



BIOTRANSFORMATION OF MIXTURES OF CHLORINATED ALIPHATIC HYDROCARBONS BY AN ACETATE-GROWN METHANOGENIC ENRICHMENT CULTURE

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(First received July 1997; accepted in revised form August 1998)

Abstract—Biotransformation of chlorinated aliphatic hydrocarbons under anaerobic conditions has received considerable attention due to the prevalence of these compounds as groundwater contaminants. However, information concerning the impact of mixtures of chlorinated compounds on their own transformation is limited. The focus of this research was to investigate the effect of combinations of chlorinated aliphatics on transformation rates and the role of toxicity to a methanogenic enrichment culture. Batch studies using combinations of carbon tetrachloride (CT), perchloroethene (PCE) and 1,1,1-trichloroethane (1,1,1-TCA) were performed with acetate as the electron donor. CT was transformed most rapidly, followed by 1,1,1-TCA and PCE. Rate coefficients for the transformation of 1,1,1-TCA and CT were significantly lower when the concentration of either compound was increased. PCE transformation was limited, and its presence did not significantly impact degradation of CT or 1,1,1-TCA. In once-fed batch reactors used to assess the potential for toxicity, a transformation limit was observed for both PCE ($1.91 \pm 0.21 \mu\text{g}$ of PCE per mg of cells) and 1,1,1-TCA ($7.84 \pm 0.34 \mu\text{g}$ of 1,1,1-TCA per mg of cells) but not CT. The low observed limit of transformation for PCE, combined with its lack of effect on the transformation of the other two compounds, suggests that the number of PCE-degrading organisms in the mixed culture is low. Transformation of repeated CT spikes has been maintained for over 400 days without supplementation with acetate, indicating that inhibition, rather than potential inactivation, was responsible for the negative impact observed when CT is present in mixtures. © 1999 Elsevier Science Ltd. All rights reserved

Key words—anaerobic, chlorinated aliphatics, kinetics, methanogenic, toxicity

INTRODUCTION

The widespread use of chlorinated aliphatic hydrocarbons as industrial solvents has led to groundwater contamination when these compounds are improperly disposed. Perchloroethene (PCE), carbon tetrachloride (CT) and 1,1,1-trichloroethane (1,1,1-TCA) represent three groups of common chlorinated aliphatic pollutants. Based on their potential as possible carcinogens, the EPA has set maximum contaminant levels (MCLs) of $5 \mu\text{g}/\text{l}$ for CT and PCE and $200 \mu\text{g}/\text{l}$ for 1,1,1-TCA (Sawyer *et al.*, 1994). To achieve these target concentration limits, suitable strategies must be developed for efficient and effective remediation. Current treatment methods such as air stripping, soil-vapor extraction or adsorption with activated carbon focus on transfer from one phase to another, necessitating further disposal decisions. Bioremediation avoids this

phase-transfer problem and holds promise for complete contaminant removal if pollutants can be mineralized or transformed to other harmless products.

Mixed and pure culture transformation of highly chlorinated ethanes, methanes and ethenes has been observed under methanogenic conditions (Bouwer and McCarty, 1983; Egli *et al.*, 1987; Klecka *et al.*, 1990). Dehalogenation can occur through a variety of mechanisms; reductive dechlorination, dihaloelimination and hydrolytic reduction are among those reactions which are thermodynamically favorable under the redox conditions present in methanogenic environments. The majority of these biotransformations were previously believed to be mediated by non-specific microbial metabolism where the substrate and pollutant are not adequately distinguished by the organism (Vogel *et al.*, 1987). A variety of studies have proven that transition metal coenzymes such as F_{430} and corrinoids, known to be abundant in methanogens (Daniels, 1993), serve as microbial components capable of facilitating

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reductive dechlorination (Krone *et al.*, 1989a,b; Gantzer and Wackett, 1991). Although such fortuitous biotransformations of chlorinated aliphatics do not provide a source of energy for growth, recent findings have shown that organisms exist which can couple the transformation of PCE to obtain energy for growth in a respiratory process (Holliger *et al.*, 1993; Scholz-Muramatsu *et al.*, 1995), including a strain which can catalyze the complete dechlorination to ethene (Maymo-Gatell *et al.*, 1997).

Despite the extensive number of studies on the biotransformation of individual chlorinated aliphatic hydrocarbons, there is little information concerning mixtures of these compounds. This general lack of knowledge is of particular importance when considering indications that approximately half of all contaminated sites may contain more than one pollutant (Westrick *et al.*, 1984). Previous studies involving combinations of chlorinated compounds have included substituted benzenes and toluenes (Ramanand *et al.*, 1993), polychlorinated biphenyls (May *et al.*, 1992; Ye *et al.*, 1992) and chlorinated benzenes and halogenated aliphatics under aerobic, nitrate-reducing, and sulfate-reducing conditions (Cobb and Bouwer, 1991). Within methanogenic enrichment cultures, Hughes and Parkin (1996) have observed that the presence of combinations of compounds can negatively influence the transformation rates of some compounds in the mixture while benefiting the transformation of others. In draw-and-fill reactors, dichloromethane (DCM) transformation was aided in the presence of 1,1,1-TCA and CF (Hughes and Parkin, 1992), but both 1,1,1-TCA and CF transformation decreased when present simultaneously in batch reactors (Hughes and Parkin, 1996). Fathepure and Vogel (1991) reported that a two-stage biofilm reactor substantially mineralized a mixture of highly chlorinated hydrocarbons. In the acetate-fed anaerobic column, trichloroethene (TCE) (80%), chloroform (CF) (32%) and hexachlorobenzene (80%) were transformed to tri- and dichlorinated metabolites. No effort was made to determine the effect of combinations of the compounds on individual transformation rates or pathways.

When multiple compounds are present, there are a number of possible factors such as toxicity and inhibition that contribute to the complexity of observed transformation phenomena. Toxicity associated with the inactivation of specific methane monooxygenases during aerobic biotransformation of chlorinated aliphatics has been studied extensively and has led to the development of a parameter termed transformation capacity (Alvarez-Cohen and McCarty, 1991a,b). Because anaerobic transformations (presumably fortuitous) of chlorinated substrates are mediated by any number of different coenzymes, the transformation capacity concept likely does not apply. However, there are indications that there may be toxicity associated

with anaerobic reductive dechlorination. Sipes and Gandolfi (1982) found that during *in vitro* microsomal studies performed under anoxic conditions, reactive intermediates formed during biotransformation of a wide range of halogenated aliphatics. Using radiolabeled parent compounds, they determined that the intermediates covalently bound to protein, lipids and DNA. This binding to macromolecules is believed to be responsible for exerting toxic effects on the liver (Goldberg, 1979). The formation of reactive intermediates during the anaerobic biotransformation of chlorinated aliphatics has been well established from *in vitro* studies with coenzymes common to anaerobic organisms. A proposed mechanism of corrinoid-catalyzed CT transformation results in the formation of tri- and dichloromethyl radical intermediates (Chiu and Reinhard, 1995). Studies by Chiu and Reinhard (1996) with vitamin B₁₂ and the iron porphyrin hematin indicate that the latter coenzyme was partially inactivated by reactive intermediates of CT transformation, though little vitamin B₁₂ inactivation was observed. Stromeyer *et al.* (1992) demonstrated that following CT transformation by aquacobalamin, portions of radiolabeled compound were found in cellular material. They speculated that this occurred as a result of the binding of a chloromethyl radical to the corrinoid. Krone *et al.* (1991) suggested that formation of highly reactive intermediates during the transformation of CT may be responsible for exerting toxic effects on anaerobes, presumably due to interactions with corrinoids involved in the acetyl-CoA pathway. In a manner similar to the toxicity associated with mammalian transformation of chlorinated aliphatics, it is possible that these microbial coenzymes serve as sites for inactivation, resulting in reduced transformation activity. Within mixed cultures, Weathers (1995) reported "toxicity quotients" of 19.5 mg of methanogenic biomass inactivated per μmol of CF transformed and 9 mg of biomass per μmol CT transformed. These parameters were calculated by determining the total mass of each compound transformed by a range of active biomass concentrations. No attempt was made to differentiate between the inactivation of particular coenzymes or entire cells.

The observation that the transformations of highly chlorinated aliphatic hydrocarbons appear to provide no direct benefit to certain organisms and often inhibit normal metabolic activity raises the possibility that these anaerobic cultures are subject to toxic effects. The potential for inactivation resulting from the transformation of one compound may be one determinant in reducing transformation rates of other contaminants. This paper addresses the prospect of toxicity in order to provide a clearer picture of prospective microbe/compound relationships that may occur when multiple pollutants are present. Without general information on the effects of mixtures of contaminants, limits are placed on

the potential application of bioremediation techniques. CT, PCE and 1,1,1-TCA were selected for investigation because they represent highly oxidized members of three main classes of chlorinated aliphatic hydrocarbons. The specific objectives of this research were to (1) determine kinetic coefficients for the transformation of combinations of CT, PCE and 1,1,1-TCA by an anaerobic mixed culture and compare them to those observed when these compounds are present alone and (2) evaluate the potential impact of toxicity on the culture by determining the maximum amount of each compound transformed under conditions similar to those in kinetic studies.

MATERIAL AND METHODS

Stock cultures

A mixed methanogenic culture was maintained in a 9-l stock reactors with a 40 day hydraulic retention time at 20°C. Acetate served as the growth substrate and was fed every other day to provide a bulk concentration of 7 mM. This dose of acetate was completely utilized within 48 h and resulted in a biomass concentration of approximately 200 mg/l as volatile suspended solids. The nutrient media consisted of the following mineral concentrations: NH₄Cl (400 mg/l), KCl (400 mg/l), MgCl₂·6H₂O (400 mg/l), (NH₄)₂HPO₄ (80 mg/l), CaCl₂·2H₂O (25 mg/l), Na₃PO₄·12H₂O (10 mg/l), KI (2.5 mg/l), CoCl₂·6H₂O (2.5 mg/l), MnCl₂·4H₂O (0.5 mg/l), NH₄VO₃ (0.5 mg/l), ZnCl₂ (0.5 mg/l), NaMoO₄·2H₂O (0.5 mg/l), H₃BO₃ (0.5 mg/l), NiCl₂·6H₂O (0.5 mg/l) and cysteine (1.0 mg/l). In addition to the nutrient media, Na₂S·9H₂O (300 mg/l) and FeCl₂·4H₂O (40 mg/l) were added separately to the feed solution to provide the necessary reducing conditions. Sodium bicarbonate was added as needed to maintain a pH between 6.7 and 7.0. The culture had previously demonstrated the ability to transform CT, CF and 1,1,1-TCA in batch reactors (Weathers, 1995; Hughes and Parkin, 1996).

Batch kinetic experiments

Kinetic experiments were conducted in 38-ml serum bottles seeded with 25 ml of cells from the stock reactors. To develop anaerobic conditions, inverted water-filled reactors were purged with N₂/Ar gas. The reactors were then capped with Teflon-lined rubber septa with care taken to minimize residual water. Aluminum crimp caps were used to seal the reactors and culture was added directly through the septa. Aliquots of the chlorinated aliphatic compounds were added via a gas-tight syringe (Hamilton) from saturated aqueous stock solutions (CT = 200 mg/l, PCE = 200 mg/l and 1,1,1-TCA = 1200 mg/l). Initial concentrations between 0 and 10 μM of CT, PCE and 1,1,1-TCA were selected for kinetic studies. Fed-cell reactors received 10 μl of glacial acetic acid to obtain an initial substrate concentration of 7 mM, while no external electron donor was added to resting-cell reactors. Media (cell-free) controls were used to account for observed changes in concentration attributable to abiotic processes such as volatility and sorption. The preparation of controls was identical to that for biotic reactors. Triplicate controls were used while duplicate biotic reactors were prepared for each treatment. All reactors were stored in a 20°C climate-control laboratory in the absence of light. Mixing was provided by a rotary shaker table (Lab-Line) set at 100 rpm.

Observed rate coefficients for each compound were determined using a pseudo-1st-order kinetic analysis which was based on a simplification of the Michaelis–Menten expression. Michaelis–Menten kinetics has been used extensively to describe enzyme-catalyzed reactions where transformation does not support growth (Sawyer *et al.*, 1994). Contaminant concentrations used in the described experiments were assumed to be much less than the Michaelis–Menten half saturation constant, yielding a rate expression that is first-order with respect to concentration. Rate coefficients were determined by plotting the natural log of the normalized concentration over time. Although 2nd-order rate coefficients can be approximated by dividing pseudo-1st-order values by the initial biomass concentration (approximately 200 mg/l), assumptions about constant biomass concentration were avoided because of the potential for toxicity from transformation. In addition, pseudo-1st-order analysis allowed for comparison between biotic reactors and abiotic controls.

Batch toxicity experiments

Batch reactors for toxicity experiments were prepared in 38-ml serum bottles identical to those used in kinetic studies. CT, PCE and 1,1,1-TCA were fed individually to a minimum of three biotic reactors and two media controls. The first dose of each chlorinated aliphatic was accompanied by either 0 or 7 mM of acetate to mimic the initial conditions of the reactors used in kinetic experiments. Once the entire set of reactors had transformed the initial dose of aliphatic below detection limits, the reactors were respiked. No acetate was added with these subsequent doses of the chlorinated compounds. Between spikes, there was no attempt to purge reactors to prevent the build-up of metabolites. Experiments were terminated when transformation ceased or slowed to a rate not substantially greater than observed abiotic losses.

An observed limit of transformation (T_L^{obs}) was determined for each compound by dividing the cumulative mass transformed ($\sum dS$) by the initial biomass concentration present in each reactor (X_0):

$$T_L^{\text{obs}} = \frac{\sum dS}{X_0}$$

Although values for this parameter were determined in a manner similar to the transformation capacity term defined by Alvarez-Cohen and McCarty (1991a), the two parameters are not the same due to the lack of evidence regarding the inactivation from reductive dechlorination. The values determined in these tests represent an upper limit on the amount of compound that can be transformed by this enrichment culture.

Chemicals

The parent compounds used during these experiments were CT (Sigma), PCE (Sigma) and 1,1,1-TCA (Aldrich). Based on expected transformation products, a number of metabolites were also analyzed: chloroform (Sigma), dichloromethane (Sigma), 1,1-dichloroethane (Sigma), trichloroethene (Sigma), *cis*-1,2-dichloroethene (Aldrich), *trans*-1,2-dichloroethene (Aldrich) and vinyl chloride (Aldrich). All purchased chemicals were either HPLC or ACS grade. Other chemicals purchased were glacial acetic acid (ACS grade, Malinkrondt), methanol (HPLC, Fisher), methane gas (Air Products) and N₂/Ar gas mix (Air Products).

Analytical methods

Headspace analysis was employed in the measurement and quantification of volatile compounds. The parent compounds and the trichlorinated metabolites were measured using a HP5890A gas chromatograph (GC)

equipped with an electron capture detector (ECD). The capillary column within this GC was 30 m with a 1.0 μm DB-5 stationary phase (Supelco). Nitrogen carrier gas at 2.5 ml/min and an ECD flow rate of 60 ml/min were used. Oven, inlet and detector temperatures were 75, 150 and 250°C, respectively. A HP5890 Series II GC was used for less-chlorinated metabolites and was set-up to provide greater sensitivity for the measurement of expected lower concentrations of these metabolites. This GC was equipped with an ECD and a 60 m capillary column with a 1.8 μm DB-VRX stationary phase (Supelco). The nitrogen carrier gas flow was 5–6 ml/min and the ECD flow was 60 ml/min. Oven, inlet and detector temperatures were 35, 150 and 250°C, respectively. Headspace samples (100 μl) were taken from the reactors using gas-tight, luer-lock syringes (Supelco).

Aqueous calibration standards were prepared in 38-ml serum bottles in a manner identical to the experimental batch reactors. Known masses of chlorinated compounds were added from methanol stock solutions to a set of reactors spanning experimental concentration ranges. A minimum of three standards from the set were run daily with a mid-range standard injected periodically to ensure that instrument output was consistent. Instrument response was plotted vs known standard concentration to develop a linear calibration curve. This relationship was then used to determine the nominal aqueous concentration within biotic and abiotic reactors. Henry's constants were used to account for partitioning of the volatile organic compounds from liquid to air in headspace analysis. Using techniques specified by Gossett (1987), the dimensionless Henry's constants (at 20°C) were 0.972 for CT, 0.552 for PCE and 0.550 for 1,1,1-TCA.

RESULTS AND DISCUSSION

Transformation of compounds when present alone

Rate coefficients for single compounds were determined in order to provide a basis for comparison to multiple compound treatments. Resting and once-fed reactors were used to assess the effect of an externally supplied electron donor on reaction rates. The transformation of each of the parent compounds at a range of initial concentrations is shown in Fig. 1. At similar initial concentrations, observed pseudo-1st-order rate coefficients for CT were approximately an order of magnitude higher than those for 1,1,1-TCA, which in turn were about an order of magnitude higher than those for PCE (Table 1). CT transformation resulted in the formation of less than equal molar amounts of chloroform, which in turn was partially transformed to dichloromethane within two days. 1,1-Dichloroethane was the major volatile product of 1,1,1-TCA transformation during the course of experiments, with formation of trace amounts of 1,1-dichloroethene. Although the transformation of PCE appeared to occur at a rate only marginally faster than that observed in abiotic controls, the formation of trichloroethene (TCE) in only the biotic reactors indicated that the culture was actively transforming PCE.

Rate coefficients for the transformation of CT and 1,1,1-TCA were significantly larger when acetate

was added to the reactors. This beneficial impact of an external electron donor in facilitating dechlorination reactions, noted extensively by past researchers (Fathepure and Boyd, 1988; Freedman and Gossett, 1989; Hughes and Parkin, 1992), is likely a result of the increased availability of reducing equivalents. Numerous *in vitro* studies with metallocoenzymes such as F_{430} and corrinoids indicate that they readily catalyze reductive dechlorination reactions when their metal centers are in reduced oxidation states (Krone *et al.*, 1989a; Lewis *et al.*, 1996). The addition of electrons presumably results in a larger percentage of coenzymes in their reduced states. Resting cells must rely on more limited endogenous electron sources to provide reducing equivalents. The addition of a growth substrate also has the potential to support the metabolic activity of a more diverse mixed culture, either through the direct use of acetate or soluble microbial products resulting from acetate metabolism. In this situation, overall dechlorination would be aided because a number of organisms with possible dechlorinating abilities would have a chance to survive in the culture.

In PCE treatments, no conclusive increase in rate coefficients was seen when acetate was added. PCE dechlorination using acetate as an electron donor has been observed for a relatively limited number of cultures, notably pure cultures of an anaerobe (Krumholz *et al.*, 1996) and a facultative aerobe (Sharma and McCarty, 1996). Smatlak *et al.* (1996) and Ballapragada *et al.* (1997) have implicated the importance of H_2 as an electron donor in cultures which have exhibited high rates of PCE dechlorination. The mixed culture used in this study was enriched with acetate, a substrate which results in the production of little or no H_2 (Gibson and Sewell, 1992). Given the lack of PCE transformation observed relative to the other compounds, the number of PCE-dechlorinating organisms within this mixed culture appears low regardless of the whether acetate or hydrogen is the preferred electron donor.

For each of the three chlorinated compounds, increasing the initial concentration resulted in lower observed rate coefficients. This change indicates the limitations inherent in using pseudo-1st-order kinetics to describe these fortuitous transformations. At higher initial concentrations, more compound is present to possibly inhibit substrate utilization and exert toxic effects. In addition, there appears to be a general decrease in instantaneous transformation rates for each compound over time based on the kinetic data. Although a portion of this rate decrease is attributable to a decrease in the contaminant concentration itself, depletion of acetate over time also may have altered transformation rates of the fortuitous substrates. These factors are not addressed in the simplified kinetic model used to derive pseudo-1st-order rate coefficients.

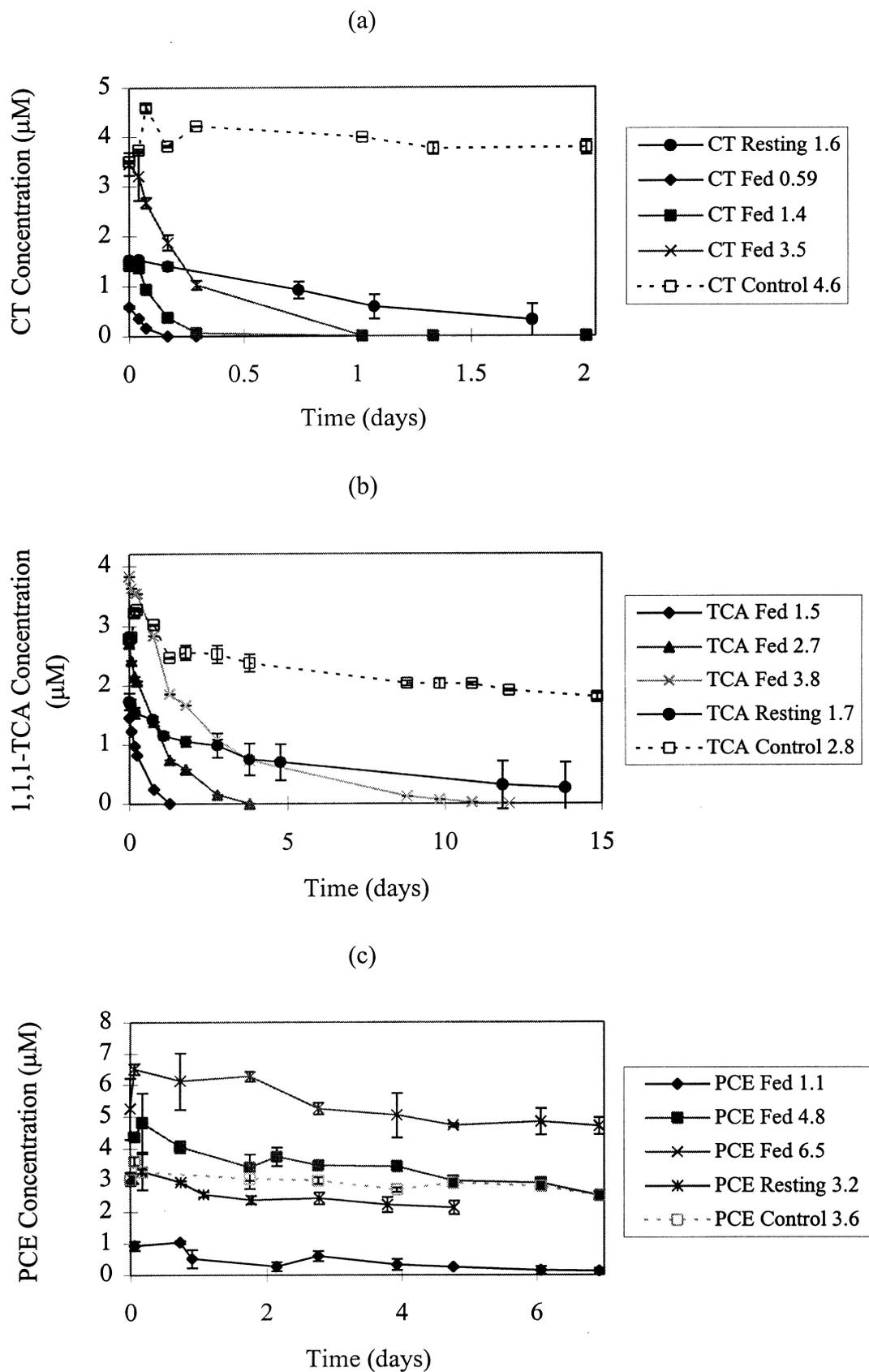


Fig. 1. Transformation of (a) CT, (b) 1,1,1-TCA and (c) PCE when present alone in resting-cell and fed-cell batch reactors (error bars represent range of data). All concentration units are μM .

Table 1. Observed pseudo-1st-order rate coefficients for transformation of single compounds in resting and fed (7 mM of acetate) batch reactors

Initial concentration (μM)	Acetate concentration (mM)	Pseudo-1st-order rate coefficient (day^{-1}) \pm range of data ($n = 2$ for biotic reactors; $n = 3$ for abiotic reactors)
CT 1.6	0	0.755 ± 0.075
CT 0.59	7	17.4 ± 2.03
CT 1.4	7	11.1 ± 0.68
CT 3.5	7	4.26 ± 0.34
CT 4.6	control	0.031 ± 0.084
PCE 3.2	0	0.084 ± 0.013
PCE 0.82	7	0.085 ± 0.002
PCE 4.8	7	0.068 ± 0.011
PCE 6.5	7	0.055 ± 0.011
PCE 3.6	control	0.042 ± 0.008
TCA 1.7	0	0.189 ± 0.082
TCA 1.5	7	2.33 ± 0.170
TCA 2.7	7	0.998 ± 0.058
TCA 3.8	7	0.445 ± 0.029
TCA 2.8	control	0.035 ± 0.003

Transformation of compounds when present in mixtures

Because of the large number of possible permutations, kinetic studies with mixtures focused on varying the concentration of two compounds (CT and PCE) while using approximately the same initial concentration of the third (1,1,1-TCA).

At the concentrations tested, CT was completely transformed within 48 h in all reactors (Fig. 2). Losses in controls were negligible over this time period. Table 2 lists pseudo-1st-order rate coefficients for the data in Fig. 2 and an additional treatment. The observed CT rate coefficient decreased significantly (at the 95% confidence level based on student-*t* test) from 11.1 to 3.89 day^{-1} when 1,1,1-TCA was added. Increasing the initial concentration of CT resulted in similar reductions in rate coefficients. The presence of PCE did not result in any statistically significant changes in CT rate coefficients beyond the effect caused by 1,1,1-TCA.

When the initial concentration of 1,1,1-TCA and CT were held constant and the initial concentration of PCE was increased from 0 to 0.35 to 0.85 μM , CT transformation was not affected (Fig. 3). This lack of impact by PCE was also observed at a higher initial concentration of CT. These results suggest that CT transformation by the mixed culture was hindered by 1,1,1-TCA and increasing CT concentrations but unaffected by the presence of PCE.

The transformation of 1,1,1-TCA followed a pattern similar to CT. Overall transformation rates were slower, but complete removal of approximately 2.0 μM of 1,1,1-TCA was observed within 5 days in all treatments (Fig. 4). The observed rate coefficients declined from 2.00 to 1.28 day^{-1} when PCE was added at 0.33 μM (Table 3). This was the only statistically significant change in rate coefficients observed when the initial concentration of PCE was increased. By comparing all other treat-

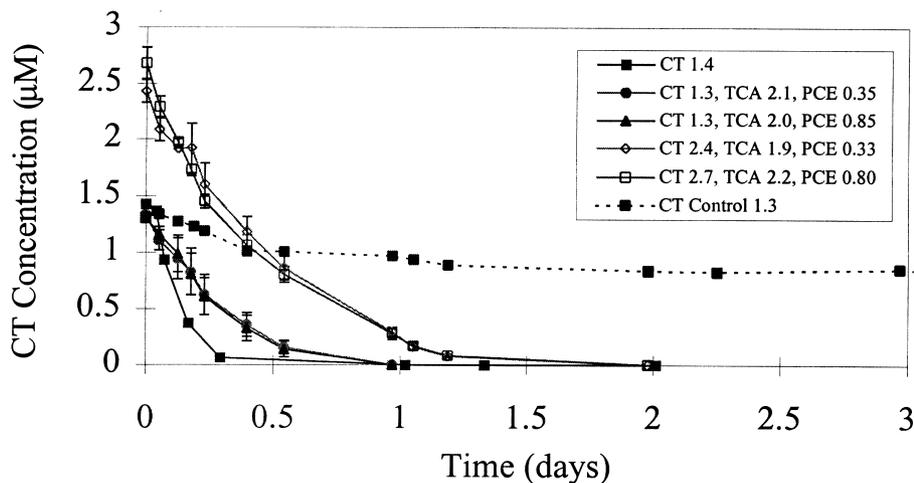


Fig. 2. Transformation of CT when present alone and in mixtures in fed-cell batch reactors (error bars represent range of data). All concentration units are μM .

Table 2. Observed pseudo-1st-order rate coefficients for carbon tetrachloride (CT) when present in mixtures in fed batch reactors^a

Initial concentration (μM)	CT pseudo-1st-order rate coefficient (day^{-1}) ± 1 range of data ($n = 2$ for biotic reactors; $n = 3$ for abiotic reactors)
CT 1.4	11.1 ± 0.34
CT 1.4, TCA 2.1	3.89 ± 0.55
CT 1.3, TCA 2.1, PCE 0.35	3.82 ± 0.89
CT 1.3, TCA 2.0, PCE 0.85	4.12 ± 0.77
CT 2.4, TCA 1.9, PCE 0.33	2.69 ± 0.10
CT 2.7, TCA 2.2, PCE 0.80	2.67 ± 0.17
CT 1.3 (control)	0.085 ± 0.01

^aA portion of these results were presented in Adamson and Parkin (1997).

ments where initial concentrations of CT and 1,1,1-TCA were held constant, it can be seen that increasing PCE had relatively little effect at the concentrations tested (Fig. 3). CT was significantly more detrimental to 1,1,1-TCA transformation, as indi-

cated by the pattern of decreased rate coefficients at higher initial CT concentrations (Fig. 5).

PCE transformation, as was seen when it was the only compound added, was limited when combinations of compounds were present (Fig. 6).

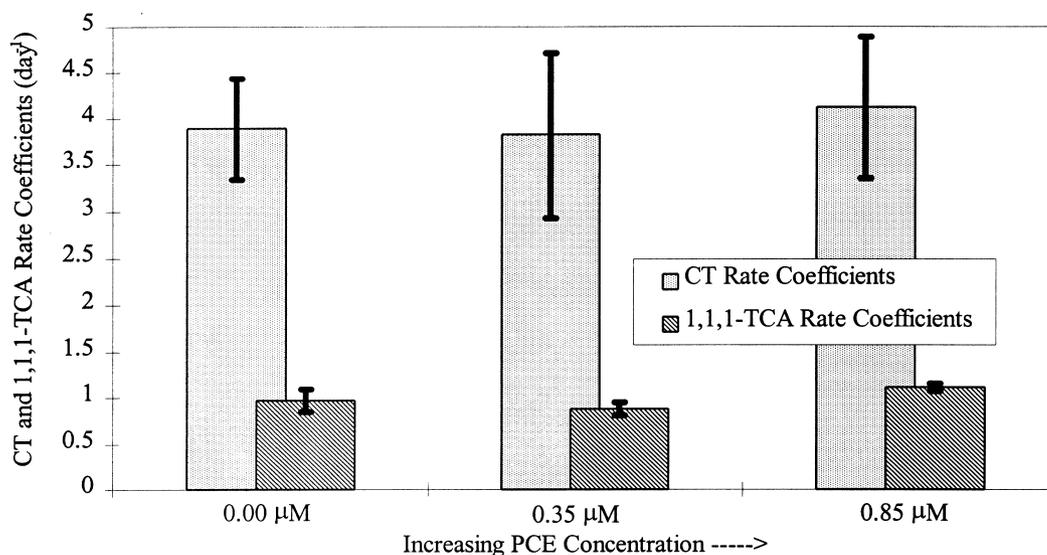


Fig. 3. Effect of increasing PCE concentration on CT and 1,1,1-TCA rate coefficients (error bars represent range of data).

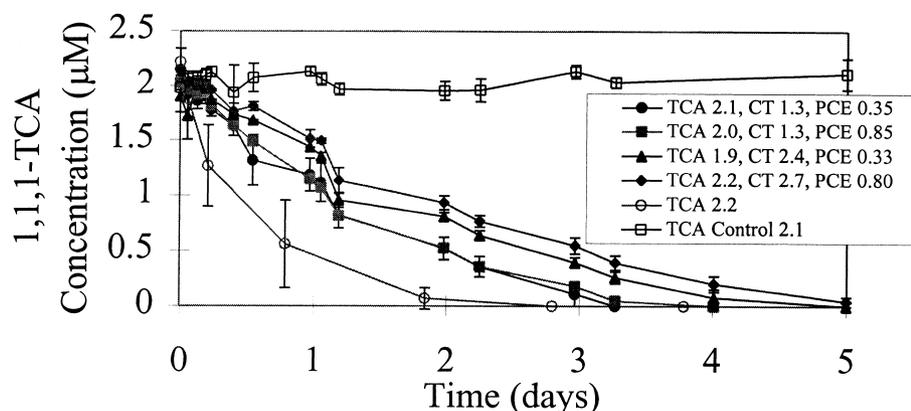


Fig. 4. Transformation of 1,1,1-TCA when present alone and in mixtures in fed-cell batch reactors (error bars represent range of data). All concentration units are μM .

Table 3. Observed pseudo-1st-order rate coefficients for 1,1,1-trichloroethane (1,1,1-TCA) when present in mixtures in fed batch reactors^a

Initial concentration (μM)	1,1,1-TCA pseudo-1st-order rate coefficient (day^{-1}) \pm range of data ($n = 2$ for biotic reactors; $n = 3$ for abiotic reactors)
TCA 2.2	2.00 ± 0.518
TCA 2.0, PCE 0.33	1.28 ± 0.036
TCA 2.4, PCE 1.1	1.24 ± 0.053
TCA 2.1, CT 1.4	0.97 ± 0.012
TCA 2.1, CT 1.3, PCE 0.35	0.88 ± 0.070
TCA 2.0, CT 1.3, PCE 0.85	1.11 ± 0.040
TCA 1.9, CT 2.4, PCE 0.33	0.69 ± 0.011
TCA 2.2, CT 2.7, PCE 0.80	0.66 ± 0.027
TCA 2.1 (control)	0.00 ± 0.001

^aA portion of these results were presented in Adamson and Parkin (1997).

Transformation was incomplete even after 10 days and TCE was the only metabolite detected. The slow degradation rate appeared to introduce a large degree of scatter into the data, resulting in relatively large differences (± 12 to 54%) between rate coefficients

between replicates (Table 4). Because of this, the majority of the observed differences in rate coefficients between treatments were not statistically significant at the 95% confidence level. The exception was the reactor set which received relatively high

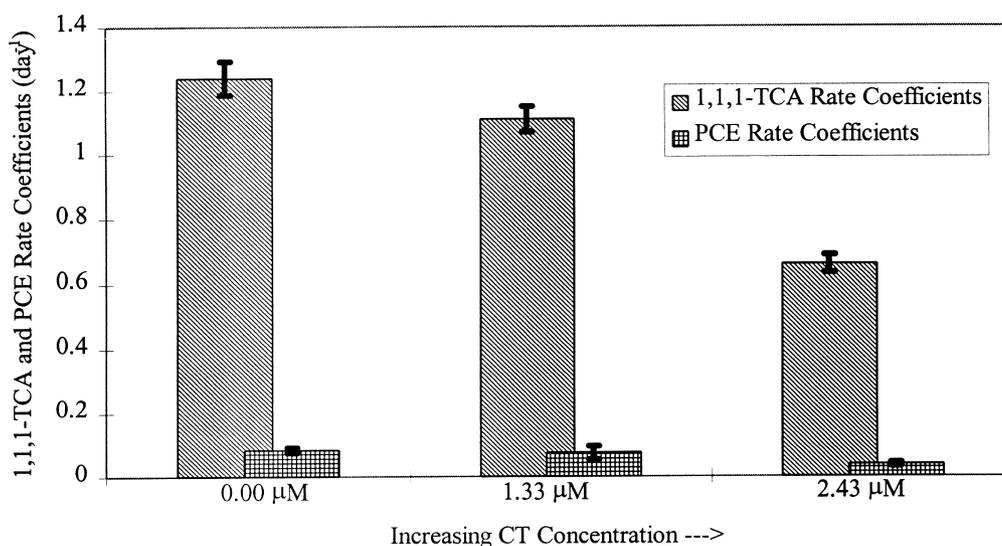


Fig. 5. Effect of increasing CT concentration on PCE and 1,1,1-TCA rate coefficients (error bars represent range of data).

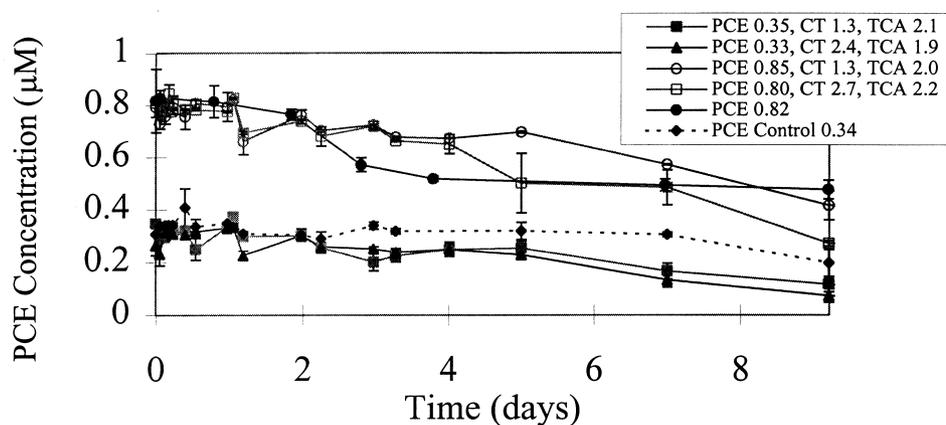


Fig. 6. Transformation of PCE when present alone and in mixtures in fed-cell batch reactors (error bars represent range of data). All concentration units are μM .

Table 4. Observed pseudo-1st-order rate coefficients for perchloroethene (PCE) when present in mixtures in fed batch reactors^a

Initial concentration (μM)	PCE pseudo-1st-order rate coefficient (day^{-1}) \pm range of data (day^{-1}) \pm range of data ($n = 2$ for biotic reactors; $n = 3$ for abiotic reactors)
PCE 0.82	0.085 ± 0.002
PCE 0.33, TCA 2.0	0.138 ± 0.034
PCE 0.35, TCA 2.1, CT 1.3	0.084 ± 0.030
PCE 0.33, TCA 1.9, CT 2.4	0.088 ± 0.018
PCE 0.85, TCA 2.0, CT 1.3	0.075 ± 0.021
PCE 0.80, TCA 2.2, CT 2.7	0.038 ± 0.005
PCE 0.34 (control)	0.017 ± 0.002

^aA portion of these results were presented in Adamson and Parkin (1997).

doses of each of the three parent compounds (PCE at $0.80 \mu\text{M}$, CT at $2.7 \mu\text{M}$ and 1,1,1-TCA at $2.2 \mu\text{M}$). The rate coefficient for this treatment was significantly different than those observed for all other treatments, including the abiotic media control. Although this suggests that PCE transformation was possibly hindered as a result of increasing the initial concentration of any of the compounds present, it is difficult to infer any general trend due to the relative lack of substantial PCE transformation by the culture.

These batch experiments indicate that, at the concentrations tested, the presence of additional compounds either decreased transformation rates (CT and 1,1,1-TCA) or had no effect (PCE). When present in mixtures, no lag period was observed prior to the initiation of transformation of any of the three compounds in resting or fed-cell reactors. This suggests that the biotransformation was mediated by factors that are either constitutive or present in sufficient amounts due to previous substrate exposure. Based on comparisons of data within Figs 2, 4 and 6, there was no evidence of diauxic effects, which indicates that the mixed culture did not preferentially transform any one of compounds in exclusion of the others. Because these transformations are believed to be fortuitous and provide no direct benefit to the organisms, increasing the number and concentration of compounds present would be expected to be detrimental to the degradation of individual compounds. This was observed with CT and 1,1,1-TCA. Competition between fortuitous substrates for active sites on mediating coenzymes is one possible factor, as well as inactivation due to the transformation of the chlorinated compounds.

Observed limits of transformation for individual compounds

To investigate the potential for inactivation, observed limits of transformation were determined in resting and once-fed batch reactors (Table 5). For each compound, the observed limit of transformation increased when acetate was provided. The addition of an external electron donor supplies a greater supply of reducing equivalents, indicating that the T_L^{obs} term is partially limited by availability of growth substrate and/or reducing equivalents.

In once-fed reactors, a limit was reached in the transformation of PCE (Fig. 7) and 1,1,1-TCA (Fig. 8), but CT transformation has continued indefinitely. Figure 9 displays the transformation of multiple spikes of CT during a time period of approximately 125 days; however, transformation has continued beyond 400 days even though no additional acetate has been supplied to the reactors. The value listed in Table 5 for CT represents the amount transformed to date. Rates have slowed considerably, with cell decay presumably a contributing factor due to the extended duration of the experiment. Based on the continued ability to transform CT, culture inactivation did not appear to occur as a result of transformation. Hashsham *et al.* (1995) observed a similar pattern during mixed culture transformation of CT, although they supplied reactors with additional growth substrate (H_2) during portions of their study. In experiments lasting approximately 20 days, Novak (1997) observed no loss of CT dechlorinating ability by an extracellular factor excreted by *Methanosarcina thermophilla*. The acetate-enrichment culture used in these experiments may have facilitated CT transformation

Table 5. Observed transformation capacities for carbon tetrachloride, perchloroethene and 1,1,1-trichloroethane for resting and fed-cell batch reactors

Compound	Transformation capacity of resting cells (μg compound/mg cells)	Transformation capacity of fed cells (μg compound/mg cells)
CT	16.9 ± 0.86	$> 146 \pm 4.9$
1,1,1-TCA	2.66 ± 0.33	7.84 ± 0.34
PCE	1.38 ± 0.48	1.91 ± 0.21

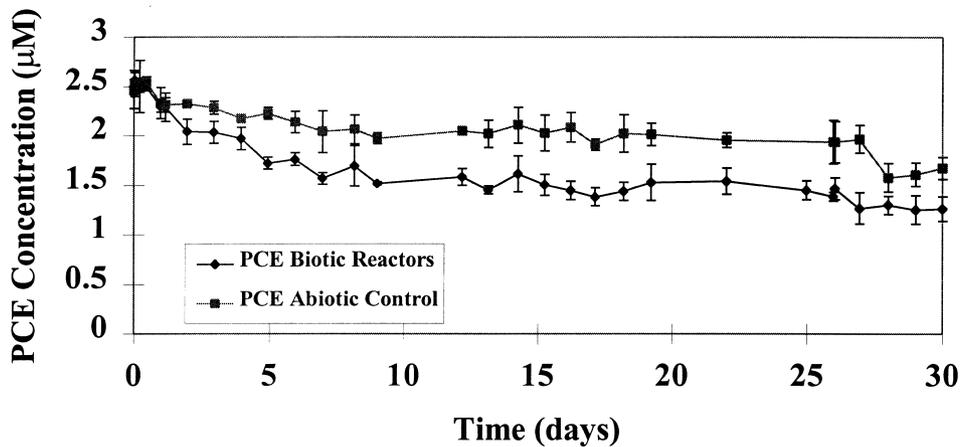


Fig. 7. PCE transformation in determination of observed transformation capacity in once-fed batch reactors (error bars represent range of data).

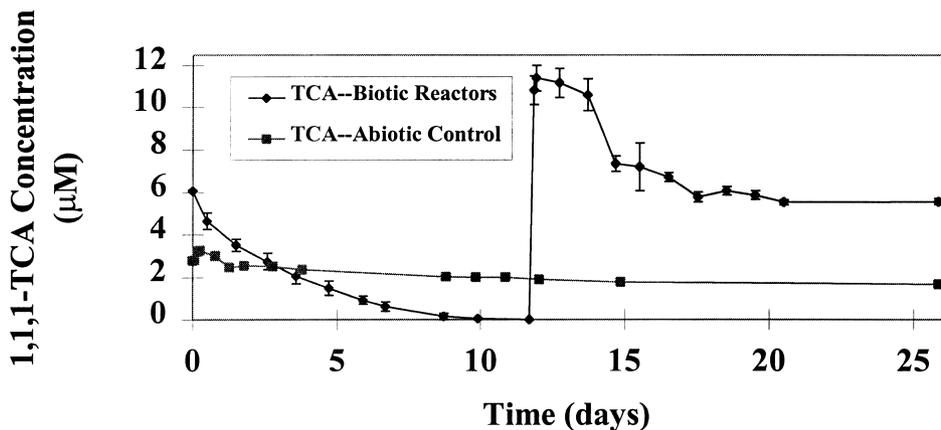


Fig. 8. 1,1,1-TCA transformation in determination of observed transformation capacity in once-fed batch reactors (error bars represent range of data).

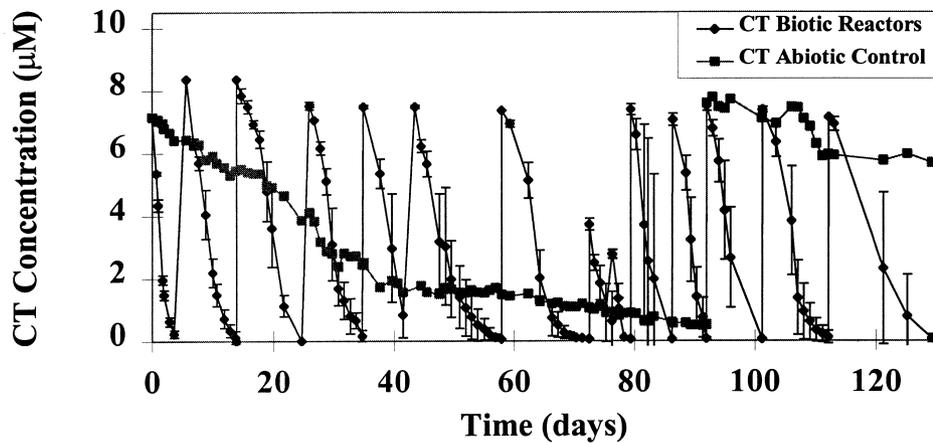


Fig. 9. CT transformation in determination of observed transformation capacity in once-fed batch reactors (error bars represent range of data).

via a similar mechanism because such a factor has characteristics consistent with metallocoenzymes.

The experimental results indicate that inactivation from CT transformation is not the primary cause of reduced transformation rates of 1,1,1-TCA in the presence of CT. The significant decrease seen in 1,1,1-TCA rate coefficients was likely due to inhibition, either through competition or a variety of other mechanisms at the enzymatic level.

Because the reducing equivalents provided in once-fed reactors greatly exceeded the amount of 1,1,1-TCA and PCE transformed, toxicity was a possible contributing factor in the observed limits of transformation. Although the differences between resting and once-fed reactors indicate that substrate availability is important in determining values for the T_L^{obs} parameter, some evidence suggests that toxicity also plays a role. When once-fed reactors which had ceased transforming a particular contaminant were supplemented with additional acetate, the total mass of contaminant transformed was substantially lower (data not reported).

However, toxicity and substrate availability were probably not the only reasons why transformation ceased in these reactors. It has been speculated that the mechanism for inactivation in reductive dechlorination reactions results from binding between bio-cellular material and radicals produced during transformation. Unless transformation occurs, these compounds will not be present to cause the inactivation of the cells. The limited PCE and 1,1,1-TCA transformation ability of the organisms in our culture perhaps minimizes the contribution of toxicity within mixtures. The observation that PCE had relatively little effect on the transformation rate of either CT or 1,1,1-TCA is probably the result of this inability of the culture to transform PCE. The observed limits of transformation for PCE and 1,1,1-TCA are possibly due to the lack of abundant numbers of suitable dechlorinating organisms and/or applicable coenzymes within the mixed culture. Therefore, the experimental values may have been reached by inactivating those organisms which were able to transform PCE or 1,1,1-TCA without affecting the remaining population of CT-degrading organisms. This is supported by the finding that reactors which had ceased transforming PCE and 1,1,1-TCA were able to degrade CT (data not reported). These reactors received no additional acetate, again suggesting that growth substrate limitation does not fully explain the limits observed for PCE and 1,1,1-TCA.

The results of the batch toxicity experiments do not provide conclusive evidence that enzyme inactivation occurs as the result of anaerobic reductive dechlorination. Although previous studies in the literature do not eliminate the possibility that inactivation occurs, the presence of multiple mediating coenzymes and a general lack of knowledge of the mechanisms involved makes establishment of such a

link problematic. Despite the similarity between the pattern observed for anaerobic 1,1,1-TCA transformation and methanotrophic cometabolism of chlorinated aliphatics, the T_L^{obs} values reported here should not be compared to previously published transformation capacities. Limits of transformation were determined in an effort to examine the potential contributing role of toxicity when mixtures of compounds are present and the values should be viewed as substrate-dependent and culture-specific.

SUMMARY

Batch kinetic studies indicated that rate coefficients for CT were higher than those for 1,1,1-TCA and PCE when the compounds were present alone and in mixtures. CT and 1,1,1-TCA were detrimental to the transformation of one another. The presence of PCE did not significantly affect the transformation of CT or 1,1,1-TCA, presumably because PCE transformation itself was slow. When experimental limits of transformation were determined, finite values were observed for PCE and 1,1,1-TCA, but not CT. Although toxic effects may have been exerted by these compounds, the results suggest that the number of PCE-degrading organisms within the mixed culture was limited. The lack of PCE transformation minimized potential inactivation and therefore did not affect the transformation of 1,1,1-TCA and CT. There was no finite limit observed for the transformation of CT, indicating that inhibition may be a more important factor in the decreased rate coefficients observed in the presence of this compound. The ability of a representative mixed culture to maintain CT transformation for an extended duration holds promise for long-term remediation of contaminated sites. Although negative impacts may be seen from the presence of competing and possibly toxic fortuitous substrates, supplementation with a suitable growth substrate may be sufficient to overcome these limitations.

Acknowledgements—This research was funded through the USEPA Great Plains/Rocky Mountain Hazardous Substance Research Center. Additional funding was provided by the Iowa Biotechnology and Byproducts Consortium. Invaluable aid with instrumentation was provided by Laboratory Director Craig Just.

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