

# Impact of Mixtures of Chlorinated Aliphatic Hydrocarbons on a High-Rate, Tetrachloroethene-Dechlorinating Enrichment Culture

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The ability of a high-rate, tetrachloroethene (PCE)-dechlorinating culture with apparent dehalorespiring activity to function in the presence of fortuitously transformed cocontaminants carbon tetrachloride (CT) and 1,1,1-trichloroethane (1,1,1-TCA) was examined. A PCE-acclimated, lactate-enrichment culture that demonstrated the ability to rapidly transform PCE to ethene in conjunction with methanogenesis was able to degrade both CT and 1,1,1-TCA despite no previous exposure to these compounds. While the presence of <math><20\ \mu\text{M}</math> of 1,1,1-TCA had little effect on PCE removal, the addition of 10–15  $\mu\text{M}$  of CT negatively impacted both the PCE and vinyl chloride (VC) transformation steps. CT and 1,1,1-TCA primarily inhibited methanogenesis before each compound was completely biotransformed. They served to further inhibit methanogenesis as well as utilization of acetate and propionate in PCE-containing treatments via increased persistence of metabolites such as VC. The inclusion of CT with PCE increased peak hydrogen concentrations from 2 to 20  $\mu\text{M}$ , suggesting that CT disrupted the ability of dechlorinating and other hydrogenotrophic organisms to maintain low hydrogen thresholds. Despite the negative impacts on multiple populations as a result of the addition of CT and 1,1,1-TCA, transformation proceeded in treatments containing all three target compounds. This indicates that the specific dechlorinating organisms within the culture were capable of either transforming these cocontaminants or remaining functional, while nonspecific organisms mediated their removal.

## Introduction

Chlorinated aliphatic hydrocarbons constitute one of the major classes of groundwater pollutants due to their widespread use and the potential for adverse health effects following exposure. Extensive research has been conducted to develop effective methods for applying bioremediation due to the potential for reducing the extent of contamination at costs lower than those associated with many traditional physical-chemical methods (1). Reductive dechlorination and other biologically mediated transformations of highly chlorinated compounds have been well-documented in laboratory studies with mixed and pure anaerobic cultures (2–5).

Early research indicated that these reactions were catalyzed by nonspecific metallocoenzymes (6) and that these

transformation resulted in no direct benefit for the mediating organism (7). There is no published information to-date that contradicts the theory that highly chlorinated ethanes and methanes such as 1,1,1-trichloroethane (1,1,1-TCA) and carbon tetrachloride (CT) are transformed solely via this type of “fortuitous” reaction (8). However, a number of recent studies have provided evidence that transformation of tetrachloroethene (PCE) and other chlorinated ethenes can be coupled to energy generation. These reductive dechlorination reactions occur through use of the chlorinated ethene as an electron acceptor in the presence of an added electron source in a process termed dehalorespiration (9). A wide variety of organisms have been isolated which are capable of energy-conserving chlorinated ethene transformations (8), although only one isolate has thus far been identified which can mediate the complete dechlorination of PCE to ethene. *Dehalococcoides ethenogenes* strain 195 has been shown to catalyze each of the reductive dechlorination steps required to produce ethene (10), while the majority of other isolates stop at *cis*-1,2-DCE (cDCE). Because these other dehalorespirers have been isolated from enrichment cultures in which ethene was the predominant endproduct, it is likely that uncharacterized organisms within these cultures facilitate the transformation of the less-chlorinated metabolites.

The increasing documentation of PCE dechlorination and ethene production within contaminated sites suggests that dehalorespirers may be prevalent in the environment (11). Acclimation of native microbes through exposure to chlorinated ethenes at these sites appears to provide a selective pressure for the development and growth of such organisms. The resulting potential for complete transformation of chlorinated ethenes to relatively harmless products has played no small role in the interest in implementing natural attenuation or enhanced bioremediation strategies at these sites.

While there are a number of pilot-scale chlorinated ethene bioremediation projects that appear to be initially successful, broader application requires addressing the problems associated with complex contaminated sites. Available data from the 1430 sites found on the National Priority List (as identified by the USEPA) in 1997 suggests that many were contaminated by more than one class of pollutant (12). For example, PCE or other chlorinated ethenes were present in at least 771 sites, while CT or one of its expected chlorinated metabolites (in particular chloroform) were present in at least 717 sites. Combined with the observation that 1,1,1-TCA was present in at least 696 sites, it is clear that a large number of sites can be characterized as containing a complex mixture of contaminants.

The feasibility of remediating such sites using currently favored methodologies is largely unexplored. Natural attenuation models designed to estimate the amount of time necessary to completely transform a plume of PCE often rely on input parameters. If these parameters are determined under the assumption that dehalorespiration is possible, it must be verified that the presence of other common pollutants has a minimal influence on the dechlorination activity and viability of dehalorespirers. In situations where bioaugmentation might be considered, it is important to examine the impact of potentially inhibitory cocontaminants on these specialized organisms as well as on the microbial community as a whole.

The objective of this study was to assess the impact of two fortuitously transformed compounds, CT and 1,1,1-TCA, on a lactate-enrichment culture which displays dehalorespiring tendencies. This mixed culture was developed via an ac-

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TABLE 1. GC/Detector/Column Conditions Used during Headspace Analysis

method	1	2	3	4	5
analyte	DCM, 1,1-DCA, tDCE, 1,1-DCE,	CA, VC, methane	hydrogen	CT, CF, 1,1,1-TCA, PCE, TCE, cDCE	ethene
detector	ECD	FID	TCD	ECD	FID
carrier gas	N <sub>2</sub> at 2–3 mL/min	N <sub>2</sub> at 2–3 mL/min	N <sub>2</sub> at 60 mL/min	N <sub>2</sub> at 2–3 mL/min	N <sub>2</sub> at 60 mL/min
column (length, inner diameter, film thickness)	DB WAX capillary (30 m, 0.320 mm, 1.8 μm)	DB WAX capillary (30 m, 0.53 mm, 1.0 μm)	Hayesep Q 80/20 packed (8 ft, 0.125 in, NA)	DB5 capillary (30 m, 0.320 mm, 0.25 μm)	HP Porapax Q packed (6 ft, 0.125 in, NA)
oven temp (°C)	35	35	120	35	55
injection temp (°C)	150	150	150	150	50
detector temp (°C)	250	250	250	250	250

climation process that resulted in rapid transformation of high concentrations of PCE to ethene. Because this transformation occurred in conjunction with complete conversion of lactate to methane, the effect of each of these contaminants on different populations in the mixed culture was examined using contaminant disappearance, substrate utilization, methane production, and hydrogen evolution as indicators. Experiments focused on batch transformation of PCE in the presence of increasing concentrations of CT and 1,1,1-TCA. The results demonstrate that while these compounds can be transformed simultaneously with PCE, their presence had a negative impact on the activity of multiple communities within the enrichment culture.

## Materials and Methods

**Chemicals.** The majority of the chlorinated ethenes were obtained as neat liquids: tetrachloroethene (Sigma), trichloroethene (TCE) (Aldrich), *cis*-1,2-dichloroethene (cDCE) (Sigma), *trans*-1,2-dichloroethene (tDCE) (Sigma), 1,1-dichloroethene (1,1-DCE) (Sigma), 1,1,1-trichloroethane (1,1,1-TCA) (Aldrich), 1,1-dichloroethane (1,1-DCA) (Chem Service), chloroethane (CA) (Sigma), carbon tetrachloride (Sigma), chloroform (CF) (Sigma), and dichloromethane (DCM) (Sigma). Vinyl chloride (VC) (Sigma) was obtained in a solution of 2000 μg/mL of methanol for use in standard preparation. All purchased chemicals were either HPLC or ACS grade. Gases purchased from Air Products, Inc. included N<sub>2</sub>, N<sub>2</sub>/CO<sub>2</sub> (80/20% v/v), methane, ethene, ethane, and H<sub>2</sub>. Liquid chemicals used were glacial acetic acid (ACS grade, Fisher), lactic acid (ACS, Fisher), propionic acid (Certified, Fisher), and methanol (Optima, Fisher).

**Analytical Methods.** Gas chromatography headspace analysis was employed in the measurement and quantification of volatile compounds. Multiple GC/detector setups were used to quantify the complete set of analytes (Table 1). All GCs were Hewlett-Packard models. Headspace samples (100 μL) were taken from the reactors using gastight, luer-lock syringes (Supelco) and injected manually.

Fatty acid concentrations were determined by direct liquid injections (100 μL) into a Hewlett-Packard model 1100 HPLC. At least 1 mL of sample was transferred to 2-mL vials using 0.45 μM Millipore syringe filters. Vials were crimped and stored for later analysis via an autosampler. Instrument flowrate was 1 mL/min with 0.001 N H<sub>2</sub>SO<sub>4</sub> as the eluent. Compounds were separated on an Alltech anion exclusion column measuring 300 mm long with an inner diameter of 7.8 mm.

Aqueous calibration standards were prepared in 38-mL serum bottles containing 25 mL of liquid volume and known masses of chlorinated aliphatics dissolved in methanol. A minimum of four standards from the set were run daily. Instrument response was plotted versus known standard concentration to develop a linear calibration curve ( $r^2 > 0.99$  in all cases). This relationship was then used to determine the nominal aqueous concentration within reactors. Henry's constants were used to account for partitioning of the volatile

organic compounds from liquid to air in headspace analysis. Dimensionless Henry's constants at 20 °C were obtained using techniques described by Gossett (13).

**Culture Enrichment and PCE-Aclimation.** The development of the PCE-acclimated lactate-enrichment culture used in this study was detailed previously (14). A stock reactor was seeded with anaerobic digester sludge with no known prior exposure to any chlorinated aliphatic hydrocarbons. The stock reactor, operated at an 80-day hydraulic retention time to maintain a biomass concentration of roughly 200 mg of volatile suspended solids per liter, served as a source for batch studies. At the time of the experiments detailed here, the stock reactor was dosed with 4.3 mM of lactate and 74 μM of PCE every 2 days. It was confirmed that this dose of PCE was completely removed with ethene as the only product before the beginning of the subsequent wasting and feeding cycle.

**Batch Reactor Preparation for Mixtures Studies.** Experiments were conducted in 38-mL serum bottles seeded with 25 mL of suspended biomass from the stock reactors. Anaerobic conditions were created by purging inverted water-filled reactors with N<sub>2</sub>/CO<sub>2</sub> gas. Reactors were then capped with Teflon-lined rubber septa while minimizing residual water. Aluminum crimp caps completed the seal. Prior to any culture transfer, it was verified that the most recent chlorinated ethene dose to a stock reactor had been completely transformed. Because of the presence of primary substrate metabolites in the stock reactor, it was necessary to measure the concentration of lactate, acetate, and propionate prior to initiation of an experiment. Cultures were added directly to the batch reactors in an anaerobic glovebox to further prevent incidental O<sub>2</sub> exposure. Reactors were purged immediately after removal from the glovebox to eliminate hydrogen contributions from the tent atmosphere (typically 1.0–2.0%). This reduced potential interference in future hydrogen measurements and also served to remove the majority of background methane. Aliquots of the chlorinated aliphatic compounds were added via a gastight syringe (Hamilton) as either neat compound (PCE) or from saturated aqueous stock solutions (CT = 800 mg/L, 1,1,1-TCA = 1440 mg/L). The initial concentration of PCE was generally set at 200 μM (nominal concentration assuming all mass present in liquid phase) to provide a basis for comparison between treatments. Initial concentrations of 1,1,1-TCA and CT were varied but were generally kept lower than 20 μM due to their potential to act as toxic substrates. Lactate was then added to the serum bottle reactors at an initial concentration of 2.14 mM. To avoid potential shifts in culture dynamics, care was taken to minimize the amount of time between culture transfer and contaminant + lactate spikes. Media (cell-free) controls were prepared in a manner identical to biotic reactors and were used to account for potential abiotic losses. All treatments were performed in duplicate, with mean values presented (standard deviations were generally less than 10%). A constant mixing speed of 160 rpm was provided by a rotary shaker table (Lab-Line).

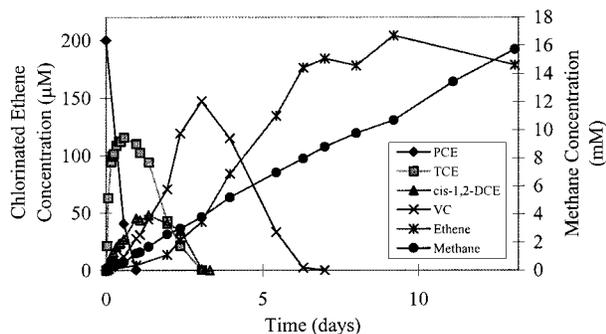


FIGURE 1. Dechlorination of 200  $\mu\text{M}$  PCE and methane production by LEC 1.

Reactors were stored in a 20 °C climate-control laboratory in the absence of light.

## Results and Discussion

**Culture Characteristics.** Following more than 1 year of acclimation, the PCE-acclimated culture (labeled LEC 1) demonstrated the ability to transform high concentrations of PCE completely to ethene in conjunction with methanogenesis (Figure 1). Lactate was supplied as the primary growth substrate, but hydrogen appeared to serve as the electron donor for dechlorination (14). The culture was sufficiently diverse to contain a significant biomass of dechlorinating organisms as well as the necessary organisms capable of mediating the various steps of lactate conversion to methane. Due to this diversity, it was difficult to determine definitively if the culture contained dehalorespirers. However, rates of PCE dechlorination observed in batch reactors containing LEC 1 were generally greater than 1  $\mu\text{M}/\text{mg}$  VSS-day. This is within an order of magnitude of rates observed in other cultures from which dehalorespiring organisms have been isolated (15, 16), and much greater than those consider. In addition, preliminary phylogenetic analysis using PCR techniques and a 16S rDNA-based probe specific for *Dehalococcoides ethenogenes* strain 195 suggested that LEC 1 contains an organism with a high similarity to strain 195 (data not reported). Based on this information, the working assumption is that the mixed culture consisted of a population of dehalorespirers in conjunction with other groups of organisms incapable of transforming chlorinated ethenes as part of a respiratory process.

**Transformation of 1,1,1-TCA and CT.** Before determining the effect of cocontaminants on PCE removal, the ability of the culture to transform 1,1,1-TCA and CT was assessed. Initial concentrations below 20  $\mu\text{M}$  were selected to ensure that these fortuitous substrates would be completely transformed and to minimize competitive inhibition relationships between PCE and either of the two compounds.

Both CT and 1,1,1-TCA were rapidly transformed despite the lack of previous exposure to these compounds (Figure 2a,b). Chloroform formed transiently from CT degradation (Figure 2c), and DCM was the major chlorinated metabolite at roughly 40% of the initial molar concentration of CT added (data not shown). DCM was slowly degraded relative to CT and CF. Transformation of 1,1,1-TCA resulted in the transient formation of 1,1-DCA (Figure 2d), followed by accumulation of CA at roughly 50% of the initial molar concentration of 1,1,1-TCA (data not shown). No noticeable transformation of CA occurred during the course of the experiment. No significant compound disappearance was observed in the cell-free controls.

The addition of both CT and 1,1,1-TCA inhibited the transformation of one another (Figure 2a,b), as expected in a mixed culture where many of the same nonspecific coenzymes may have been mediating individual fortuitous

transformations (7). In treatments where PCE was included, removal of CT and 1,1,1-TCA was also slower than when single compounds were present. PCE increased the persistence of DCM and 1,1-DCA, thus reducing the amount of CA observed during the course of the experiment.

**PCE Transformation in the Presence of CT and/or 1,1,1-TCA.** The addition of CT significantly impacted two steps within the overall dechlorination process (Figure 3a): (1) PCE transformation to TCE and (2) VC transformation to ethene. An initial CT concentration of 10.6  $\mu\text{M}$  increased the amount of time required to remove PCE (from 1 to 3 days) and to convert all VC to ethene (from 6 to 11 days). Increasing the initial concentration of CT further inhibited these two steps and resulted in the transient formation of larger concentrations of VC. The amount of TCE and cDCE formed in the presence of CT was actually lower than that observed in treatments where CT was omitted (Figure 3b).

The inhibition of the PCE dechlorination step suggests that the initial attack of the chlorinated ethene molecule by the mediating enzyme(s) was negatively impacted by the presence of CT. It is unknown whether specific enzymes involved in dehalorespiration are capable of transforming CT, but there is a possibility that such enzymes would be subject to interactions with CT. Despite the fact that the initial concentration of CT selected for these treatments was much less than the concentration of PCE added, enzyme affinity for CT may have been high. While this may have reduced catalysis of PCE transformation, it did not appear to impact TCE and cDCE transformation. Release of TCE and cDCE from the active site of the enzyme may have been such that these compounds were subject to further reductive dechlorination once they were in the vicinity of dehalogenases. In addition, CT may have negatively impacted different enzymes involved in the dehalogenation process. For example, *D. ethenogenes* strain 195 contains two distinctly different dehalogenases: PCE-RDase catalyzes the reductive dechlorination of PCE, while cDCE and TCE are transformed by TCE-RDase (17). The slower dechlorination of PCE by LEC 1 in the presence of CT, in combination with the tentative identification of *D. ethenogenes* in LEC 1, suggests that inhibition of particular steps in the process may have occurred.

The negative impact of the presence of CT on VC dechlorination is consistent with other studies indicating that this step is particularly important in the overall conversion of PCE to ethene. The transformation of VC as the rate-limiting step has been observed for both mixed cultures (2, 16) and *Dehalococcoides ethenogenes* strain 195 (10). The latter organism transforms VC via a fortuitous mechanism, so it would not be surprising for CT to inhibit this step. Because *Dehalococcoides ethenogenes* strain 195 is the only isolate to-date which mediates each step in the dechlorination of PCE to ethene, it is apparent that organisms involved in the latter steps may be more fastidious than currently characterized dehalorespirers and therefore more subject to disruption in the presence of a potentially inhibitory substrate such as CT.

When 1,1,1-TCA was added in combination with PCE, there was little effect on PCE transformation (data not shown). This lack of impact exerted by 1,1,1-TCA was again noticeable when all three compounds (PCE + CT + 1,1,1-TCA) were added to treatments (Figure 4). There was a slight (but insignificant at the 95% confidence level) increase in the removal times for both PCE and VC in the presence of 1,1,1-TCA, but, in general, the data presented in Figure 4 mirrors that in Figure 3a.

Transformation of all three compounds occurred simultaneously with no apparent lag phase or diauxic effects. While it is assumed that a variety of organisms within the mixed culture were capable of fortuitously transforming CT and

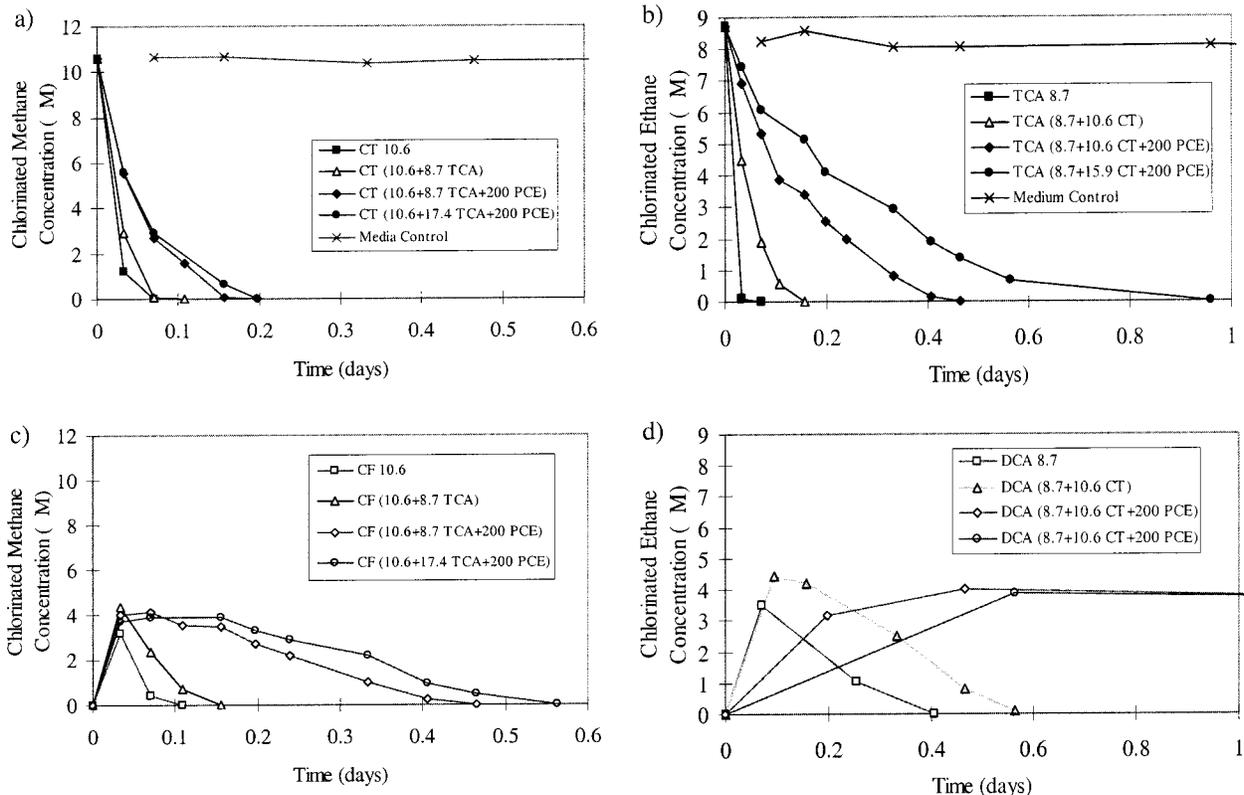


FIGURE 2. Dechlorination of (a) CT and (b) 1,1,1-TCA in treatments containing single compounds and mixtures. Formation and dechlorination of (c) CF and (d) 1,1-DCA in treatments containing single compounds and mixtures. Numbers in legend represent initial concentrations of parent compounds in  $\mu\text{M}$ .

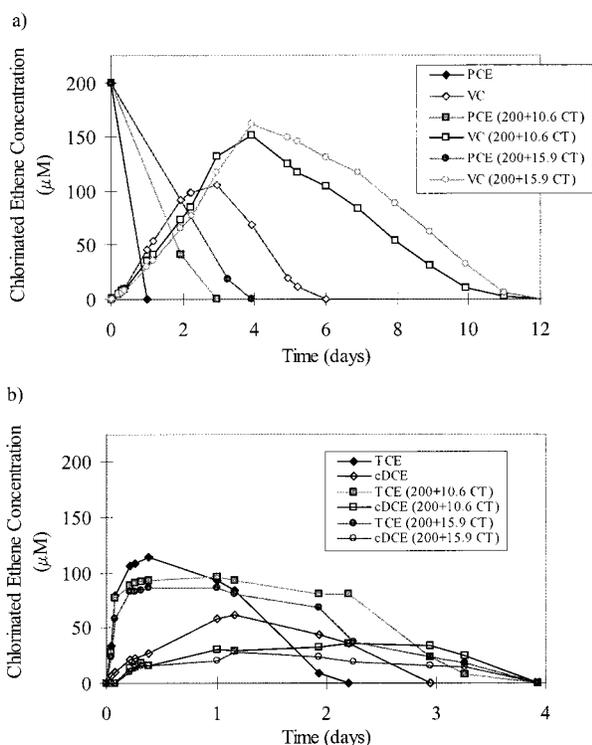


FIGURE 3. Dechlorination of (a) PCE and VC and (b) TCE and cDCE in treatments containing PCE and PCE + CT. Numbers in legend represent initial concentration of parent compounds in  $\mu\text{M}$ .

1,1,1-TCA, it is unclear whether these compounds were transformable by dehalorespiring organisms. In batch reactors seeded with an unacclimated lactate-enrichment culture

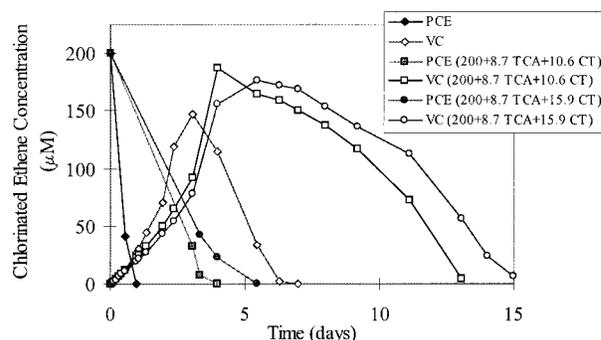


FIGURE 4. Dechlorination of PCE and VC in treatments containing PCE and PCE + CT + 1,1,1-TCA. Numbers in legend represent initial concentration of parent compounds in  $\mu\text{M}$ .

from a stock reactor operated similarly to the one containing LEC 1 but without PCE addition, transformation rates were significantly slower than those observed in LEC 1-seeded reactors (data not shown). Because neither of these cultures had prior exposure to CT or 1,1,1-TCA, it would be expected that each would contain similar populations of organisms capable of fortuitously transforming these compounds. Therefore, it appears that PCE-transforming organisms within LEC 1 possessed the ability to rapidly transform CT and 1,1,1-TCA as well. The possibility of using these compounds as electron acceptors in an energy-conserving process was indeterminable; 1,1,1-TCA appeared to be a more likely candidate than CT based on its noninhibitory role in PCE transformation.

**Methane Production.** The effect of the target contaminants on nondehalorespiring organisms within the mixed culture was first examined by noting methane production rates in various treatments. Acetoclastic and hydrogenotrophic

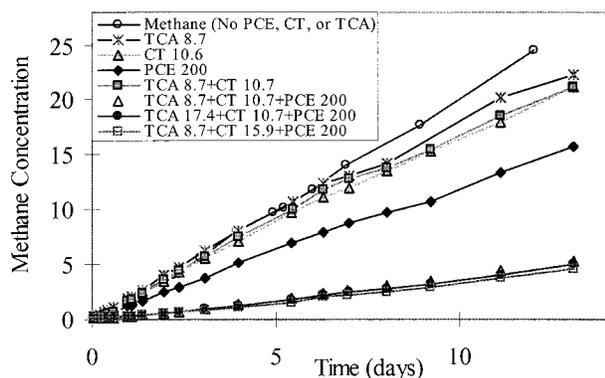


FIGURE 5. Methane production in treatments containing single compounds and mixtures. Numbers in legend represent initial concentration of parent compounds in  $\mu\text{M}$ .

methanogenesis were assumed to be the only sources of methane. At the time these experiments were conducted, reactors contained background concentrations of 8.7 mM of propionate and 5.3 mM of acetate, in addition to the 2.1 mM of lactate supplied as a primary growth substrate.

As displayed in Figure 5, treatments containing only CT and/or 1,1,1-TCA produced methane at nearly identical rates of 1.6–1.7 mM/day. Both compounds inhibited methane production when compared to unamended controls, but only while they were still present in systems; the rapid transformation of each minimized the impact on overall methane levels. Because CT and 1,1,1-TCA were transformed within 24 h in treatments containing these single compounds, methane production in these treatments occurred at a rate similar to a control system which was not spiked with chlorinated compounds. The major inhibitory metabolite appeared to be CF since no methane production was observed while this compound was present, but long-term methanogenesis was relatively unaffected due to the rapid transformation of CF. 1,1-DCA appeared to be inhibitory based on the slight increase in the methane production rate following the complete removal of this chlorinated ethane. Because no similar increase was observed when DCM was completely transformed in CT-dosed treatments, DCM was not judged to be inhibitory to methanogenesis (data not shown).

When PCE was the only compound present, the methane production rate (1.21 mM/day) was statistically significantly inhibited (roughly 27%) even after all metabolites were converted to ethene. The inhibition of methanogenesis was not surprising since this role has been widely reported for PCE and VC (2, 18), but the sustained impact of exposure and/or transformation on methane production suggests that some type of toxic inactivation may have occurred. Another possible explanation lies in the increased proportion of electrons routed away from methanogenesis in the presence of an alternate electron acceptors such as chlorinated ethenes. However, the magnitude of the difference in cumulative methane production between treatments with and without PCE was too large (45–50 mequiv of methane per liter versus 1.6 mequiv of chlorinated ethenes supplied per liter) for this to fully explain the discrepancy. One possibility was that the high concentrations of ethene that formed in the PCE-spiked systems proved inhibitory to methanogenesis; Schink et al. (19) demonstrated that this compound served this role in mixed methanogenic cultures.

The addition of all three compounds resulted in similar methane production rates of 0.41–0.45 mM/day when averaged over the course of the experiment. The lowered production rates were attributed to a combination of persistence of both parent compounds and chlorinated

metabolites. As noted previously, the inhibitory metabolite 1,1-DCA persisted through the duration of the experiment. VC in excess of 150  $\mu\text{M}$  also formed, further contributing to the decrease in methanogenesis.

**Fatty Acid Utilization.** The initial concentrations of 8.7 mM of propionate and 5.3 mM of acetate were determined by measuring the amount of each fatty acid within the commingled aliquot of LEC 1 that was used to seed the series of reactors. Once this aliquot had been separated into 25-mL reactors, 2.1 mM of lactate was added to each system. These three values represent the day 0 fatty acid concentrations listed in Table 2. Because measurement of fatty acids following the initiation of the experiment required sacrificing the seeded reactors, samples were taken only at the end of the experiment (day 13). As expected from the methane production data, acetate utilization was inhibited by all three compounds due to increased persistence of each when present in mixtures. Propionate utilization was moderately affected by CT and 1,1,1-TCA and may have been inhibited only when these compounds were still present in systems. The addition of PCE significantly inhibited propionate utilization relative to treatments where PCE was omitted. The formation of chlorinated metabolites from the transformation of PCE, CT, and 1,1,1-TCA may have contributed to the observed inhibition of propionate and acetate utilization, but this possibility was not directly investigated. Lactate was completely removed in all treatments, but differences in the extent of transformation were not determined due to the lack of intermediate time measurements.

**Hydrogen Evolution.** The formation and utilization of hydrogen was monitored because of its importance as an electron donor for dechlorination for this culture. The fermentation of lactate as a primary growth substrate was expected to result in a rapid spike in hydrogen concentration followed by a decrease to a steady-state concentration (20). This basic pattern was observed for all treatments, although the exact profile depended significantly on the compounds present (Figure 6). Hydrogen formation occurred at roughly the same rate regardless of treatment, suggesting that initial hydrogen availability was relatively unaffected by the presence of multiple contaminants. The treatment containing only PCE demonstrated the lowest maximum hydrogen concentration (<2  $\mu\text{M}$ ). This supports previous studies indicating that dehalorespirers are capable of thriving at low hydrogen levels and can generate energy at lower thresholds than hydrogenotrophic methanogens or homoacetogens (21, 22). The addition of CT and/or 1,1,1-TCA significantly increased both the magnitude of the hydrogen peak (up to 20  $\mu\text{M}$ ), and the time elapsed until the maximum  $\text{H}_2$  level was reached. CT was much more inhibitory than 1,1,1-TCA. It appeared that hydrogen utilization did not begin until CT was completely removed from the systems, suggesting that this compound (along with its metabolites) interfered with the culture's ability to maintain low hydrogen concentrations. Although the presence of CT may have impacted all hydrogenotrophic organisms, the fact that PCE inhibited methanogenesis but not hydrogen utilization indicates that CT may have disrupted the ability of dehalorespirers to maintain low hydrogen levels. The inability for PCE-dechlorinating organisms to obtain electrons from hydrogen is a potential factor for the reduced PCE removal rates observed in the presence of CT.

**Electron Equivalent Distribution.** Determining a precise electron balance was difficult due to the excess of reducing equivalents present in the stock reactor at the time the culture was transferred to batch reactors. The amount of electrons routed to methane production (based on 8 mequiv per mmol of methane) was compared to the amount of methane predicted to form based on observed changes in substrate concentrations (assuming all equivalents were routed to

TABLE 2. Batch Substrate Utilization, Methane Production, and Percentage Electron Equivalents Routed to Dechlorination

treatment	200 $\mu\text{M}$ PCE	8.7 $\mu\text{M}$ TCA	10.6 $\mu\text{M}$ CT	8.7 $\mu\text{M}$ TCA + 10.6 $\mu\text{M}$ CT	200 $\mu\text{M}$ PCE + 8.7 $\mu\text{M}$ TCA + 10.6 $\mu\text{M}$ CT	200 $\mu\text{M}$ PCE + 17.4 $\mu\text{M}$ TCA + 10.6 $\mu\text{M}$ CT	200 $\mu\text{M}$ PCE + 8.7 $\mu\text{M}$ TCA + 15.9 $\mu\text{M}$ CT
day 0 concentrations (mM) lactate, propionate, acetate	2.1, 8.7, 5.3	2.1, 8.7, 5.3	2.1, 8.7, 5.3	2.1, 8.7, 5.3	2.1, 8.7, 5.3	2.1, 8.7, 5.3	2.1, 8.7, 5.3
day 13 concentrations (mM) lactate, propionate, acetate	0, 2.6, 1.8	0, 0, 0.5	0, 0, 0.6	0, 0, 0.5	0, 4.7, 10.0	0, 4.5, 10.4	0, 4.3, 10.8
actual methane (mM)	15.7	22.3	21.2	21.2	5.38	5.33	5.85
actual methane (mequiv/L)	125.8	178.5	169.3	169.4	43.0	42.6	46.8
predict. methane (mequiv/L) <sup>a</sup>	139.2	183.9	185.6	185.8	43.9	43.9	44.1
% eq routed to dechlorination <sup>b</sup>	3.6	ID <sup>c</sup>	ID <sup>c</sup>	ID <sup>c</sup>	4.9	4.8	4.6

<sup>a</sup> Based on provision of (12, 14, 8) mol of equivalents per mol of (lactate, propionate, acetate) converted. <sup>b</sup> Calculated as the ratio of equivalents routed to dechlorination to the maximum equivalents from hydrogen production. <sup>c</sup> Incomplete dechlorination.

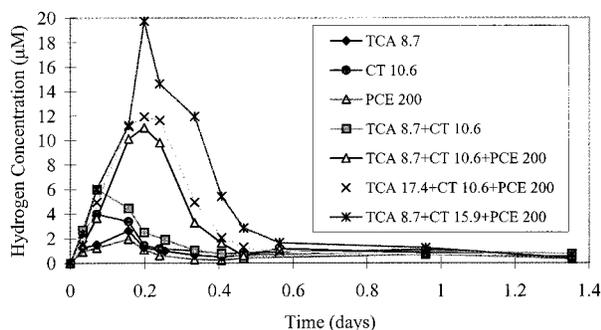


FIGURE 6. Hydrogen evolution in treatments containing single compounds and mixtures. Numbers in legend represent initial concentration of parent compounds in  $\mu\text{M}$ .

methanogenesis). The actual methane formed was generally within 85–106% of the total predicted by stoichiometric conversion (Table 2).

The active acetoclastic ability exhibited by the culture, combined with the presence of large amounts of acetate, complicated the calculation of electron equivalents routed to dechlorination. As a donor of two electron equivalents per mole, hydrogen can be used for dechlorination, methane production, or homoacetogenesis. However, there was no way to differentiate the methane formed by hydrogenotrophic methanogens from the amount formed by acetoclastic organisms; only the former (as well as homoacetogens) would likely compete for the reducing equivalents of importance to dehalorespirers. In addition, available substrate concentration data was limited to day 0 and day 13. Because conversion of PCE to ethene was nearly complete by day 13 in all treatments containing all three chlorinated aliphatics, a conservative estimate can be obtained using the observed changes in fatty acid concentrations over 13 days. For example, the system spiked with PCE (200  $\mu\text{M}$ ) + 1,1,1-TCA (8.7  $\mu\text{M}$ ) + CT (10.6  $\mu\text{M}$ ) was also able to convert 2.1 mM of lactate and 4.0 mM of propionate over the course of 13 days. Based on known stoichiometry, fermentation of 1 mM of lactate produces a maximum of 2 mM of  $\text{H}_2$ , while 3 mM of  $\text{H}_2$  is formed from the fermentation of 1 mM propionate (20). Therefore, the maximum amount of hydrogen produced in the system described above would be 16.2 mM or 32.4 mequiv/L. Complete dechlorination of PCE and its chlorinated metabolites to ethene required 1.6 mequiv/L. The ratio of the electron equivalents used for dechlorination with the maximum available from hydrogen production provides an estimate of the percentage routed to dechlorination.

Table 2 lists values calculated in this manner for the treatments containing all three contaminants. The percentage of electron equivalents routed to dechlorination ranged between 4.6 and 4.9% for these treatments. In the system containing PCE only, complete conversion to ethene occurred

within 6 days, meaning that any hydrogen produced from lactate or propionate fermentation from day 6 to day 13 was not used for dechlorination. Therefore, using the method described above to calculate a value for the percentage of equivalents routed to dechlorination represents a conservative approach in this case. The value of 3.6% is lower than that obtained for treatments containing all three contaminants, suggesting that the presence of CT and 1,1,1-TCA increased the efficiency of utilization of reducing equivalents via the inhibition of competing pathways. However, this potential advantage would be more than outweighed by the increased persistence of all chlorinated ethenes when present in mixtures.

**Significance of Results.** The combination of substrate utilization data and observed dechlorination patterns demonstrates that multiple organisms within a mixed culture are impacted by the presence of these contaminants. The potential syntrophic role of nondechlorinating organisms within PCE-dechlorinating enrichment cultures has been noted previously (14), so the inhibition of any population has the potential to affect dechlorination. As the results of this study illustrate, the addition of CT and 1,1,1-TCA can inhibit methanogenesis while disrupting hydrogen utilization and PCE dechlorination by dehalorespiring organisms. However, it is notable that although overall PCE dechlorination was inhibited in treatments containing CT (and 1,1,1-TCA, to a lesser degree), transformation of each compound still proceeded. The high-rate, PCE-dechlorinating organisms within this enrichment culture had enzyme systems capable of either transforming these cocontaminants or remaining functional, while other nonspecific organisms mediated their removal. Relying on the actions of other organisms while maintaining viability is particularly important given the diverse microbial ecology which characterizes aquifers and other contaminated sites. These findings are promising with respect to the feasibility of remediating complex sites. They suggest that natural attenuation and enhanced bioremediation based on the assumed presence of dehalorespirers are still reasonable strategies even if sites contain a variety of chlorinated solvents.

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