**The Effects of Co-contaminants and Native Wetland Sediments on the
Activity and Dominant Transformation Mechanisms of a 1,1,2,2-
Tetrachloroethane (TeCA)-Degrading Enrichment Culture**

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ABSTRACT

Bioremediation strategies, including bioaugmentation with chlorinated ethene-degrading enrichment cultures, have been successfully applied in the cleanup of subsurface environments contaminated with tetrachloroethene (PCE) and/or trichloroethene (TCE). However, these compounds are frequently found in the environment as components of mixtures that may also contain chlorinated ethanes and methanes. Under these conditions, the implementation of bioremediation may be complicated by inhibition effects, particularly when multiple dehalorespirers are present. We investigated the ability of the 1,1,2,2-tetrachloroethane (TeCA)-dechlorinating culture WBC-2 to biotransform TeCA alone, or a mixture of TeCA plus PCE and carbon tetrachloride (CT), in microcosms. The microcosms contained electron donors provided to biostimulate the added culture and sediment collected from a wetland where numerous "hotspots" of contamination with chlorinated solvent mixtures exist. The dominant TeCA biodegradation mechanism mediated by the WBC-2 culture in the microcosms was different in the presence of these wetland sediments than in the sediment-free enrichment culture or in previous WBC-2 bioaugmented microcosms and column tests conducted with wetland sediment collected at nearby sites. The co-contaminants and their daughter products also inhibited TeCA biodegradation by WBC-2. These results highlight the need to conduct biodegradability assays at new sites, particularly when multiple contaminants and dehalorespiring populations are present.

KEYWORDS: 1,1,2,2-tetrachloroethane, carbon tetrachloride, tetrachloroethene, contaminated wetlands, *Dehalococcoides*, *Dehalobacter*

1. INTRODUCTION

Improper disposal of chlorinated aliphatic compounds that have been extensively used as degreasing agents, dry cleaning solvents, and precursors in the production of industrial and agricultural chemicals and products has led to widespread contamination of groundwater. Considerable progress has been made in identifying and characterizing microbial populations that can respire the chlorinated ethenes, i.e., tetrachloroethene (PCE), trichloroethene (TCE), and their daughter products. As a result, bioremediation of PCE- and TCE-contaminated groundwater has been successfully implemented at many sites. In situ bioremediation of these sites frequently involves biostimulation to increase the availability of electron donors and/or bioaugmentation with enrichment cultures containing populations that are able to dehalorespire parent compounds and/or chlorinated daughter products to overcome limitations on naturally occurring processes. In particular, bioremediation of chlorinated ethene-contaminated sites focuses on increasing the availability of H_2 and the abundance of *Dehalococcoides mccartyi* strains (Löffler et al., 2013) that utilize H_2 in the dehalorespiration of daughter products 1,2-dichloroethene (*cis* and *trans* isomers, DCE) and vinyl chloride (VC) to prevent these compounds from accumulating.

Mixtures of chlorinated aliphatic compounds, including not just chlorinated ethenes, but also chlorinated ethanes such as 1,1,2,2-tetrachloroethane (TeCA) and chlorinated methanes such as tetrachloromethane (carbon tetrachloride, CT) and trichloromethane (chloroform, CF) occur frequently in aquifers and shallow groundwater resources throughout the U.S. (Moran et al., 2007). In recent years, bacteria that can mediate anaerobic metabolism of chlorinated ethanes and methanes have been identified. For example, dehalorespiration of CF to dichloromethane (DCM) (Grostern et al., 2010; Lee et al., 2012) and fermentation of DCM (Mägli et al., 1998; Justicia-Leon et al., 2012; Lee et al., 2012) by *Dehalobacter* strains have been demonstrated. As

65 shown in Figure 1, anaerobic biodegradation of TeCA can proceed via either dichloroelimination
 66 to DCE, which may be mediated by several dehalorespiring strains, or via hydrogenolysis to

Figure 1. Potential pathways for the anaerobic biodegradation of TeCA, PCE, and CT. Dashed arrows indicate hydrogenolysis. Heavy arrows indicate dichloroelimination reactions.

1,1,2-trichloroethane (TCA), for example by *Dehalobacter* populations (Manchester et al., 2012). However, complete biodegradation and detoxification of TeCA apparently requires consortia containing multiple dehalorespiring populations.

When a mixture of chlorinated aliphatic compounds is present, the implementation of bioremediation may be complicated by inhibition effects. In particular, CF (Edwards and Grbic-Galic, 1994; Griffin et al., 2004) and chlorinated ethenes (Cupples et al., 2004; Yu and Semprini, 2004; Yu et al., 2005; Grostern and Edwards, 2006; Grostern et al., 2009; Lai and Becker, 2013) have been shown to inhibit the dehalorespiration of other chlorinated aliphatic compounds. These interactions may be complex and compounded in mixed cultures containing different dehalorespiring strains (Lai and Becker, 2013). Thus, it is very difficult to predict the outcome of bioaugmentation of field sites when multiple contaminants and dehalorespiring populations are involved on the basis of laboratory studies of enrichment cultures maintained on a single chlorinated substrate. Not only may inhibition effects occur in the field, but the bioaugmented dehalorespiring populations may potentially be impacted by non-biological soil constituents or compete with each other or native populations for growth substrates (including the contaminants themselves) (Becker, 2006; Becker and Seagren, 2009; Lai and Becker, 2013). These ecological interactions may affect the outcome of bioremediation, and may be especially complex when the biodegradation pathways of different contaminants intersect, as is the case for TeCA and PCE (Fig. 1).

In this study, we evaluated the effects of two co-contaminants (CT and PCE) and native wetland sediment obtained from a contaminated site on the activity and dominant TeCA transformation mechanisms in the mixed microbial culture WBC-2. WBC-2 was enriched and maintained on a mixture of TeCA, 1,1,2-trichloroethane (TCA), and *cis*-1,2-

dichloroethene and is able to completely dechlorinate these compounds to ethene (Jones et al., 2006; Manchester et al., 2012). We found that the dominant TeCA biodegradation mechanism mediated by the bioaugmented WBC-2 culture was different in the presence of these wetland sediments, than in the sediment-free enrichment culture or in previous bioaugmented microcosms and column tests conducted with wetland sediment from nearby sites (Lorah et al., 2008). Co-contaminants also significantly impacted TeCA biodegradation by WBC-2 in these sediment microcosms, although not in previous tests (Lorah et al., 2008). The inhibition effects of the contaminants and their daughter products on the interactions and activities of the dehalorespiring populations in WBC-2 were complex and highlight the need to conduct biodegradability assays at new sites, particularly when multiple contaminants and dehalorespiring populations are present.

2. MATERIALS AND METHODS

2.1. Enrichment culture

The WBC-2 enrichment culture was derived from wetland sediment and groundwater collected in the West Branch Canal Creek area, which is located in a freshwater tidal wetland in the Edgewood Peninsula of Aberdeen Proving Ground, Maryland (Jones et al., 2006). The WBC-2 culture was selected because of its ability to degrade TeCA and key daughter products (TCA and *cis*-1,2-dichloroethene) at a high rate, and it was maintained as previously described (Jones et al., 2006). Analysis of clone libraries generated for the WBC-2 culture and targeted qPCR analyses have revealed several sequences related to known dehalorespiring genera including *Dehalobacter*, *Dehalococcoides*, and *Dehalogenimonas* (Jones et al., 2006; Lorah et al., 2008; Manchester et al., 2012). The WBC-2 culture was incubated statically and in the dark at 19°C.

2.2. Microcosm preparation

Microcosms were prepared in an anaerobic glove box (85% N₂/10% CO₂/5% H₂; Coy Laboratories, Grass Lake, MI) using groundwater and sediment collected as described in the Supplementary Information. Groundwater was purged with O₂-free N₂ for an hour before being used to prepare a slurry consisting of groundwater and sediment combined in a 1.5:1 (v/v) ratio. The slurry was manually sieved (No. 4 sieve, 4.76-mm) and dispensed in 45-mL aliquots to 72-mL serum bottles (Wheaton). The average bulk density of the sediment at the surface of seep locations in the West Branch Canal Creek region was 0.35 g/mL (Majcher et al., 2006). Thus, each microcosm contained approximately 0.0432 L water (V_w), 0.027 L headspace (V_g), and 6.3 g (dry weight) sediment (M_X). The bottles were sealed with grey Teflon septa (Fisher) and aluminum crimp caps. The serum bottles containing slurry were flushed with 80% N₂/20% CO₂ after being removed from the anaerobic glove box to remove H₂, which could serve as an electron donor. The WBC-2 culture was obtained from Elizabeth J. P. Jones (United States Geological Survey) and was derived from sediment collected from the West Branch Canal Creek region and enriched on a mixture of TeCA, TCA, and *cis*-1,2-dichloroethene using lactate as the electron donor under anaerobic conditions, as described by Jones et al. (2006).

A total of six pairs of viable microcosms were prepared as summarized in Table 1. Three pairs of microcosms were amended with 5 mg/L (30 µM) TeCA as the sole contaminant. To evaluate the effect of biostimulation on TeCA natural attenuation, one pair was also amended with 5 mM ethanol and 5 mM lactate, which had previously been shown to be effective electron donors for the WBC-2 culture (Jones et al., 2006) and in wetland microcosms constructed with sediment obtained at nearby wetland sites (Lorah et al., 2008). The electron donor concentrations were selected based on previous studies with the WBC-2 culture (Jones et al., 2006) and to

ensure that transformation of the chlorinated organic compounds would not be electron donor-limited. The usefulness of biostimulation plus bioaugmentation at enhancing natural attenuation was evaluated by aseptically adding 2.7 mL (~10% of the groundwater volume) of the WBC-2 culture plus 5 mM ethanol and 5 mM lactate to the third pair of TeCA-amended microcosms. The effectiveness of bioaugmentation as a stand-alone engineered bioremediation approach was not evaluated because previous studies showed that the WBC-2 culture was not effective in similar seep sediments at the site without exogenous electron donors (Lorah et al., 2008). The septa on these microcosms were quickly changed in the anaerobic glove box on day 24, but otherwise the microcosms remained sealed for the duration of the experiment.

Table 1. Design of duplicate microcosm and control treatments.

Treatment Name ¹	Constituents
TIB	TeCA + Sediment + Groundwater
TBS	TeCA + Sediment + Electron Donors + Groundwater
TBA	TeCA + Sediment + Electron Donors + WBC-2 + Groundwater
MIB	TeCA, PCE, and CT + Sediment + Groundwater
MBS	TeCA, PCE, and CT + Sediment + Electron Donors + Groundwater
MBA	TeCA, PCE, and CT + Sediment + WBC-2 + Electron Donors + Groundwater
MAC	TeCA, PCE, and CT + Sediment + Groundwater + Formaldehyde and Autoclaving Regimen
MWC	TeCA, PCE, and CT + Water

¹Naming convention: T = TeCA added as sole contaminant, M= multiple contaminants (TeCA, PCE, and CT) added, IB=intrinsic bioremediation (no electron donors or enrichment culture added), BS=biostimulation (lactate and ethanol added as electron donors), BA=bioaugmentation (WBC-2 enrichment culture added), AC = autoclaved control, and WC=water control.

To evaluate the effect of the co-contaminants PCE and CT, which were observed in the surface water at the sediment collection site (3-1E), on natural attenuation of TeCA and the effectiveness of biostimulation or bioaugmentation plus biostimulation, three pairs of

microcosms were amended with a mixture of TeCA, PCE, and CT. One pair (MIB) of mixed volatile organic compound (VOC) microcosms received no other amendments to simulate an intrinsic bioremediation (IB) scenario. A second pair (MBS) of mixed VOC microcosms was amended with 5 mM ethanol and 5 mM lactate to evaluate the effectiveness of biostimulation alone. The other pair of mixed VOC microcosms (MBA) received 5 mM of each electron donor plus 2.7 mL of the WBC-2 culture to assess the effects of biostimulation plus bioaugmentation.

Based on VOC concentrations detected at seep locations, the target aqueous concentrations of VOCs in the microcosms were 5 mg/L TeCA (30 μ M), 3 mg/L PCE (18 μ M), and 4 mg/L CT (26 μ M). VOC abundance in the microcosms is reported in terms of total mass (M_T) [M] in this study due to its partitioning into the gas, aqueous, and sediment phases, according to:

$$M_T = C_W (H_C V_g + V_w + K_d M_X) \quad (1)$$

where C_w is the aqueous VOC concentration [M L^{-3}], H_C is the dimensionless Henry's constant, K_d is the soil-water partition coefficient [$\text{L}^3 \text{M}^{-1}$], and M_X [M] is the dry mass of sediment. The detection limits of the individual chlorinated VOCs were ≤ 50 nmol/microcosm. The H_C and K_d values used in this study are reported in Table S-1 (Supplementary Information).

Microcosms and controls were incubated statically and in the dark at 19°C. The addition of VOCs and electron donors to the microcosms, preparation of controls, and analytical methods are described in the Supplementary Information.

3. RESULTS and DISCUSSION

3.1. TeCA as a single contaminant

Transformation of 75 to 100% of the added TeCA to potential daughter products (Fig. 1) was observed in all of these microcosms except in TIB1. In comparison, no TeCA daughter products were detected in the autoclaved sediment controls, and TeCA persisted in the autoclaved sediment and water controls for 80 days (Supplementary Information). Less than 14% of the TeCA in TIB1 was converted to TCA, DCE, VC, ethene (ETH), and ethane (ETA), and TeCA was still detectable at 91 d. Therefore, the data obtained from the TIB1 microcosm are not shown or discussed further.

The un-amended or intrinsic bioremediation TIB2 microcosm and biostimulated microcosms (TBS1 and TBS2) that were amended with TeCA as the sole contaminant performed similarly. The TIB2 data are shown as a representative data set in Figure 2. The TBS microcosm data followed similar trends and are provided in the Supplementary Information. TeCA was detectable in these microcosms for 35 to 78 d and initially appeared to undergo biotransformation solely via hydrogenolysis to TCA (as shown for TIB2 in Fig. 2A). While minor amounts of ethane accumulated in these microcosms and could result from successive hydrogenolysis of TCA (Fig. 1), 1,2-dichloroethane (DCA) and chloroethane (CA) were never detected in any of the microcosms amended with TeCA as the sole contaminant, and TCA was apparently transformed primarily via dichloroelimination to VC (as shown for TIB2 in Fig. 2B). In the TBS microcosms, the average amount of TeCA degraded via hydrogenolysis (approximated as the total mol of TCA plus VC) ranged from approximately 8 to 23% through day 35, while up to 46% of the TeCA underwent hydrogenolysis in TIB2 (see Supplemental Information). Methane levels were generally stable (varied by ≤ 2 μmol compared to the level measured on day 0 in TIB2 or day 6 in the TBS microcosms) until TeCA and TCA were

completely degraded (as shown for TIB2 in Fig. 2C), suggesting that these compounds inhibited methanogenic populations or that reduction of these compounds diverted electron equivalents away from methanogenesis.

Between days 30 and 56, the dominant biodegradation pathway in the TIB2 and TBS microcosms shifted to dichloroelimination of TeCA to DCE (as shown for TIB2 in Fig. 2B). The average amount of DCE measured on day 83 accounted for approximately 62% of the TeCA initially added to the TBS microcosms (see Supplemental Information). In a previous study, hydrogenolysis of TeCA to TCA in groundwater microcosms appeared to be dependent on the availability of acetate, whereas dichloroelimination of TeCA to DCE occurred independently of acetate (Aulenta et al., 2006). In the current study, the shift from TeCA hydrogenolysis to dichloroelimination occurred earliest in the TIB2 microcosm. Acetate was presumably produced from the fermentation of lactate added to the TBS (and TBA) microcosms, and the increased availability of acetate in the TBS microcosms may explain why the transition to TeCA dichloroelimination was somewhat delayed in these microcosms relative to the TIB2 microcosm.

The VC concentration increased concomitantly with the level of DCE in TIB2 (Fig. 2B) and TBS1 (Supplemental Information). VC can be produced via the dichloroelimination of TCA or hydrogenolysis of DCE (Fig. 1). However, the amounts of TCA and VC that accumulated in TIB2 and the TBS microcosms were similar, and VC levels did not increase when decreases in DCE were observed. Both of these observations suggest that VC was primarily produced via dichloroelimination of TCA. Substantial amounts ($\geq 0.01 \mu\text{mol}$) of ethene also were never detected in TIB2 or the TBS microcosms, suggesting that strains that can dehalorespire DCE or

Figure 2. Biodegradation of TeCA in TIB2: (A) TeCA, TCA, and ethane; (B) DCE, VC, and ethene; and (C) methane and hydrogen.

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224 VC were absent or present in very low numbers in the site 3-1E sediment.

225 The addition of the WBC-2 culture to the bioaugmentation (TBA) microcosms

226 substantially increased both the rate and extent of TeCA biotransformation in the wetland

227 sediment (Fig. 3). TeCA was degraded to below the detection limit within 17 d (Fig. 3A). The

228 WBC-2 culture is capable of degrading TeCA and lesser chlorinated ethanes via both the
229 hydrogenolysis and dichloroelimination pathways; however, dilutions of the culture that were fed
230 TeCA produced substantially more *trans*-1,2-DCE than TCA (Jones et al., 2006). Therefore, it
231 was somewhat surprising that the TeCA added to the bioaugmented microcosms appeared to
232 exclusively undergo hydrogenolysis to TCA. The VC produced from dichloroelimination of
233 TCA in the TBA microcosms was rapidly depleted (Fig. 3B). The decrease in VC concentrations
234 between days 24 and 39 coincided with increasing ethene levels, which suggests that
235 bioaugmentation with the WBC-2 culture provided a critical number of VC-respiring
236 *Dehalococcoides* cells that were deficient in site 3-1E sediment.
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Figure 3. Biodegradation of TeCA in the TBA microcosms: (A) TeCA, TCA, and ethane; (B) DCE, VC, and ethene; and (C) methane and hydrogen. Data points represent the average concentration in duplicate microcosms. Error bars represent ± 1 standard deviation.

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It can be difficult to assign specific functions to individual populations within mixed cultures carrying out biodegradation. This is particularly true when more than one contaminant

transformation pathway may be active and the pathways share intermediates, as in the anaerobic biodegradation of TeCA (Fig. 1). Nevertheless, it is interesting to note that the shift from hydrogenolysis to dihaloelimination in the TIB2 (Fig. 2) and TBS microcosms coincided with the accumulation of approximately 0.4 μmol VC. This suggests that VC was inhibitory to the population carrying out hydrogenolysis of TeCA, and thus the accumulation of ≥ 0.4 μmol VC caused the shift to a dihaloelimination biodegradation mechanism. Interestingly, several studies have shown inhibition of *Dehalobacter* strains by VC (Grostern and Edwards, 2006; Grostern et al., 2009; Lai and Becker, 2013), and hydrogenolysis of TeCA to TCA has been attributed to a *Dehalobacter* strain (Manchester et al., 2012). In contrast, VC levels spiked above the 0.4 μmol level only briefly and after TeCA had been completely degraded in the TBA microcosms (Fig. 3B). Presumably, by keeping VC levels generally low in the TBA microcosms, the *Dehalococcoides* population in WBC-2 prevented inhibition of the population carrying out hydrogenolysis of TeCA.

H_2 concentrations in the TBA microcosms typically ranged between approximately 10 and 25 nM and did not fall below 5 nM (Fig. 3C). Similar H_2 levels were observed in the other pairs of TeCA-amended microcosms, and H_2 concentrations did not appear to be influenced by the biodegradation of specific chlorinated compounds in any of the microcosms. While H_2 thresholds associated with various terminal electron-accepting processes are not constant and can vary from site to site depending on environmental factors and microbial kinetic characteristics, the levels measured in this study are more characteristic of methanogenic and sulfate-reducing systems (Löffler and Sanford, 2005), whereas H_2 concentrations in electron donor-limited dehalorespiring systems are typically less than 1 nM (Löffler and Sanford, 2005). Thus, the H_2 data suggest that the dehalorespiring populations were not limited by electron donor availability.

The H₂ data also provide further evidence that the rapid degradation of TeCA and VC in the TBA microcosms is attributable, at least in part, to the bioaugmentation of critical dechlorinating populations, rather than to the addition of electron donors alone.

Little methane was produced during the period of active dechlorination of TeCA and its daughter products (days 0—39; Fig. 3C). After the dechlorination phase, methane levels increased substantially in the TBA microcosms, suggesting that TeCA and/or its daughter products inhibited or outcompeted methanogens for reducing equivalents. The absence of methanogenesis while chlorinated ethanes and ethenes were present in all TeCA-amended microcosms is in contrast with the WBC-2 culture, in which methanogenesis and dechlorination occurred concomitantly.

The microcosm results clearly demonstrate that bioaugmentation with the WBC-2 culture can be used to enhance the intrinsic biodegradation of TeCA. However, individual populations, including the organisms that carried out dihaloelimination of TeCA to DCE and methanogens, appeared to be much less active in the TBA microcosms than in the WBC-2 enrichment culture by itself. Thus, the behavior of complex cultures and their effects on contaminant fate in situ may not be entirely predictable based on their activity in laboratory enrichments in the absence of native soils/sediments and, perhaps, native microbial populations.

3.2. TeCA with Co-contaminants.

The results obtained with the microcosms amended with a mixture of TeCA, PCE, and CT showed that the presence of other contaminants can also affect the behavior of individual populations within an enrichment culture. Most importantly, while bioaugmentation with WBC-2 was clearly required to achieve complete dechlorination in the TeCA-amended microcosms, the

287 presence of the co-contaminants (or their daughter products) delayed the biodegradation of
288 TeCA and influenced the dominant TeCA transformation pathway.

289 In particular, CT had a major impact on the biotransformation processes that occurred in
290 the microcosms. CT was rapidly transformed in all of the microcosms (Fig. 4A). The products of
291 CT transformation apparently were primarily non-chlorinated compounds because only a minor
292 amount of CT was converted via hydrogenolysis to CF in the microcosms (Fig. 4B). However,
293 electron donor addition clearly accelerated the overall rate of CT removal and promoted
294 accumulation of CF and DCM in the MBS and MBA microcosms (Figs. 4B and 4C). CT was not
295 detectable in these microcosms by day 6. CT was also transformed to non-chlorinated
296 compounds and minor amounts of CF (< 150 nmol) and DCM (<450 nmol) in the autoclaved
297 sediment controls (Supplementary Information). However, the rate of CT transformation was
298 much slower in the autoclaved sediment controls than in the microcosms and complete removal
299 of CT was not observed in the controls until day 30. This indicates that viable microorganisms
300 contributed to the rapid transformation of CT in the microcosms. These results are consistent
301 with the results of an earlier study involving microcosms constructed with wetland sediment
302 collected from non-seep areas, which showed that rate constants for CT degradation were about
303 twice as high in live microcosms compared to sterile controls (Lorah et al., 2008).

304 Hydrogenolysis of CF to DCM under anaerobic conditions can occur via cometabolic
305 processes, as summarized by Cappelletti et al. (2012), or via dehalorespiration mediated by
306 *Dehalobacter* populations (Grostern et al., 2010; Lee et al., 2012). The small amount of CF that
307 accumulated in the MIB microcosms most likely underwent cometabolic transformation, and the
308 lack of DCM production in these microcosms suggests oxidative transformation of CF to CO₂
309 may have occurred (Becker and Freedman, 1994). The addition of electron donors apparently

Figure 4. Summary of biotransformation results for the MIB, MBS, and MBA microcosms amended with a mixture of CT, PCE, and TeCA: (A), CT; (B), CF; (C) DCM; (D) PCE; (E) DCE; and (F) TeCA.

stimulated reduction dechlorination of CF to DCM in the MBS microcosms, as noted above (Figs. 4B and 4C). However, the DCM was not transformed further and significant amounts of

CF persisted in these microcosms for over 80 days. Thus, hydrogenolysis of CF to DCM also presumably occurred via a cometabolic process in the MBS microcosms. In contrast, CF was completely removed by day 32 in MBA2. CF biodegradation in MBA2 proceeded exclusively via hydrogenolysis to DCM and presumably occurred via dehalorespiration carried out by the *Dehalobacter* population in WBC-2. Whereas DCM was not degraded in the MBS microcosms, by day 60, the DCM level began decreasing steadily in the MBA2. Chloromethane (CM) was not detected in MBA2 (or any other microcosms), suggesting that DCM may have been fermented, as observed in several previous studies (Mägli et al., 1998; Justicia-Leon et al., 2012; Lee et al., 2012). Similar amounts of CF were produced in the MBA1 and MBA2 microcosms. However, only a portion of the CF in MBA1 was transformed to DCM, which persisted. In this respect, the performance of microcosm MBA1 was more similar to that of the MBS microcosms than to the MBA2 microcosm. It is not uncommon for replicate environmental microcosms to exhibit variable behavior (Nielsen and Christensen, 1994), especially when they contain material as heterogeneous as the wetland sediment used in the current study. In any case, the results suggest the CF-respiring *Dehalobacter* population in the WBC-2 culture was not active in MBA1.

High levels of PCE persisted in the MIB microcosms (Fig. 4D). Thus, the gradual accumulation of DCE that began in these bottles after day 48 (Fig. 4E) was likely produced through dihaloelimination of TeCA. Complete biodegradation of PCE was observed by day 64 in MBS2, whereas PCE persisted in MBS1 throughout the incubation period. These results suggest that native PCE-respiring bacteria were present in the seep site sediments, but may have been limited by electron donor availability. In fact, several PCE-respiring bacteria have been detected in West Branch Canal Creek sediments, including *D. mccartyi* 195, *Desulfuromonas michiganensis* BB1, and *Desulfuromonas chloroethenica* (Lorah et al., 2003). However, the

distribution of these populations exhibited a high degree of spatial variability, consistent with the results obtained with the MBS microcosms. The abundance of *Desulfuromonas* in the in situ wetland sediments ranged from non-detect to 10^4 cells/g, and the abundance of *Dehalococcoides* ranged from non-detect to 10^6 cells/g (Lorah et al., 2003).

In the current study, biotransformation of PCE was first observed in MBA2, in which PCE was completely removed by day 32. The onset of PCE degradation in MBA2 coincided with the onset of CF removal (and accumulation of DCE). In MBA1, biotransformation of PCE to DCE occurred later than in MBA2 and was completed by day 54. It is likely that the respiration and removal of CF by a *Dehalobacter* population in the added WBC-2 culture alleviated inhibition of the PCE-respiring population in MBA2. In contrast, the persistence of CF delayed the onset of PCE biotransformation in MBA1 and MBS2. Previous studies have also shown that CF inhibits PCE- and TCE-respiring populations (Bagley et al., 2000; Maymó-Gatell et al., 2001).

While any inhibition of PCE-respiration appeared to be temporary, the persistence of DCE in the MBA microcosms suggests that CF permanently inhibited, and/or was toxic to, the DCE-respiring population(s) in the WBC-2 culture (*Dehalococcoides* and/or *Dehalogenimonas*). Likewise, Duhamel et al. (2002) observed an accumulation of *cis*-1,2-dichloroethene and VC in chlorinated ethene-respiring cultures exposed to CF. Similar, but less extensive, inhibition of DCE degradation was observed in WBC-2-bioaugmented column experiments containing a mixture of commercial wetland sediment and compost (Lorah et al., 2008). The columns were initially supplied with TeCA and later PCE, TCE, CT and CF were added to the influent as co-contaminants. Degradation of the parent compounds when supplied as a mixture was not inhibited in the WBC-2 bioaugmented columns, compared to the degradation of these

compounds when supplied to the column as the sole contaminant. However, addition of high CF concentrations did slow DCE and VC degradation and caused greater accumulation of these daughter products within the column than was observed in the absence of CF or CT. Despite these inhibition effects, DCE and VC were completely degraded upstream of the column outlet (Lorah et al., 2008). There are several important differences in the experimental conditions in the microcosms used in the current study versus the flow-through columns. First, the seep sediments used in the current study were collected from a different location compared to the sediments used in the column study, and thus there were undoubtedly variations in the structures and functions of the native sediment microbial communities in the two studies. Second, in a column study, different microbial activities tend to be spatially separated. In the column studies of Lorah et al. (2008), it is possible that CF was transformed near the column inlet, thereby allowing PCE and DCE transformation to proceed downstream. Differences in microbial communities, the potential for spatial segregation of key populations and biotransformations in columns, or a combination of both factors, could explain why the toxicity/inhibition effects observed in the microcosm study reported here are more pronounced than in other studies with the WBC-2 culture (Lorah et al., 2008; Majcher et al., 2009). Nevertheless, batch microcosm assays like the ones conducted with contaminant mixtures in this study may reveal the potential for inhibition effects that could be important during remediation of field sites.

TeCA persisted at high levels in the MIB microcosms throughout the incubation period (Fig. 4F). TeCA was also detectable throughout this time period in the MBS and MBA1 microcosms. Traces of TCA were detected in all of the microcosms after day 10; however, TCA levels remained below 0.1 μmol in the MIB microcosms and were even lower in the MBS and MBA microcosms (data not shown). The levels of VC, which is formed via dihaloelimination of

TCA, were below 0.2 μmol in all microcosms (data not shown). This confirms that transformation of TeCA via hydrogenolysis to TCA did not occur to a significant extent in any of the microcosms. These results contrast with the findings obtained with the microcosms amended with TeCA alone. Biotransformation of TeCA initially occurred via hydrogenolysis in the TIB2 and TBS microcosms, and hydrogenolysis appeared to be the sole TeCA biodegradation mechanism active in the TBA microcosms, as noted above. Inhibition of TeCA-degrading populations by VC was presumably not a factor in the microcosms amended with the contaminant mixture. However, unlike in other microcosm and column experiments with WBC-2 (Lorah et al., 2008), CF appears to have significantly affected TeCA-biodegradation, just as it impacted PCE and DCE biotransformation, because CF and TeCA persisted for approximately 50 days or more in all of the microcosms except MBA2. Complete biodegradation of TeCA was observed by day 43, approximately 10 days after CF was depleted, in MBA2. Thus, it appears that CF caused reversible inhibition of the TeCA dihaloelimination pathway. The hydrogenolysis of TeCA to TCA may have been permanently inhibited by CF, because this process was not activated in the MBA2 microcosm after CF was depleted, despite the fact that bioaugmentation with WBC-2 promoted hydrogenolysis of TeCA to TCA in the TBA microcosms. As noted above, both hydrogenolysis of CF and TeCA are likely carried out by *Dehalobacter* in the WBC-2 enrichment culture. Interestingly, a *Dehalobacter* reductive dehalogenase has been shown to catalyze hydrogenolysis of both CF and 1,1,1-trichloroethane (Tang and Edwards, 2013).

3.3. Implications for Bioremediation Practice

The results of this study have important implications for bioremediation practice. They demonstrate that bioaugmentation of contaminated sediments or subsurface environments with enrichment cultures whose activities are well-characterized under ideal laboratory conditions

may not yield the expected results due to the effects of native sediment/soils or co-contaminants on key populations. In the current study, the WBC-2 consortium, which is known to biodegrade TeCA and TCE and their daughter products to ethene (Jones et al., 2006; Lorah et al., 2008), was able to convert all of the added CF to DCM. However, CF and/or DCM appeared to inhibit the biodegradation of DCE by the WBC-2 culture, which contains DCE-respiring *Dehalococcoides* and *Dehalogenimonas* populations (Manchester et al., 2012). DCE is an intermediate in the biodegradation pathways of both TeCA and PCE (Figure 1). Thus, it may be more challenging to achieve complete detoxification of TeCA, PCE, and TCE if CT or CF is present as a co-contaminant. If biostimulation is implemented along with bioaugmentation, the problem may be exacerbated because the addition of electron donors increased the amount of CT that was abiotically dechlorinated to CF.

One possible strategy for bioremediation of sites contaminated with mixtures of chlorinated methanes, ethanes, and ethenes would be addition of vitamin B₁₂ to mediate biotransformation of CT and CF (Becker and Freedman, 1994; Hashsham et al., 1995; Guerrero-Barajas and Field, 2005) without the accumulation of lesser chlorinated methanes that could potentially inhibit biodegradation of chlorinated ethanes or ethenes. Addition of vitamin B₁₂ has been successfully implemented in field-scale bioremediation studies, e.g., (Sorel et al., 2001). Following treatment with vitamin B₁₂, bioaugmentation with a culture such as WBC-2 that can detoxify chlorinated ethanes and ethenes could be implemented in conjunction with biostimulation for sites that are electron donor-limited.

Even in the absence of co-contaminants, the activity of enrichment cultures may be affected by soils or sediments at a given site. For example, based on laboratory studies conducted with the WBC-2 enrichment culture in the absence of soil or sediment, dihaloelimination was

expected to be the predominant TeCA transformation mechanism (Jones et al., 2006; Manchester et al., 2012). Dichloroelimination also was the predominant TeCA transformation mechanisms in WBC-2 bioaugmented microcosm and column experiments with wetland and seep sediment collected close to the site used in the experiments reported here, as well as in bioaugmentation experiments with mixtures of commercial wetland sediment and compost (Lorah et al., 2008). When WBC-2 was added to different wetland sediments in the current study, conversion of TeCA occurred rapidly but solely via hydrogenolysis to TCA. Methanogenesis was also inhibited by TeCA in the bioaugmented microcosms, despite the concomitant biodegradation of TeCA and production of methane observed in the sediment-free WBC-2 culture in the laboratory. These results highlight the importance of testing the feasibility of bioaugmentation using soil or sediment from a given site so that the dominant biodegradation pathways and metabolic processes can be predicted.

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