). This comparison of summer 1995 and summer 2000 data indicates that degradation rates are consistent over time, at least when comparing samples collected during the same season, as was observed with the TeCA-amended microcosms conducted in May–June 1996 and March–April 1999 with sediment collected from site WB30 (figs. 4 and 7b).

Table 5. *Degradation rates for 1,1,2,2-tetrachloroethane (TeCA) and trichloroethene (TCE) estimated from field data in July–August 1995 and June–July 2000 along upward flowpaths in the wetland sediments, using TeCA and TCE concentrations normalized with chloride and an estimated linear flow velocity of 0.9 meters per year*

[TCE degradation rates in 1995 with a linear flow velocity of 0.9 meters per year (m/yr) were reported previously in Lorah and others (2001); r^2, correlation coefficient]

		TeCA Degradation Rate			TCE Degradation Rate		
Site	Flowpath [A]	Rate constant (day^{-1})	Half-life (days)	r^2	Rate constant (day^{-1})	Half-life (days)	r^2
July–August 1995 Piezometers							
WB23	WB23D to -23B	0.0071 ± .0005	98	0.99	0.012	58	1.00
WB26	WB26F to -26C	0.0067 ± .0017	103	.94	0.011 ± .007	63	.68
WB27	WB27D to -27A	0.0085 ± .0007	82	.99	0.009 ± .0009	77	.94
WB30	WB30E to -30C	0.0065 ± .0040	107	.69	0.011 ± .002	63	.99
June–July 2000 Multi-level Sampler							
WBM30	WBM30E to -30A	0.0076 ± .0003	91	.99	0.0065 ± .0008	107	.97

[A] Sequence of piezometers used to calculate rates at each site. See figures 1 and 3.

Daughter Compound Production and Degradation

Laboratory microcosm experiments can assist in understanding the observed distributions of chlorinated VOCs in the wetland porewater. It should be noted that microcosms were constructed using sediment from relatively shallow depths in the wetland, and the microbial communities and biodegradation processes could vary with depth. Changes in redox conditions in the microcosms, however, generally reflected the observed changes in porewater chemistry along an upward flow gradient in the wetland sediment (figs. 13 and 38). Along upward flowpaths, concentrations of the reduced form of dissolved iron (ferrous iron) generally reached a peak before methane did (fig. 38). Methane concentrations then peaked as ferrous iron concentrations decreased or remained about the same (fig. 38). Similarly, an early increase in iron concentrations in the microcosms resulted in higher dissolved iron than methane concentrations, whereas methane concentrations were higher at the end of the experiment (fig. 13). Thus, the changes in the microcosms over incubation time may be representative of redox conditions and biodegradation along a flowpath. Distributions of TeCA and its daughter compounds in wetland porewater along vertically upward flowpaths are shown at sites WB23 and WB30 (fig. 39). These distributions are shown as percentages of the initial TeCA present in the aquifer immediately underlying the wetland sediment at each site. The aquifer concentrations were obtained from multi-level sampler points WBM23G and WBM30G, screened at a depth of 3.9 m (figs. 1 and 3). Although TCE can be an abiotic daughter product of TeCA, TCE also was used and disposed of as a waste in the study area (Lorah and others, 1997). The majority of the TCE in the wetland porewater probably is the result of its disposal as a waste product because the microcosms indicated that only 3 to 11 percent TCE resulted from TeCA degradation in these sediments (table 4 and fig. 11b).

The most obvious difference in the distributions of daughter compounds in the wetland sediments at sites WB23 and WB30 is the higher percentage of VC at all depths at site WB30 (fig. 39). When calculated relative to the amount of TeCA present in the aquifer, the TCE concentrations were about 30 percent higher at site WB30 than at site WB23 (fig. 39). The higher percent TCE at site WB30, however, does not account for the difference in VC, which was as high as 55 percent. The distribution of daughter products observed in the field, therefore, is consistent with the pattern

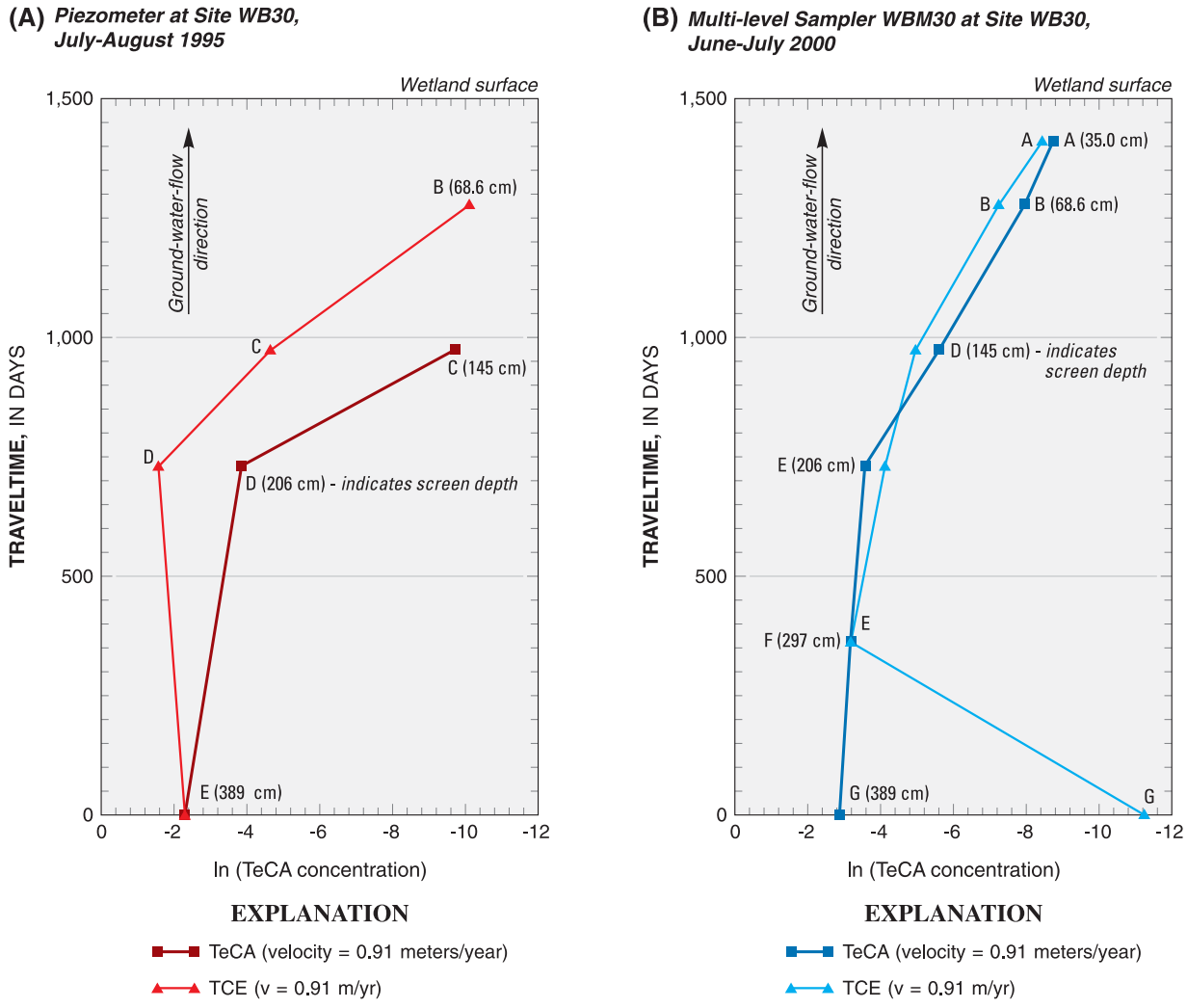

Figure 37. Natural log of 1,1,2,2-tetrachloroethane (TeCA) concentrations [ln(C)] (normalized by chloride concentrations) and traveltime in wetland porewater **(A)** from piezometer site WB30 in July-August 1995 and **(B)** from a multi-level sampler (WBM30) at the same site in June-July 2000. *[Traveltime calculated using linear flow velocity (v) of 0.91 meters per year.]*

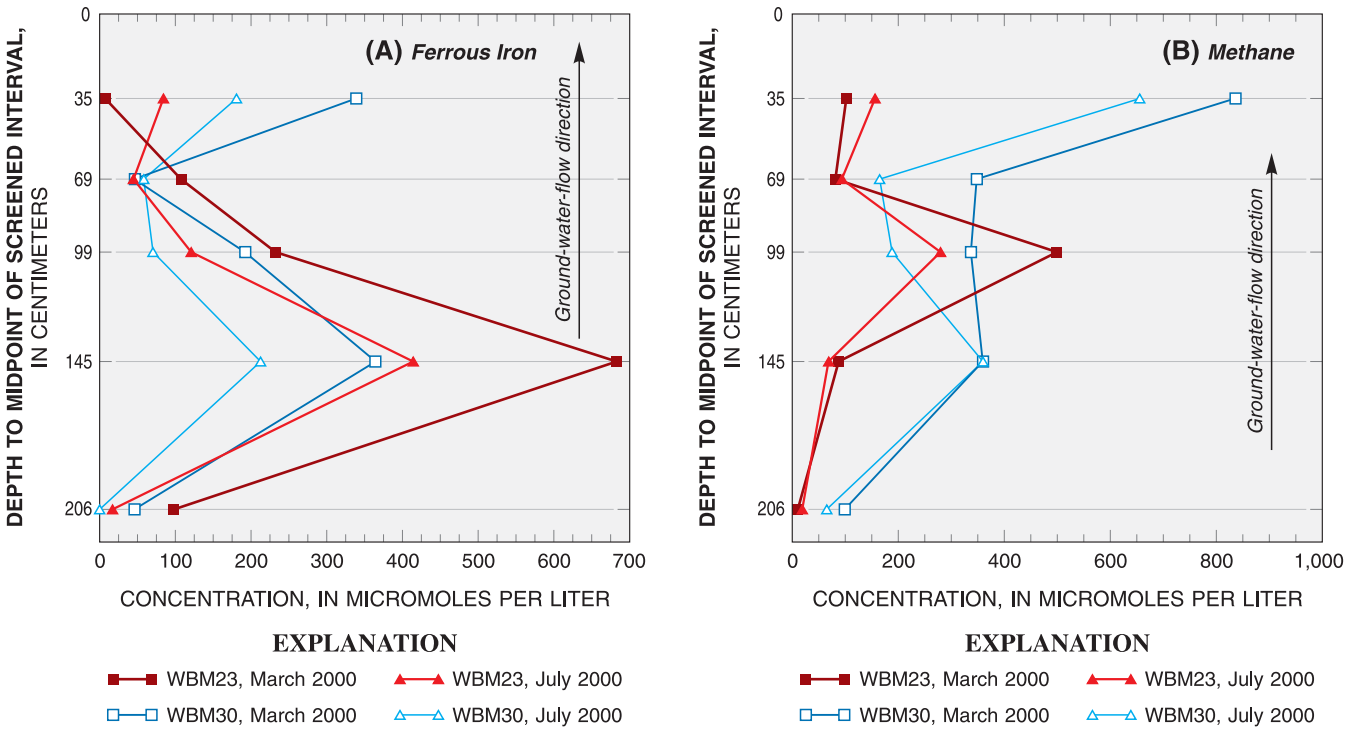

Figure 38. Concentrations of (**A**) ferrous iron and (**B**) methane in wetland porewater collected from multi-level samplers (WBM23 and WBM30) at sites WB23 and WB30, June-July 2000.

of higher VC production observed in the microcosms constructed with sediment from site WB30. In addition, VC decreased from over 55 percent to less than 5 percent near the surface of the wetland sediments at site WB30 (WBM30C to WB30B in fig. 39), which is consistent with the greater capacity for anaerobic degradation of VC that was observed in the WB30 microcosms (figs. 17 and 18). In contrast, the percentage of VC remained constant along the upward flowpath at site WB23, although the percentage of TeCA removed before a depth of 46 to 69 cm was reached was about the same at the two sites (fig. 39). The amount of TeCA lost along the flowpath at site WB23 was not accounted for by an equivalent increase in daughter products (fig. 39). If a higher percentage of 12DCA was produced at this site from TeCA degradation as observed in the microcosms, the 12DCA was degraded as rapidly as it was produced. Overall, field measurements indicated a lower prevalence of 12DCA in the wetland porewater than of 12DCE or VC (Lorah and others, 1997). Molecular analyses of the microbial communities with depth in the wetland sediment at these sites would provide a better understanding of the spatial heterogeneity in the biodegradation processes and of the correlation between laboratory and field results.

Microbial Communities

All the bacterial peaks that were identified from the microcosm analyses as potentially involved in the degradation pathways of TeCA were present in the surficial wetland sediment samples collected from the contaminated sites along transects A-A′ and C-C′ (fig. 40) and at the background site WB19 (fig. 41). These include bacterial species or groups at base pair sizes 86, 90, 170, and 198 (figs. 40 and 41). Bacterial diversity and biomass were substantially higher in transect A-A′ compared to transect C-C′ in March and August 1999 (fig. 40). Concentrations of chlorinated VOCs in shallow wetland porewater were lower in transect A-A′ compared to transect C-C′, whereas methane concentrations were higher in transect A-A′ (Lorah and others, 1997). Microcosm experiments showed that higher microbial biomass and higher methanogen activity were associated with higher TeCA and daughter compound degradation rates in the wetland sediments, indicating that the lower VOC concentrations along transect A-A′ could be at least partly due to higher degradation rates. Hydrogeologic factors, including the thinner wetland sediments and the occurrence of a focused discharge area that cause lower residence time of the contaminants, likely contribute to the higher VOC concentrations along transect C-C′ (Lorah and others, 1997; Lorah and Olsen, 1999b). It is possible that toxic effects of the generally higher contaminant concentrations along transect C-C′ than transect A-A′ reduced the biomass and diversity of the microbial community in this area of the wetland (fig. 40).

The frequency and depth of tidal flooding on the wetland sediments also could affect the microbial diversity and biomass along the transects. Restriction of oxygen influx and other physiochemical effects on the sediment with more

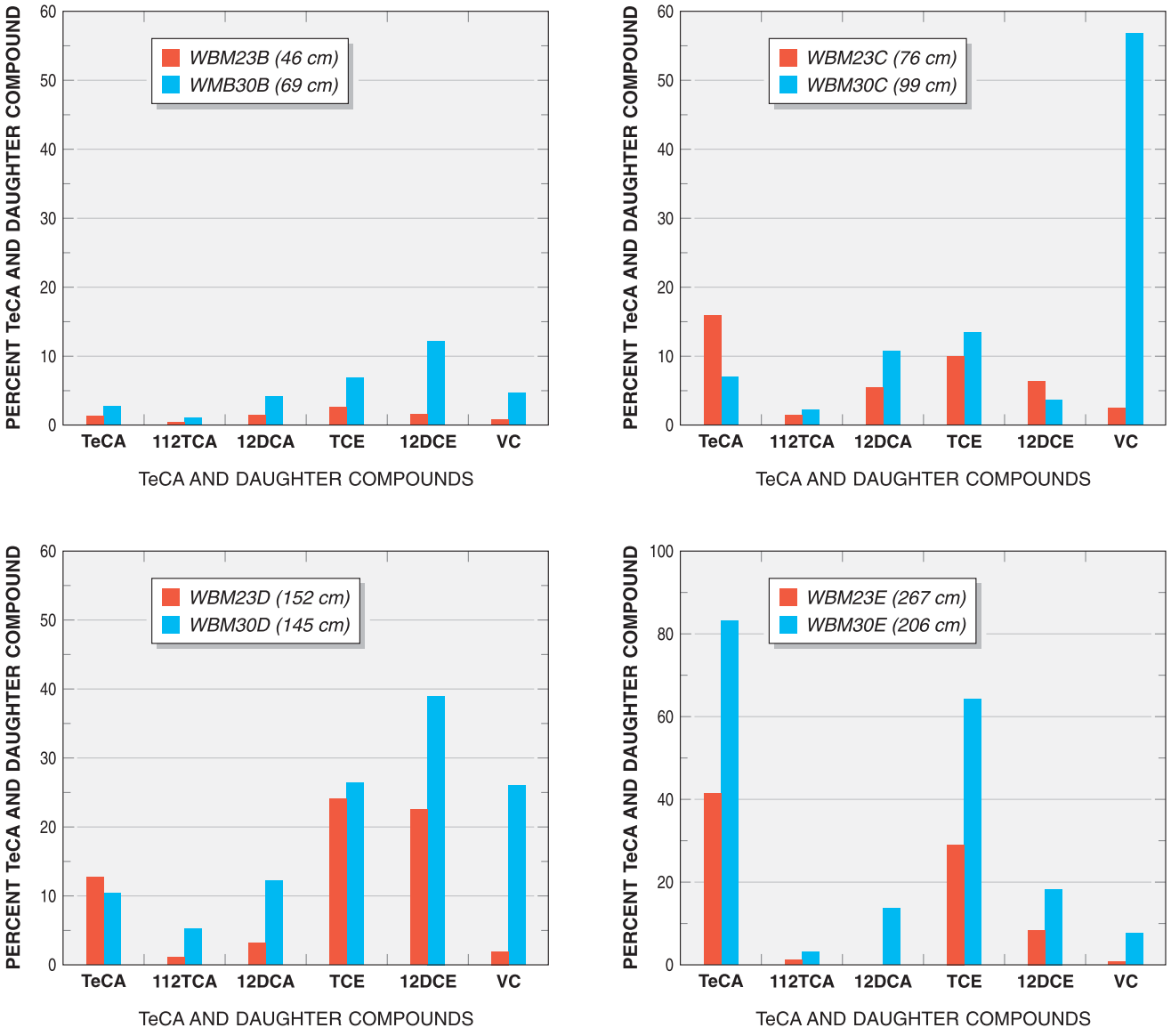

Figure 39. Distributions of 1,1,2,2-tetrachloroethane (TeCA) and daughter compounds in wetland porewater collected from multi-level samplers (WBM23 and WBM30) at sites WB23 and WB30, June-July 2000. *(Note that TCE is a parent compound from disposal sources in the field. Distributions were calculated as percent of the initial TeCA in the aquifer in multi-level samples WBM23G and WBM30G, screened at a depth of 390 cm.)*

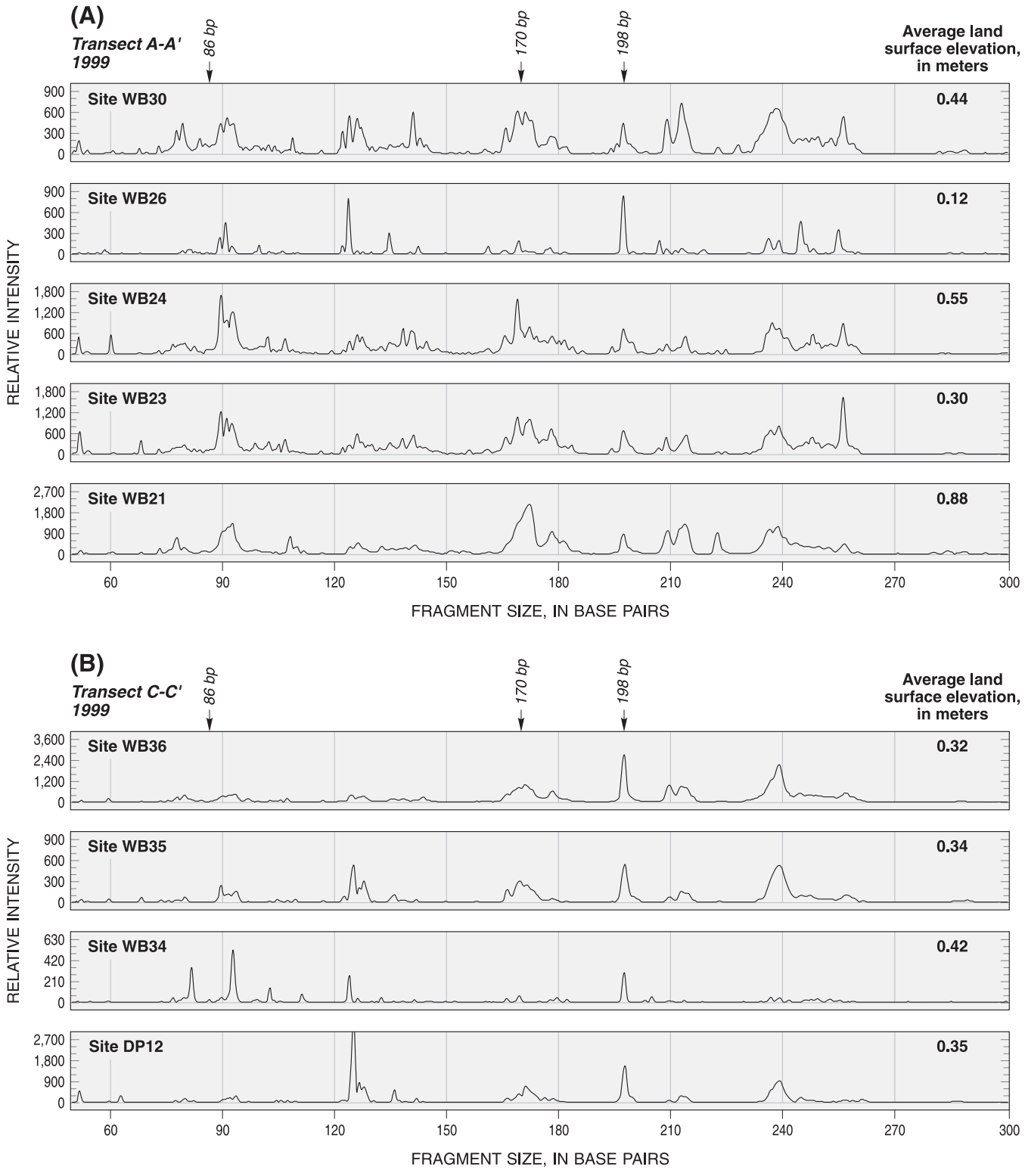

Figure 40. Bacteria terminal-restriction fragment length polymorphism profiles in the surficial wetland sediment along **(A)** transect A-A' and **(B)** transect C-C', 1999.

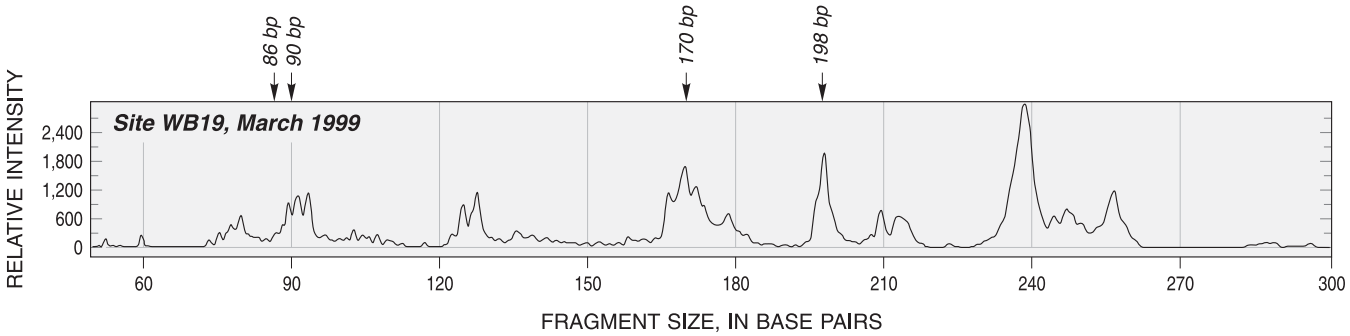

Figure 41. Bacteria terminal-restriction fragment length polymorphism profile in surficial wetland sediment at background site WB19, March 1999.

frequent and higher flooding levels could decrease microbial biomass and diversity. In addition, relatively low concentrations of VOCs (maximum of 50.2 µg/L) that could have a toxic effect on the microbial community were measured in the surface water (Phelan and others, 2001). Chloroform, the most common surface-water contaminant at this site, has been shown to be highly toxic to methanogenic and dehalorespiring microorganisms (Scholten and others, 2000; Weathers and Parkin, 2000; Maymó-Gatell and others, 2001). Except for site WB26, the land-surface elevation of the wetland was higher along transect A-A′ than transect C-C′ (Olsen and others, 1997; Spencer and others, 2000), which would result in less frequent and lower tidal inundation along transect A-A′ (fig. 40). Field observations have shown that transect C-C′ is more consistently flooded at all piezometer sites in the wetland. Site WB26, which is on the bank of the creek channel and has the lowest land-surface elevation, was the only site along transect A-A′ that showed bacterial biomass and diversity as low as those measured along transect C-C′ (fig. 40).

The proportion of Methanosarcinaceae, the group that includes all acetate-utilizing methanogens, was between 10.8 and 14.9 percent of the total methanogens in surficial wetland sediment samples collected along transect A-A′ in March 1999 (table 6). Increases in the proportion of Methanosarcinaceae in microcosms constructed with WB30 sediment compared to those constructed with WB23 sediment were linked to greater VC degradation. As observed in the microcosm sediments, the percent of Methanosarcinaceae was higher in the surficial wetland sediment samples from site WB30 than site WB23 (table 6). The percent Methanosarcinaceae was similar at site WB30 and the background site WB19, indicating that the relative proportion of these methanogens is not related to the presence of the contaminants. Although data are available for only two sites along transect C-C′, it is interesting to note that the lowest percent Methanosarcinaceae measured for both transects occurred at one of these sites (table 6). Site WB36 had 6.1 percent Methanosarcinaceae, compared to 10.8 to 14.9 percent in other samples from transects A-A′ and C-C′ (table 6). It is possible that the generally higher concentrations of daughter products, especially VC, observed in the wetland porewater along transect C-C′ compared to transect A-A′ (Lorah and others, 1997; Olsen and others, 1997; Spencer and others, 2002) could be due in part to the lower proportion of Methanosarcinaceae in the methanogen community. Additional molecular analyses of sediment samples along transect C-C′ would be needed to confirm this association, however.

Table 6. *Abundance of Methanosarcinaceae as a percentage of the total methanogen community in surface grab samples of wetland sediment, March 1999*

[Transect and site locations are shown in fig. 1]

Transect	Site number	Percent Methanosarcinaceae
Background site	WB19	14.1
A-A′	WB23	10.8
A-A′	WB24	11.5
A-A′	WB30	14.9
C-C′	WB35	13.2
C-C′	WB36	6.1

Implications for Natural Attenuation and Remediation

Understanding TeCA and TCE degradation pathways is necessary in determining whether natural attenuation, or *in situ* bioremediation, of the chlorinated VOCs discharging to the wetlands sediments is a feasible ground-water remediation method. The rapid TeCA degradation rates in wetland sediments observed in this study indicate that natural attenu-

ation is a feasible remediation method in the anaerobic wetland sediments. The general consistency between TeCA and TCE degradation rates calculated from laboratory microcosm experiments and from field data indicates that the rapid anaerobic degradation rates measured in the laboratory are reasonable. The first-order rate constant of 0.053 per day (half-life of 13 days) estimated for the TeCA-amended microcosms without MeOH is high compared to biodegradation rates reported in the literature for chlorinated solvents in sand aquifers. Although no other TeCA degradation rates from field measurements are reported in the literature, degradation rates are available for TCE under a range of conditions. For example, anaerobic TCE biodegradation in microcosms constructed with sandy aquifer sediments at Picatinny Arsenal, New Jersey, and at a Superfund site at St. Joseph, Michigan, ranged from 0.0001 to 0.003 per day, which corresponds to half-lives of 230 to 6,930 days (Rifai and others, 1995). Because of the commonly slow degradation of chlorinated solvents in sand aquifers, Wiedemeier and others (1998) recommend that batch microcosm studies should last from 12 to 18 months to evaluate rates for natural attenuation studies, compared to the 1 to 1.5 months used in this study with wetland sediments. The high degradation rates in the wetland sediments reported from the initial study at this APG wetland site led to the construction or plans for construction of wetlands at several sites for the purpose of remediation of chlorinated VOCs (Richard and others, 2002; Kassenga and Pardue, 2002; Lorah and others, 2002).

Although both laboratory and field data showed that degradation rates are slower in the wetland sediments in the summer compared to late winter/early spring and fall, TCE and TeCA degradation rates that were calculated using wetland porewater samples collected in the summer gave half-lives in the range of 60 to 100 days, which still is considerably faster degradation than reported for TCE in sand aquifers (Rifai and others, 1995). The observation of lower summer degradation rates is contrary to the previously hypothesized lower degradation rates in the winter because of the colder temperatures (Lorah and others, 1997). Field measurements of temperature and VOC concentrations with depth in the wetland sediment indicate that winter temperature decreases are substantial only in the upper 0.15 m, whereas concentrations of TeCA and its anaerobic daughter compounds have decreased to below or near detection levels before this shallow depth is reached (Lorah and others, 2000). Temperature, therefore, apparently is not a major factor affecting degradation rates in these wetland sediments. Based on field measurements, the higher concentrations of daughter VOCs in the summer (without increase in breakthrough of parent VOCs to the wetland porewater) is due to a higher mass flux of parent VOCs from the aquifer to the wetland sediments, which occurs as a delayed effect of high spring-time recharge to the aquifer upgradient of the wetland (Lorah and others, 2000). The seasonal laboratory microcosm experiments presented earlier in this report indicate that lower biodegradation rates, caused by a lower microbial biomass, also could be a factor in the relatively high concentrations of VOCs in the wetland porewater in the summer. Because the microcosm experiments and microbial community analyses were conducted using surficial wetland sediments (from the upper 25 cm), it is unknown whether the lower biodegradation rates also occur at greater depths. However, the relatively slow degradation rates, lower microbial biomass, and higher daughter compound concentrations observed in the summer compared to late winter/early spring and fall indicate that monitoring during the summer months would be especially crucial in long-term monitoring plans.

The microbial groups or species represented by the TRFLP peaks that were identified as potentially involved in degradation of TeCA and its daughter products in the microcosm experiments were present at all sites where wetland sediment samples were collected for molecular analyses (fig. 40). Although microbial biomass and diversity were substantially lower along transect C-C′ than transect A-A′, all of the identified microbial groups or species were present along transect C-C′. Field data along both transects have shown that natural attenuation is effective in reducing VOC concentrations to below detection levels before the wetland surface is reached (Lorah and others, 1997; Lorah and Olsen, 1999a, b). In addition, degradation of TeCA in microcosms constructed with sediment from the background site (WB19, fig. 23) occurred without a lag and at a similar rate as microcosms constructed with sediment from the two contaminated sites (fig. 5). Field data showed that the same identified TRFLP peaks were present at the uncontaminated and contaminated sites (figs. 40 and 41). The combined microcosm, field, and microbial analyses, therefore, indicate that the capacity to degrade TeCA is widespread in these wetland sediments and that similar degradation could be expected if contaminants discharged to a previously uncontaminated area of the wetland. In previous studies, the abundance of organic matter (and thus of substrates to support dechlorination) has been hypothesized to be a relatively ubiquitous characteristic of wetland sediments that could result in an ubiquitous ability of wetland sediments at APG and elsewhere to degrade chlorinated solvents like TeCA and TCE (Lorah and others, 1997; Lorah and Olsen, 1999a, b). However, TeCA and TCE degradation rates in wetland sediments from this site at APG were compared to freshwater wetland sediments in a forested swamp in the New Jersey Pine Barrens in a recent field and laboratory study (Lorah and others, 2002). In anaerobic laboratory microcosms amended with the same initial TeCA or TCE concentration, the TeCA or TCE degraded to below the detection level in less than 25 days in the APG wetland sediment, whereas degradation was negligible over a 35-day period in the New Jersey wetland sediment (Lorah and others, 2002). Although 12DCE was detected in field samples of the New Jersey wetland porewater, VC was not detected. TeCA and TCE degradation, therefore, were substantially lower and incomplete in the New Jersey wetland sediments, which contained a higher organic carbon content than the APG wetland sediments (Lorah and others, 2002). Differences in the microbial communities are the likely cause of the differences in degrada-

tion rates and completeness (Lorah and others, 2002). Although the abundance of organic substrates is an important factor in increasing the rates of degradation compared to sand aquifers, the occurrence of degradation is dependent on the presence of specific microorganisms. Similarly, addition of organic substrates and nutrients to an anaerobic sand aquifer at a test site at Dover Air Force Base, Delaware could not stimulate the indigenous microorganisms to degrade TCE beyond cDCE (Harkness and others, 1999; Ellis and others, 2000). TCE dechlorination beyond cDCE was achieved only after injection to the aquifer test site of a microbial enrichment culture that was originally started with sediment from another site where dechlorination to ethene did occur (Harkness and others, 1999; Ellis and others, 2000). Evaluation of microbial communities by the molecular techniques used in this study would be an effective method of evaluating the feasibility of natural attenuation at new wetland sites that are natural discharge areas for chlorinated solvents, and in designing constructed wetlands for remediation of these contaminants. Definitive identification of the important species/groups noted in this study and construction of gene probes would simplify use of these molecular techniques.

The feasibility of natural attenuation as a remediation method depends on the fate of 12DCE and VC, the toxic compounds that laboratory experiments and field measurements show are the predominant, persistent daughter products (figs. 7–9 and 39). Although substantial spatial variation in degradation rates of TeCA was not observed in the laboratory experiments or in field estimates, both laboratory and field data indicate that spatial variation in the production and degradation of VC does occur. The substantially higher production of VC at site WB30 compared to site WB23 could hinder the use of natural attenuation as a remediation method, if the conditions leading to higher VC production were widespread in the wetland. The fact that the same sediments that produced high VC also showed a greater capacity to degrade VC, however, alleviates this problem. Geochemical analyses of the microcosm experiments indicated that VC production from both 112TCA and 12DCE is linked to methanogenic activity, although microbial community analysis indicated that bacteria rather than methanogens were associated with the 112TCA degradation reaction. Microcosm experiments amended with BES and enrichment culture experiments also indicated that VC production from 112TCA and 12DCE could occur without methane production. However, because the enrichment cultures were inoculated with methanogenic microcosm slurry, the possibility that methanogens enhance degradation of these compounds without producing methane cannot be excluded. VC degradation seemed to be more strongly associated with methanogenesis than was VC production, as shown by the negligible VC degradation in the BES-amended microcosms and the rapid VC degradation in methanogenic VC/sediment enrichments compared to BES- and FeOOH-amended enrichments. The strong relation observed between VC degradation and relatively high abundance of acetotrophic methanogens suggests that complete degradation of TeCA requires methanogenesis. However, during periods of lower methanogenic activity, there was an increase in significance of other VC degradation pathways. This is evidenced by the fact that VC degradation occurred in the summer when low methanogenic activity was observed, although TeCA degradation rates were lower (figs. 5, 8, and 13). This non-methanogenic VC degradation most likely was through the action of dehalorespiring bacteria, such as the *Dehalococcoides* or *Desulfuromonas* species that were detected at this site using specific primers. Seasonal shifts in the microbial species or groups that seem to be involved in degradation also occurred for the production of VC from 112TCA dichloroelimination. Thus, the adaptable and diverse microbial community of the wetland sediments seems to provide compensation for changing spatial and seasonal conditions and allows for continued effective natural attenuation throughout the year.

The diversity of microorganisms involved in degradation of TeCA in these wetland sediments provides options to increase biodegradation rates in areas such as seeps, where high concentrations of VOCs (up to 50,000 µg/L) have been measured near the wetland surface (Phelan and others, 2001). Possible enhancements to increase biodegradation include "biostimulation," which involves adding substances such as nutrients to stimulate indigenous microorganisms present at a site, and "bioaugmentation," which involves adding microorganisms to a site that is lacking in one or more microbial species/groups needed for biodegradation. It is unclear from the laboratory and field data presented here whether the acetrotrophic methanogens are directly involved in VC degradation or are indirectly involved by enhancing the degradation capability of dehalorespiring bacteria or of acetogens, as Bradley and Chapelle (2000) suggested. If acetotrophic methanogens are indirectly involved, enhancing either their population or enhancing the bacteria that they are supporting could be a possible remediation option. Molecular analysis of the microbial communities in the VC-degrading enrichment experiments could provide definitive evidence of whether their role is direct or indirect. VC/sediment enrichment experiments showed that WB23 and WB30 wetland sediments had equally great capacities to degrade high concentrations of VC after pre-exposure to the contaminants and prolonged incubation under methanogenic conditions. The presence of microorganisms necessary for degradation, therefore, may be widespread in the wetland sediment, but populations may need to be enhanced in some cases. Enhancing the acetotrophic methanogen population in the wetland sediments where enhanced degradation rates might be needed could be more practical than enhancing the population of dehalorespiring bacteria.

Summary and Conclusions

Defining biodegradation rates and processes is a critical part of assessing the feasibility of monitored natural attenuation as a remediation method for ground water containing organic contaminants because biodegradation is commonly the primary destructive process for these contaminants. During 1998–2001, the U.S. Geological Survey conducted a microbial study at a freshwater tidal wetland at Aberdeen Proving Ground (APG), Maryland, where an ongoing investigation of natural attenuation of chlorinated volatile organic compounds (VOCs) in the wetland sediments began in 1992. In this microbial study, a combination of geochemical analyses and molecular biology techniques were used to investigate factors controlling the occurrence of the different anaerobic degradation pathways of 1,1,2,2-tetrachloroethane (TeCA) and to characterize the microbial communities that potentially are important in these degradation reactions. Laboratory microcosm and enrichment experiments were used to investigate the effects of spatial and seasonal variability, redox conditions, substrate type, and toxicity on the degradation reactions. Field measurements then were used to compare degradation rates, distributions of daughter products, and microbial community profiles to laboratory results and to discuss the implications on natural attenuation in the wetland. Geochemical analyses included measurements of VOCs and redox-sensitive constituents. Microbial analyses included terminal-restriction fragment length polymorphism (TRFLP) analysis of PCR (polymerase chain reaction)-amplified bacterial 16S rDNA and *mcr*A (methylcoreductase gene) to obtain profiles of the bacterial and methanogen communities, respectively. These genetic analyses commonly are referred to as "DNA fingerprinting." An intense microbial study to support the natural attenuation investigation at this site was warranted because of the expected complex pathways of TeCA degradation, the limited previous information available on degradation of this contaminant in ground water, and the expected large variability in biodegradation rates and processes in a dynamic, surficial wetland system. A better understanding of factors controlling production and degradation of toxic intermediate daughter compounds, particularly vinyl chloride (VC), was needed to evaluate the effectiveness of natural attenuation in the wetland sediments.

Anaerobic microcosm experiments conducted with wetland sediment collected from two different sites (WB23 and WB30) and during three different seasons (March–April 1999, July–August 1999, and October–November 2000) showed little spatial variability but high seasonal variability in TeCA degradation rates. Seasonal evaluations of degradation rates included microcosms incubated at 19 °C (degrees Celsius) during the three periods and a duplicate set of microcosms in March–April 1999 that was incubated at 5 °C. For the March–April 1999 and October–November 2000 microcosms incubated at 19 °C, initial first-order rate constants (generally calculated from TeCA loss in the first 12 days) ranged between 0.10 ± 0.01 and 0.16 ± 0.05 per day (half-lives of 4.3 to 6.9 days) for degradation of TeCA, whereas rate constants of 0 ± 0.03 and 0.06 ± 0.03 per day were obtained in the July–August 1999 microcosms. Microbial community profiles showed that low microbial biomass and microbial diversity in the summer could account for these unexpectedly low degradation rates. Although it is unknown if these low summertime rates are a recurrent event, competition for nutrients by growth of the *Phragmites* (common reed) that dominates wetland vegetation could reduce microbial biomass during the summer. Degradation of TeCA also was observed in the March–April 1999 microcosms incubated at 5 °C, indicating that biodegradation would continue during cold winter temperatures in the wetland sediment. About 50 percent of the initial TeCA in solution was converted to daughter products within the 35-day incubation period at 5 °C, whereas complete loss of the added TeCA occurred within 21 days in duplicate microcosms incubated at 19 °C.

Simultaneous production of 1,2-dichloroethene (12DCE) and 1,1,2-trichloroethane (112TCA) from dichloroelimination and hydrogenolysis of TeCA, respectively, occurred without a lag in all live microcosm experiments that were conducted. Although this initial TeCA degradation occurred at a similar rate in the microcosms constructed with WB23 and WB30 sediments, VC production and degradation in the microcosms showed substantial spatial variability (variability associated with the location from which the sediment was collected for microcosm construction). The two major pathways of VC production were from dichloroelimination of 112TCA and from hydrogenolysis of 12DCE that was produced by dichloroelimination of TeCA. Abiotic production of trichloroethene (TCE) was not higher than 11 percent, indicating that hydrogenolysis of TCE is not a substantial source of VC in the wetland porewater. The TeCA- and 112TCA-amended microcosms constructed with WB30 sediment showed approximately twice as much VC production from 112TCA dichloroelimination as those constructed with WB23 sediment. VC degradation, however, also was substantially higher in the TeCA-amended microcosms constructed with WB30 sediment than those constructed with WB23 sediment. In microcosms constructed with WB30 sediment, between 30 to 96 percent of the VC was degraded within the 35-day incubation period, whereas 0 to 14 percent of VC was degraded in the microcosms constructed with WB23 sediment. Addition of methanol to microcosms enhanced VC degradation in both WB23 and WB30 wetland sediments. Enrichment experiments amended with VC showed that the spatial difference in VC degradation was negligible after prolonged incubation under methanogenic conditions at high initial VC concentrations. Production of VC in enrichment cultures with 112TCA was linear, whereas production of VC in enrichment cultures with 12DCE was exponential and occurred after a lag.

Addition of an inhibitor of methanogenic activity (BES, or 2-bromoethanesulfonic acid) slightly lowered TeCA deg-

radation rates but did not inhibit production of 112TCA, 12DCA, and 12DCE. VC and chloroethane production, however, were substantially inhibited in the BES-amended microcosms. The lower VC production in the BES-amended microcosms is consistent with the association noted between onset of methane production and of VC production from 112TCA dichloroelimination in many of the microcosms. Inhibition of methanogenesis by addition of BES also completely inhibited 12DCE and VC degradation in the microcosms and inhibited VC degradation in enrichment experiments with VC-amended sediment. VC degradation in enrichment experiments with VC-amended sediment also was inhibited under iron-reducing conditions.

Pre-exposure of the wetland sediment to TeCA was not required for degradation of TeCA, as shown by equally rapid TeCA degradation rates in microcosms constructed with wetland sediment from the contaminated sites WB23 and WB30, and from an uncontaminated site WB19. Pre-exposure of sediments to VC, however, substantially increased VC degradation rates in enrichment experiments.

A microbial consortium, rather than one individual microorganism or microbial group, is involved in the degradation of TeCA, as indicated by the occurrence of multiple degradation pathways and the variability in daughter product distributions in the microcosms with the site or season from which the sediment was collected. To identify possible critical microbial species or groups involved in degradation of TeCA, shifts in the TRFLP profiles (DNA fingerprints) of the microbial communities in the microcosm sediments over time and among different experiments were compared to shifts in the daughter product distributions to note consistent associations. Changes in degradation pathways observed from spatial and seasonal variability were linked to changes in the microbial communities. A bacterial peak at 90 base pair (bp) fragment length was linked to TeCA hydrogenolysis to 112TCA. Increases in intensity of a few bacterial species were observed in killed controls that showed recovery from the formaldehyde by the end of the experiment. The only daughter product observed in these killed controls was 12DCE from TeCA dichloroelimination, and growth of species represented by 198 and 170 bp fragment lengths were associated with the production of 12DCE. Dichloroelimination of 112TCA to VC also was associated with growth of this 198 bp bacterial peak in March–April 1999 and October–November 2000 microcosms, whereas an 86 bp or the 170 bp bacterial peak was associated with 112TCA dichloroelimination in the July–August 1999 experiment. Hydrogenolysis of 12DCE to VC was associated with a CO_2-utilizing methanogen at 307 bp in the March–April 1999 and October–November 2000 microcosm experiments, whereas methanogen biomass was low in the July–August 1999 experiments.

Production of VC that occurred without methane production in the July–August 1999 microcosms constructed with sediment from WB30 and in 112TCA-amended and 12DCE-amended enrichment cultures indicated the involvement of other microorganisms in VC production in the wetland sediments. The immediate, linear production of VC in the 112TCA-amended enrichment cultures indicated a cometabolic reaction. In contrast, the exponential increase of VC in 12DCE-amended enrichment cultures after an initial lag indicated growth of a microbial species or group, possibly the dehalorespiring bacteria. Molecular analyses showed the presence of known dehalorespiring bacteria of the *Dehalococcoides* group (*Dehalococcoides ethenogenes* and *Dehalococcoides* sp. strain FL2) and of the acetate-oxidizing *Desulfuromonas* group (*Desulfuromonas* sp. strain BB1 and *Desulfuromonas chloroethenica*) in microcosm slurry from WB30, but not WB23. The lower activity of methanogens in the July–August 1999 microcosms may have allowed the activity of the dehalorespiring bacteria to increase because methanogens compete with dehalorespiring bacteria for hydrogen as a substrate. Both hydrogen, which is a favored substrate for *Dehalococcoides*, and acetate, which is a favored substrate for *Desulfuromonas*, stimulated VC production in enrichment experiments with 12DCE-amended cultures. Three times as much VC, however, was produced with hydrogen than with acetate in these enrichment experiments, indicating a greater population of hydrogen-utilizing than acetate-utilizing bacteria in the sediment. Ethene, which is an end product of dechlorination of VC by *Dehalococcoides ethenogenes*, was produced only in enrichments supplied with hydrogen.

The higher VC degradation in microcosms constructed with WB30 sediment compared to WB23 sediment and in microcosms amended with methanol showed a marked association with an increase of acetotrophic methanogens, a group of methanogens that can utilize acetate as a substrate. The apparent link between VC degradation and acetotrophic methanogens observed in these microcosm experiments may support the theory that VC mineralization under anaerobic conditions begins with oxidation by acetogens and that acetotrophic methanogens enhance degradation, although the involvement of acetogens in the experiments reported here is purely speculative. The microcosm and enrichment culture experiments indicate that VC can be produced and degraded by more than one reaction pathway and associated microorganisms in the wetland sediments, and the pathway that is dominant can change with changing seasons, location in the wetland, redox conditions, or substrate type.

Estimates of TeCA and TCE degradation rates using field data of porewater concentrations and flow velocity in the wetland sediments showed that the rapid anaerobic degradation rates measured in the laboratory are reasonable. Half-lives for TeCA and TCE estimated from field data were in the range of 60 to 100 days, which agrees well with laboratory estimates considering the inherent differences in the laboratory and field systems. The relative proportions of daughter products in the wetland porewater also are consistent with laboratory microcosm experiments, which showed 12DCE and VC were the predominant, persistent daughter compounds from TeCA degradation. In addition, the porewater chemistry showed higher production of VC in the wetland sediment at site WB30 than at site WB23, as was

observed in the microcosm experiments. Molecular analyses of grab samples of surficial wetland sediment showed that all of the microbial species or groups linked to TeCA degradation in the microcosm experiments were present in all the sediment samples. Microbial biomass and diversity were lowest in an area of the wetland where porewater VOC concentrations are highest (transect C-C′), indicating that the higher VOC concentrations could result from lower degradation rates. The lower microbial biomass and diversity along transect C-C′ could be caused from toxicity effects of the contaminants, or possibly from differences in frequency and duration of tidal inundation. Land-surface elevation is lower along transect C-C′ than along A-A′, causing greater and more frequent tidal inundation, which would affect redox and other physiochemical conditions in the surficial wetland sediments. A toxicity effect also could be associated with tidal inundation because low concentrations of VOCs, including chloroform, were measured in the surface water.

The rapid TeCA and daughter product degradation measured in the wetland sediments observed in this study confirm that natural attenuation is a feasible remediation method in the anaerobic wetland sediments. The adaptable and diverse microbial community that appears to be involved in TeCA degradation in the wetland sediments provides compensation for changing spatial and seasonal conditions and allows natural attenuation to be effective throughout the year. The lower degradation rates in the summer, however, indicate that ground-water and surface-water monitoring during the summertime is especially crucial. Definitive identification of the microbial species involved in TeCA degradation by completion of genetic analyses of the enrichment experiments would allow development of gene probes that could be used for long-term monitoring of the presence of critical microbial species over time in the wetland sediment, and for evaluating the potential for natural attenuation at other contaminated sites. Definitive identifications also would assist in evaluating possible enhancements, including "biostimulation" or "bioaugmentation," that could be used to increase biodegradation rates in areas such as seeps where higher concentrations of VOCs have been measured at the wetland surface. Because anaerobic TeCA degradation encompasses chlorinated ethane and ethene pathways, results of this study are transferable to other sites at APG and elsewhere that have TeCA, TCE, or their anaerobic degradation products as contaminants.

References Cited

Baedecker, M.J., and Cozzarelli, I.M., 1992, The determination and fate of unstable constituents in contaminated groundwater, *in* S. Lesage and R.E. Jackson (eds.), Groundwater Contamination and Analysis at Hazardous Waste Sites: New York, Marcel Dekker, p. 425–461.

Bagley, D.M., and Gossett, J.M., 1990, Tetrachloroethene transformation to trichloroethene and *cis*-1,2-dichloroethene by sulfate-reducing enrichment cultures: Applied and Environmental Microbiology, v. 56, no. 8, p. 2,511–2,516.

Belay, Negash, and Daniels, Lacy, 1987, Production of ethane, ethylene, and acetylene from halogenated hydrocarbons by methanogenic bacteria: Applied and Environmental Microbiology, v. 53, no. 7, p. 1,604–1,610.

Bradley, P.M., and Chapelle, F.H., 1996, Anaerobic mineralization of vinyl chloride in Fe(III)-reducing aquifer sediments: Environmental Science and Technology, v. 30, no. 6, p. 2,084–2,086.

_____ **1998**, Microbial mineralization of VC and DCE under different terminal electron accepting conditions: Anaerobe, v. 4, p. 81–87.

_____ **1999a**, Methane as a product of chloroethene biodegradation under methanogenic conditions: Environmental Science and Technology, v. 33, no. 4, p. 653–656.

_____ **1999b**, Role for acetotrophic methanogens in methanogenic biodegradation of vinyl chloride: Environmental Science and Technology, v. 33, no. 19, p. 3,473–3,476.

_____ **2000**, Acetogenic microbial degradation of vinyl chloride: Environmental Science and Technology, v. 34, no. 13, p. 2,761–2,763.

Bradley, P.M., Chapelle, F.H., and Lovley, D.R., 1998, Humic acids as electron acceptors for anaerobic microbial oxidation of vinyl chloride and dichloroethene: Applied and Environmental Microbiology, v. 64, no. 8, p. 3,102–3,105.

Brunk, C.F., Avaniss-Aghajani, E., and Brunk, C.A., 1996, A computer analysis of primer and probe hybridization potential with bacterial small-subunit rRNA sequences: Applied and Environmental Microbiology, v. 62, p. 872–879.

Capone, D.G., and Kiene, R.P., 1988, Comparison of microbial dynamics in marine and freshwater sediments: Contrasts in anaerobic catabolism: Limnology and Oceanography, v. 33, p. 725–749.

Chapelle, F.H., 1993, Ground-water microbiology and geochemistry: New York, John Wiley & Sons, 424 p.

Chapelle, F.H., and Bradley, P.M., 1998, Selecting remediation goals by assessing the natural attenuation capacity of groundwater systems: Bioremediation Journal, v. 2, nos. 3 & 4, p. 227–238.

Chen, Chun, Puhakka, J.A., and Ferguson, J.F., 1996, Transformations of 1,1,2,2-tetrachloroethane under methanogenic conditions: Environmental Science and Technology, v. 30, no. 2, p. 542–547.

Clement, B.G., Kehl, L.E., DeBord, K.L. and Kitts, C., 1998, Terminal restriction fragment patterns (TRFPs), a rapid, PCR-based method for the comparison of complex bacterial communities: Journal of Microbiological Methods, v. 31, p. 135–142.

Dyer, Linda Jo, Lorah, M.M., and Burris, D.R., 2002, Effect of sampling method on measured porewater concentrations in a wetland contaminated by chlorinated solvents, *in* K.W. Nehring and S.E. Brauning (eds.), Proceedings of the Second International Conference on Wetlands & Remediation, September 5–6, 2001, Burlington, Vermont: Columbus, Ohio, Battelle Press, p. 33–40.

Ellis, D.E., Lutz, E.J., Odom, J.M., Buchanan, R.J., Jr., Lee, M.D., Bartlett, C.L., Harkness, M.R., and Deweerd, K.A., 2000, Bioaugmentation for accelerated *in situ* anaerobic bioremediation: Environmental Science and Technology, v. 34, no. 11, p. 2,254–2,260.

Fathepure, B.Z., and Boyd, S.A., 1988, Dependence of tetrachloroethylene dechlorination on methanogenic substrate consumption by Methanosarcina sp. strain DCM: Applied and Environmental Microbiology, v. 54, p. 2,976–2,980.

Fennel, D.E., Carroll, A.B., Gossett, J.M, and Zinder, S.H., 2001, Assessment of indigenous reductive dechlorinating potential at a TCE-contaminated site using microcosms, polymerase chain reaction analysis, and site data: Environmental Science and Technology, v. 35, no. 9, p. 1,830–1,839.

Fennel, D.E., and Gossett, J.M., 1998, Modeling the production of and competition for hydrogen in a dechlorinating culture: Environmental Science and Technology, v. 32, p. 2,450–2,460.

Flynn, S.J., Löffler, F.E., and Tiedje, J.M., 2000, Microbial community changes associated with a shift from reductive dechlorination of PCE to reductive dechlorination of *cis*-DCE and VC: Environmental Science and Technology, v. 34, no. 6, p. 1,056–1,061.

Gantzer, C.J., and Wackett, L.P., 1991, Reductive dechlorination catalyzed by bacterial transition-metal coenzymes: Applied and Environmental Microbiology, v. 55, p. 2,144–2,151.

Harkness, M.R., Bracco, A.A., Brennan, M.J., Jr., Deweerd, K.A., and Spivack, J.L., 1999, Use of bioaugmentation to stimulate complete reductive dechlorination of trichloroethene in Dover soil columns: Environmental Science and Technology, v. 33, no. 7, p. 1,100–1,109.

Holliger, Christof, Schraa, Gosse, Stams, A.J.M., and Zehnder, A.J.B., 1993, A highly purified enrichment culture couples the reductive dechlorination of tetrachloroethene to growth: Applied and Environmental Microbiology, v. 59, no. 9, p. 2,991–2,997.

Jafvert, C.D., and Wolfe, N. Lee, 1987, Degradation of selected halogenated ethanes in anoxic sediment-water systems: Environmental Toxicology and Chemistry, v. 6, p. 827–837.

Kassenga, G.R., and Pardue, J.H., 2002, Fate of TCE in constructed and natural wetland mesocosms, *in* K.W. Nehring and S.E. Brauning (eds.), Proceedings of the Second International Conference on Wetlands & Remediation, September 5–6, 2001, Burlington, Vermont: Columbus, Ohio, Battelle Press, p. 25–32.

Kästner, Matthias, 1991, Reductive dechlorination of tri- and tetrachloroethylenes depends on transition from aerobic to anaerobic conditions: Applied and Environmental Microbiology, v. 57, no. 7, p. 2,039–2,046.

Klein, A., Allmansberger, R., Bokranz, M., Knaub, S., Muller, B. and Muth, E. 1988, Comparative analysis of genes encoding methylcoenzyme M reductase in methanogenic bacteria: Molecular and General Genetics, v. 213, p. 409–420.

Krumholz, L.R., Sharp, Richard, and Fishbain, S.S., 1996, A freshwater anaerobe coupling acetate oxidation to tetrachloroethylene dehalogenation: Applied and Environmental Microbiology, v. 62, no. 11, p. 4,108–4,113.

Liang, Li-Nuo, and Grbic´-Galic´, D., 1993, Biotransformation of chlorinated aliphatic solvents in the presence of aromatic compounds under methanogenic conditions: Environmental Toxicology and Chemistry, v. 12, p. 1,377–1,393.

Löffler, F.E., Sanford, R.A., and Tiedje, J.M., 1996, Initial characterization of a reductive dehalogenase from *Desulfitobacterium chlororespirans* Co23: Applied and Environmental Microbiology, v. 62, no. 10, p. 3,809–3,813.

Löffler, F.E., Sun, Qing, Li, Jieran, and Tiedje, J.M., 2000, 16S rRNA gene-based detection of tetrachloroethene-dechlorinating *Desulfuromonas* and *Dehalococcoides* species: Applied and Environmental Microbiology, v. 66, no. 4, p. 1,369–1,374.

Lorah, M.M. and Clark, J.S., 1996, Contamination of ground water, surface water, and soil, and evaluation of selected ground-water pumping alternatives in the Canal Creek area of Aberdeen Proving Ground, Maryland: U.S. Geological Survey Open-File Report 95–282, 318 p.

Lorah, M.M., Olsen, L.D., Smith, B.L., Johnson, M.A., and Fleck, W.B., 1997, Natural attenuation of chlorinated volatile organic compounds in a freshwater tidal wetland, Aberdeen Proving Ground, Maryland: U.S. Geological Survey Water-Resources Investigations Report 97–4171, 95 p.

Lorah, M.M., and Olsen, L.D., 1999a, Degradation of 1,1,2,2-tetrachloroethane in a freshwater tidal wetland: Field and laboratory evidence: Environmental Science and Technology, v. 33, no. 2, p. 227–234.

_____ 1999b, Natural attenuation of chlorinated volatile organic compounds in a freshwater tidal wetland: Field evidence of anaerobic biodegradation: Water Resources Research, v. 35, no. 12, p. 3,811–3,827.

Lorah, M.M., Olsen, L.D., and Johnson, J.C., 2000, Temporal dynamics of biogeochemical processes in a contaminated wetland, Aberdeen Proving Ground, Maryland: American Geophysical Union 2000 Spring Meeting, Washington, D.C., May 30–June 3, 2000. Eos, Transactions, American Geophysical Union, v. 81, no. 19, p. S197.

Lorah, M.M., Olsen, L.D., Capone, D.G., and Baker, J.E., 2001, Biodegradation of trichloroethylene and its anaerobic daughter products in freshwater wetland sediments: Bioremediation Journal, v. 5, no. 2, p. 101–118.

Lorah, M.M., Burris, D.R., and Dyer, L.J., 2002, Efficiency of natural attenuation of chlorinated solvents in two freshwater wetlands, *in* K.W. Nehring and S.E. Brauning (eds.), Proceedings of the Second International Conference on Wetlands & Remediation, September 5–6, 2001, Burlington, Vermont: Columbus, Ohio, Battelle Press, p. 9–16.

Lorah-Devereux, M.M., 1999, Natural attenuation of chlorinated volatile organic compounds in a freshwater tidal wetland: College Park, Maryland, University of Maryland, Ph.D. Dissertation, 210 p.

Lovley, D.R., and Phillips, E.J.P., 1987, Rapid assay for microbially reducible ferric iron in aquatic sediments: Applied and Environmental Microbiology, v. 53, no. 7, p. 1,536–1,540.

Luton, P.E., 1996, A study of landfill methanogens: Liverpool, England, Liverpool John Moores University, PhD Dissertation, [variously, paged].

Maymó-Gatell, Xavier, Anguish, Timothy, and Zinder, S.H., 1999, Reductive dechlorination of chlorinated ethenes and 1,2-dichloroethane by "*Dehalococcoides ethenogenes*" 195: Applied and Environmental Microbiology, v. 65, no. 7, p. 3,108–3,113.

Maymó-Gatell, Xavier, Chien, Yueh-Tyng, Gossett, J.M., and Zinder, S.H., 1997, Isolation of a bacterium that reductively dechlorinates tetrachloroethene to ethene: Science, v. 276, p. 1,568–1,571.

Maymó-Gatell, Xavier, Nijenhuis, Ivonne, and Zinder, S.H., 2001, Reductive dechlorination of *cis*-1,2-dichloroethene and vinyl chloride by "*Dehalococcoides ethenogenes*": Environmental Science and Technology, v. 35, no. 3, p. 516–521.

Maymó-Gatell, Xavier, Tandoi, Valter, Gossett, J.M., and Zinder, S.H., 1995, Characterization of an H_2-utilizing enrichment culture that reductively dechlorinates tetrachloroethene to vinyl chloride and ethene in the absence of methanogenesis and acetogenesis: Applied and Environmental Microbiology, v. 61, no. 11, p. 3,928–3,933.

McCarty, P.L., and Semprini, Lewis, 1994, Ground-water treatment for chlorinated solvents, *in* R.D. Norris and others, Handbook of bioremediation: Ann Arbor, Michigan, Lewis Publishers, p. 87–116.

Mitsch, W.J., and Gosselink, J.G., 1993, Wetlands (2d Edition): New York, John Wiley & Sons, 722 p.

National Research Council, 2000, Natural attenuation for groundwater remediation: Washington, D.C., National Academy Press, 274 p.

Novak, P.J., Daniels, L., and Parkin, G.F., 1998, Rapid dechlorination of carbon tetrachloride and chloroform by extracellular agents in cultures of *Methanosarcina thermophila*: Environmental Science and Technology, v. 32, no. 20, p. 3,132–3,136.

Olsen, L.D., Lorah, M.M., Marchand, E.H., Smith, B.L., and Johnson, M.A., 1997, Hydrogeologic, water-quality, and sediment-quality data for a freshwater tidal wetland, West Branch Canal Creek, Aberdeen Proving Ground, Maryland, 1992–96: U.S. Geological Survey Open-File Report 97–560, 267 p.

Oremland, R.S., and Capone, D.G., 1988, Use of "specific" inhibitors in biogeochemistry and microbial ecology, *in* K.C. Marshall (ed.), Advances in microbial ecology: New York, Plenum Press, v. 10, p. 285–383.

Pankow, J.F., and Cherry, J.A., 1996, Dense chlorinated solvents: Portland, Oregon, Waterloo Press, 522 p.

Pavlostathis, S.G., and Zhuang, Ping, 1991, Transformation of trichloroethylene by sulfate-reducing cultures enriched from a contaminated subsurface soil: Applied Microbiology and Biotechnology, v. 36, p. 416–420.

Peijnenburg, Willie, Eriksson, Lennart, de Groot, Arthur, Sjostrom, Michael, and Verboom, Hans, 1998, The kinetics of reductive dehalogenation of a set of halogenated aliphatic hydrocarbons in anaerobic sediment slurries: Environmental Science & Pollution Research, v. 5, no. 1, p. 12–16.

Phelan, D.J., Olsen, L.D., Senus, M.P., and Spencer, T.A., 2001, Assessment of volatile organic compounds in surface water at Canal Creek, Aberdeen Proving Ground, Maryland, November 1999–September 2000: U.S. Geological Survey Open-File Report 01–292, 49 p.

Ravi, V., Chen, J., Wilson, J.T., Johnson, J.A., Gierke, W., and Murdie, L., 1998, Evaluation of natural attenuation of benzene and dichloroethanes at the KL Landfill: Bioremediation Journal, v. 2, nos. 3 & 4, p. 239–258.

Richard, D.E., Connell, Doug, Berns, J.J., and Hirl, P.J., 2002, Assessment of a reconstructed wetland for remediation of chlorinated solvents, *in* K.W. Nehring and S.E. Brauning (eds.), Proceedings of the Second International Conference on Wetlands & Remediation, September 5–6, 2001, Burlington, Vermont: Columbus, Ohio, Battelle Press, p. 117–124.

Rifai, H.S., Borden, R.C., Wilson, J.T., and Ward, C.H., 1995, Intrinsic bioattenuation for subsurface restoration, *in* R.E. Hinchee and others (eds.), Intrinsic bioremediation: Columbus, Ohio, Battelle Press, p. 1–29.

Rose, D.L., and Schroeder, M.P., 1995, Methods of analysis by the U.S. Geological Survey National Water-Quality Laboratory—Determination of volatile organic compounds in water by purge and trap capillary gas chromatography/mass spectrometry: U.S. Geological Survey Open-File Report 94–708, 26 p.

Schanke, C.A., and Wackett, L.P., 1992, Environmental reductive elimination reactions of polychlorinated ethanes mimicked by transition-metal coenzymes: Environmental Science and Technology, v. 26, no. 4, p. 830–833.

Scholten, J.C.M., Conrad, R., and Stams, A.J.M., 2000, Effect of 2-bromo-ethane sulfonate, molybdate and chloroform on acetate consumption by methanogenic and sulfate-reducing populations in freshwater sediment: FEMS Microbiology Ecology, v. 32, p. 35–42.

Schwarzenbach, R.P., Gschwend, P.M., and Imboden, D.M., 1993, Environmental Organic Chemistry: New York, John Wiley & Sons, 681 p.

Semprini, Lewis, Kitanidis, P.K., Kampbell, D.H., and Wilson, J.T., 1995, Anaerobic transformation of chlorinated aliphatic hydrocarbons in a sand aquifer based on spatial chemical distributions: Water Resources Research, v. 31, no. 4, p. 1,051–1,062.

Smatlak, C.R., Gossett, J.M, and Zinder, S.H., 1996, Comparative kinetics of hydrogen utilization for reductive dechlorination of tetrachloroethene and methanogenesis in an anaerobic enrichment culture: Environmental Science and Technology, v. 30, no. 9, p. 2,850–2,858.

Spencer, T.A., Olsen, L.D., Lorah, M.M., and Mount, M.M., 2000, Water-quality and water-level data for a freshwater tidal wetland, West Branch Canal Creek, Aberdeen Proving Ground, Maryland, October 1998–September 1999: U.S. Geological Survey Open-File Report 00–282, 184 p.

Spencer, T.A., Phelan, D.J., Olsen, L.D., and Lorah, M.M., 2002, Water-quality data for a freshwater tidal wetland, West Branch Canal Creek, Aberdeen Proving Ground, Maryland, November 1999—May 2001: U.S. Geological Survey Open-File Report 01-420, 295 p.

Tomassoni, Guy, 2000, A federal statutory/regulatory/policy perspective on remedial decision-making with respect to ground-water/surface-water interaction, *in* Proceedings of the ground-water/surface-water interactions workshop: EPA/542/R–00/007, p. 13–14.

U.S. Environmental Protection Agency, 1998, Announcement of the drinking water contaminant candidate list: Federal Register, v. 63, no. 40, March 2, 1998, p. 10,273–10,287.

_____ 2001, National Primary Drinking Water Regulations, Office of Water, March 2001: Available at *http://www.epa.gov/safewater/mcl.html.*

Vogel, T. M., Criddle, C.S., and McCarty, P.L., 1987, Transformations of halogenated aliphatic compounds: Environmental Science and Technology, v. 21, no. 8, p. 722–736.

Vogel, T.M., and McCarty, P.L., 1985, Biotransformation of tetrachloroethylene to trichloroethylene, dichloroethylene, vinyl chloride, and carbon dioxide under methanogenic conditions: Applied and Environmental Microbiology, v. 49, p. 1,080–1,083.

Weathers, L.J., and Parkin, G.F., 2000, Toxicity of chloroform biotransformation to methanogenic bacteria: Environmental Science and Technology, v. 34, no. 13, p. 2,764–2,767.

Wiedemeier, T.H., Swanson, M.A., Moutoux, D.E., Gordon, E.K., Wilson, J.T., Wilson, B.H., Kampbell, D.H., Hansen, J.E., Haas, P., and Chapelle, F.H., 1998, Technical protocol for evaluating natural attenuation of chlorinated solvents in ground water: United States Environmental Protection Agency, EPA/600/R–98/128, September 1998, Available at *http://www.epa.gov/ada/reports.html*.

Yager, R.M., Bilotta, S.E., Mann, C.L., and Madsen, E.L., 1997, Metabolic adaptation and in situ attenuation of chlorinated ethenes by naturally occurring microorganisms in a fractured dolomite aquifer near Niagara Falls, New York: Environmental Science and Technology, v. 31, no. 11, p. 3,138–3,147.