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Bacterial Dehalogenation of Mixtures of 1,2-Dichloroethane, 1,2-Dichloropropane, and 1,1,2-Trichloroethane by Dehalogenimonas

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BACTERIAL DEHALOGENATION OF MIXTURES OF 1,2-DICHLOROETHANE, 1,2-DICHLOROPROPANE, and 1,1,2-TRICHLOROETHANE BY DEHALOGENIMONAS

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ABSTRACT

The goal of the research presented here was to assess the ability of two bacterial strains, *Dehalogenimonas lykanthroporepellens* BL-DC-9^T and *Dehalogenimonas* strain IP3-3, to reductively dehalogenate four contaminant mixtures comprised of combinations of the chlorinated solvents 1,2-dichloroethane (1,2-DCA), 1,2-dichloropropane (1,2-DCP), and 1,1,2-trichloroethane (1,1,2-TCA). Both of these bacteria were first isolated from contaminated groundwater at the PetroProcessors of Louisiana, Inc. Superfund site located near Baton Rouge, LA and are of interest because of their novel abilities to transform halogenated alkanes.

In previous work (Yan *et al*., 2009, Bowman *et al*., 2012), it was demonstrated that both strain IP3-3 and *D. lykanthroporepellens* BL-DC-9^T were able reductively dehalogenate 1,2-dichloroethane, 1,2-dichloropropane, and 1,1,2-trichlorethane when present as individual contaminants. The effects of these compounds as co-contaminant mixtures have not been thoroughly examined until now. Whether transformation of multiple contaminants occurs concurrently (i.e., both compounds transformed simultaneously) or sequentially (i.e., one compound transformed before the other) has important implications when assessing fate and transport of these contaminants in the environment.

The experimental protocol involved inoculation of the strains into anaerobic media that had previously been amended with all three possible binary (i.e., two-component) mixtures as well as a three-component mixture. Duplicate batch serum bottles were repeatedly sampled at multiple time steps to assess contaminant transformation over time. Chlorinated solvent depletion and daughter product formation was determined by analyzing both the aqueous and gas headspace concentrations via gas chromatography.

Preferential dechlorination of 1,1,2-TCA over both 1,2-DCA and 1,2-DCP was observed for both strains. In the combination of 1,2-DCP and 1,1,2-TCA, 1,2-DCP was not converted to propene until the 1,1,2-TCA aqueous concentration was relatively low (i.e., in the tenths of a mM range). Similarly, when 1,2-DCA and 1,1,2-TCA were present as co-contaminants, 1,2-DCA was not converted to ethene until the aqueous 1,1,2-TCA concentration were lower (i.e., <0.1 mM). When the strains were exposed to a combination of 1,2-DCA and 1,2-DCP, both compounds were utilized concurrently over a comparably large rang

CHAPTER 1: INTRODUCTION AND LITERATURE REVIEW

This chapter contains a literature review regarding previous studies on reductive dehalogenation of chlorinated hydrocarbons mixtures of by various microbial communities (Section 1.1) followed by an outline of the thesis (Section 1.2).

1.1 Literature Review

1.1.1 Prevalence of Chlorinated Solvents

Chlorinated alkanes are widely produced chemical solvents that are used in a number of industrial applications (De Wilderman and Verstraete, 2003). These compounds are also common environmental pollutants found at a large number of superfund sites and other contaminated sites throughout the United States (National Priorities List). This is the result of large scale production of these compounds, combined with historically unregulated management practices of waste sites (De Wilderman and Verstraete, 2003; EPA, 2000). At least half of the sites listed on the National Priorities List (NPL) contain chlorinated alkanes as constituents present in co-contaminant mixtures (Westrick *et al*., 1984). Currently there are at least 329 sites contain dichloroethane and trichloroethane as co-contaminants. At least 87 sites contain dichloropropane and dichloroethane as co-contaminants, and at least 71 sites contain dichloropropane and trichloroethane as co-contaminants (EPA, 2012). Furthermore, there are at least 67 sites listed on the NPL contaminated by mixtures that include dichloroethane, dichloropropane, and trichloroethane as co-contaminants (isomers not specified) (EPA, 2012).

Anaerobic reductive dehalogenation, a process in which microorganisms utilize halogenated organics as electron acceptors, is thermodynamically favorable under anoxic or reducing conditions (De Wilderman and Verstraete, 2003). For undefined mixed cultures, a relatively limited number of anaerobic reductive dehalogenation studies involving chlorinated

solvent mixtures have been reported previously (Adamson and Parkin, 1999; Adamson and Parkin, 2000; Aulenta *et al*., 2005; Grostern and Edwards, 2006; Jones *et al*., 2006). Due to the fact that virtually all previous studies have employed different microbial consortia that were acclimated to different environmental conditions and tested different multi-contaminant mixtures, drawing generalizations about the results is complicated or impossible. Nevertheless, the following subsection summarizes previous studies as a means of setting the stage for the research described in this thesis.

1.1.2 Microbial Solvent Mixture Degradation

Adamson and Parkin (1999, 2000) tested carbon tetrachloride (CT), 1,1,1-trichloroethane (1,1,1-TCA), and perchloroethene (PCE) mixtures using two anaerobic mixed cultures. The first mixed culture originated from three anaerobic biofilters that had previously been fed chlorinated aliphatics (Hughes and Parkin, 1996; Adamson and Parkin, 1999). The stock inoculum culture was maintained in a batch reactor provided only with acetate as a growth substrate. There was no exposure to the chlorinated compounds prior to experimentation. When the culture was exposed to a mixture of CT and 1,1,1-TCA only, both compounds decreased the transformation rates of one another (when compared to the transformation rates of the compounds when present alone). The presence of PCE as a co-contaminant had no effect of on the transformation rates of 1,1,1-TCA or CT. When all three compounds were introduced, however, the transformation rates of 1,1,1-TCA and CT decreased, while the PCE transformation rate remained the same as when present alone. Though the rates were affected for 1,1,1-TCA and CT, all three compounds were transformed simultaneously, with no evidence of diauxic-type (i.e., sequential) utilization. No information regarding the microbial community structure was reported.

Secondly, Adamson and Parkin (2000) tested an anaerobic PCE-acclimated enrichment culture. This culture was originally seeded with anaerobic digester sludge that had no known prior exposure to chlorinated aliphatic hydrocarbons. The culture was maintained in a batch reactor that was provided with lactate and hydrogen as electron donors, and was fed PCE for over a year prior to mixture experiments. Similar to what was observed by Adamson and Parkin (1999), simultaneous addition of CT and 1,1,1-TCA inhibited the transformation rates of one another (compared to the transformation rates of the compounds present alone). The PCE transformation rate was mostly unaffected by the presence of CT or 1,1,1-TCA. Also, transformation of all compounds occurred simultaneously, without any indication of diauxic effects. The makeup of the microbial community was also not investigated. For both Adamson and Parkin (1999, 2000) studies, two completely independently collected anaerobic mixed cultures, with differing enrichment conditions, behaved in a similar manner when exposed to the same mixture.

More recently, Aulenta *et al*. (2005) experimented with degrading a mixture of 1,1,2,2-tetrachloroethane (1,1,2,2-TeCA) and PCE on two independently collected anaerobic mixed cultures. The first mixed culture, referred to as 'sediment enrichment culture', was seeded with contaminated anoxic sediment from a brackish industrial canal in the northeast of Italy. The culture was enriched by addition PCE and H_2 gas via the headspace (Aluenta *et al*., 2002). It was previously shown that the sediment enrichment culture contained *Dehalococcoides* (Aluenta 2004). *Dehalococcoides* is a genus that was formally described only recently (Loffler *et al*., 2012), but the name of which has been used extensively for quite some time. (Maymo-Gatell *et al.*, 1997). When the culture was exposed to a mixture of 1,1,2,2-TeCA and PCE, the sediment enrichment culture did not degrade 1,1,2,2-TeCA. Furthermore, 1,1,2,2-TeCA adversely affected

the overall transformation rate of PCE, more specifically the conversion of vinyl chloride (VC) to ethene.

The second culture reported by Aulenta *et al* (2005), designated 'soil enrichment culture', was obtained from a microcosm constructed of aquifer material and groundwater from a 1,1,2,2-TeCA and TCE contaminated site in northern Italy (Aulenta *et al*., 2005). The microcosm previously demonstrated the ability to dechlorinate both 1,1,2,2-TeCA and TCE to ethene. No microbial community analysis was reported for the soil enrichment culture. When the soil enrichment culture was exposed to a mixture of PCE and 1,1,2,2-TeCA, both compounds were transformed to ethene concurrently and at the same rate as when PCE or 1,1,2,2-TeCA was present alone. In contrast to what Adamson and Parkin (1999, 2000) observed, Aluenta *et al*.'s two independently collected, mixed microbial communities responded in different ways when exposed to the same mixture (i.e., PCE and 1,1,2,2-TeCA).

Grostern and Edwards (2006) attempted to degrade a mixture of 1,1,1-TCA and trichloroethene (TCE) using two anaerobic mixed cultures referred to as 'MS' and 'KB-1'. MS was cultured from anoxic groundwater and solids from an industrial area contaminated with 1,1,1-TCA and TCE. It was shown that MS contained organisms most closely related to *Dehalobacter* species. Prior to exposure to contaminant mixtures, the MS culture was incapable of degrading TCE. Initially, the MS culture was tested on 1,1,1-TCA alone, where it degraded 1,1,1-TCA to dichloroethane (1,1-DCA) first, then to chloroethane (CA). When exposed to a mixture of 1,1,1-TCA and TCE, the MS culture was able to degrade 1,1,1-TCA to 1,1-DCA, with no further degradation to CA occurring in the presence of TCE.

KB-1 is an anaerobic mixed culture that was previously maintained on TCE and methanol and is known to include *Dehalococcoides* (Duhamel *et al*., 2004). KB-1 previously

demonstrated the ability to degrade TCE to ethene when TCE was supplied as a single contaminant. Furthermore, it was demonstrated that KB-1 did not have the ability to degrade 1,1,1-TCA. When exposed to a mixture of 1,1,1-TCA and TCE, KB-1 did not degrade 1,1,1-TCA. Additionally, degradation of TCE stalled at *cis*-dichloroethene (*cis*DCE) and VC, with no ethene production occurring when 1,1,1-TCA was added.

Lastly, a culture combining KB-1 and MS was created. The combined culture was also exposed to a mixture of 1,1,1-TCA and TCE. Sequentially, TCE was converted to VC, but not further, until all of the 1,1,1-TCA was converted to 1,1-DCA. Then, VC was converted to ethene. Finally, after all of the VC was converted to ethene, dechlorination of 1,1-DCA to CA went to completion. Interestingly, diauxic growth, which did not occur when the cultures were present alone, was observed when the two communities were combined. The results obtained after combining the cultures provides a clear example of how changes in microbial community structure can affect degradation patterns for chlorinated contaminant mixtures.

Jones *et al*. (2006) tested a mixture of 1,1,2,2-TeCA, 1,1,2-TCA, and *cis*-DCE on an anaerobic mixed culture. The anaerobic mixed culture, designated 'WBC-2' was isolated from 1,1,2,2-TeCA contaminated anoxic wetland sediment and groundwater. The culture was enriched on 1,1,2,2-TeCA prior to exposure to contaminant mixtures. Analysis of the microbial community via construction of a 16S rRNA gene clone library and qPCR revealed that WBC-2 contained a high proportion of *Clostridium* (37%) and *Acetobacteria* (26%) sequences, with some *Dehalobacter* (2%) sequences (Jones *et al.*, 2006). Further molecular analysis conducted on WBC-2 enrichment subcultures at a later date revealed that *Dehalococcoides, Dehalobacter* and *Dehalogenimonas* were present at proportionally high abundances, with their distribution varying as a function of electron acceptor (Manchester *et al.*, 2012). When WBC-2 was exposed

to the chlorinated mixture of 1,1,2,2-TeCA, 1,1,2-TCA, and *cis*-DCE, all three compounds were completely dechlorinated to ethene and ethane simultaneously, with no stalling at intermediates. Also, there was no apparent inhibition or preferential utilization of one compound over the others (Jones *et al.,* 2006). The simultaneous complete dechlorination of the alkanes and alkene contrasts with what Grostern and Edwards (2006) reported.

The variable results reported in the literature may be due, at least in part, to differences in the microbial community structure present in the various cultures tested and the regulatory systems present in different microbial species.

1.1.3 Background of the genus *Dehalogenimonas*

Dehalogenimonas lykanthroporepellens BL-DC-9^T was isolated from groundwater collected from the Brooklawn portion of the PetroProcessors of Louisiana, Inc. (PPI) Superfund site (Yan *et al*., 2009a; Moe *et al*., 2009). More recently, *Dehalogenimonas* strain IP3-3 was isolated from groundwater collected from the Scenic Highway portion of the PPI Superfund site, approximately two miles from where *D. lykanthroporepellens* was first isolated (Bowman *et al*., 2012). The groundwater from which both strains were isolated contained a mixture of chlorinated solvents that included 1,1,2-trichloroethane (1,1,2-TCA), 1,2-dichloroethane (1,2-DCA), and 1,2-dichloropropane (1,2-DCP) (Bowman *et al*., 2006; Yan *et al.,* 2009a; Bowman *et al*., 2012). The chlorosolvent concentrations in the groundwater where each strain was isolated are listed in Table 1.1 (*D. lykanthroporepellens* BL-DC-9^T) and Table 1.2 (*Dehalogenimonas* strain IP3-3).

Table 1.1 Chlorosolvent concentrations measured in groundwater from well W-1024-1 at the PPI site from which *D. lykanthroporepellens* BL-DC-9^T was isolated (Bowman *et al.*, 2006)

	Concentration mg/L (mM)	
Analyte	Range	Average*
1,1,2,2-tetrachloroethane	30.1-89.2	57.3 (0.341 mM)
1,1,2-trichloroethane	239-530	367 (2.75 mM)
1,2-dichloroethane	363-756	540 (5.46 mM)
1,2-dichloropropane	54.5-83.9	67.3 (0.596 mM)
Vinyl Chloride	33.1-75.2	52.2 (0.835 mM)
Hexachlorobutadiene	5.26-7.89	6.58 (0.0252 mM)

*n=3

Table 1.2 Contaminant concentrations measured in groundwater used for isolation of *Dehalogenimonas* strain IP3-3 (Bowman *et al.*, 2012)

Analyte*	Well ID no. IP-3
1,2-dichloroethane	2.30 mg/L
1,2-dichloropropane	2.46 mg/L
cis-1,2-dichloroethene	2.26 mg/L
trans-1,2-dichloroethene	0.23 mg/L
1,1,2,2-tetrachloroethane	0.11 mg/L
tetrachloroethene	1.14 mg/L
1,1,2-trichloroethane	1.36 mg/L
trichloroethene	1.43 mg/L
Vinyl chloride	2.42 mg/L

*Analyzed using US EPA Method 624

In experiments testing various chlorinated compounds as individual contaminants, both *D. lykanthroporepellens* BL-DC-9^T and strain IP3-3^T have been demonstrated to anaerobically dehalogenate 1,1,2-TCA, 1,2-DCA, and 1,2-DCP via what appears to be a dihaloelimation reaction mechanism (simultaneous removal of chlorine atoms from adjacent carbon atoms with concurrent formation of a C-C double bond) to final products of vinyl chloride, ethene, and propene, respectively (Yan *et al*., 2009a; Bowman *et al*., 2012). Based on phenotypic and phylogenic characteristics, strain IP3-3 has been proposed as the type strain of a new species in the genus *Dehalogenimonas* with a proposed name of *D. alkenigignens* (Bowman *et al.*, 2012).

Because *D. alkenigignens* is not yet a validly published species, in this thesis, the strain is simply referred to as strain IP3-3.

While previously tested for abilities to dechlorinated various chlorinated compounds provided as sole electron acceptors (i.e., single contaminants), it has not yet been determined, however, what happens when these strains are simultaneously provided with multiple contaminants. Thus, it is not known whether these bacteria are able to concurrently transform multiple chlorinated alkanes, or whether one or more compounds is preferentially transformed with other compounds persisting longer, as would be the case for diauxic type growth. Such data is also presently lacking in the literature for other pure cultures able to reductively dehalogenate chlorinated alkanes [e.g., *Dehalococcoides mccartyi* strain 195^T (formerly referred to as "Dehalococcoides ethenogenes", Loffler *et al.,* 2012), *Desulfitobacterium dichloroeliminans* strain DCA1, (De Wildeman *et al.*, 2003), or *Desulfitobacterium* sp. Y51, (Suyama *et al.*, 2001)].

1.2 Thesis Organization

Chapter 2 of this thesis describes the materials, experimental procedures, and analytical techniques applied to study the effects of mixtures on *Dehalogenimonas* spp. Dechlorination. Chapter 3 contains figures and descriptions of the data collected throughout the course of the experiments and a discussion of the results. Chapter 4 contains overall conclusions and recommendations for future research. Chapter 5 contains a list of references cited throughout the thesis. Appendix A contains graphs depicting results from the individual batch serum bottles used in the binary and tertiary mixture experiments.

CHAPTER 2: MATERIALS AND METHODS

2.1 Chemicals

1,2-dichloroethane (≥99%), 1,2-dichloropropane (≥99%), 1,1,2-trichloroethane (≥96%), propene (≥99%), and ethene (≥99.5) were purchased from Sigma-Aldrich (St. Louis, MO). Vinyl Chloride (1000 ppm_v) was purchased from Supelco (Bellefonte, PA).

2.2 Medium Preparation

Anaerobic basal media was prepared as described by Sung *et al*., (2003). The anaerobic medium contained the following constituents (per liter): NaCl, 1.0 g; $MgCl_2 \cdot 6H_20$, 0.4 g; $CaCl_2 \cdot 6H_2O$, 0.1 g; KH_2PO_4, 0.2 g; NH_4Cl, 0.25 g; KCl, 0.5 g; resazurin, 0.001 g; L-cysteine hydrochloride, 0.25 g; sodium acetate 0.0041 g (0.05 mmol); sodium pyruvate, 0.0055 g (0.05 mmol); sodium lactate, 0.0057 g (0.05 mmol), 0.7 mL (5 mmol); non-chelated trace element solution, 1.0 mL (Kuever *et al.*, 2005); and 1.0 mL selenite-tungstate solution (Alain *et al.*, 2002). After the medium was autoclaved and cooled under a N_2 atmosphere, the following components were added aseptically from sterile stock solutions; 30 mL of $NaHCO_3$ solution (84 g/L autoclaved under N_2 atmosphere), 10 mL of titanium citrate solution (24 g/L), 0.1 mL vitamin solution (40 mg/L 4-aminobenzoic acid, 10 mg/L D(+)-biotin, 100 mg/L nicotinic acid, 50 mg/L calcium D (+)-pantothenate, 150 mg/L pyridoxine dihydrochloride, filter-sterilized and stored in 10 mM pH 7.1 sodium phosphate buffer at 10°C), 0.1 mL thiamin solution (1 g/L thiamine chloride, filter sterilized and stored in 25 mM pH 3.4 sodium phosphate buffer at 10°C), 0.1 mL vitamin B12 solution (500 mg/L, filter sterilized and stored in distilled water at 10°C). The pH of the medium was adjusted to 7.0-7.5 with 1 M NaOH (autoclaved).

Aliquots of the sterile basal medium (200 mL) was dispensed aseptically into sterile 545 mL serum bottles (Wheaton) and sealed with butyl-rubber stoppers and aluminum crimp caps.

Gas headspace of the serum bottles was flushed with a filter sterilized H_2/N_2 gas mixture (80%/20%, v/v).

2.3 Binary and Tertiary Mixture Experimental Design

Duplicate bottles were spiked with a combination of either 1,1,2-TCA/1,2-DCP, 1,1,2-TCA/1,2-DCA, or 1,2-DCA/1,2-DCP, (filter sterilized, neat compounds), to reach a final target aqueous phase concentration of approximately 1 mM per individual compound following dissolution and equilibration. A fourth mixture, comprised of 1,1,2-TCA/1,2-DCA/1,2-DCP was prepared identically except that the target aqueous phase concentration was 0.5 mM per chlorinated compound. Duplicate bottles received a 6% (v/v) inoculum of either strain IP3-3, or *D. lykanthroporepellens* BL-DC-9^T, previously grown with 1.5 – 2 mM 1,2-DCP, and incubated until at least 50% of the initial mass of 1,2-DCP had been dechlorinated. Abiotic negative controls were prepared following the exact same procedure except that they did not receive any inoculum. The cultures were incubated at 30 °C in the dark without shaking. At regular time intervals, the duplicate bottles were sampled and analyzed via gas chromatography for 1,1,2-TCA, 1,2-DCA, 1,2-DCP and their degradation products (i.e., vinyl chloride, ethene, and propene).

To further assess the effect of 1,1,2-TCA on 1,2-DCA dechlorination, duplicate bottles were spiked with 1,2-DCA as the sole electron acceptor or a combination of 1,1,2-TCA and 1,2-DCA, but with the 1,1,2-TCA at a higher and lower concentration than in the initial experiments (target concentration of 1,2-DCA = 1 mM, target concentrations of 1,1,2-TCA = 1.5 mM and 0.75 mM). Bottles then received a 6% (v/v) inoculum of strain IP3-3, or *D. lykanthroporepellens* BL-DC-9^T, previously grown with 1.5 – 2 mM 1,2-DCP, and incubated until at least 50% of the initial mass of DCP had been dechlorinated. The cultures were incubated at 30°C in the dark. At

regular time intervals, the duplicate bottles were sampled and analyzed via gas chromatography for 1,1,2-TCA, 1,2-DCA, and their degradation products (i.e., vinyl chloride and ethene).

To assess whether the trends observed in the 1,1,2-TCA/1,2-DCA mixtures were influenced by inoculum growth conditions (i.e., growth on 1,2-DCP prior to inoculation), further experiments were conducted with inoculum grown on 1,2-DCA. Briefly, duplicate bottles were spiked with a mixture comprised of 1,1,2-TCA/1,2-DCA to reach a target final aqueous phase concentration of 1 mM each. The bottles then received a 6% (v/v) inoculum of strain IP3-3, or *D. lykanthroporepellens* BL-DC-9^{T}, previously grown with 1.5 – 2 mM 1,2-DCA, and incubated until at least 50% of the initial mass of 1,2-DCA had been dechlorinated. The cultures were incubated at 30°C in the dark without shaking. At regular time intervals, the duplicate bottles were sampled and analyzed via gas chromatography for 1,1,2-TCA, 1,2-DCA, and their potential degradation products (i.e., vinyl chloride and ethene).

2.4 Analytical Methods

Chlorinated solvents and degradation product concentrations were measured using an HP model 6890 gas chromatograph (GC) as described by Yan *et al* (2009a). Briefly, the GC was equipped with a flame ionization detector and GS-GasPro capillary column (60 m × 0.32 mm I.D., J&W P/N 113-4362) with helium as carrier gas with a flow of 3.0 mL/min. Gas headspace samples collected in 100 µL gas-tight glass syringes (Hamilton, Baton Rouge, LA) were introduced via direct injection. Aqueous samples were purged with helium in a Tekmar 3000 Purge and Trap with sample introduction to the GC using a Tekmar 2016 concentrator. Separate gas-headspace samples and aqueous-phase samples were analyzed from each serum bottle at each time step. The mass of each volatile compound per serum bottle was calculated as the sum of the liquid and gas concentrations times volumes.

Liquid standard solutions were prepared by dissolving known volumes of neat compound into 160 mL of water in sealed serum bottles with no headspace. After the solutions were mixed and allowed to equilibrate overnight, 0.5 mL aliquots were loaded into the purge and trap autosampler and analyzed as described above. Serial dilutions of vinyl chloride, ethene, and propene were made by injecting known volumes into sealed 160 mL serum bottles. The diluted gas standards were measured via direct injection. Calibration curves were prepared by plotting peak area versus concentration.

CHAPTER 3: RESULTS AND DISCUSSION

3.1 Binary Mixture of 1,2-Dichloroethane (1,2-DCA) and 1,2-Dichloropropane (1,2-DCP)

In tests to assess the potential effects of a combination of 1,2-DCA and 1,2-DCP, the experimentally measured aqueous-phase concentrations of 1,2-DCA at t=0 ranged from 1.09 to 1.16 mM (108 to 115 mg/L) with a total quantity of 243 to 259 µmoles 1,2-DCA per bottle (accounting for both aqueous and gas phases). For 1,2-DCP, the starting aqueous phase concentrations ranged from 1.02 to 1.12 mM (115 to 127 mg/L) with a total quantity of 1,2-DCP ranging from 273 to 290 µmoles/bottle (again accounting for both the aqueous and gas phases). Thus, at the start of the experiment, the concentration of 1,2-DCA and 1,2-DCP were each close to the target level of 1 mM in the aqueous phase.

The quantity of 1,2-DCA, 1,2-DCP, ethene, and propene in µmoles per bottle plotted versus time in days for serum bottles inoculated with each of the two strains (either strain IP3-3 or *D. lykanthroporepellens* strain BL-DC-9^T) can be seen in Figure 3.1. Each data point in Figure 3.1 represents the average of duplicate bottles. Error bars represent one standard deviation. Data for each of the replicate bottles plotted separately is shown in Figure A.1.

For strain IP3-3, the quantity of propene measured at t=6.83 days (the third time bottles were analyzed) was 26.5±2.4 µmoles/bottle (mean±standard deviation), which accounts for greater than 5% of the 1,2-DCP initially present at t=0. At t=8.88 days, the next time samples were analyzed, propene had further increased to 92.8±16.1 µmoles/bottle (mean±standard deviation), with a corresponding aqueous-phase concentration of 0.33±0.04 mM (37.3±4.51 mg/L) 1,2-DCP remaining. At the same time step (i.e., 8.88 days), ethene, the terminal product of 1,2-DCA dechlorination by strain IP3-3, had increased to 33.4±6.42 µmoles/bottle. This quantity of ethene corresponds to greater than 5% of the initial 1,2-DCA present at t=0.

Thereafter, 1,2-DCP and 1,2-DCA concurrently decreased and propene and ethene concurrently increased until essentially all (>99%) of the parent compounds (i.e., 1,2-DCP and 1,2-DCA) were consumed by day 18.0, the end of the experiment. The aqueous-phase 1,2-DCA concentration at the time when >5% of the starting 1,2-DCP had been transformed to propene (day 6.83) was 1.03±0.05 mM (102±4.95 mg/L).

For strain IP3-3 experiments, mass balance calculations indicated that the mass of 1,2-DCA and ethene present at the end (i.e., t=18.0 days), or removed via sampling at intermediate time steps, accounted for 88.6±4.80% of that present at the beginning of the experiment. Likewise, mass balance calculations for the mass of 1,2-DCP and propene showed 71.6±7.70% was recovered at the end (i.e., t=18.0 days), or removed via sampling. The fraction of compounds removed via sampling accounted for ≤1.1% of the contaminants present at t=0. In abiotic (uninoculated) negative controls, the quantity of ethene observed was ≤7.03 μmoles/bottle which is ≤2.82% of the starting 1,2-DCA molar mass. Likewise, the quantity of propene was ≤2.14 μmoles/bottle which is ≤0.78% of the starting 1,2-DCP molar mass. The overall mass balances for 1,2-DCA and 1,2-DCP in negative controls were 71.5±4.2% and 58.3±0.97% respectively. The same set of uninoculated negative controls was used for both strains [data collected at t=42.9 days].

Bottles inoculated with *D. lykanthroporepellens* strain BL-DC-9^{T} followed a pattern similar to that observed with strain IP3-3. Propene corresponding to greater than 5% of the starting 1,2-DCP was observed on day 4.04 (the second time samples were collected), and ethene corresponding to greater than 5% of the starting 1,2-DCA was observed on day 6.00, the next sample analysis.

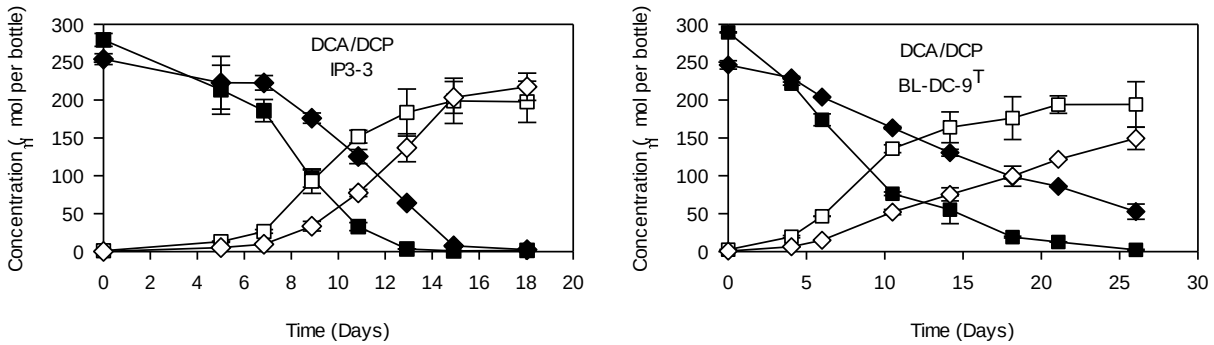

Figure 3.1. Experimentally measured 1,2-DCA (closed diamonds), 1,2-DCP (closed squares), ethene (open diamonds), and propene (open squares) as a function of time in serum bottles inoculated with strain IP3-3 (left) or *D. lykanthroporepellens* BL-DC-9^T (right). Target starting concentrations were 1 mM for the parent compounds. Each data point represents the mean calculated average from duplicate bottles. Error bars represent one standard deviation.

Thereafter, 1,2-DCP and 1,2-DCA concurrently decreased and propene and ethene concurrently increased throughout the remainder of the incubation interval. At the time step when propene corresponding to greater than 5% of the starting 1,2-DCP was first observed (i.e., day 4.04), the aqueous-phase concentration of 1,2-DCA was 0.99±0.01 mM (98.0±1.00 mg/L). At the time step when ethene corresponding to greater than 5% of the starting 1,2-DCA was first observed (i.e., day 6.00), the aqueous-phase concentration of 1,2-DCP was 0.61±0.03 mM (69.0±3.39 mg/L).

For *D. lykanthroporepellens* BL-DC-9^T, mass balance calculations indicated that the mass of 1,2-DCA and ethene present at the end (i.e., t=26.0 days), or removed via sampling at intermediate time steps, accounted for 83.1±11.9% of that present at the beginning of the experiment. Also, mass balance calculations for the mass of 1,2-DCP and propene showed 67.9±10.3% was recovered at the end (i.e., t=26.0 days), or removed via sampling. The fraction of compounds removed via sampling accounted for ≤1.20% of the contaminants present at t=0.

For both strains, appreciable ethene production (i.e., >5% of the starting 1,2-DCA on a molar basis) was first observed when the mean aqueous phase concentration of 1,2-DCP was at least 0.61±0.03 mM (69.0±3.39 mg/L). For both strains, appreciable propene production (i.e., >5% of the starting 1,2-DCP) was first observed when the aqueous-phase 1,2-DCA concentration was at least 1.03±0.05 mM (102±4.95 mg/L). Thus, when 1,2-DCA and 1,2-DCP were present at aqueous concentrations on the order of 0.6 to 1 mM, the compounds were degraded concurrently.

3.2 Binary Mixture of 1,2-Dichloroethane (1,2-DCA) and 1,1,2-Trichloroethane (1,1,2-TCA)

In tests to assess the effects of a combination of 1,2-DCA and 1,1,2-TCA on strain IP3-3 and *D. lykanthroporepellens* BL-DC-9^T, the experimentally measured aqueous phase concentrations of 1,2-TCA at t=0 ranged from 0.56 to 0.86 mM (74.7 to 114 mg/L) with the total quantity of 1,1,2-TCA ranging from 126 to 189 µmoles/bottle. For 1,2-DCA, the t=0 aqueous phase concentrations ranged from 0.67 to 1.10 mM (66.3 to 109 mg/L) with 154 to 243 µmoles/bottle 1,2-DCA total.

The total quantities of 1,2-DCA, 1,1,2-TCA, ethene, and VC in µmoles per bottle versus time plotted for both strain IP3-3 and *D. lykanthroporepellens* BL-DC-9^T inoculated serum bottles can be seen in Figure 3.3. Data for each of the replicate bottles plotted separately is shown in Appendix A, Figure A.3.

For strain IP3-3 inoculated bottles, the quantity of VC measured at the second time step (i.e., t=3.13 days) was 36.8±3.81 µmoles/bottle, which accounts for greater than 20% of the quantity of 1,1,2-TCA present at t=0. By t=5.79 days (the fourth sampling), the quantity of VC had further increased to 116±2.11 µmoles/bottle, with a corresponding 1,1,2-TCA aqueous phase concentration of 0.04±0.02 mM (5.34±2.67 mg/L) remaining. At the same time step (i.e., t=5.79

days) the ethene quantity had increased to 14.21±1.89 μmoles/bottle, which corresponds to greater than 5% of the 1,2-DCA measured at t=0. It should be noted that during the initial portion of the study, the average concentration of 1,2-DCA increased (Fig. 3.2). This trend was observed in both inoculated replicate bottles (see Fig. A.3), as well as abiotic negative controls. As further discussed in Section 3.3, a comparatively small amount of abiotic production of 1,2-DCA (on the order of 20 micromoles per bottle) from 1,2-TCA was also observed in mixture experiments involving 1,2-DCP and 1,1,2-TCA. In these latter studies (described in Section 3.3), the abiotic transformation of 1,1,2-TCA to 1,2-DCA was more readily apparent due to the fact that no 1,2-DCA was initially spiked into the bottles. Abiotic transformation of polychlorinated alkanes in the presence of vitamin B12 (which was present in the basal medium of the present study) and titanium citrate (which was employed as a reducing agent in the present study) under reducing conditions has been reported previously (Schanke and Wackett., 1992). Following t=5.79 days, the quantity of both 1,2-DCA and 1,1,2-TCA decreased at the same time, with a concurrent increase in ethene and VC.

Due to abiotic transformation of 1,1,2-TCA to 1,2-DCA (as further discussed in Section 3.3), the mass balance was calculated as the sum of 1,2-DCA, 1,1,2-TCA, ethene, and VC on a molar basis. This same mass balance calculation approach was applied to all other mixtures containing 1,1,2-TCA. For strain IP3-3, the total mass of these compounds (i.e., 1,2-DCA, 1,1,2-TCA, ethene, and VC) present at the end (i.e., t=19.8 days), or removed via sampling, accounted for 115 ± 15.8% of that present at the beginning of the experiment. The fraction of compounds removed via sampling accounted for ≤2.06% of the contaminants present at t=0. In abiotic negative controls, the quantity of the daughter product VC, was ≤10.1 μmoles/bottle, which is ≤8.11% of the starting quantity of 1,1,2-TCA. The quantity of ethene recorded in negative

controls accounted for ≤0.09% of starting 1,2-DCA quantity measured. In abiotic negative controls, the mean concentration of 1,2-DCA increased from 187±69.3 to 230±8.21 µmoles/bottle indicating abiotic transformation of 1,1,2-TCA to 1,2-DCA. The overall mass balance recovery for 1,2-DCA and 1,1,2-TCA in abiotic negative controls was 104±32.8%. The same abiotic negative controls were used for both strains.

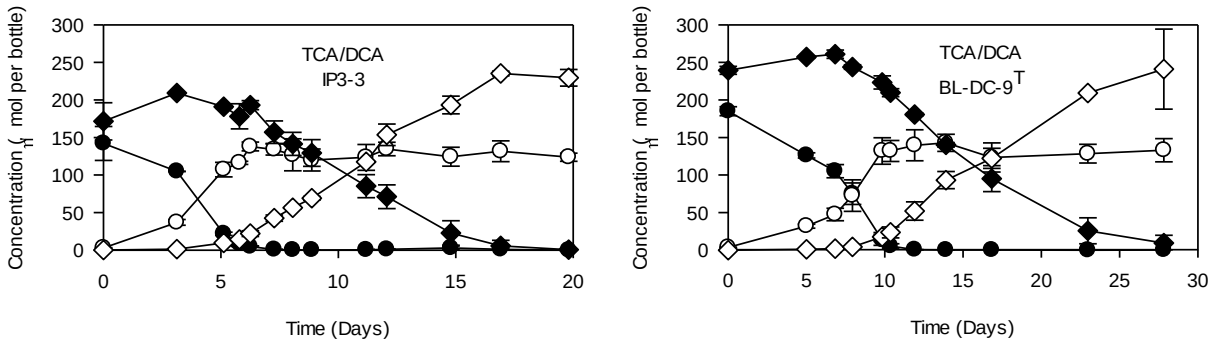

Figure 3.2. Experimentally measured 1,2-TCA (closed circles), 1,2-DCA (closed diamonds), vinyl chloride (open circles), and ethene (open diamonds) as a function of time in serum bottles inoculated with strain IP3-3 (left) or *D. lykanthroporepellens* BL-DC-9^T (right). Target starting concentrations were 1 mM for the parent compounds. Each data point represents the mean calculated average from duplicate bottles. Error bars represent one standard deviation

The same general trend observed for strain IP3-3 was also observed for *D. lykanthroporepellens* BL-DC-9^T. At the third time step (i.e., t=6.83 days), VC equivalent to greater than 20% of the starting 1,1,2-TCA was observed. Similar to IP3-3 inoculated bottles, the 1,2-DCA quantity increased in both replicates during the initial portion (i.e., from t=0 to t=6.83 days) of the experiment. The quantity of ethene measured at the fifth time step (i.e., t=9.83 days) was 17.6±5.87 µmoles/bottle, which corresponds to greater than 5% both the starting and maximum quantity of 1,2-DCA measured. At the same time step (i.e., t=9.83 days), the aqueous phase concentration of the remaining 1,1,2-TCA was 0.06±0.04 mM (8.00±5.33 mg/L). Thereafter, the quantity of 1,2-DCA and 1,1,2-TCA decreased with concurrent production of ethene and VC.

The mass balance for *D. lykanthroporepellens* BL-DC-9^T inoculated serum bottles was calculated as the sum of 1,1,2-TCA, 1,2-DCA, VC, and ethene. The total mass of these compounds present at the end (i.e., t=27.8 days), or removed via sampling, accounted for 91.0 ± 11.2% of that present at the beginning of the experiment. The fraction of compounds removed via sampling accounted for ≤1.60% of the contaminant present at t=0.

For both strains, appreciable VC production (i.e., greater than 20% of the starting 1,1,2-TCA on a molar basis) was first observed when the mean aqueous phase concentration of 1,2-DCA was at least 1.17±0.01 mM (115±1.10 mg/L). For both strains, appreciable ethene production (i.e., >5% of the starting 1,2-DCA quantity recorded) was first observed when the aqueous-phase 1,1,2-TCA concentration was markedly lower with 1,1,2-TCA dechlorination mostly complete [remaining concentration was at least 0.06±0.04 mM (8.00±5.36 mg/L)]. Thus, it appears that when 1,1,2-TCA and 1,2-DCA were initially present at approximately equal concentrations on the order of 1 mM each, the compounds were degraded in a largely sequential manner, with 1,2-DCA not converted to ethene until the aqueous 1,1,2-TCA concentration was relatively low (i.e., <0.1 mM).

3.2.1 Further Experiments on the Binary Mixture of 1,2-Dichloroethane and 1,1,2-Trichloroethane

Additional experiments were designed to further characterize the effect of 1,1,2-TCA on the dechlorination of 1,2-DCA. In the first set of replicate bottles (Fig. 3.3, first row), 1,2-DCA was supplied as the sole electron acceptor, with no addition of 1,1,2-TCA. The 1,2-DCA aqueous phase concentrations at t=0 in all bottles were quite consistent, ranging from 1.02 to 1.18 mM (101 to 117 mg/L), corresponding to a range of 226 to 258 µmoles/bottle total (accounting for both gas-phase and aqueous-phases). In the second set of replicate bottles (Fig. 3.3, second row),

the t=0 aqueous phase concentration of 1,1,2-TCA ranged from 0.65 to 0.71 mM (86.7 to 94.7 mg/L), with the total quantity of 1,1,2-TCA ranging from 142 to 158 µmoles/bottle (again accounting for both gas-phase and aqueous phases). In the third set of duplicate bottles (Fig. 3.3, third row), the t=0 aqueous phase concentration of 1,1,2-TCA ranged from 1.32 to 1.44 mM (176 to 192 mg/L), with a range of 287 to 311 µmoles/bottle total. For each strain, bottles were inoculated from the same culture, at the same time.

Figure 3.3 (left column) displays the mean 1,2-DCA, 1,1,2-TCA, ethene, and VC quantity in µmoles per bottle versus time for bottles inoculated with strain IP3-3. Data for individual replicate bottles is shown in Figure A.5. In bottles amended with 1.08 mM (average aqueous concentration) 1,2-DCA only and strain IP3-3, nearly all of the starting 1,2-DCA (> 93%, corresponding to >238 µmoles/bottle) had been converted to ethene by the fourth time step (i.e., t=12.3 days). In bottles amended with both 1,2-DCA and 0.67 mM (average aqueous concentration) 1,1,2-TCA at the same time step (i.e., t=12.3 days) the maximum quantity of ethene (accounting for both replicates) was 109 µmoles/bottle, which corresponds to no more than 43% conversion of the starting 1,2-DCA to ethene. In bottles amended with 1,2-DCA and 1.38 mM (average aqueous concentration) 1,1,2-TCA at the same time step (i.e., t=12.3 days), the maximum quantity of ethene present (i.e., 10.1 µmoles/bottle) accounted for less than 4% of the 1,2-DCA present at t=0. The presence of 1,1,2-TCA at the concentrations tested had on obvious negative effect on 1,2-DCA dechlorination.

Figure 3.3 (right most column) displays the mean quantity of 1,2-DCA, 1,1,2-TCA, ethene, and VC versus time for bottles inoculated with *D. lykanthroporepellens* BL-DC-9^{T}. Data for individual replicated bottles is shown in Figure A.6.

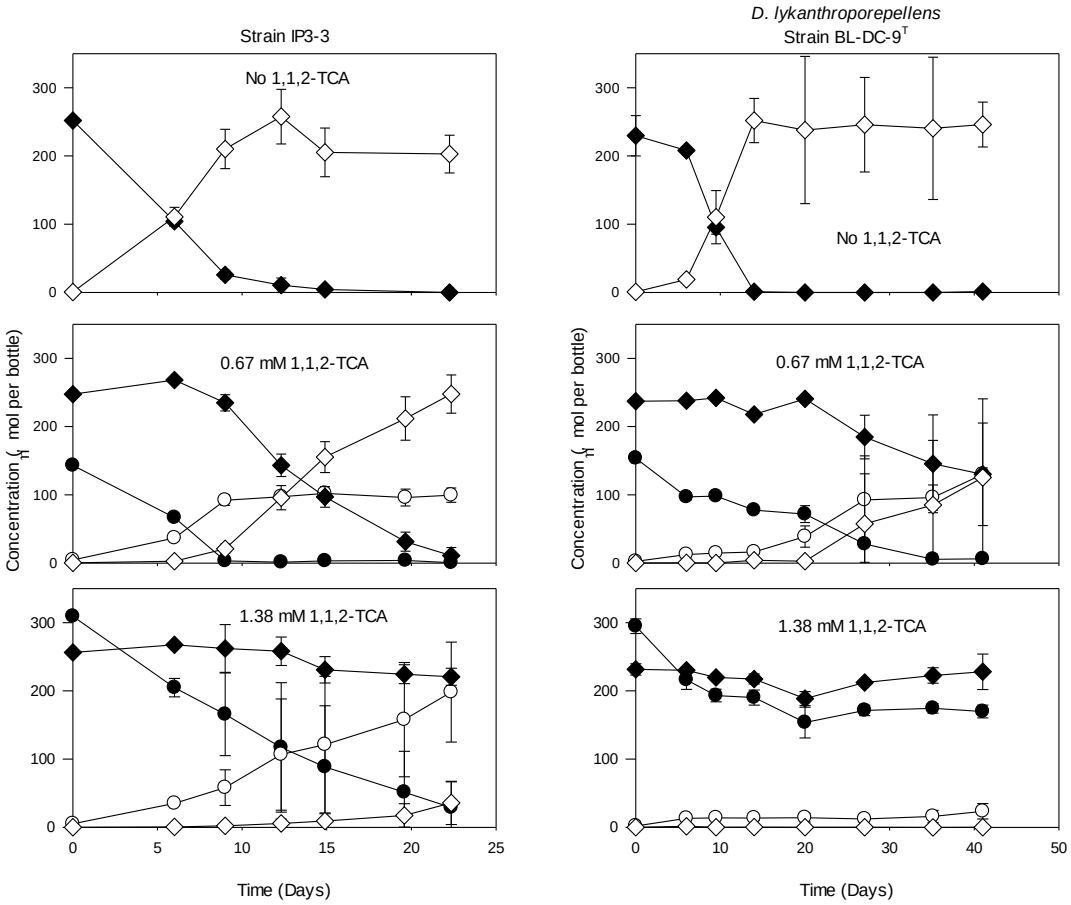

Figure 3.3. Experimentally measured 1,2-TCA (closed circles), 1,2-DCA (closed diamonds), vinyl chloride (open circles), and ethene (open diamonds) as a function of time in serum bottles inoculated with strain IP3-3 (left) or *D. lykanthroporepellens* BL-DC-9^T (right). Each data point represents the mean calculated average from duplicate bottles. Row 1 displays bottles amended with 1,2-DCA only. Rows 2 and 3 display bottles amended with a mixture of 1,2-DCA and 1,1,2-TCA (1,1,2-TCA average starting aqueous concentration displayed on title. Error bars represent one standard deviation

For *D. lykanthroporepellens* BL-DC-9^T, the same general trend that was observed for strain IP3-3 was also observed. Mass balance calculations revealed that greater than 109% (>274 μmoles/bottle) of the starting quantity of 1,2-DCA was recovered as ethene by t=14.0 days in bottles amended with 1,2-DCA only. For bottles amended with 1,2-DCA and 0.67 mM 1,1,2-TCA, the quantity of ethene present at t=14.0 days accounted for less than 2.70% (<6.47

µmoles/bottle) of the starting 1,2-DCA. In the bottles amended with 1,2-DCA and 1.38 mM TCA, the quantity of ethene present at t=14.0 days accounted for less than 0.02% (<0.27 µmoles/bottle) of the starting 1,2-DCA. As with strain IP3-3, the presence of 1,1,2-TCA at both concentrations tested had an obvious negative effect on 1,2-DCA dechlorination.

For both strains, when 1,2-DCA was present as the sole electron acceptor, the ethene concentration consistently increased, and accounted for nearly all (>93%) of the starting 1,2-DCA by t=14.0 days. Furthermore, in corresponding time steps at and before t=14.0 days, the quantity of ethene in bottles provided with both 1,2-DCA and 1,1,2-TCA was consistently lower than the quantity of ethene in bottles amended with 1,2-DCA only. Similarly to what was observed in the original binary 1,2-DCA/1,1,2-TCA mixtures experiment, when the aqueous 1,2-TCA was present at 0.67±0.02 mM (89.4±2.6 mg/L), appreciable 1,2-DCA dechlorination (>%5 of the starting 1,2-DCA) was not observed until the 1,1,2-TCA dechlorination was nearly complete [≤0.05 mM remaining in any replicate (Figure A.5 and Figure A.6, middle rows)] When 1,1,2-TCA was present at an aqueous concentration of 1.38±0.05 mM (184±6.67 mg/L), 1,1,2-TCA persisted until the end of the experiment [Maness (2012) showed that high chlorinated alkane concentrations could possibly become inhibitory to the *Dehalogenimonas* reductive dechlorination mechanism], which seemingly suppressed 1,2-DCA dechlorination to ethene. In fact, at the end of the experiments (i.e., t=22.4 for strain IP3-3 and t=47.3 for *D. lykanthroporepellens* BL-DC-9^{T}), the maximum amount of ethene measured in bottles spike with the highest concentration of 1,1,2-TCA (i.e., 1.38±0.05 mM) was 58.6 µmoles/bottle, which accounted for only 23% of the starting 1,2-DCA. This provides additional experimental support for the notion that 1,1,2-TCA is largely degraded prior to 1,2-DCA when present as a mixture.

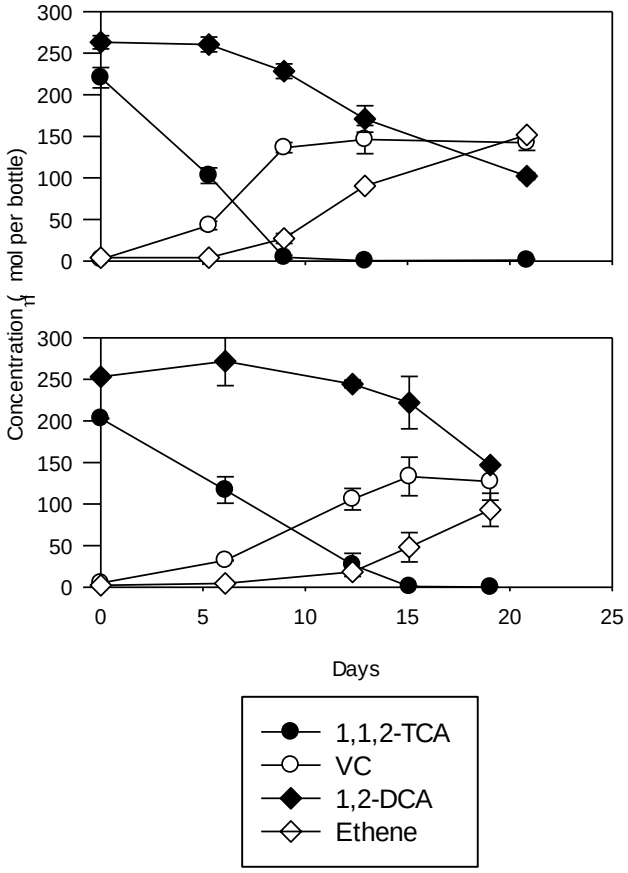

Figure 3.4. Experimentally measured 1,1,2-TCA, 1,2-DCA, vinyl chloride, and ethene as a function of time in serum bottles inoculated with strain IP3-3 (top) or *D. lykanthroporepellens* BL-DC-9^T (bottom). Inoculum was grown on 1,2-DCA rather than 1,2-DCP. Target starting concentration was 1 mM per compound. Each data point represents the mean calculated average from duplicate bottles. Error bars represent one standard deviation.

Additional experiments were conducted to test whether the seemingly preferential dechlorination of 1,1,2-TCA over 1,2-DCA was influenced by the fact that the inoculum was grown on 1,2-DCP rather than 1,2-DCA prior to inoculation. In these tests, identical 1,1,2-TCA/1,2-DCA mixture tests were conducted (i.e., same target starting concentrations as the binary mixtures experiments); however, the inoculum was grown on 1,2-DCA rather than 1,2-DCP. As shown in Figure 3.4, no variances from the previously discussed results were observed for either strain. 1,1,2-TCA dechlorination to VC was consistently observed earlier than

dechlorination of 1,2-DCA to ethene, and appreciable production of ethene, accounting for >5% of the starting 1,2-DCA, was not observed until 1,1,2-TCA concentration were low [≤ 0.17 mM (22.7 mg/L) in the aqueous phase].

3.3 Binary Mixture of 1,2-Dichloropropane (1,2-DCP) and 1,1,2-Trichlorethane (1,2-TCA)

To assess the effects of a combination of 1,2-DCP and 1,1,2-TCA on strain IP3-3 and *D. lykanthroporepellens* BL-DC-9^T, the experimentally measured aqueous-phase concentrations of 1,2-TCA at t=0 ranged from 0.51 to 0.90 mM (68.0 to 120 mg/L) with the total quantity of 1,2-TCA ranging from 113 to 198 µmoles/bottle. For 1,2-DCP, t=0 aqueous phase concentrations ranged from 0.63 to 1.11 mM (71.2 to 125 mg/L) with a total quantity of 170 to 288 µmoles 1,2-DCP per bottle.

The quantity of 1,2-DCP, 1,1,2-TCA, propene, and VC versus time plotted for both the strain IP3-3 and *D. lykanthroporepellens* BL-DC-9^T inoculated serum bottles are shown in Figure 3.5. Also depicted in Figure 3.5 is the quantity of 1,2-DCA per bottle. Data for each replicate bottle plotted separately are shown in Appendix A, Figure A.2.

In strain IP3-3 inoculated serum bottles, the quantity of VC measured at t=3.13 days (the second time the bottles were measured) was 20.5±1.98 µmoles/bottle which corresponds to >5% of the starting 1,1,2-TCA measured at t=0. At t=6.25 days (the fourth time samples were collected), the quantity of VC had further increased to 58.8±11.4 µmoles/bottle, with 56.5±11.3 µmoles 1,1,2-TCA remaining per bottle, corresponding to an aqueous 1,1,2-TCA concentration of 0.26±0.06 mM (34.7±8.00 mg/L). At the same time step (i.e., t=6.25 days), the quantity of propene had increased to 14.25±4.72 µmoles/bottle, which corresponds to greater than 5% of the

initial 1,2-DCP measured at t=0. Subsequently, the quantity of both 1,2-DCP and 1,1,2-TCA simultaneously decreased, with a concurrent increase in the daughter products propene and VC.

Though not intentionally spiked into the bottles and detected at only trace levels at t=0 (1.90±0.36 μmoles/bottle), 1,2-DCA was transiently observed in strain IP3-3 inoculated bottles (initially increasing and subsequently decreasing). 1,2-DCA was also consistently observed in uninoculated negative controls (present at levels ranging from 2.66 to 19.39 μmoles/bottle in all measurements after t=0) . The maximum quantity of 1,2-DCA measured in the inoculated bottles was 18.7±3.16 μmoles/bottle at t=8.06. At the same time step (i.e., t=8.06 days), the quantity of ethene was observed to be 1.59±0.26 μmoles/bottle, which is greater than 5% of the maximum 1,2-DCA concentration recorded. At the same time step (i.e., t=8.06 days), the aqueous 1,1,2-TCA concentration was measured to be 0.06±0.02 mM (7.40±2.35 mg/L) and the aqueous-phase 1,2-DCP concentration was measured to be 0.44±0.12 mM (49.2±13.0 mg/L).

For the strain IP3-3 inoculated serum bottles, the sum of 1,2-DCA, 1,1,2-TCA, ethene and VC present at the end (i.e., t=16.3 days), or removed via sampling, accounted for 131±2.76% of that present at the beginning of the experiment. Likewise, mass balance calculations for the mass of 1,2-DCP and propene showed 120±11.9% was recovered at the end (i.e., t=16.3 days), or removed via sampling. The fraction of compounds removed via sampling accounted for ≤5.63% of the contaminants present at t=0. In abiotic negative controls, the quantity of the VC was ≤9.65 μmoles/bottle, which is ≤5.58% of the 1,1,2-TCA. The quantity of propene was ≤0.07 μmoles/bottle, which is ≤0.02% of the starting 1,2-DCP. The overall mass balances for 1,2-DCP and 1,1,2-TCA in abiotic negative controls were 77.9±16.8% and 71.4±19.3% respectively. The maximum quantity of 1,2-DCA observed was 19.4 μmoles/bottle. The maximum quantity of ethene observed in negative controls was 0.23 μmoles/bottle, which corresponds to 1.17% of the

maximum 1,2-DCA concentration observed in negative controls [data collected at t=21 days]. The same set of negative controls was used for both strains.

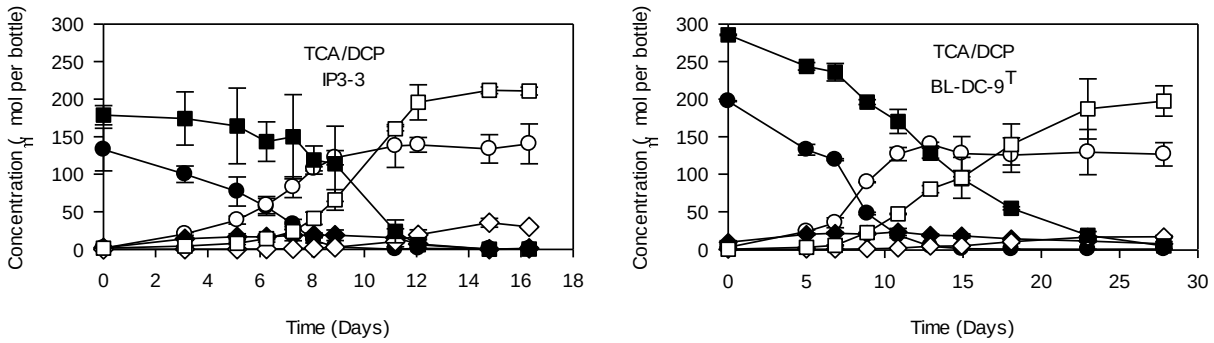

Figure 3.5. Experimentally measured 1,2-TCA (closed circles), 1,2-DCA (closed diamonds), 1,2-DCP (closed squares), vinyl chloride (open circles), ethene (open diamonds), and propene (open squares) as a function of time in serum bottles inoculated with strain IP3-3 (left) or *D. lykanthroporepellens* BL-DC-9^T (right). Target starting concentrations were 1 mM for the parent compounds. Each data point represents the mean calculated average from duplicate bottles. Error bars represent one standard deviation

D. lykanthroporepellens BL-DC-9^T inoculated serum bottles followed a pattern similar to that observed with strain IP3-3. VC corresponding to greater than 5% of the starting 1,1,2-TCA was observed on day 5.00 (the second time samples were collected), and propene corresponding to greater than 5% of the starting 1,2-DCP was observed on day 8.88 (the fourth time samples were collected). Thereafter, 1,2-DCP and 1,1,2-TCA concurrently decreased, with concomitant production of propene and VC throughout the duration of the incubation interval. At the time when VC production was observed to be greater than 5% of the starting 1,1,2-TCA, the aqueous phase 1,2-DCP concentration was 0.90±0.06 mM (101.69±6.78 mg/L). At the time step when propene corresponding to greater than 5% of the starting 1,2-DCP was first observed (i.e., t=8.88 days), the aqueous phase concentration of 1,1,2-TCA was 0.22±0.01 mM (29.4±1.33 mg/L).

Abiotic production of 1,2-DCA from 1,1,2-TCA was also observed in the *D. lykanthroporepellens* BL-DC-9^T inoculated serum bottles. The average maximum concentration observed in the inoculated bottles was 23.1±4.73 µmoles/bottle at t=10.9. At the same time step (i.e., t=10.9 days), the quantity of ethene measured was 1.46±0.13 µmoles/bottle, which corresponds to greater than 5% of the maximum 1,2-DCA quantity recorded in the biotic bottles. At the same time step (i.e., t=10.9 days), the aqueous 1,1,2-TCA concentration was observed to be 0.08±0.01 mM (7.94±5.19 mg/L) and the aqueous 1,2-DCP concentration was observed to be 0.62±0.06 mM (70.3±6.31 mg/L).

Mass balance calculations for *D. lykanthroporepellens* BL-DC-9^T indicated that the mass of 1,1,2-TCA, 1,2-DCA, VC, and ethene present at the end (i.e., t=27.8 days), or removed via sampling, accounted for 73.3±7.93% of that present at the beginning of the experiment. Likewise, mass balance calculations for the mass of 1,2-DCP and propene showed 71.8±6.45% was recovered at the end (i.e., t=16.3 days), or removed via sampling. The fraction of compounds removed via sampling accounted for ≤1.14% of the contaminant present at t=0.

For both strains, appreciable VC production from 1,1,2-TCA dechlorination (i.e., >5% of the starting 1,1,2-TCA on a molar basis) was observed prior to appreciable dechlorination of 1,2-DCP to propene. Further, VC production in an amount greater than 5% of the starting 1,1,2-TCA was observed when the mean aqueous phase concentration of 1,2-DCP was at least 0.90±0.06 mM (101±6.55 mg/L). Thus, it is clear that 1,1,2-TCA dechlorination is possible even in the presence of relatively high 1,2-DCP concentrations. For both strains, appreciable propene production (i.e., >5% of the starting 1,2-DCP) was first observed when the aqueous-phase 1,1,2-TCA concentration was at least 0.26±0.06 mM (34.7±8.00 mg/L), and the reductive dechlorination of both compounds proceeded concurrently at lower concentrations,

demonstrating that both compounds can be simultaneously dechlorinated by these *Dehalogenimonas* strains when the contaminant concentrations are sufficiently low.

Although the intended purpose was to investigate only a mixture of 1,1,2-TCA and 1,2-DCP (and 1,2-DCA was therefore not initially added to the bottles), 1,2-DCA was also present in the mixture due to abiotic formation from 1,1,2-TCA. The abiotically produced 1,2-DCA was dechlorinated by both strains. In IP3-3 inoculated serum bottles, the formation of the 1,2-DCA daughter product, ethene, in amounts greater than 5% of the maximum 1,2-DCA quantity observed, was first detected when the aqueous phase concentrations of 1,1,2-TCA and 1,2-DCP were 0.06±0.02 and 0.44±0.12 mM (7.41±2.35 and 49.2±13.0 mg/L) respectively. Likewise, in *D. lykanthroporepellens* BL-DC-9^T inoculated serum bottles, formation of ethene in amounts greater than 5% of the maximum 1,2-DCA recorded, was first detected when the aqueous phase concentration of 1,1,2-TCA and 1,2-DCP were 0.08±0.01 and 0.62±0.06 mM (10.7±1.98 and 70.3±6.31 mg/L) respectively. In latter experiments, the three-component mixture (i.e., 1,2-DCA, 1,2-DCP, and 1,1,2-TCA) was studied in further detail (Section 3.4).

3.4 Tertiary Mixture of 1,2-Dichlorethane (1,2-DCA), 1,2-Dichloropropane (1,2-DCP), and 1,1,2-Trichlorethane (1,1,2-TCA)

For the three-component mixture experiment, the target starting concentration was 0.5 mM each 1,2-DCA, 1,2-DCP, and 1,1,2-TCA. Considering both strains, the experimentally measured aqueous phase concentrations of 1,2-TCA at t=0 ranged from 0.42 to 0.45 mM (56.0 to 60.0 mg/L), corresponding to 90.7 to 98.8 µmoles per bottle total (considering both aqueous and gas phases). For 1,2-DCA, the starting aqueous phase concentrations ranged from 0.56 to 0.75 mM (55.4 to 74.2 mg/L), with the total quantity of 1,2-DCA ranging from 122 to 140 µmoles per bottle. For 1,2-DCP, the t=0 aqueous phase concentrations ranged from 0.53 to 0.58 mM

(59.9 to 65.5 mg/L), with a total quantity of 1,2-DCP ranging from 136 to 151 µmoles per bottle. Thus, the experimentally measured concentrations at t=0 were close to the target levels.

The mean concentrations of 1,2-DCA, 1,2-DCP, 1,1,2-TCA, ethene, propene, and VC plotted versus time for both strain IP3-3 and *D. lykanthroporepellens* BL-DC-9^T inoculated serum bottles can be seen in Figure 3.6. Data for each of the replicate bottles plotted separately is shown in Appendix A, Figure A.4.

For bottles inoculated with strain IP3-3, the quantity of VC measured at t=7.00 days was 41.1±2.25 µmoles per bottle, with a remaining aqueous concentration of 1,1,2-TCA of 0.13±0.03 mM (17.3±4.00 mg/L). This quantity of VC accounts for greater than 40% of the 1,1,2-TCA initially measured at t=0. At the same time step (i.e., t=7.00 days), the quantity of propene was 13.6±1.36 µmoles per bottle, which accounts for greater than 5% of the starting 1,2-DCP. At t=9.13 days (the third time samples were taken), the quantity of VC and propene had increased to 66.5±9.25 and 56.4±13.4 µmoles per bottle respectively. The corresponding aqueous concentration remaining of 1,1,2-TCA and 1,2-DCP were 0.01±0.01mM (1.33±1.33 mg/L) and 0.19±0.08 mM (21.5±9.04 mg/L) respectively. At the same time step (i.e., t=9.13 days), the quantity of ethene had increased to 21.7±9.01 µmoles per bottle, which corresponds to greater than 5% of the starting quantity of 1,2-DCA. Thereafter, 1,2-DCA, 1,2-DCP, and 1,1,2-TCA concurrently decreased, with concomitant production of ethene, propene, and VC.

The mass balance for 1,2-DCA and 1,1,2-TCA was calculated as the sum of 1,1,2-TCA, 1,2-DCA, VC, and ethene. This was necessary because, as pointed out in Sections 3.2 and 3.3, there was some abiotic transformation of 1,1,2-TCA to 1,2-DCA. Consequently, mass balance recovery calculated solely on the basis of the parent compound 1,1,2-TCA and the biologically

produced final product of VC would underestimate recovery of the starting 1,1,2-TCA, and recovery calculated solely on the basis of the parent compound 1,2-DCA and the biologically produced final product of ethene would overestimate recovery of the starting 1,2-DCA. For strain IP3-3, the total mass of the four compounds (1,1,2-TCA, 1,2-DCA, VC, and ethene) observed at the end (i.e., t=22.6 days), or removed via sampling, accounted for 71.8±6.51% of that present at the beginning of the experiment. Likewise, mass balance calculations for 1,2-DCP and propene showed 66.0±2.13% was recovered at the end (i.e., t=22.6 days), or removed via sampling. The fraction of compounds removed via sampling accounted for ≤0.74% of the contaminants present at t=0. In abiotic (uninoculated) negative controls the quantity of the daughter product VC was ≤15.5 μmoles per bottle, which was ≤16.1% of the starting 1,2-TCA. The quantity of the daughter product ethene was ≤0.00 μmoles per bottle, which was less than 0% of the starting 1,2-DCA. The quantity of the daughter product propene was ≤0.03 μmoles per bottle, which was ≤0.02% of the starting molar mass of 1,2-DCP. The same set of negative controls was used for both strains.

For bottles inoculated with *D. lykanthroporepellens* strain BL-DC-9^{T}, a pattern similar to strain IP3-3 was observed. VC corresponding to greater than 30% of the starting 1,1,2-TCA was observed at t=4.10 days. At the next time step when samples were analyzed (i.e., t=7.00), the quantity of ethene and propene had increased to greater than 5% of the initial quantity of 1,2-DCA and 1,2-DCP present at t=0. Thereafter, 1,2-DCA, 1,2-DCP, and 1,1,2-TCA concurrently decreased with simultaneous production of ethene, propene, and VC. At the time step when VC corresponding to greater than 20% of the starting 1,1,2-TCA was first observed (i.e., t=4.10 days), the aqueous concentrations of 1,2-DCA and 1,2-DCP were 0.509±0.002 mM (50.3±0.19 mg/L) and 0.36±0.03 (40.7±3.38 mg/L) respectively.

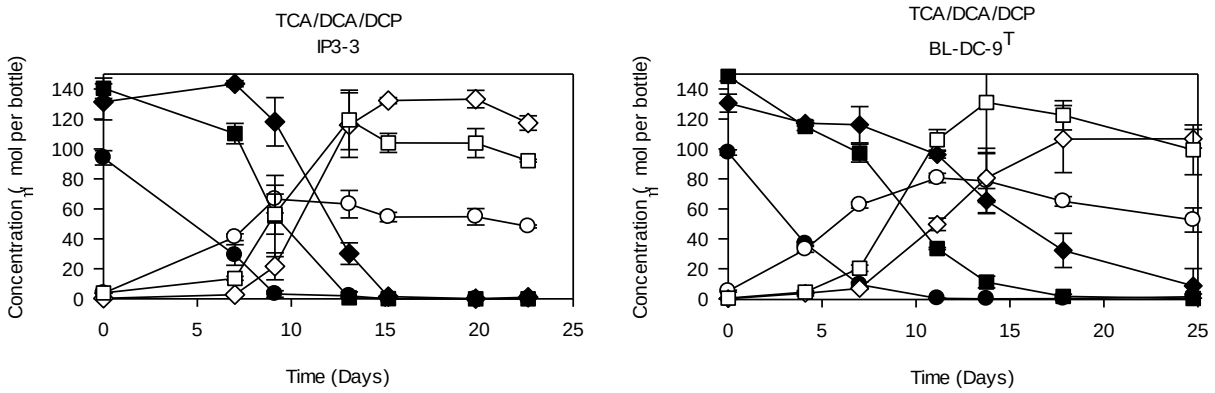

Figure 3.6. Experimentally measured 1,2-TCA (closed circles), 1,2-DCA (closed diamonds), 1,2-DCP (closed squares), vinyl chloride (open circles), ethene (open diamonds), and propene (open squares) as a function of time in serum bottles inoculated with strain IP3-3 (left) or *D. lykanthroporepellens* BL-DC-9^T (right). Target starting concentrations were 0.5 mM for the parent compounds. Each data point represents the mean calculated average from duplicate bottles. Error bars represent one standard deviation

At the time step where both ethene and propene were measured to be greater than 5% of the maximum 1,2-DCA and starting 1,2-DCP concentration, the aqueous-phase concentrations of 1,2-DCA, 1,2-DCP, and 1,1,2-TCA were 0.50±0.06 mM(49.4±6.14 mg/L), 0.32±0.03 mM (36.6±3.62 mg/L), and 0.044±0.004 mM (5.87±0.53 mg/L) respectively.

Mass balance calculations for *D. lykanthroporepellens* BL-DC-9^T indicated that the mass of 1,1,2-TCA, 1,2-DCA, VC, and ethene present at the end (i.e., t=24.8 days), or removed via sampling, accounted for 73.6±14.1% of the 1,1,2-TCA and 1,2-DCA present at the beginning of the experiment. Likewise, mass balance calculations for the mass of 1,2-DCP and propene showed 67.6±9.15% was recovered at the end (i.e., t=24.8 days), or removed via sampling. The fraction of compounds removed via sampling accounted for ≤0.86% of the contaminants present at t=0.

For both strains, appreciable VC production (i.e., >30% of the starting 1,1,2-TCA on a molar basis) was first observed when the mean aqueous-phase concentration of 1,2-DCA was at least 0.64±0.002 mM (63.3±0.197 mg/L), and the mean 1,2-DCP aqueous concentration was at

least 0.40±0.01 mM (45.2±1.13 mg/L). Appreciable propene production (>5% of the starting 1,2-DCP on a molar basis) was first observed when the mean aqueous concentration of 1,2-DCA was at least 0.64±0.002 mM (63.3±0.197 mg/L) and the mean aqueous concentration of 1,1,2-TCA was at least 0.10±0.03 mM (17.8±4.15 mg/L). Appreciable ethene production was first observed when the aqueous concentration of 1,2-DCP was at least 0.32±0.03 mM (36.6±3.38 mg/L) and the 1,1,2-TCA aqueous concentration was at least 0.044±0.004 mM (5.87±0.57 mg/L). As observed in the binary mixtures, appreciable vinyl chloride production was observed earlier than appreciable production of propene and ethene. 1,2-DCP and 1,1,2-TCA were degraded concurrently, with both strains dechlorinating these compounds at the same time when concentrations were on the order of 0.1 to 0.4 mM. Likewise, both strains consumed 1,2-DCA and 1,2-DCP concurrently when the contaminants were present at concentrations on the order of 0.3 to 0.6 mM., Appreciable production of ethene, the final product of 1,2-DCA reductive dechlorination was not observed until the 1,1,2-TCA concentration was relatively low (i.e., <0.1 mM), making the utilization pattern appear to be largely sequential rather than concurrent.

3.5 Discussion and Implications

Table 3.1 displays the half reactions and net change in Gibbs free energy for the reduction reactions of the chlorinated alkanes dehalogenated in this study. In theory, all of the compounds of interest can be reduced in energy yielding reduction reactions due to the negative ΔG value calculated from the half reactions.

When comparing the values of net change in Gibbs free energy, it is apparent that bacteria such as *Dehalogenimonas,* stand to gain more energy by reducing 1,1,2-TCA than by reducing either 1,2-DCA or 1,2-DCP. Thus, the preferential use of 1,1,2-TCA over 1,2-DCA or 1,2-DCP when they are present as a mixture may be advantageous to microorganisms.

Table 3.1 Thermodynamics of reduction half reactions for the chlorinated compounds of concern (data from De Wildeman and Verstraete, 2003)

Electron Acceptor	**Half Reaction**	***ΔG(KJ)/Electron**
1,1,2-Trichloroethane	$Cl_2HC–CH_2Cl + 2e- \rightarrow ClHC=CH_2 + 2Cl–$	-78.1
1,2-Dichloroethane	$ClH_2C–CH_2Cl + 2e- \rightarrow H2C=CH2 + 2Cl–$	-71.4
1,2-Dichloropropane	$ClH_2C–CHCl–CH_3 + 2e- \rightarrow H_2C=CH–CH_3 + 2Cl–$	-69.6

*calculated assuming pH=7, $[Cl^-]$=1 M, T=25 °C

The experimental observation that 1,1,2-TCA was preferentially dechlorinated over 1,2-DCA is consistent with the notion that the bacteria would use the most thermodynamically favorable reaction. From a thermodynamic perspective, the experimental observation that 1,2-DCA and 1,2-DCP were reductively dechlorinated more-or-less concurrently is not surprising given their close Gibbs free energy values.

The toxicity of non-chlorinated solvents (e.g., alcohols, alkanes, and aromatic hydrocarbons) has been found previously to be correlated to hydrophobicity measured in terms of the log of the octanol/water partition coefficients (i.e., log K_{ow}) (Sikkema *et al.,* 1995). Compounds with a log K_{ow} in the range of 1.5 to 4 are generally toxic to microorganisms (Inoue and Horikoshi, 1991; Sikkema *et al.,* 1994), with maximum toxicity occurring for compounds when the log K_{ow} was between 2 and 4 (Kieboom and de Bont, 2000). Bowman *et al*. (2009) reported that the adverse effects of 1,2-DCA, 1,1,2-TCA, and PCE on hydrogen production by a variety of *Clostridium* species was consistent with these previous observations regarding the correlation between log K_{ow} and toxicity for non-chlorinated solvents. 1,2-DCA (log Kow 1.48) (Alvarez & Illman, 2006) had the least effect on hydrogen production, followed by 1,1,2-TCA (log Kow 2.47) (Alvarez & Illman, 2006), and PCE (log Kow 2.60) (Alvarez & Illman, 2006). Maness (2012) demonstrated a similar relationship when assessing the ability of *D. lykanthroporepellens* BL-DC-9^T and strain IP3-3 to reductively dechlorinate high concentrations of 1,2-DCA, 1,2-DCP, and 1,1,2-TCA. Maness (2012) reported that for equal molar

concentrations, 1,1,2-TCA (log K_{ow} 2.47, Alvarez and Illman, 2006) had a more adverse effect than both 1,2-DCP (log K_{ow} 2.0, Alvarez and Illman, 2006) and 1,2-DCA (log k_{ow} 1.48, Alvarez and Illman, 2006). In the results presented in this thesis, 1,1,2-TCA dechlorination was consistently observed prior to both 1,2-DCA and 1,2-DCP dechlorination. A plausible explanation for this dechlorination pattern is that the organisms may have been responding to environmental stresses induced by the higher toxicity of 1,1,2-TCA relative to 1,2-DCP and 1,2-DCA. In other words, the microorganisms may have preferentially removed the most toxic compound first.

A better understanding of bacterial dehalogenation progression has important implications for modeling cleanup of halogenated solvents at the PPI site and many other sites. For example, some fate and transport models, such as BIOSCREEN, utilize a lumped kinetic degradation parameter that assumes concurrent transformation of all present contaminants (USEPA, 1996). If diauxic growth is occurring, the models could greatly under predict the spread of a contaminant, due to the fact that an assumed degradation rate is applied in a spatial domain where, in fact, no transformation is occurring. Alternatively, the accuracy of models such as BIOCHLOR has the potential to be improved. BIOCHLOR can model multiple independent zones of biodegradation (USEPA, 2000). For the case of diauxic growth of the tested compounds, manipulation of the rate coefficients in individual degradation zones would more closely simulate actual contaminant transformation patterns. For example, input of a non-preferentially degraded compound (e.g. 1,2-DCA) rate coefficient as zero in the first zone, when a more "preferred" compound (e.g. 1,1,2-TCA) is present, and then inputting a non-zero rate coefficient in zone two once the "preferred" compound has been degraded to concentrations below a certain threshold. The fact that *Dehalogenimonas* spp. shows signs of both concurrent

and preferential transformation may allow these models to be more accurately applied in the future.

As a basis for comparison to actual site data, the groundwater in the well where *Dehalogenimonas* BL-DC-9^T was first isolated contained a combination of 1,2-DCA, 1,2-DCP and 1,1,2-TCA at average concentrations of 540 mg/L (5.46 mM), 67.3 mg/L (0.60 mM), and 367 mg/L (2.75 mM) respectively (Bowman *et al.,* 2006). While experiments described in this thesis did not specifically test a mixture of contaminant at these concentrations and there were additional contaminants present in the groundwater (see Table 1.1), and there were many differences between the site groundwater geochemistry and the media formulation employed here, the results from this study suggest that if either strain tested was present as the only dehalogenating bacterium at this site, the microorganisms would degrade this mixture of contaminants at the concentrations measured in succession, beginning with 1,1,2-TCA, then 1,2-DCP, followed by 1,2-DCA. In the presence of such high concentrations of 1,1,2-TCA, it is plausible that dechlorination of 1,2-DCP and 1,2-DCA may not occur or my occur only at a slow rate. On the other hand, the contaminant concentrations in the well where strain IP3-3 was first isolated for 1,2-DCA, 1,2-DCP, and 1,1,2-TCA were 2.30 mg/L (0.02 mM), 2.46 mg/L (0.02 mM), and 1.36 mg/L (0.01 mM) respectively (Bowman *et al*, 2012). Results from the experiments reported here suggest that if either *Dehalogenimonas* strain was presented with this combination of compounds at the concentrations observed, all three compounds would likely be degraded concurrently.

CHAPTER 4: OVERALL CONCLUSIONS AND RECOMMENDATIONS FOR FUTURE WORK

4.1 Conclusions and Implications

Both *Dehalogenimonas lykanthroporepellens* BL-DC-9^T and *Dehalogenimonas* strain IP3-3 were shown to be able to dehalogenate 1,1,2-TCA, 1,2-DCA, and 1,2-DCP when present as co-contaminant mixtures. The pathways by which the reductive dehalogenation occurred for contaminants present in mixtures appears to be identical that observed by Yan *et al* (2009) and Bowman *et al.* (2012) for dehalogenation of single contaminants (i.e., an exclusively dihaloelimation reaction mechanism) by these strains. At the contaminant concentrations tested here, appreciable 1,1,2-TCA dechlorination to vinyl chloride was consistently observed prior to 1,2-DCA dechlorination in binary and tertiary mixtures. Appreciable dechlorination of 1,2-DCA to ethene was not observed until 1,1,2-TCA concentrations were low (on the order of 0. 1 mM or lower). In contrast, 1,2-DCA and 1,2-DCP were utilized concurrently and the combination of 1,2-DCP and 1,1,2-TCA were utilized concurrently over a comparatively wider range of concentrations.

These results have practical implications with respect to the decision-making process in site remediation. For example, the PPI Superfund site where both strains were isolated contained a co-contaminant combination of 1,2-DCA, 1,2-DCP, and 1,1,2-TCA. Results from this study suggest that the high concentration (i.e., 5.46 mM) of 1,1,2-TCA present in the DNAPL source zone at the Brooklawn portion of the site may inhibit transformation of 1,2-DCA and 1,2-DCP. Furthermore, results suggest that the lower 1,1,2-TCA concentration (i.e., 0.02 mM) at the Scenic Highway portion of the site would likely allow for concurrent degradation of all three

contaminants tested (i.e., 1,2-DCA, 1,2-DCP, and 1,1,2-TCA) by both *Dehalogenimonas* strains evaluated here.

Though it remains to be seen whether other as-yet uncharacterized bacteria exhibit similar dechlorination patterns, the research presented here provides an important step in the characterization of bacteria able to reductively dehalogenate chlorinated aliphatic alkanes, a class of compounds that remains relatively poorly understood in terms of their biodegradation pathways and processes.

4.2 Future Work

Throughout the progression of the experiments in this thesis, many ideas for future work came to light. One direction for future research would be further study into the mechanism behind the phenomena observed in the 1,1,2-TCA and 1,2-DCA binary mixture. Though speculation over preference based on higher energy yield is presented in Section 3.5, it remains largely unknown why the organisms preferentially degraded 1,1,2-TCA over 1,2-DCA when present as a mixture. *Dehalogenimonas* is a relatively recently described genus, and little is known about the biological mechanisms that mediate the dihaloelimation reactions. Further studies into dehalogenase gene expression could shed some light on the mechanisms behind this phenomenon. For example, it is possible that both 1,1,2-TCA and 1,2-DCA are transformed by the same enzyme and the enzyme has higher affinity for 1,1,2-TCA than for 1,2-DCA. Alternately, there are many putative reductive dehalogenases in the recently sequenced genome of *D. lykanthroporepellens* BL-DC-9^{T} (Siddaramappa *et al.,* 2012), and the transformation of each halogenated compound may be mediated by different enzymes regulated by yet-to-be elucidated genetic-level control mechanisms.

Future research could also include similar mixture experiments, but with compounds other than those present in the experiments described in this thesis. In both groundwater wells where the *Dehalogenimonas* strains presented here were first isolated, 1,2-DCA, 1,2-DCP, and 1,1,2-TCA were not the sole co-contaminants present (Table 1.1 and Table 1.2). In particular, further exploration into the effects of mixtures of compound that are unable to be dechlorinated by *Dehalogenimonas* (i.e., chloroform or PCE) with compounds that are known to be dechlorinated by the microorganisms (i.e., chlorinated alkanes and propanes) would have practical implications for understanding potential constraints on contaminant biotransformation by these bacteria at the PPI Superfund site and other sites around the world.

Practical applications of these strains in real-world bioaugmentation remediation approaches would benefit from further studies to determine how these organisms will perform under site specific groundwater conditions. The experiments presented in this thesis were conducted under relatively ideal growth conditions (i.e., temperature of 30°C, high hydrogen concentration in the gas headspace, and readily available nutrient supply) compared to those found at most if not all contaminated sites. In essence, the present experiments may poorly mimic site conditions. Further examination of how the genus *Dehalogenimonas* perform under actual site conditions (i.e., ambient ground water temperatures, nutrients at limiting concentrations, and in mixed cultures) would aid in decision-making related to the use of these microorganisms as a tool in various bioremediation strategies.

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APPENDIX: INDIVIDUAL BOTTLE GRAPHS

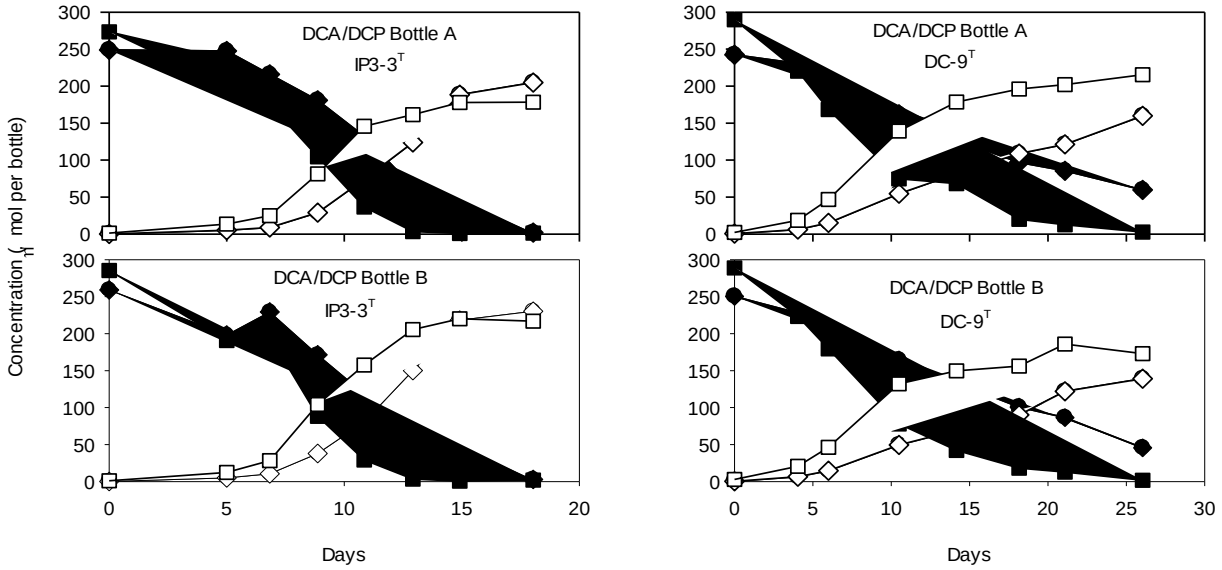

Figure A.1. Individual bottle analysis of experimentally measured 1,2-DCA (closed diamonds), 1,2-DCP (closed squares), ethene (open diamonds), and propene (open squares) as a function of time in serum bottles inoculated with strain IP3-3 (left) or *D. lykanthroporepellens* BL-DC-9^T (right). Target starting concentrations were 1 mM for the parent compounds. Each data point represents the mean calculated average from duplicate bottles. Error bars represent one standard deviation.

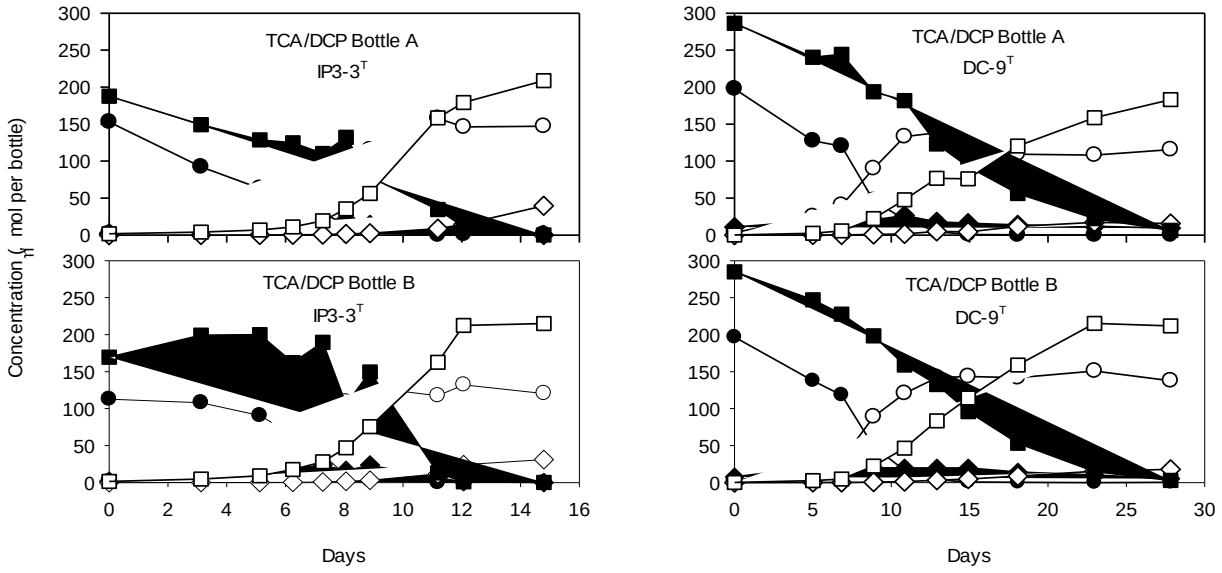

Figure A.2. Individual bottle analysis of experimentally measured 1,2-TCA (closed circles), 1,2-DCA (closed diamonds), 1,2-DCP (closed squares), vinyl chloride (open circles), ethene (open diamonds), and propene (open squares) as a function of time in serum bottles inoculated with strain IP3-3 (left) or *D. lykanthroporepellens* BL-DC-9^T (right). Target starting concentrations were 1 mM for the parent compounds. Each data point represents the mean calculated average from duplicate bottles. Error bars represent one standard deviation

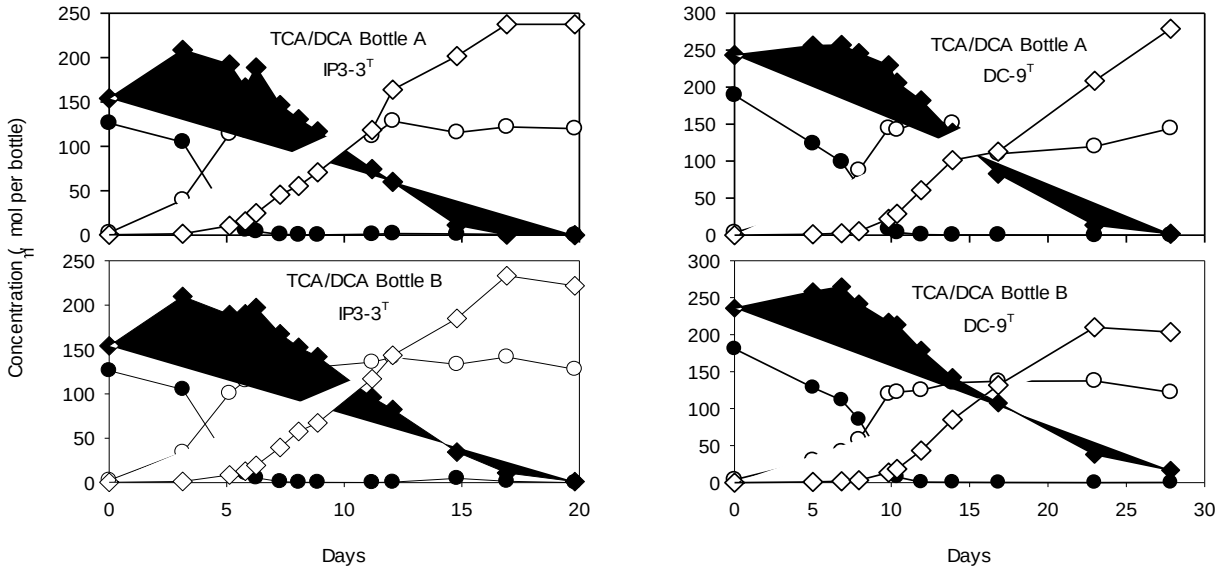

Figure A.3. Individual bottle analysis of experimentally measured 1,2-TCA (closed circles), 1,2-DCA (closed diamonds), vinyl chloride (open circles), and ethene (open diamonds) as a function of time in serum bottles inoculated with strain IP3-3 (left) or *D. lykanthroporepellens* BL-DC-9^T (right). Target starting concentrations were 1 mM for the parent compounds. Each data point represents the mean calculated average from duplicate bottles. Error bars represent one standard deviation

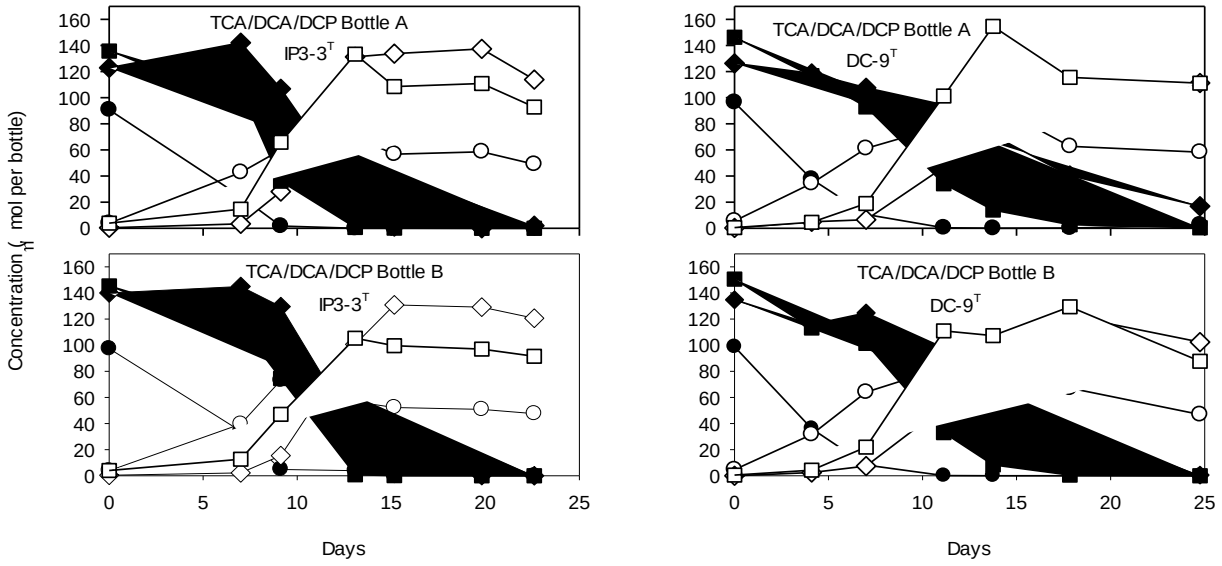

Figure A.4. Individual bottle analysis of experimentally measured 1,2-TCA (closed circles), 1,2-DCA (closed diamonds), 1,2-DCP (closed squares), vinyl chloride (open circles), ethene (open diamonds), and propene (open squares) as a function of time in serum bottles inoculated with strain IP3-3 (left) or *D. lykanthroporepellens* BL-DC-9^T (right). Target starting concentrations were 1 mM for the parent compounds. Each data point represents the mean calculated average from duplicate bottles. Error bars represent one standard deviation.

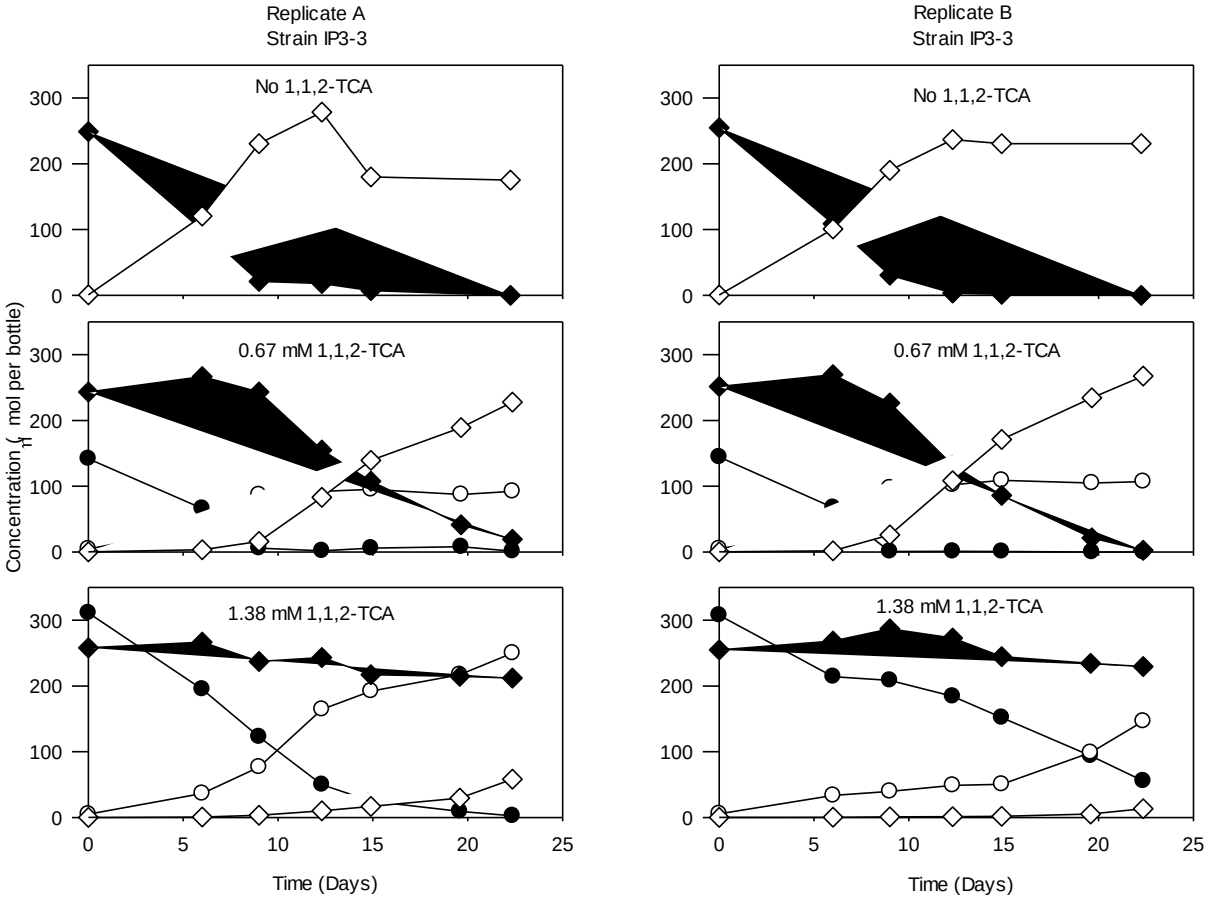

Figure A.5. Experimentally measured 1,2-TCA (closed circles), 1,2-DCA (closed diamonds), vinyl chloride (open circles), and ethene (open diamonds) as a function of time in replicate serum bottles inoculated with strain IP3-3. Row 1 displays bottles amended with 1,2-DCA only. Rows 2 and 3 display bottles amended with a mixture of 1,2-DCA and 1,1,2-TCA (1,1,2-TCA average starting aqueous concentration for displayed on title)

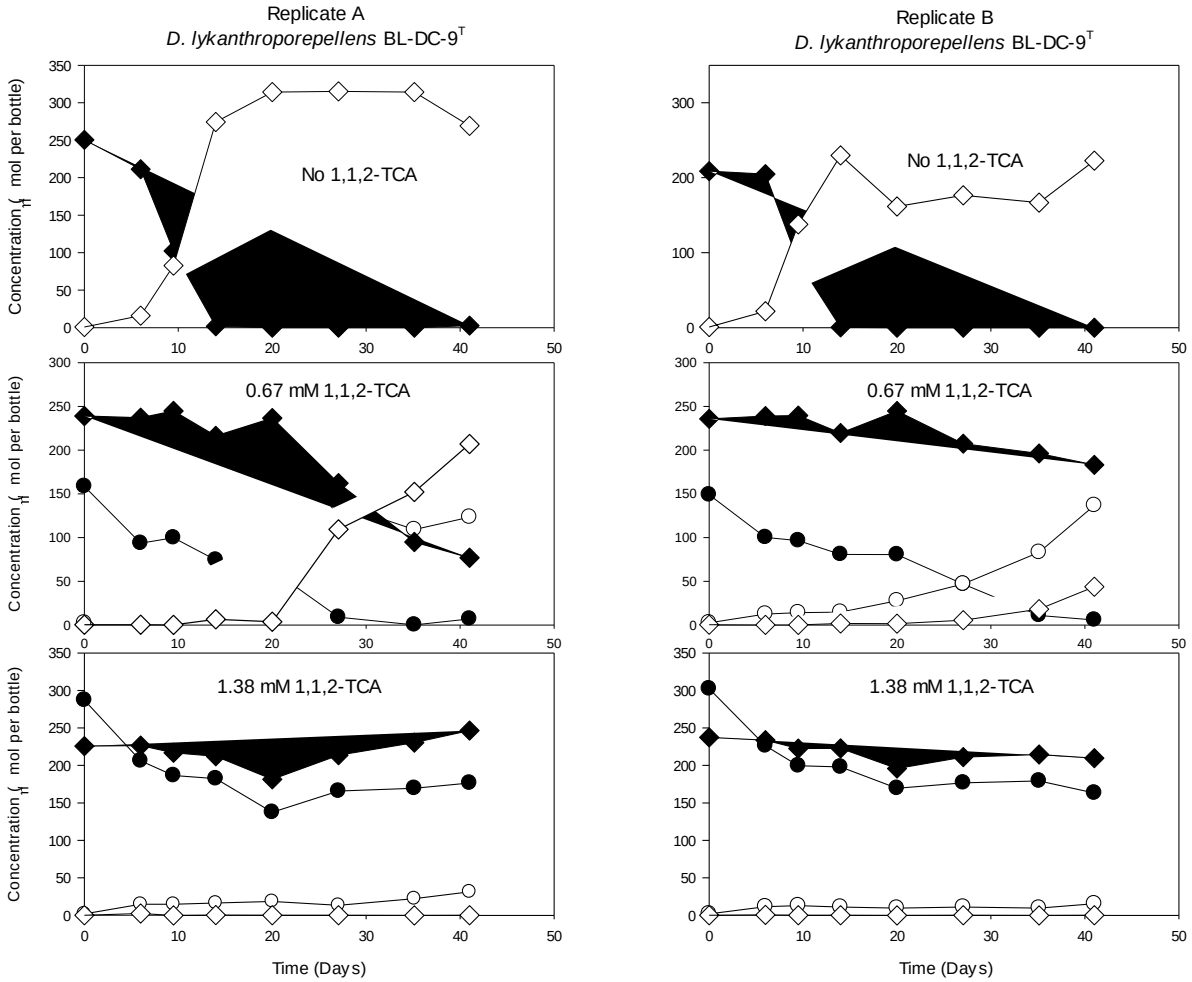

Figure A.6. Experimentally measured 1,2-TCA (closed circles), 1,2-DCA (closed diamonds), vinyl chloride (open circles), and ethene (open diamonds) as a function of time in replicate serum bottles inoculated with strain IP3-3. Row 1 displays bottles amended with 1,2-DCA only. Rows 2 and 3 display bottles amended with a mixture of 1,2-DCA and 1,1,2-TCA (1,1,2-TCA average starting aqueous concentration for displayed on title)

VITA

Jacob Dillehay was born in Lake Charles, LA on April 7th, 1987. He was raised in Iowa, LA. He is the son of Jeffrey and Tracie Dillehay. He currently resides in Jarreau, LA on False River. Jacob graduated from Iowa High School in Iowa, LA in May, 2005. He then moved to Baton Rouge, LA to attend LSU and pursue an undergraduate degree in Civil Engineering. Due to his passion for the outdoors and the environment, in his third semester, he changed his major to Environmental Engineering. After taking two undergraduate courses taught by Dr. William Moe, he approached the professor about a job. Dr. Moe agreed to take him on as an undergraduate student worker. While working in the lab, the need to learn more pushed him to want to obtain a higher level degree. In May 2010, Jacob graduated from LSU with a B.S. degree in Environmental Engineering. He entered graduate school in the following August under the same Dr. William Moe. Upon the completion and defense of this thesis, Jacob will have completely all the requirements for a Master's degree in Civil Engineering, and will collect his diploma in December of 2012.