Environ. Sci. Technol. **2002**, *36,* 2171−2177

Cometabolism of *cis*-1,2-Dichloroethene by Aerobic Cultures Grown on Vinyl Chloride as the Primary Substrate

MATTHEW F. VERCE,† CLAUDIA K. GUNSCH,‡ ANTHONY S. DANKO, AND DAVID L. FREEDMAN*

Department of Environmental Engineering and Science, Clemson University, Clemson, South Carolina 29634

An aerobic enrichment culture was grown on vinyl chloride (VC) as the sole source of carbon and energy. In the absence of VC, the enrichment culture cometabolized *cis*-1,2-dichloroethene (cDCE) and, to a lesser extent, *trans*-1,2-dichloroethene (tDCE), beginning with oxidation to the corresponding DCE-epoxides. When provided with VC (1.3 mM) and cDCE (0.2−0.3 mM), the enrichment culture cometabolized repeated additions of cDCE for over 85 days. Cometabolism of repeated additions of tDCE was also demonstrated but at a lower ratio of nongrowth substrate to VC. VC-grown *Pseudomonas aeruginosa* MF1 (previously isolated from the enrichment culture) also readily cometabolizes cDCE, with an observed transformation capacity ($T_{c,obs}$) of 0.82 μmol of cDCE/mg of total suspended solids (TSS). When provided with VC and cDCE, MF1 did not begin cometabolizing cDCE until nearly all of the VC was consumed. The presence of cDCE reduces the maximum specific rate of VC utilization. A kinetic model was developed that describes these phenomena via Monod parameters for substrate and nongrowth substrate, plus inactivation and inhibition coefficients. MF1 did not show any cometabolic activity on tDCE or trichloroethene and very limited activity on 1,1-DCE ($T_{c,obs} = 2 \times 10^{-5}$ μmol/mg TSS). Above 40 μM, tDCE and TCE noticeably increased the maximum specific rate of VC utilization, even though neither compound was consumed during or after VC consumption. High concentrations of 1,1-DCE (950 μM) completely inhibited VC biodegradation. As there is currently no evidence for aerobic biodegradation of cDCE as a sole source of carbon and energy, the results of this study provide a potential explanation for in situ disappearance of cDCE when the only other significant substrate available is VC. It is fortuitous that the VC-grown cultures tested exhibit their highest cometabolic activity toward cDCE, because it is the predominant DCE isomer formed during anaerobic reductive dechlorination of trichloroethene and tetrachloroethene.

Introduction

Anaerobic reductive dechlorination of tetrachloroethene and trichloroethene (TCE) often leads to accumulation of *cis*-1,2-dichloroethene (cDCE) and vinyl chloride (VC). VC presents the greatest concern because it is a known carcinogen. As contaminated groundwater containing cDCE and VC migrates away from a source area, electron-acceptor conditions may transition from anaerobic to aerobic, due to either engineered or naturally occurring conditions. Disappearance of cDCE and VC at the aerobic fringe of contaminant plumes has been documented in field (*1*, *2*) and microcosm studies (*3*−*5*) of natural attenuation. Currently, several explanations exist for this observation. First, methane generated in the anaerobic source area that migrates with (or somewhat ahead of) the daughter products can be used as a primary substrate for the cometabolism of VC and cDCE under aerobic conditions downgradient. Both VC and cDCE are readily consumed by methanotrophs (*6*−*13*). Second, ethene and ethane formed from reductive dechlorination of VC in the anaerobic zone can be used as primary substrates for the cometabolism of cDCE (*14*) and VC (*14*−*18*) under aerobic conditions. Third, VC can be degraded by aerobes that use it as a growth substrate (*19*−*21*). No organisms have been isolated thus far with the ability to use cDCE as a growth substrate.

Another possible explanation for disappearance of VC and cDCE at the aerobic fringe of a contaminant plume is cometabolism of cDCE by organisms using VC as a primary substrate. *Mycobacterium aurum* L1 was the first VC-grown organism reported to cometabolize DCEs (*20*). Initial oxidation rates of cDCE, *trans*-1,2-dichloroethene (tDCE), and 1,1-dichloroethene (1,1-DCE) were 50−80% lower than the initial VC oxidation rate. However, the apparent ability of *M. aurum* L1 to cometabolize DCEs is somewhat obscured by the statement that degradation of polychlorinated ethenes in the absence of VC was "not significant" (*20*). Furthermore, cDCE cometabolism in the presence of VC was not characterized.

The objective of this study was to evaluate the ability of VC-grown cultures to transform cDCE, tDCE, 1,1-DCE, and TCE either as growth substrates or as nongrowth substrates following growth on VC. Preliminary tests were conducted with a VC-grown enrichment culture, followed by similar tests with a pure culture of strain MF1 that was isolated from the enrichment culture. The effect of polychlorinated ethenes on the kinetics of VC utilization by MF1 were also evaluated.

Materials and Methods

Chemicals and Medium. VC gas (99.5%, containing <0.5% phenol to inhibit polymerization), cDCE (97%), tDCE (98%), and 1,1-DCE (99%) were obtained from Aldrich. TCE (99.5%) was obtained from Fisher. All other chemicals used were of reagent grade. cDCE, tDCE, 1,1-DCE, and TCE were added as either neat compounds or saturated water solutions. The minimal salts medium (MSM) described by Hartmans et al. (*22*) was used but with the amount of $(NH_4)_2SO_4$ reduced to 0.67 g/L. No vitamins or other complex growth factors were added to the MSM. cDCE- and tDCE-epoxide were chemically synthesized by reacting the parent compounds (1.1 g) with 3-chloroperoxybenzoic acid (1.6 g) dissolved in chloroform-*d* (6 mL) and heating (50−60 °C) for 8−12 h (*23*).

Analytical Methods. Consumption of VC, DCEs, and TCE was monitored by gas chromatographic analysis of headspace samples (0.1-mL sample), as previously described (*21*, *24*). The gas chromatograph (GC) response was calibrated to give

* Corresponding author phone: (864) 656-5566; fax: (864) 656-0672; e-mail: dfreedm@clemson.edu.

† Present address: Environmental Restoration Division, Lawrence Livermore National Laboratory, Livermore, CA 94550.

‡ Present address: Department of Civil Engineering, University of Texas, Austin, TX 78712.

10.1021/es011220v CCC: $22.00
Published on Web 04/02/2002

the total mass of the compound in that bottle (*25*). Assuming the headspace and aqueous phases were in equilibrium, the total mass present was converted to an aqueous-phase concentration

$$C_l = \frac{M}{V_l + H_c V_g} \quad (1)$$

where C_l = concentration in the aqueous phase (μM); M = total mass present (μmol/bottle); V_l = volume of the liquid in the bottle (L); V_g = volume of the headspace in the bottle (L); and H_c = Henry's constant ((mol·m^{-3} gas concentration)/(mol·m^{-3} aqueous concentration)), calculated for 23 °C (*25*). Aqueous-phase detection limits ranged from 10 nM for 1,1-DCE to 50 nM for cDCE. The validity of assuming equilibrium between headspace and aqueous phases was verified during kinetic experiments (see the following discussion).

cDCE- and tDCE-epoxide were identified by analysis of headspace samples with a GC/mass spectrometer (MS) system, consisting of a Hewlett-Packard 5890 GC with a 60-m 1-μm DB-5 column, a Ruska Instruments thermal desorption trap for kryofocusing of samples, and a 5988 Hewlett-Packard MS. Confirmation of epoxide formation by the VC-grown enrichment culture exposed to cDCE and tDCE involved purging the headspace of a serum bottle with nitrogen through a solution containing 0.094M 4-(4-nitrobenzyl)-pyridine in ethylene glycol. Aliquots of the solution were then combined with 0.1 M Tris-HCl buffer and acetone, heated at 37 °C, and analyzed in a scanning UV/visible spectrophotometer. The 4-(4-nitrobenzyl)pyridine and epoxide reaction product absorbs at 550−570 nm (*26*). Chemically synthesized cDCE- and tDCE-epoxide were used to validate the GC/MS and 4-(4-nitrobenzyl)pyridine procedures.

Standard methods (*27*) were used to determine total suspended solids (TSS).

Culture Maintenance and Kinetic Experiments. A VC-grown enrichment culture (*18*) was maintained in a 2.5-L glass bottle (Bellco Biotechnology) capped with a gray butyl rubber septum (30-mm), held in place with a screw cap. Maintenance consisted of pH adjustment (7.0 ± 0.1) with 8 M NaOH, purging the headspace with oxygen, and adding VC (100 mL) every 3−5 days. After the consumption of the VC, aliquots were periodically removed and replaced with MSM. Samples (100 mL) were placed in serum bottles (160 mL) to evaluate cDCE and tDCE biodegradation in the presence and absence of VC. The bottles were capped with Teflon-faced rubber septa. Autoclaved controls (121 °C for 15 min) and water controls (WC) were used to evaluate abiotic losses.

Strain MF1 (isolated from the aforementioned enrichment culture) was grown in a VC-fed reactor operated in a semicontinuous draw-and-fill batch mode, as previously described (*21*). The VC provided contains a trace level of phenol (see previous discussion), but MF1 is unable to use phenol as a sole carbon and energy source (*21*). The slow growth rate of MF1 necessitated that the reactor be run at a relatively long retention time (140 days). The TSS concentration in the reactor averaged 162 mg/L. After the consumption of VC, reactor effluent samples (25 mL) were distributed to serum bottles (70 mL) for the kinetic experiments with cDCE, tDCE, 1,1-DCE, and TCE in the presence and absence of VC. Bottles were agitated on a gyratory shaker table (150 rpm) between headspace sampling.

The endogenous decay coefficient for cometabolic activity (b_c) was determined for cDCE in the same manner described by Chang and Criddle (*28*) (details provided in Supporting Information).

Kinetic Modeling. A model was developed to describe the ability of MF1 to cometabolize cDCE, both in the presence and absence of growth substrate (VC). For a completely mixed batch system, the coupled differential equations for substrate and nongrowth substrate use, respectively, are

$$-\frac{dS}{dt} = \left(k_S - k_{S,C}\left(\frac{C_0}{C_0 + K_I}\right)\right)\left(\frac{S}{K_S\left(1 + \frac{C}{K_C}\right) + S}\right)X \quad (2)$$

$$-\frac{dC}{dt} = \left(k_C' - k_{inact}(C_0 - C)\right)\left(\frac{K_S}{K_S + S}\right)\left(\frac{C}{K_C\left(1 + \frac{S}{K_S}\right) + C}\right)X \quad (3)$$

where S is the growth substrate concentration (μM); k_S is the maximum specific growth substrate utilization rate (μmol of substrate/mg of TSS/d); K_S is the Monod half-saturation coefficient for growth substrate (μM); C is the nongrowth substrate concentration (μM); C_0 is the initial nongrowth substrate concentration (μM); k_C' is the theoretical maximum specific nongrowth substrate utilization rate (μmol of nongrowth substrate/mg of TSS/d); K_C is the Monod half-saturation coefficient for nongrowth substrate (μM); $k_{S,C}$ is the specific rate of substrate inactivation (μmol of nongrowth substrate/mg of TSS/d); K_I is a nongrowth substrate inhibition coefficient (μM); k_{inact} is the specific rate of nongrowth substrate inactivation (L/mg TSS/d); and X is the biomass concentration (mg of TSS/L). The increase in biomass concentration during kinetic experiments was estimated to be less than 2%, permitting the use of initial biomass concentrations (X_0) in place of X in eqs 2 and 3.

Parameter estimates were obtained with a stepwise approach described by Chang and Criddle (*28*). Parameters k_S and K_S were determined previously in experiments using only VC (*21*). Parameters k_C', K_C, and k_{inact} were obtained simultaneously by fitting eq 3 to depletion data from cultures fed only cDCE. Parameters $k_{S,C}$ and K_I were obtained simultaneously by fitting eqs 2−3 to depletion curves from additional cultures fed VC plus cDCE. The effect of mass transfer on parameter estimation was evaluated with a previously described method (*29*), by comparing solutions of eqs 2−3 to solutions of similar equations that include mass transfer. For example, the impact of mass transfer on k_C', K_C, and k_{inact} was evaluated by comparing the solution to eq 3 without growth substrate present to the following equation:

$$-\frac{dC}{dt} = \left(k_C' - k_{inact}(C_0 - C)\right)\left(\frac{C}{K_C + C}\right)X - K_L a(C - C_{act}) \quad (4)$$

where $K_L a$ is the mass transfer coefficient for cDCE (17.6 ± 2.47 h^{-1}) and C_{act} is the actual liquid-phase concentration of cDCE experienced by the culture (μM). $K_L a$ for VC was measured previously (*21*). A similar analysis was performed to assess the impact of mass transfer on estimation of parameters $k_{S,C}$ and K_I. In all cases, solutions including mass transfer became indistinguishable from solutions assuming equilibrium before the first data points for VC and cDCE were collected, indicating that mass transfer did not affect the estimation of parameters.

Fitting of model equations to substrate depletion data was performed numerically using the software Aquasim 2.0 (*30*). Fitting was done in a simultaneous manner, in which all available depletion curves were fitted together to obtain one set of parameters, rather than fitting each curve individually and reporting an arithmetic average. A reiterative weighting procedure (*31*) was used to obtain an adequate fit of the cDCE-only data. Standard deviations of parameter

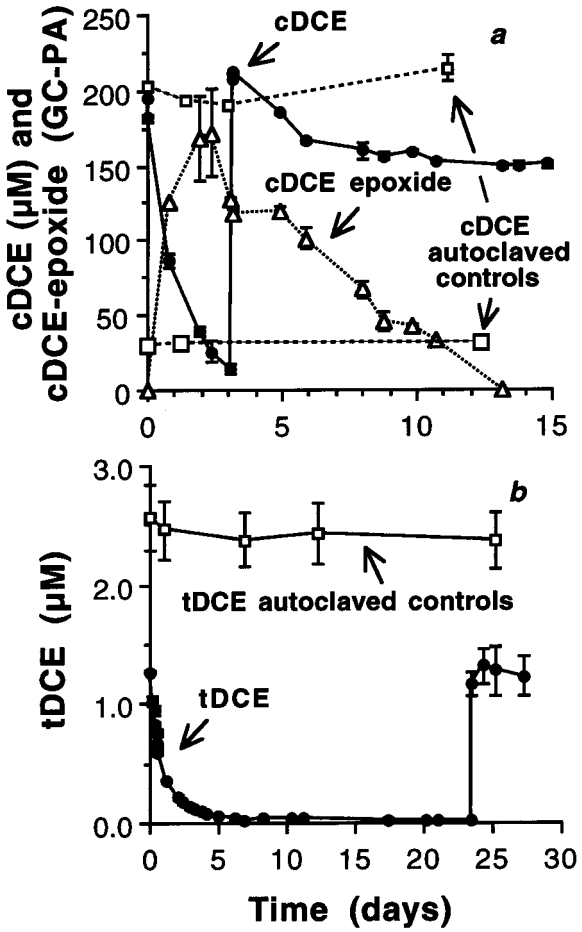

FIGURE 1. Cometabolism of cDCE (a) and tDCE (b) by the VC-grown enrichment culture. A standard was not available for quantification of the cDCE-epoxide. Error bars represent one standard deviation of replicate bottles.

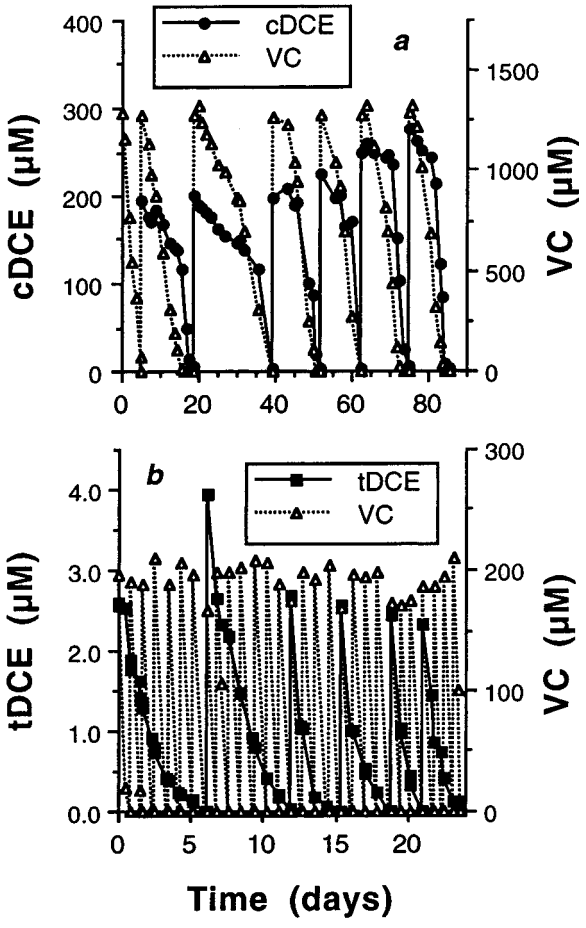

FIGURE 2. Sustained cometabolism of cDCE (a) and tDCE (b) by the VC-grown enrichment culture when VC is simultaneously provided as the growth substrate. Results for one bottle are shown in each panel; duplicates behaved similarly.

estimates and correlation matrix elements were also computed by Aquasim.

Results

Enrichment Culture. The enrichment culture was maintained on VC as a sole source of carbon and energy for over 3 years prior to the present experiments. Samples were distributed to duplicate serum bottles and fed either cDCE or tDCE. Subcultures fed cDCE rapidly consumed one addition but did not completely consume a second (Figure 1a). By comparison, the enrichment culture transformed a much smaller amount of tDCE (Figure 1b). When higher initial amounts of cDCE and tDCE were added than shown in Figure 1, similar amounts of both compounds were consumed, leaving a residual amount of cDCE and tDCE in the bottles (data not shown). The autoclave controls demonstrated that the disappearance of cDCE and tDCE was a biotic process and that the Teflon-faced rubber septa adequately retained cDCE and tDCE. The enrichment culture did not transform 1,1-DCE (130 μM) or TCE (170 μM) after 13 days, as compared to abiotic losses (data not shown).

Concurrent with cDCE biodegradation was accumulation of a volatile compound, subsequently identified as cDCE-epoxide. Identification required a much higher headspace concentration than that shown in Figure 1a. This was achieved by growing the enrichment culture on VC in a 2.5-L bottle to a biomass concentration of approximately 740 mg of TSS/L, centrifuging the biomass (20 min at 16 000*g*), resuspending the biomass in MSM (100 mL) in a serum bottle (160 mL), and adding a high dose of cDCE (6.1−12.2 mM). Once most of the cDCE was consumed, headspace samples were analyzed by GC/MS. The resulting spectra matched what has been reported for chemically synthesized and biologically produced cDCE-epoxide (*32*). Further confirmation of cDCE-epoxide in the headspace of the serum bottle was obtained by trapping the contents in 4(4-nitrobenzyl)pyridine. The reaction product absorbed at 550−570 nm, consistent with chemically synthesized cDCE-epoxide.

tDCE-epoxide was not detectable when low amounts of tDCE were transformed by the enrichment culture (Figure 1b). However, when a high biomass concentration was used along with a high initial concentration of tDCE (as described previously in Results for cDCE), formation of tDCE-epoxide was confirmed by GC/MS and the headspace trapping procedure with 4(4-nitrobenzyl)pyridine.

Simultaneous addition of cDCE and VC was also examined. The enrichment culture consumed repeated additions of both compounds (Figure 2a). Over an 85 day period, 143 μmol of cDCE and 1420 μmol of VC were biodegraded. Autoclaved controls indicated minimal loss of VC with the Teflon-faced septa (8.0% in 38 days). Experiments with simultaneous additions of VC and tDCE yielded similar results, although a much lower amount of tDCE was consumed relative to VC (Figure 2b). Over a 24 day period, 2.17 μmol of tDCE and 913 μmol of VC were biodegraded. These results demonstrated the potential to sustain cDCE and tDCE cometabolism when the enrichment culture was maintained on VC as a growth substrate.

Cometabolism of cDCE by MF1. The kinetics of cDCE cometabolism were examined further with VC-grown strain

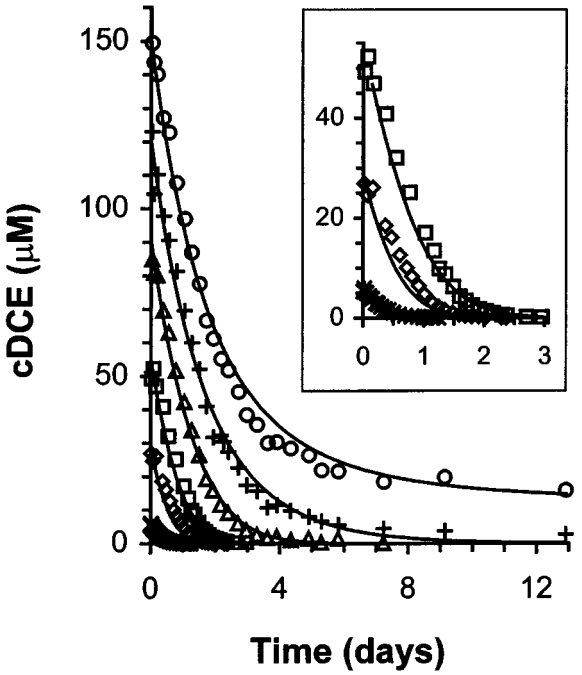

FIGURE 3. cDCE cometabolism by VC-grown MF1. These data were used simultaneously to determine k_C', K_C, and k_{inact}. Solid lines represent the fit of eq 3. The observed transformation capacity was determined from bottles with incomplete cDCE utilization. The inset provides an expanded view of the data and model fit for the three lowest cDCE concentrations.

TABLE 1. Summary of Kinetic Parameter Estimates for Metabolism of VC and Cometabolism of cDCE by MF1

parameter	value
k_S (μmol of VC/mg of TSS/d)	0.41 ± 0.003[a]
K_S (μM VC)	0.26 ± 0.037[a]
k_C' (μmol of cDCE/mg of TSS/d)	0.44 ± 0.011
K_C (μM cDCE)	22.0 ± 0.8
$k_{S,C}$ (μmol of VC/mg of TSS/d)	0.31 ± 0.002
K_I (μM cDCE)	1.0 ± 0.15
k_{inact} (L/mg of TSS/d)	$0.0032 \pm (9 \times 10^{-5})$

[a] From Verce et al. (*21*).

MF1, isolated from the VC enrichment culture (*21*). Consumption of cDCE in the absence of VC by strain MF1 is shown in Figure 3. Fitting of eq 3 to these data permitted estimation of k_C', K_C, and k_{inact} (Table 1). Some, but not all, off-diagonal elements of the resulting correlation matrix were greater than 0.9, indicating that estimates of k_C', K_C, and k_{inact} were not highly correlated (*33*). The finite amount of cDCE that can be cometabolized by strain MF1, expressed as an observed transformation capacity ($T_{c,obs}$), is 0.82 ± 0.06 μmol of cDCE/mg of TSS.

When fed VC and cDCE, strain MF1 did not begin consuming cDCE until virtually all of the VC was utilized (Figure 4). This is consistent with MF1 having a much smaller half-saturation coefficient for VC than for cDCE (i.e., $K_C > K_S$, Table 1), indicating that VC should effectively out-compete cDCE for consumption. The presence of cDCE reduces the rate of VC utilization, as indicated by cultures with and without cDCE present (Figure 4). The complete kinetics model (eqs 2−3) was fit to depletion curves from cultures fed VC plus cDCE to obtain estimates of $k_{S,C}$ and K_I (Table 1), using the values for k_C', K_C, and k_{inact} determined with cDCE-only data. Parameters $k_{S,C}$ and K_I were not correlated, as indicated by correlation matrix elements less than 0.9 (*33*).

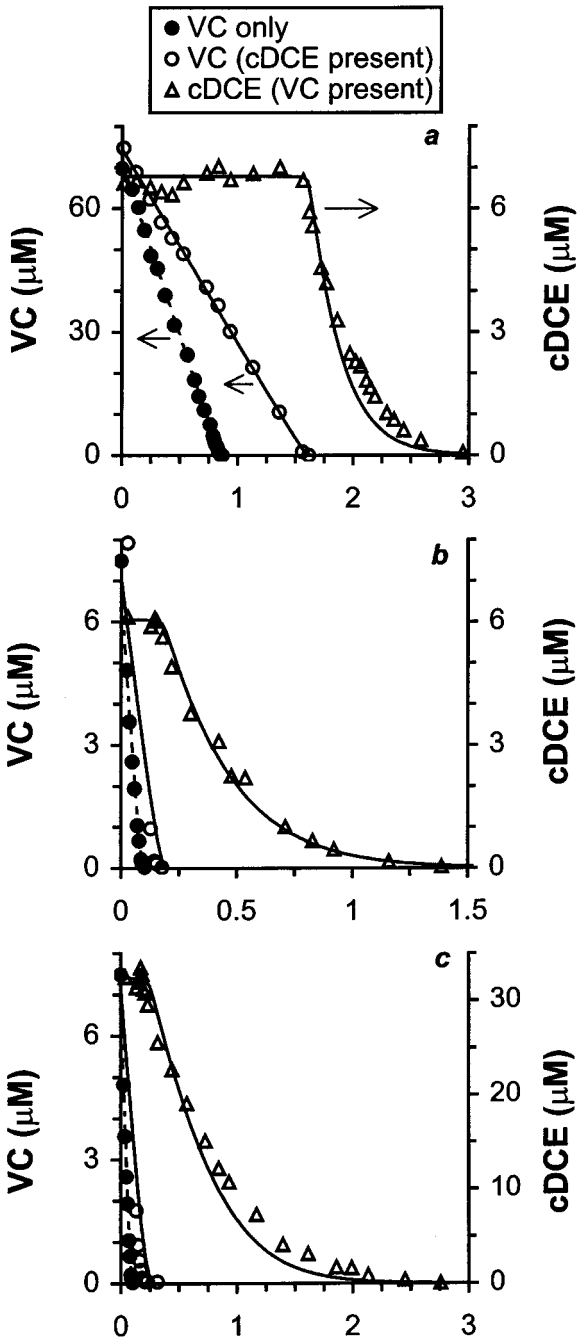

FIGURE 4. Metabolism of VC and cometabolism of cDCE by MF1 when (a) $[VC]_o \gg [cDCE]_o$; (b) $[VC]_o \approx [cDCE]_o$; and (c) $[VC]_o \ll [cDCE]_o$. Data from these plus three other bottles were used simultaneously to determine $k_{S,C}$ and K_I. Solid lines represent the fit of eqs 2−3. The dashed lines represent the fit of the Monod model when VC is present as the sole substrate.

Initial zeroth-order rates of cDCE cometabolism by MF1 were measured just after the culture finished consuming VC ($r_{C(0)}$) and after increasing amounts of time in the absence of VC ($r_{C(t)}$), to determine the endogenous decay coefficient for nongrowth substrate activity (b_C) (Figure 5). The resulting value of value of b_C is 0.041 ± 0.038 h^{-1}. Similar experiments were not performed with tDCE, 1,1-DCE, and TCE because MF1 exhibits comparatively little or no activity on them (see the following section).

Evaluation of tDCE, 1,1-DCE, and TCE Cometabolism by MF1. Cometabolism of tDCE (1−90 μM), 1,1-DCE (5−20 μM), and TCE (5−12 μM) by VC-grown MF1 was examined in the absence of VC. In contrast to cDCE, MF1 exhibited no

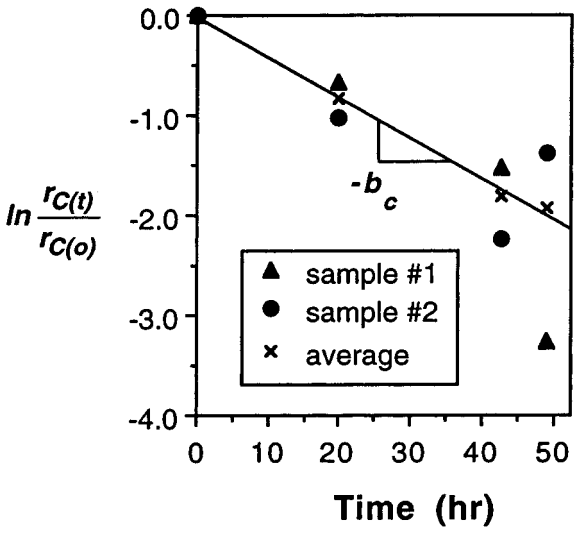

FIGURE 5. Determining the endogenous decay coefficient for cDCE activity (b_c) for VC-starved MF1.

cometabolic activity with tDCE or TCE at any of the concentrations tested. Some activity was observed with 1,1-DCE, but the transformation capacity was only 2×10^{-5} μmol of 1,1-DCE/mg of TSS (see Supporting Information for representative results).

Transformation of tDCE, 1,1-DCE, and TCE was also tested in the presence of VC to determine if VC consumption would facilitate cometabolic activity and if the presence of these compounds impacted the kinetics of VC utilization. During and shortly after VC consumption, little or no cometabolism of tDCE (15−800 μM), 1,1-DCE (8−950 μM), and TCE (8−800 μM) was observed (see Supporting Information for representative results). However, at tDCE and TCE concentrations above 40 μM, their presence increased the maximum specific rate of VC utilization by 13−42% (statistically significant, $\alpha = 0.05$), even though tDCE and TCE were not consumed during or after VC consumption (see Supporting Information for representative results). On the other hand, high concentrations of 1,1-DCE (950 μM) completely inhibited VC biodegradation. For most of the experiments, the presence of tDCE, 1,1-DCE, or TCE did not have a statistically significant impact on the K_S for MF1's utilization of VC.

Discussion

The findings reported here demonstrate that microorganisms grown aerobically on VC as a primary substrate are capable of cometabolizing cDCE and, to a lesser extent, tDCE and 1,1-DCE. VC-supported cometabolism of polychlorinated ethenes was first suggested in studies with *M. aurum* L1 based on initial oxidation rates of 43, 36, 14, and <1 μmol/d/mg of cells for cDCE, tDCE, 1,1-DCE, and TCE, respectively (*20*). However, nongrowth substrate depletion curves were not provided for L1, and no experiments were conducted in which VC and the nongrowth substrates were added at the same time. Our results conclusively demonstrate the phenomenon of VC serving as a primary substrate in support of cometabolism, especially with respect to cDCE. The observed transformation capacity for cDCE by VC-grown MF1 is 14−43% of values reported for cDCE by methanotrophic cultures (*8*, *10*). The transformation yield for MF1 ($T_y = T_{C,obs} \times Y_{obs} = 0.0026$ μmol of cDCE/μmol of VC) is considerably lower than the comparable value for an ethene-grown enrichment culture (*14*), in part because of the lower yield for VC as a growth substrate.

Both MF1 and L1 utilize an alkene monooxygenase to initiate catabolism of VC (*20*, *21*). Conversion of cDCE and tDCE by the VC enrichment culture to their respective epoxides is consistent with this pathway in MF1. Given the substrate range of other alkene monooxygenases, such as that used by propylene-grown *Xanthobacter* strain Py2 (*34*, *35*), cometabolism of polychlorinated ethenes may be expected. However, the alkene monooxygenase possessed by VC-grown MF1 shows a distinct preference for cDCE, while L1 and Py2 show high levels of cometabolic activity with both cDCE and tDCE. Py2 also readily cometabolizes TCE, while MF1 and L1 show no activity on it. Several butane grown organisms also cometabolize cDCE but not tDCE (*36*, *37*).

It is not yet known why the VC-grown enrichment culture (from which MF1 was isolated) exhibits a low level of cometabolic activity on tDCE, while MF1 shows none. One possible explanation examined is a rapid loss of cometabolic activity in the absence of primary substrate, a process quantified by the endogenous decay coefficient for cometabolic activity (b_C). MF1 loses its capacity to cometabolize cDCE nearly twice as fast as a methanotrophic enrichment culture that cometabolizes TCE (*28*). However, a rapid loss of tDCE cometabolic capacity does not account for MF1's lack of activity on this compound. In experiments where VC and tDCE were added, VC was readily metabolized (which should have increased the capacity for cometabolism), but there was still no activity on tDCE, either during or after VC consumption. In addition to different levels of activity on tDCE, the enrichment culture and MF1 also differ in their growth rates on VC; the enrichment grows approximately 2 times faster.

Although MF1 showed little or no cometabolic activity on tDCE, 1,1-DCE, and TCE, these compounds did have a noticeable impact on the maximum rate of VC utilization at concentrations above approximately 40 μM (see Supporting Information). The inhibitory effect of 1,1-DCE is consistent with other studies that have demonstrated the toxicity of this compound or its metabolites (*34*, *38*), and MF1 does exhibit a low level of cometabolic activity toward this compound. The mechanism by which tDCE and TCE increase the maximum rate of VC metabolism, without themselves being transformed, is not yet known.

The batch depletion kinetics of cDCE by MF1 are characterized by several features. First, in the absence of VC, the highest observed rate of cDCE utilization (0.26 μmol of cDCE/mg of TSS/d based on the highest amount shown in Figure 3b) was lower than the fitted value for k_C'. Thus, k_C' represents a "theoretical" maximum specific nongrowth substrate utilization rate rather than an observed rate typified by Monod kinetics. This suggests that MF1 suffers inactivation that inhibits cDCE use before it can reach the maximum theoretical rate (k_C'). Although the nature of this inactivation is not known, it is more likely to be due to a depletion of reducing power than to toxicity of reactive intermediates. cDCE epoxide, observed in the headspace of the enrichment culture, has the longest half-life of all chloroethene epoxides (*23*) and is presumably the least toxic. Inactivation due to consumption of cDCE is described by the subterm "$k_{inact}(C_0 - C)$" in eq 3, which was adapted from a previously developed model (*39*). The k_C' value obtained for MF1 is 2 orders of magnitude lower than the initial oxidation rate reported for VC-grown L1 (*20*). Even higher maximum specific rates have been reported for methanotrophic cometabolism of cDCE (e.g., refs *8* and *13*), although a much lower rate has been observed with an ammonia oxidizer (*40*).

Second, when VC and cDCE are present together, cDCE decreases the rate of VC use. The ability of the model to adequately describe this phenomenon is due to subterm "$C_0/(C_0 + K_I)$" in eq 2, which acts as a "switch" to decrease the maximum substrate utilization rate by an amount $k_{S,C}$ when cDCE is present. The low value of K_I (Table 1) indicates

that this inhibition occurs even at very low initial concentrations of cDCE.

Third, cDCE use is completely inhibited by VC. Preferential use of a growth substrate prior to consumption of a nongrowth substrate is different from the more common pattern of simultaneous consumption of growth substrate and nongrowth substrate. Another pattern, in which the nongrowth substrate is consumed preferentially to the growth substrate, has also been observed (*16*, *41*). Thus, it appears there is a spectrum of substrate and nongrowth substrate consumption patterns. VC inhibition of cDCE use is captured by subterm "$K_S/(K_S + S)$" in eq 3, which is essentially zero for all values of S because of the low value of K_S. Competitive inhibition is incorporated in the present model and others (*28*, *42*−*45*) by subterms "$1 + C/K_C$" and "$1 + S/K_S$" in eqs 2−3, respectively. However, these terms by themselves were not enough to fully describe the interactions observed between the substrate and nongrowth substrate. The high degree of correlation between k_C' and K_C, as well as between k_C' and k_{inact}, indicates that unique estimates may not have been obtained for these parameters. Nevertheless, the fitted value for K_C is comparable to those for methanotrophic cometabolism of cDCE, based on studies that used a similar experimental approach (*8*, *13*). Correlation between kinetic parameters, which has been reported for other models of cometabolism kinetics (*28*, *46*), was minimized by estimating $k_{S,C}$ and K_I separately from K_C, k_C', and k_{inact}.

As there is currently no evidence for aerobic biodegradation of cDCE as a sole source of carbon and energy, the results of this study provide a potential explanation for in situ disappearance of cDCE when the only other significant substrate available is VC. It is fortuitous that VC-grown MF1 and L1 exhibit their highest cometabolic activity toward cDCE, because cDCE is the predominant dichloroethene isomer formed during anaerobic reductive dechlorination and is the final dechlorination product of several halorespiring organisms (*47*). The inability of known VC-grown organisms to biotransform TCE further emphasizes the value of sequential anaerobic and aerobic conditions for achieving complete in situ removal of polychlorinated ethenes, especially via monitored natural attenuation.

Acknowledgments

Ana Isabel Carvalho, Joana Pinto de Sousa, Paulo Malho Guedes, Nuno Miguel G. Coelho, and Luis Miguel M. Silva, Catholic University of Portugal, worked tirelessly to complete the experiments with the VC-grown enrichment culture. The assistance of Kim Ivey in the collection of MS data is greatly acknowledged. Meghna Swamy assisted in the evaluation of mass transfer effects. This research was supported in part by a grant from the U.S. Environmental Protection Agency.

Supporting Information Available

The procedures for determining the endogenous decay coefficient for cometabolic activity, data for evaluating tDCE, 1,1-DCE, and TCE cometabolism by MF1, and the effect of tDCE, 1,1-DCE, and TCE on the maximum specific rate of VC metabolism by MF1. This material is available free of charge via the Internet at http://pubs.acs.org.

Literature Cited

(1) Cox, E.; Edwards, E.; Lehmicke, L.; Major, D. In *Intrinsic Bioremediation*; Hinchee, R. E., Wilson, J. T., Downey, D. C., Eds.; Battelle Press: Columbus, OH,1995; pp 223−232.
(2) Edwards, E. A.; Cox, E. E. In *In Situ and On-Site Bioremediation*; Alleman, B. C., Leeson, A., Eds.; Battelle Press: Columbus, OH, 1997; pp 261−265.
(3) Bradley, P. M.; Chapelle, F. H. *Anaerobe* **1998**, *4*, 81−87.
(4) Davis, J. W.; Carpenter, C. L. *Appl. Environ. Microbiol.* **1990**, *56*, 3878−3880.
(5) Freedman, D. L.; Danko, A. S.; Verce, M. F. *Water Sci. Technol.* **2001**, *43*, 333−340.
(6) Anderson, J. E.; McCarty, P. L. *Appl. Environ. Microbiol.* **1997**, *63*, 687−693.
(7) Castro, C. E.; Riebeth, D. M.; Belser, N. O. *Environ. Toxicol. Chem.* **1992**, *11*, 749−755.
(8) Chang, H. L.; Alvarez-Cohen, L. *Appl. Environ. Microbiol.* **1996**, *62*, 3371−3377.
(9) Dolan, M. E.; McCarty, P. L. *Environ. Sci. Technol.* **1995**, *29*, 1892−1897.
(10) Dolan, M. E.; McCarty, P. L. *Environ. Sci. Technol.* **1995**, *29*, 2741−2747.
(11) Fogel, M. M.; Taddeo, A. R.; Fogel, S. *Appl. Environ. Microbiol.* **1986**, *51*, 720−724.
(12) Oldenhuis, R.; Vink, R. L. J. M.; Janssen, D. B.; Witholt, B. *Appl. Environ. Microbiol.* **1989**, *55*, 2819−2826.
(13) Oldenhuis, R.; Oedzes, J. Y.; van der Waarde, J. J.; D. B. Janssen, D. B. *Appl. Environ. Microbiol.* **1991**, *57*, 7−14.
(14) Koziollek, P.; Bryniok, D.; Knackmuss, H. J. *Arch. Microbiol.* **1999**, *172*, 240−246.
(15) Verce, M. F.; Ulrich, R. L.; Freedman, D. L. *Environ. Sci. Technol.* **2001**, *35*, 4242−4251.
(16) Verce, M. F.; Freedman, D. L. *Biotechnol. Bioeng.* **2001**, *71*, 274−285.
(17) Freedman, D. L.; Herz, S. D. *Water Environ. Res.* **1996**, *68*, 320−328.
(18) Freedman, D. L.; Verce, M. F. In *In Situ and On-Site Bioremediation*; Alleman, B. C., Leeson, A., Eds.; Battelle Press: Columbus, OH, 1997; Vol. 3, pp 255−260.
(19) Hartmans, S.; de Bont, J.; Tramper, J.; Luyben, K. *Biotechnol. Lett.* **1985**, *7*, 383−388.
(20) Hartmans, S.; de Bont, J. A. M. *Appl. Environ. Microbiol.* **1992**, *58*, 1220−1226.
(21) Verce, M. F.; Ulrich, R. L.; Freedman, D. L. *Appl. Environ. Microbiol.* **2000**, *66*, 3535−3542.
(22) Hartmans, S.; Kaptein, A.; Tramper, J.; de Bont, J. A. M. *Appl. Microbiol. Biotechnol.* **1992**, *37*, 796−801.
(23) Janssen, D. B.; Grobben, G.; Hoekstra, R.; Oldenhuis, R.; Witholt, B. *Appl. Microbiol. Biotechnol.* **1988**, *29*, 392−399.
(24) Freedman, D. L.; Gossett, J. M. *Appl. Environ. Microbiol.* **1989**, *55*, 2144−2151.
(25) Gossett, J. M. *Environ. Sci. Technol.* **1987**, *21*, 202−208.
(26) Barbin, A.; Brésil, H.; Croisy, A.; Jacquignon, P.; Malaveille, C.; Montesan, R.; Bartsch, H. *Biochem. Biophys. Res. Commun.* **1975**, *67*, 596−603.
(27) American Public Health Association. *Standard Methods for the Examination of Water and Wastewater*, 18th ed.; APHA: Washington, DC, 1989.
(28) Chang, W.; Criddle, C. S. *Biotechnol. Bioeng.* **1997**, *56*, 492−501.
(29) Smatlak, C. R.; Gossett, J. M.; Zinder, S. H. *Environ. Sci. Technol.* **1996**, *30*, 2850−2858.
(30) Reichert, P. *Concepts Underlying a Computer Program for the Identification and Simulation of Aquatic Systems*; Report No. CH-8600; Schriftenreihe der EAWAG, Swiss Federal Institute for Environmental Science and Technology (EAWAG): Dübendorf, Switzerland, 1994.
(31) Neter, J.; Kutner, M. H.; Nachtsheim, C. J.; Wasserman, W. *Applied Linear Statistical Models*; Irwin: Chicago, IL, 1996.
(32) Vlieg, J. E. T. v. H.; de Koning, W.; Janssen, D. B. *Appl. Environ. Microbiol.* **1996**, *62*, 3304−3312.
(33) Beck, J. V.; Arnold, K. J. *Parameter Estimation in Engineering and Science*; Wiley and Sons: New York, 1977.
(34) Ensign, S.; Hyman, M.; Arp, D. *Appl. Environ. Microbiol.* **1992**, *58*, 3038−3046.
(35) Ensign, S. A. *Appl. Environ. Microbiol.* **1996**, *62*, 61−66.
(36) Hamamura, N.; Page, C.; Long, T.; Semprini, L.; Arp, D. J. *Appl. Environ. Microbiol.* **1997**, *63*, 3607−3613.
(37) Kim, Y.; Arp, D. J.; Semprini, L. *J. Environ. Eng.* **2000**, *126*, 934−942.
(38) Rasche, M. E.; Hyman, M. R.; Arp, D. J. *Appl. Environ. Microbiol.* **1991**, *57*, 2986−2994.
(39) Ely, R. L.; Williamson, K. J.; Hyman, M. R.; Arp, D. J. *Biotechnol. Bioeng.* **1997**, *54*, 520−534.
(40) Vannelli, T.; Logan, M.; Arciero, D. M.; Hooper, A. B. *Appl. Environ. Microbiol.* **1990**, *56*, 1169−1171.
(41) Bagley, D. M.; Gossett, J. M. *Appl. Environ. Microbiol.* **1995**, *61*, 3195−3201.
(42) Broholm, K.; Christensen, T. H.; Jensen, B. K. *Water Res.* **1992**, *26*, 1177−1185.
(43) Saéz, P. B.; Rittmann, B. E. *Biodegradation* **1993**, *4*, 3-21.

(44) Chang, H.-L.; Alvalez-Cohen, L. *Environ. Sci. Technol.* **1995**, *29*, 2357−2367.
(45) Ely, R. L.; Williamson, K. J.; Guenther, R. B.; Hyman, M. R.; Arp, D. J. *Biotechnol. Bioeng.* **1995**, *46*, 218−231.
(46) Smith, L. H.; Kitanidis, P. K.; McCarty, P. L. *Biotechnol. Bioeng.* **1997**, *53*, 320−331.
(47) Holliger, C.; Schumacher, W. *Antonie van Leeuwenhoek* **1994**, *66*, 239−246.

Received for review August 21, 2001. Revised manuscript received February 26, 2002. Accepted February 27, 2002.
ES011220V