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Product distribution during transformation of multiple contaminants by a high-rate, tetrachlorethene-dechlorinating enrichment culture

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Abstract

Radiolabeled tetrachloroethene (PCE) and carbon tetrachloride (CT) were added to batch systems containing a lactate-enrichment culture displaying apparent dehalorespiration abilities to analyze the influence of mixtures on product distribution. Both CT and PCE were readily dechlorinated, although significant carbon disulfide (CS_2) formation was observed during CT transformation. Calculated 1,2-^{14}C-PCE recoveries for biotic treatments were between 91 and 104%, but an inability to recover products such as CS_2 led to lower recoveries of ^{14}C-CT (55 to 62%). While the majority of activity in ^{14}C-CT-spiked treatments was recovered in the volatile fraction, $^{14}CO_2$ increased significantly over time. 1,2-^{14}C-PCE was primarily recovered in volatile and non-strippable fractions, but a significant increase in $^{14}CO_2$ relative to cell-free controls suggested that the presence of a non-specific dechlorination pathway complementing dehalorespiration. The addition of both CT and PCE inhibited the transformation of the individual compounds and reduced the percentages recovered as $^{14}CO_2$. However, the magnitude of these reductions was not severe and appeared to be the result of slower overall transformation rather than a complete inhibition of mineralization pathways.

Introduction

The application of anaerobic bioremediation for the treatment of chlorinated aliphatic hydrocarbons (CAH) has received considerable attention due to its potential to inexpensively remove contamination without extensive soil intrusion. In part, this is a result of the presence of native anaerobic organisms capable of mediating reactions where the contaminant serves as an electron acceptor. While the addition of a suitable electron donor and nutrients might be necessary to allow transformation to proceed, the catalytic role of the CAH may alleviate the need for the injection of other electron acceptors. For example, aerobic treatment strategies tend to be design- and cost-limited by the ability to deliver sufficient oxygen to the contaminated zone (McCarty & Semprini 1994).

Reductive dechlorination of even mono-chlorinated compounds is thermodynamically favorable, and the attractiveness of the recently-elucidated dehalorespiration process is that organisms have shown the ability to derive energy from these reactions (Holliger & Schraa 1994). However, reductive dechlorination is far from the only mechanism by which microbes can transform CAHs. Hydrolysis, elimination, and even oxidation products have been identified in microbe-driven reactions (Egli et al. 1987, 1988, 1990; Galli & McCarty 1989; Schwartzenbach et al. 1993). Identification of organisms with as-yet unknown metabolic pathways continues with the development of more innovative analytical techniques (Madsen 1998). Abiotic transformation of CAHs, with or without electron transfer, can yield numerous other endproducts (Criddle & McCarty 1991; Kriegman-King & Reinhard 1992, 1994). Because of the presence of various reducing agents and natural organic material, both biotic and abiotic processes would be expected to occur at varying degrees *in situ* (Schwartzenbach et al. 1993).

The further acceptance of biological remediation technologies at sites contaminated with chlorinated solvents may be hindered by the potential for the formation of undesirable byproducts (McCarty & Semprini 1994). The fact that multiple contaminants are present at many of these sites further complicates the situation, potentially impacting both removal rates and transformation pathways (Reardon 1994). A previous study examining the transformation of PCE by an acclimated lactate- enrichment culture (LEC 1) in the presence of mixtures indicated that transformation proceeded through reductive dechlorination to roughly stoichiometric amounts of ethene (Adamson & Parkin 2000). The addition of 1,1,1-trichloroethane had little impact on the extent of dechlorination, but CT decreased the rate of conversion of PCE. CT was completely removed, but the reductive dechlorination products that resulted could not fully account for the original dose. Different microbial populations were impacted to varying degrees by the presence of multiple CAHs, suggesting that particular transformation pathways mediated by certain organisms could be inhibited and result in a shift in product distribution.

In the present study, a mass-balance approach was employed to determine product distribution in systems containing multiple contaminants. LEC 1 was used as a seed culture for batch experiments with radiolabeled compounds. Analytical methods allowed for the separation of the original dose of contaminant into volatile, non-strippable, and $^{14}CO_2$ fractions after varying periods of exposure. In light of the demonstrated negative impact of CT on PCE dechlorination rates by LEC 1, the experiments described were designed to test the hypothesis that the presence of CT would result in a shift in the product distribution during PCE transformation. Both compounds are commonly found at contaminated sites, and the fact that each is capable of being transformed by a number of different organisms via multiple mechanisms reinforced their selection as suitable targets for investigation.

Materials and methods

Radiochemicals and chemicals

Neat, radiolabeled PCE (250 μCi, radiochemical purity = 98.8% as determined by HPLC using a Supelco Discovery C18 column at 35 °C) and CT (100 μCi, radiochemical purity > 97% as determined by gas liquid chromatography using a Porapak QS Durapak Porasil C column at 180 °C) were obtained from Sigma and DuPont, respectively. Both carbons on the ethene were labeled (1,2-^{14}C). The specific activities listed by the manufacturers were 4.0 mCi/mmol for ^{14}C-CT and 5.1 mCi/mmol for 1,2-^{14}C-PCE. Neat compounds were transferred via glass pipette to separate 2-mL vials containing 1 mL of methanol, and these were sealed and refrigerated for storage.

Activity was generally determined by transferring 100-μL aliquots to 20-mL glass liquid scintillation (LS) vials containing 10 mL of Scinti-Verse cocktail (Fisher) followed by counting using a Beckman 6000IC model liquid-scintillation counter (LSC). Counting proceeded for 15 minutes or until a fractional error of 0.5% at the 95% confidence level was reached. The LSC used an internal correction mechanism to record efficiency. Different sharp-tip syringes (Hamilton) were used for CT and PCE to ensure that cross- contamination was minimal. Care was taken to rinse each 3 to 5 times with methanol to prevent compound carryover between uses.

Liquid chemicals, including all CAHs, were either ACS or HPLC grade. Previously-described headspace analysis techniques using gas chromotographs equipped with ECD/FID/TCD capabilities were used to determine concentrations of volatile compounds (Adamson & Parkin 2000), including quantification of carbon disulfide (CS_2) using a GC with a DB WAX column connected to an ECD (approximate detection limit = 0.1 μM). Headspace samples (100 μL) were taken from the reactors using gas-tight, luer-lock syringes (Supelco) and injected manually.

Source culture

Biotic reactors were seeded with LEC 1. Organisms are maintained in a reduced mineral medium at a biomass concentration of approximately 200 mg/L of volatile suspended solids (VSS). The culture has demonstrated the ability to rapidly transform PCE (at rates between 0.5 and 2 μM PCE/mg VSS/day) to roughly stoichiometric amounts of ethene in conjunction with methanogenesis (Adamson & Parkin 2000), and preliminary analysis (conducted at Cornell University) suggests the presence of a dehalorespiring population. Following DNA extraction, PCR analysis using both universal primers and primers specific for *Dehalococcoides ethenogenes* was performed. The complete protocol followed in this analysis was detailed by Fennell et al. (2000). Sequences of PCR products were compared to available SSU rDNA se-

quences in a BLAST search in an attempt to determine similarity with characterized organisms.

Fractionation experiments

Anaerobic, 38-mL serum bottles (Wheaton) were seeded with 25-mL aliquots of LEC 1. Reactors were purged prior to the addition of substrate, non-radiolabeled compound, and radiolabeled compound. Biotic reactors received 2.1 mM of lactate and 7 mM of acetate as a growth substrate and carbon source, respectively. Abiotic reactors containing cell-free reduced mineral medium (prepared with $FeC_{12}{\cdot}H_2O$ (40 mg/L) and $Na_2S{\cdot}H_2O$ (300 mg/L) as a reductant) were used to ascertain losses resulting from non-microbial processes. Three experiments were conducted, with each using two basic initial treatments carried out in duplicate reactors:

Biotic Set 1: ^{14}C-CT alone (6.2 μM) and ^{14}C-CT (6.2 μM) + PCE (200 μM)

Biotic Set 2: 1,2-^{14}C-PCE alone (60 μM) and 1,2-^{14}C-PCE (60 μM) + CT (6.2 μM)

Abiotic Set: ^{14}C-CT alone (6.2 μM) and 1,2-^{14}C-PCE alone (60 μM)

In each set, duplicate reactors were sacrificed after 6, 24, or 72 hours and the radiolabel separated into different fractions.

The target initial activities were 0.3 μCi and 1.0 μCi for 1,2-^{14}C-PCE and ^{14}C-CT-spiked reactors, respectively. While an initial liquid sample was removed within 30 minutes following the addition of radiolabeled compound, the resulting activity measurement was not used in mass balances calculations due to perceived incomplete mass transfer and the potential for rapid product formation. Instead, calculated initial activities were used in these calculations. The appropriateness of this decision was verified after determining that activities measured in reductant-free medium controls following extended time for equilibration were similar (error $< 5\%$) to the calculated values (data not shown).

Headspace samples were taken regularly throughout the course of experiments to determine concentrations of volatile compounds. A final headspace sample was taken immediately before reactor breakdown at 6, 24, or 72 hours. The pH was then lowered below 4 via the addition of 2 mL of 2 N HCl. The reactors were then attached to a stripping apparatus designed to separate both the CO_2 and volatile fractions by vacuum pumping ambient air through the reactors and into a series of traps. The volatile fraction was trapped on Orbo® tubes consisting of activated carbon encased in glass columns. The CO_2 fraction was trapped in 2 25-mL test tubes filled with 10 mL of NaOH through which air was pulled for approximately 70 minutes.

Radiolabeled compound left in the reactor was referred to as "non-strippable Residue" (NSR) because it withstood extensive removal efforts. While it was assumed that much of this portion was soluble, it would also contain any radiolabel that had become associated with cells or sorbed onto precipitates in the media. To differentiate between these two possibilities, a further fractionation step was performed via centrifugation ($16000g$) for 40 minutes. Activity was then determined in the supernatant and in the resuspended pellet (solids-associated fraction).

The ^{14}C concentration attributable to the volatile fraction was determined after the Orbo® tubes were broken open and the contents analyzed using an R. J. Harvey Biological Material Oxidizer (model OX-600). The oxidizer converted all volatile radiolabeled compound trapped on the activated carbon to CO_2 by combusting the material at 900 °C for three to four minutes in a stream of oxygen at a flowrate of 350 mL/min. Nitrogen (350 mL/min) was used as a purge gas to prevent interference from ambient oxygen. The combustion products passed through a series of catalysts at 700 °C, and the generated CO_2 was then captured in an external trap filled with 15 mL of Carbon-14 liquid scintillation cocktail (R. J. Harvey). Initial controls were run to correct for potential background concentrations and to determine instrument efficiency. Mannitol was spiked with 1,2-^{14}C-PCE and ^{14}C-CT from the methanol stock solutions and then combusted; the resulting activity was compared to a solution of Carbon-14 cocktail spiked with identical volumes of the stock solutions. The ratio of the two values (0.82) was the capture efficiency during combustion and was used as a correction factor for the comparison of the volatile fraction with the other fractions.

Results and discussion

Culture characterization

Preliminary phylogenetic analysis performed at Cornell University using a 16S rDNA-based molecular probe specific for *Dehalococcoides ethenogenes* has

indicated that LEC 1 contains an organism with high similarity to this dehalorespirer. The primers used (PCEA and PCEB) consisted of sequences unique to *D. ethenogenes*, and using these primers in previous PCR analyses has not resulted in the formation of a PCR product except in cultures where *D. ethenogenes* was known to exist (Fennell et al. 2000). A PCR product was obtained following DNA extraction of LEC 1, and gel electrophoresis indicated a strong band with size similar to *D. ethenogenes* (data not shown). The sequence of the 626 base PCR product was used in a BLAST search, and the most similar sequence was determined to be *D. ethenogenes* with a 96% identity (603 of 626 bases identical). Coupled with the high observed rates of dechlorination by LEC 1, this suggests the presence of the dehalorespirer *D. ethenogenes* within the mixed culture. This is the only organism known to reductively dechlorinate both higher chlorinated ethenes and VC, although it is not capable of energy conservation when VC is used as an electron acceptor (Maymo-Gatell et al. 1997).

Parent compound transformation

Throughout the GC monitoring period, duplicate reactors generally displayed similar transformation profiles. In addition, reactors that were spiked identically but sacrificed at different time points behaved in a like manner, allowing reasonable comparisons between treatments.

Biotic Set 1 (^{14}C-CT): Figure 1a shows the transformation of CT in reactors spiked with ^{14}C-CT. CT was transformed within 24 hours, with the rapid formation of chloroform (CF) and dichloromethane (DCM). CF was non-detectable after 72 hours. DCM reached a concentration of 2.7 μM, but further transformation was not observed during this period. Carbon disulfide, a known volatile product formed during CT transformation in abiotic systems (Kriegman-King & Reinhard 1992), was observed at concentrations up to 1.4 μM in this treatment (23% of the added CT). The transformation pattern was similar in reactors spiked with ^{14}C-CT and PCE (200 μM initial concentration) (Figure 1b). The removal time for CT was roughly the same as the reactor set that did not receive PCE, and the peak concentration of CF was similar. However, greater than 1 μM of CF remained after 72 hours, resulting in the formation of less DCM. The amount of CS^2 that formed was also less than that observed in the reactor set spiked with CT only.

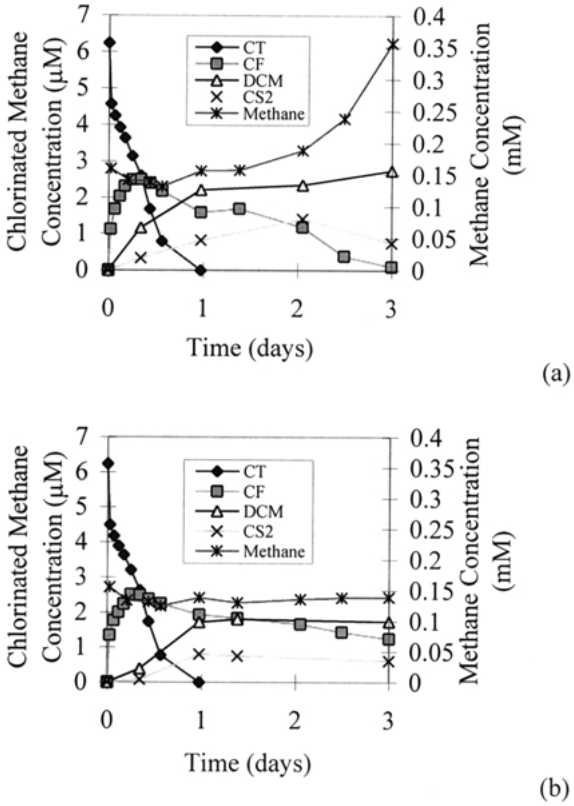


Figure 1. CT transformation and metabolite formation in Biotic Set 1 spiked with (a) ^{14}C-CT, and (b) ^{14}C-CT and PCE (200 μM initial concentration).

Biotic Set 2 (1,2-14C-PCE): In reactors spiked with 1,2-^{14}C-PCE and 60 μM of non-radiolabeled PCE, extensive dechlorination was observed at this lower initial dose (Figure 2a). No PCE or TCE was present after 72 hours, and less than 5 μM of cDCE remained. The primary metabolites detected at this point were VC (34 μM) and ethene (29 μM). A mass balance on the volatile compounds was determined by summing the detectable chlorinated metabolites and ethene at each sampling point. The sum was lower than 60 μM for the first 24 hours, suggesting that mass transfer was incomplete or that products other than volatile, chlorinated metabolites formed during this period. The sum of metabolites generally increased over time and reached a value greater than 60 μM by 72 hours.

This pattern for the sum of the volatile compounds was also observed in treatments containing CT and 1,2-^{14}C-PCE, but the transformation of PCE was significantly inhibited in the presence of CT (Figure 2b). There was a rapid decrease in PCE concentration

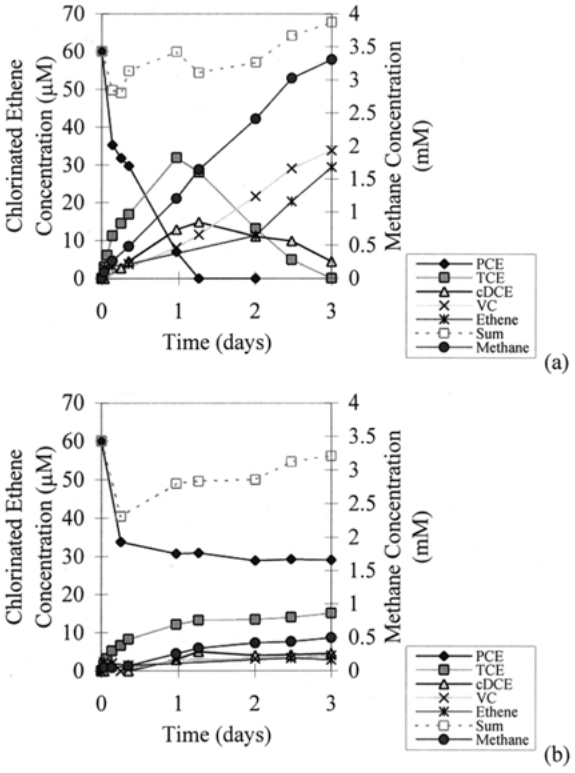


Figure 2. PCE transformation and metabolite formation in Biotic Set 2 spiked with (a) 1,2-^{14}C-PCE (60 μM initial concentration), and (b) 1,2-^{14}C-PCE (60 μM initial concentration) + CT (6.2 μM initial concentration).

within the first 24 hours with a simultaneous increase in TCE concentration. However, TCE formation eventually slowed (peak concentration of 15 μM), resulting in the formation of moderate amounts of cDCE (5 μM), VC (4 μM), and ethene (3 μM) after 72 hours. Removal of the entire dose of PCE was not observed prior to the sacrifice of the last reactor set. The formation of lower peak concentrations of TCE and cDCE in the presence of CT was consistent with previously-described results (Adamson & Parkin 2000), but the short-term nature of this study did not allow sufficient time for more extensive dechlorination to VC. While the fact that severe inhibition of chlorinated ethene transformation was observed following the initial burst of activity is a concern, it must be noted that significant amounts of CF had formed during this period. CT transformation in Biotic Set 2 was nearly identical to that shown in Figure 1b for Biotic Set 1; greater than 1 μM of CF was present throughout the majority of the monitoring period. Short-term inhibition of PCE transformation by CF has been observed previously,

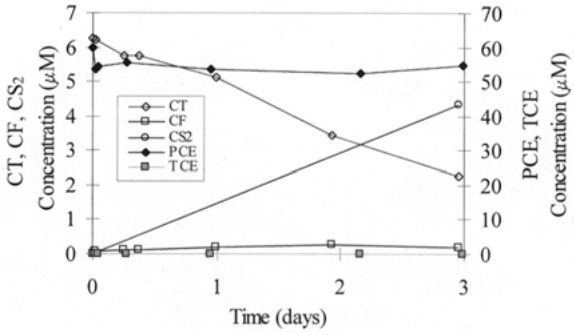


Figure 3. CT and PCE transformation and metabolite formation in Abiotic Set.

and an eventual recovery of activity following complete CF transformation would be expected based on those results (Adamson & Parkin 2000). In the prior study, the addition of CT (10.6 μM) increased the time required for complete removal of PCE (200 μM) and all chlorinated metabolites from 6 to 11 days.

Abiotic Set. The PCE concentration changed little over time in cell-free reactors containing only reduced mineral medium, and no chlorinated ethene metabolites were detected (Figure 3). However, a steady loss of CT was observed over the course of 72 hours, with only 64% of the original dose of 6.2 μM remaining when the reactors were sacrificed. While a small amount of CF was observed in this treatment, CS^2 was the major product and roughly accounted for the amount of CT removed on a stoichometric basis. The abiotic formation of CS^2 from CT was not surprising since it has been seen previously in systems containing iron sulfides (Kriegman-King & Reinhard 1992). The expected species of iron sulfide present in the reduced medium would be mackinawite (FeS) and perhaps some pyrite (FeS_2) (Rickard 1969). Both species have been shown to result in the formation of CS_2 when exposed to CT, either through nucleophilic attack or a redox-disproportionation reaction (Kriegman-King & Reinhard 1992, 1994). Systems reduced with S^{2-} plus cysteine have also yielded CS_2 as an abiotic transformation product (Lewis et al. 1996).

Previous results demonstrated that no CT transformation or CS_2 formation was observed in cell-free mineral media in which FeS was omitted (data not reported). In a trial designed to assess whether the production of CS_2 was a function of the sulfide concentration, the peak CS_2 concentration in a biotic reactor containing normally-prepared reduced media was roughly twice that observed in a treatment consisting of media with half the normal reductant

Table 1. Mass balances in LEC1-seeded batch reactors spiked with radiolabeled PCE (Biotic Set 2). Non-radiolabeled PCE concentration was 60 μM and non-radiolabeled CT concentration (when added) was 6.2 μM. Values are the sum of volatile, CO_2, and non-strippable fractions, and represent the average of duplicates ± range of data.

Treatment	Time of reactor sacrifice (h)	Mass balance (%) ± range of data
1,2-^{14}C-PCE	6	100 ± 16
1,2-^{14}C-PCE + CT	6	99 ± 1.1
1,2-^{14}C-PCE	24	100 ± 21
1,2-^{14}C-PCE + CT	24	103 ± 6.1
1,2-^{14}C-PCE	72	91 ± 6.0
1,2-^{14}C-PCE + CT	72	104 ± 3.2

Table 2. Mass balances in LEC 1-seeded batch reactors spiked with radiolabeled CT (Biotic Set 2). Non-radiolabeled CT concentration was 6.2 μM and non-radiolabeled PCE concentration (when added) was 200 μM. Values are the sum of volatile, CO_2, and non-strippable fractions, and represent the average of duplicates ± range of data.

Treatment	Time of reactor sacrifice (h)	Mass balance (%) ± range of data
^{14}C-CT	6	57 ± 0.03
^{14}C-CT + PCE	6	62 ± 1.2
^{14}C-CT	24	60 ± 1.0
^{14}C-CT + PCE	24	55 ± 2.4
^{14}C-CT	72	61 ± 7.0
^{14}C-CT + PCE	72	62 ± 2.0

concentration (data not reported). This provided evidence that the formation of this undesirable compound was linked to the background sulfide concentration, and that the impact of CS_2 could be minimized by adjusting the media composition.

Mass balances

To determine recoveries using ^{14}C analysis, the amount of radiolabeled compound in the individual fractions (CO_2, volatile, and NSR) following reactor sacrifice were summed. This value was then divided by the target initial activity, with the mass balance expressed as a percentage.

The mass balances ranged between 91 and 104% in the 1,2-^{14}C-PCE treatments in Biotic Set 2 (Table 1), indicating that a majority of the transformation products were captured in the fractionation process. Duplicates behaved similarly, resulting in low relative standard deviations (1.1–21%). As noted previously, the measured initial activities (data not reported) were not used because incomplete mass transfer was a concern. The observation that the sum of chlorinated ethenes was low during the first 12 hours of headspace sampling (Figure 2) indicates that a sample taken so shortly (30 minutes) after addition of 1,2-^{14}C-PCE was not likely to be representative.

The mass recoveries in the ^{14}C-CT treatments in Biotic Set 1 were generally lower than those calculated for the 1,2-^{14}C-PCE treatments. Values ranged between 55% and 62% (Table 2), but deviations between duplicates were not excessively large (0.3–7.0%). The low mass balances reflect-in part-the inability to recover $^{14}CS_2$ during the fractionation process selected for these experiments. This product is only minimally soluble in water but does not adsorb to the activated carbon used to trap volatile compounds. However, CS_2 concentrations were able to be quantified using GC headspace analysis, and the CT transformation profiles in Figure 1 demonstrate that significant amounts were present in these treatments. The production of any other similarly uncaptured volatile radiolabeled compounds would have further contributed to the low observed recoveries.

While the values were substantially lower than 100%, mass balances obtained for the reduced medium control treatments spiked with ^{14}C-CT (67–75%) were slightly higher than those obtained for the ^{14}C-CT treatments in Biotic Set 1 (Table 3). GC analysis determined that there was significant loss over the course of 72 hours with unrecoverable CS_2 as the only identified product. Mass balances in the 1,2-^{14}C-PCE-spiked reactors in the Abiotic Set ranged from 89 to 97%. In reductant-free media controls, recovery of the calculated initial activity ranged from 94 to 99% (data not reported). This indicated that transformation to uncaptured products – rather than an inability to recover product from the reactor systems – was responsible for the mass recoveries of lower than 100% obtained in the Biotic Sets and the Abiotic Set.

Product distribution

The fractionation process allowed for the separation of added radiolabeled compounds into $^{14}CO_2$, NSR, and volatile components at different points in time of the experiment. Paired two-sample, student t-tests were used to determine whether differences in concentra-

Table 3. Mass balances in abiotic control reactors spiked with ^{14}C-CT or 1,2-^{14}C-PCE (Abiotic Set). Non-radiolabeled CT concentration was 6.2 μM and non-radiolabeled PCE concentration (when added) was 60 μM. Values are the sum of volatile, CO_2, and non-strippable fractions, and represent the average of duplicates ± range of data.

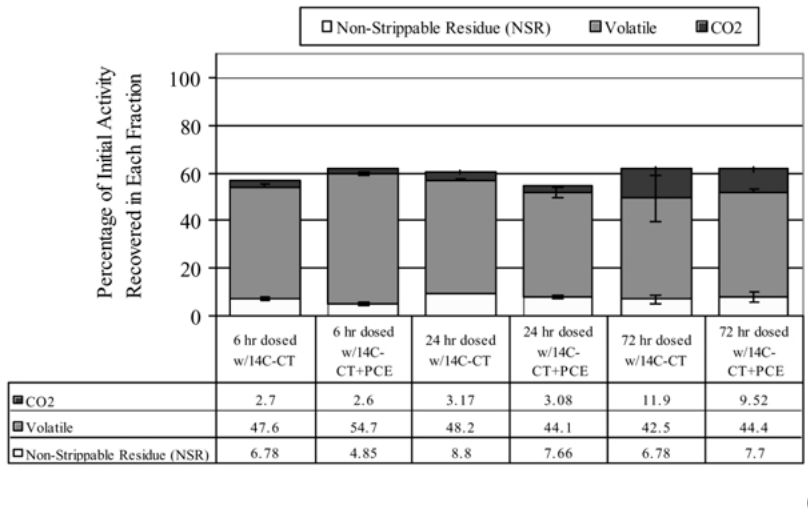
Treatment	Time of reactor sacrifice (h)	Mass balance (%) ± range of data
1,2-^{14}C-PCE	6	89 ± 3.9
^{14}C-CT	6	67 ± 3.8
1,2-^{14}C-PCE	24	97 ± 5.6
^{14}C-CT	24	75 ± 0.02
1,2-^{14}C-PCE	72	97 ± 2.4
^{14}C-CT	72	74 ± 0.3

tion between treatments and differences in concentration over time were statistically significant at the 95% confidence level.

1,2-^{14}C-PCE. At each of the time intervals, the majority of the radiolabel in Biotic Set 2 was found in the volatile and NSR fractions (Figure 4b). The latter fraction was unexpectedly large because headspace analysis indicated that most of the original PCE dose could be accounted for by summing the parent compound and metabolites. The probability that the NSR fraction represented PCE that had crossed the cell membrane or sorbed to cell surfaces or precipitates present in the medium was low based on the proportion of the radiolabel recovered in the supernatant. Following separation of the NSR fraction (Table 4), activities in the supernatant were generally an order-of-magnitude higher than those obtained from resuspension of the pellet. Although it did not appear to be solids-associated, the NSR fraction did decrease over time. The mean values of the NSR fractions after 6 hours were significantly higher those obtained after 72 hours. This indicates that while a portion of 1,2-^{14}C-PCE was not in a volatile (and strippable) form, the NSR fraction was likely still bioavailable and subject to transformation. But while the transformation profiles in Figure 2 suggest the decrease in the NSR fraction may have corresponded with a transfer to a more volatile form over time, the observed increases in the volatile fraction over time were generally not statistically significant. Because of this, the low chlorinated ethene "sum" values obtained in the first 6 hours were most likely due to incomplete partitioning of the PCE between gas and liquid phases rather than some type of conversion of NSR. It did appear that the addition of CT slightly decreased the amount of PCE that was found in the NSR fraction; the mean values in the 6-hour treatments were significantly less at the 95% confidence level.

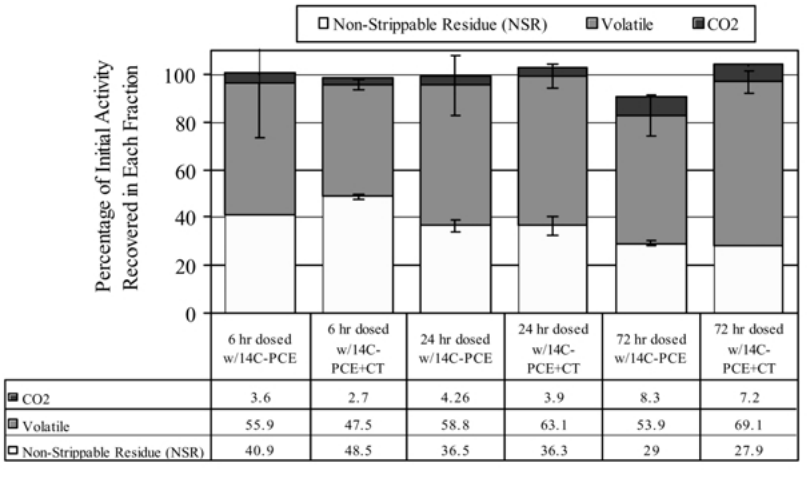
While the volatile and NSR fractions accounted for a majority of the recovered activity, there was PCE-derived CO_2 observed in all treatments. Furthermore, this amount increased significantly over time, with nearly three times as much $^{14}CO_2$ in the 72-hour reactors than in the 6-hour reactors. The formation of CO_2 during anaerobic transformation of PCE has not been widely reported in the literature, presumably because reductive dechlorination is the most studied pathway. There are no studies to-date which have demonstrated CO_2 production during the transformation of PCE by a pure culture (Holliger et al. 1999). Vogel & McCarty (1985) reported between 20 and 30% conversion of both PCE and VC to CO_2 in mixed methanogenic cultures. Using similar initial conditions, Freedman & Gossett (1989) observed only minimal CO_2 formation from radiolabeled PCE, and even this minor production was in doubt due to a perceived contamination problem within the original stock solution. The percentage of radiolabel found in CO_2 in our study exceeded 8%, a figure considerably greater than the radiochemically-determined 1.2% impurity level in the stock 1,2-^{14}C-PCE, making it unlikely that conversion of ^{14}C contaminants was responsible for the observed increase.

A more recent set of studies provides evidence of a potential explanation for CO_2 formation in anaerobic mixed cultures. Bradley & Chapelle (1999a, b, 2000) demonstrated that biological transformation of 1,2-^{14}C-VC in anaerobic sediment microcosms resulted in the immediate production of roughly equal amounts of $^{14}CO_2$ and $^{14}CH_4$. In part because of the transient appearance of acetate, it was proposed that VC was transformed via oxidative acetogenesis by an unknown species. This acetate was then metabolized by acetoclastic methanogens, resulting in the formation of CO_2 and CH_4. The overall reaction describing this process is thermodynamically favorable, and the presence of acetoclastic methanogens in LEC 1 suggests that this pathway is a reasonable explanation for the $^{14}CO_2$ found in these experiments. In the 72-hour treatment set containing 1,2-^{14}C-PCE, headspace analysis indicated that the majority of the added PCE had been transformed beyond cDCE. Although $^{14}CO_2$ formation in this treatment represented up to 10% of the initial activity, the presence of 29 μ M of ethene



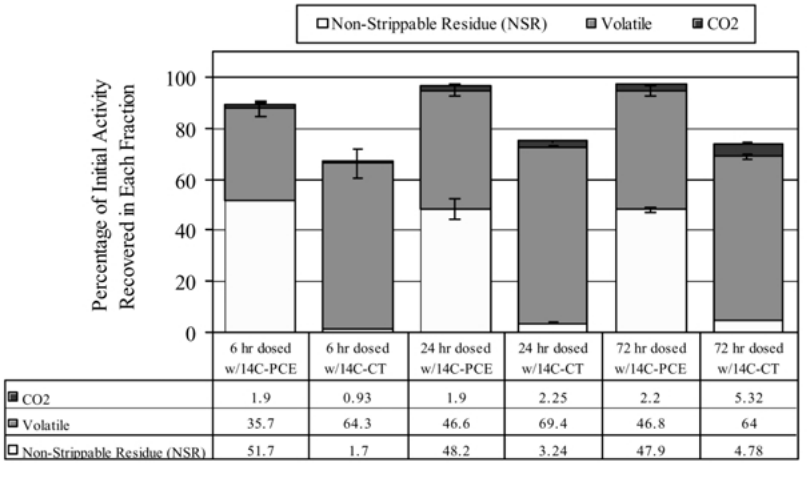
	6 hr dosed w/14C-CT	6 hr dosed w/14C-CT+PCE	24 hr dosed w/14C-CT	24 hr dosed w/14C-CT+PCE	72 hr dosed w/14C-CT	72 hr dosed w/14C-CT+PCE
CO2	2.7	2.6	3.17	3.08	11.9	9.52
Volatile	47.6	54.7	48.2	44.1	42.5	44.4
Non-Strippable Residue (NSR)	6.78	4.85	8.8	7.66	6.78	7.7

(a)



	6 hr dosed w/14C-PCE	6 hr dosed w/14C-PCE+CT	24 hr dosed w/14C-PCE	24 hr dosed w/14C-PCE+CT	72 hr dosed w/14C-PCE	72 hr dosed w/14C-PCE+CT
CO2	3.6	2.7	4.26	3.9	8.3	7.2
Volatile	55.9	47.5	58.8	63.1	53.9	69.1
Non-Strippable Residue (NSR)	40.9	48.5	36.5	36.3	29	27.9

(b)



	6 hr dosed w/14C-PCE	6 hr dosed w/14C-CT	24 hr dosed w/14C-PCE	24 hr dosed w/14C-CT	72 hr dosed w/14C-PCE	72 hr dosed w/14C-CT
CO2	1.9	0.93	1.9	2.25	2.2	5.32
Volatile	35.7	64.3	46.6	69.4	46.8	64
Non-Strippable Residue (NSR)	51.7	1.7	48.2	3.24	47.9	4.78

(c)

Figure 4. Product distribution in (a) Biotic Set 1 treatments spiked with ^{14}C-CT, (b) Biotic Set 2 treatments spiked with 1,2-^{14}C-PCE, and (c) Abiotic Set treatments spiked with 1,2-^{14}C-PCE or ^{14}C-CT. Error bars represent range of data from duplicate reactors.

Table 4. Separation of NSR fraction into supernatant and solid-associated fractions in reactors spiked with 1,2-^{14}C-PCE or ^{14}C-CT. Mass balance was calculated by dividing the activity in the NSR fraction by the sum of the activities in the supernatant and solid-associated fractions.

Treatment	Activity in NSR fraction (μCi)	Activity recovered in supernatant (μCi)	Activity recovered in resuspended pellet (μCi)	Mass balance on NSR fraction (%)
^{14}C-CT (Biotic Set 1)				
6 CT	0.068	0.027	0.031	91
6 CT + PCE	0.048	0.021	0.018	73
24 CT	0.088	0.032	0.035	76
24 CT + PCE	0.077	0.029	0.036	85
72 CT	0.068	0.031	0.031	86
72 CT + PCE	0.077	0.033	0.024	82
1,2-^{14}C-PCE (Biotic Set 2)				
6 PCE	0.123	0.121	0.012	102
6 PCE + CT	0.145	0.140	0.013	118
24 PCE	0.109	0.104	0.012	106
24 PCE + CT	0.109	0.115	0.012	117
72 PCE	0.087	0.079	0.010	108
72 PCE + CT	0.084	0.089	0.010	105
Abiotic Set				
6 CT	0.017	0.010	Not determined	Not determined
6 PCE	0.155	0.150	0.007	102
24 CT	0.032	0.015	Not determined	Not determined
72 CT	0.048	0.019	0.016	73
72 PCE	0.144	0.153	0.007	111

after 72 hours suggested that reductive dechlorination remained the dominant transformation pathway.

In all cases, treatments containing CT in addition to PCE resulted in lower activities recovered as $^{14}CO_2$. The presence of CT clearly inhibited the overall extent of transformation, and considerably less VC had formed after 72 hours in the treatment set containing CT. If the production of $^{14}CO_2$ was linked to VC transformation, then the formation of a lower amount of CO_2 in this treatment (or any containing CT) is not surprising. This is directly supported by the observation of an increase in CO_2 over time as transformation of PCE proceeded. But because only 5 μM of VC had formed and large amounts of PCE, TCE, and cDCE remained in the 1,2-^{14}C-PCE + CT treatment, the relative proportion of CO_2 was unexpectedly high. Roughly 7.2% of the initial activity was recovered as $^{14}CO_2$ in this treatment, only slightly less than the 8.3% recovered in the 1,2-^{14}C-PCE treatment. This suggests that the proposed VC degradation pathway to CO_2 and CH_4 was not completely inhibited by the presence of CT.

^{14}C-CT. Numerous other studies have documented anaerobic biotransformation of ^{14}C-CT. As expected, treatments containing this compound were characterized by increased $^{14}CO_2$ production over time, in part via the substitutive or biooxidative transformation of CT and CF (Figure 4a). Past research has established that single organisms possess the ability to both reductively dechlorinate and biooxidize chlorinated methanes (Egli et al. 1988, 1990; Boyd & Mikesell 1990; Stromeyer et al. 1992), and the prevalence of methanogens and other anaerobes in LEC 1 presumably was responsible for the formation of a portion of the $^{14}CO_2$. Although the volatile fraction accounted for the majority of activity recovered in each treat-

ment, this fraction decreased over time, as did the non-captured product CS_2, due to the production of $^{14}CO_2$.

The increase in $^{14}CO_2$ over time corresponded to more complete transformation of CT and CF. In reactors containing only ^{14}C-CT, all CT and CF was transformed within 72 hours, resulting in significant CO_2 production. Conversely, the inclusion of PCE in ^{14}C-CT treatments contributed to slower CT and CF transformation, and, as a consequence, there was a statistically significant difference between the CO_2 fractions in the two 72-hour treatments. However, the difference between these values was not large (9.5% for ^{14}C-CT + PCE treatment vs. 11.9% for ^{14}C-CT only) and therefore did not suggest an obvious shift away from the substitutive pathway in the presence of PCE. Additional CO_2 formation would be expected if CF transformation had been allowed to proceed to completion in the ^{14}C-CT + PCE treatment, and this would have narrowed the gap between the two treatments. Mineralization of CT, CF, DCM, and CS_2 (Kriegman-King & Reinhard 1992, 1994) to CO_2 is considered positive degradation of the original contaminant. CS_2 is toxic and its formation in groundwater is a concern, but its concentration eventually decreased in both treatments. Furthermore, abiotic controls indicated that CS_2 formation was an artifact of the initial sulfide concentration. Provided sufficient time for acclimation of DCM-degrading organisms (Magli et al. 1998), CT may have been completely transformed to innocuous products.

The formation of non-volatile or soluble products in biotic and abiotic systems during CT transformation has been noted in numerous previous studies (Lewis et al. 1996, Stromeyer et al. 1992; Bagley & Gossett 1995; Fathepure & Vogel 1991). Such compounds would be recovered in the NSR fraction in our experiments with ^{14}C-CT, and indeed represented between 4.8 and 8.8% of the initial activity depending on the treatment. However, there was no apparent trend between treatments that suggested a connection with the presence of PCE or elapsed time. Compared to the treatments containing 1,2-^{14}C-PCE alone, there was considerably less activity recovered in the NSR fraction in reactors spiked with ^{14}C-CT alone. Roughly equal activities were recovered in supernatant and solids-associated fractions, and resulting mass balances on the NSR fraction indicated a reasonable overall recovery (73–91%). The presence of precipitates and other solid surfaces (such as FeS in the mineral medium) has been shown to influence the removal of CT (Kriegman-King & Reinhard 1992, 1994), and any non-volatile products resulting from these interactions would be recovered in the NSR fraction if they formed in our systems. Other potential contributors to the solids-associated fraction include quenched radical intermediates formed during the biotransformation of CT in both CF- and CO_2-yielding pathways (Criddle & McCarty 1991) and biomass production from metabolism of $^{14}CO_2$.

Abiotic Set. In the ^{14}C-CT abiotic controls, small but increasing amounts of CO_2 were observed over time (up to 5.3% of the initial activity) (Figure 4c). Because of the significant CS_2 formation in these reactors (Figure 3), it was not surprising to see some $^{14}CO_2$. In anaerobic systems with sulfide as the reductant, hydrolysis of CS_2 is thought to be the primary pathway by which CO_2 is abiotically produced (Kriegman-King & Reinhard 1992). The volatile fraction changed little over time, with the only statistically significant decrease occurring between the 24-hour and 72-hour treatments, despite the expectation that it would steadily decrease over time due to the formation of CS_2. The low mass recovery for the 6-hour reactor set (67%) argues that the volatile fraction at this time point was underestimated. The NSR fraction decreased significantly from 6 to 72 hours, but its contribution never exceeded 4.8% of the initial activity. The high percentage of the NSR radiolabel found in the solids-associated fraction in the 72-hour treatment (Table 4) suggests that sorption of ^{14}C-CT or products may have occurred.

Analysis of the abiotic controls containing 1,2-^{14}C-PCE determined that there was little change in any of the fractions over time, with a slight decrease in the NSR fraction the only one that proved statistically significant. This pattern matched the lack of transformation observed in this set. Furthermore, less than 3% of the initial activity was recovered as $^{14}CO_2$ in any reactor. Therefore, the CO_2 activity within these reactors represented a background concentration that was a function of either the capture technique or contamination of the stock solution. Because this percentage was significantly less than that observed in biotic reactors, the production of PCE-derived CO_2 appeared to be linked to the presence of organisms within LEC 1 capable of mediating these reactions. Like the biotic reactors spiked with 1,2-^{14}C-PCE, large portions of the radiolablel were recovered in the NSR fraction, and the large majority of this was recovered in the supernatant portion (Table 4). While the high NSR fraction raised concerns that contaminants

in the radiolabeled stock solution were responsible for a portion of this fraction, this is not consistent with the 98.8% radiochemical purity for 1,2-^{14}C-PCE that was established prior to initiation of experiments. In addition, the percentage of activity found in the NSR fraction was somewhat less in FeS-free media controls (data not reported), suggesting that there was some type of interaction with FeS occurring during the fractionation process that contributed to the NSR fraction. While the NSR fraction represents unaccounted-for and unidentified activity, the PCE profile in Figure 3 suggests that it did not form as an abiotic product of PCE transformation.

Conclusions

Phylogenetic analysis indicated that an organism with high similarity to *D. ethenogenes* is present in LEC 1. This organism and other dehalorespirers have been shown to transform PCE strictly through reductive dechlorination. The observed formation of $^{14}CO_2$ during transformation of 1,2-^{14}C-PCE was not consistent with the presence of *D. ethenogenes* as the sole dechlorinating organism within LEC 1. Bradley & Chapelle (2000) have proposed that within mixed cultures, uncharacterized organisms are capable of converting VC to acetate, which is further metabolized to CH_4 and CO_2 during acetoclastic methanogenesis. LEC 1 appears to contain both a specific dehalorespiring population and a non-specific dechlorinating population capable of transforming PCE to both ethene and CO_2 in the same environment (although ethene is the dominant end-product).

Mixtures of CT and PCE lowered overall transformation rates of the individual compounds over the course of the experiments described here. The addition of CT had little impact on the amount of 1,2-^{14}C-PCE found in NSR and volatile fractions, but slightly reduced the activity recovered as $^{14}CO_2$ after 72 hours by 13%. Similarly, the presence of PCE resulted in less $^{14}CO_2$ formation from ^{14}C-CT but did not noticeably impact the amount found in NSR and volatile fractions. Because the formation of $^{14}CO_2$ was tied to biological activity, the slower transformation caused by the addition of inhibitory cocontaminants is proposed as the explanation for the decreased CO_2 production observed when both CT and PCE were present. However, these decreases in CO_2 production were small enough (13–20% over 72 hours) to suggest that the product distributions were converging to similar profiles even in the presence of a competing or inhibitory compound. Thus, the addition of mixtures would not cause a shift to a new product distribution, but rather slows progress to the expected product distribution as transformation proceeds to completion (Adamson & Parkin 2000). These results indicate that both mineralization and dehalorespiration of CAHs can occur even at sites with complex contaminant profiles.

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