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Scientific Investigations Report 2007–5165

U.S. Department of the Interior
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Conversion Factors 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

Other abbreviated units of measure: Water temperature, chemical concentration, and other chemical and physical properties of constituents are given in metric units. Water temperature in degrees Celsius (°C) can be converted to degrees Fahrenheit (°F) by use of the following equation:

$$°F = 1.8\ (°C) + 32$$

Specific conductance is given in microsiemens per centimeter at 25 degrees Celsius (µS/cm at 25°C).

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

Abbreviations

APG	Aberdeen Proving Ground
CERCLA	Comprehensive Environmental Response, Compensation, and Liability Act of 1980
CF	Chloroform
*cis*12DCE	*cis*-1,2-dichloroethene
CT	Carbon tetrachloride
DNAPL(s)	dense non-aqueous-phase liquid(s)
GC/FID	Gas chromatography/flame ionization detector
GC/MS	Gas chromatography/mass spectrometry
NWQL	National Water Quality Laboratory
PCE	Tetrachloroethene
RPDs	Relative percent differences
TCE	Trichloroethene
TeCA	1,1,2,2-tetrachloroethane
*trans*12DCE	*trans*-1,2-dichloroethene
USGS	U.S. Geological Survey
VC	Vinyl chloride
VOC(s)	Volatile organic compounds
12DCA	1,2-dichloroethane
12DCE	Total of *cis*-1,2-dichloroethene and *trans*-1,2-dichloroethene
112TCA	1,1,2-trichloroethane

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by Michelle M. Lorah, Emily H. Majcher, Elizabeth J. Jones, and Mary A. Voytek

Abstract

Chlorinated solvents, including 1,1,2,2-tetrachloroethane, tetrachloroethene, trichloroethene, carbon tetrachloride, and chloroform, are reaching land surface in localized areas of focused ground-water discharge (seeps) in a wetland and tidal creek in the West Branch Canal Creek area, Aberdeen Proving Ground, Maryland. In cooperation with the U.S. Army Garrison, Aberdeen Proving Ground, Maryland, the U.S. Geological Survey is developing enhanced bioremediation methods that simulate the natural anaerobic degradation that occurs without intervention in non-seep areas of the wetland. A combination of natural attenuation and enhanced bioremediation could provide a remedy for the discharging ground-water plumes that would minimize disturbance to the sensitive wetland ecosystem. Biostimulation (addition of organic substrate or nutrients) and bioaugmentation (addition of microbial consortium), applied either by direct injection at depth in the wetland sediments or by construction of a permeable reactive mat at the seep surface, were tested as possible methods to enhance anaerobic degradation in the seep areas. For the first phase of developing enhanced bioremediation methods for the contaminant mixtures in the seeps, laboratory studies were conducted to develop a microbial consortium to degrade 1,1,2,2-tetrachloroethane and its chlorinated daughter products under anaerobic conditions, and to test biostimulation and bioaugmentation of wetland sediment and reactive mat matrices in microcosms. The individual components required for the direct injection and reactive mat methods were then combined in column experiments to test them under ground-water-flow rates and contaminant concentrations observed in the field. Results showed that both direct injection and the reactive mat are promising remediation methods, although the success of direct injection likely would depend on adequately distributing and maintaining organic substrate throughout the wetland sediment in the seep area.

For bioaugmentation, two mixed anaerobic cultures, named the "West Branch Consortia" (WBC-1 and WBC-2), were developed by enrichment of wetland sediment collected from two contaminated sites in the study area where rapid and complete reductive dechlorination naturally occurs. WBC are capable of degrading 1,1,2,2-tetrachloroethane, 1,1,2-trichloroethane, 1,2-dichloroethane, tetrachloroethene, trichloroethene, *cis*- and *trans*-1,2-dichloroethene, and vinyl chloride to the non-chlorinated end-products ethene and ethane. In addition, the column experiments showed that the consortia could completely degrade carbon tetrachloride and chloroform, although they were not grown on these contaminants. No other cultures are known that can degrade the broad mixture of chlorinated alkanes, alkenes, and methanes as shown for WBC. WBC-2 (suspended in the culture media) is capable of complete dechlorination of 50 micromolar 1,1,2,2-tetrachloroethane to ethene in 1 to 2 days with little transient accumulation of chlorinated daughter products. Only about 5 percent of the clones sequenced from WBC-1 and WBC-2 were related to dechlorinating bacteria that have been studied previously in culture, indicating the presence of unknown dechlorinators. *Dehalococcoides* spp. comprised about 1 percent of WBC-1 and WBC-2, which is minor compared to the population size of about 30 percent in other dechlorinating consortia for chlorinated alkenes. Although both WBC-1 and WBC-2 showed efficient degradation in laboratory tests in this study, long-term cultivation of WBC-1, which was developed using hydrogen as the organic substrate, was determined to be infeasible. Thus, WBC-2, cultivated with lactate as the organic substrate, would be used in future tests.

Nutrient (ammonia and phosphate mixture) addition to anaerobic microcosms constructed with wetland sediment and ground water collected from the study area showed some enhancement in the degradation rate of 1,1,2,2-tetrachloroethane, but degradation of 1,1,2,2-tetrachloroethane's anaerobic daughter compounds (1,1,2-trichloroethane, 1,2-dichloroethane, trichloroethene, 1,2-dichloroethene, and vinyl chloride) was not enhanced.

Similarly, nutrient amendment did not enhance degradation of 1,1,2,2-tetrachloroethane or its anaerobic daughter products in microcosms constructed with mixtures of BionSoil (a dairy-derived compost) and commercial peat that were evaluated as potential reactive mat materials. Biostimulation by addition of organic donor (lactate) also did not enhance 1,1,2,2-tetrachloroethane degradation in microcosms with wetland sediment and BionSoil/peat mixtures compared to unamended microcosms. In contrast, microcosms constructed with wetland sediment or the compost/peat mixture and augmented with the developed anaerobic mixed culture in media showed 1,1,2,2-tetrachloroethane biodegradation rates that were two to three times higher than those in controls amended with culture media alone. In addition, bioaugmentation substantially enhanced degradation of the chlorinated daughter compounds, with typically a maximum of about 20 to 30 percent of the initial 1,1,2,2-tetrachloroethane added in the bioaugmented microcosms recovered as daughter products compared to 50 to 100 percent in the unamended microcosms during a 42-day incubation. Bioaugmentation with WBC-2 was equally effective in enhancing 1,1,2,2-tetrachloroethane degradation in the mixture of BionSoil compost and peat as observed in wetland sediment from the study area. Wetland sediment from the study area and bioaugmented BionSoil/peat also was able to rapidly degrade carbon tetrachloride and chloroform to non-chlorinated end-products. Out of five additional composts tested in microcosms with WBC-2, only two others provided a suitable environment for the culture, indicating the need to test individual composts before use in a bioremediation effort.

Column tests were used to (1) combine biostimulation and bioaugmentation to simulate direct injection in seep sediment, and to combine the matrix zones conceptualized for the permeable reactive mat (zero-valent iron (ZVI) and organic layer with an overlying bioaugmented organic layer), (2) better simulate the bioremediation methods under field conditions, including continuous ground-water flow at measured rates and increased contaminant concentrations, and (3) evaluate possible adverse water-quality effects that could result from the proposed near-surface bioremediation methods in the wetland. The enriched, mixed microbial culture did not appear to stimulate enhanced biodegradation of site contaminants in the seep sediment columns without the adequate addition of continuous, soluble electron donor. With the addition of ethanol, methanogenic conditions were rapidly established and efficient degradation of 1,1,2,2-tetrachloroethane, as well as tetrachloroethene and trichloroethene, was observed in the columns. Degradation efficiency was highly sensitive to changes in the ethanol dosing and indicated that success of direct injection may be largely dependent on the ability to adequately distribute electron donor.

The bioaugmented microbial culture stimulated enhanced degradation of 1,1,2,2-tetrachloroethane in both matrices for the reactive mat (organic mix and zero-valent iron/organic mix) without lactate and then, with improved efficiency, with lactate addition. A zero-valent iron layer underlying the bioaugmented organic mix layer was tested primarily because of the high carbon tetrachloride and chloroform concentrations in some seeps— these compounds typically are toxic to many microorganisms but can be degraded abiotically in zero-valent iron. In both matrices, 1,1,2,2-tetrachloroethane and all associated daughter compounds were typically removed within 46 centimeters (cm) along the column. In the presence of chloroethenes and chloromethanes, rapid 1,1,2,2-tetrachloroethane degradation was maintained, and all daughter compounds were removed within the 76-centimeter column length in both sediment matrices. Thus, the microbial community established within each column matrix after bioaugmentation with WBC was able to facilitate complete degradation of the chloroethene (tetrachloroethene and trichloroethene) and chloromethane (carbon tetrachloride and chloroform) co-contaminants and associated daughter compounds without any substantial toxicity effects. The overall comparable degradation efficiencies in the mix and iron-organic mix columns indicate that zero-valent iron would not be a necessary component of an organic-based, bioaugmented reactive mat, even in seep areas with high chloromethane concentrations (up to 25 micromolar was tested). Neither organic-based matrix is likely to cause any adverse effects on water quality. The results of this laboratory study show the broad dechlorinating capabilities of the developed WBC and support the feasibility of applying the consortia in the field for enhanced bioremediation of ground-water discharge seep areas, either by direct injection at depth in the wetland sediment or by construction of a reactive, flow-through mat on the seep surface.

Introduction

In cooperation with the U.S. Army Garrison, Aberdeen Proving Ground (APG), Maryland, the U.S. Geological Survey (USGS) previously conducted an extensive field and laboratory study of natural attenuation processes occurring as plumes of chlorinated volatile organic compounds (VOCs) discharge to a freshwater tidal wetland along West Branch Canal Creek (Lorah and others, 1997; Lorah and Olsen, 1999a, 1999b; Phelan and others, 2002; Lorah and others, 2003; Lorah, Spencer, and McGinty, 2005) (figs. 1a-c). This study showed that the wetland sediments could support efficient dechlorinating microorganisms for chlorinated alkanes and alkenes and that anaerobic biodegradation was a dominant natural attenuation process for the VOCs in the discharging ground water in the wetland sediments. Concentrations of VOCs measured in shallow porewater at all sites in the natural attenuation study area generally decreased to below detection levels before land surface was reached at all times of the year, indicating that monitored natural attenuation could be an effective ground-water remedy at this site. In anaerobic microcosms constructed with wetland sediment collected at two different sites and at different times of the

year, the parent contaminant 1,1,2,2-tetrachloroethane (TeCA) degraded rapidly with an average half-life of 13 days (Lorah and Voytek, 2004). Trichloroethene (TCE), another major parent contaminant in the natural attenuation study area, had a half-life between 2 and 15 days in anaerobic microcosms (Lorah and others, 1997; Lorah and others, 2001). Degradation by reductive dechlorination reactions was complete to non-chlorinated end-products for both TeCA and TCE. Continued microcosm and enrichment experiments during 1999–2001 defined some of the factors controlling degradation reactions and evaluated microbial communities associated with these reactions (Lorah and others, 2003; Lorah and Voytek, 2004). The involvement of a number of diverse species or groups of bacteria and methanogens apparently were needed to achieve complete degradation of TeCA (Lorah and Voytek, 2004).

Although ground-water and microcosm data showed complete degradation of the VOCs in the natural attenuation study in the West Branch Canal Creek area, detections of parent VOCs in the surface water throughout the study indicated that natural attenuation is not effective over the entire wetland and creek (Phelan, Olsen, and others, 2001). The apparently conflicting ground-water and surface-water data led to the hypothesis that seeps existed where ground water discharging upward from the aquifer has insufficient residence time in the wetland porewater to allow degradation to occur before land surface is reached. Consequently, USGS

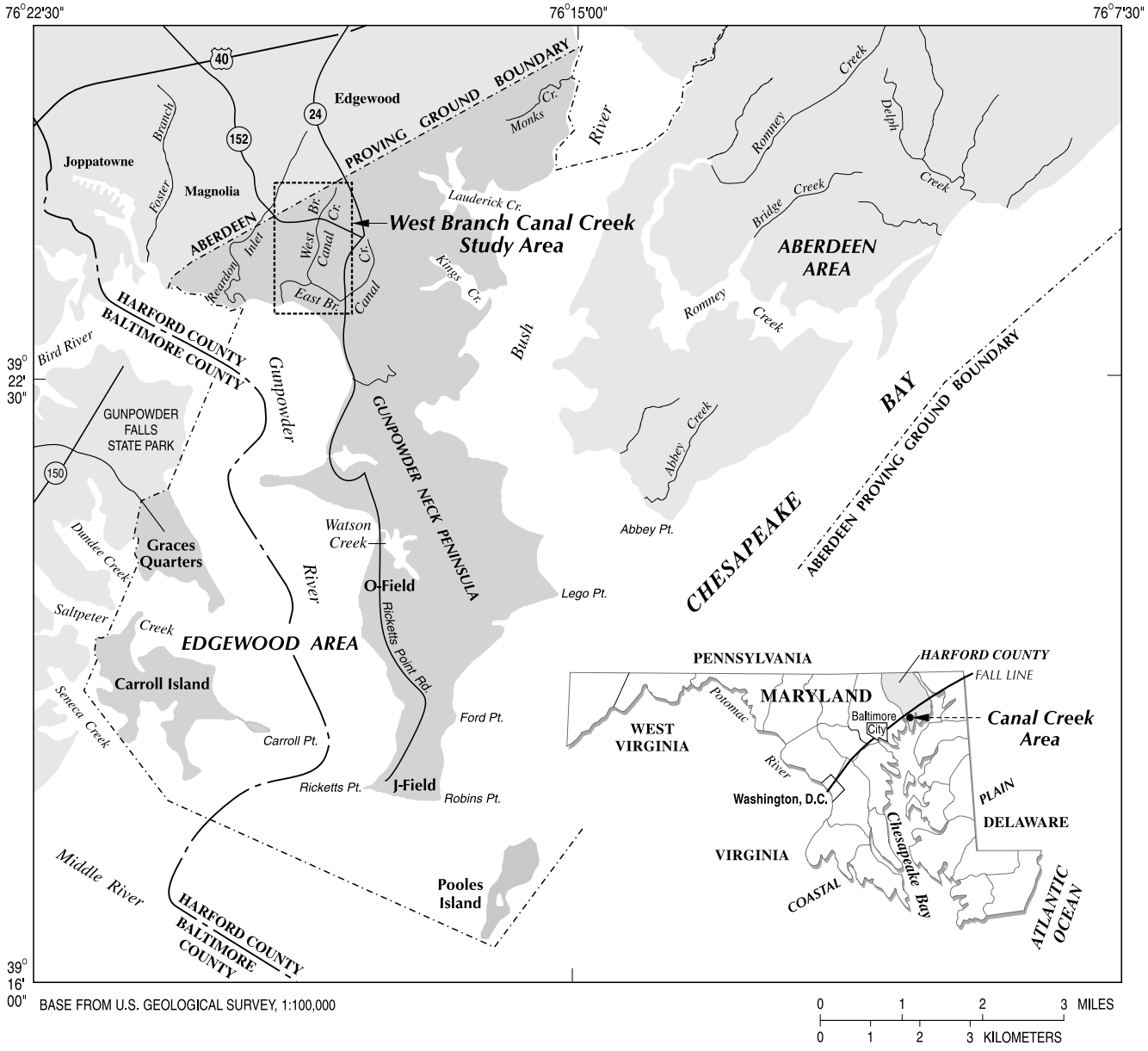


Figure 1a. Location of the West Branch Canal Creek study area, Aberdeen Proving Ground, Maryland (modified from Majcher and others, 2007).

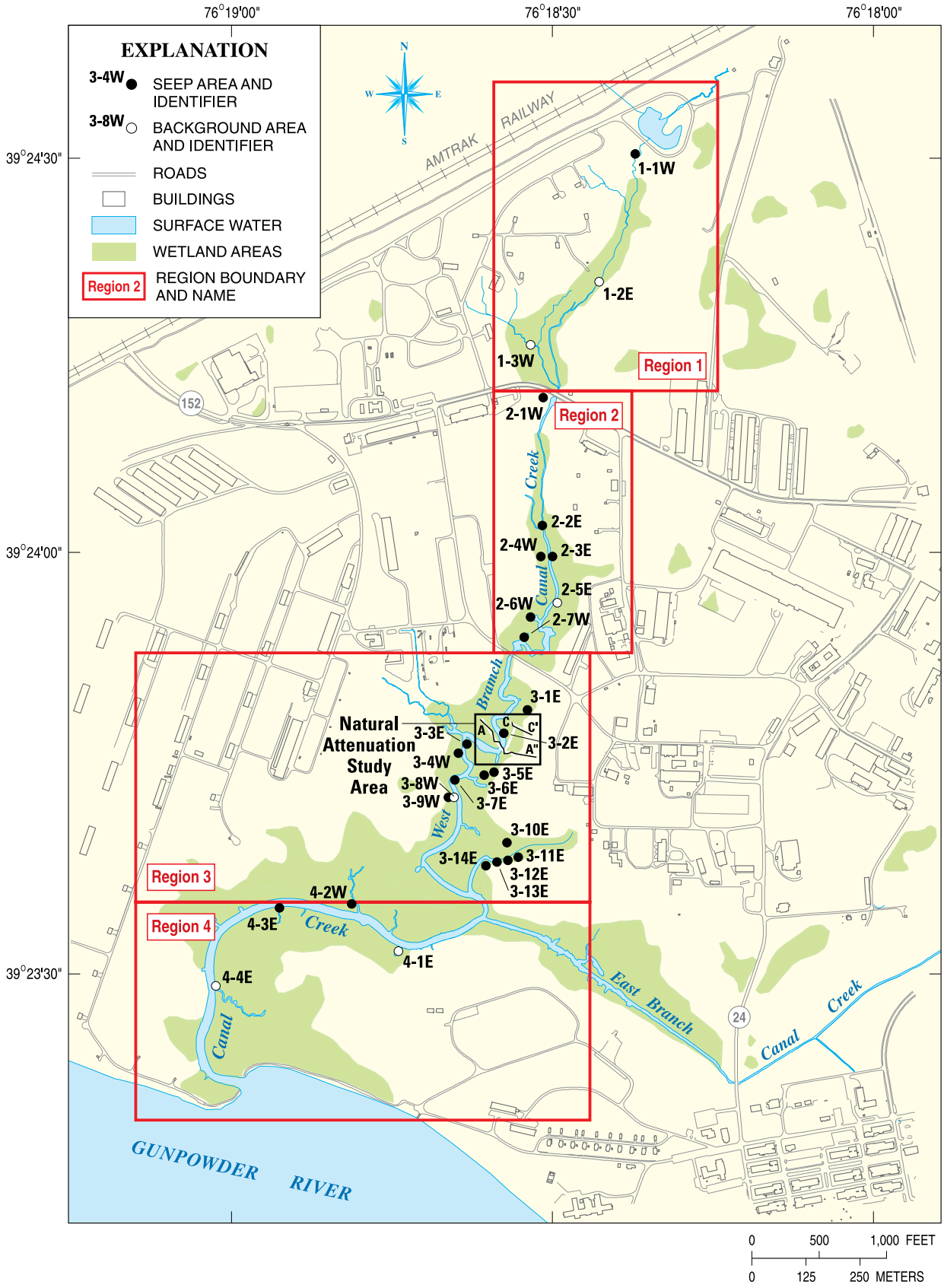


Figure 1b. Location of natural attenuation and seep study areas, West Branch Canal Creek study area (modified from Majcher and others, 2007).

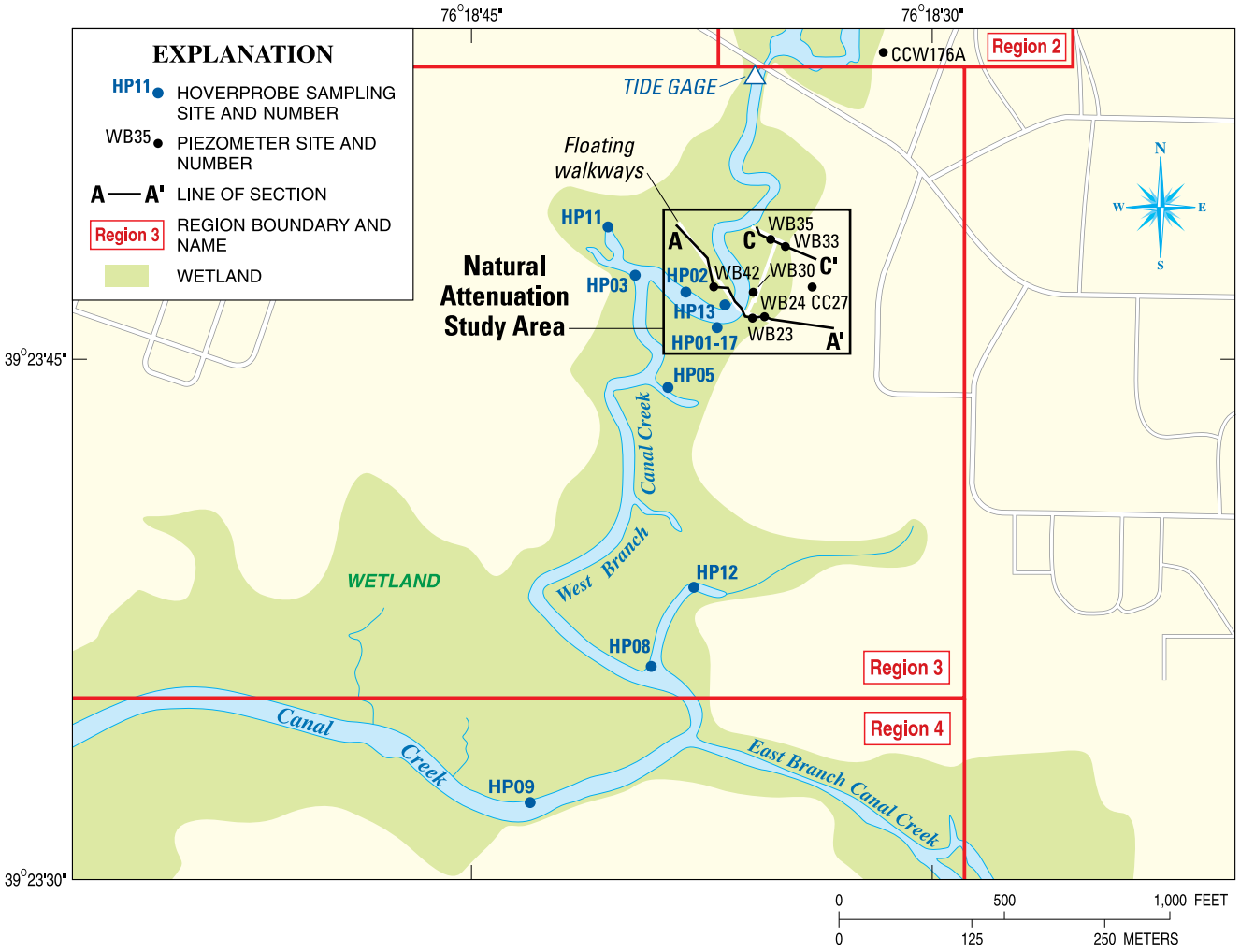


Figure 1c. Location of the hoverprobe sampling sites, selected piezometers, and lines of sections A–A′ and C–C′, West Branch Canal Creek study area (modified from Phelan, Senus, and Olsen, 2001).

began two concurrent investigations in 2002— a remedial investigation to locate and characterize seeps in the West Branch Canal Creek wetland area, and a feasibility study to develop and test enhanced bioremediation methods for contaminated seep areas. Laboratory work completed for the first phase of the feasibility study is described in this report, including (1) development of wetland sediment-derived microbial consortia to degrade the suite of chlorinated alkanes and alkenes found in the West Branch Canal Creek area, and (2) microcosm and column experiments conducted to evaluate two selected enhanced bioremediation methods. *In situ* bioremediation technologies were selected and developed for testing, because of the efficiency of the microbial degradation reactions observed in the previous study and because their application was expected to cause minimal disturbance to the wetland ecosystem.

The goal of the selected technologies is to treat the contaminants at or near the point of discharge in the seep area by increasing the efficiency of anaerobic biodegradation reactions in this ground-water/surface-water interface zone. Although laboratory experiments have shown that aerobic degradation processes also are important for the major chlorinated daughter products produced from TeCA and TCE degradation, the higher chlorinated parent VOCs at the site did not biodegrade aerobically (Lorah and others, 1997; 2001). The selected technologies utilize biostimulation (addition of electron donor, nutrients, or both to stimulate microbial activity) and bioaugmentation (addition of microorganisms) to increase diversity, abundance, and activity of dechlorinating microorganisms. In the first bioremediation method that was bench-tested in this study, a permeable reactive mat was developed to replace the surficial seep sediments with commercially available organic- and nutrient-rich materials that would be bioaugmented with the microbial consortium. The reactive mat is similar in concept to permeable reactive barrier technologies that have been deployed vertically for remediation of chlorinated VOCs in subsurface ground-water environments where horizontal ground-water flow

predominates. The reactive mat developed for seep areas would instead be placed horizontally at the wetland surface, because ground-water-flow directions are predominantly vertical. Bioaugmentation would be a critical component of the reactive mat because (1) the matrix consists of non-native organic materials that could not be expected to contain the appropriate dechlorinating microbial community for the site contaminants, and (2) a high rate of dechlorinating activity is needed in this near-surface application to achieve a thin mat that would not alter hydrologic conditions.

In the second technology tested, existing wetland sediment in the seep area would be enhanced by direct injection of constituents to increase microbial activity (biostimulation), with or without addition of additional microorganisms (bioaugmentation). Treatment of the contaminated ground water would begin at depth in the direct-injection method, increasing biodegradation rates along the natural upward flowpath through the wetland sediments in the seep area. Although biostimulation and bioaugmentation have been tested in sand aquifers to stimulate reductive dechlorination of chlorinated VOCs (Major and others, 2002), their application in natural wetland sediments has not been previously reported. For both the reactive mat and direct-injection technologies, initial laboratory testing was needed to determine the best substances and materials to use, to quantify biodegradation rates with selected materials, and to identify and adjust for possible secondary reactions from added substances that could impact the surface ecosystem or water quality.

The reported studies of bioaugmentation to enhance reductive dechlorination have occurred at sites contaminated principally with chlorinated alkenes, rather than chlorinated alkanes such as TeCA. Microbial consortia are commercially available for anaerobic degradation of tetrachloroethene (PCE), TCE, and the lower chlorinated alkenes 1,2-dichloroethene (12DCE) and vinyl chloride (VC), but none are currently available for degradation of chlorinated alkanes. For this study, two consortia were developed using microbiological "enrichment" techniques to select for microbial species from the wetland sediment that have the ability to degrade the contaminants of interest under anaerobic conditions. The two consortia differed primarily in the substrates used to maintain their growth, and the ease with which the consortium could be propagated to large volumes for use in the field tests. Wetland sediment was used from two sites in the previously studied area of the wetland, where the existing microbial communities were known to be capable of degrading TeCA, TCE, and their chlorinated daughter products to completion (Lorah and others, 2003; Lorah and Voytek, 2004). During the enrichment process, the targeted contaminants are continually amended to the sediment as it is transferred into culture medium, until the medium:sediment ratio is high and the microbial species remaining are those most likely to be directly or indirectly involved in degradation of these contaminants. Other microbial species die during the enrichment process because the contaminants are toxic to them, the electron donor/acceptors that they require are not available in the culture medium, or the anaerobic environment is not suitable for them.

This report describes the development and testing of the microbial consortia derived from the wetland sediments in the West Branch Canal Creek area, and the results of laboratory experiments designed to develop the reactive mat and direct-injection bioremediation technologies for the area. In addition to chlorinated alkane and alkene degradation, abiotic and biotic degradation of chlorinated methanes, including carbon tetrachloride (CT) and chloroform (CF), was evaluated because more recently discovered plumes in the West Branch Canal Creek wetland area had high concentrations of these contaminants (Phelan, Senus, and Olsen, 2001; Phelan and others, 2002). Initial characterization of seeps in the wetland area after consortia development had begun indicated that chlorinated methanes were major contaminants at some seeps along with the chlorinated alkanes and alkenes (Majcher and others, 2007). Results from the laboratory phase of this enhanced bioremediation study were used to design the bioreactive mat, which the USGS deployed for a field pilot test beginning in October 2004, and a direct-injection test, which began in August 2005. In addition to addressing critical needs for ground-water remediation in the West Branch Canal Creek wetland area, the consortia development and laboratory tests presented here enhance the understanding of reductive dechlorination processes and could aid in the application of bioremediation at other sites, including the use of constructed wetlands to treat chlorinated VOCs in ground water (Pardue and others, 2000; Pardue, 2002).

Purpose and Scope

The purpose of this report is to describe (1) the development of anaerobic microbial consortia derived from wetland sediments in the West Branch Canal Creek area and enriched to efficiently degrade a suite of chlorinated VOCs, and (2) the results of laboratory microcosm and column experiments conducted to evaluate the reactive mat and direct-injection technologies for enhanced bioremediation of ground-water contamination in seep areas of the wetland. For development of the consortia, enrichment experiments were conducted using wetland sediment from sites where the existing microbial communities were known from previous studies to be able to degrade TeCA, TCE, and their chlorinated daughter products to completion. Because of uncertainty in requirements to develop and maintain a consortium to degrade the wide suite of contaminants in the ground water in the West Branch Canal Creek area, two different enrichment and electron donor feeding procedures were used to develop two consortia, named WBC-1 and WBC-2, in 2002 and 2003, respectively. Laboratory experiments were performed in three different matrices to address the different objectives of the study: (1) batch experiments to evaluate and refine the activity of the consortia were done using culture medium as

the matrix, (2) microcosm and column experiments to evaluate the direct-injection technology were done using natural wetland or seep sediment from the study area as the matrix, and (3) microcosm and column experiments to evaluate the reactive mat technology were done using commercially available, engineered components or mixtures.

To evaluate the direct-injection technology, initial microcosm experiments focused on evaluating the effects of nutrient (ammonium and phosphate mixture) biostimulation on degradation of TeCA in the wetland sediments. These microcosms were constructed with wetland sediment collected from three sites and during two different periods— one in early June 2002 during early stages of plant growth in the wetland, and the second in September 2002 after the high plant growth and activity of the summer had declined. TeCA degradation and the potential effect of CT and CF as co-contaminants were evaluated in microcosms conducted for the direct-injection technology. Biostimulation with lactate as an electron donor also was tested using wetland sediment collected from one site. When development of WBC-1 was completed in September 2002, the effect of bioaugmentation with WBC-1 was tested in microcosms with wetland sediment from the three sites and compared to the nutrient biostimulation experiments. These nutrient biostimulation and bioaugmentation experiments were repeated with wetland sediment collected from the same three sites in March 2003 to determine reproducibility of the results.

To evaluate the reactive mat technology, different mixtures of commercially available peat, sand, and compost were evaluated in microcosms, with and without addition of WBC-1 or WBC-2, from September 2003 through August 2004. Degradation of TeCA was evaluated in all bioaugmented microcosms, and degradation of a mixture of chlorinated alkanes, alkenes, and methanes was evaluated in one experiment. Microcosms were also conducted with iron metal to evaluate its potential use in the reactive mat for abiotic degradation of the chlorinated methanes (CT and CF). To obtain degradation rates more applicable to field deployment of the direct-injection and reactive mat technologies, flow-through column experiments were operated from November 2003 through June 2004. Three sets of duplicate columns were operated— one set prepared with seep sediment; one prepared with a mixture of peat, sand, and compost; and one containing an iron metal layer overlain with the mixture of peat, sand, and compost. Implications of the results of the consortium development and microcosm and column experiments for enhanced bioremediation of ground water in the West Branch Canal Creek wetland area are discussed.

Background on Enhanced Bioremediation Technologies for Reductive Dechlorination

Reductive dechlorination refers to biologically mediated degradation of chlorinated organic compounds, where the chlorinated compound serves as an electron acceptor for catabolic or cometabolic reactions (Alexander, 1999). The three major classes of chlorinated VOCs— chlorinated alkenes such as PCE and TCE, chlorinated alkanes such as TeCA, and chlorinated methanes such as CT— degrade by sequential reductive dechlorination reactions where a chlorine atom is replaced with a hydrogen atom in each reaction (figs. 2a,b). Dichloroelimination, where two chlorines are replaced with hydrogen in a single reaction, also is a major reductive dechlorination pathway for the chlorinated alkanes (Lorah and Olsen, 1999a; Lorah and Voytek, 2004) (fig. 2a). Reductive dechlorination requires the presence of an electron donor, which can be a carbon substrate or hydrogen gas (H_2), and occurs under anaerobic conditions. Lack of sufficiently reducing conditions or lack of sufficient natural organic matter in aquifer materials generally are believed to be major causes of incomplete reductive dechlorination of chlorinated VOCs under natural ground-water conditions, causing accumulation of intermediate daughter compounds such as 12DCE and VC (National Research Council, 2000). Initial development and application of *in situ* enhanced bioremediation technologies for reductive dechlorination of VOCs in ground water focused on biostimulation—addition of carbon substrates or H_2 to generate reducing conditions and stimulate existing microorganisms in the contaminated zone to degrade the VOCs. Nutrient amendments, most commonly nitrogen and phosphorus, often have been added along with donor amendments during biostimulation because of an expected increase in demand with rapid microbial growth and the low natural availability in many ground-water systems (Lee and others, 1998).

The discovery of bacteria known as halorespirers or dehalorespirers, that can couple energy from reductive dechlorination to growth, led to more recent application of bioaugmentation as an enhanced bioremediation approach for chlorinated VOCs (Maymó-Gatell and others, 1997; Löffler and others, 1999). Bioagumentation approaches for chlorinated solvents have been suggested where the indigenous bacteria were unable to completely dechlorinate the VOCs to non-chlorinated end-products, or where the time to achieve dechlorination with biostimulation methods was longer than desired (Ellis and others, 2000; Lendvay and others, 2003). Reductive dechlorination rates by dehalorespirers are reported to be substantially faster than rates of cometabolic reductive dechlorination by sulfate reducers, methanogens, or acetogens (Löffler and others, 1999). A carbon substrate usually is added in conjunction with bioaugmentation to achieve reducing conditions in the ground water and to provide carbon for cell growth (Lee and others, 1998). The types of *in situ* enhanced bioremediation technologies developed for chlorinated VOCs in ground water are briefly summarized here, focusing on applications that used organic solids as substrates and on tests near the ground-water/surface-water interface.

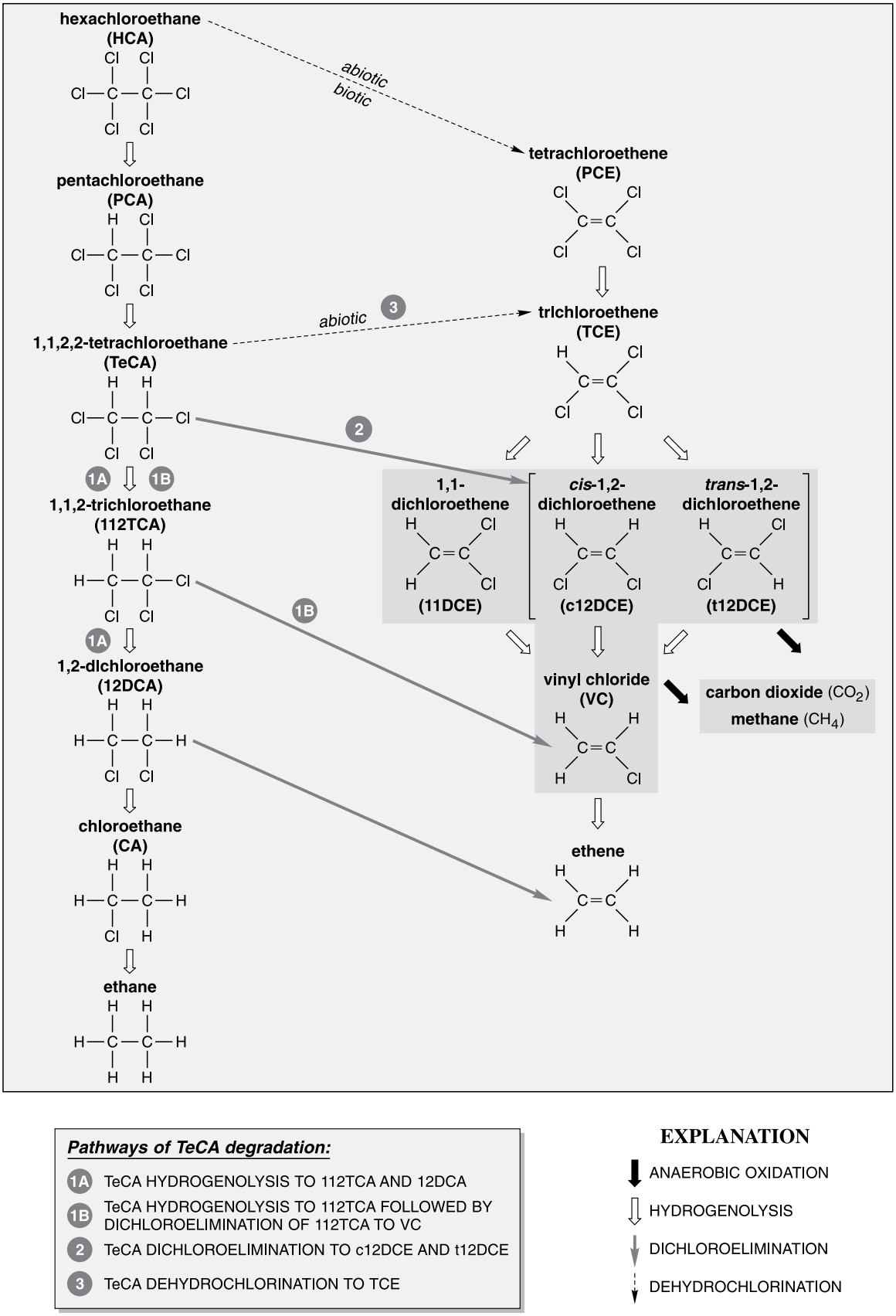


Figure 2a. Anaerobic degradation pathways for major chlorinated alkanes and alkenes observed in ground water in the West Branch Canal Creek wetland area (modified from Lorah and others, 1997).

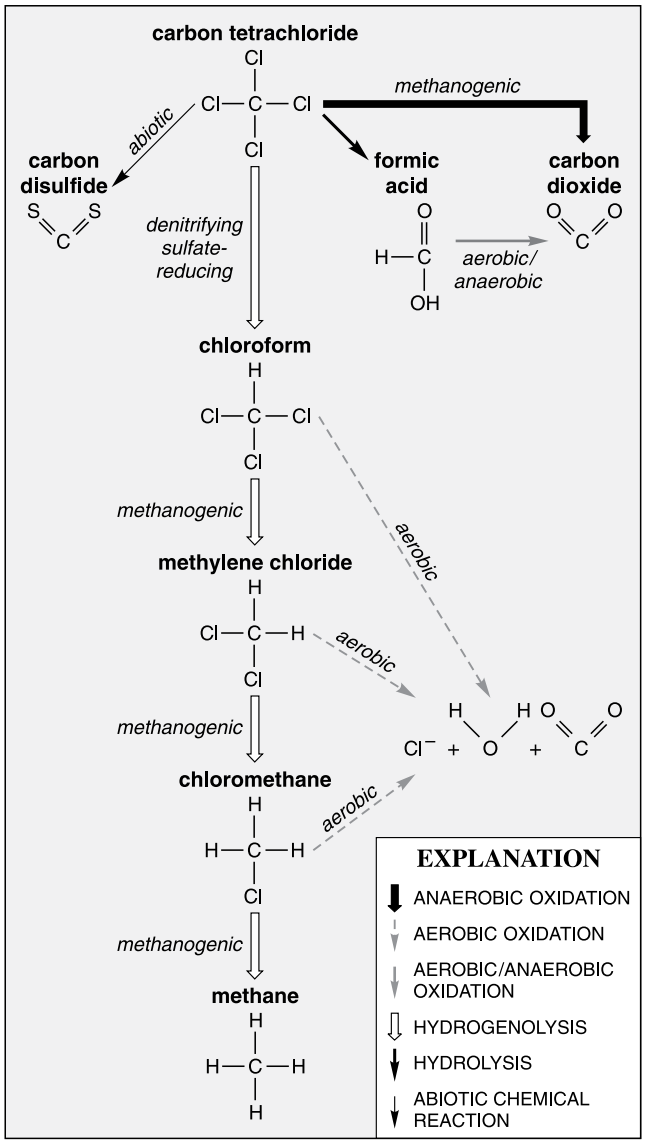


Figure 2b. Anaerobic and aerobic degradation pathways for chlorinated methanes observed in ground water in the West Branch Canal Creek wetland area (from Majcher and others, 2007).

Biostimulation

Biostimulation technologies that have been developed differ primarily in the type and delivery method of an electron donor to support reductive dechlorination of the VOCs in the ground water. Organic substrates can be delivered as a (1) dissolved phase, including volatile fatty acids, sugars, and alcohols; (2) slow-release compound, including vegetable oils and HRC™ (a proprietary polylactate ester); or (3) solid phase, including chitin, mulch and compost, and peat (Parsons Engineering Science, Inc., December 2002). A survey of 93 sites that had pilot- or full-scale-application of enhanced bioremediation by reductive dechlorination found that HRC™, molasses, lactate, and edible vegetable oils were the most commonly used organic substrates (Parsons Engineering Science, Inc., December 2002). Direct application of H_2 gas, a favored substrate of some of the known dehalorespirers characterized to date, also has been investigated for stimulation of *in situ* reductive dechlorination (Newell and others, 2001). Fermentation of organic substrates, however, can also release H_2 and has the advantage of providing carbon for microbial growth and metabolic products to support other microorganisms that directly or indirectly degrade the VOCs. Most reported biostimulation field tests in aquifers have been at sites where PCE or TCE were the major VOCs, although several sites with 1,1,1-trichloroethane or 12DCA have been reported (Dyer and others, 2003).

Delivery systems for dissolved phase donors most often have consisted of multiple injection wells installed in a line to traverse the plume, or of single or multiple injection and extraction wells installed in various recirculation configurations (McCarty, 2002). Recirculation has been used to assist in hydraulic control and mixing of the substrates with the contaminated ground water. Similar methods have been used for delivery of slow-release compounds, but generally involve single or less-frequent applications than the continuous or pulsed injections generally used for delivery of dissolved phase donors.

Solid phase delivery systems that have been tested include emplacement of mulch, compost, or peat in permeable reactive barriers, which are installed perpendicular to the direction of ground-water flow in the aquifer (Parsons Engineering Science, Inc., December 2002). Peat provided a source of primary substrates for reductive dechlorination of PCE and TCE in anaerobic laboratory microcosms that were inoculated with aquifer sediments or activated sludges and used to test the concept of a peat biobarrier in aquifers (Kao and Lei, 2000). Although complete PCE and TCE removal was observed in the anaerobic microcosms with peat, degradation of the daughter product VC was relatively slow (Kao and Lei, 2000). Peat biobarriers also have been tested in the laboratory as a technology for degradation of benzene (Yerushalmi and Guiot, 2001) and gasoline (Yerushalmi and others, 1999) under low dissolved-oxygen conditions. Recent microcosm experiments were used to test a similar biobarrier concept for bioremediation of PCE, where sludge cake from domestic wastewater treatment and cane molasses from sugar industry waste were used as the organic substrates (Kao and others, 2003). In a field pilot test in a shallow TCE-contaminated aquifer at Offutt Air Force Base, Nebraska, a tree and leaf mulch generated onsite was mixed with sand (50:50 ratio) and installed in a 1-ft (foot)-thick, 23-ft-deep, and 100-ft-long biobarrier (Aziz and others, 2001). Reducing conditions were generated in the initially aerobic aquifer downgradient of the biobarrier. A mean TCE removal of 70 percent was observed downgradient of the biobarrier over a 31-month test period, and production of 12DCE occurred (Aziz and others, 2001; Gonzales and others, 2002). Later sampling, however, showed a substantial decrease in the removal efficiency of

the mulch biobarrier. In a second field pilot test at Offutt Air Force Base, mulch was placed as a surface amendment, relying on recharge water to leach organic substrates from the mulch and into the contaminated shallow ground water. Reducing conditions in the ground water downgradient of the mulch surface amendment did not proceed past nitrate reduction, however, and TCE reductive dechlorination was not significantly greater than losses in a control plot from natural attenuation (Groundwater Services, Inc., 2001).

Bioaugmentation

Bioaugmentation for reductive dechlorination of chlorinated solvents has been investigated in laboratory studies and in several pilot-scale tests in aquifers (Ellis and others, 2000), including a sand aquifer near a ground-water/surface-water interface by Lake Huron (Lendvay and others, 2003). Interest in bioaugmentation for anaerobic remediation of chlorinated solvents was initiated by the identification of bacteria able to obtain energy for growth by the reduction of the chlorinated ethenes PCE, TCE, and 12DCE (Maymó-Gatell and others, 1997). More recently, bacteria that can gain energy from reduction of 12DCE and VC to ethene have been identified, including the *Dehalococcoides* species strain VS (Müeller and others, 2004) and *Dehalococcoides* isolate BAV1 (He and others, 2003). A mixed microbial consortium typically has been used in bioaugmentation applications for chlorinated VOCs to achieve complete dechlorination to ethene, ethane, or other non-chlorinated compounds.

The first successful pilot test of bioaugmentation for anaerobic degradation of chlorinated ethenes was conducted at a TCE-contaminated aquifer at Dover Air Force Base, Delaware, using the Pinellas culture (Ellis and others, 2000). The Pinellas culture was enriched from the Department of Energy's Pinellas site in Largo, Florida, and contains *Dehalococcoides ethenogenes*. Lactate was first injected into the initially aerobic aquifer to obtain reducing conditions; however, TCE was only reduced to 12DCE in this biostimulation phase. After addition of the Pinellas culture to the pilot test site, TCE dechlorination to VC and ethene was observed (Ellis and others, 2000). Another mixed culture containing *Dehalococcoides ethenogenes* and closely related strains was enriched from a TCE-contaminated site in Ontario, Canada and designated as KB-1. KB-1 has been used successfully in field tests of bioaugmentation (Duhamel and others, 2002; Major and others, 2002). In a pilot test in an aquifer at Kelly Air Force Base, Texas, PCE and TCE were not degraded past 12DCE after 173 days of addition of electron donors, but were degraded completely after bioaugmentation with KB-1. Genetic testing of bacteria in ground water and soil samples from the bioaugmented plot showed that the *Dehalococcoides* sp. or spp. from the culture were distributed throughout the site (Major and others, 2002). Lendvay and others (2003) conducted a comparison of bioaugmentation and biostimulation in a PCE-contaminated plume using side-by-side test plots in a sand aquifer at the Bachman Road Residential Wells Site. A sediment-free, anaerobic dechlorinating culture was enriched from aquifer sediment collected at different locations and depths inside the plume and used for bioaugmentation at the site. Bioagumentation increased the *Dehalococcoides* population by at least an order of magnitude and caused a three-fold decrease in the time required to achieve PCE dechlorination compared to the biostimulation plot (Lendvay and others, 2003).

A pilot test of bioaugmentation for *in situ* remediation of CT in ground water under nitrate-reducing conditions was done using *Pseudomonas stutzeri* KC from a culture grown aerobically on site (Dybas and others, 1998). When a pH greater than 7.6 and effective acetate delivery was maintained in the test grid, CT concentrations in the ground water decreased by about 65 percent without significant accumulation of chlorinated daughter VOCs. Initial laboratory column experiments showed that biostimulation with acetate by itself (without injection of *Pseudomonas stutzeri* KC) was not effective in decreasing CT concentrations in sediments from the Schoolcraft, Michigan test site (Dybas and others, 1998).

No reports of bioaugmentation field tests for the chlorinated alkanes were found, although bench tests with ground water contaminated with 1,2-dichloroethane (12DCA) have been reported (De Wildeman, Diekert, and others, 2003; De Wildeman and others, 2004). These lab experiments utilized an anaerobic bacterium *Desulfitobacterium dichloroeliminans* strain DCA1 that was recently isolated from sediment beneath an industrial storage tank containing 12DCA and produced in 10-L (liter) bottles (De Wildeman and others, 2004). *Desulfitobacterium dichloroeliminans* is the only reported dehalorespirer that completely dechlorinates 12DCA by dichloroelimination to ethene (fig. 2), although *Dehlaococcoides ethenogenes* converts 12DCA to about 99 percent ethene and 1 percent VC (Maymó-Gatell and others, 1999). Higher chlorinated alkanes, including TeCA and 1,1,2-trichloroethane (112TCA), however, were not degraded by this bacterium (De Wildeman, Diekert, and others, 2003). In laboratory microcosms containing ground water at a neutral pH, bioaugmentation with 5 percent by volume of *Desulfitobacterium dichloroeliminans* strain DCA1 completely degraded 40 mg/L (milligrams per liter) of 12DCA to ethene within 3 to 14 days at temperatures between 13 and 28°C (degrees Celsius).

Constructed Wetlands

Interest in the use of constructed wetlands for remediation of chlorinated VOCs grew after the natural attenuation study in the West Branch Canal Creek wetland area showed complete removal of the contaminants before discharge to land surface or a tidal creek occurred (Lorah and others, 1997; Lorah and Olsen, 1999a, 1999b). The efficiency of natural attenuation indicated that upflow treatment wetlands could be constructed to intercept chlorinated solvent plumes in areas where wetlands were not naturally present (Ferraro

and others, 2000; Pardue and others, 2000). Bench-scale soil column experiments were conducted to investigate potential peat substrate mixtures for use in a pilot-scale treatment wetland for a Superfund site in Connecticut (Kassenga and others, 2003). A mixture of sand, commercial peat, and BionSoil (a compost derived from dairy waste) was determined to show the most suitable hydraulic properties and the highest degradation rates for a mixture of chlorinated ethenes and ethanes compared to a peat-sand mixture (Kassenga and others, 2003). Contaminated ground water from the site was used in this study to seed the peat substrate mixture with microorganisms capable of degrading the chlorinated VOCs.

Several pilot-scale treatment wetlands for chlorinated VOC ground-water plumes have been reported, although information documenting the processes operating in these wetland systems and their performance is lacking. A passive upflow wetland system with four parallel treatment cells has been operating since October 1998 in Hillsdale, Michigan, to intercept ground water contaminated with metal-cleaning solvents (Haberl and others, 2003). Two additional constructed wetlands (one full-scale) at Fort Edward, New York, and one at Offutt Air Force Base, Nebraska, are cited for treatment of chlorinated VOCs (Interstate Technology and Regulatory Council, 2003). A full-scale reconstruction of a wetland along the shore of a lake in Minnesota was reported as a remediation design for ground-water discharge that contained low concentrations of chlorinated VOCs (Richard and others, 2001). Two field-scale, vertical-flow constructed wetland cells were constructed in 2000 at Wright-Patterson Air Force Base, Ohio to treat ground water that contained low concentrations of chlorinated VOCs (Clemmer, 2003; BonDurant, 2004). Bioaugmentation to enhance microbial degradation was not used in construction of these reported treatment wetlands.

Background on Ground-Water Contaminants and Anaerobic Degradation Processes

Numerous manufacturing and research and development plants for chemical warfare agents and explosives once operated in the uplands east of West Branch Canal Creek, and used chlorinated solvents extensively as raw products in some manufacturing processes, decontaminating agents, cleaners, and degreasers (Lorah and Clark, 1996). The initial natural attenuation study in the West Branch Canal Creek wetland area focused on extensive characterization of the contaminant distribution and natural attenuation processes along two transects through the wetlands area (A–A′ and C–C′ transects in fig. 1c) (Lorah and others, 1997; Lorah and Olsen, 1999a, 1999b; Phelan and others, 2002). TeCA and TCE were the major parent contaminants in the aquifer on the eastern side of the creek along these transects. TeCA concentrations in the aquifer ranged from about 100 to 4,000 µg/L (micrograms per liter), whereas TCE concentrations were in the range of 50 to 500 µg/L along the eastern side of the transects (Phelan and others, 2002). CT and CF were present in this area in lower concentrations, ranging from 10 to 100 µg/L (Phelan and others, 2002). Along the section of the A transect extending on the western side of the creek (fig. 1c), however, CT and CF concentrations were as high as 2,000 to 3,000 µg/L in the aquifer (Phelan and others, 2002). To assist in defining the areal extent of contamination within the wetland area, additional drilling and ground-water-quality profiling was done along the creek channel south of the A transect using the USGS hoverprobe (Phelan, Senus, and Olsen, 2001). This water-quality profiling found concentrations of CT and CF in the aquifer as high as 30,900 and 23,100 µg/L, respectively. Relatively high PCE and hexachloroethane (HCA) concentrations (maximum of 6,500 and 10,000 µg/L) also were characteristic of the CT- and CF-dominated plume found in the aquifer along and south of the A transect (Phelan, Senus, and Olsen, 2001), whereas these compounds had low or undetectable concentrations in the TeCA-dominated plume along the A and C transects (Spencer and others, 2000; Spencer and others, 2002).

In the winter of 2002, the USGS identified 28 seep areas in the West Branch Canal Creek wetland area and began delineation and characterization of these seeps concurrent with bioremediation laboratory testing presented here (Majcher and others, 2007) (fig. 1b). Passive-diffusion (peeper) sampling methods were used to characterize contamination of the shallow ground water at all identified seeps, and six seep sites in Regions 2 and 3 were targeted for additional characterization (fig. 1b). Concentrations of chlorinated VOCs were highest in shallow ground water in seeps in Regions 2 and 3 and included VOCs previously detected in the wetland area. Some seeps in these regions contained a mixture of VOCs similar to those reported from the natural attenuation study in the TeCA-dominated plumes along the A and C transect east of the creek channel, whereas some seeps contained a mixture of VOCs similar to the CT- and CF-dominated plume previously identified. Within Region 3, samples analyzed from seep 3–4W contained the most significant concentrations of chlorinated VOCs in the shallow ground-water investigation during 2002, which were confirmed in 2003. Shallow ground-water contamination in this seep is dominated by CF and CT (both detected in excess of 10,000 µg/L), followed by TCE and PCE (maximum of both VOCs near 4,000 µg/L). Methylene chloride, 12DCE, VC, TeCA, pentachloroethane, and HCA also were detected in the seep area, although concentrations were one to two orders of magnitude lower than these other VOCs. Concentrations of the VOCs in the seep porewater generally do not decrease as land surface is approached. These results differ from what is typically observed in peepers deployed along the transects located just downgradient of monitoring well CC27 in the USGS wetland study area.

All the chlorinated alkanes, alkenes, and methanes observed in ground water of the West Branch wetland area can degrade by reductive dechlorination reactions under anaerobic conditions (figs. 2a,b). Microcosm and enrichment

experiments during 1995–2001 defined some of the factors controlling degradation of TeCA and TCE and evaluated microbial communities associated with these reactions in the wetland sediment (Lorah and Olsen, 1999a; Lorah and others, 2001; Lorah and others, 2003; Lorah and Voytek, 2004). Both TeCA and TCE degradation produced 12DCE and VC as the daughter compounds that showed the greatest transient accumulation in anaerobic microcosms. These daughter products also were the VOCs detected in the highest concentrations in the wetland porewater during the natural attenuation study (Lorah and Olsen, 1999b; Lorah and Voytek, 2004). TCE undergoes hydrogenolysis to sequentially produce 12DCE (predominantly the *cis*- isomer), VC, and ethene, whereas TeCA can undergo both hydrogenolysis and dichloroelimination (also called β-elimination) reactions (fig. 2a). Simultaneous hydrogenolysis and dichloroelimination of chlorinated alkanes was observed in wetland microcosms with TeCA and 112TCA for the West Branch Canal Creek study area (Lorah and Olsen, 1999a; Lorah and others, 2003) and in studies with a key enzyme, methyl-CoM reductase, utilized by methanogens (Holliger and others, 1992). 12DCE, including both the *cis*- and *trans*-isomers, is produced from dichloroelimination of TeCA; VC is produced from dichloroelimination of 112TCA, which is a hydrogenolysis product of TeCA, and from hydrogenolysis of the 12DCE produced by dichloroelimination (fig. 2a). Abiotic dehydrohalogenation of TeCA to TCE (fig. 2a) accounted for only about 3 percent of the TeCA amended to wetland microcosms for the West Branch Canal Creek study area. Other studies with anaerobic pond and stream sediments showed TeCA dichloroelimination to 12DCE as the sole degradation pathway (Jafvert and Wolfe, 1987; Peijnenburg and others, 1998).

Ethene, a non-chlorinated and non-toxic end-product of TeCA and TCE degradation (fig. 2a), was observed infrequently and only in trace quantities in the field and anaerobic laboratory microcosms for the West Branch Canal Creek area, although no chlorinated daughter compounds generally were detectable at the end of microcosm experiments (Lorah and Olsen, 1999a; Lorah and Voytek, 2004). Later enrichment experiments, however, showed that ethene is produced but is rapidly mineralized to carbon dioxide under anaerobic conditions in the wetland sediment (Lorah and others, 2003). Ethene can be produced from hydrogenolysis of 12DCE and from dichloroelimination of 12DCA. Previous microcosm and enrichment studies with wetland sediment from the West Branch Canal Creek have shown transient accumulation of 12DCA without chloroethane production, indicating that the dichloroelimination pathway could be predominant. Production of chloroethane and subsequent rapid hydrogenolysis to ethanol, however, could have occurred (Lorah and Voytek, 2004). Ethene has been reported as the primary anaerobic daughter compound from 12DCA during cometabolic degradation by pure cultures of methanogenic and sulfate-reducing bacteria (Klecka and others, 1998) and of a homoacetogenic bacterium (De Wildeman, Neumann, and others, 2003).

Anaerobic degradation of PCE occurs by hydrogenolysis to TCE, and microbial consortia previously reported to degrade TCE can also degrade PCE. Similarly, the higher chlorinated alkanes detected in the seep porewater, including pentachloroethane and HCA, have been reported to degrade by hydrogenolysis and dichlorelimination reactions, as observed for TeCA (Schanke and Wackett, 1992; Miller and others, 1998; van Eekert and others, 1999) (fig. 2a). Pentachloroethane and HCA dichloroelimination in microcosms with anaerobic lake water produced TCE and PCE, respectively, as dominant daughter products (Miller and others, 1998). Hydrogenolysis of the pentachloroethane and HCA also occurred, producing sequentially lower chlorinated alkanes. As observed with TeCA degradation in anaerobic wetland sediments at APG (Lorah and Olsen, 1999a), abiotic dehydrohalogenation of pentachloroethane and hexachloroethane was a minor reaction in these lake water experiments (Miller and others, 1998). The higher chlorinated VOCs have a higher oxidation state and thus should degrade more rapidly by reductive dechlorination reactions than TCE or TeCA (Vogel and others, 1987; van Eekert and others, 1999). Development of the dechlorinating consortia in this study, therefore, focused on degradation of TeCA and its anaerobic daughter products, including 12DCE and VC, which appeared to be rate-limiting in previous studies.

The chlorinated methane CT also degrades by hydrogenolysis reactions, sequentially producing CF, methylene chloride (also called dichloromethane), and ultimately methane (fig. 2b). Since CT and CF are known to have been used and disposed of together during manufacturing operations in the Canal Creek area at APG (Lorah and others, 1997), the high CF observed in the aquifer and seep porewater in the wetland area likely represents a parent contaminant, rather than a degradation product. Acetogenic bacteria and methanogenic *Methanosarcina* strains have been shown to generate carbon dioxide as a primary end-product of CT and CF hydrogenolysis, rather than methane (Egli and others, 1988; Mikesell and Boyd, 1990). Chloromethane generally is not observed as an intermediate of methylene chloride or occurs only in trace concentrations (Davis and others, 2003). Reductive dechlorination of CT can be microbially mediated or occur abiotically in the presence of ZVI or microbial cofactors such as vitamin B_{12} under anaerobic conditions (Davis and others, 2003). Methanogens and some acetogens that have been shown to be capable of rapid CT degradation naturally contain elevated levels of vitamin B_{12}. Although abiotic reductive hydrolysis of CT is slow in typical groundwater solutions, laboratory studies have shown that abiotic degradation rates can be greatly enhanced in the presence of iron- and sulfide-bearing compounds and minerals and in the presence of ferrous iron and (or) humic acid (Devlin and Müller, 1999; Davis and others, 2003). Cultures of *Methanosarcina thermophila* secreted an extracellular factor that resulted in a greatly enhanced ability to degrade CT and

CF by hydrogenolysis when grown on elemental iron (Novak and others, 1998). In laboratory and field experiments under sulfate-reducing conditions and in the presence of active iron sulfide precipitation, CT transformation to CF and carbon disulfide (CS_2) occurred (Devlin and Müller, 1999). Other potential non-chlorinated degradation products of CT reduction include carbon monoxide and formate through a reductive elimination reaction (Davis and others, 2003) (fig. 2b). Microbially mediated and abiotic degradation of CT and CF were examined in this current bioremediation study.

Methods

Enrichment of wetland sediment for development of the dechlorinating consortia, WBC-1 and WBC-2, was done at the same time as the biostimulation microcosms were conducted in 2002 and 2003, respectively (fig. 3, table 1). Anaerobic microcosm and column experiments were performed by USGS personnel in Baltimore, Maryland, whereas enrichment experiments and microbial community analyses were performed by USGS National Research Program personnel in Reston, Virginia. Initial biostimulation experiments were designed to test the effect of nutrient stimulation on anaerobic VOC degradation (table 1), because limiting nutrient concentrations were hypothesized to cause slower degradation rates measured previously in microcosms with wetland sediment collected during the summer (Lorah and others, 2003). The high organic carbon content of the natural wetland sediments indicated that substrate (electron donor) concentrations may not be a limiting factor. Initial substrate testing was instead focused on tests with the consortia during their development to select the most suitable for growth and maintenance. Limited testing with organic carbon (lactate) addition was later done in microcosms (table 1) and compared to previous experiments done with methanol-amended microcosms (Lorah and others, 2003). Anaerobic microcosms to test the effect of bioaugmentation were done with wetland sediment in 2003 and with potential reactive mat materials in 2003 and 2004 (table 1). Column experiments were begun in 2003 after the first series of microcosms with a bioaugmented peat/compost mixture. Due to difficulties later encountered with availability of the BionSoil compost selected for

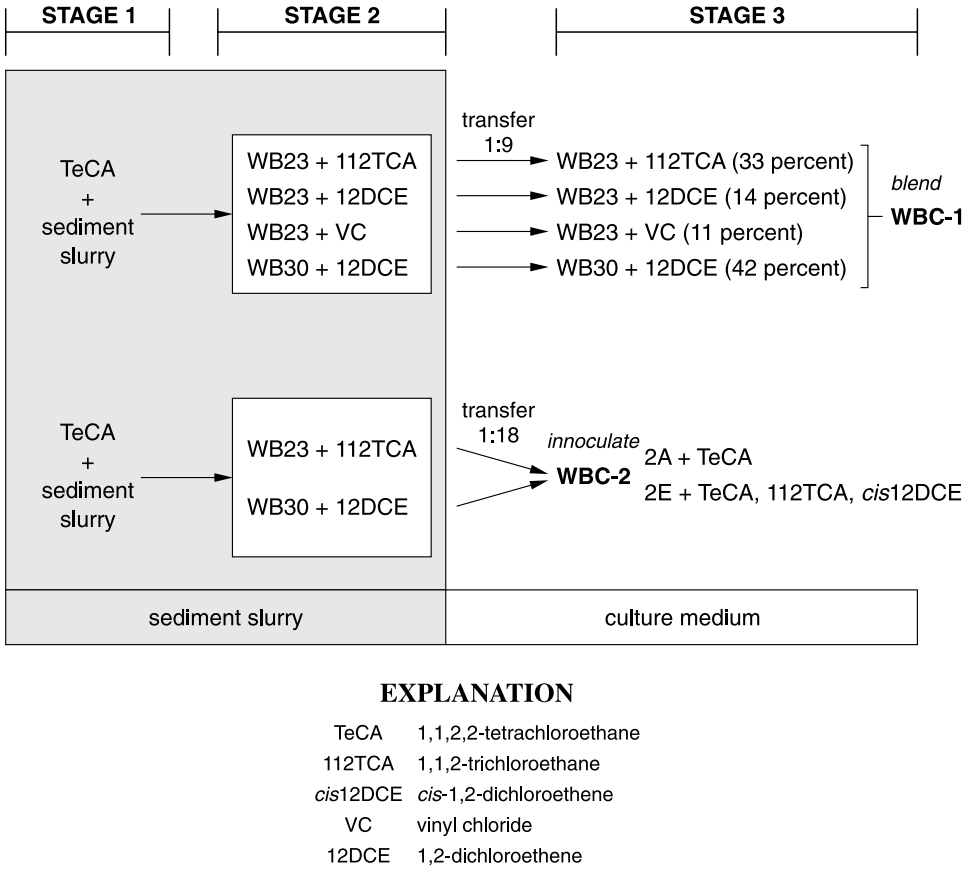


Figure 3. Schematic of West Branch Consortia (WBC-1 and WBC-2) development methods.

testing in these first microcosms and column experiments, additional microcosms were conducted in 2004 to compare biodegradation in bioagumented BionSoil/peat mixtures with other selected compost mixtures (tables 1 and 2). General microcosm preparation and incubation methods were the same in the 2002–04 experiments as those methods reported previously for anaerobic microcosms (Lorah and others, 1997; Lorah and others, 2003), but details for specific treatments are given here. Geochemical analyses of water samples collected from the microcosm and column experiments were the same as those reported for previous microcosm and ground-water sample analyses (Spencer and others, 2000; Lorah and others, 2003; Lorah, Spencer, and McGinty, 2005). Geochemical microbial analyses conducted at the Reston USGS laboratory are the same as those previously reported (Lorah and others, 2003; Lorah and Voytek, 2004).

Microbial Consortia Development

The microbial consortia were developed using an enrichment process, where original sediment/ground-water slurry is enriched in microbial species capable of degrading the target contaminants by repeated amendments with the contaminants and by sequential dilutions into anaerobic culture medium to decrease the amount of sediment remaining. After repeated incubation with the contaminants and transfers to culture medium, a mostly sediment-free matrix composed of microbial biomass suspended in culture medium can be achieved. A sediment-free matrix is desirable to decrease growth of non-dechlorinating microorganisms that could utilize substrates from the sediment and compete with the dechlorinating organisms, and to assist in propagating stable large volumes of culture for field bioaugmentation applications. This enrichment process reduces the different types of microbes in the original wetland sediment until the species remaining are those most likely to be directly or indirectly involved in degradation of these contaminants. Other microbial species die during the enrichment process because TeCA or its daughter products are toxic to them, the electron donor or acceptors that they require are not available in the culture medium, or the anaerobic environment is not suitable for them.

To begin the enrichment process for WBC-1 and WBC-2 development, wetland sediment was collected from sites WB23 and WB30 within the natural attenuation study area of the West Branch Canal Creek area (fig. 1c) and prepared using the same methods as those for anaerobic microcosms reported below and in previous studies (Lorah and Olsen, 1999a; Lorah and others, 2003; Lorah and Voytek, 2004). Sediments for WBC-1 and WBC-2 were collected in May 2002 and March 2003, respectively. Sediment from each site was mixed with wetland ground water collected from piezometer WB24B (fig. 1c), amended with TeCA (7.2 μM/micromolar, or 1,200 μg/L), and incubated anaerobically for 1 month in duplicate 1-L serum bottles without headspace (fig. 3). Aliquots (100 mL/milliliters) of the most active sediment slurries were transferred to 160-mL serum bottles with a nitrogen headspace, amended with selected daughter compounds of TeCA for 1 to 2 months, and then transferred to 100-mL anaerobic culture medium. These initial culture medium bottles contained 10 percent by volume of the sediment slurry. The culture medium consists of a bicarbonate solution (2.5 g/L (grams per liter) $NaHCO_3$) to buffer to pH of 6.8, and added nutrients (0.5 g/L of NH_4Cl and $NaPO_4$), trace minerals, and vitamins that are commonly needed for microbial growth.

WBC-1 was prepared using a two-step process that was considered most likely to ensure that each step of both the hydrogenolysis and dichloroelimination pathways to degrade TeCA would be enriched (figs. 2a and 3). Before transfer to culture medium, slurries used to prepare WBC-1 were enriched using *cis* and *trans*12DCE (multiple feedings totaling 50 and 90 μM, respectively), 112TCA (8 μM), or VC (3.4 μM). The same compound used for slurry enrichment was added to the culture medium bottles after transfer to culture medium (fig. 3). Acetate (5 mM/millimolar) and H_2 (60 kPa/kilopascal overpressure) were added to the culture medium bottles as substrates. After incubating these culture enrichments for 2 to 3 months, the bottles were combined to form WBC-1 (fig. 3). WBC-1 was maintained by feeding TeCA (25 μM), acetate (5 mM), and H_2 (20 kPa added to the headspace approximately every other day); acetate was added only periodically as a carbon source after the first 2 months. Batches of WBC-1 were maintained in the Reston USGS laboratory in lots of 1 L. WBC-1 was transferred with a 10-percent inoculum to fresh medium 3 months after its formation; thereafter, fresh medium was added to WBC-1 only to replace culture volumes removed for activity tests or microcosms.

A simplified procedure was used during development of WBC-2 using only two types of sediment slurry enrichments— a slurry of WB30 sediment incubated with 12DCE and a slurry of WB23 sediment incubated with 112TCA (fig. 3). These two types of sediment slurry enrichments were selected on the basis of previous results demonstrating that 12DCE-reducing organisms were most readily enriched from WB30 sediment, whereas 112TCA- and VC-reducing organisms were most readily enriched from WB23 (Lorah and others, 2003). Inocula from these enriched slurries were mixed directly during the first transfer to culture medium, unlike WBC-1, where slurries were further enriched in separate batches of media with a single TeCA daughter compound before being combined into the mixed consortium. WBC-2 was maintained by feeding lactate (1.5 mM) as a carbon/electron donor substrate and adding TeCA (25 μM), 112TCA (50 μM), and 12DCE (50 μM) 2 times a week. Batches of WBC-2 were maintained in the Reston USGS laboratory in lots of 1-2 L. Some 2-L batches of WBC-2 containing 1 to 5 percent sediment were stored long-term (1 year or more) at 19°C with monthly addition of only TeCA (50 μM). These cultures could be returned to full activity within 2 weeks by increasing the feeding rate (refeeding at

each depletion) and adding electron donor (1 mM lactate). WBC-2 was not transferred after initial development (as WBC-1 was after 3 months) but was instead allowed to grow relatively slowly. WBC-2 was diluted only by replacing medium when culture volumes were removed for experiments. In general, cultures were diluted by no more than 50 percent at any one time, although occasionally a transfer of 10 or 20 percent to fresh medium was made.

After initial development and testing by the USGS, portions of each consortium were sent to SiREM Laboratories, a wholly owned subsidiary of Geosyntec Consultants, located in Guelph, Ontario, Canada (Geosyntec Consultants, Inc., 2004). SiREM Laboratories was contracted by the U.S. Army to further propagate the cultures to quantities required for USGS field tests. Three 100-mL vials of WBC-1 were sent to SiREM laboratory in April 2003, and two vials of WBC-2 culture were sent in August 2003. All culture activity tests and microcosms reported here were conducted using batches of the consortia maintained in the Reston USGS laboratory, although the columns were bioaugmented initially with batches of consortia received from SiREM laboratories.

Dechlorinating Activity Characterization

Dechlorinating activity of the consortium was monitored after development by analysis of the headspace over the cultures for VOCs and methane. Headspace was sampled using a gas-tight syringe and injected into one of three gas chromatography (GC) systems at the Reston USGS laboratory. The WBC-1 samples were analyzed for methane, ethene, ethane, TeCA, 112TCA, 12DCA, TCE, the total of *cis*12DCE and *trans*12DCE, and VC using an Antek GC with a Flame Ionization Detector (FID) and a glass column packed with Supelco 60/80 Carbopack C, with 0.3 percent carbowax 20M/0.1 percent H_3PO_4 at 40°C ramping to 100°C after 3 minutes at 30°C per minute. WBC-2 samples were analyzed for TeCA, 112TCA, 12DCA, TCE, *cis*12DCE and *trans*12DCE using a Hewlett-Packard model 5890 series II GC/FID after separation at 100°C on a VOCOL (Supelco) capillary column (30 m/meters X 0.53 mm/millimeters). Methane, ethene, ethane, and VC in WBC-2 were analyzed using a Shimadzu model GC-17A with FID after separation on a Rt Q-Plot (Restek) column (30m X 0.32 mm) with a temperature program of 100°C for 5 min, ramping to 200°C at 20°C/min. Headspace concentrations were converted to dissolved concentrations of VOC by applying Henry's law.

The stimulation of dechlorination by electron donors was tested on both WBC-1 and WBC-2 immediately after consortium development. All electron donor tests were performed in duplicate on sub-samples removed from the primary culture vessels. Aliquots (10 mL) of the cultures were transferred anaerobically to 28-mL pressure tubes (Bellco Glass) filled with N_2/CO_2 (80:20). WBC-1 was evaluated for dechlorination of VOCs in three electron donor treatments: H_2 (60 kPa), acetate (5 mM), or no electron donor. The VOCs tested for each electron donor included TeCA (44 μM), 112TCA (164 μM), *cis*12DCE (750 μM), *trans*12DCE (190 μM), added as a vapor from a headspace equilibrated with neat solvent, or VC (4.2 μM) from a pressurized cylinder. WBC-2 was evaluated for dechlorination of VOCs in the following electron donor treatments: propionate (10 mM); succinate (3 mM); lactate (3mM); pyruvate (3 mM); yeast extract (2 g/L) plus proteose peptone (5 g/L); benzoate (3 mM); formate (10 mM), with or without yeast extract (0.5 g/L) added as a carbon source; acetate (10 mM); H_2 (20 kPa overpressure, added 3 times during the incubation) and with or without acetate (1 mM) added as a carbon source; whey (5 g/L); and a control with no electron donor added. VOCs tested included *cis*12DCE (approximately 1 mM), 112TCA (approximately 0.5 mM), or VC (4.2 μM). All treatments were monitored by sampling the headspace for analysis by gas chromatography/flame ionization detector (GC/FID), as described above. Due to the poor partitioning of TeCA into the headspace, water samples from the TeCA treatments for WBC-1 also were analyzed using GC/MS in the Baltimore USGS laboratory.

Cells were harvested by transferring culture to bottles flushed with N_2/CO_2 (80:20), centrifuging, and washing the pellet with anaerobic bicarbonate buffer (2.5 g/L $NaHCO_3$ sparged with N_2/CO_2, 80:20). The resulting pellet of cells was homogenized and resuspended (0.05 – 0.5 mL) in 10-mL anaerobic bicarbonate buffer to achieve cell densities varying from 0.5 to 5 times the original cell density of the culture in separate batch tests. For each cell density and for a control without cells, 112TCA, *cis*12DCE, or *trans*12DCE was added as a vapor from headspace equilibrated with neat solvent. H_2 (60 kPa) was added as an electron donor. The cell suspensions were incubated at 19°C and headspace samples were collected for measurement of chlorinated VOCs and methane (see below).

Molecular Characterization

Immediately after consortia development and transfer to medium, sub-samples were taken for molecular characterization of the microbial community. Cultures were monitored over time and through subsequent transfers to fresh medium. Bacterial and methanogen communities were characterized using a polymerase chain reaction (PCR) procedure of amplifying targeted DNA (16S rDNA and *mcr*A for bacteria and methanogens, respectively) and performing terminal restriction fragment length polymorphism (TRFLP) analysis on the PCR products, as previously reported for wetland sediment and microcosm slurry analyses (Lorah and others, 2003; Lorah and Voytek, 2004). Primers specific for *Dehalococcoides* spp. and dechlorinating strains of *Desulfuromonas* were used to determine the abundance of these two known reductive dechlorinating bacteria in the consortium by quantitative PCR analysis (qPCR). DNA copy number was determined by real time PCR using the quantitect SYBR green real time PCR kit (Qiagen, Chatsworth, California) and the Opticon real time PCR system

Table 1. Treatments used in microcosm experiments 2002–04.

All matrices were mixed with WB24B wetland ground water. All composts were mixed with commercial peat and sand, respectively, in ratios of 40:40:20 by volume before mixing with WB24B water. Where more than one matrix was tested in separate treatments in an experiment, the different site numbers where the wetland sediment was collected or the different compost names are listed in the "Matrix" column, and indicated in brackets in the experiment name. See Table 2 for descriptions of the composts. For microcosms amended with volatile organic compounds (VOCs), the planned concentrations (at day 0) are indicated in micrograms per liter. TeCA, 1,1,2,2-tetrachloroethane; CF, chloroform; CT, carbon tetrachloride; VOC mix, a mixture added to give planned day 0 concentrations (in micrograms per liter) of about 1,500 TeCA, 1,200 1,1,2-trichloroethane, 600 *trans*-1,2-dichloroethene, 700 *cis*-1,2-dichloroethene, 600 CT, and 400 CF; %, percent; --, not applicable. Nutrient amendment consisted of 0.2 g/L (grams per liter) ammonium phosphate monobasic. All culture used for the bioaugmented experiments was grown and maintained in Reston USGS laboratory.]

Experiment type	Experiment name	Experiment date	Matrix	Planned VOC (day 0)	Amendments	Culture used
Wetland sediment	[23,30,35]T.6/02	June-July 2002	WB23, WB30, WB35	TeCA (1,200)	--	--
Wetland sediment	[23,30,35]T.9/02	Sept-Oct 2002	WB23, WB30, WB35	TeCA (1,200)	--	--
Wetland sediment	[23,30,35]T.3/03	March-April 2003	WB23, WB30, WB35	TeCA (1,500)	--	--
Wetland sediment/compost	BT.9/03	Sept-Oct 2003	Bion	TeCA (1,500)	--	--
Nutrient biostimulation	[23,30,35]TN.6/02	June-July 2002	WB23, WB30, WB35	TeCA (1,200)	nutrient	--
Nutrient biostimulation	[23,30,35]TN.9/02	Sept-Oct 2002	WB23, WB30, WB35	TeCA (1,200)	nutrient	--
Donor biostimulation	23TO.9/03	Sept-Oct 2003	WB23	TeCA (1,500)	lactate	--
Donor biostimulation	BTO.9/03	Sept-Oct 2003	Bion	TeCA (1,500)	lactate	--
Co-contaminant	[23,30]CT.6/02	June-July 2002	WB23, WB30	CT (500)	--	--
Bioaugmentation/co-contaminant	BTVC2.9/03	Sept-Oct 2003	Bion	TeCA (1,500)+ VOC mix	--	WBC2 (first mix)
Bioaugmentation	[23,30,35]TC1.9/02	Sept-Oct 2002	WB23, WB30, WB35	TeCA (1,200)	--	WBC1 (first mix)
Bioaugmentation	[23,30,35]TC1.3/03	March-April 2003	WB23, WB30, WB35	TeCA (1,500)	--	WBC1 (first mix)
Bioaugmentation	BTC1.9/03	Sept-Oct 2003	Bion	TeCA (1,500)	--	WBC1
Bioaugmentation	BTC2.9/03	Sept-Oct 2003	Bion	TeCA (1,500)	--	WBC2 (first mix)
Bioaugmentation	BT2C2.9/03	Sept-Oct 2003	Bion	high TeCA (15,000)	--	WBC2 (first mix)
Bioaugmentation	[B,Pa,Tlc,Po,Lg,Cr] TC2.7/04	July 2004	Bion, Paygro, TLC, Poultry, Leafgro, Crab	TeCA (1,500)	--	WBC2
Bioaugmentation (retest)	TlcTC2.7/04	August 2004	TLC	TeCA (1,500)	--	WBC2
Media control	[30,35]TM.9/02	Sept-Oct 2002	WB23, WB30, WB35	TeCA (1,200)	culture media	--
Media control	[23,30,35]TM.3/03	March-April 2003	WB23, WB30, WB35	TeCA (1,500)	culture media	--
Media control	BTM.9/03	Sept-Oct 2003	Bion	TeCA (1,500)	culture media	--

Table 1. Treatments used in microcosm experiments 2002–04.—Continued

All matrices were mixed with WB24B wetland ground water. All composts were mixed with commercial peat and sand, respectively, in ratios of 40:40:20 by volume before mixing with WB24B water. Where more than one matrix was tested in separate treatments in an experiment, the different site numbers where the wetland sediment was collected or the different compost names are listed in the "Matrix" column, and indicated in brackets in the experiment name. See Table 2 for descriptions of the composts. For microcosms amended with volatile organic compounds (VOCs), the planned concentrations (at day 0) are indicated in micrograms per liter. TeCA, 1,1,2,2-tetrachloroethane; CF, chloroform; CT, carbon tetrachloride; VOC mix, a mixture added to give planned day 0 concentrations (in micrograms per liter) of about 1,500 TeCA, 1,200 1,1,2-trichloroethane, 600 *trans*-1,2-dichloroethene, 700 *cis*-1,2-dichloroethene, 600 CT, and 400 CF; %, percent; --, not applicable. Nutrient amendment consisted of 0.2 g/L (grams per liter) ammonium phosphate monobasic. All culture used for the bioaugmented experiments was grown and maintained in Reston USGS laboratory.]

Experiment type	Experiment name	Experiment date	Matrix	Planned VOC (day 0)	Amendments	Culture used
Live control	[23,30,35]LC.6/02	June-July 2002	WB23, WB30, WB35	none	--	--
Live control	[23,30,35]LC.9/02	Sept-Oct 2002	WB23, WB30, WB35	none	--	--
Live control	[23,30,35]LC.3/03	March-April 2003	WB23, WB30, WB35	none	--	--
Live control	BLCC2.9/03	Sept-Oct 2003	Bion	none	--	WBC2 (first mix)
Live control	[Le,Cr]LC.8/04	August 2004	Leafgro, Crab	none	--	--
Sterile, wetland sediment	S[23,30,35]T.6/02	June-July 2002	WB23, WB30, WB35	TeCA (1,200)	1 % formaldehyde	--
Sterile, wetland sediment	S[23,30,35]T.9/02	Sept-Oct 2002	WB23, WB30, WB35	TeCA (1,200)	1 % formaldehyde	--
Sterile, nutrient biostimulation	S[23,30,35]TN.6/02	June-July 2002	WB23, WB30, WB35	TeCA (1,200)	nutrient; 1 % formaldehyde	--
Sterile, nutrient biostimulation	S[23,30,35]TN.9/02	Sept-Oct 2002	WB23, WB30, WB35	TeCA (1,200)	nutrient; 1 % formaldehyde	--
Sterile, co-contaminant	S30TCF.6/02	June-July 2002	WB30	TeCA (1,200) +CF (50)	1 % formaldehyde	--
Sterile, co-contaminant	S[23,30]CT.6/02	June-July 2002	WB23, WB30	CT (500) (AE)	1 % formaldehyde	--
Sterile, bioaugmentation	S[23,30]CT.9/02	Sept-Oct 2002	WB23, WB30	TeCA (1,200)	1 % formaldehyde	WBC1 (first mix)
Sterile, bioaugmentation	SBTC1.9/03	Sept-Oct 2003	Bion	TeCA (1,500)	1 % formaldehyde	WBC1
Sterile, bioaugmentation	SBTC2.9/03	Sept-Oct 2003	Bion	TeCA (1,500)	1 % formaldehyde	WBC2 (first mix)
Sterile, bioaugmentation	SBT2C2.9/03	Sept-Oct 2003	Bion	high TeCA (15,000)	1 % formaldehyde	WBC2 (first mix)
Sterile, compost	S[Le,Cr]TC2.8/04	August 2004	Leafgro, Crab	TeCA (1,500)	1 % formaldehyde	--

Table 2. Characteristics of composts used in microcosm experiments.

[C, carbon; N, nitrogen; P, phosphorus; --, unknown]

Compost name	Source	Moisture content (percent)	pH	Organic matter (percent)	C:N ratio	Total N (percent)	P (percent)
BionSoil (New York)	organic dairy waste	77	6.2	40	--	1	0.5
Chesapeake Green (New Earth Services, Delaware)	poultry, clam shells, wood chips	27	5.5	--	13	1.1	.61
Chesapeake Blue (New Earth Services, Delaware)	crab shells, wood chips	31	7.7	--	12	0.66	.82
Paygro Composted Cow Manure (Missouri)	dairy waste	--	--	--	--	--	--
Tender Loving Compost (TLC) (New York)	dairy waste	--	--	--	--	--	--
Leafgro (Maryland)	municipal yard waste	--	--	--	--	--	--

(MJ Research). The 16S rDNA based primers used to target *Dehalococcoides* were dhc730f, 5'-GCG GTT TTC TAG GTT GTC-3' and dhc1350r, 5'- CAC CTT GCT GAT ATG CGG -3' (Bunge and others, 2001). The *Desulfuromonas* primers (designed for specificity to *Desulfuromonas* sp. strain BB1 and *D. chloroethenica*) and conditions were previously described (Löffler and others, 2000). Methanogens were quantified using *mcr*A primers (Luton and others, 2002). A standard curve was determined using values of serial dilutions of plasmid containing the dhc or *mcr*A amplified fragment, or the *Desulfuromonas* sp. strain BB1 amplicon of known concentration (and thus copy number), and the samples were plotted against that curve to determine abundance. Calculations of cell numbers were based on one 16S rDNA copy per cell for *Dehalococcoides,* and three *mcr*A copies per cell for methanogens.

WBC-1 was cloned and sequenced for molecular characterization immediately after its initial development and transfer to culture medium, whereas WBC-2 was cloned and sequenced 11 months after its development. The culture batch that was used for WBC-1 cloning and sequencing had 10 percent by volume of sediment slurry, whereas the WBC-2 culture batch had about 0.08 percent sediment slurry after 11 months and periodic transfers and dilutions with fresh medium. Microbial members of the consortia were characterized by cloning and sequencing the bacterial 16S rDNA and methanogen *mcr*A amplicons. Amplicons were purified using the wizard PCR purification kit (Promega, Madison, Wisconsin) and cloned using the TA cloning kit or the Topo TA cloning kit for sequencing (Invitrogen, San Diego, California).

Colonies were picked and 16S rRNA and *mcr*A gene clone fragments (133 and 48, respectively) were recovered using vector primers and *mcr*A primers, respectively, using PCR. For the bacterial 16S rDNA characterization, the PCR products were reamplified using 46f and 519r primers. All PCR amplicons were digested with restriction enzymes (6 μL/microliters of PCR product with 2.5U each of MspI and HinPI) according to manufacturer's instructions (Promega, Madison, Wisconsin). Restriction fragments were analyzed by size-separating fragments on a 3.5-percent metaphor agarose gel, and restriction fragment length polymorphism (RFLP) patterns were distinguished and the frequency with which each pattern occurred was determined. It should be noted that the frequency of clones in the library may not correspond directly to relative phylotype numbers in the culture due to differences in the number of 16S rDNA copies per cell. Representative clones for each pattern were selected for sequencing. Amplicons to be sequenced were purified with the wizard PCR purification system (Promega, Madison, Wisconsin), and cycle sequencing was performed on both strands using big dye v3.1 (Applied Biosystems, Foster City, California) and run on a ABI310 genetic analyzer (Applied Biosystems, Foster City, California). Sequences were edited and assembled using Sequencher (Gene Codes, Ann Arbor, Michigan). Closest phylogenetic relatives were determined by BLASTn search of the NCBI database (http://www.ncbi.nlm.nih.gov/BLAST/).

Microcosm Experiments

Laboratory methods used for microcosm and column tests are described in this section.

Biostimulation and Bioaugmentation Tests

Biostimulation experiments to examine nutrient effects on anaerobic degradation of TeCA were conducted with wetland sediment collected before and after peak vegetation growth (early June and September 2002, respectively) (table 1). Saturated wetland sediment from the upper peat unit was collected from a depth of 0 to 25 cm below land surface near three piezometer sites in the natural attenuation study area —WB23, WB30, and WB35 (fig. 1c). The sediment was placed in mason jars with minimum airspace for transport to the laboratory, where the jars were placed in an anaerobic glove box.

All live microcosms were prepared without headspace in 162-mL bottles using a 1.5:1 volumetric ratio of unfiltered wetland ground water (from piezometer WB24B at site WB24, screened at a depth of 92 to 107 cm below land suface) to sediment as described previously (Lorah and others, 1997; Lorah and others, 2003), whereas formaldehyde-killed controls were constructed in 70-mL bottles using the same volumetric ratio. For each sediment, live (no formaldehyde), TeCA-amended microcosms were prepared with and without the addition of nutrients. Nutrient amendment was done by adding ammonium phosphate monobasic to the water during sediment slurry mixing to achieve a concentration of 0.2 g/L. Due to expected losses caused by sorption during the first 24 hr (hours) (Lorah and others, 1997; Lorah and Olsen, 1999a), the microcosms were spiked on day 0 with a TeCA concentration about 2.4 times greater than the desired initial (day 1) aqueous concentration, which was about 3.0 μM, or 500 μg/L. TeCA was added to the microcosms from stock solutions of neat solvent (Supelco, Bellefonte, Pennsylvania) mixed in deionized water (Lorah and others, 2003). Water controls, prepared by placing anaerobic deionized water in 70-mL serum bottles without headspace and amending with TeCA, were used to verify initial concentrations added to microcosms and to monitor for potential volatilization from or sorption to the bottles. Unamended live controls that contained sediment-water slurry but did not contain TeCA, nutrients, or formaldehyde also were prepared for each sediment type in 70-mL serum bottles.

When the bioaugmentation microcosm experiments were conducted in 2003–04, culture media controls also served as nutrient biostimulation controls because the culture media used for WBC-1 and WBC-2 contained similar concentrations of ammonia and phosphate as the nutrient-amended microcosms prepared in 2002 (table 1). These culture media controls were prepared by adding autoclaved culture media that did not contain microorganisms to the sediment-water slurry in the microcosms. Microcosm experiments also were performed to test the effect of substrate (electron donor) biostimulation on TeCA degradation. Lactate was added to the biostimulated microcosms to achieve a concentration of 10 mM. Two sets of substrate-biostimulated microcosms were prepared—one with wetland sediment from site WB23 and one with the organic matrix initially selected for the reactive mat. The organic matrix consisted of a commercially available peat (Mohican Peat) and compost (BionSoil) mixed with sand in a volumetric ratio of 40/40/20 (table 2). This selection was based on column experiments reported by Kassenga and others (2003) where BionSoil mixed with a similar commercial peat provided a suitable matrix for dechlorinating microorganisms contained in contaminated ground water circulated through the columns. Since the compost/peat mixture was not water-saturated, the volumetric ratio of water (from piezometer WB24B) to sediment was adjusted to 3:1 to prepare these microcosms, compared to the 1.5:1 ratio used for microcosms prepared with water-saturated wetland sediment taken from the study area. All bioaugmented microcosms were constructed without headspace in 70-mL serum bottles.

Bioaugmented microcosms were first done in September–October 2002 to test the efficiency of WBC-1 in wetland sediment from the same three sites used for the nutrient biostimulated microcosms (table 1). The microcosms were inoculated by replacing 10 percent of the water volume with the consortium. No organic donor was added to the bioaugmented microcosms because the wetland sediment or compost/peat mixtures were expected to provide sufficient donor. Because seasonal changes had been observed previously in the degradation efficiency of the natural microbial community in the wetland sediment, these bioaugmented microcosms were repeated with wetland sediment collected in early March 2003. In September–October 2003, microcosm experiments were conducted to test the degradation efficiency of the consortia in the BionSoil/peat mixture. Degradation of TeCA with WBC-1 was compared to degradation with the newly developed WBC-2. Microcosms to test the effect of high concentrations of TeCA (approximately 10 times the TeCA concentration previously tested) also were prepared in the September–October 2003 experiments with BionSoil/peat (table 1).

In 2004, additional bioaugmented microcosms were prepared to evaluate the effect of compost type on the degradation efficiency of WBC-2 (tables 1 and 2). BionSoil became unavailable after the initial microcosms were completed, and other composts had to be selected for compatibility with WBC-2 as a matrix for the reactive mat (table 2). The Paygro and TLC composts were from dairy waste, similar to the BionSoil, whereas the other composts made from crab, poultry, and municipal yard wastes were selected from local sources that would have lower transportation costs to the pilot test site.

All microcosm bottles were stored upside down in the dark and incubated at 19°C. Duplicate bottles were sacrificed for sampling at various time intervals. Water samples were withdrawn from the top of the microcosms with a syringe after sediment was allowed to settle when turned upright for sampling. Supernatant pH was measured with a commercial, multi-line meter that was calibrated prior to each sampling event using two standard solutions. For all experiments, water samples were analyzed in the Baltimore USGS laboratory for VOCs by purge-and-trap capillary GS/MS, methane by

GC with an FID, ferrous iron (0.2-micron-filtered sample) by the bipyridine method on a Bausch and Lomb Spectronic 21 spectrophotometer, and ammonia (unfiltered sample) by CHEMetrics (Calverton, Virginia) System 1000 colorimetric method, as described in earlier studies (Lorah and others, 2003; Lorah, Burris, and Dyer, 2005). For the biostimulated and bioaugmented microcosms in 2002 and 2003, selected samples also were analyzed for chloride, sulfate, nitrate, and phosphate by ion chromatography in the Reston USGS laboratory. In 2004, 0.45-micron-filtered samples from the compost/peat microcosms were analyzed for phosphate using the CHEMetrics molybdenum blue/stannous chloride method. Sediment slurry samples also were collected from microcosm bottles and immediately frozen for potential microbial community analyses using TRFLP and qPCR methods.

Degradation rates were calculated assuming first-order kinetics. Degradation rate constants, *k*, were estimated from the slope of a linear regression of the natural logarithm of the aqueous concentration remaining in solution against time since the start of the experiment. After the rate constant was estimated, degradation rates (μM/day) were calculated by use of equation 1:

$$\text{RATE} = kC_i \quad (1)$$

where

k is the first-order rate constant (day^{-1}),

and

C_i is the initial concentration (μM).

Comparison of live and formaldehyde-killed microcosms in these tests and previous microcosms has consistently shown that sorption losses of the added VOC are substantial in the first 1 to 3 days of the experiments (Lorah and others, 1997; Lorah and others, 2003). Thus, degradation rates were calculated using the day 1 or day 3 analyses as the initial concentrations after comparison with the sterile (formaldehyde-killed) controls. After this initial period, removal rates in the sterile controls were within the standard error for the calculated degradation rates in the live microcosms.

Co-Contaminant Tests

Since several seep areas were found to contain high concentrations of CT, CF, and varying concentrations of TeCA degradation products in addition to TeCA, a microcosm that contained a mixture of VOCs was constructed in the September–October 2003 experiments with BionSoil/peat (table 1). The mixed-VOC treatment was amended to give concentrations of 5.0 μM TeCA, 7.3 μM 112TCA, 5.7 μM *cis*12DCE, 4.4 μM *trans*12DCE, 1.3 μM CT, and 3.0 μM CF on day 0. CT and CF degradation in these experiments with bioaugmented BionSoil/peat was compared to June–July 2002 microcosms, in which the ability of the natural microbial community in the wetland sediments to degrade CT was examined (table 1). Microcosms were constructed with wetland sediment from sites WB30 and WB23 and amended only with CT to give an initial (day 0) concentration of 7.5 μM, or 1,150 μg/L; the day 1 concentration was expected to be about 500 μg/L after sorption. Microcosms were stored, sampled, and analyzed as described above for the biostimulation and bioaugmentation tests.

Zero-Valent Iron Tests

Porewater at some seep locations contained greater than 10 mg/L of CF and CT. These contaminants, particularly CF, have been shown to have a toxic effect on the complete degradation of chlorinated ethenes and an inhibitory effect on methanogenesis (Bagley and others, 2000; Weathers and Parkin, 2000). Therefore, ZVI filings were considered as an addition to the reactive mat matrix to facilitate degradation of chlorinated methanes detected in high concentrations in porewater of some seep locations within West Branch Canal Creek. In February–March 2003, an anaerobic microcosm was conducted with commercially available ZVI filings to determine the potential degradation rate of CF at two concentrations (4.2 and 42 μM) with and without wetland sediment, to identify the fate of the associated chlorinated daughter products under these conditions, and to investigate the reactivity and water-quality effects of the organic-rich sediments and site porewater with ZVI. Controls (iron and porewater only, CF and porewater only) were used to measure interaction of the site porewater and ZVI and to document any VOC losses over the course of the experiment.

Dry ZVI filings were added to 162-mL serum bottles in a ratio of 0.078 m^2/mL (square meters per milliliter) of total volume (Gillham and O'Hannesin, 1994) and incubated under ambient conditions. The ZVI commonly used in permeable barrier walls (ETI CC-1004) with an average surface area of 1.5 m^2/g (square meters per gram) (S. O'Hannesin, Connolly-GPM, Chicago, Illinois, oral commun., December 2002) was obtained from Connolly-GPM (Chicago, Illinois) and used in the experiment, unwashed. Porewater or wetland sediment slurry was added to the serum bottles containing ZVI to leave no headspace under a nitrogen atmosphere. Porewater was collected from piezometer WB24B and shallow wetland sediment was collected from sie WB23 as described in the previous section. Wetland sediment slurry was comprised of sediment and porewater in a volumetric ratio of 1.5:1, as previously used in microcosm experiments (Lorah and others, 1997; 2003). A stock solution was made from neat CF (Supelco, Bellefonte, Pennsylvania) and to obtain the desired starting concentration, and aliquots were added to the serum bottles using a gas-tight syringe before sealing the bottles with aluminum crimp caps. All bottles were stored sideways on a shaker in the dark under ambient temperatures until sampled (1 to 29 days). Duplicate bottles were prepared for each treatment and sacrificed for chemical analyses at each of six or seven time steps, depending on the treatment.

At each time step (except the final time step), supernatant from duplicate treatment bottles was withdrawn for analyses of pH, VOCs, methane, soluble (ferrous) iron, and sulfide. At selected time steps, supernatant was also collected for analyses of anions (including chloride and sulfate), ammonia, and alkalinity. Initial pH in treatment bottles ranged from 6.35 to 6.8 and increased to as high as 8.7 in the non-sediment-containing treatments by the end of the experiment. All analyses were performed as described in the previous section for the biostimulation and bioaugmentation microcosms, except ferrous iron (0.2-µm/micrometer filtered sample) was analyzed using colorimetric methods with the CHEMetrics 1000 System. Alkalinity was measured using end-point titration with 0.00252 N hydrochloric acid.

Degradation rates were calculated assuming first-order kinetics (eq. 1), as typically done for abiotic iron reduction rates (Gillham and O'Hannesin, 1994; Matheson and Tratnyek, 1994).

Column Experiments

The contaminant mixtures and concentrations detected in seep porewater are difficult to replicate in microcosm experiments. Therefore, columns were used to more accurately simulate the seep conditions in the laboratory. The specific objectives of the column study were to evaluate in three organic-based matrices: (1) TeCA degradation pathways and rates, (2) TeCA daughter compound degradation and fate, (3) redox-sensitive conditions, (4) water quality, and (5) co-contaminant degradation under bioaugmentation and biostimulation conditions. Columns were constructed with wetland sediment from a seep area as one of the organic-based matrices to evaluate enhanced bioremediation by *in situ* biostimulation and bioaugmentation of existing sediment at a site. Other columns were constructed with matrices planned for the reactive mat to evaluate this remedial option. Seep sediment columns were also utilized as a natural wetland matrix "control" for comparisons to the reactive mat matrices.

Flow-Through Column Construction

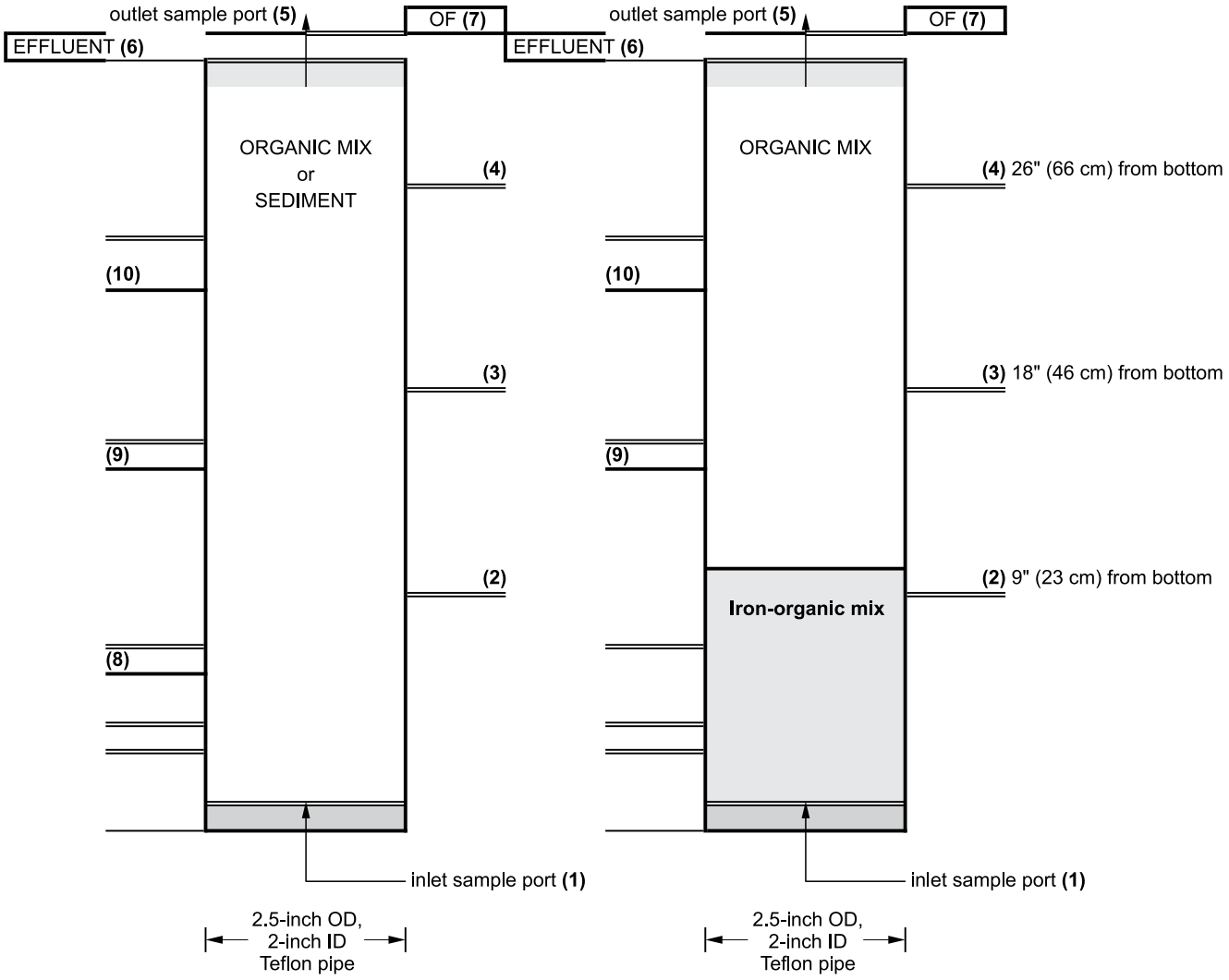
A total of six flow-through column systems were constructed to circulate chilled, nitrogen-purged, site porewater spiked with chlorinated VOCs through various organic-rich matrices. Columns were sized to allow operation at field-estimated porewater velocities and a reasonable hydraulic residence time in order facilitate sample collection along the length of the column. A schematic of the column design is shown in figure 4. The columns were constructed of 1.3-cm-thick Teflon pipe with an inner diameter of 5.1 cm and length of 76.2 cm. Teflon plates (2.5 cm thick) sealed the bottom and top of the column using a Viton gasket. A Lexan plate was placed about half-way along the outside column length and four all-threaded rods anchored each corner of the bottom, middle, and top plates for column stability. Three injection ports that had 0.6-cm Teflon tubing extending to the axial center of the column were placed on one side of each column at 15, 36, and 53 cm above the base during column packing. Three sampling ports with 0.318-cm Teflon tubing were placed on the opposite side of each column at 23, 46, and 66 cm above the base. Two additional sampling ports were at the influent and effluent three-way valves. Columns were operated continuously in an upflow configuration using two low-flow, peristaltic pumps equipped with four drive heads. Wherever possible, Teflon, nylon, or stainless steel tubing, fittings, stopcocks, valves, and connectors were used to prevent any adsorption of VOCs to column components. Tedlar bags were used to store influent and effluent. Samples were collected from each Tedlar bag at the beginning and end of the time it provided influent to the columns to verify that VOC losses were minimal over time.

Column Matrix Materials, Packing, and Operation

Three sets of duplicate flow-through columns were constructed— one set with wetland sediment from seep 3–7E, a second set with a 40:20:20 (percent by volume) mixture of commercially available compost, peat, and sand (referred to as the organic mix columns, M-A and M-B), and the third set with a ZVI mixture (from 0 to 25.4 cm) under a layer of the organic mix (from 25.4 to 76.2 cm) (referred to as the iron-organic mix columns, FM-A and FM-B). The ZVI mixture contained 60 percent by volume ZVI (Connelly GPM, Inc., Chicago, Illinois) and 40 percent by volume of the organic mixture (40:20:20 compost, peat, and sand).

At the time of the column experiment start-up, no piezometers had been installed at the seep sites to obtain water for circulation through the columns. Site porewater was collected instead from the same piezometer (WB24B) as that used for the microcosms. Water was collected on a weekly basis and purged with nitrogen to remove any oxygen introduced during collection prior to circulation into the columns. Water was transferred to Tedlar bags using a peristaltic pump in the nitrogen atmosphere, and injected with a specified aliquot of stock VOCs (and ethanol or lactate stock solution when needed) using a gas-tight syringe. The Tedlar bag was placed on a shaker for about 60 minutes. Stock VOCs were prepared from neat chemicals (Supelco, Bellefonte, Pennsylvania) and boiled, ultra-pure (18 megaohm) deionized (UPDI) water. Stock ethanol solution for use as a carbon substrate was prepared from neat ethanol (Sigma) or 60-percent lactate solution (Sigma) and UPDI water to the desired concentration. The bag(s) were then placed in a small, darkened refrigerator equipped with an inlet port that allowed the delivery of low-flow nitrogen and an outlet port that allowed for delivery of site water to the columns via Teflon tubing. Depending on sampling frequency and column treatments, Tedlar bags were exchanged every 1 to 5 days.

Seep sediment was collected from seep location 3–7E in the upper 60 cm of the wetland sediments (fig. 1b). Sediment was placed in mason jars and packed to leave minimal airspace



RIGHT SIDE PORTS (all columns)

1	Along inlet sample line
2	sampling port 9" (23 cm) from bottom of column
3	sampling port 18" (46 cm) from bottom of column
4	sampling port 26" (66 cm) from bottom of column (4" from top)
5	Along outlet sample line
6	effluent bag
7	overflow bag (OF)

LEFT SIDE PORTS

8	Injection port (not for iron-organic mix column), 6" from bottom
9	Injection port 14" (36 cm) from bottom
10	Injection port 21" (53 cm) from bottom

Figure 4. Schematic of column test design.

and then jars were stored under a nitrogen atmosphere until columns were packed (about 1 week). Sediment from this seep location was selected since it had a variety of VOCs detected in the field including chlorinated ethanes, ethenes, and methanes, representative of most other seep locations throughout Canal Creek. Subsequent physical property analysis of nearby surface grab samples of these sediments showed a slightly lower than average total organic carbon content (average 7.2 percent), but typical pH (4.5 to 6) and water content (228 to 322 percent) compared to the other seeps (Majcher and others, 2007). Since sediments were collected and stored under field conditions, no additional porewater was mixed into the sediment during column packing. A total of 1,628 g (grams) of sediment was homogenized and placed in each column (S-A, S-B).

The organic mix was comprised of three commercially available products reported previously for use in model mesocosms and constructed wetlands (Kassenga and others, 2003; Kassenga and others, 2004)— BionSoil, Mohican peat, and medium-sized drilling sand. BionSoil is a compost derived from organic dairy manure that was given a U.S. Environmental Protection Agency Class A Standard (Easton and Petrovic, 2004) (table 2). Mohican peat is a reed sedge peat harvested in Lucas, Ohio. Its physical properties were similar to that of the Latimer peat described by Kassenga and others (2003) (J. Pardue, Louisiana State University, written commun., August 2003). Its porosity was estimated to be 92 percent, and its water content was greater than 200 percent as received. A medium-sized sand (#0 drilling sand, diameter range from 0.23 to 2 mm, Ricci Brothers Sand, Port Norris, New Jersey) was sieved to remove the finest 1 percent, rinsed with deionized water until clear, and baked overnight. The porosity of this sand was estimated to be 43 percent. The BionSoil, Mohican peat, and sand, respectively, were mixed in a ratio of 40:40:20 percent by volume to result in a saturated density greater than water, as described by Kassenga and others (2003), and an estimated porosity of 79 percent. A total of 1,398 g of the mixture was packed into each of the two mix (M-A, M-B) columns, to give the desired final saturated density of 1.2 g/cm^3 (grams per cubic centimeter).

The ZVI filings incorporated in the FM columns were the same as those used in the ZVI microcosm tests described in a previous section. A total of 556 g of the ZVI filings were hand-mixed with 289 g of organic mix to comprise the bottom third of each column. A total of 938 g of organic mix was added to each column to fill the remaining two-thirds of the columns.

Approximately 2.5 cm and between 2.5–4 cm of medium-sized sand were placed at the bottom and top of each column, respectively, to promote an even distribution of influent and effluent. Once columns were packed, pumps were brought on-line to begin filling the porespace using water without added VOCs. Pumps were calibrated to result in approximately 0.2 mL/minute effluent flow rate within the first several weeks of column operation. This operational flow rate was selected on the basis of field estimates of seepage flux from three contaminated seeps at West Branch Canal Creek (Majcher and others, 2007) and was in the range of flow rates (and corresponding velocity through the columns) reported for other column studies in the literature (Harkness and others, 1999; Kassenga and others, 2003). The resulting hydraulic residence time through the different matrices ranged from 3.2 (S columns) to 4.1 (M and FM columns) days. The total porosity of the M and FM columns, estimated by weight before and after columns were water-filled, was 68 and 51 percent, respectively.

Column Treatments, Sample Collection, and Analyses

Porewater was circulated through the column matrices for 18 to 74 days, or 3.2 to 18 column pore volumes, prior to addition of VOCs (Appendixes A1–A3). Chlorinated VOCs were added to the column influent and operated for a total of 191 days under different treatment conditions. Major treatments throughout the duration of the seep sediment columns included introduction of TeCA in the influent at a target concentration of 5 mg/L; bioaugmentation with WBC-1; amendment with ethanol as an electron donor; re-bioaugmention with WBC-2 (lot WBC-2E, fig. 3); and introduction of chloroethene co-contaminants (PCE and TCE) (Appendix A1). Major treatments for the organic mix (M-A and M-B) and iron-organic mix columns (FM-A and FM-B) were the same as for the sediment columns (S-A and S-B), except that chloromethane (CT and CF) were also evaluated as co-contaminants of the TeCA (Appendix A, tables A2, A3).

Influent electron donor (ethanol or lactate) concentrations were started at 10 mM, but were then adjusted to between 0 and 5 mM, depending on methane concentrations and resulting shifts in contaminant degradation (Appendixes A1–A3). Bioaugmentation of the columns was initially done with WBC-1 that had been diluted in culture media to 15 percent by volume. Out of concern that the diluted WBC-1 would not degrade the VOCs efficiently, all B columns were re-bioaugmented with undiluted WBC-2. The A columns for each matrix pair were not re-bioaugmented so that they could be compared with the B columns to determine if re-bioaugmentation enhanced degradation rates. Except for the re-bioaugmentation of S-B, the seep sediment columns were operated as duplicates throughout the experiment. The organic mix and iron-organic mix column pairs also were treated differently with the addition of co-contaminants (Appendixes A2 and A3). The effects of chloroethene (PCE and TCE) co-contaminants with TeCA were evaluated in the M-A and FM-A columns between days 132 and 184. After 184 days of operation of the M-A and FM-A columns, a CF concentration of 8 mg/L was added to evaluate short-term co-contaminant effects of chloromethanes in columns not acclimated to CF. The chloromethanes CT and CF were added, along with TeCA, to the M-B and FM-B columns, respectively, after 107 and 128 days of column operation, and chloroethene

co-contaminants were not added. CT and CF concentrations were gradually increased in the M-B and FM-B columns (from 1 mg/L to greater than 10 mg/L over 81 days) in an attempt to minimize their possible toxicity effects to the culture.

The same method was used for each column bioaugmentation. For the sediment and organic mix columns, three injections of 20 mL of culture followed by 5–10 mL of WB24B water (without added VOCs) were added to each column through the injection ports. For the iron-organic mix columns, two injections of 20 mL of culture followed by 5–10 mL of influent site ground water were added through two injection ports within the organic mix zone of these columns. The culture was injected at a rate twice that of the normal circulation rate through the columns, or approximately 20 mL/hour. Columns were inoculated from the bottom-most injection port first, then upwards. Each pair of columns was inoculated simultaneously at the equivalent port. During injection, the effluent port (76.2 cm) of each column was left open, but the influent port (0 cm) was closed to prevent any loss of column porewater in the downward direction. Following bioaugmentation, column influent remained disconnected for 24 hours to allow culture to become stable in the column matrix before inflow was resumed.

Sampling typically was conducted during every other pore volume and near the end of the test, every pore volume (Appendixes A1–A3). Sample collection for all analytes of interest was staggered to minimize sample volumes from the column porewater during any single event. Therefore, a single sample event was considered to be within the limits of 1 to 1.5 column pore volume (up to 4 days) to limit the total removal of porewater to less than 2 percent of the column pore volume. Samples were collected from the top of the column, or theoretically the least contaminated sample (76.2 cm), to the bottom of the column, or theoretically the most contaminated sample (0 cm). Glass-on-glass, luer-lock syringes were attached to sampling ports and were allowed to fill to the desired volume at the operational flow rate by closing the effluent port following collection of the first sample. Effluent bags were disconnected from the columns during this time and were sampled.

On each sample-collection date, the total volume, pH, and flow rate of the effluent was measured. Samples for analysis of VOCs, methane, ethane, and ethene, and ferrous iron were collected during each sampling event from 3 to 5 ports per column. During some sampling events, samples for analysis of anions (sulfate, nitrate, nitrite, and phosphate), cations (trace metals), sulfide, ammonia, dissolved organic carbon (DOC), and alkalinity were collected from 2 to 3 ports per column. Effluent pH was measured with a commercial meter that was calibrated prior to each sampling event using two standard solutions. VOCs and dissolved methane were analyzed by purge-and-trap GC/MS and GC-FID, respectively at the on-site laboratory, as described in the previous section. Samples for ferrous iron, sulfide, and ammonia were analyzed as described for the microcosms. Anion (0.2-µm filtered) and cation (0.2-µm filtered and acidified with nitric acid) samples were analyzed by ion chromatography at the USGS laboratory in Reston, Virginia. Samples for DOC analyses were filtered through a 0.2-µm Ion Chromatography (IC) Acrodisc filter (Pall Corporation, East Hills, New York) and acidified with 1:2 phosphoric acid prior to analyses by high temperature, non-catalytic combustion (Qian and Mopper, 1996).

Microbial Consortia Development and Characterization

Enrichment of microorganisms endogenous to the West Branch Canal Creek wetland sediments was successful in developing two consortia, WBC-1 and WBC-2, capable of degrading TeCA and its chlorinated ethane and ethene daughter products under anaerobic conditions. Development of both consortia began with an initial enrichment of wetland sediment with TeCA, followed by separate enrichments in sediment with either a chlorinated ethane intermediate (such as 112TCA) or a chlorinated ethene intermediate (such as 12DCE) of TeCA degradation (fig. 3). The next stages of development and maintenance of the two consortia, however, had three major differences: (1) WBC-1 was formed in a culture medium matrix after further cultivating the separate sediment enrichments for several months, whereas WBC-2 was formed by immediately inoculating the separate sediment enrichments into a culture medium matrix with TeCA as the only VOC added; (2) WBC-1 was developed and maintained using amendments of H_2 and acetate as electron donors, whereas WBC-2 was developed and maintained with lactate as the electron donor; and (3) WBC-1 was maintained using only TeCA as the added electron acceptor, whereas WBC-2 was amended with TeCA, 112TCA, and 12DCE as electron acceptors. The dechlorinating efficiencies and possible effects of the differing development techniques for the two cultures are discussed in this section. Clone libraries constructed for both consortia to characterize their microbial composition also are compared.

Dechlorinating Activity

After the initial formation of the consortia in culture medium, both WBC-1 and WBC-2 were able to degrade TeCA and its intermediate chlorinated ethene and ethane degradation compounds (figs. 5a–f). Although TeCA was amended to the consortia, TeCA concentrations could not be monitored by the headspace analysis used on these initial culture batch tests. When only TeCA was amended to WBC-1, ethene was the primary daughter compound observed in the headspace (data not shown). Amendment of WBC-1 and WBC-2 with only *cis*12DCE led to VC production in batch tests; ethene production also was observed in WBC-1 (figs. 5a–b). Amendment of WBC-1 with 112TCA led to transient accumulation of VC and 12DCA, followed by nearly

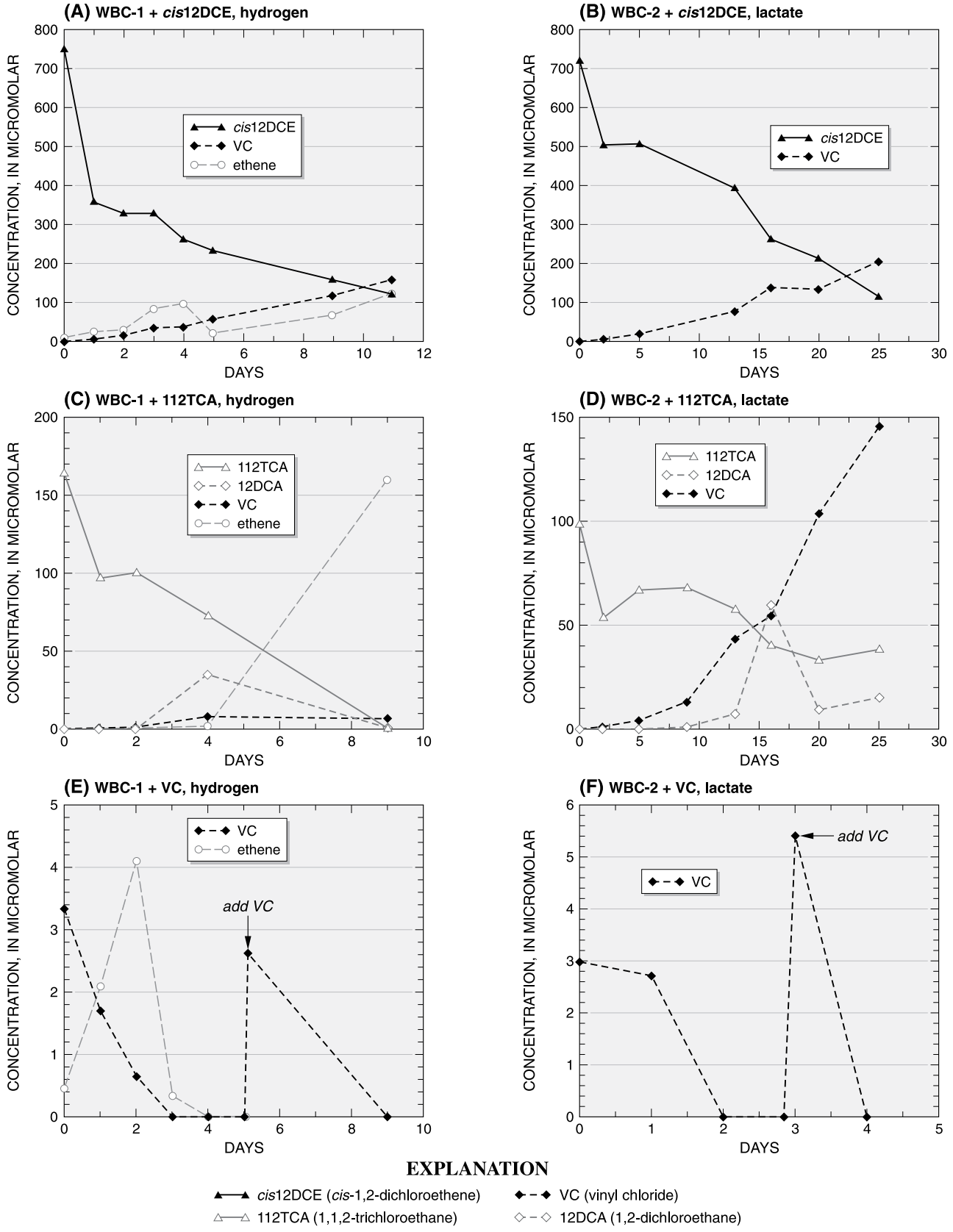


Figure 5. Dechlorination activities of WBC-1 and WBC-2 on selected test compounds immediately after their development in culture media: *(A, B)* amendment with *cis*-1,2-dichloroethene (*cis*12DCE), *(C, D)* amendment with 1,1,2-trichloroethane (112TCA), and *(E, F)* amendment with vinyl chloride (VC).

stochiometric conversion to ethene (fig. 5c). Amendment of WBC-2 with 112TCA led to production of 12DCA and VC (fig. 5d). Although 12DCA concentrations decreased by the end of the 112TCA-amended batch test, VC concentrations remained high (fig. 5d). Both consortia rapidly degraded VC, however, when it was the only VOC added (figs. 5e–f). Methane was produced in both consortia.

Selecting appropriate electron donors was crucial in growing and maintaining the consortium. H_2 and acetate were selected for WBC-1 development because they were reported as favored electron donors of known dechlorinating bacteria such as *Dehalococcoides ethenogenes*, *Dehalobacter restrictus*, *Sulfurospirillum multivorans*, *Desulfitobacterium frappieri* (H_2) and *Desulfuromonas chloroethenica* and *Desulfuromonas* sp. strain BB1 (acetate). After initial formation of WBC-1, its dechlorinating ability with either H_2 or acetate as an electron donor was compared to treatments that received no additional electron donor. Although TeCA degradation was observed in all three electron donor treatments, greater removal of TeCA and its intermediate daughter compounds was observed in the H_2 and no donor added treatments than with acetate added (fig. 6). A similar pattern of electron donor preference was observed when WBC-1 was amended with the daughter compounds 112TCA, *cis*12DCE, *trans*12DCE, and VC in separate batch treatments (data not shown). On the basis of these donor test results, only H_2 was added regularly to maintain and grow WBC-1, and acetate was added infrequently to provide a carbon source. The initial batch of WBC-1 was transferred (10 percent) to fresh medium after 3 months, and activity tests were repeated. These activity tests showed that the dechlorinating efficiency of WBC-1 had improved over this period (data not shown) compared to initial activity tests after forming the consortium (figs. 5a,c,e).

Dechlorinating activity of WBC-1, however, began to decline after 12 months of continued propagation in culture media in the Reston USGS laboratory. After 18 months of cultivation, WBC-1 degradation of TeCA, 112TCA, 12DCE, VC, and 12DCA had declined substantially (selected activity tests shown in figs. 7a,c,e). TeCA was degraded predominantly to the abiotic dechlorination product TCE after 18 months. The batch of WBC-1 maintained at SiREM Laboratories also showed efficient degradation during the first several months of scaling up to larger quantities, but 20-L batches of WBC-1

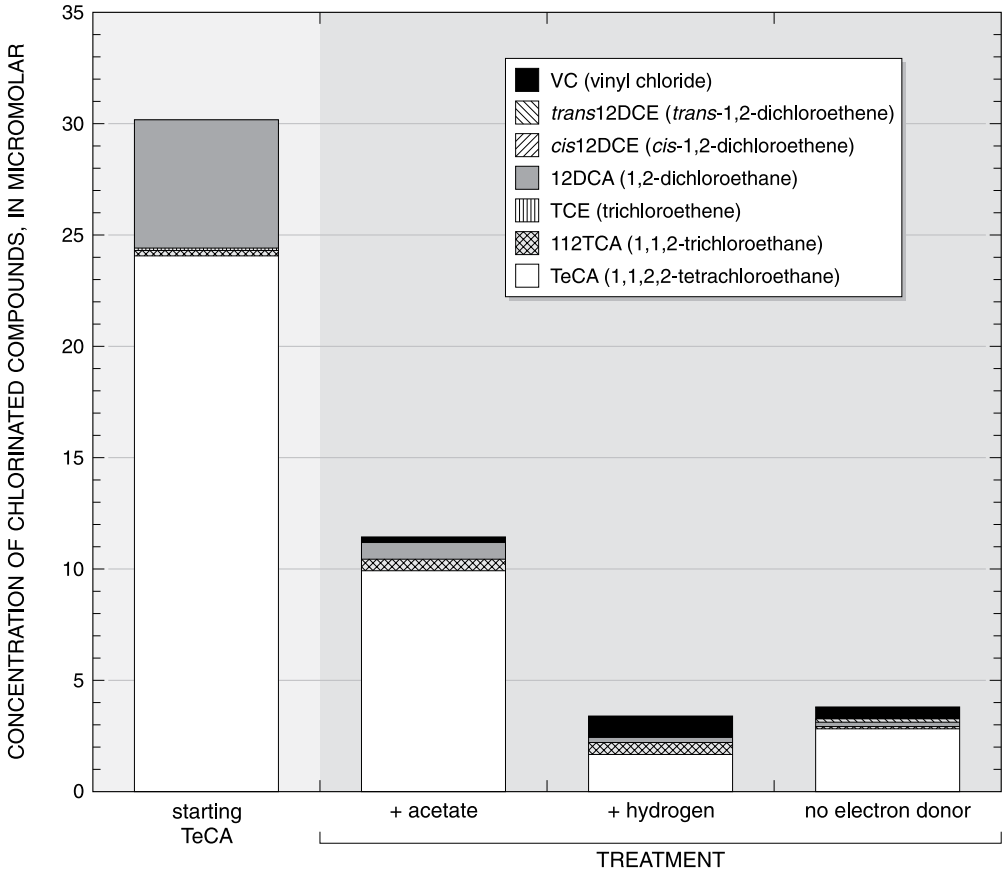


Figure 6. Distribution of 1,1,2,2-tetrachloroethane (TeCA) and its chlorinated daughter compounds in batch tests with WBC-1 amended with TeCA and either acetate, hydrogen (H_2), or no additional electron donor.

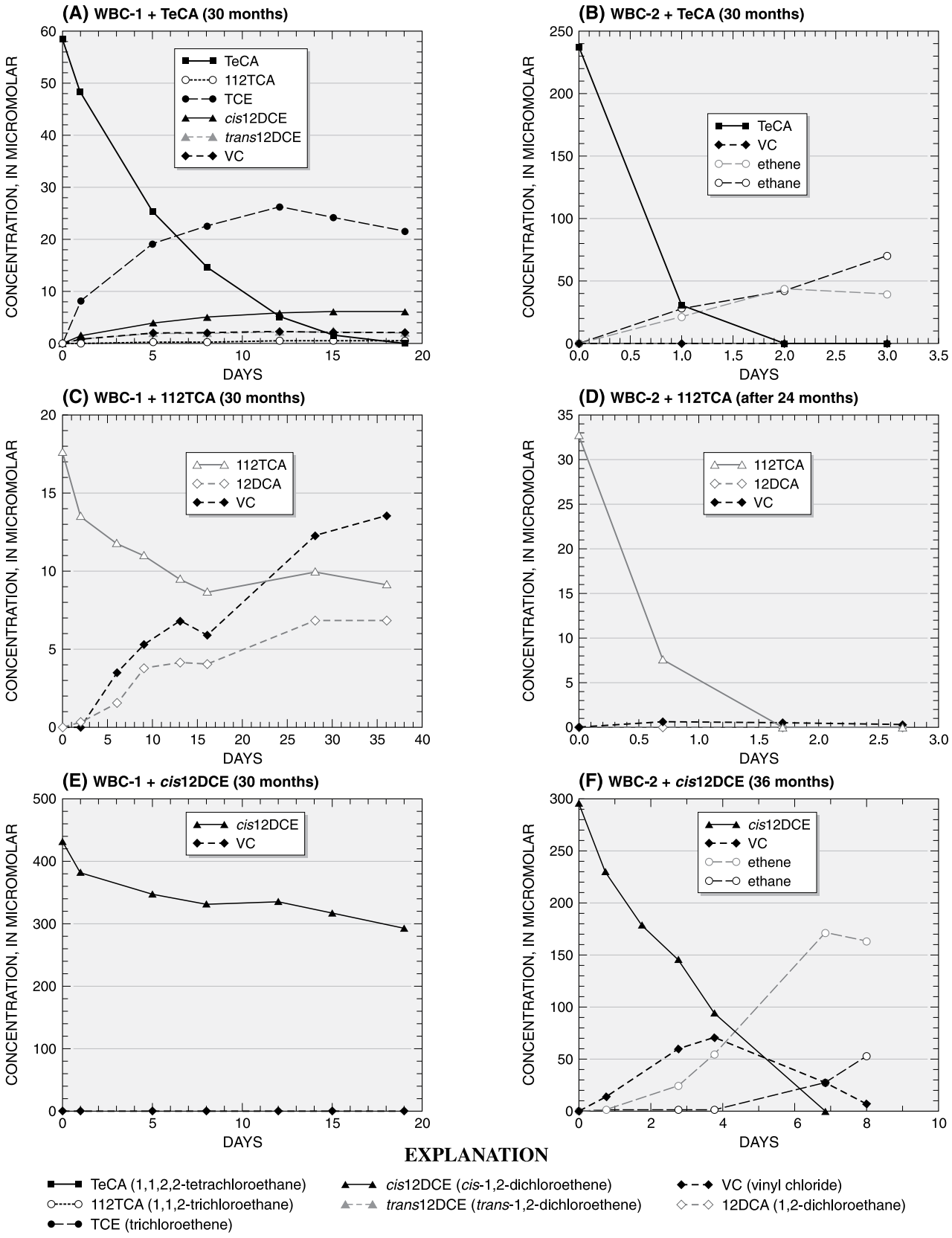


Figure 7. Dechlorination activities of WBC-1 and WBC-2 on selected test compounds after long-term growth and maintenence of the consortia in culture media (30 months for WBC-1 and 18–24 months for WBC-2): *(A, B)* amendment with 1,1,2,2-tetrachloroethane (TeCA), *(C, D)* amendment with 1,1,2-trichloroethane (112TCA), and *(E, F)* amendment with *cis*-1,2-dichloroethene (*cis*12DCE). (Note that ethene and ethane were not analyzed if they are not shown.)

lost dechlorinating activity about a year after the initial 100-mL culture batches of the consortium had been received (Geosyntec Consultants, Inc., 2004).

Long-term maintenance and growth of WBC-2 was successful both in the Reston laboratory (figs 7b,d,f) and at SiREM Laboratories (Geosyntec Consultants, Inc., 2004). A major difference between the two consortia was the electron donor selected for addition to the consortium. To select a donor, the degradation efficiency of WBC-2 was tested on a greater variety of electron donors than WBC-1, using replicate batch tests amended with *cis*12DCE, 112TCA, or VC (table 3). Dechlorinating activities of WBC-2 on these three test compounds were compared to select an electron donor that could support degradation of each major component of the TeCA degradation pathway. Lactate best supported dechlorination of each of these test compounds (table 3), and was selected as the electron donor for cultivation of WBC-2. As observed with WBC-1, acetate did not stimulate reductive dechlorination in WBC-2. H_2 addition greatly enhanced degradation of VC by WBC-2, but did not enhance degradation of *cis*12DCE or 112TCA. Yeast extract (alone or in various combinations) greatly stimulated reductive dechlorination of *cis*12DCE and 112TCA; however, high intermediate concentrations of VC were produced from these compounds, and yeast extract did not stimulate degradation of VC (table 3). In addition, yeast extract could potentially stimulate excessive growth of unwanted microorganisms (De Wildeman, Neumann, and others, 2003). Propionate has been suggested as a good source of slow release of H_2 to support dechlorinators (He and others, 2002), but propionate was ineffective in supporting dechlorination by WBC-2.

In contrast to H_2, lactate can be used directly by some microorganisms as an electron donor and carbon source, in addition to potentially being converted to acetate, H_2, or other electron donors. Thus, lactate is more likely to support a more diverse microbial community than H_2. Most previous studies that have shown H_2 as a suitable electron donor for dechlorinating bacteria were developed to degrade chlorinated ethenes rather than chlorinated ethanes. H_2 also was a suitable electron donor for the recently isolated 12DCA dehalorespirer, *Desulfitobacterium dichloroeliminans* strain DCA1, although it does not degrade the higher chlorinated alkanes investigated for WBC-1 and WBC-2 (De Wildeman, Diekert, and others, 2003). The poor solubility of H_2 also may have been a factor in the decline of the dechlorinating activity of WBC-1, especially in high volumes of media where the microorganisms may have had limited access to the H_2.

WBC-2 dechlorination activity was significantly greater after 18–24 months of cultivation and dilution of the original sediment to 0.1 percent in the Reston USGS laboratory compared to the initial measurements of dechlorination activity (figs. 5 b,d,e and 7 b,d,e). WBC-2 rapidly degraded TeCA to ethene and ethane with little intermediate accumulation of chlorinated daughter products, indicating a tight coupling of the degradation reactions (fig. 7b). Of the 470 µmol (micromoles) of TeCA added, 410 µmol were recovered as the total of ethene and ethane after 3 days. When *cis*12DCE was added as the only VOC, degradation by WBC-2 was slower than when a similar concentration of TeCA was added; however, complete conversion of the *cis*12DCE to ethene and ethane was observed within 8 days (fig. 7f). WBC-2 dechlorination activity on 112TCA showed

Table 3. Relative degradation efficiency of *cis*-1,2-dichloroethene (*cis*12DCE), 1,1,2-trichloroethane (112TCA), and vinyl chloride (VC) in microcosms with WBC-2 in culture medium amended with various electron donors.

[Treatments were amended with 1 millimolar (mM) of *cis*12DCE, 0.5 mM of 112TCA, or 4.2 µM of VC. Dechlorination was compared to the control test that received no electron donor: -, dechlorination not greater than no donor control; +, greater than no donor control; ++, removal at least 50 percent complete after 27 days for *cis*12DCE and 112TCA, or after 3 days for VC; +++, complete removal of *cis*12DCE and 112TCA after 27 days, or of VC after 3 days; NT, not tested.]

Electron donor	*cis*-1,2-Dichloroethene	1,1,2-Trichloroethane	Vinyl chloride
Propionate	+	-	-
Succinate	+	+	-
Lactate	++	+++	+++
Pyruvate	+	++	+++
Yeast extract and Proteose peptone	+++	++	-
Benzoate	-	+	NT
Formate	+	++	-
Acetate	-	-	-
H_2	-	-	+++
Whey	-	-	NT
None	-	-	-

low transient accumulation of 12DCA and VC, but ethene and ethane were not analyzed in this test (fig. 7d). The production of ethene and ethane in the consortia confirms the fate of VC in the wetland sediments, which was unknown in previous microcosms with wetland sediment where VC degradation was observed without ethene or ethane accumulation (Lorah and Voytek, 2004).

WBC-2 was routinely amended with a mixture of TeCA, 112TCA, and *cis*12DCE during its growth and maintenance. An example of the dechlorination activity of WBC-2 on this mixture is shown in figure 8. Rapid simultaneous degradation of the mixture of chlorinated ethenes and ethanes was observed, producing ethene and ethane as the detected end-products. Regular amendments with this mixture of VOCs, compared to only adding TeCA, was another major difference between WBC-2 and WBC-1 that could have affected the long-term survival of the consortium. Activity was retained in 2-L batches of WBC-2 culture with 1–5 percent sediment stored long-term (1 year) at 19°C, with monthly addition of TeCA (50 μM). These cultures could be activated for use or transfer in 1–2 months by increasing the feeding rate (refeeding at each depletion) with TeCA and adding electron donor (1 mM lactate), resulting in a culture capable of complete dechlorination of 50 μM TeCA to ethene in 1–2 days. Active cultures were maintained by feeding 50 μM TeCA and 1 mM lactate, or 25 μM TeCA, 50 μM *cis*12DCE, 50 μM 112TCA, and 1.5 mM lactate 2 times per week.

The degradation activity of WBC-2 culture maintained on the mixture of TeCA, 112TCA, and 12DCE also was tested with PCE and TCE, two common co-contaminants in the seep ground water. WBC-2 degraded TCE (14 μM), 12DCE (14 μM), and TeCA (10 μM) within 5 days (fig. 9). PCE degradation was slower, with some of the 5 μM added still detected after a week. 112TCA degradation apparently was inhibited by these co-contaminants with the initial 112TCA added remaining after 7 days (fig. 9).

Dechlorination activity was correlated to cell density. When cells from WBC-1 (taken when the culture was fully active) were harvested and washed to remove extracellular factors and then resuspended in bicarbonate buffer at cell densities varying from 1 to 5 times that in the maintained consortia, the degradation rates of *cis*12DCE increased with increasing cell density (fig. 10a). Concentrations of the intermediate daughter product VC decreased with increasing cell density during the reduction of 12DCE (fig. 10a). VC accumulation was minimal at 5 times cell density, presumably because its rate of degradation equaled its rate of production. The presence of active cells was required for reductive dechlorination to occur, as no decrease in parent compound concentrations or increase in daughter compounds was observed in the absence of cells. A similar relation of culture activity to cell density was observed by the increasing methane production with increasing cell density (fig. 10b). Similar increases in dechlorination activity with increasing cell density were observed for tests amended with *trans*12DCE and 112TCA (data not shown) as for *cis*12DCE (fig. 10a). However, 112TCA was degraded only to 12DCA by the washed cell suspension, rather than simultaneous reduction to 12DCA and VC as observed in the unwashed WBC-2 consortium.

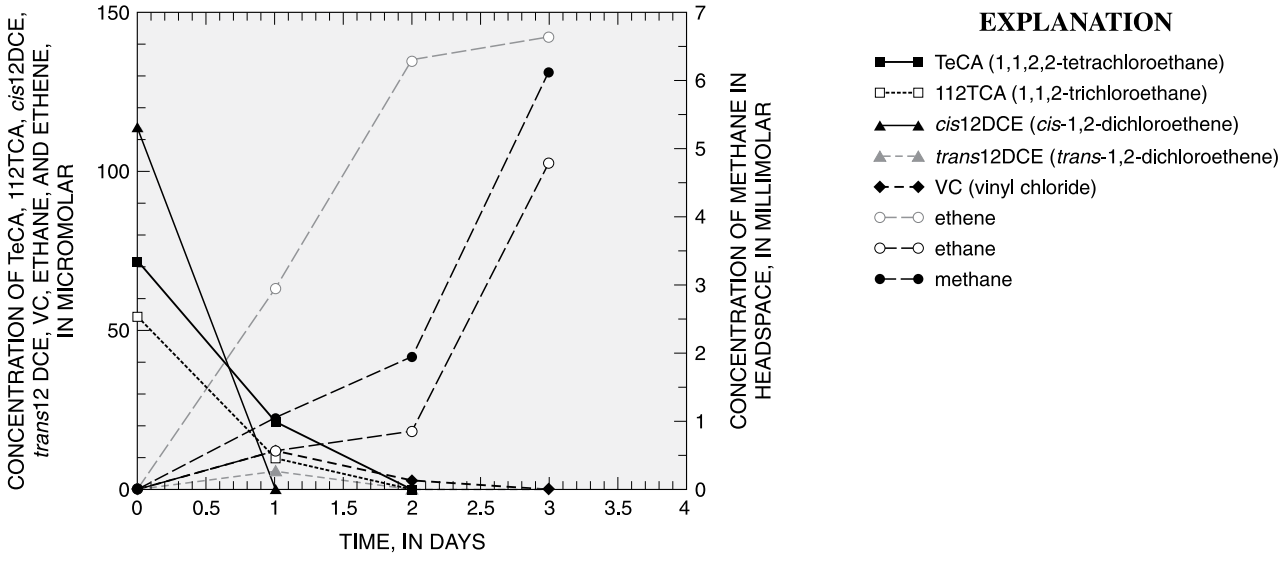


Figure 8. Dechlorination activity of WBC-2 amended with a mixture of 1,1,2,2-tetrachloroethane (TeCA), 1,1,2-trichloroethane (112TCA), and *cis*-1,2-dichloroethene (*cis*12DCE) after long-term growth and maintenance of the consortia in culture media.

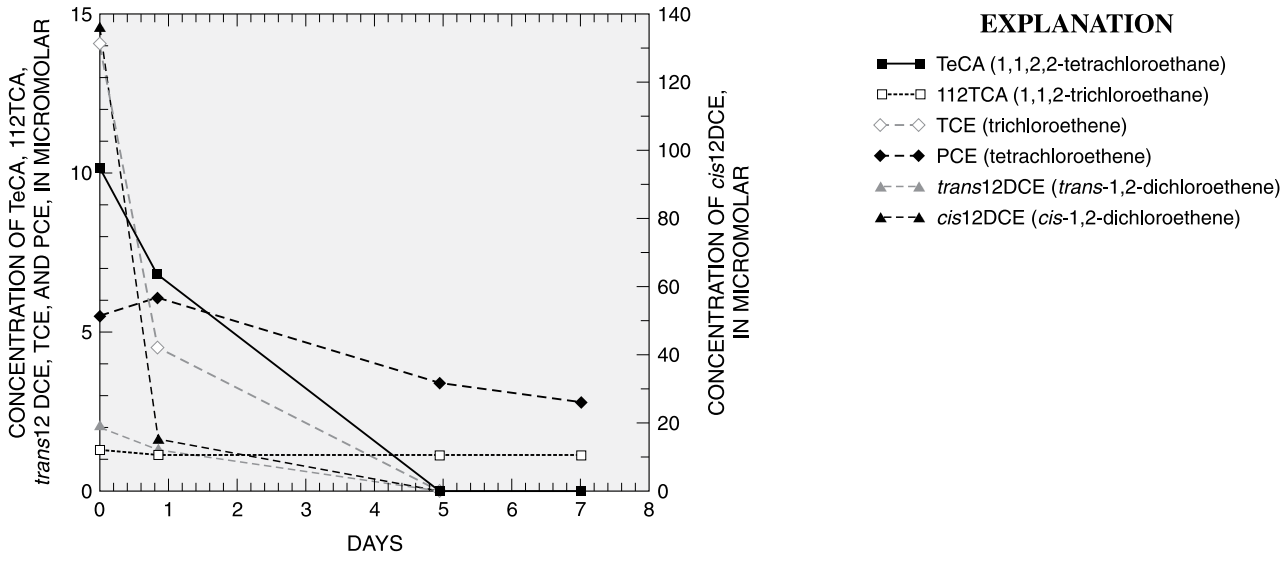


Figure 9. WBC-2 degradation of of 1,1,2,2-tetrachloroethane (TeCA), 1,1,2-trichloroethane (112TCA), and *cis*-1,2-dichloroethene (*cis*12DCE) with co-contaminants tetrachloroethene (PCE) and trichloroethene (TCE).

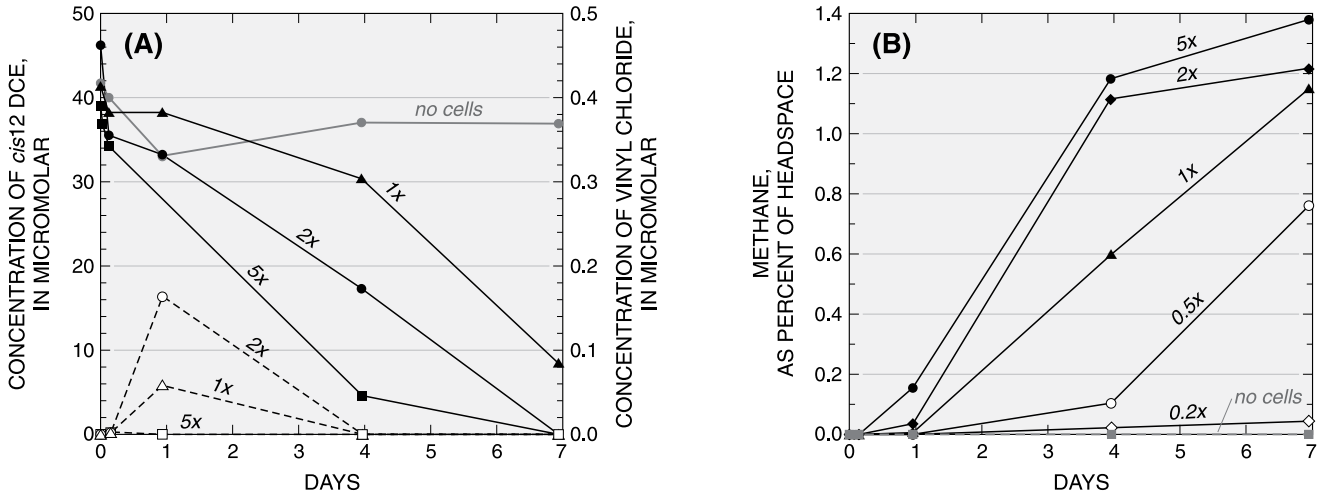


Figure 10. *(A)* dechlorination of *cis*-1,2-dichloroethene (*cis*12DCE), and *(B)* production of methane at various cell densities (0, 0.2x, 0.5x, 1x, 2x, and 5x) of WBC-1. Dashed lines in *A* are vinyl chloride (VC) concentrations. [Note that 0.2x and 0.5x cell density tests were analyzed for chlorinated compounds and methane; these *cis*12DCE concentrations are not shown because they were not significantly different from the no cells control (0 cell density). Activity tests for *trans*-1,2-dichloroethene (*trans*12DCE) were done at the same cell densities and gave similar results as for the *cis*12DCE above.]

Molecular Characterization

The timing of DNA sampling for construction of the clone libraries differed for the two consortia, resulting in a clone library for WBC-2 that represents a more highly enriched culture in which the original sediment inoculum had been greatly diluted compared to the WBC-1 clone library. The first clone library was constructed from DNA sampled immediately after the culture batches were mixed together to form WBC-1. The second was constructed from WBC-2 DNA sampled after repeated transfers over an 11-month period had diluted the amount of sediment in the culture and obtained a more highly enriched culture.

Both libraries were dominated by Clostridiales (20 percent in WBC-1; 63 percent in WBC-2). Diversity in the WBC-1 clone library was higher than in WBC-2 (figs. 11a–d), probably due to the early phase of the enrichment/dilution process. As shown in table 4, less than 5 percent of the clones in each library were more or less related to dechlorinating bacteria that have been studied in culture, although in general, WBC-1 included members that were more similar to known dechlorinators than WBC-2.

A number of studies have suggested that complete degradation of chlorinated aliphatic hydrocarbons requires the presence of specific microorganisms. Since *Dehalococcoides* includes the only known species capable of complete

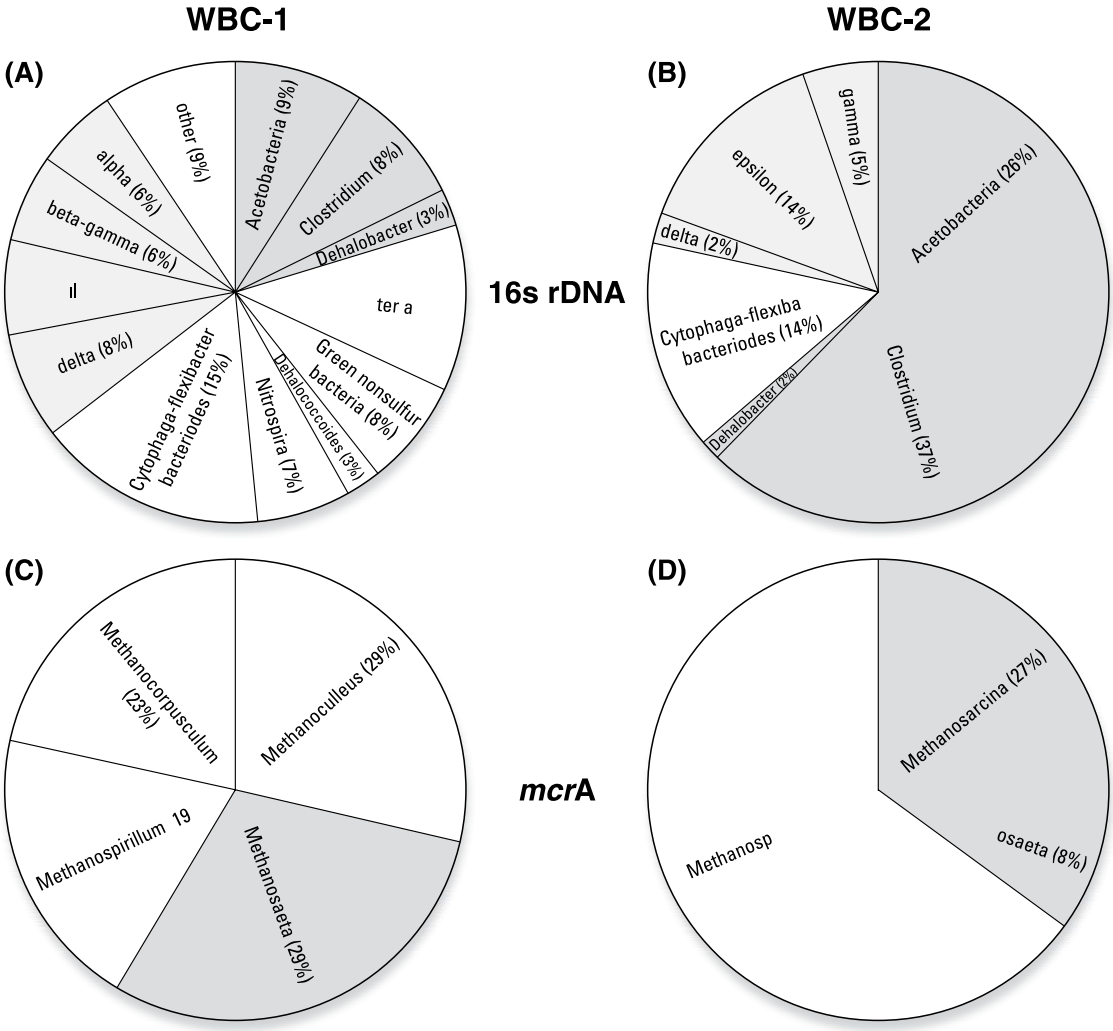


Figure 11. Distribution among phylogenetic groups for *(A, B)* bacteria (16s rDNA) and *(C, D)* methanogens (*mcr*A) for clones sequenced from WBC-1 and WBC-2. The dark gray shadings for the 16s rDNA clone library indicate bacteria in the Clostridiales groups, while the lighter gray shadings indicate Proteobacteria. The dark gray shadings for the mcrA clone library indicate acetate-utilizing methanogens.

Table 4. Dechlorinating isolates related to WBC-1 and WBC-2 clones.

[Relatedness given as percentage of shared bases in 16s rDNA between 46f and 519r.]

Dechlorinating isolate	Clone library	Relatedness, in percent	Representation in library
Dehalobacter restrictus	WBC-1	98	3
	WBC-2	91	2
Sulfurospirillum multivorans	WBC-1	0	0
	WBC-2	84	3
Dehalococcoides sp. strain FL2	WBC-1	99	3
	WBC-2	0	0

biodegradation of chlorinated ethenes to ethene, there has been specific interest in using molecular approaches to identify and enumerate this organism at contaminated sites (Hendrickson and others, 2002). Although *Dehalococcoides* spp. were not detected in the WBC-2 clone library, it was determined that about 1 percent of the consortium was *Dehalococcoides* sp. by comparing qPCR values with total cell counts (Jones and others, 2006). Thus, *Dehalococcoides* spp. represent a minor part of the populations of WBC-1 and WBC-2 relative to other dechlorinating consortia, in which they represent more than 30 percent of the bacterial population (Duhamel and others, 2002; Richardson and others, 2002; Gu and others, 2004). Furthermore, *Dehalococcoides* counts by qPCR did not increase in the WBC-2 culture as the efficiency of dechlorination improved in the consortium (data not shown), indicating that *Dehalococcoides* is not a good indicator of WBC-2's ability to dechlorinate chlorinated ethanes and ethenes. The dechlorinating *Desulfuromonas* sp. was undetectable in both WBC-1 and WBC-2 using the qPCR technique.

Although 95 percent of the WBC-2 clones were not related to dechlorinating bacteria that have been studied in isolation, they were related to bacterial clones that have been observed at other dechlorinating sites (table 5). The phylogenetic types observed in the WBC-1 clone library (such as the GNS and Nitrospira) are also types commonly associated with dechlorination (Lowe and others, 2002; Gu and others, 2004). Earlier studies indicated that dechlorination of TeCA and its daughter products could not be attributed to a single organism (Lorah and Voytek, 2004); therefore, it is not surprising that enrichment for TeCA degradation resulted in a consortium of organisms rather than a single dominant organism.

Clostridiales dominated the WBC-2 consortium. Gerritse and others (1999) noted that the ability to metabolize chlorinated compounds appears to be particularly pronounced in the Clostridium-Bacillus subphylum of the gram-positive bacteria. For example, *Desulfitobacterium frappieri* grew using PCE or TCE as a terminal electron acceptor with lactate as an electron donor, but could not ferment lactate (Gerritse and others, 1999). Although the role of Clostridia in WBC-2 might be limited to fermentation of lactate to supply H_2 to dechlorinating bacteria, these organisms also were abundant in the WBC-1 culture that was fed only acetate and H_2. (Dolfing, 2000) suggested that the fermentation of chlorinated compounds is thermodynamically possible. Although this has yet to be demonstrated, the Clostridiales, known for their fermentation capabilities, would be good candidates for this reaction.

Another numerically important Clostridiales genus in the clone libraries is *Acetobacterium*. Acetobacteria are homoacetogens, able to grow by converting hydrogen and carbon dioxide to acetate, although growth by fermentation has also been observed (De Wildeman, Neumann, and others, 2003). Acetobacteria therefore could subsist in the culture without deriving energy from dechlorination. Acetobacteria are among the groups of microorganisms that produce corrinoid factors, which may be important in promoting dechlorination. In addition, an *Acetobacterium* sp. related to *A. wieringae* was isolated that obtains energy for growth from dechlorination of 12DCA to ethene, although this strain lost its ability to dechlorinate after about 10 transfers (De Wildeman, Neumann, and others, 2003).

Phylogeny of clones identified in the consortia indicate that in addition to fermentation and homoacetogenesis, the processes of sulfur-cycling, methanogenesis, and chemoautotrophy also may support populations in the culture. Maintaining diversity in the culture may be an advantage, as failed attempts to maintain dechlorinating cultures have often been attributed to unknown nutritional requirements (Maymó-Gatell and others, 1999; Richardson and others, 2002). The WBC-1 clone library, however, exhibited greater diversity than the WBC-2 library, but WBC-1 could not be maintained. Part of the enrichment process involves the selection of organisms that can tolerate cultivation. With WBC-2, a culture was developed that can be maintained and grown, and that can degrade both chlorinated ethanes and ethenes simultaneously without apparent inhibition. The following microcosm and column experiments also indicate that WBC-2 can degrade CT and CF.

Table 5. WBC-2 clones, frequency within the clone library, and significant relations.

[TCE, trichloroethene; PCB, polychlorinated biphenyl; PCE, tetrachloroethene; 12DCA, 1,2-dichloroethane; CA, chloroethane; VC, vinyl chloride.]

Frequency, as percent	Phylogenetic group (putative division)	Accession number	Source
37	**Clostridiales**: uncult. *Clostridium* sp.	AY667266	TCE-dechlorinating community in a contaminated basaltic aquifer (closest relative).
26	**Clostridiales**: *Acetobacteria*	X96955	*Acetobacterium wieringae* isolate.
		AY185326, AY185312	1,2-Dichloropropane-dechlorinating enrichment.
		AJ535706	Mixed culture that dechlorinates tri-chlorodibenzo-p-dioxin.
		AJ488081	Chlorobenzene-degrading consortium.
		AF479584	A. *malicum* strain HAAP-1.
2	**Clostridiales**: *Dehalobacter sp*.	AF422637	TCE-reducing community.
		AJ009454	Trichlorobenzene-degrading consortium.
		AJ249096, AJ278164	1,2 -Dichloropropane-degrading populations.
		AY754830	PCB-degrading sediment culture.
		AY754830	1,2-Dichloropropane-dechlorinating bioreactor population.
14	**CFB group**: Uncult. *Bacteroidetes*	AY217446	TCE-dechlorinating community (closest relative).
		AY754840	PCB-degrading community.
		AJ488070	Chlorobenzene-degrading consortia.
		AJ306738	1,2-Dichloropropane-dechlorinating mixed culture.
Less than 1	**Delta Proteobacteria**	AF223382	*Trichlorobacter thiogenes*, isolate that reductively dechlorinates trichloroactic acid.
		AF447133	PCE-dechlorinating community.
		AY667270	TCE-dechlorinating community in a contaminated basaltic aquifer.
		AB186851	Polychlorinated dioxin-dechlorinating community.
		AY221614	Soil contaminated with metals and organic solvents.
1.5	**Delta Proteobacteria**	AJ012591	Sulfate-reducing bacteria.
		AY548775	Desulfobulbus
		AF050526	*Syntrophus,* in aquifer contaminated with 12DCA, CA, VC.
		.	Trichlorobenzene-transforming consortium AEM65:283-286.
14	**Epsilon Proteobacteria**	AB030592	Petroleum-contaminated ground water.
		AY692045	Arcobacter, anaerobic biofilms.
			Member of 2-bromophenol-dehalogenating consortium.
5	**Gamma Proteobacteria**	AY321589	*Pseudomonas stutzeri* (closest relative).
		AY017341	Chlorate-reducing isolate, *Pseudomonas chloritidismutans* (very close relative).
		AJ544240	Aerobic PCE dechlorination.

Microcosm Experiments

The microcosms completed during 2002–04 were generally preliminary steps in designing the enhanced bioremediation pilot tests in the wetland and, thus, were kept simple and consistent. The focus for most tests (tables 1 and 6) was on evaluating degradation of approximately the same initial TeCA concentration under the same conditions, except for one variable such as the addition of nutrients (nutrient biostimulation), addition of an organic donor (donor biostimulation), addition of WBC-1 or WBC-2 (bioaugmentation), or a change in the matrix to test commercial mixtures considered for the reactive mat pilot test. Although possible co-contaminant effects were addressed in several treatments, concentrations were kept relatively low because toxicity effects can be magnified in closed batch systems. To evaluate biostimulation and bioaugmentation effects for the direct-injection pilot test, wetland sediment from sites within the natural attenuation wetland area was used because seep delineation and characterization occurred concurrently with this initial laboratory phase. Seep sediment matrix and higher concentrations of mixed VOCs were tested later in the column experiments after the seep areas were characterized and a pilot test site was selected. Discussion of the microcosms in the following sections is grouped according to the process that the test was designed to evaluate—biostimulation, bioaugmentation, co-contaminant degradation and effects, and ZVI effects on enhancing biodegradation of chlorinated VOCs.

Biostimulation for Enhanced Bioremediation

Nutrient and donor limitation is a common cause of low microbial growth and activity in ground water. Although the vegetation in wetlands might be expected to supply abundant nutrients and organic carbon to the sediment, the form of the nutrient or organic carbon and competition with the plants and other microorganisms may limit their availability to the contaminant-degrading microorganisms. Nutrient uptake during plant growth was hypothesized to be a factor in the observed decrease in TeCA degradation rates during the summer in previous studies in the natural attenuation area (Lorah and others, 2003; Lorah, Spencer, and McGinty, 2005). An ammonium-phosphate solution was used for nutrient biostimulation microcosms in June–July 2002 and September–October 2002 (table 1). In the 2003 microcosms, the media control treatments, which had only the culture media used to grow and maintain WBC-1 and WBC-2, had the same ammonium-phosphate concentration as the nutrient-amended micrososms in September–October 2002. Thus, nutrient biostimulation in the 2003 microcosms was evaluated using the media controls.

Overall, wetland sediment collected from three sites showed no enhancement in degradation of TeCA or its daughter products with nutrient biostimulation compared to unamended microcosms (figs. 12–14). Except for WB23 sediment in the June–July 2002 microcosm, TeCA degradation rates showed only a slight increase or a decrease compared to unamended sediment (fig. 12). Seasonal changes in TeCA degradation rates were observed with the wetland sediment as seen in earlier studies, with the lowest degradation rates occurring with wetland sediment collected in late August 2002 (September–October 2002 microcosms) and the highest rates occurring with sediment collected in early March 2003 (fig. 12). Addition of nutrients to the microcosms did not change this seasonal pattern, indicating that nutrient uptake by plants is not a factor in the lower degradation rates in the summer.

The total production of chlorinated daughter products was about the same in the nutrient-amended and unamended microcosms, and complete degradation did not occur within the 35-day experiments (fig. 13). Time courses for the individual daughter products in representative microcosms are shown in fig. 14. The daughter product distributions were similar in the nutrient biostimulated and unamended sediment, with TeCA degradation initiated with simultaneous production of 12DCE (dichloroelimination pathway, fig. 2a) and 112TCA (hydrogenolysis pathway) as observed in earlier studies (Lorah and others, 2003). Higher concentrations of 12DCA were observed with nutrient biostimulation than those observed in the unamended sediment, however (fig. 14). In addition, low concentrations of chloroethane were detected in the nutrient biostimulated sediment (data not shown), although it was not detected in the unamended microcosms in these or previous experiments (Lorah and others, 1997; Lorah and others, 2003). The higher concentrations of 12DCA and chloroethane could indicate that nutrient biostimulation increased the relative proportion of TeCA degrading by the hydrogenolysis degradation pathway (fig. 2a), or decreased the degradation rate of 12DCA and chloroethane.

Nutrient biostimulation in the BionSoil/peat mixture increased the TeCA degradation rate compared to the unamended wetland sediment or the unamended BionSoil/peat, but the daughter product distribution was unaffected (figs. 12 and 15). TeCA degradation in the BionSoil/peat mixture was initiated with simultaneous production of 12DCE and 112TCA as observed in the wetland sediment from the natural attenuation study area. Relatively high 12DCA and chloroethane production was observed in both the unamended and nutrient-amended BionSoil/peat mixture, unlike the wetland sediment microcosms where only the nutrient amendment gave higher 12DCA and chloroethane concentrations.

TeCA degradation was unexpected in this unamended microcosm because neither the BionSoil nor the commercial peat was known to have been previously exposed to chlorinated VOCs. It is possible that microorganisms capable of degrading the contaminants were introduced to the BionSoil mixture with the contaminated ground water (WB24B) used to mix the microcosm slurry. TeCA degradation rates and daughter product distributions in microcosms constructed

Table 6. Rate constants for 1,1,2,2-tetrachloroethane (TeCA) degradation in microcosms with different matrices and amendments.

[Note that all concentrations and rates are for TeCA, except those under the subheading "CT degadation," which are for carbon tetrachloride. C_o, concentration of TeCA or CT on day 0; C_i, concentration of TeCA or CT on first day of indicated time period that was used to calculate rates. Normalized rates were calculated with C_i=3.0 micromoles for comparison among treatments, except for BT2C2.9/03, which had a high initial concentration and is indicated as "NA" (not applicable).]

Treatment name	C_o (micromolar)	C_i (micromolar)	Rate constant (per day)	Half-life (days)	Correlation coefficient (r^2)	Time period (days)	Rate (micromolar per day)	Normalized rate (micromolar per day)
				Unamended				
23T.6/02	7.08	3.40	.19 ± .02	3.6	0.96	1–21	0.65	0.57
30T.6/02	7.08	2.40	0.17 ± 0.01	4.1	.97	3–30	.41	.51
35T.6/02	7.08	2.90	0.11 ± 0.01	6.3	.93	1–26	.32	.33
23T.9/02	6.36	3.70	0.13 ± 0.01	5.3	.97	1–21	.48	.39
30T.9/02	6.36	2.20	0.07 ± 0.01	9.9	.92	3–12	.15	.21
35T.9/02	6.36	2.30	0.06 ± 0.005	12	.96	1–25	.14	.18
23T.3/03	8.75	5.84	0.064 ± 0.0043	11	.97	3–30	.37	.19
30T.3/03	8.75	7.76	0.085 ± 0.007	8.2	.97	3–21	.66	.26
35T.3/03	8.75	4.91	0.071 ± 0.0087	9.8	.94	3–21	.35	.21
BT.9/03	8.36	2.46	0.10 ± 0.005	6.9	.99	1–16	.25	.30
				Biostimulation				
23TN.6/02	7.08	2.80	0.26 ± 0.07	2.7	.77	1–16	.73	.78
30TN.6/02	7.08	2.20	0.11 ± 0.005	6.3	.99	1–26	.24	.33
35TN.6/02	7.08	2.50	0.15 ± 0.009	4.6	.98	1–16	.38	.45
23TN.9/02	6.36	3.20	0.10 ± 0.008	6.9	.96	1–21	.32	.30
30TN.9/02	6.36	2.60	0.06 ± 0.004	12	.97	3–30	.16	.18
35TN.9/02	6.36	3.00	0.07 ± 0.01	9.9	.87	1–16	.21	.21
BTO.9/03	8.36	2.68	0.12 ± 0.01	5.6	.98	1–16	.33	.37
				Bioaugmentation				
23TC1.9/02	6.65	5.80	0.27± 0.02	2.6	.98	1–21	1.57	.81
30TC1.9/02	6.65	6.60	0.13 ± 0.007	5.3	.98	1–30	.86	.39
35TC1.9/02	6.65	5.20	0.18 ± 0.02	3.8	.96	1–12	.94	.54
23TC1.3/03	8.75	4.98	0.20 ± 0.068	3.4	.82	3–12	1.00	.60
30TC1.3/03	8.75	3.89	0.188 ± 0.028	3.7	.96	3–16	.73	.56
35TC1.3/03	8.75	4.75	0.13 ± 0.045	5.2	.9	3–9	.62	.39
BTC1.9/03	8.36	2.06	0.25 ± 0.049	2.7	.90	1–12	.52	.76
BTC2.9/03	8.36	2.32	0.19 ± 0.031	3.7	.95	1–9	.44	.56
BTC2.7/04	8.04	3.96	0.13 ± 0.008	5.4	.99	1–12	.51	.39
BT2C2.9/03	83.6	30.7	0.06 ± 0.01	11	.92	1–12	1.99	NA
CrTC2.7/04	8.04	5.20	0.14 ± 0.01	5.0	.99	1–12	.72	.42
LgTC2.7/04	8.04	4.70	0.21 ± 0.028	3.3	.94	1–16	.99	.63
PoTC2.7/04	8.04	4.04	0.14 ± 0.013	5.1	.98	1–12	.55	.41

Table 6. Rate constants for 1,1,2,2-tetrachloroethane (TeCA) degradation in microcosms with different matrices and amendments. —Continued

[Note that all concentrations and rates are for TeCA, except those under the subheading "CT degadation," which are for carbon tetrachloride. C_o, concentration of TeCA or CT on day 0; C_i, concentration of TeCA or CT on first day of indicated time period that was used to calculate rates. Normalized rates were calculated with C_i=3.0 micromoles for comparison among treatments, except for BT2C2.9/03, which had a high initial concentration and is indicated as "NA" (not applicable).]

Treatment name	C_o (micromolar)	C_i (micromolar)	Rate constant (per day)	Half-life (days)	Correlation coefficient (r^2)	Time period (days)	Rate (micromolar per day)	Normalized rate (micromolar per day)
Bioaugmentation (continued)								
PaTC2.7/04	8.04	4.31	0.024 ± 0.009	29	0.80	1–16	0.10	0.07
TlcTC2.7/04	8.04	3.43	0	NA	NA	NA	.00	.00
TlcTC2.8/04	6.45	3.43	0.03 ± 0.006	26	.91	1–14	.09	.08
Media control								
30TM.9/02	6.36	3.60	0.04 ± 0.005	17	.92	3–25	.14	.12
35TM.9/02	6.36	3.50	0.11 ± 0.009	6.3	.97	1–16	.39	.33
23TM.3/03	8.75	5.22	0.056 ± 0.0045	12	.96	3–30	.29	.17
30TM.3/03	8.75	4.61	0.124 ± 0.032	5.6	.79	3–21	.57	.37
35TM.3/03	8.75	5.41	0.079 ± 0.017	8.8	.88	3–16	.43	.24
BTM.9/03	8.36	2.37	0.16 ± 0.018	4.3	.95	1–16	.38	.48
Co-contaminant								
BTVC2.9/03	8.36	3.17	0.27 ± 0.045	2.6	.92	1–16	.86	.81
CT degradation								
23CT.6/02	7.50	1.30	1.38	0.5	1	1–3	1.8	4.14
30CT.6/02	7.50	2.00	2.5	.3	1	1–3	5	7.50
S23CT.6/02	7.50	.88	.66	1	1	1–3	.58	1.98
S30CT.6/02	7.50	1.40	0.73± 0.15	1	.96	1–6	1	2.19
BTVC2.9/03	4.10	4.10	2.6	.3	1	0–3	11	7.80

with 0.2 μm-filtered ground water, however, were comparable to those constructed without filtering the ground water (data not shown). In earlier studies, the first reaction steps in TeCA degradation occurred under a wide range of conditions and indicated that the microorganisms initiating these reactions are ubiquitous in wetland sediments (Lorah and others, 2003).

TeCA degraded at similar rates in the BionSoil/peat mixture and the wetland sediment collected from the natural attenuation area, although complete degradation to non-chlorinated end-products did not occur (figs. 12b and 15a). About 100 percent of the initial TeCA concentration in solution at day 1 was remaining as chlorinated daughter products at day 33 in the BionSoil peat mixture microcosms that were unamended (TeCA addition only) (fig. 15a).

Donor-stimulated (lactate addition) microcosms were constructed with wetland sediment from site WB23 and with the BionSoil/peat mixture (fig. 15b). The donor-stimulated WB23 microcosms (23TO) showed such a high methane production rate (fig. 16b) that sampling was stopped after 2 weeks because the tops started to come off the serum bottles. The first 2 weeks showed relatively high 12DCA and chloroethane production in the WB23 donor-stimulated microcosm (data not shown), as observed in the nutrient-stimulated microcosms with the wetland sediment (fig. 14c). Donor stimulation did not enhance degradation of TeCA in the BionSoil/peat mixture compared to the unamended microcosm (figs. 15a–b).

Sterile controls, both unamended and nutrient-amended, with the BionSoil and with wetland sediment from the three

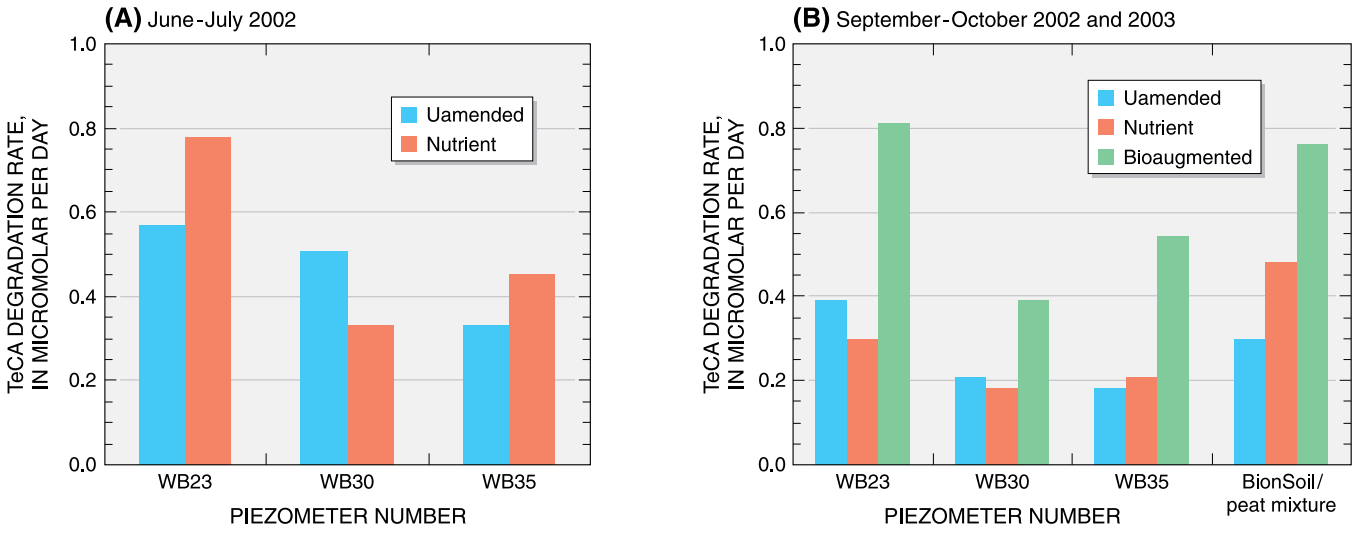


Figure 12. 1,1,2,2-tetrachloroethane (TeCA) degradation rates in unamended microcosms with wetland sediment or compost compared to nutrient-amended and bioaugmented microcosms, *(A)* June–July 2002 and *(B)* September–October 2002 and 2003. [All rates were calculated using the rate constants in table 6 and an initial TeCA concentration of 3.0 micromolar.]

sites showed only low production of the abiotic daughter compound TCE (less than 3 percent of the initial TeCA added), and sometimes 12DCE production near the end of the microcosm experiments. 12DCE produced in previous microcosms with the wetland sediment has been attributed to some rebound in microbial activity in the formaldehyde-treated controls with time (Lorah and others, 2003).

Redox constituents and nutrients, including methane, ferrous iron, sulfate, ammonia, and phosphate, were analyzed in the unamended sediment and biostimulated microcosms (data not shown). Phosphate and ammonia concentrations were variable among wetland sediment from the three sites and typically were similar in the unamended and nutrient-amended microcosms. Phosphate and ammonia can sorb to the sediment and interact with iron minerals (Scudlark and Church, 1989; Hogan and others, 2004). The fact that unamended and nutrient-amended microcosms had similar concentrations indicates that these sediment interactions are probably the major control on the nutrient concentrations in solution. Methane concentrations increased with time in the unamended, nutrient-amended, and donor-amended microcosms constructed with wetland sediment from the three sites, with the donor-amended microcosm showing substantially higher methane production than the other treatments (microcosms with WB23 and WB35 sediment shown in figs. 16a–d). The nutrient-amended microcosms for WB23 and WB35 showed an apparent inhibition in methane production in the September–October 2002 microcosms compared to the June–July 2002 microcosms. The nutrient addition, however, may not have been a factor in this decrease in methane because the live control (no TeCA or other amendments) or TeCA-amended microcosms also showed this seasonal decrease in methane concentrations (figs. 16a–d). Methane concentrations in the microcosms constructed with the BionSoil/peat mixture did not show an increase in methane production from organic donor amendment compared to unamended microcosms, as observed in the wetland sediment microcosm amended with organic donor (figs. 16b, 17a). Instead, the unamended, nutrient-amended, and donor-amended microcosms with the BionSoil showed about the same methane concentrations over time (fig. 17a).

Overall, nutrient and donor stimulation did not enhance degradation of the chlorinated VOCs in wetland sediment from the study area or in the commercial BionSoil/peat mixture. Biostimulation of the wetland sediment seemed to cause the TeCA degradation to shift to a predominantly hydrogenolysis pathway, similar to that observed in the unamended and biostimulated BionSoil microcosms, without increasing the total removal of chlorinated daughter compounds. The similar VOC distributions in these biostimulated microcosms indicate that only a more ubiquitous bacterial community (present in the commercial peat and the study area wetland sediment) was stimulated, rather than the specific dechlorinating community that is known to be capable of completely degrading TeCA in the wetland area. TRFLP analyses (data not shown) of the peat used in the BionSoil/peat mixture showed similar bacterial diversity as the wetland sediment from sites WB23 and WB30, indicating that the current TRFLP technique with general primers is not able to distinguish this dechlorinating community.

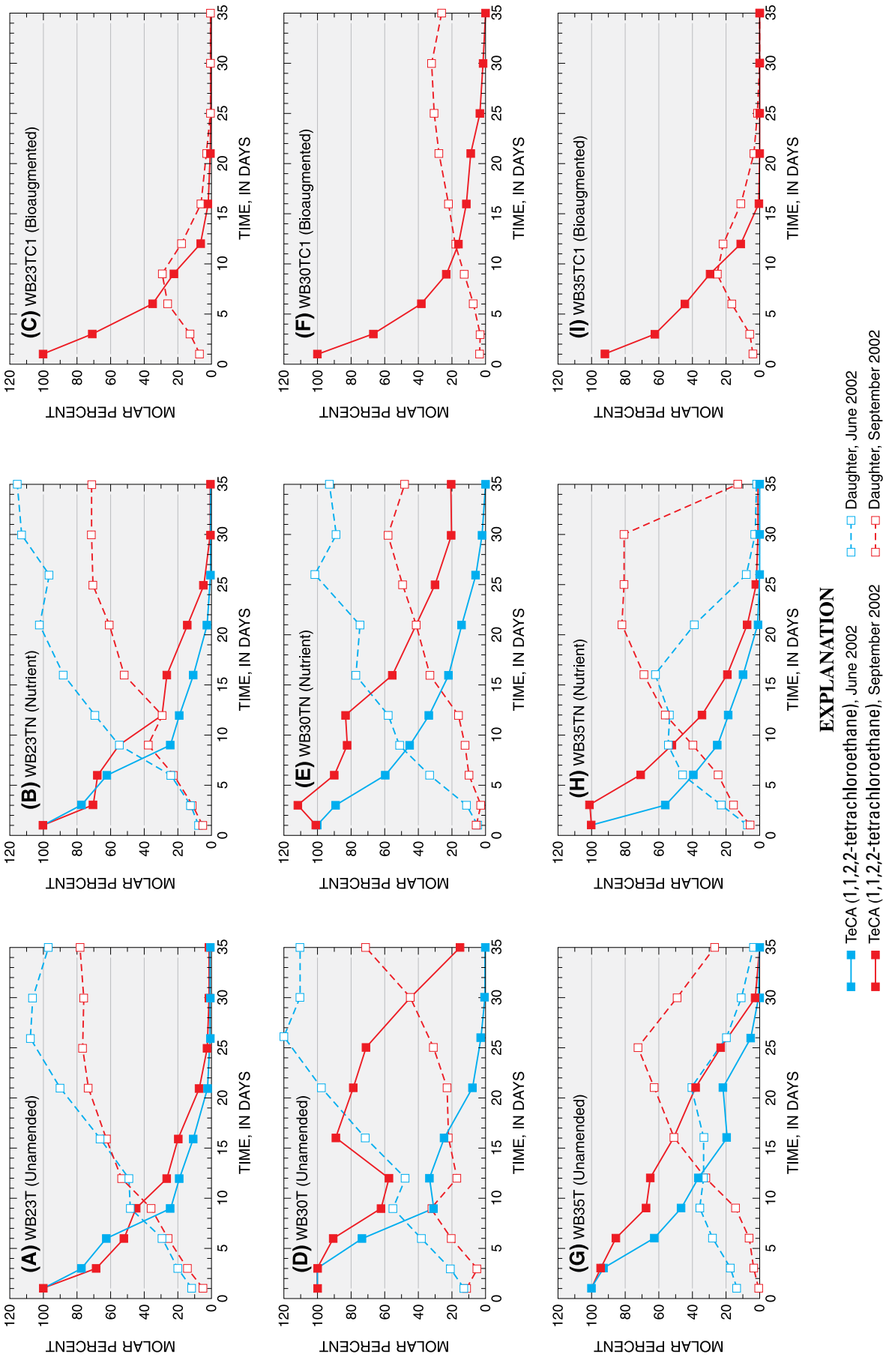


Figure 13. Removal of 1,1,2,2-tetrachloroethane (TeCA) and total production of chlorinated daughter compounds in unamended sediment, nutrient-amended, and bioaugmented microcosms with wetland sediment from sites *(A, B, C)* WB23, *(D, E, F)* WB30, and *(G, H, I)* WB35, June and September 2002. [Concentrations are shown as molar percent of the initial TeCA measured in solution.]

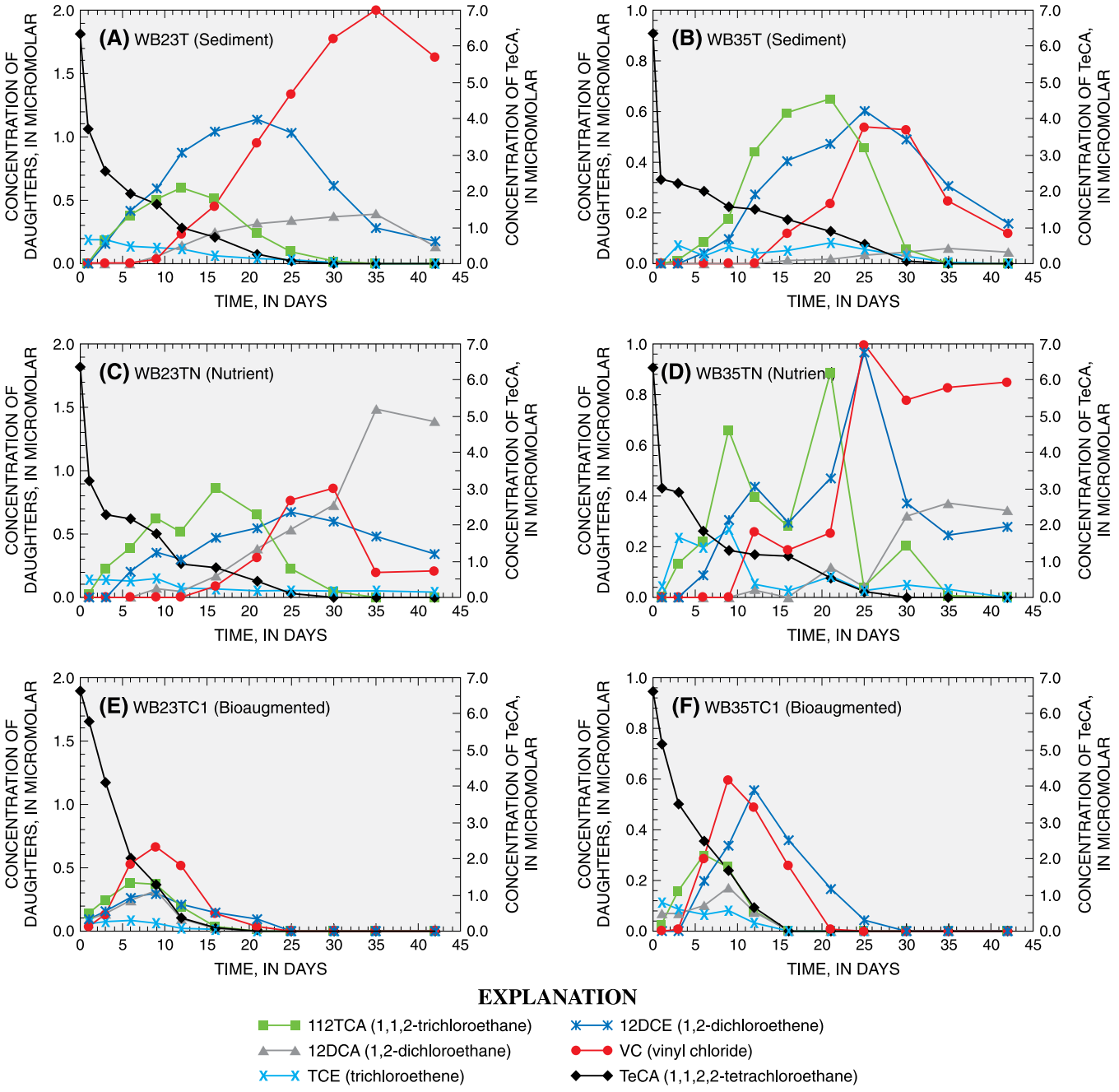


Figure 14. Removal of 1,1,2,2-tetrachloroethane (TeCA) and distribution of chlorinated daughter compounds in selected *(A, B)* unamended sediment, *(C, D)* nutrient-amended, and *(E, F)* bioaugmented microcosms with wetland sediment from sites WB23 and WB35, September–October 2002.

Bioaugmentation for Enhanced Bioremediation

Bioaugmentation by replacing only 10 percent of the water volume in the microcosms with WBC-1 or WBC-2 substantially enhanced degradation of TeCA and its anaerobic daughter products in both the wetland sediment and commercial compost/peat mixtures. TeCA degradation rates were 2 to 3 times higher in the bioaugmented sediment and BionSoil microcosms than in the associated unamended or biostimulated treatments in September–October 2002 and March 2003 (fig. 12b and table 6). BionSoil/peat mixtures bioaugmented with the newly developed WBC-2 culture in September–October 2033 showed similar TeCA degradation rates to those bioaugmented with WBC-1 (table 6). Chlorinated daughter products were removed within 25 days in the bioaugmented microcosms, whereas daughter products

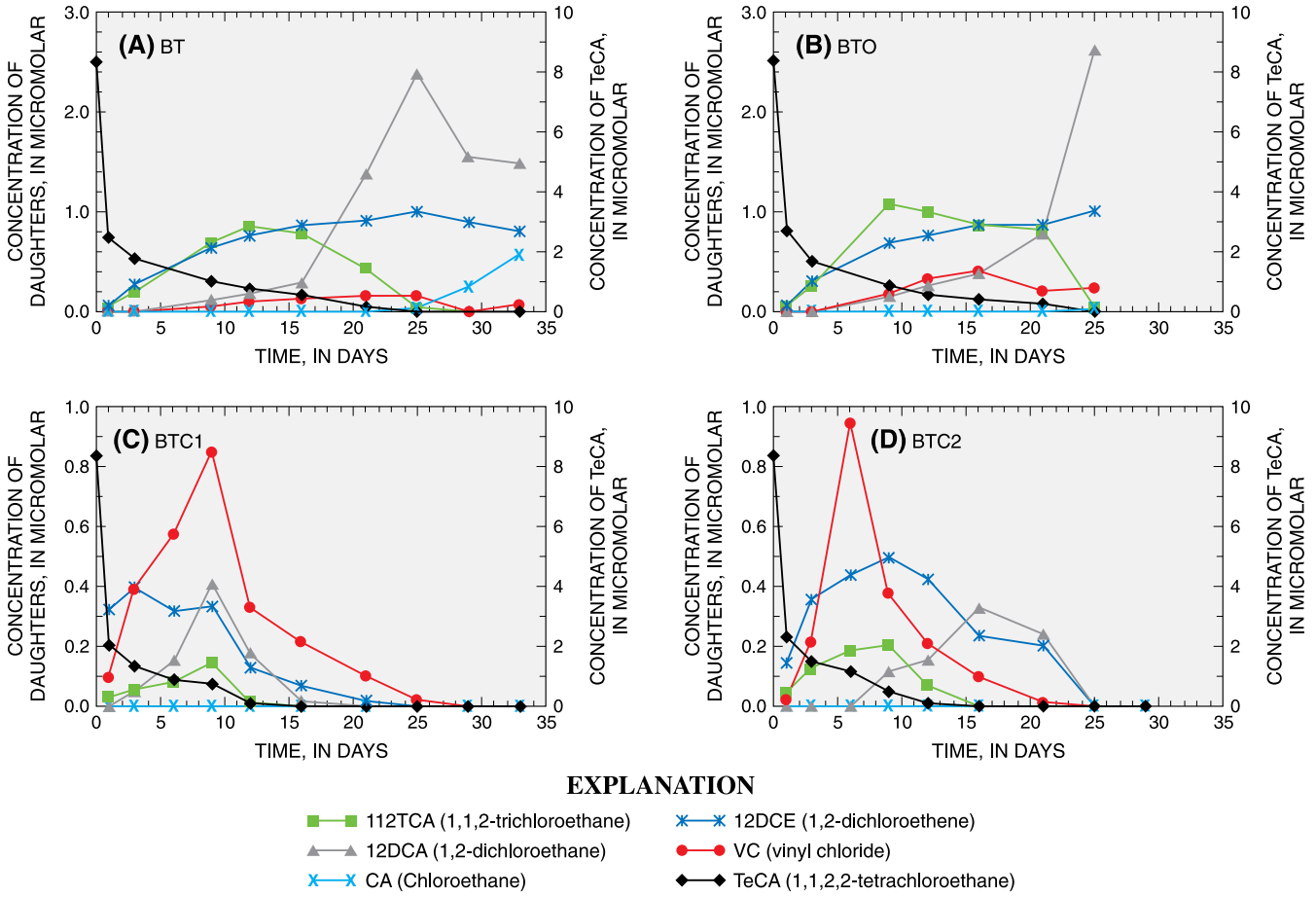


Figure 15. Removal of 1,1,2,2-tetrachloroethane (TeCA) and distribution of chlorinated daughter compounds in *(A)* unamended sediment (BT), *(B)* lactate-amended (BTO), and *(C, D)* WBC-1- or WBC-2-bioaugmented (BTC1 or BTC2) microcosms constructed with BionSoil/peat mixture, September–October 2003.

were still remaining in the unamended sediment after 35 days (figs. 13–14). The chlorinated daughter products were degraded completely within 25 days in both the WBC-1 and WBC-2 bioaugmented microcosms constructed with the BionSoil/peat mixture, although a delay in the peak production and removal of 12DCE and 12DCA was observed in the WBC-2 bioaugmented microcosm compared to the one bioaugmented with WBC-1 (figs. 15c–d). This delay may reflect that the older WBC-1 culture had more time for growth of the dechlorinating population. Thus, WBC-1 initially contained an efficient dechlorinating community, although it could not be maintained during later transfers and growth.

Less accumulation of total chlorinated daughter products was observed in the bioaugmented microcosms—typically a maximum of about 20 to 30 percent of the initial TeCA added in the bioaugmented microcosms compared to 50 to 100 percent in the unamended microcosms (figs. 13 and 15). The same chlorinated daughter products were observed in the bioaugmented microcosms constructed with the wetland sediment and BionSoil, including 112TCA, 12DCA, 12DCE (*cis*- and *trans*-isomers), and VC (figs. 14e–f and 15c–d). 12DCA had a higher concentration in the bioaugmented BionSoil microcosm than microcosms constructed with the wetland sediment, but VC was the predominant chlorinated daughter product in both matrices (figs. 14e–f and 15c–d). Chloroethane was not observed in the bioaugmented microcosms. Thus, the relative predominance of the alkane hydrogenolysis pathway that was observed in the BionSoil unamended microcosm and in the nutrient-amended microcosms was not observed in the bioaugmented microcosms, indicating that WBC-2 was controlling the degradation rather than the native community in the commercial peat. All daughter product concentrations peaked about the same time early in the microcosm experiments, whereas a delay in VC and 12DCA production, respectively, occurred in the unamended microcosms constructed with the wetland sediment and BionSoil (figs. 14 and 15). Ethene was not detected in the bioaugmented microcosms, although it is the major product of TeCA degradation in the stock bottles of the WBC-1 and WBC-2 culture in medium. The substantially

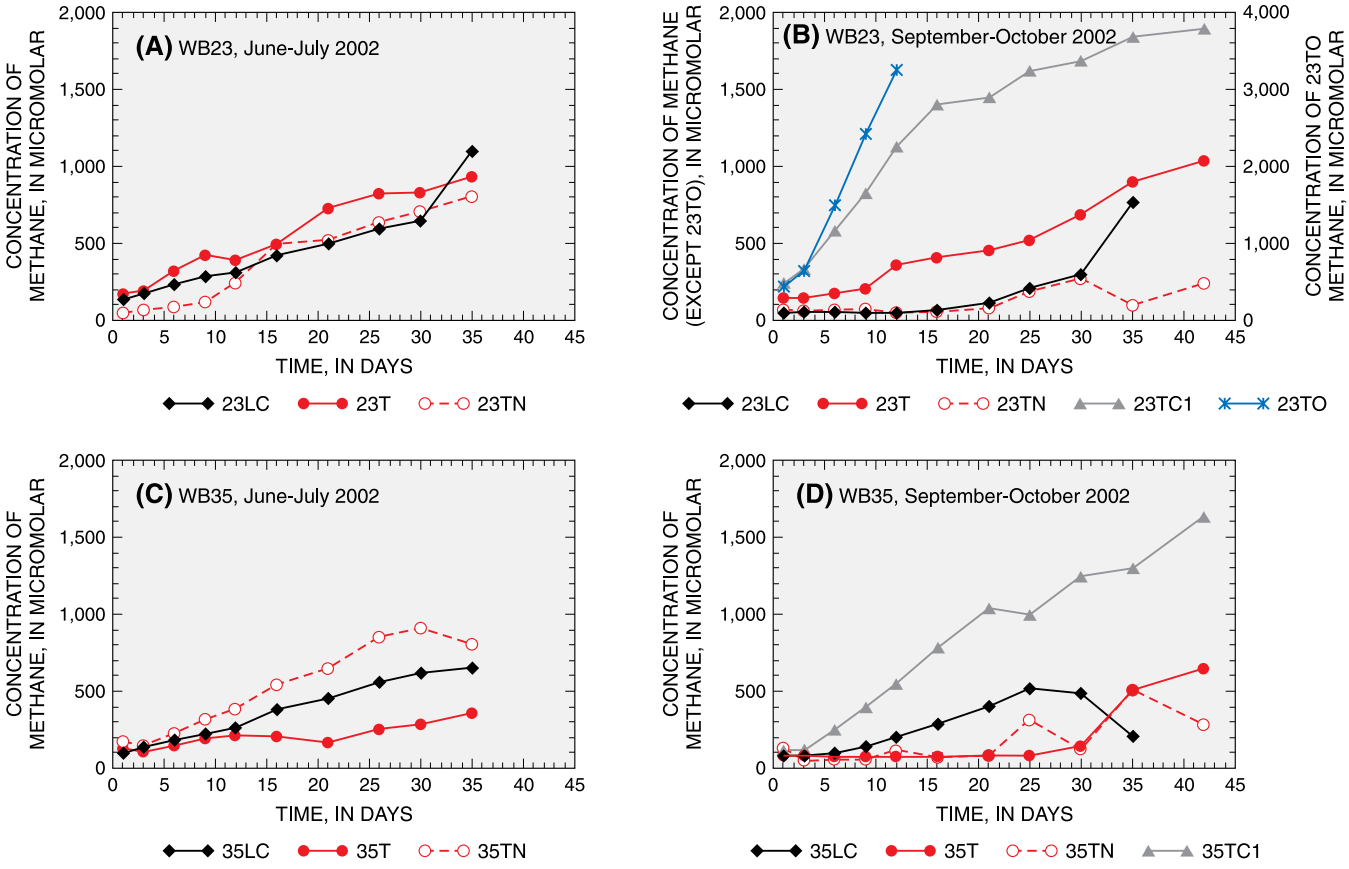


Figure 16. Methane concentrations in microcosms constructed with wetland sediment from sites *(A, B)* WB23 and *(C, D)* WB35, comparing live controls (LC), unamended (T), nurient amended (TN), organic donor amended (TO), and bioaugmented (TC1) treatments, June–July 2002 and September–October 2002. [See table 1 for treatment amendments.]

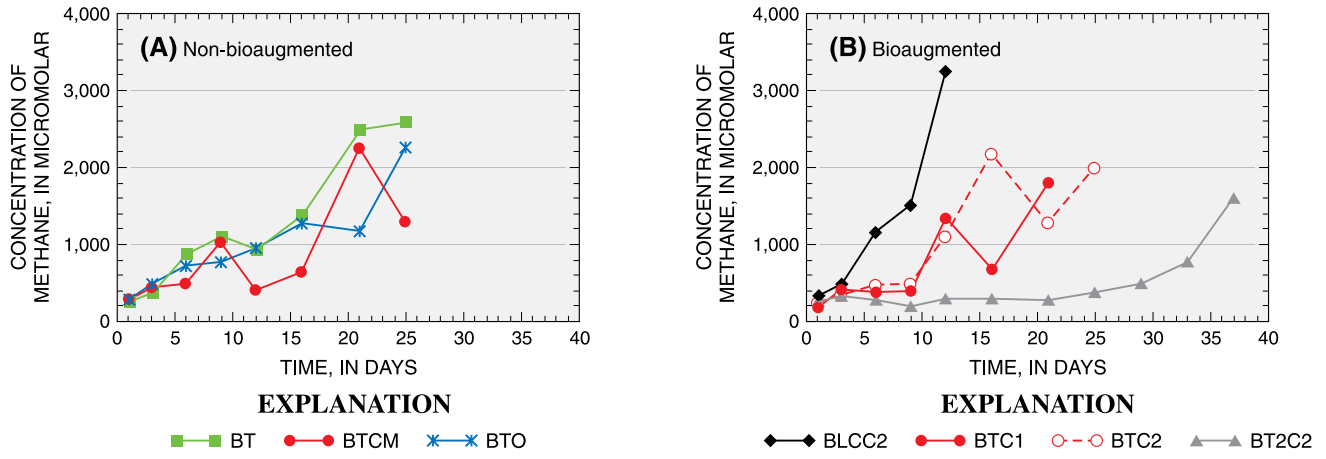


Figure 17. Methane concentrations in *(A)* non-bioaugmented and *(B)* bioaugmented microcosms constructed with BionSoil/peat mixture. [See table 1 for microcosm treatment descriptions.]

lower TeCA concentrations that were added to the sediment microcosms than in the culture stocks, however, probably allowed ethene degradation to maintain a similar rate as ethene production (Lorah and Voytek, 2004).

In one set of bioaugmented microcosms constructed with the BionSoil/peat mixture, the TeCA concentration added at day 0 (83.6 μM) was approximately 10 times higher than typically added to the microcosms (table 6). Although TeCA concentrations decreased to non-detectable concentrations within 15 days, production of chlorinated daughter products was initially inhibited in the microcosms with high TeCA compared to the bioaugmented microcosms with about 8 μM TeCA (figs. 15c–d and 18). Comparison with the sterile control indicated that sorption decreased TeCA concentrations to about 30 μM in the first day of the experiment before 112TCA, VC, and 12DCE production was observed in the live bioaugmented microcosm (fig. 18). 12DCE concentrations were substantially higher than other daughter compounds, accounting for about 90 percent of the initial TeCA in solution. In contrast, more rapid degradation in the bioaugmented microcosms amended with low TeCA (about 8 μM) only resulted in a total accumulation of chlorinated daughter products of approximately 20 molar percent of the initial TeCA in solution (figs. 15c–d). In addition, more than 90 percent of the 12DCE in the high TeCA microcosms was the *trans* isomer, whereas all other microcosms showed predominantly *cis*12DCE. The daughter product distribution in the high TeCA microcosms compared to the low TeCA microcosms indicated that microorganisms involved in hydrogenolysis of TeCA to 112TCA, production of *cis*12DCE, and degradation of 12DCE to VC were inhibited by the high TeCA concentrations or by the lack of methanogenic conditions in this microcosm. Only low concentrations of 12DCE (about 2 μM) were observed in the sterile control for the high TeCA microcosms, indicating that its production in the live microcosm was biotic.

BionSoil became unavailable after the initial microcosms were completed, therefore, other composts were tested for compatibility with WBC-2 as a matrix for the reactive mat (table 2). These composts were mixed with peat and sand from the sources and in the same ratios as the earlier BionSoil microcosms. The dairy-derived Paygro and TLC composts were expected to be most similar to the BionSoil, but both showed the lowest TeCA removal in the microcosms compared to the BionSoil (fig. 19a). In fact, no chlorinated daughter products were produced in the TLC compost mixture, indicating that WBC-2 was not active in this matrix (fig. 19b). The microcosm with the TLC mixture was repeated, but the same negative results were obtained (tables 1 and 6). Although daughter production was observed in the Paygro mixtures, little degradation was observed within 25 days (fig. 19b). The crab compost (Chesapeake Blue) mixture and the yard-waste-derived compost (Leafgro) showed rapid degradation of TeCA and its anaerobic daughter products that was similar to WBC-2 activity observed in the BionSoil mixture (figs. 19a–b).

Earlier studies with the dechlorinating community in the natural wetland sediment have shown that methane production not only indicates that appropriate reducing conditions are present for degradation, but that the methanogens are involved (directly or indirectly) in the degradation reactions (Lorah and Voytek, 2004). Similar results were found for the bioaugmented wetland sediment. Methane production typically was high in the bioaugmented microcosms with wetland sediment (23TC1 and 35TC1 for September–October 2002 in figs. 16b,d, for example) compared to the sediment amended only with TeCA (23T and 35T). At the end of

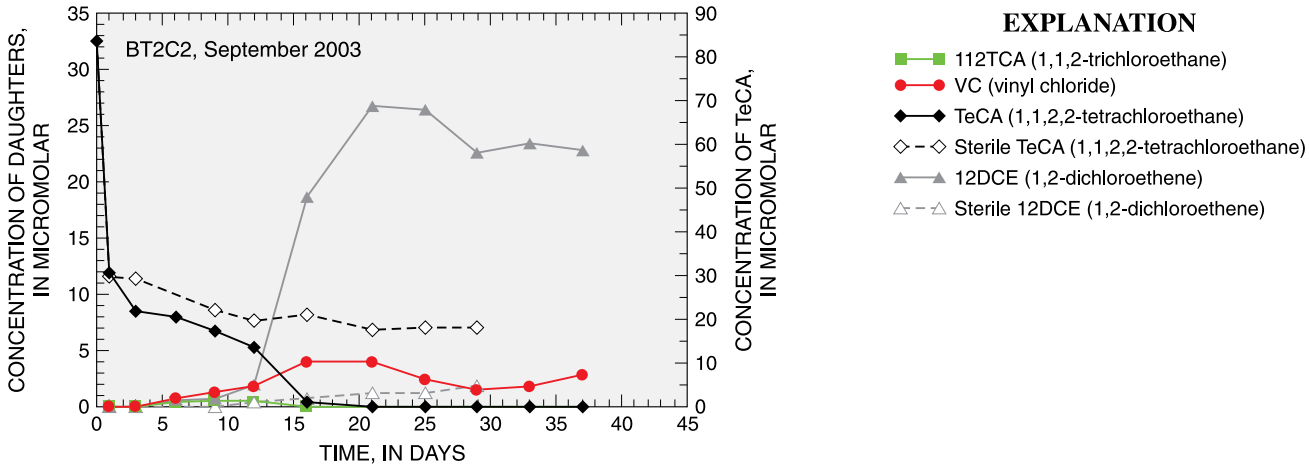


Figure 18. Removal of 1,1,2,2-tetrachloroethane (TeCA) and distribution of chlorinated daughter compounds in WBC-2-bioaugmented microcosms constructed with BionSoil/peat mixture and amended with high initial TeCA concentration, September–October 2003.

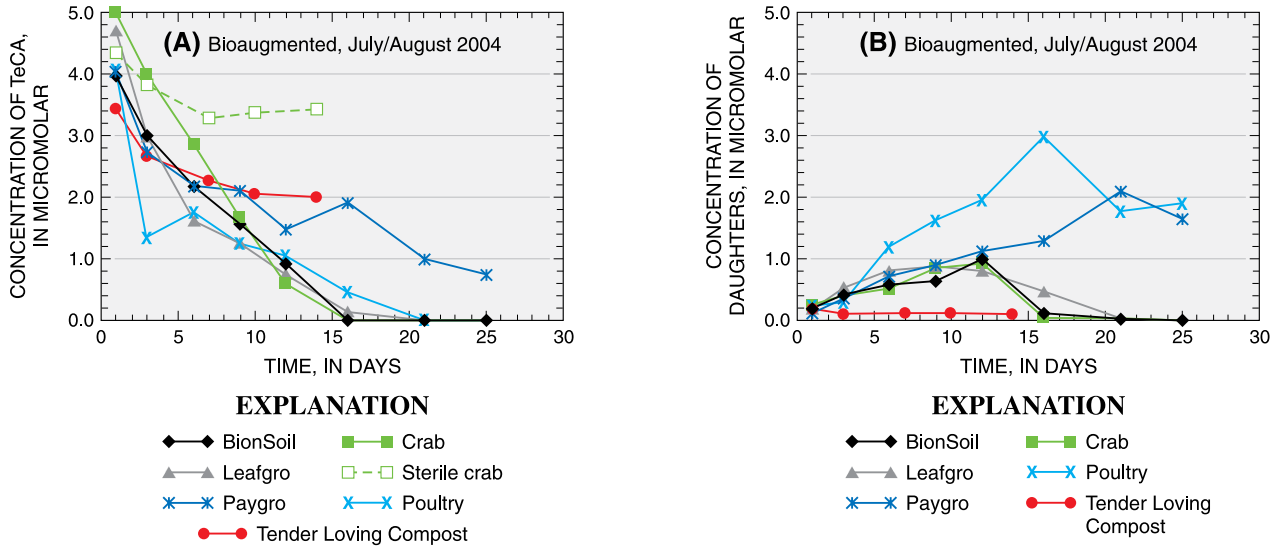


Figure 19. *(A)* Degradation of 1,1,2,2-tetrachloroethane (TeCA), and *(B)* production of daughter compounds in selected bioaugmented microcosms with different compost mixtures, July and August 2004. [See table 2 for compost characteristics.]

the experiment, methane concentrations were 2 to 3 times higher in the bioaugmented sediment than in the sediment amended only with TeCA and no culture. In the March 2003 experiment, methane production was slow for the first 10 days in the unamended and bioaugmented sediment; the relatively lower TeCA and daughter product degradation rates in these bioaugmented microcosms (data not shown) appeared to be associated with the lack of methanogenesis. A higher accumulation of total chlorinated daughter products (up to 60 percent of the initial TeCA) was observed in these bioaugmented microcosms than in the September–October 2002 bioaugmented microcosms.

In the BionSoil/peat mixtures, methane production was highest in a control microcosm that was bioaugmented with WBC-2 but not amended with TeCA (BLCC2 in fig. 17b). Addition of about 8 µM TeCA lowered the methane concentrations in the biougmented (BTC1 and BTC2) and non-bioaugmented (BT) microcosms to about 2,000–2,500 µM in 20 to 25 days, compared to more than 3,000 µM methane by day 12 in BLCC2. Partial inhibition of methanogenesis by TeCA has been observed in previous microcosms (Lorah and others, 2003). Methane production was low until about day 25 in the BionSoil microcosms that were amended with high initial TeCA concentrations (83.6 µM). The lack of methanogenic conditions in this microcosm may be associated with the limited diversity of daughter products and the low degradation of 12DCE (fig. 18). Methane was not produced in the TLC compost mixture, which also did not support TeCA degradation (figs. 19a–b and 20).

In summary, bioaugmentation was successful in enhancing degradation of TeCA and its chlorinated alkane and alkene daughter products, and WBC-2 performed equally well in the wetland sediment from which it was

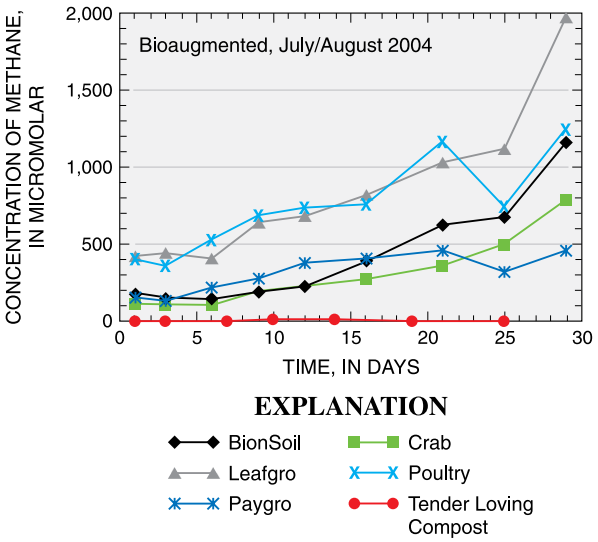


Figure 20. Methane concentrations in bioaugmented microcosms with different compost mixtures, July and August 2004. [See table 2 for compost characteristics.]

derived and in several commercial compost/peat mixtures. Thus, the microcosms indicated that bioaugmentation could be successful in both direct-injection and reactive mat bioremediation technologies. Although degradation rates were substantially enhanced in the bioaugmented microcosms compared to the unamended or biostimulated microcosms, the later column experiments indicated that degradation rates could have been increased by also adding some organic donor along with the culture in the bioaugmented microcosms.

Methane analyses of the microcosms indicated that the best enhanced degradation rates can be obtained when methanogenic conditions are present prior to amendment with the WBC culture.

Co-Contaminant Effects and Degradation

Earlier studies in and near the natural attenuation area (Lorah and others, 1997; Phelan and others, 2002) and the concurrent seep delineation study showed that the chloromethanes CT and CF were common co-contaminants with TeCA. Although CT concentrations as high as about 200 µg/L (1.3 µM) entered the wetland sediment in the natural attenuation area, CT and CF were detected infrequently and in low concentrations in the wetland porewater, indicating that the chloromethanes were degraded rapidly (Lorah and others, 1997). CT-amended microcosms constructed with wetland sediment from sites WB23 and WB30 for this study showed that CT and CF degraded more rapidly than TeCA in the natural wetland community from which the WBC cultures were derived (fig. 21a shows WB30; WB23 was the same). CT degraded with an estimated half-life of 0.3 to 0.5 days for the two sediments, compared to about 4 days for TeCA degradation in these sediments in June–July 2002 (table 6). The 8 µM of CT added to the microcosms at day 0 was undetectable by day 3, with about 10 percent of the CT accumulated as CF. Comparison to the sterile controls indicated that the CT removal and CF production with this first amendment of VOCs was partly abiotic, although estimated degradation rates in the sterile controls were less than half those in the live microcosms (table 6). Low concentrations of methylene chloride (maximum of about 0.2 µM, or about 2.5 percent of the added CT) also were observed in the live microcosms but not in the sterile controls (figs. 21a–b). Chloromethane, another chlorinated daughter product reported for CT, was not detected in the microcosms. A lack of methylene chloride accumulation and chloromethane production also was observed in field sampling of ground water and porewater at Canal Creek (Lorah and others, 1997).

Re-amendment with 8 µM CT at day 23 again degraded within 3 days, although higher accumulation of CF and methylene chloride occurred than during the first amendment. An increased rate of methane production was observed after the second CT amendment and may indicate that methane also was an end-product of the CT degradation (fig. 22). CT did not degrade completely in the sterile controls after the second amendment, and CF production was low (fig. 21b).

The bioaugmented BionSoil/peat microcosms that were amended with a mixture of VOCs also showed rapid removal of CT and CF, along with the added TeCA, 112TCA, 12DCA, and 12DCE (figs. 23a–b). The approximately 4 µM each of CT and CF added at day 0 was removed from the live microcosms within 3 and 10 days, respectively. Although CF removal appeared to take longer than observed in the CT-amended wetland sediment microcosms that were not bioaugmented with WBC-2 (fig. 21a), CF was likely being produced from the CT as the initial CF added was being degraded. Methylene chloride was observed, but only accounted for about 10 percent of the total molar concentrations of CT and CF added to the microcosm (fig. 23b).

Methane production was relatively slow in the microcosms amended with the mixture of VOCs compared to bioagumented BionSoil microcosms amended only with TeCA (figs. 17 and 23b), and does not indicate that methane was an end-product of the CT and CF degradation. Although sorption caused concentrations of all the added VOCs to decrease substantially within the first day in the sterile control for the mixed-VOC microcosm, only CT was completely

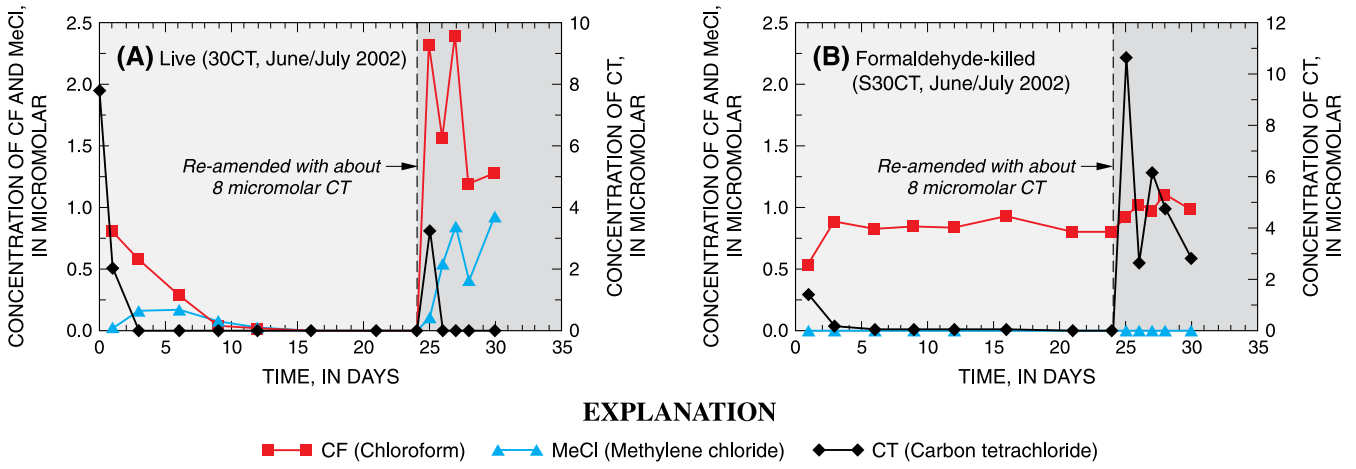


Figure 21. Degradation of carbon tetrachloride (CT) in *(A)* live and *(B)* formaldehyde-killed control microcosms with wetland sediment from site WB30, June–July 2002.

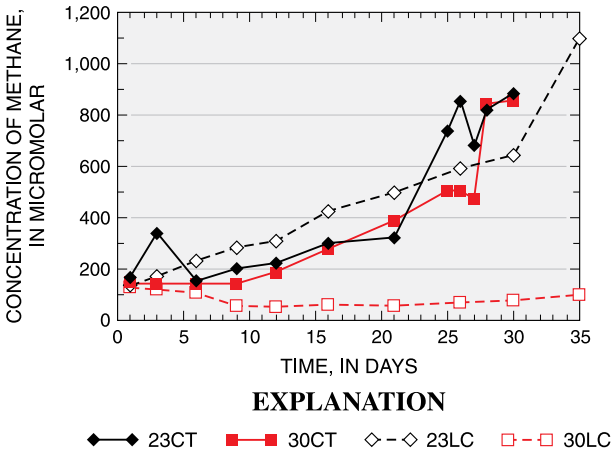
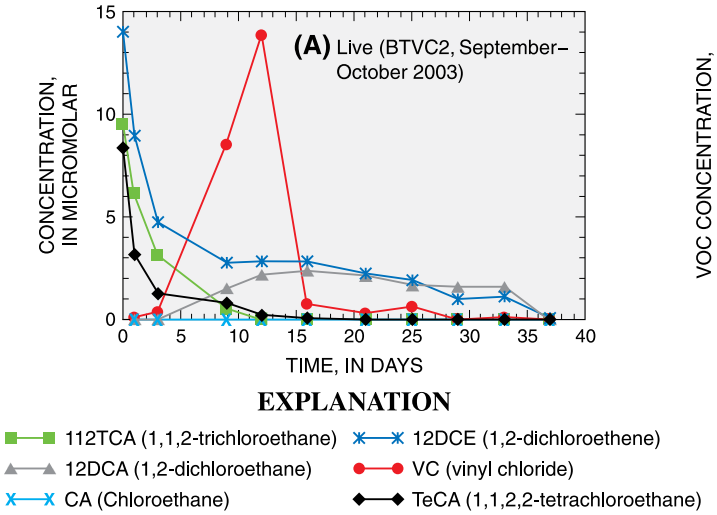
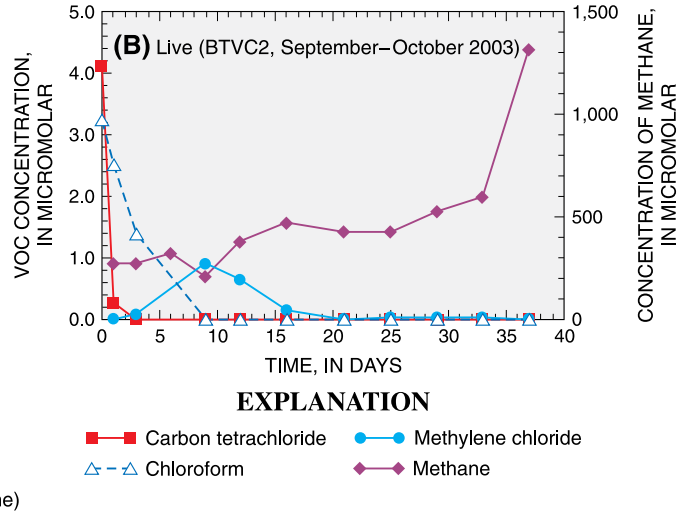


Figure 22. Methane concentrations in microcosms with wetland sediment from WB23 and WB30, unamended (LC) and amended (CT) with carbon tetrachloride.





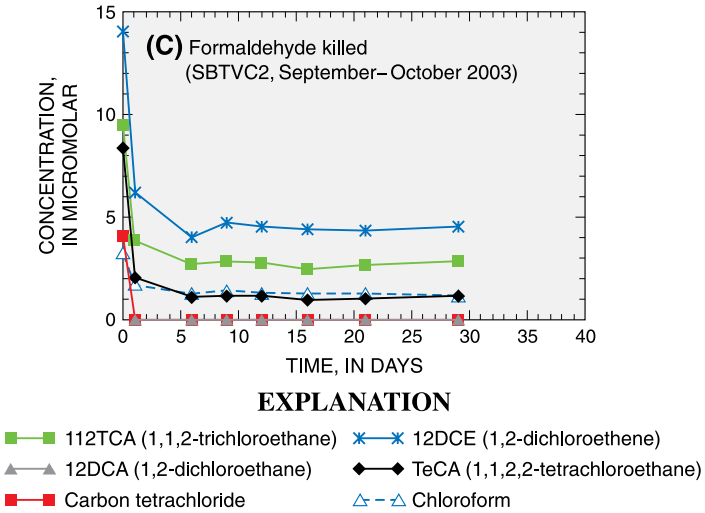


Figure 23. Degradation of 1,1,2,2-tetrachloroethane (TeCA) and a mixture of co-contaminant volatile organic compounds (VOCs) in live *(A,B)* (BTVC2.9/03) and *(C)* formaldehyde-killed control (SBTCV2.9/03) microcosms with BionSoil/peat mixture, September–October 2003.

removed. Thus, CT degradation may have been largely abiotic, as was observed in the CT-amended wetland sediment microcosms (figs. 21b and 23c). TeCA degradation rate was unaffected by the mixture of VOCs—the half-life was 2.6 days in the mixed VOC microcosm compared to 3.7 days in the TeCA-amended BionSoil microcosm that was bioaugmented with WBC-2 (table 6; figs. 15d and 23a). All VOCs were degraded in the mixed VOC-amended microcosm, indicating that WBC-2 applied to sediment could efficiently degrade a combination of chlorinated alkanes, alkenes, and methanes. In contrast, chloromethanes have been shown to be toxic to many bacteria (Weathers and Parkin, 2000), and degradation of chlorinated ethenes by known dehalorespirers has been reported to be inhibited by the presence of chlorinated ethanes and CF (Duhamel and others, 2002; Aulenta and others, 2005; Grostern and Edwards, 2006). The total concentration of mixed VOCs evaluated in the microcosm was low, however, especially after sorption by the organic-rich sediment assisted in reducing the total concentration to about 20 μM by day 1.

Zero-Valent Iron for Enhanced Bioremediation

Previous studies with granular ZVI have been shown to degrade the chlorinated parent ethanes, ethenes, and methanes present at the West Branch Canal Creek seep sites via abiotic reduction according to pseudo-first order degradation (Gillham and O'Hanessin, 1994; Matheson and Tratnyek, 1994). Further, an enhancement of abiotic degradation of CT and CF has been observed when ZVI was coupled with anaerobic biodegradation by mixed and pure dechlorinating cultures that utilized cathodic hydrogen produced by the corrosion of the ZVI as a substrate (Weathers and others, 1997; Novak and others, 1998). While the rate of abiotic degradation is directly related to the surface area to volume ratio of ZVI (Gillham and O'Hannesin, 1994), when abiotic and biodegradation were used together, equivalent or greater rates were achieved with less surface area to volume ratios in the presence of live cells (Novak and others, 1998; Weathers and others, 1997). Of the parent, chlorinated compounds present at West Branch Canal Creek, CF has the least reactivity (lowest rate of degradation reported) with ZVI and the greatest potential to adversely affect the ethane- or ethene-degrading microorganisms. Degradation rates of CT, TeCA, TCE, and PCE with ZVI are several orders of magnitude greater than that of CF; therefore, microcosms were focused on CF specifically.

In microcosms carried out over a period of 16 to 29 days, the degradation of CF and its daughter compounds were determined at concentrations of 4.2 and 42 μM (500 and 5,000 μg/L), with and without the presence of wetland sediment slurry. Abiotic degradation of 4.2 and 42 μM CF with ZVI alone resulted in CF rate constants of 0.48 and 0.55 $days^{-1}$, respectively. These rate constants result in removal rates of 1.68 and 19.1 μM/day (micromolar per day), respectively. Gillham and O'Hanessin (1994) reported a rate constant for CF of 0.87 $days^{-1}$ with ZVI that had been treated to remove sulfides and other minerals on the surface, with a resulting rate of removal of CF equal to 14.7 μM/day. In the presence of sediment slurry, a reduction in the overall CF rate of removal was observed, with rates equal to 2.3 and 2.88 μM/day for 4.2 and 42 μM, respectively. The observed decrease in the overall rate of removal indicates that the presence of sediment may impede the abiotic surface interaction of the ZVI filings and CF.

Dechlorination of daughter compound methylene chloride (or dichloromethane) was detected in both sediment-free (figs. 24a–b) and sediment (figs. 24c–d) treatments at both CF concentrations. To a lesser extent, chloromethane was also detected in the sediment-free treatments at both concentrations of CF (about 3 percent of total added mass, figs. 24a–b). Despite the reduction in overall removal rate of CF in the presence of sediment, complete removal of methylene chloride was observed only in the sediment treatments. In the sediment-free treatments, methylene chloride was not removed through the duration of the experiment, consistent with results previously reported (Matheson and Tratnyek, 1994), and accounted for a maximum of about 9 to 16 percent of the total added mass for the 4.2 and 42 μM CF concentrations, respectively. With sediment, transient methylene chloride accumulation reached a maximum of nearly one-third of the added mass for both concentrations, indicating the degradation mechanism may be at least partly biotic due to activity of native microorganisms in sediment. This increased proportion of methylene chloride was observed by Weathers and others (1997) who reported cometabolism of CF by hydrogen-consuming methanogens in the presence of ZVI and a mixed, methanogenic culture. The hypothesis that biological activity contributes to the degradation in the batch system is further supported by the observation that methane concentrations were one to two orders of magnitude greater in treatments that contained sediment (fig. 25a) compared to sediment-free (fig. 25b) treatments. An increase in methane concentrations inversely corresponded to increased CF concentrations (fig. 25b). This increase may be a result of the inhibition of some methanogen groups in the native sediment, but does not appear to inhibit those capable of degrading methylene chloride. The further degradation of methylene chloride to carbon dioxide, acetic acid, methane, and a non-strippable residue under methanogenic conditions was attributed to DCM-oxidizers and acetogens by Freedman and Gossett (1991). A lack of methylene chloride accumulation and chloromethane production also was observed in microcosms with wetland sediment (fig. 21a) and in ground water and porewater in the West Branch Canal Creek area (Lorah and others, 1997).

Water-quality effects of the ZVI with and without sediment were evaluated over the duration of the experiment by periodic measurements of pH, dissolved iron, and sulfate. An expected increase in pH due to the iron corrosion was observed in the sediment-free treatments from 6.8 to a maximum 8.7. The buffering capacity of the wetland sediment minimized the increase in pH for the sediment-containing

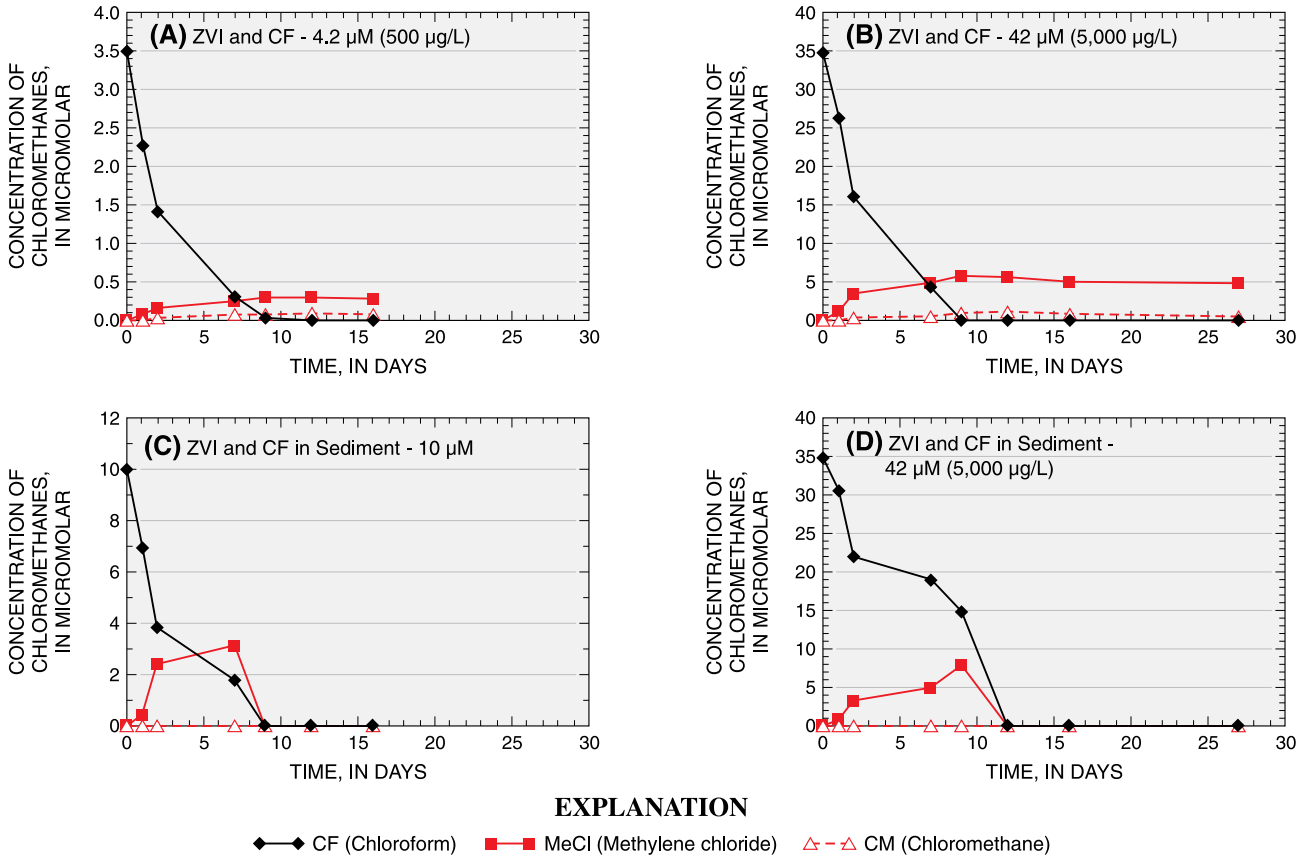


Figure 24. Degradation of chloroform (CF) and associated daughter compounds by zero-valent iron (ZVI) at two concentrations, with and without wetland sediment in microcosm experiments *(A)* ZVI plus 4.2 µM (micromolar) CF, *(B)* ZVI plus 42 µM CF, *(C)* ZVI plus sediment plus 10 µM CF, and *(D)* ZVI plus sediment plus 42 µM CF.

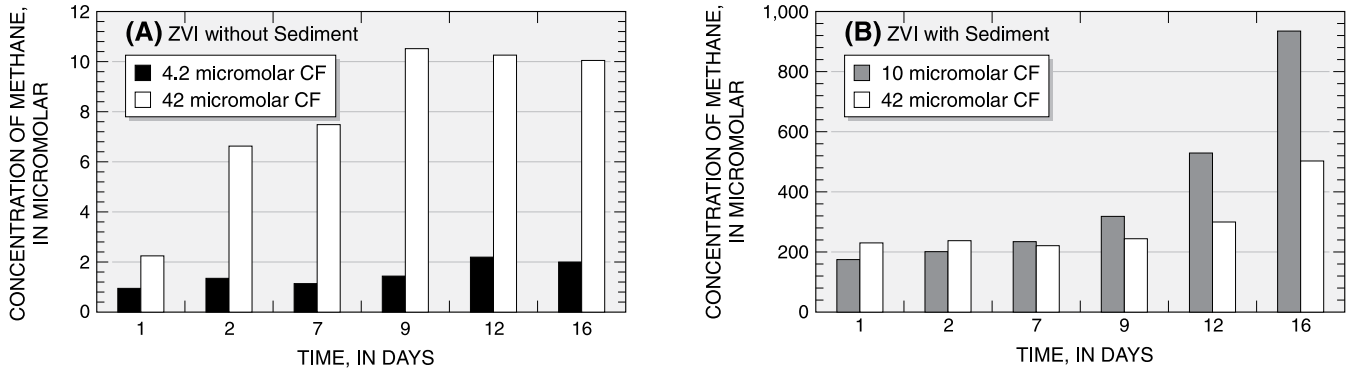


Figure 25. Effect of degradation of chloroform (CF) at two initial concentrations on methane production in microcosms with *(A)* zero-valent iron (ZVI) without sediment, and *(B)* ZVI with sediment.

treatments, which increased from 6.8 to a maximum of 7.3. This increased buffering capacity was evident in the alkalinity measured near the end of the experiment in the sediment treatments. Alkalinity (measured as calcium carbonate) for the sediment-free treatments ranged from 54 to 66 mg/L, whereas the sediment treatments ranged from 258 to 288 mg/L.

Dissolved iron (likely present as ferrous iron under the reducing conditions) was measured during each time step through day 16 under all treatment conditions. In the sediment-free treatments, maximum concentrations of dissolved iron were detected at day 1 sampling and declined to non-detectable levels at day 16. At the higher CF concentration of 5,000 µg/L, dissolved iron concentrations (631 µM) were not significantly greater than those detected with the starting CF concentration of 500 µg/L (618 µM). This may be a result of the peak in iron concentrations occurring before day 1 in the case of the higher CF concentrations. Further, the observed decline in iron concentrations over time may be due to the precipitation of secondary mineral phases, as reported by Vikesland and others (2003). In sediment treatments, the overall magnitude of iron concentrations was less and did not exhibit the same pattern over time when compared to the sediment-free treatments. Iron concentrations increased over time during the experiment and did not decline by 16 days. An increase in iron concentrations was observed to be related to the increase in CF concentrations (data not shown). Under the strongly reducing conditions with the sediment, sulfate reduction was apparent from the overall decrease in sulfate concentrations from about 2–2.25 mg/L in non-sediment treatments to 0.15 mg/L in sediment treatments.

ZVI, particularly when coupled with biological processes observed in the native wetland, appeared to be an effective additive to facilitate the rapid degradation of chlorinated methanes, such as CF. These microcosms indicated that for seep areas where chloromethanes are detected in concentrations above the reported toxicity to chlorinated ethene biodegradation, ZVI may be an appropriate addition to the reactive matrix. Therefore, ZVI was tested further in columns for the reactive matrix.

Column Experiments

After microcosms were used to evaluate individual components needed for the proposed enhanced bioremediation methods in the seep areas, column tests were used to (1) combine the components, including combined biostimulation and bioaugmentation and the combined matrix zones conceptualized for the permeable reactive mat (ZVI and organic layer with an overlying bioaugmented organic layer), (2) better simulate the bioremediation methods under field conditions, including continuous ground-water flow at measured rates and increased contaminant concentrations, and (3) evaluate the possible adverse water-quality effects that could result from the proposed near-surface bioremediation methods in the wetland. The results of the column experiments are discussed in relation to the two specific enhanced bioremediation methods proposed for the seep areas—direct injection at depth in the wetland sediments, and application of a permeable reactive mat at the seep surface.

Direct Injection for Enhanced Bioremediation

In some areas of the wetland where natural attenuation is not efficient, the direct injection of organic substrate or a dechlorinating, mixed microbial culture at depth may enhance biodegradation processes along the vertical discharge pathway. An injection at depth within the wetland sediment could enhance biodegradation due to an increased microbial population density, an increased soluble electron donor concentration, or a combination of the two. Throughout the tests with the seep sediment columns (S-A and S-B), ground-water influent spiked with VOCs was continuously circulated through the seep sediment columns to determine the effects of bioaugmentation, donor addition, and co-contaminants under various conditions (Appendix A1). Degradation pathways and rates, fate of daughter compounds, redox conditions, and water-quality effects were evaluated prior to bioaugmentation (P), after bioaugmentation and prior to donor addition (BA), after bioaugmentation and donor addition (BABS), and second bioaugmentation of B columns and continued donor addition (2BABS). The results of these column experiments with wetland sediment from a seep area were used to evaluate the feasibility of direct injection in the wetland sediments as an enhanced bioremediation method.

Effect of Bioaugmentation and Donor Addition

1,1,2,2-Tetrachloroethane Degradation Pathways and Rates

Previous investigations have shown that anaerobic biodegradation of TeCA can proceed through the chlorinated ethanes via hydrogenolysis or through chlorinated ethenes via dichloroelimination (Chen and others, 1996; Lorah and Olsen, 1999a; Lorah and Voytek, 2004). Since natural DOC in the wetland sediments was shown to provide sufficient substrate for complete dechlorination of TeCA in previous microcosms and field studies in the natural attenuation area (Lorah and Olsen, 1999a; Lorah and Voytek, 2004) and in bioagumented microcosms completed during this study (figs. 14e–f), no donor was initially added to the column influent, nor was it immediately added following the initial bioaugmentation of the columns. Measured DOC concentrations in the effluent from the seep sediment columns averaged 4.3±0.9 mg/L, which is about one-third to half of the previously reported concentrations in wetland sediments

from the natural attenuation area (Lorah and others, 1997). During these two treatment scenarios, degradation of influent TeCA concentrations of about 30 µM was incomplete, with a reduction of only 40 to 45 percent of the total added mass. No observable change in the TeCA degradation was apparent following the initial bioaugmentation of the columns. Similarly, degradation rates were consistent during these two treatments with an average rate constant of 0.16±0.04 day^{-1}. This rate is equal (within the standard error) to the rates of 0.13±0.01 and 0.19±0.02 day^{-1} calculated from non-bioaugmented microcosms constructed with WB23 sediment on two dates (23T in table 6). Mass balance of the total added chloroethanes was poor during these treatments until day 65. Degradation that did occur appeared to favor hydrogenolysis of TeCA to 112TCA during these treatments, similar to results observed in the seep ground water under natural conditions (Majcher and others, 2007). In contrast, the bioaugmented BionSoil/peat microcosms with high TeCA concentration degraded TeCA primarily to 12DCE (fig. 18).

Ethanol (initially at a concentration of 10 mM) was added to the column influent starting at day 70 based on previous evaluation of multiple electron donors (Geosyntec Consultants, Inc., 2006). An increase in effluent DOC concentrations was measured with the ethanol addition, and a corresponding increase in the TeCA removal to near 100 percent by day 100 was observed (fig. 26b). Rates of TeCA removal increased following ethanol addition by an order of magnitude over the previous treatments without donor to 1.95±0.42 day^{-1} (table 7). It is likely that both TeCA hydrogenolysis and dichloroelimination pathways were active, but TeCA hydrogenolysis improved such that dichloroelimination products (mainly *cis*12DCE) were the main degradation product observed by day 93 (figs. 26a–b). This distribution of daughter products differed from the organic donor (lactate) biostimulated microcosms constructed with wetland sediment and the BionSoil/peat mixture, which predominantly showed an increase in the hydrogenolysis pathway with higher accumulation of 12DCA and chloroethane observed than in unamended or bioaugmented microcosms without donor addition (figs. 14–15). The apparent differences in reaction pathways in the column and microcosms could be attributed to the difference between flowing and static conditions, where accumulation of by-products from donor fermentation may have enhanced different bacteria or methanogen species.

Ethanol concentrations were adjusted on the basis of the detected magnitude of dissolved methane concentrations throughout the column operation. The brief, inadvertent removal of ethanol from column influent starting on day 138 resulted in an almost immediate decline in efficient degradation of TeCA via hydrogenolysis to 112TCA and a measurable decline in the TeCA degradation rate. Reintroduction of ethanol on day 154 rapidly restored the efficient TeCA removal so that dichloroelimination products dominated the contaminant profile though the column. The rapid response to ethanol concentrations indicates that the efficiency of the degradation facilitated by the microbial community within the sediment columns is largely dependent on the supply of electron donor and the presence of methanogenic conditions. These observations reinforce previous laboratory and field evidence of the importance of methanogenic conditions in complete efficient TeCA degradation, which was linked specifically to acetotrophic methanogens (Lorah and others, 2003; Lorah and Voytek, 2004). The fact that high methane production without efficient TeCA degradation was stimulated in the donor-biostimulated microcosms (figs. 15b,16b, and 17a) may indicate that hydrogenotrophic methanogens, rather than acetotrophic methanogens, were stimulated. It is also possible, however, that the relation to methane production is indirect. For example, the homoacetogens (*Acetobacterium*), a group identified in the WBC-2 clones, have a syntrophic relationship with methanogens in many anaerobic environments, and include species known to dechlorinate chlorinated solvents through both co-metabolic and growth-related processes (De Wildeman, Neumann, and others, 2003).

Prior to sampling on day 111, the S-A column was bioaugmented for a second time. No apparent increase in TeCA degradation (average rate constant 2.4±0.53 day^{-1}) was observed following the addition of WBC-2 when compared to S-B. TeCA was already being efficiently removed by 23 cm along the column, however, so no apparent increase was anticipated for TeCA degradation (figs. 26c–d).

Daughter Compound Degradation and Fate

Primary degradation products detected during the P and BA treatments included 112TCA, and to a lesser extent, both the *cis*- and *trans*-isomers of 12DCE, confirming that both hydrogenolysis and dichloroelimination pathways are active in the sediments. During these early treatments, complete removal of these daughter compounds was not observed through the length of the column, and they were detected in the effluent. Only minimal VC and ethene were detected after 65 days of operation. The overall mass balance during these events was initially poor, but improved after day 51. Following the addition of ethanol to the influent, *cis*12DCE, followed by VC and *trans*12DCE, were the predominant chlorinated daughter products (fig. 26b). These daughter compounds were transient through the length of the column, with simultaneous, maximum concentrations typically detected at the first sampling port 23 cm along the column (fig. 26b). With additional pore volumes, the magnitude of the transient accumulation of these daughter products decreased, with VC and ethene comprising the majority of the mass. This shift to the lesser chlorinated products at 23 cm along the column indicates that the actual peak of the daughter compound accumulation is likely between the influent and first ports, making it difficult to discern the predominant mechanism of reduction (hydrogenolysis or dichloroelimination). This

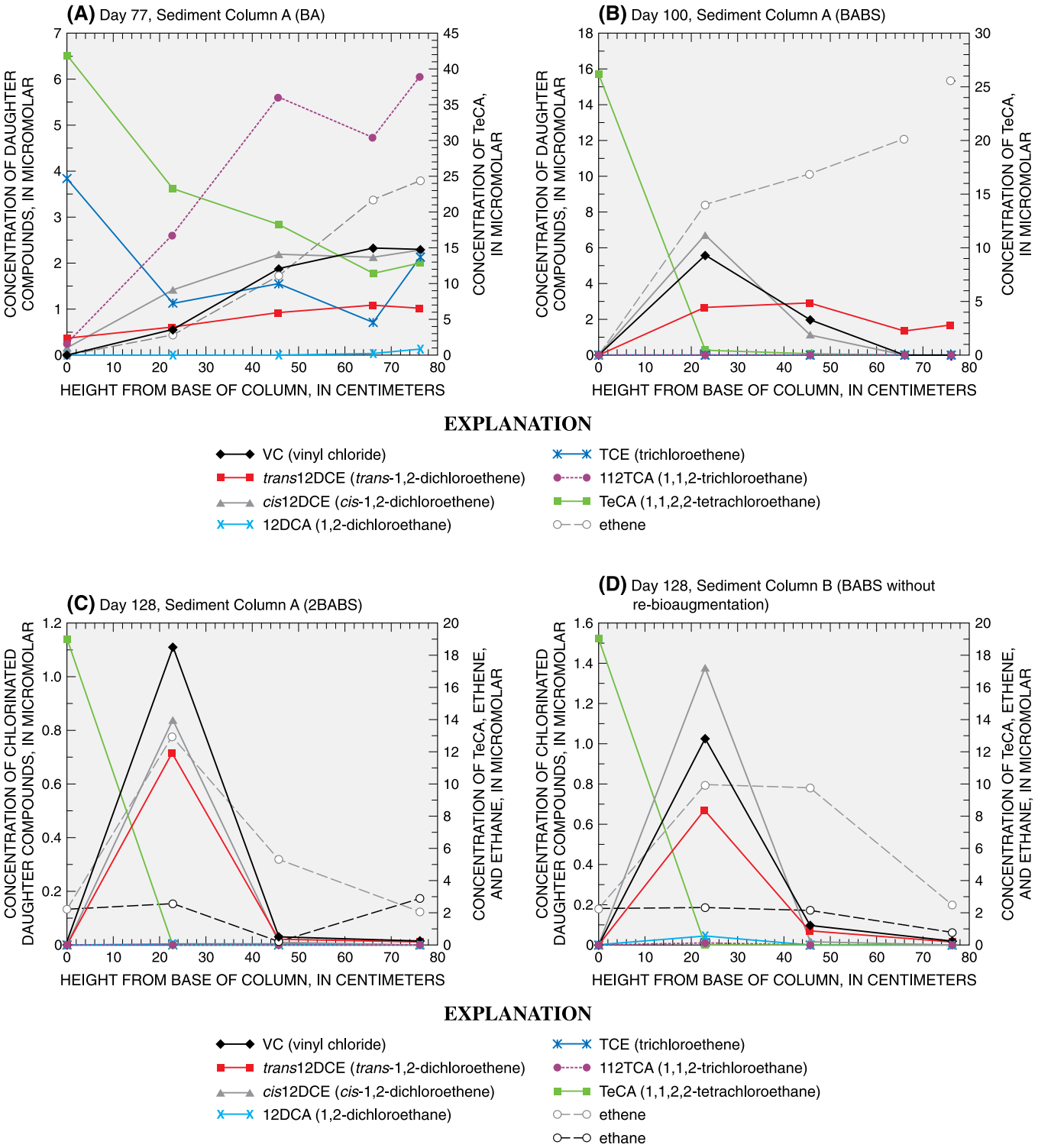


Figure 26. Contaminant profiles of 1,1,2,2-tetrachloroethane (TeCA) and its daughter compounds through sediment columns during various treatments: *(A)* bioaugmentation (BA), *(B)* bioaugmentation and biostimulation (BABS), *(C)* re-bioaugmentation (2BABS), and *(D)* no re-bioaugmentation (BABS).

Table 7. Degradation rate constants and rates for 1,1,2,2-tetrachloroethane in seep 3-7E sediment columns under different treatment conditions.

[μM, micromolar; --, not applicable]

Treatment	Rate Constant (day^{-1})	Half-life (days)	Rate (μM /day)
Bioaugmentation (BA)			
Mean	0.159	4.4	5.25
Median	.162	4.3	5.24
Standard deviation	.038	--	1.4
Biostimulation and bioaugmentation (BABS)			
Mean	1.95	0.36	47.2
Median	1.94	.36	42.3
Standard deviation	.424	--	24.9
Re-bioaugmentation (2BABS)			
Without chloroethene addition			
Mean	2.4	.29	55.9
Median	2.42	.29	54.1
Standard deviation	.531	--	9.95
Chloroethene addition			
With re-bioaugmentation			
Mean	2.96	.23	107
Median	2.55	.27	86.8
Standard deviation	1	--	46.1
Without re-bioaugmentation			
Mean	2.59	.27	83.6
Median	2.65	.26	86
Standard deviation	.343	--	24.8

enhancement in rates through both pathways is likely the cause for the loss of mass balance during this period of the test. Throughout the remaining length of the column, a decrease in ethene and subsequent increase in ethane production was observed for the duration of the test.

Although the degradation rate of TeCA did not appear to increase as a result of the additional bioaugmentation, it did appear to further increase the removal efficiency for the daughter compounds (figs. 26c,d). It should be noted that this difference in column performance was observed after the columns temporarily lost degradation efficiency, apparently due to the decrease in ethanol addition, and then regained it with the reintroduction of ethanol. The transient accumulation of chlorinated daughter compounds ranged from 2 to 6 percent in the re-bioaugmented column (fig. 26c) compared to 12 to 36 percent in the column that was bioaugmented once (fig. 26d). The transient accumulation of *cis*12DCE and VC along with the production of ethene that was observed in the bioaugmented columns following biostimulation is consistent with previous enrichment studies with the natural wetland sediments at the site (Lorah and others, 2003; Lorah and Voytek, 2004) and with laboratory tests with WBC-1 and WBC-2 in media (figs. 7–8).

Redox

Despite the naturally elevated TOC concentrations in the seep sediments, there was insufficient DOC in the column (operated at field discharge rates) to establish highly reducing, methanogenic conditions without the addition of electron donor (such as ethanol). Concentrations of ferrous iron (greater than 1 mg/L), sulfide (less than the detection limit of 100 μg/L), and methane (less than the detection limit) are indicative of iron-reducing conditions in the columns prior to ethanol addition. Methane was detected in the upper sampling ports of the columns after day 58; however, concentrations were less than 500 μg/L and therefore the sediments were still considered to be iron-reducing. Ammonia did not accumulate throughout the column length.

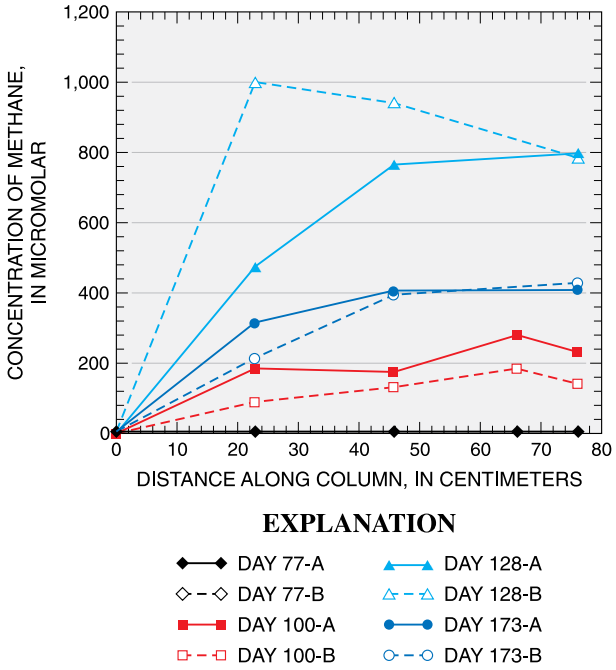


Figure 27. Methane concentrations in column tests with seep sediment during various treatments.

Following the addition of ethanol to the columns on day 70, methanogenic conditions were rapidly established (fig. 27). Methane concentrations increased to greater than 31 µM throughout the column by day 90, and to 1,250 µM by day 104. Subsequently, ethanol concentrations were lowered to 5 mM and then to 1 mM. Ethanol was removed from the influent between days 132 and 154, resulting in a decline in methane concentrations by an order of magnitude. As discussed in the previous sections, a corresponding decline in the degradation efficiency of TeCA and its daughters was observed. In a previous study with natural wetland sediments from West Branch Canal Creek, enhancement in degradation of VC was associated with growth of acetotrophic methanogens (Lorah and Voytek, 2004).

Water Quality

The pH of the column porewater effluent was consistent throughout the duration of column operation. The pH of sediment columns S-A and S-B, respectively, averaged 6.71±0.14 and 6.74±0.15 after VOCs were introduced. This pH is favorable to biodegradation processes. Specific conductance was more variable throughout the test with values of 699±124 µS/cm (microsiemens per centimeter) in column S-A and 691±109 µS/cm in column S-B. This variability is largely attributed to an increase in specific conductance observed in both columns after ethanol was added to the influent. Alkalinities of the column effluent during the pre-bioaugmentation, bioaugmentation, and donor addition treatments were consistent with average bicoarbonate concentrations in columns S-A and S-B, respectively, of 176±15 mg/L and 202±14 mg/L. These values are typical of those previously reported in the wetland sediments (Olsen and others, 1997).

Co-Contaminant Effects and Degradation

Both PCE and then PCE and TCE were added to the sediment column influent to determine the effect of co-contaminants on TeCA degradation, and to evaluate the degradation of the co-contaminants themselves. There was no apparent effect of the addition of the chloroethenes on the degradation of TeCA (table 7, fig. 28a). A concentration of 6 µM (1 mg/L) PCE was rapidly degraded in both columns with greater than 90 percent removal by the first sampling port by day 166 (event 27), and 99 percent removal by day 181 (event 29). Similarly, with the addition of 7.6 µM (1 mg/L) TCE, greater than 90 percent removal was measured 23 cm along the column, despite the generation of TCE from PCE degradation. These co-contaminants appeared to have no adverse effects through the end of the column operation (35 and 46 days for TCE and PCE, and PCE, respectively).

PCE degradation rate constants were lower than those measured for TeCA in the columns, averaging 2.2±.0.60 day^{-1}, but remained greater than those reported for PCE or TCE in aquifers by two to three orders of magnitude (Aziz and others, 2000; Washington and Cameron, 2001). Similar to TeCA, there did not appear to be an observable enhancement of PCE or TCE degradation in the A column that had been re-bioaugmented (figs. 28a–b). Degradation of TCE, despite its production from PCE reductive dechlorination, could be estimated with first-order kinetics (r^2 ranged from 0.80 to 0.99), indicating that it was consumed as rapidly as it was produced in the first 23 cm of the column. The average TCE rate constant (3.2±0.49 day^{-1}) was greater than PCE degradation.

TeCA, PCE, and TCE can all produce the same daughter compounds, making it difficult to discern their sources. The degradation of lesser chlorinated daughter compounds (such as 12DCE and VC) appeared to proceed more rapidly in column A than in column B, possibly due to the additional bioaugmentation of column B (figs. 28a–b). The establishment and maintenance of highly reducing conditions had the greatest effect on PCE and TCE degradation efficiency, as observed for TeCA degradation. The degradation efficiency of parent and daughter compounds was correlated with methane concentrations. Thus, the sediment column tests indicate that direct injection of donor and culture could result in complete removal of parent and daughter chlorinated VOCs within a shallow depth of wetland sediments, but that its success as an enhanced bioremediation method would be largely dependent on the ability to adequately distribute electron donor through the low permeability wetland sediments.

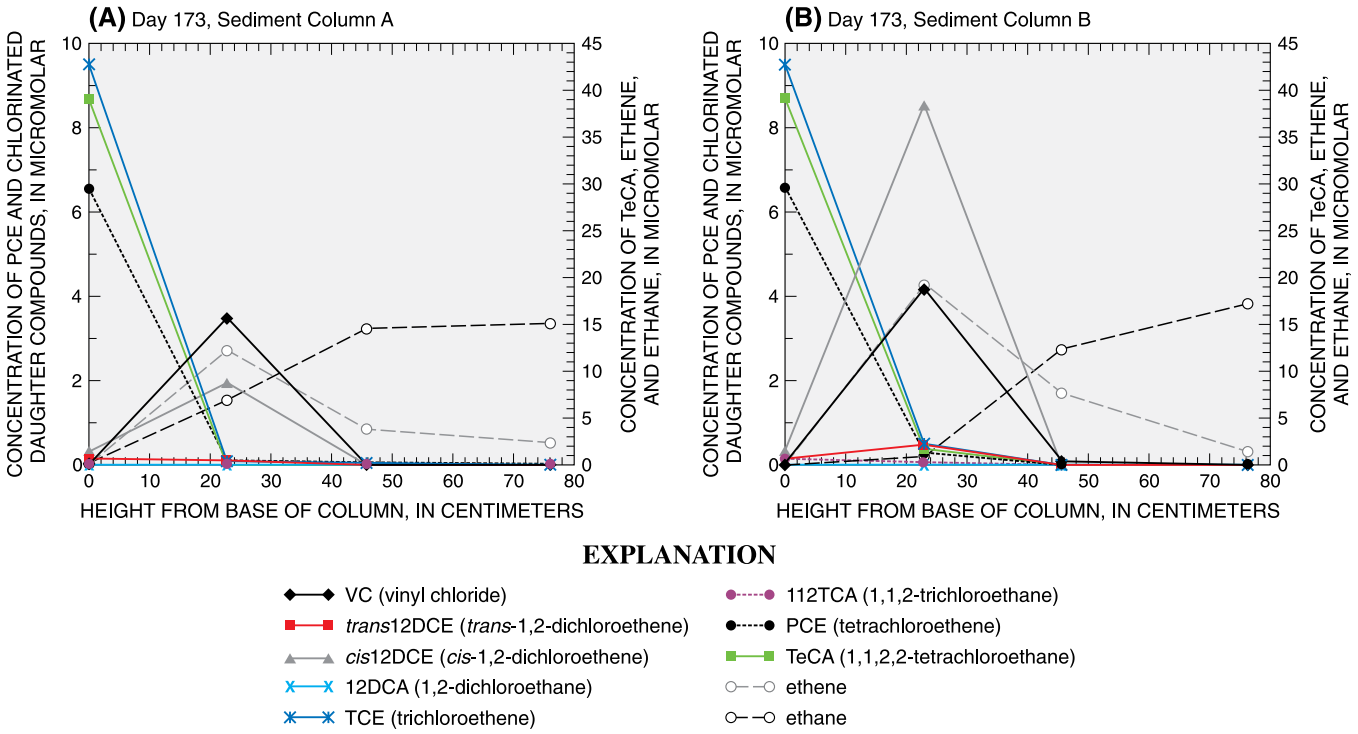


Figure 28. Effects of co-contaminants on 1,1,2,2-tetrachloroethane (TeCA) and daughter compound degradation in seep sediment columns: *(A)* re-bioaugmentation, and *(B)* no re-bioaugmentation.

Reactive Mat for Enhanced Bioremediation

Throughout the duration of the column test, groundwater influent was continuously circulated through the organic mix and iron-organic mix columns to determine the effects of bioaugmentation, donor addition, and co-contaminants under various treatment conditions (Appendixes A2 and A3). Degradation pathways and rates, fate of daughter compounds, redox conditions, and water-quality effects were evaluated prior to bioaugmentation (P), after bioaugmentation and prior to donor addition (BA), after bioaugmentation and donor addition (BABS), and second bioaugmentation of M-A and FM-A columns, and continued donor addition (2BABS). Results of different treatments were also compared to results from the sediment columns to better understand how the two reactive mat matrices may perform compared to native wetland seep sediment.

Effect of Bioaugmentation and Donor Addition

1,1,2,2-Tetrachloroethane Degradation Pathways and Rates

Both organic-based matrices (organic and iron-organic mix) facilitated removal of TeCA as well as or better than the native sediment under the various treatment conditions. Unlike in the native sediment columns, TeCA was largely removed (90 to 100 percent) in the organic and iron-organic mix columns prior to bioaugmentation and biostimulation (P treatment). All added TeCA mass was removed within the iron-containing zone of the iron-organic mix columns, whereas mass removal in the organic mix columns took place over the length of the entire column (figs. 29a,b). Some of the enhanced TeCA removal compared to the sediment columns was likely attributable to adsorption; greater sorption of TeCA was observed in the BionSoil microcosms than in microcosms with wetland sediment. Less than 10 percent of total added TeCA mass was recovered as daughter compounds, and neither ethene nor ethane was detected in the organic mix columns during the P treatment. Recovery of added TeCA mass as daughter compounds was higher in the iron-organic mix columns than the organic mix columns during the P treatment because some immediate abiotic degradation occurred, as expected, in the ZVI zone of the iron-organic mix columns (fig. 29c). Both ethene and ethane were detected after 8 days of TeCA addition to the iron-organic mix columns during this initial P phase. The daughter compounds that were detected in both the organic mix and the iron-organic mix columns during the P phase indicated that TeCA degradation was occurring through hydrogenolysis to 112TCA and dichloroelimination to *cis*12DCE (partly abiotically in the iron-organic mix columns).

TeCA removal in the organic mix columns improved following bioaugmentation (day 20–22), so that complete

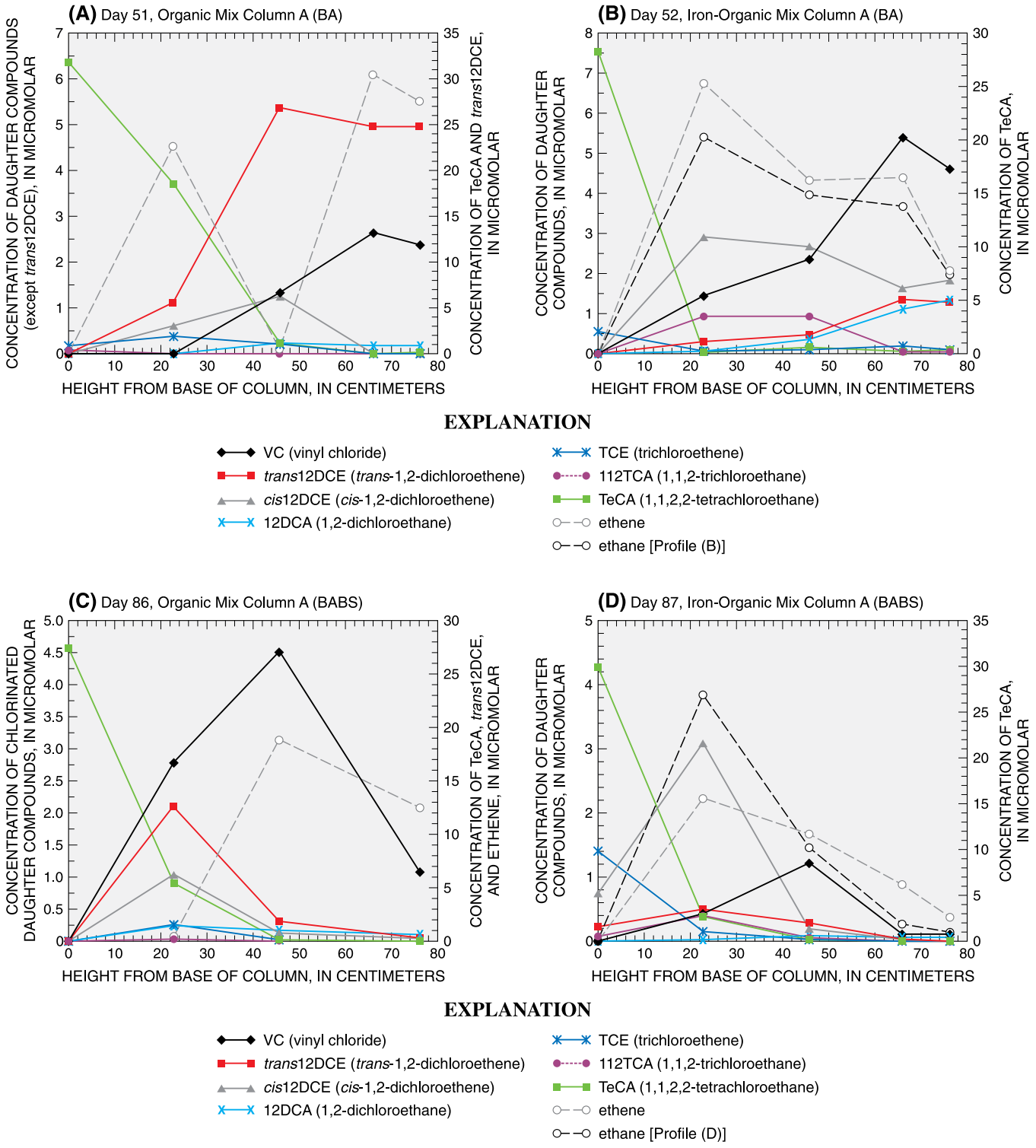


Figure 29. Contaminant profiles of 1,1,2,2-tetrachloroethane (TeCA) and its daughter compounds through organic mix and iron-organic mix columns under different treatment conditions *(A)* bioaugmented (BA) organic mix, *(B)* bioaugmented (BA) iron-organic mix, *(C)* biostimulated and bioaugmented (BABS) organic mix, and *(D)* biostimulated and bioaugmented (BABS) iron-organic mix.

removal was consistently observed within 46 cm by day 51 (fig. 29a). With the first addition of lactate (10 mM at day 93) as an electron donor to the influent, TeCA removal in the organic mix column was immediately improved to near 100 percent within 23 cm along the column. In the iron-organic mix columns, TeCA continued to be completely removed before the first sampling port at 23 cm during the BABS treatment. TeCA rate constants of 2.66±0.96 day^{-1} and 3.14±1.68 day^{-1}, respectively, were calculated in the organic and iron-organic mix columns during the BABS treatment (table 8).

Lactate addition to the influent combined with re-bioaugmentation (2BABS), did appear to increase the rates of TeCA removal in the organic mix column compared to the BA phase, but not to the BABS phase (table 8, figs. 30a,b). Re-bioaugmentation (2BABS) of the organic zone of the iron-organic mix columns on day 94 caused a slight increase in the rate constant to 5.90±0.48 day^{-1}, compared to 3.14±1.68 day^{-1} in the BABS phase (table 8, fig. 30d).

Daughter Compound Degradation and Fate

Although TeCA was removed prior to bioaugmentation in both the organic and iron-organic mix matrices, the chlorinated daughter compounds were not completely removed throughout the length of the columns. Following initial bioaugmentation of the organic mix columns after 20 days (BA treatment), dichloroelimination daughter product *trans*12DCE, and to a lesser extent, *cis*12DCE and VC, were predominant. This distribution of daughter compounds is similar to that observed in the BionSoil microcosms that were amended with high TeCA concentrations (fig. 18). High concentrations of *trans*12DCE relative to *cis*12DCE also have been observed previously in microcosms with wetland sediment from the West Branch Canal Creek area and have been partly attributed to slower degradation of the *trans*12DCE compared to the *cis*-isomer (Lorah and Olsen, 1999a). In addition, transient accumulation of *trans*12DCE occurs in the stocks of WBC-2 culture maintained at the Reston USGS laboratory (fig. 6)

Table 8. Degradation rate constants and rates for 1,1,2,2-tetrachloroethane in organic mix and iron-organic mix columns under different treatment conditions.

[µM, micromolar; --, not applicable]

Treatment	Organic Mix		Iron-Organic Mix	
	Rate Constant (day^{-1})	Rate (µM /day)	Rate Constant (day^{-1})	Rate (µM /day)
Bioaugmentation (BA)				
Mean	1.87	51.2	3.82	101
Median	1.92	48.8	3.94	112
Standard deviation	0.24	9.5	0.72	25.8
Biostimulation and bioaugmentation (BABS)				
Mean	2.66	85.4	3.14	94.5
Median	--	--	--	--
Standard deviation	.96	28.1	1.68	51.0
Re-bioaugmentation (2BABS) (includes chloroethene addition)				
Mean	3.43	75.1	5.90	141
Median	3.55	76.0	6	135
Standard deviation	.72	22.8	.48	33.0
Chloromethane addition				
Mean	1.99	46.0	2.74	69.3
Median	1.82	41.8	2.33	64.4
Standard deviation	.72	13.6	.72	21.1

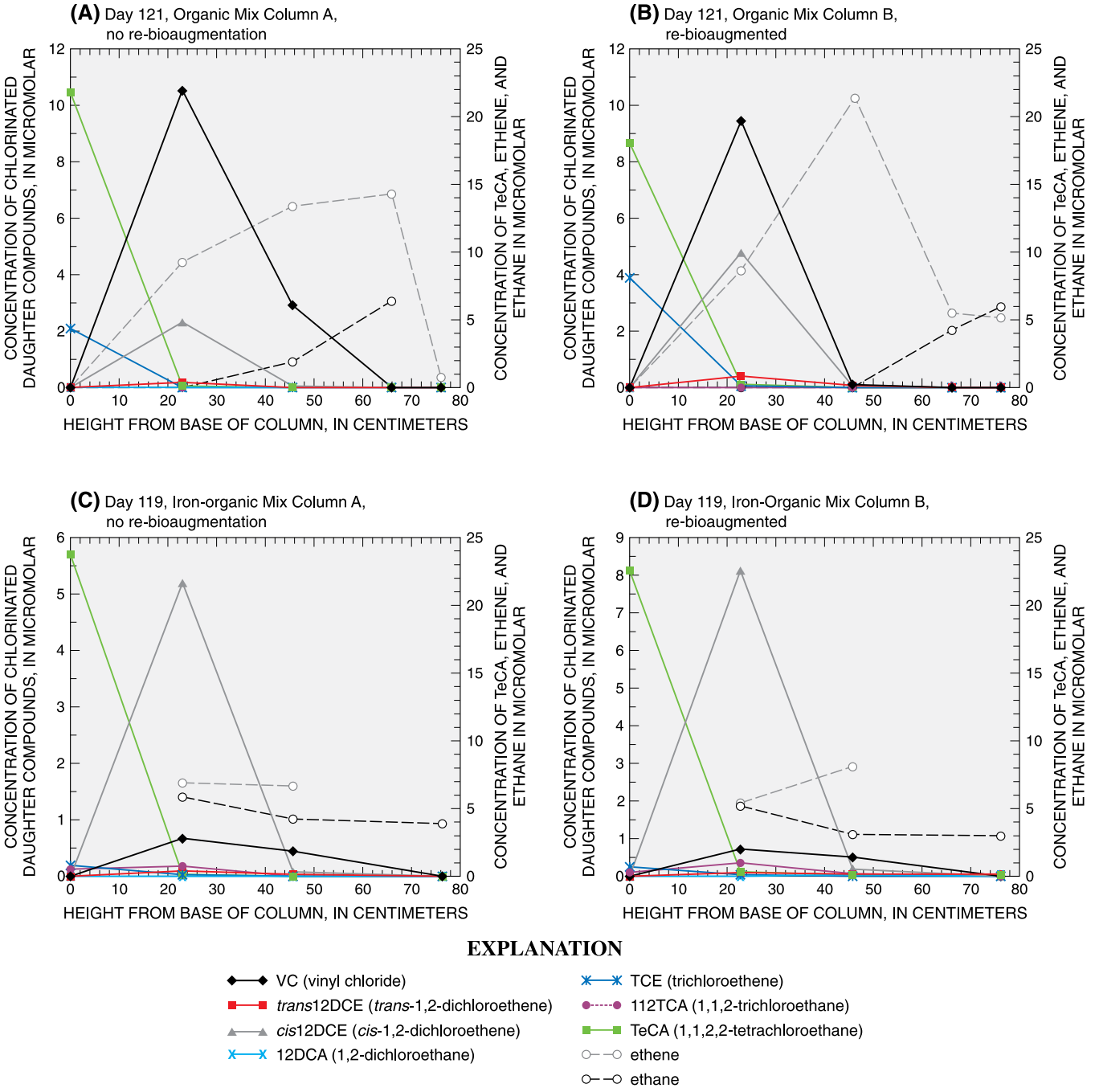


Figure 30. Effect of additional bioaugmentation on contaminant profiles of 1,1,2,2-tetrachloroethane (TeCA) and its daughter compounds through *(A, B)* organic mix and *(C, D)* iron-organic mix columns.

and Geosyntec laboratories (Geosyntec Consultants, Inc., 2006). Although not as frequently as *cis*12DCE, *trans*12DCE has been observed as a primary degradation product of chlorinated ethenes under certain situations and was associated with a *Dehalococcoides* or *Dehalobacter* organism (Griffin and others, 2004). The high accumulation of TeCA dichloroelimination daughter compounds relative to other chlorinated products during the BA phase could indicate either a shift to higher production through this dichloroelimination pathway or an improvement in the efficiency of 112TCA degradation and its daughter products (fig. 2a). Mass balance improved by day 51 during the BA phase with the consistent detection of ethene. Unlike the seep sediment columns, organic substrate addition was not necessary to achieve complete degradation of the chlorinated daughter products in organic mix columns. Consistent production and subsequent removal of *trans*- and *cis*12DCE, and VC occurred throughout the column length by day 72 of the BA phase, prior to addition of organic substrate (figs. 29c–d).

With the addition of lactate to the organic mix column influent on day 93, degradation rates of daughter compounds increased with a corresponding shift from *trans*12DCE as the predominant daughter produced and degraded, to mostly *cis*12DCE, and subsequently to VC. This shift was accompanied by detection of ethane by day 114 and a subsequent decrease in mass balance, likely due to the continued degradation of ethene and ethane through the remainder of the column. Further improvement in the efficient removal of lesser chlorinated daughter compounds was observed with the second bioaugmentation of the B column on day 107, after which only minimal transient production of VC, ethene, and ethane was detected (fig. 30b).

In the iron-organic mix columns, bioaugmentation of the organic zone after 8 days of operation resulted in the simultaneous production and subsequent decline of *cis*12DCE, 112TCA, ethene, and ethane, with maximum concentrations detected 23 cm above the base of the column (figs. 29b, d). Some VC mass remained in the column effluent through day 52 (fig. 29c), whereas the chlorinated daughter compounds *cis*12DCE and 112TCA were not removed below detection limits within the column until day 80 (fig. 29d).

The addition of lactate (10 mM) to the iron-organic mix column influent beginning on day 80 resulted in no apparent change in daughter compound degradation. After additional bioaugmentation of the organic zone of the iron-organic mix column on day 94, 112TCA declined as a degradation product (fig. 30c). Under mixed iron-reducing and methanogenic conditions, only minor accumulation of 112TCA was commonly observed in the field and in microcosms with wetland sediment where natural attenuation was shown to be efficient (Lorah and Voytek, 2004; Lorah, Spencer, and McGinty, 2005). In addition to strictly biological factors, abiotic enhancements due to iron sulfide and other biogenic minerals (Gander and others, 2002) cannot be discounted as a possible reason for the improved removal rates in the iron-organic mix columns.

Redox

Effluent DOC concentrations were higher in the organic and iron-organic mix columns than those in the sediment columns or those measured in the field. During periodic measurements between events 8 and 15, DOC averaged 28.1±6 mg/L and 32.8±7 mg/L for organic and iron-organic mix column effluent, respectively. These DOC concentrations appeared to be adequate to establish methanogenic conditions prior to the addition of VOCs and to maintain these conditions throughout the test. Although methane concentrations varied throughout the various treatments, at least some part of the column remained methanogenic.

Prior to and following bioaugmentation, as well as following lactate addition and re-bioaugmentation, ferrous iron concentrations were relatively low, with concentrations in the organic and iron-organic mix column effluent, respectively, typically ranging from 2–4 mg/L and 0.5–3.6 mg/L. In general, ferrous iron concentrations increased through the length of the column. Sulfide was not consistently detected in either the organic or iron-organic matrix columns. When it was detected, it was near the detection limit and decreased through the column length to below detection limits. The decline in sulfide may indicate the formation of ferrous sulfide precipitates or other complex iron cycling in the highly organic sediments. Iron sulfide precipitates have been implicated in the decline of porespace of reactive media (Vikesland and others, 2003), but also in the improved rates of transformation of some chlorinated VOCs due to the formation of biogenic minerals (Gander and others, 2002; McCormick and others, 2002).

An increase in methane production was observed throughout the organic mix and iron-organic mix column lengths following the addition of lactate starting on days 93 and 80, respectively, in the two columns (figs. 31a, d). As was the case with the sediment columns, once methane concentrations increased substantially, lactate concentration in the influent was reduced (Appendixes A2 and A3). Unlike the sediment columns, lactate was never removed from the influent; however, the methane concentrations appeared to be more consistent in these matrices compared to the sediment columns, and therefore less susceptible to dramatic shifts in dechlorination efficiency. Re-bioaugmentation did not result in an increase in methane concentrations in the B columns of the organic or iron-organic mix. No adverse effects resulting from possible competition between dechlorinators and methanogens were observed over the duration of the test, consistent with long-term results reported by Fennell and others (1997).

Water Quality

The pH of column effluent remained consistent in both matrices throughout the duration of the column operation. Effluent pH from the organic matrix averaged 6.80±0.11 and 6.78±0.11 in the A and B columns, respectively, similar to values measured in wetland sediment. Effluent pH from the iron-organic matrix averaged 7.17±0.13 and 7.23±0.13,

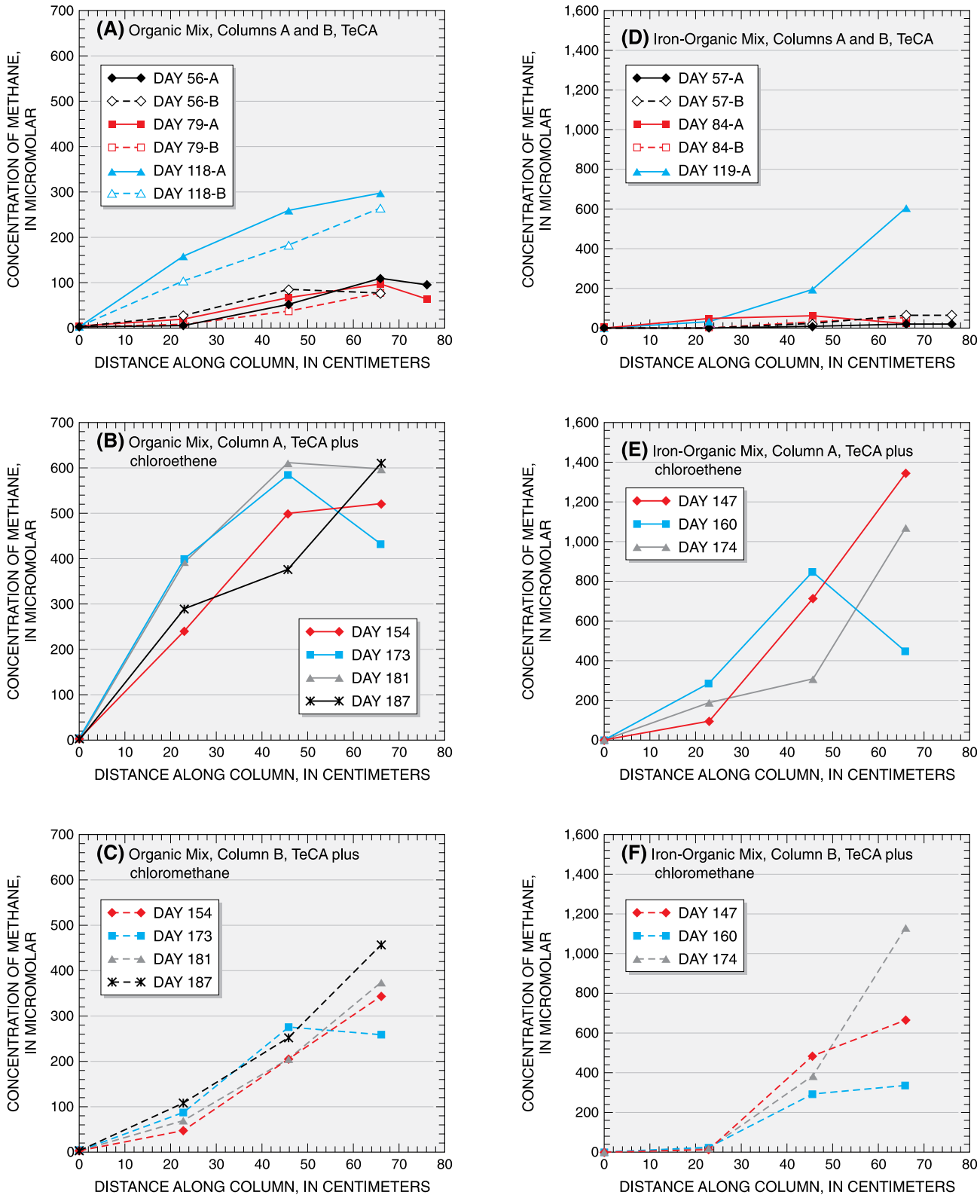


Figure 31. Methane concentrations in *(A, B, C)* organic mix and *(D, E, F)* iron-organic mix columns amended with 1,1,2,2-tetrachloroethane (TeCA), TeCA plus chloroethenes, or TeCA plus chloromethanes.

respectively, in the A and B columns. The slight increase in pH compared to the organic mix columns, which was likely due to production of hydroxyl ions from iron corrosion, did not inhibit reductive dechlorination. Laboratory tests with WBC-2 have shown degradation of the chlorinated ethanes and ethenes up to a pH of 9 (Geosyntec Consultants, Inc., 2006).

Similar to the sediment columns, greater variability was observed in the specific conductance than in the pH over the duration of the column test. Specific conductance in the A and B columns, respectively, averaged 935±314 and 994±371 µS/cm for the organic mix, and 922±344 and 905±343 µS/cm for the iron-organic mix. Variability in the conductance values was attributed to the addition of lactate to the influent, as increased conductance corresponded to events where lactate concentrations were greater than 1 mM.

Elevated concentrations of nutrients (nitrate, ammonia, and phosphate) were observed in the porewater early in the column operation and resulted from the use of organic materials (commercial compost and peat) in the reactive matrix. Nitrate increased over the column length from below detection limits (less than 0.25 mg/L) to a maximum of 1.0 and 1.6 mg/L, respectively, in the organic and iron-organic mix columns. Ammonia effluent concentrations ranged from 0 to 3.75 mg/L. A similar ammonia concentration range was observed in the influent concentrations, therefore, the ammonia may not be associated with release from the reactive matrix. Phosphate increased roughly an order of magnitude in both matrices over the length of the column. Maximum concentrations detected in the porewater were 64 and 42 mg/L, respectively, in the organic and iron-organic mix columns. Nutrient cycling with plants in the native wetland, however, would be expected to remove much of this phosphate in the natural wetland environment.

Co-Contaminant Effects and Degradation

To simulate the mixtures of chlorinated solvents present in the seep areas and their effect on degradation in the reactive mat matrices, column tests were performed adding chlorinated ethenes (PCE and TCE) and chlorinated methanes (CT and CF) to the influent water. Overall, the bioaugmented microbial community established within the reactive mat mixtures was able to facilitate rapid degradation of TeCA, with and without the presence of chloroethene and chloromethane co-contaminants, and also to facilitate the rapid degradation of the co-contaminants themselves. The comparable degradation efficiency in the organic and iron-organic mix columns indicates that ZVI would not be a necessary component of an organic-based, bioaugmented reactive mat, even in seep areas with high chloromethane concentrations (up to 25 µM total was tested). The column test results are detailed in the following sections.

Chloroethenes

Chloroethenes (PCE and TCE) were added to column A of the organic mix (M-A) and iron-organic mix (FM-A), respectively, starting on days 145 and 132 and continuing through the end of the column operation. During the PCE and subsequent TCE addition, TeCA degradation continued to proceed through the dichloroelimination pathway at rates similar to or more rapid than those observed prior to chloroethene addition in both matrices (table 8, figs. 32a-b). Since TeCA degradation was nearly exclusively degrading via dichloroelimination, the chlorinated daughter compounds are common between TeCA, PCE, and TCE. The primary measured daughter compound, VC (and to a lesser extent, *cis*12DCE), continued to be detected at a maximum concentration 23 cm along the column and removed through the length of the column. Final degradation products ethene and ethane continued to be detected throughout the column in the presence of PCE and TCE, and all chlorinated daughter compunds were removed through the column length in both matrices.

Methanogenic conditions and efficient VOC degradation were maintained throughout the period of co-contaminant addition in both matrices (figs. 31b, e). As was observed with the sediment columns, chlorinated ethenes were removed at rates lower than those calculated for TeCA in the reactive mat matrices. PCE removal was slower than TeCA removal in tests with WBC-2 in culture media (fig. 9). Both PCE and TCE appeared to degrade according to first-order kinetics in both reactive matrices tested in the columns, despite the generation of TCE during PCE degradation. PCE reaction rate constants were similar in the M-A and FM-A columns, estimated as 1.4±.0.15 day^{-1} and 1.6±0.57 day^{-1}, respectively (table 9). As observed in the sediment columns, TCE rate constants in reactive mat matrices were greater than PCE rate constants, and were approximately equal for the three matrices—3.2±0.49 day^{-1} in the seep sediment compared to 3.0±0.54 and 2.4±0.03 day^{-1} in the reactive mat marices (table 9).

Chloromethanes

Chloromethanes (CF and CT) were added to M-B and FM-B, respectively, starting on days 128 and 101, and continuing through the end of the column operation, with incremental increases in concentration from a starting concentration of 3 µM to a maximum of 220 µM total CT and CF (fig. 33). TeCA degradation continued to proceed efficiently in these columns, with ethene and ethane the only daughter products detected in the column effluent. As concentrations increased to the maximum added, TeCA removal appeared to be correlated to the complete removal of CF in the influent along the column. In the organic and iron-organic mix columns, the degradation rate constants of TeCA with the added chloromethanes were comparable to the

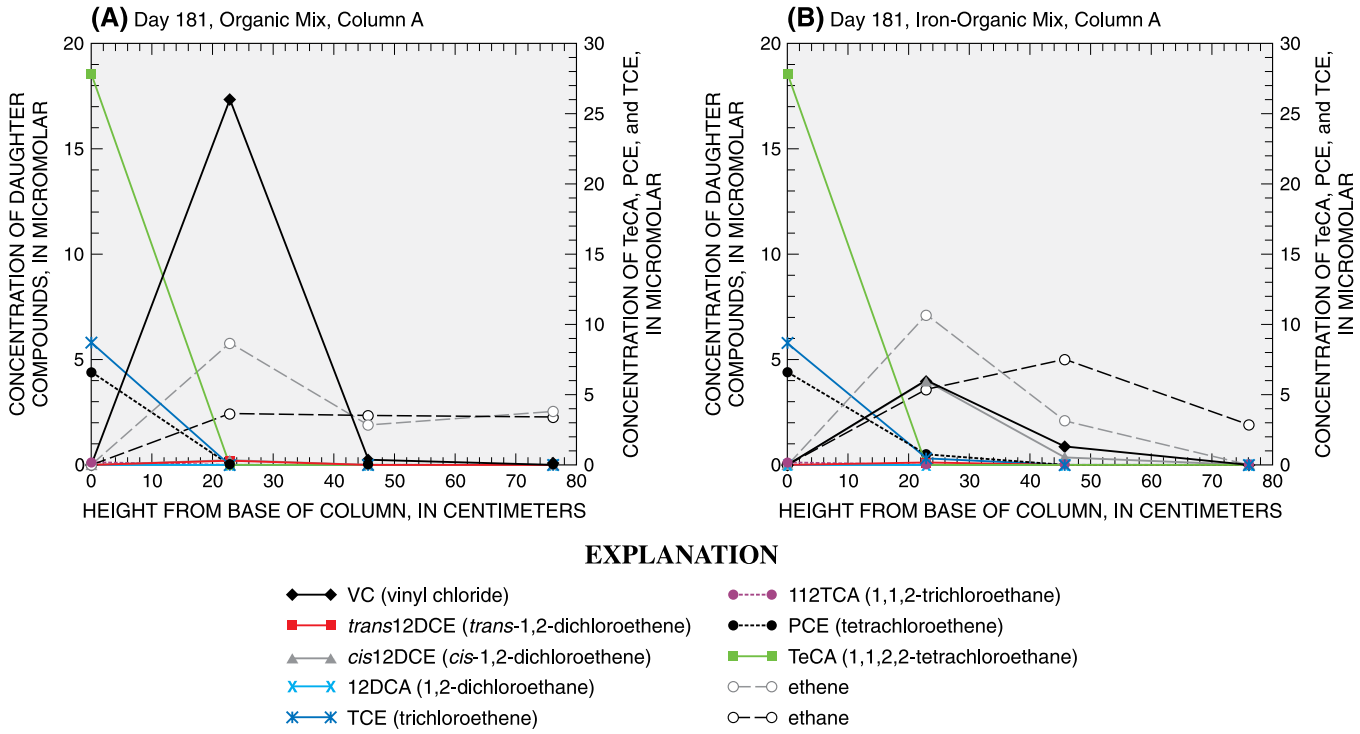


Figure 32. Effects of the chloroethene co-contaminants tetrachloroethene (PCE) and trichloroethene (TCE) on degradation of 1,1,2,2-tetrachloroethane (TeCA) in *(A)* organic mix and *(B)* iron-organic mix columns (column A).

Table 9. Co-contaminant degradation rate constants in organic mix and iron-organic mix columns under different treatment conditions.

[--, not applicable]

Co-Contaminant	Organic mix		Iron-organic mix	
	Mean rate constant (day^{-1})	Standard deviation	Mean rate constant (day^{-1})	Standard deviation
Chloroform (CF)	2.31	0.42	1.44	0.18
Carbon tetrachloride (CT)	2.83	.8	--	--
Tetrachloroethene (PCE)				
without chloromethanes	1.4	.15	1.63	.57
with chloromethanes	0.766	.05	1.19	.09
Trichloroethene (TCE)				
without chloromethanes	3.04	.54	2.43	.03
with chloromethanes	.72	--	1.12	--

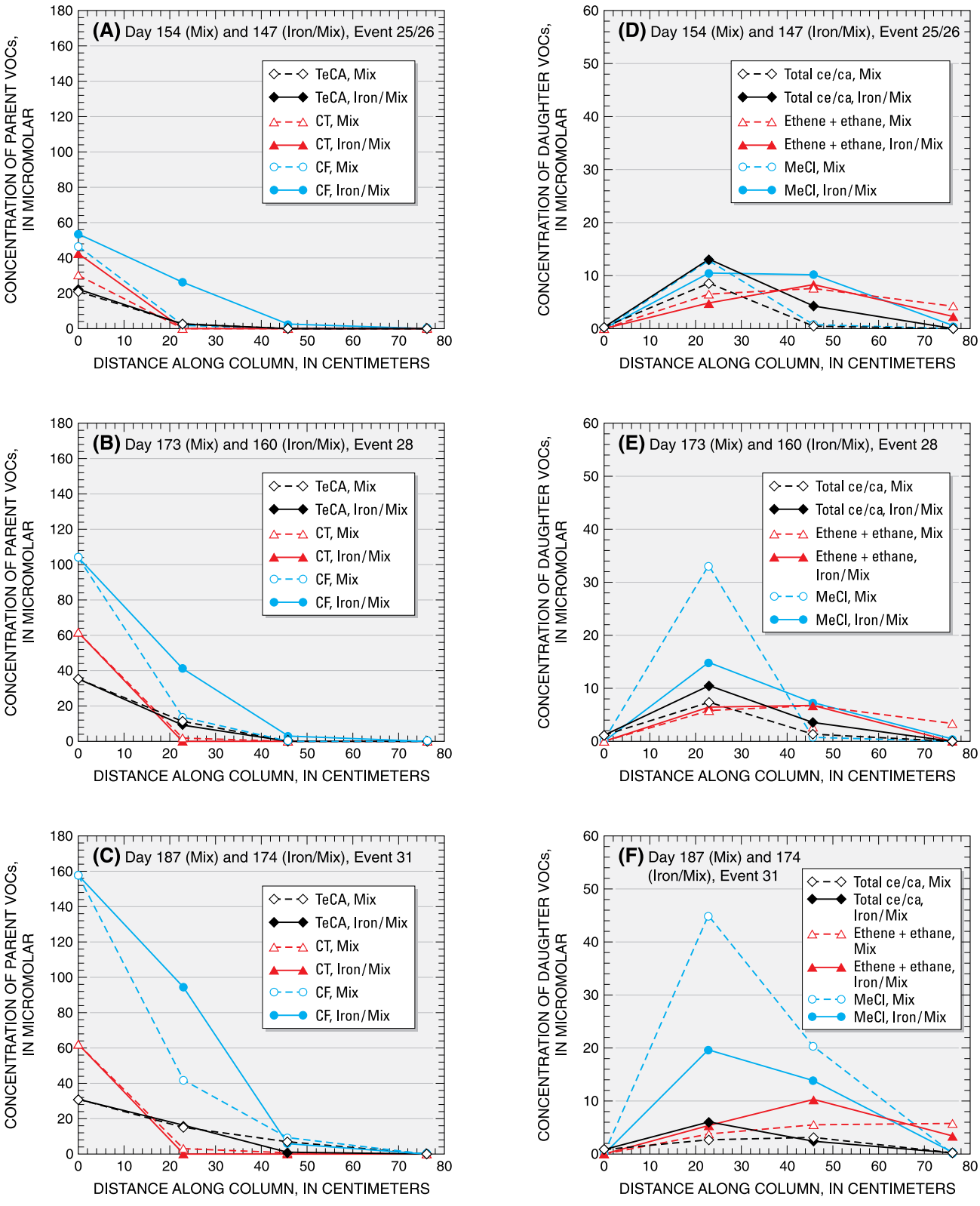


Figure 33. Degradation of *(A, B,* and *C)* 1,1,2,2-tetrachloroethane (TeCA) while carbon tetrachloride (CT) and chloroform (CF) were incrementally increased, and distribution of *(D, E,* and *F)* daughter volatile organic compounds (VOCs) shown as the total of chlorinated ethenes and ethanes (total ce/ca), the non-chlorinated daughters ethene plus ethane, and methylene chloride (MeCl) in organic mix (Mix) and iron-organic mix (Iron/Mix) columns.

bioaugmented (BA) and biostimulated/bioaugmented (BABS) rate constants, but were less than those calculated in the re-bioaugmented columns (tables 8 and 9). All added TeCA mass was removed within 23 cm in the column.

An effect of chloromethane addition was observed in the daughter compound removal rates and methane concentrations in both matrices. In the M-B column, re-emergence of *trans*12DCE (and to a lesser extent, *cis*12DCE) as transient daughter compounds coincided with the addition of chloromethanes to the influent (total chlorinated daughter products shown in fig. 33). In some cases, the complete removal of chlorinated daughter compounds from the column porewater was not as rapid as was observed prior to chloromethane addition, with high concentrations occurring at 46 to 68 cm along the column (figs. 33d,f). A corresponding decrease in methane concentrations, particularly in the lower portions of the column, resulted from the addition of chloromethanes (fig. 31e). Methanogenic conditions (methane greater than 31 µM), however, were maintained throughout all events.

CF also was added at a concentration of 62 µM to columns A of the mix and iron-organic mix (M-A and FM-A) near the end of the test (following addition of TCE and PCE), and concentrations were monitored for the final two sampling events (data not shown). In the M-A and FM-A columns, TeCA degradation rates decreased by about 25 percent with the addition of CF to the influent (table 9). More than 90 percent of the added TeCA still was removed within the bottom one-third of the columns, however. A decrease in daughter compound removal also was observed, with VC remaining in the column effluent of both matrices. These results are similar to those observed by Kaseros and others (2000) at an order of magnitude less CF added. They attributed temporary inhibition to the lack of acetic acid formation from ethanol (Kaseros and others, 2000). This CF toxicity is consistent with that observed by Weathers and Parkin (2000) to unacclimated, methanogenic bacteria. A corresponding decrease in methane concentrations was not observed in the M-A and FM-A columns with addition of the chloromethanes, which may indicate that over time, complete degradation of TeCA and the chlorinated ethenes could have been restored in these columns to the efficiency observed in the B columns. Bagley and others (2000) could not establish PCE degradation in the presence of CT and CF, but dechlorination in columns already degrading PCE could be restored over time with exposure to CT and CF.

In both reactive matrices, apparent first-order rate constants were calculated for CT and CF, despite the generation of CF from the concurrent degradation of CT (table 9). Both matrices degraded the chlorinated methanes through anaerobic reductive dechlorination, with some detection of the lesser chlorinated compound methylene chloride (fig. 33) and, in the case of the iron-organic mix columns, chloromethane. Chloromethane was consistently detected but only accounted for less than 1 percent of the total added mass in the iron-organic mix columns (data not shown). In the mix columns, an average CT rate constant was calculated to be 2.8±0.8 day^{-1}; however, the rate of removal could not be quantified in the iron-organic mix columns since all CT was consistently removed before the first sampling port at 23 cm along the column length. The CF degradation rate constants were 2.3±0.42 day^{-1} in the organic mix columns and 1.4±0.18 day^{-1} in the iron-organic mix columns (table 9). Despite the generation of CF from the degradation of CT, the added concentration of CF was linearly correlated to the rate of CF removal in both matrices (figs. 34a–b).

Both CT and CF appeared to degrade through a common reductive dechlorination pathway, generating primarily

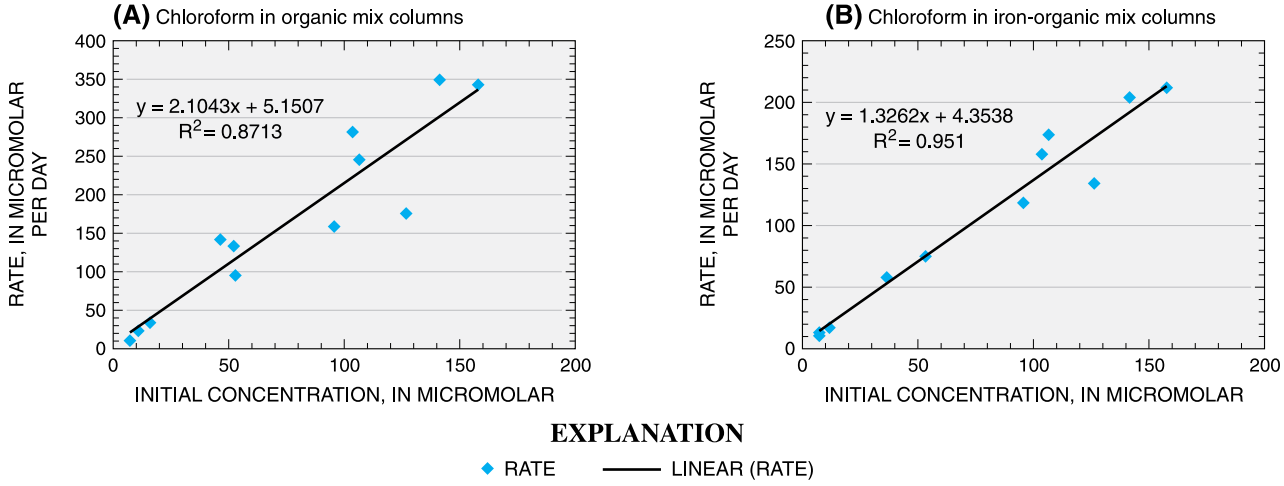


Figure 34. Correlation between rate of chloroform removal and initial concentration in: *(A)* organic mix columns, and *(B)* iron-organic mix columns.

methylene chloride (detected) and possibly carbon dioxide or other non-volatile products such as carbon disulfide (deBest and others, 1998). In the organic mix matrix, a maximum of 20 to 30 percent of the total added mass of CT and CF was detected as methylene chloride within the first 23 cm of the column (fig. 33). Less transient accumulation of methylene chloride was measured in the iron-organic mix columns compared to the organic mix columns. This likely indicates that less overall methylene chloride was produced in the iron-organic mix than the organic mix matrix, because methylene chloride cannot be degraded abiotically with ZVI (Gillham and O'Hannesin, 1994). Although chlorinated methanes were not added to the sediment columns, the transient methylene chloride accumulated in the organic mix columns was similar to that observed in the CF- and ZVI-amended microcosms (with and without sediment, figs. 24a–d), and to that observed in microcosms with WB23 and WB30 sediment (fig. 21a). Despite the greater accumulation of methylene chloride without ZVI, the microorganisms present in the wetland sediment and in the bioaugmentation culture appear to be capable of biodegrading CF and methylene chloride to below detection limits within the length of the column. CF and CT degradation was previously reported in the presence of particular methanogens, two of which are part of the Methanosarcina group (Novak and others, 1998), known to be an important group in the WBC. Degradation of methylene chloride under methanogenic conditions to carbon dioxide, acetic acid, methane, and a non-strippable residue has been attributed to DCM-oxidizers and acetogens (Freedman and Gossett, 1991).

Summary and Conclusions

Chlorinated solvents, including 1,1,2,2-tetrachloroethane (TeCA), tetrachloroethene, trichloroethene, carbon tetrachloride (CT), and chloroform (CF), are reaching land surface in localized areas of focused ground-water discharge (seeps) in a wetland and tidal creek in the West Branch Canal Creek area, Aberdeen Proving Ground, Maryland. Although natural attenuation has been shown to be an effective ground-water remediation method in much of the wetland, biostimulation and bioaugmentation are being tested as possible methods to enhance anaerobic degradation in the localized seep areas. The U.S. Geological Survey, in cooperation with the U.S. Army Garrison, Aberdeen Proving Ground, Maryland, conducted laboratory studies as the first phase of developing enhanced bioremediation methods for the contaminant mixtures in the seeps. This laboratory phase included development of a microbial consortium to degrade TeCA and its chlorinated daughter products under anaerobic conditions, microcosm experiments to test biostimulation and bioaugmentation in individual matrices, and column experiments designed to combine the individual components and test them under field ground-water-flow and contaminant conditions. The two specific enhanced bioremediation methods evaluated were direct injection at depth in the wetland sediments in the seep area, and a permeable reactive mat that can be applied at the ground-water/surface-water interface in the seep area. Matrices tested for the reactive mat included commercially available compost and peat mixtures, and zero-valent iron that potentially could be mixed with the organic materials for the first mat layer in areas of high chloromethane (CT and CF) concentrations.

For bioaugmentation, a mixed anaerobic culture was developed by enrichment of wetland sediment collected from two sites in the study area where rapid and complete reductive dechlorination occurs. The "West Branch Consortia" (WBC-1 and WBC-2) are capable of degrading TeCA, trichloroethene, *cis*- and *trans*-1,2-dichloroethene, 1,1,2-trichloroethane, 1,2-dichloroethane, and vinyl chloride to the non-chlorinated end-products ethene and ethane. Although either H_2 and acetate (WBC-1) or lactate alone (WBC-2) supported dechlorination activity, only lactate supported long-term cultivation of a population capable of complete dechlorination. WBC-2 is capable of complete dechlorination of 50 micromolar TeCA to ethene in 1–2 days with little transient accumulation of chlorinated daughter products. Active WBC-2 cultures were maintained by feeding 50 micromolar TeCA and 1 millimolar lactate, or 25 micromolar TeCA, 50 micromolar *cis*-1,2-dichloroethene, 50 micromolar 1,1,2-trichloroethane, and 1.5 millimolar lactate 2 times per week. The degradation activity of WBC-2 culture maintained on the mixture of TeCA, 1,1,2-trichloroethane, and *cis*-1,2-dichloroethene also was tested with tetrachloroethene and trichloroethene, two common co-contaminants in the seep ground water. WBC-2 degraded trichloroethene (14 micromolar), *cis*-1,2-dichloroethene (14 micromolar), and TeCA (10 micromolar) within 5 days, although tetrachloroethene degradation was slower than degradation of the other parent compounds.

About 5 percent of the clones sequenced from WBC-1 and WBC-2 were related to dechlorinating bacteria that have been studied in culture. *Dehalococcoides* was not dominant in the culture, comprising only about 1 percent of the microbial population. Clostridiales, including Acetobacteria, comprised 65 percent of the bacterial clones in WBC-2, with *Cytophaga-Flavobacterium-Bacteroides* (14 percent), and epsilon Proteobacteria (14 percent) also numerically important. A *Clostridium* sp. closely related to an uncultured member of a trichloroethene-degrading consortium was the numerically dominant bacterial member of WBC-2 after 11 months of enrichment in culture. Methanogens identified in the consortia were members of the Order Methanomicrobiales, which includes acetoclastic methanogens.

To test the effect of biostimulation by nutrient (ammonia and phosphate mixture) addition, anaerobic microcosms to measure TeCA degradation were constructed with wetland sediment and ground water collected from three sites and on three different dates. Although nutrient addition enhanced the degradation rate of TeCA by nearly 40 percent for

some microcosms compared to controls that did not receive added nutrients, degradation of TeCA's anaerobic daughter compounds (1,1,2-trichloroethane, 1,2-dichloroethane, trichloroethene, 1,2-dichloroethene, and vinyl chloride) was not enhanced by nutrient addition. Similarly, nutrient amendment did not enhance degradation of TeCA or its anaerobic daughter products in microcosms constructed with mixtures of BionSoil (a dairy-derived compost) and commercial peat. Biostimulation by addition of organic donor (lactate) also did not enhance TeCA degradation in microcosms with wetland sediment and BionSoil/peat mixtures. A shift in the TeCA degradation pathway toward more 1,2-dichloroethane and chloroethane production was observed in the donor-stimulated microcosms relative to unamended microcosms, and degradation of these daughter products was minimal within the 35-day experiments.

To test the effect of bioaugmentation, microcosms constructed with wetland sediment from the three sites were amended with TeCA and the developed anaerobic mixed culture in media (suspended culture addition was 10 percent by volume of the microcosm solution). TeCA biodegradation rates were about 2–3 times higher in the bioaugmented microcosms than in controls amended with culture media alone. In addition, bioaugmentation substantially enhanced degradation of the chlorinated daughter compounds, with typically a maximum of about 20 to 30 percent of the initial TeCA added in the bioaugmented microcosms recovered as daughter products, compared to 50 to 100 percent in the unamended microcosms. Rapid biodegradation of TeCA and its chlorinated daughter compounds in the bioaugmented microcosms was associated with greater methane production than in microcosms without the added culture. Bioaugmentation, therefore, was more effective than biostimulation. Bioaugmentation with WBC-2 was equally effective in enhancing TeCA degradation in wetland sediment from the study area and in the mixture of commercial BionSoil and peat. Out of five additional composts (mixed with the same peat as in the BionSoil mixtures) tested in microcosms with WBC-2, two composts, one derived from crab waste and one from municipal yard waste, also provided a suitable environment for the culture. These two composts showed similar TeCA and daughter product degradation efficiency to the BionSoil. Wetland sediment from the study area and bioaugmented BionSoil/peat also was able to rapidly degrade CT and CF to non-chlorinated end-products, with little transient accumulation of methylene chloride.

Column tests were used to (1) combine biostimulation and bioaugmentation to simulate direct injection in seep sediment, and to combine the matrix zones conceptualized for the permeable reactive mat (zero-valent iron and organic layer with an overlying bioaugmented organic layer), (2) better simulate the bioremediation methods under field conditions, including continuous ground-water flow at measured rates and increased contaminant concentrations, and (3) evaluate possible adverse water-quality effects that could result from the proposed near-surface bioremediation methods in the wetland. The enriched, mixed microbial culture did not appear to stimulate enhanced biodegradation of site contaminants in the seep sediment columns without the adequate addition of continuous, soluble electron donor. With the addition of ethanol, methanogenic conditions were rapidly established and efficient degradation of TeCA, as well as tetrachloroethene and trichloroethene, was observed in the columns. Degradation efficiency was highly sensitive to changes in the ethanol dosing. Degradation rates of parent and daughter compounds in the sediment columns were adequate to allow complete removal of these chlorinated volatile organic compounds within a shallow depth of wetland sediments, indicating that field trials of the technique are worthwhile. Success of direct injection may be largely dependent on the ability to adequately deliver and distribute electron donor through the low permeability wetland sediments.

The bioaugmented microbial culture stimulated enhanced degradation of TeCA in both matrices for the reactive mat (organic mix and iron/organic mix) without lactate, and with improved efficiency, with lactate addition. In both matrices, TeCA and all associated daughter compounds were typically removed within 46 centimeters along the column. In the presence of chloroethenes and chloromethanes, rapid TeCA degradation was maintained in both matrices. TeCA daughter compound removal varied with increasing amounts of chloromethanes. A measurable decline in the removal efficiency was observed and different isomers of 1,2-dichloroethene were produced in the mix (*trans*-isomer) and iron-organic mix (*cis*-isomer) columns. Nonetheless, all daughter compounds were removed within the 76-centimeter column length in both matrices. The bioaugmented microbial community within each column matrix was able to facilitate degradation of the chloroethene (tetrachloroethene and trichloroethene) and chloromethane (CT and CF) co-contaminants and associated daughter compounds, showing that the developed culture has an unusual ability to degrade a wide range of chlorinated solvents without substantial toxicity effects. The overall comparable degradation efficiency in the mix and iron-organic mix columns indicates that zero-valent iron would not be a necessary component of an organic-based, bioaugmented reactive mat, even in seep areas with high chloromethane concentrations (up to 25 micromolar total was tested). The addition of a zero-valent iron layer to the reactive mat could provide a safeguard against loss in degradation efficiency with potential decline in microbial activity in the colder winter months, however. Neither organic-based matrix is likely to cause any adverse effects to water quality. Results of this laboratory study showed the broad dechlorinating capabilities of the developed microbial consortia, and support both direct injection and the newly conceived reactive mat as promising remediation methods for ground water discharging to seeps in the wetland.

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Appendix A

Appendix A1. Sampling and operation of column experiments-- Sediment columns A and B (S-A and S-B).

[Amendments given in column influent were continued until a change is noted in the comments. Major treatments noted in comments include: P, addition of contaminants but prior to bioaugmentation or biostimulation; BA, bioaugmentation with culture; BABS, biostimulation by addition of organic donor to bioaugmented columns; 2BABS, re-bioaugmentation and continued biostimulation; VOCs, volatile organic compounds; DOC, dissolved organic carbon; VFA, volatile fatty acids; TeCA, 1,1,2,2-tetrachloroethane; PCE, tetrachloroethene; TCE, trichloroethene; mg/L, milligrams per liter; mM, millimolar]

Event	Date	Days from VOCs amendment	Number of pore volumes	Samples	Comments
0	2/2/2004 2/4/2004	0		VOCs Methane, iron, ammonia	Background samples **P**: Begin TeCA amendment (5 mg/L).
1	2/6/2004	2	0.62	VOCs	
2	2/8/2004 2/10/2004	4 6	1.24 1.86	Anions, cations, DOC, alkalinity VOCs	
3	2/17/2004	13	4.02		**BA**: Bioaugmented with WBC-1 on 2/11/05. No samples collected for event 3.
4	2/21/2004	17	5.26		No samples collected for event 4.
5	2/23/2004 2/25/2004 3/4/2004	19 21 29	5.88 6.50 8.98	Methane, iron VOCs Methane, iron	
6	3/1/2004 3/4/2004	26 29	8.05 8.98	VOCs Anions, cations, DOC, alkalinity	High TeCA detected (about 10 mg/L).
7	3/8/2004 3/10/2004	33 35	10.22 10.84	VOCs Methane, iron	High TeCA detected (about 10 mg/L); no TeCA added.
8	3/12/2004 3/15/2004 3/17/2004	37 40 42	11.46 12.38 13.00	VOCs Anions, cations, DOC, alkalinity Methane, iron	
9	3/19/2004 3/22/2004 3/24/2004	44 47 49	13.62 14.55 15.17	VOCs Anions, cations, DOC, alkalinity Methane, iron, VFA, hydrogen	Restart TeCA amendment (5 mg/L).
10	3/26/2004 3/29/2004 3/31/2004	51 54 56	15.79 16.72 17.34	VOCs Anions, cations, DOC, alkalinity Methane, iron, hydrogen	
11	4/2/2004 4/7/2004	58 63	17.96 19.50	VOCs Methane, iron (ferrous), ammonia, sulfide	
12	4/9/2004 4/14/2004	65 70	20.12 21.67	VOCs Methane, iron (total, ferrous), ammonia, sulfide	**BABS**: Begin donor amendment (ethanol,10 mM).
13	4/16/2004	72	22.29	VOCs	
14	4/21/2004 4/23/2004 4/26/2004	77 79 82	23.84 24.46 25.39	VOCs Methane, iron (total, ferrous), ammonia, sulfide Anions, cations, DOC, alkalinity	

Appendix A1. Sampling and operation of column experiments-- Sediment columns A and B (S-A and S-B).—Continued

[Amendments given in column influent were continued until a change is noted in the comments. Major treatments noted in comments include: P, addition of contaminants but prior to bioaugmentation or biostimulation; BA, bioaugmentation with culture; BABS, biostimulation by addition of organic donor to bioaugmented columns; 2BABS, re-bioaugmentation and continued biostimulation; VOCs, volatile organic compounds; DOC, dissolved organic carbon; VFA, volatile fatty acids; TeCA, 1,1,2,2-tetrachloroethane; PCE, tetrachloroethene; TCE, trichloroethene; mg/L, milligrams per liter; mM, millimolar]

Event	Date	Days from VOCs amendment	Number of pore volumes	Samples	Comments
15	4/28/2004 4/30/2004	84 86	26.01 26.63	Methane, iron (total, ferrous), ammonia, sulfide VOCs	
16	5/4/2004 5/7/2004	90 93	27.86 28.79	Methane, ammonia, sulfide VOCs, iron (total and ferrous)	
17	5/11/2004 5/14/2004	97 100	30.03 30.96	Methane, ammonia, sulfide VOCs, iron (total and ferrous)	Ethanol reduced to 5 mM.
18	5/18/2021 5/21/2004	104 107	32.20 33.13	Methane, ammonia, sulfide VOCs, iron (total and ferrous)	**2BABS**: Re-bioaugmented S-B on 5/24 with WBC-2e.
19	5/25/2004 5/28/2004	111 114	34.37 35.29	Methane, ammonia, sulfide VOCs, iron (total and ferrous)	
20	6/1/2004 6/4/2004	118 121	36.53 37.46	Methane, ammonia, sulfide VOCs, iron (total and ferrous)	Ethanol reduced to 1 mM.
21	6/8/2004 6/11/2004	125 128	38.70 39.63	Methane, ammonia, sulfide VOCs, iron (total and ferrous)	
22	6/15/2004	132	40.87	VOCs, methane	Ethanol removed from column influent.
23	6/21/2004	138	42.72	VOCs, methane	
24	6/28/2004	145	44.89	VOCs, methane	
25	7/7/2004	154	47.68	VOCs, methane	Co-contaminant of 1 mg/L PCE added with TeCA (5 mg/L). Ethanol added (1 mM).
26	7/13/2004	160	49.54	VOCs, methane	
27	7/19/2004	166	51.39	VOCs, methane	
28	7/26/2004	173	53.56	VOCs, methane	Co-contaminant of 1 mg/L TCE added with 5 mg/L TeCA and 1 mg/L PCE. Ethanol increased to 2 mM.
29	8/3/2004	181	56.04	VOCs, methane	
30	8/6/2004	184	56.97	VOCs, methane	
31	8/9/2004	187	57.89	VOCs, methane	

Appendix A2. Sampling and operation of column experiments—Organic mix column A (M-A) and iron/mix column A (FM-A).

[Amendments given in column influent were continued until a change is noted in the comments. Major treatments noted in comments include: P, addition of contaminants but prior to bioaugmentation or biostimulation; BA, bioaugmentation with culture; BABS, biostimulation by addition of organic donor to bioaugmented columns; VOCs, volatile organic compounds; DOC, dissolved organic carbon; VFA, volatile fatty acids; TeCA, 1,1,2,2-tetrachloroethane; PCE, tetrachloroethene; TCE, trichloroethene; CF, chloroform; mg/L, milligrams per liter; mM, millimolar]

Event	Date	Column M-A		Column FM-A		Samples	Comments
		Days from VOCs amendment	Number of pore volumes	Days from VOCs amendment	Number of pore volumes		
	11/19/2003						Circulation of porewater began to M-A
0	2/2/2004		18			VOCs	Baseline; no VOCs added to influent.
	2/4/2004	0		0		Methane, iron, ammonia	**P**: Begin TeCA amendment to M-A (5 mg/L). FM-A repacked; no VOCs added.
1	2/6/2004	2	0.49			VOCs	
2	2/8/2004	4	.98			Anions, cations, DOC, alkalinity	
	2/10/2004	6	1.46			VOCs	
3	2/17/2004	13	3.17		3.17	VOCs, methane, iron, ammonia	Begin TeCA amendment to FM-A (5 mg/L).
4	2/21/2004	17	4.15	4	0.98	VOCs	
5	2/23/2004	19	4.63	6	1.46	Methane, iron	
	2/25/2004	21	5.12	8	1.95	VOCs	**BA**: Bioaugmentation of M-A and FM-A with WBC-1.
6	3/1/2004	26	6.34	13	3.17	VOCs	
	3/4/2004	29	7.07	16	3.90	Anions, cations, DOC, alkalinity, methane, iron	
7	3/8/2004	33	8.05	20	4.88	VOCs	
	3/10/2004	35	8.54	22	5.37	Methane, iron, hydrogen	
8	3/12/2004	37	9.02	24	5.85	VOCs	
	3/15/2004	40	9.76	27	6.59	Anions, cations, DOC, alkalinity	
	3/17/2004	42	10.24	29	7.07	Methane, iron, hydrogen	
9	3/19/2004	44	10.73	31	7.56	VOCs	
	3/22/2004	47	11.46	34	8.29	Anions, cations, DOC, alkalinity	
	3/24/2004	49	11.95	36	8.78	Methane, iron, VFA, hydrogen	
10	3/26/2004	51	12.44	38	9.27	VOCs	
	3/29/2004	54	13.17	41	10.00	Anions, cations, DOC, alkalinity	
	3/31/2004	56	13.66	43	10.49	Methane, iron, hydrogen	
11	4/2/2004	58	14.15	45	10.98	VOCs	
	4/7/2004	63	15.37	50	12.20	Methane, iron (ferrous), ammonia, sulfide	

Appendix A2. Sampling and operation of column experiments—Organic mix column A (M-A) and iron/mix column A (FM-A).—Continued

[Amendments given in column influent were continued until a change is noted in the comments. Major treatments noted in comments include: P, addition of contaminants but prior to bioaugmentation or biostimulation; BA, bioaugmentation with culture; BABS, biostimulation by addition of organic donor to bioaugmented columns; VOCs, volatile organic compounds; DOC, dissolved organic carbon; VFA, volatile fatty acids; TeCA, 1,1,2,2-tetrachloroethane; PCE, tetrachloroethene; TCE, trichloroethene; CF, chloroform; mg/L, milligrams per liter; mM, millimolar]

Event	Date	Column M-A		Column FM-A		Samples	Comments
		Days from VOCs amendment	Number of pore volumes	Days from VOCs amendment	Number of pore volumes		
12	4/9/2004	65	15.85	52	12.68	VOCs	
	4/14/2004	70	17.07	57	13.90	Methane, iron (total, ferrous), ammonia, sulfide	
13	4/16/2004	72	17.56	59	14.39	VOCs	
14	4/21/2004	77	18.78	64	15.61	VOCs	
	4/23/2004	79	19.27	66	16.10	Methane, iron (total, ferrous), ammonia, sulfide	
	4/26/2004	82	20.00	69	16.83	Anions, cations, DOC, alkalinity	
15	4/28/2004	84	20.49	71	17.32	Methane, iron (total, ferrous), ammonia, sulfide	
	4/30/2004	86	20.98	73	17.80	VOCs, VFA	
16	5/4/2004	90	21.95	77	18.78	Methane, ammonia, sulfide	
	5/7/2004	93	22.68	80	19.51	VOCs, iron (total and ferrous)	**BABS**: Begin donor amendment (lactate, 10mM).
17	5/11/2004	97	23.66	84	20.49	Methane, ammonia, sulfide	
	5/14/2004	100	24.39	87	21.22	VOCs, iron (total and ferrous)	
18	5/18/2021	104	25.37	91	22.20	Methane, ammonia, sulfide	
	5/21/2004	107	26.10	94	22.93	VOCs, iron (total and ferrous)	
19	5/25/2004	111	27.07	98	23.90	Methane, ammonia, sulfide	
	5/28/2004	114	27.80	101	24.63	VOCs, iron (total and ferrous)	
20	6/1/2004	118	28.78	105	25.61	Methane, ammonia, sulfide	Lactate reduced to 5 mM.
	6/4/2004	121	29.51	108	26.34	VOCs, iron (total and ferrous)	
21	6/8/2004	125	30.49	112	27.32	Methane, ammonia, sulfide	
	6/11/2004	128	31.22	115	28.05	VOCs, iron (total and ferrous)	
22	6/15/2004	132	32.20	119	29.02	Methane, VOCs	Lactate reduced to 1 mM.
23	6/21/2004	138	33.66	125	30.49	Methane, VOCs	Co-contaminant of 1.0 mg/L PCE added with 5 mg/L TeCA .
24	6/28/2004	145	35.37	132	32.20	Methane, VOCs	
25	7/7/2004	154	37.56	141	34.39	Methane, VOCs	
26	7/13/2004	160	39.02	147	35.85	Methane, VOCs	

Appendix A2. Sampling and operation of column experiments—Organic mix column A (M-A) and iron/mix column A (FM-A).—Continued

[Amendments given in column influent were continued until a change is noted in the comments. Major treatments noted in comments include: P, addition of contaminants but prior to bioaugmentation or biostimulation; BA, bioaugmentation with culture; BABS, biostimulation by addition of organic donor to bioaugmented columns; VOCs, volatile organic compounds; DOC, dissolved organic carbon; VFA, volatile fatty acids; TeCA, 1,1,2,2-tetrachloroethane; PCE, tetrachloroethene; TCE, trichloroethene; CF, chloroform; mg/L, milligrams per liter; mM, millimolar]

Event	Date	Column M-A		Column FM-A		Samples	Comments
		Days from VOCs amendment	Number of pore volumes	Days from VOCs amendment	Number of pore volumes		
27	7/19/2004	166	40.49	153	37.32	Methane, VOCs	Co-contaminant of 1 mg/L TCE added with 1 mg/L PCE and 5 mg/L TeCA.
28	7/26/2004	173	42.20	160	39.02	Methane, VOCs	Lactate increased to 2 mM.
29	8/3/2004	181	44.15	168	40.98	Methane, VOCs	
30	8/6/2004	184	44.88	171	41.71	Methane, VOCs	Co-contaminants added 8.0 mg/L CF, 1.0 mg/L TCE, 1.0 mg/L PCE, and 5 mg/L TeCA.
31	8/9/2004	187	45.61	174	42.44	Methane, VOCs	
32	8/13/2004	191	46.59	178	43.41	Methane, VOCs	

Appendix A3. Sampling and operation of column experiments—Organic mix column B (M-B) and iron/mix column B (FM-B).

[Amendments given in column influent were continued until a change is noted in the comments. Major treatments noted in comments include: P, addition of contaminants but prior to bioaugmentation or biostimulation; BA, bioaugmentation with culture; BABS, biostimulation by addition of organic donor to bioaugmented columns; 2BABS, re-bioaugmentation and continued biostimulation. VOCs, volatile organic compounds; DOC, dissolved organic carbon; VFA, volatile fatty acids; TeCA, 1,1,2,2-tetrachloroethane; PCE, tetrachloroethene; TCE, trichloroethene; CF, chloroform; CT, carbon tetrachloride; mg/L, milligrams per liter; mM, millimolar]

Event	Date	Column M–B		Column FM–B		Samples	Comments
		Days from VOCs amendment	Number of pore volumes	Days from VOCs amendment	Number of pore volumes		
0	2/2/2004		18			VOCs	Baseline
	2/4/2004	0		0		Methane, iron, acetate, ammonia	**P**: First TeCA amendment to M-B (5 mg/L).
1	2/6/2004	2	0.49			VOCs	
2	2/8/2004	4	0.98			Anions, cations, DOC, alkalinity	
	2/10/2004	6	1.46			VOCs	
3	2/17/2004	13	3.17		3.17	VOCs, methane, iron, acetate, ammonia	Begin TeCA amendment to FM-B (5 mg/L).
4	2/21/2004	17	4.15	4	0.98	VOCs	
5	2/23/2004	19	4.63	6	1.46	Methane, iron, acetate, hydrogen	
	2/25/2004	21	5.12	8	1.95	VOCs	
6	3/1/2004	26	6.34	13	3.17	VOCs	
	3/4/2004	29	7.07	16	3.90	Anions, cations, DOC, alkalinity, methane, iron	**BA**: Bioaugmentation of M-B and FM-B with WBC-1.
7	3/8/2004	33	8.05	20	4.88	VOCs	
	3/10/2004	35	8.54	22	5.37	Methane, iron, hydrogen	
8	3/12/2004	37	9.02	24	5.85	VOCs	
	3/15/2004	40	9.76	27	6.59	Anions, cations, DOC, alkalinity	
	3/17/2004	42	10.24	29	7.07	Methane, iron, hydrogen	
9	3/19/2004	44	10.73	31	7.56	VOCs	
	3/22/2004	47	11.46	34	8.29	Anions, cations, DOC, alkalinity	
	3/24/2004	49	11.95	36	8.78	Methane, iron, VFA, hydrogen	
10	3/26/2004	51	12.44	38	9.27	VOCs	
	3/29/2004	54	13.17	41	10.00	Anions, cations, DOC, alkalinity	
	3/31/2004	56	13.66	43	10.49	Methane, iron, hydrogen	
11	4/2/2004	58	14.15	45	10.98	VOCs	
	4/7/2004	63	15.37	50	12.20	Methane, iron (ferrous), ammonia, sulfide	
12	4/9/2004	65	15.85	52	12.68	VOCs	
	4/14/2004	70	17.07	57	13.90	Methane, iron (total, ferrous), ammonia, sulfide	

Appendix A3. Sampling and operation of column experiments—Organic mix column B (M-B) and iron/mix column B (FM-B).—Continued

[Amendments given in column influent were continued until a change is noted in the comments. Major treatments noted in comments include: P, addition of contaminants but prior to bioaugmentation or biostimulation; BA, bioaugmentation with culture; BABS, biostimulation by addition of organic donor to bioaugmented columns; 2BABS, re-bioaugmentation and continued biostimulation. VOCs, volatile organic compounds; DOC, dissolved organic carbon; VFA, volatile fatty acids; TeCA, 1,1,2,2-tetrachloroethane; PCE, tetrachloroethene; TCE, trichloroethene; CF, chloroform; CT, carbon tetrachloride; mg/L, milligrams per liter; mM, millimolar]

Event	Date	Column M–B		Column FM–B		Samples	Comments
		Days from VOCs amendment	Number of pore volumes	Days from VOCs amendment	Number of pore volumes		
13	4/16/2004	72	17.56	59	14.39	VOCs	
14	4/21/2004	77	18.78	64	15.61	VOCs	
	4/23/2004	79	19.27	66	16.10	Methane, iron (total, ferrous), ammonia, sulfide	
	4/26/2004	82	20.00	69	16.83	Anions, cations, DOC, alkalinity	
15	4/28/2004	84	20.49	71	17.32	Methane, iron (total, ferrous), ammonia, sulfide	VFA sample
	4/30/2004	86	20.98	73	17.80	VOCs	
16	5/4/2004	90	21.95	77	18.78	Methane, ammonia, sulfide	
	5/7/2004	93	22.68	80	19.51	VOCs, iron (total and ferrous)	**BABS**: Begin donor amendment (lactate, 10mM).
17	5/11/2004	97	23.66	84	20.49	Methane, ammonia, sulfide	
	5/14/2004	100	24.39	87	21.22	VOCs, iron (total and ferrous)	Begin CT amendment (1 mg/L) in addition to TeCA (5 mg/L).
18	5/18/2021	104	25.37	91	22.20	Methane, ammonia, sulfide	
	5/21/2004	107	26.10	94	22.93	VOCs, iron (total and ferrous)	**2BABS**: M-B and FM-B re-bioaugmented with WBC-2 on 5/24.
19	5/25/2004	111	27.07	98	23.90	Methane, ammonia, sulfide	
	5/28/2004	114	27.80	101	24.63	VOCs, iron (total and ferrous)	
20	6/1/2004	118	28.78	105	25.61	Methane, ammonia, sulfide	
	6/4/2004	121	29.51	108	26.34	VOCs, iron (total and ferrous)	Lactate reduced to 5 mM.
21	6/8/2004	125	30.49	112	27.32	Methane, ammonia, sulfide	
	6/11/2004	128	31.22	115	28.05	VOCs, iron (total and ferrous)	Begin CF amendment (1 mg/L).
22	6/15/2004	132	32.20	119	29.02	Methane, VOCs	Lactate reduced to 1 mM. CF increased to 5 mg/L.
23	6/21/2004	138	33.66	125	30.49	Methane, VOCs	Begin adding 5 mg/L CF and 5 mg/L CT.
24	6/28/2004	145	35.37	132	32.20	Methane, VOCs	
25	7/7/2004	154	37.56	141	34.39	Methane, VOCs	
26	7/13/2004	160	39.02	147	35.85	Methane, VOCs	Increased both CF and CT to 10 mg/L.

Appendix A3. Sampling and operation of column experiments—Organic mix column B (M-B) and iron/mix column B (FM-B).—Continued

[Amendments given in column influent were continued until a change is noted in the comments. Major treatments noted in comments include: P, addition of contaminants but prior to bioaugmentation or biostimulation; BA, bioaugmentation with culture; BABS, biostimulation by addition of organic donor to bioaugmented columns; 2BABS, re-bioaugmentation and continued biostimulation. VOCs, volatile organic compounds; DOC, dissolved organic carbon; VFA, volatile fatty acids; TeCA, 1,1,2,2-tetrachloroethane; PCE, tetrachloroethene; TCE, trichloroethene; CF, chloroform; CT, carbon tetrachloride; mg/L, milligrams per liter; mM, millimolar]

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		Days from VOCs amendment	Number of pore volumes	Days from VOCs amendment	Number of pore volumes		
27	7/19/2004	166	40.49	153	37.32	Methane, VOCs	
28	7/26/2004	173	42.20	160	39.02	Methane, VOCs	Lactate increased to 2 mM.
29	8/3/2004	181	44.15	168	40.98	Methane, VOCs	Increased CF to16 mg/L (CT still 10 mg/L).
30	8/6/2004	184	44.88	171	41.71	Methane, VOCs	Increased CF to18 mg/L (CT still 10 mg/L).
31	8/9/2004	187	45.61	174	42.44	Methane, VOCs	Decreased CF to 15 mg/L and CT to 5 mg/L.
32	8/13/2004	191	46.59	178	43.41	Methane, VOCs	

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