

Methanotrophic Chloroethene Transformation Capacities and 1,1-Dichloroethene Transformation Product Toxicity

MARK E. DOLAN* AND
PERRY L. MCCARTY†

Department of Civil Engineering, Stanford University,
Stanford, California 94305

For a mixed methanotrophic culture grown under copper deficiency, the relative transformation capacities of the chloroethenes with 10 mM formate present was in the order from highest to lowest: 1,2-*trans*-dichloroethylene (t-DCE); 1,2-*cis*-dichloroethylene (c-DCE); vinyl chloride (VC); trichloroethylene (TCE); and 1,1-dichloroethylene (1,1-DCE). Respective values were 4.8, 3.6, 2.3, 0.85, and 0.13 μmol transformed per mg of cells. Chloroethenes with asymmetric chlorine distributions had lower transformation capacities, probably due to higher transformation product toxicity. While 1,1-DCE itself was not toxic at the concentrations evaluated (up to 1 mg/L), its transformation products were highly toxic. Aquifer microcosms transformed up to 4.8 mg/L VC with no apparent toxic effects, but when both VC and 1,1-DCE were present, about 75% less transformation of VC and a marked decrease in methane oxidation rate resulted because of 1,1-DCE transformation product toxicity. About 25 times more VC was transformed in the soil microcosms per unit of methane consumed than in aqueous batch tests.

Introduction

Groundwater contamination by chloroethenes has resulted from their widespread use and disposal in the environment. Under anaerobic conditions, the more highly chlorinated chloroethenes (TCE, c-DCE, t-DCE, and 1,1-DCE) can be reductively dehalogenated to form VC (1, 2), a known human carcinogen. However, aerobic methanotrophic oxidation of chloroethenes is also possible and has been the subject of many studies (1-4). Methane monooxygenase (MMO), an enzyme system with broad substrate specificity, can initiate the oxidation of most chloroethenes leading to the formation of the respective epoxides (5, 6). Chloroethene epoxides are chemically unstable and degrade to a variety of products, some of which can be toxic to the transforming organisms (5, 7-9). Such transformation product toxicity (TPT) also exists with chloroethene cometabolic transfor-

mation by toluene (10, 11), ammonia (12, 13), alkene (14, 15), and isoprene utilizers (16). In both enzyme and whole cell studies, the degree of toxicity has been found to be proportional to the amount of chloroethene transformed (5, 7, 9, 14). The most suspect transformation products include either radical or carbocation intermediates, the epoxides themselves, or acyl chlorides, resulting from the thermal rearrangement of the epoxides or from atomic migration while still in the active site of the enzyme. Information is lacking on the effect of TPT from chloroethene transformation on organisms in general, on the extent of chloroethene transformation, and on mixtures of chloroethenes. Such information was sought in this study through aqueous batch transformation tests. In addition, microcosms with aquifer material from a Superfund site in St. Joseph, MI, were used to study TPT with VC and 1,1-DCE, alone and in mixtures.

Materials and Methods

Aqueous Batch Tests. Stock solutions of chloroethenes, except VC, were prepared by adding a specific mass of each chloroethene to a 62-mL glass bottle filled with demineralized water and sealed with a Teflon-lined Mininert cap. TCE (99.0% purity), t-DCE (98.5% purity), c-DCE (96% purity), and 1,1-DCE (99.0% purity) were obtained from Alltech Associates Inc. (Deerfield, IL). VC stock solutions were prepared by equilibrating VC gas (99.5% purity, Fluka Chemicals, Buchs, Switzerland) with demineralized water in a glass barrel syringe with a Teflon plunger, followed by transfer to glass vials with Mininert caps.

Aqueous batch transformation experiments were conducted in a 20 °C environmental chamber using 62-mL clear glass bottles sealed with Teflon-lined Mininert caps containing 23.8 mL of mineral media with composition as described by Fogel *et al.* (3), except no copper was added. For the experiments with formate added, 250 μL of the mineral media was replaced with 250 μL of a 1.0 M sodium formate in demineralized water solution to give a final concentration (cell culture included) of 10 mM formate. Chloroethene stock solution was added by a gas-tight syringe through the Mininert closures; the culture bottles were placed upside down on a Lab-Line circular-action shaker table and shaken throughout the study at 200-300 rpm. After 15 min, initial headspace samples for chloroethene analyses were taken. Then 1.25 mL of mixed methanotrophic cell culture, obtained from a 10-L continuous gas-feed reactor (10% methane in air at 280 mL/min) and operated with a 9-day detention time as described previously (7), was spiked by syringe through the Mininert closure. Headspace samples were taken periodically with a 200 μL gas-tight Hamilton syringe for chloroethene analysis. Specific chloroethene transformation rates were determined from the change in total chloroethene mass with time divided by the total cell dry mass in the bottle.

In subsequent methane and formate oxidation studies to evaluate cell activity after chloroethene transformation, the above bottles were removed from the shaker table after approximately 30 h, uncapped, and purged for 5 min with nitrogen gas followed by reintroduction of air into the headspace. The bottles were then recapped and either 3

* Telephone: (415) 723-0315; Fax: (415) 725-8662. E-mail address: mdolance@leland.stanford.edu

† Silas H. Palmer Professor of Civil Engineering.

mL of methane gas or 50 μ L of 1.0 M sodium formate in demineralized water was injected into the bottles. Initial 300 μ L headspace or 100 μ L-aqueous samples were taken and analyzed for methane or formate followed by replacement on the shaker table and periodic sampling over the next 24 h to monitor methane and formate consumption.

Analytical Procedures for Aqueous Batch Tests. Chloroethene concentrations were determined by headspace analysis, and total mass in each bottle was calculated using known solution and headspace volumes and dimensionless Henry's constants for the respective compound (0.92 for VC, 0.88 for 1,1-DCE, 0.31 for t-DCE, 0.13 for c-DCE, and 0.30 for TCE at 20 °C) (17). From 20 to 100 μ L of headspace gas was withdrawn using a Hamilton gas-tight syringe and analyzed on a Hewlett-Packard 5890 gas chromatograph equipped with a 30-m J&W Scientific DB-624 megabore column, using either a HNU Systems Model PI 52-02A photoionization detector or an OI Corporation Model 4420 electrolytic conductivity detector, with data integration on a Series 900 PE Nelson analytical interface.

Methane and oxygen concentrations were determined by injection of 400 μ L of headspace gas taken with a Precision-lok gas-tight syringe into a Fisher Model 25V gas partitioner equipped with a thermal conductivity detector using helium as the carrier gas.

Formate concentration was determined by dilution of a 100- μ L sample in 5 mL of demineralized water, filtration through a 0.2- μ m membrane filter (Gelman Sciences Inc., Ann Arbor, MI), and analysis of filtrate on a Dionex Series 4000i ion chromatograph equipped with an HPIC AS4A Dionex ion pak column and a conductivity detector using a borate solution as eluent.

Culture density, as total suspended solids (TSS), was determined at the end of transformation experiments by filtration of a specific volume of suspended culture through a 0.2- μ m membrane filter. The filter was dried overnight in a 105 °C oven and cooled, and the change in weight was compared to controls consisting of filters with the same volume of mineral media passed through them.

Microcosm Tests. Twelve microcosms consisting of 15-mL test tubes filled with aquifer material and fitted with a fluid exchange system to allow sequential batch testing (18) were prepared using aquifer material aseptically obtained from a Superfund site in St. Joseph, MI (19). Microcosm pore fluids were exchanged at a flow rate of about 2.5 mL/min using a syringe pump (Sage Instruments, Division of Orion Research Inc., Cambridge, MA) with two 100-mL glass-barrel gas-tight syringes and adjustable Teflon-lined plungers (Spectrum, Houston, TX). Feed solutions consisted of methane- and oxygen-saturated groundwater spiked with stock solutions of VC and 1,1-DCE. Groundwater obtained from an uncontaminated zone at the St. Joseph site (19) was filter-sterilized using a 0.2- μ m membrane filter before being placed in two autoclaved glass bottles and purged with either oxygen or methane gas (99.9% purity, Liquid Carbonics, Chicago, IL) to create gas-saturated solutions. The VC stock solution was prepared in a gas-tight 50-mL glass-barreled syringe by saturating 35 mL of demineralized water with VC gas (99.9% purity, Fluka Chemicals, Buchs, Switzerland). A 1,1-DCE stock solution was prepared by adding a specific volume of 1,1-DCE (99% purity, Alltech Associates Inc., Deerfield, IL) to a 62-mL glass vial filled with demineralized water and sealed with a Teflon-lined Mininert cap.

The microcosms were operated in sequential batch mode with effluent collected for analysis in a manner to prevent the loss of volatile components (18). Samples were kept in 2-mL glass vials sealed with Teflon-lined septa and open-holed screw caps and stored in a 4 °C room until analyzed. During each exchange, an initial effluent sample was collected as the first 3.25 mL of solution was passed through the microcosm and was considered to represent conditions in the soil pore water just prior to the exchange. A final effluent sample was collected after 20 mL of feed solution had been passed through the microcosm, representing the influent or initial conditions for the subsequent incubation period.

Analytical Procedures for Microcosm Tests. Each effluent sample was analyzed within 48 h of the exchange for VC, 1,1-DCE, dissolved oxygen (DO), and methane. In general, 5–50 μ L was first removed through the 2-mL sample vial septa to analyze for VC and 1,1-DCE. For DO analysis, 300 μ L was then removed, followed by the removal of 500 μ L for methane analysis.

VC and 1,1-DCE concentrations, were determined by adding the 5–50- μ L aqueous sample, after dilution in 4 mL of demineralized degassed water, to a Tekmar Model 4000 purge-and-trap system. This consisted of an ALS autosampler connected to a Hewlett Packard 5890A gas chromatograph (GC) equipped with a 70-m J&W Scientific DB-624 megabore column and a Tracor electrolytic conductivity detector with data integration using a Series 900 Perkin Elmer Nelson analytical interface.

Dissolved oxygen (DO) concentrations were determined by dilution of a 300 μ L sample in 300- μ L of air-saturated DI water and analysis on a Hansatech Model CB1-D mini-DO probe.

Methane concentration was determined by injection of a 500- μ L sample into a 9-mL serum vial sealed with a rubber sleeve stopper. After inverting and shaking vigorously for 1 min, a 300- μ L headspace sample was removed with a Hamilton gas-tight syringe and analyzed for methane on a Hewlett Packard 5730A GC using a 5 ft by 1/8 in. Supelco 60/80 Carbosieve packed column at 120 °C with a flame ionization detector.

Results

Chloroethene Transformation in Aqueous Batch System.

In order to determine the relative transformation capacity of a resting mixed methanotrophic culture for the five chloroethenes studied, 22 bottles were prepared with a combination of mineral media, cells (125 mg/L of TSS), formate, and chloroethene. Since the objective of the test was to determine relative chloroethene transformation capacities (T_c) for the mixed methanotrophic culture, the cells used came from one harvest of the growth reactor and were used within 2 h. For each chloroethene, one bottle had no formate, one bottle had formate, and two bottles were controls (no cells added). An additional two bottles were prepared containing only mineral media and cells. The initial chloroethene concentration desired was that which would exhaust the transformation ability of the cells within 24 h, but would leave 25–50% of the chloroethene untransformed. On this basis and from experimentation, initial aqueous concentrations for the bottles without formate were 0.79, 6.73, 8.59, 10.5, and 11.5 mg/L for 1,1-DCE, VC, TCE, c-DCE, and t-DCE, respectively. In the same respective order, initial aqueous concentrations in the formate, fed bottles were 0.77, 14.8, 35.4, 46.8, and 60.0 mg/L.

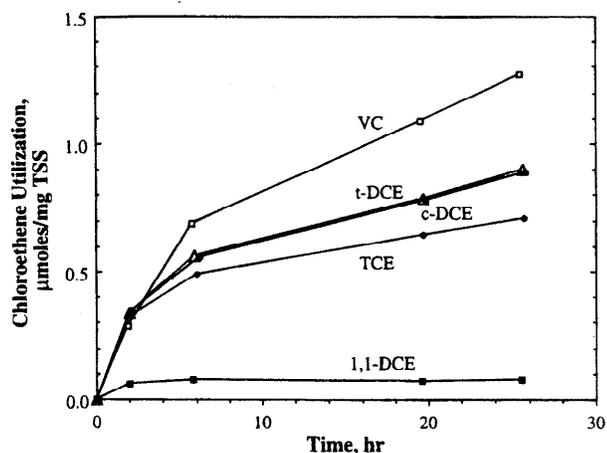


FIGURE 1. Chloroethene utilization by a mixed methanotrophic culture in the absence of formate.

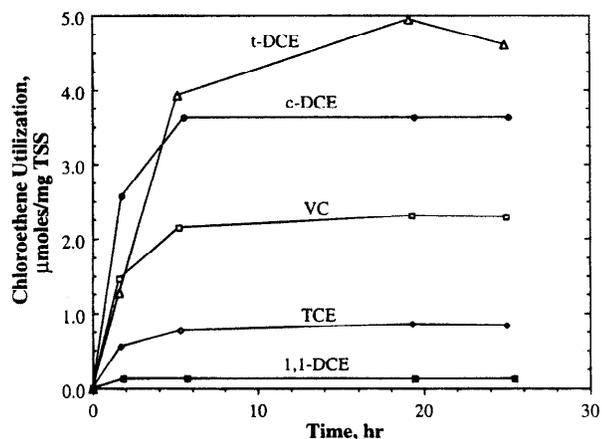


FIGURE 2. Chloroethene utilization by a mixed methanotrophic culture in the presence of 10 mM formate.

The relative rates of chloroethene utilization in the absence of formate is indicated in Figure 1. In all cases except 1,1-DCE, the capacity of the organisms to transform chloroethenes was not expended within the first 26 h. Over the first few hours, transformation occurred at a relatively high rate but was followed by a slower transformation rate for the duration of the experiment. VC was transformed to the greatest extent, on a molar basis, whereas 1,1-DCE was the least transformed. Transformation of 1,1-DCE essentially stopped after the first few hours. Figure 2 illustrates the transformation of chloroethenes in the presence of 10 mM formate. Unlike the bottles with no formate, the full transformation capacity of the cells appears to have been expended within the first 5–10 h. Transformation did not cease due to the absence of formate as greater than 65% of the 10 mM formate added remained at the end of the 26-h test. In all cases, except possibly with 1,1-DCE, the presence of formate enhanced the initial rate of transformation as well as the amount of chloroethene ultimately transformed in the 26-h test.

Due to the number of bottles tested, the time required for sample analysis, and the desire to use one batch of freshly harvested cells, the data presented in Figures 1 and 2 are the results of single determinations. However, the transformation tests were repeated employing duplicate bottles testing one chloroethene at a time. The results were very similar to those reported above with slow continual transformation in the absence of formate and rapid initial transformation followed by inactivation after about 10 h in

TABLE 1

Specific Rates of Methane Utilization for Cells after Exposure to Chloroethenes for 24 h

chloroethene	formate added (mM)	chloroethene added (μ g)	chloroethene transformed (μ g)	methane utilization rate ($\text{mg mg}^{-1} \text{d}^{-1}$)
none	0	0	0	0.350
none	0	0	0	0.410
VC	0	400	250	0.330
t-DCE	0	460	275	0.280
c-DCE	0	360	270	0.049
1,1-DCE	0	46	24	0.039
TCE	0	340	290	0.320
VC	10	880	450	0.066
t-DCE	10	2400	1450	0.061
c-DCE	10	1600	1100	0.038
1,1-DCE	10	45	40	0.055
TCE	10	1400	350	0.190

the formate-amended bottles. Duplicates were in good agreement with each other with standard deviations of less than 10% of the mean in all cases. The relative rates of chloroethene utilization in non-formate-fed bottles and the order of transformation capacities in formate-fed bottles were the same as those in Figures 1 and 2. T_c values showed a 10–30% decrease from those above for VC and the three isomers of DCE, and a 50% increase for TCE. These differences were most likely due to temporal variations in activity within the growth reactor.

In an attempt to determine the possible extent of cell inactivation due to chloroethene TPT, specific methane utilization rates were measured at the end of the 26-h experiment as described previously. The bottles were purged of chloroethenes and volatile intermediates, and 3 mL of methane was added to produce a headspace concentration of approximately 6% methane. Methane disappearance was monitored over 24 h, and specific methane utilization rates were computed for each sample as well as for the two bottles that had contained only mineral media and cells (Table 1). The samples without formate had specific methane utilization rates similar to those of cells not exposed to chloroethenes, with the exception of c-DCE and 1,1-DCE for which the rates were much lower. Thus, the transformation capacity of the cells with the latter two chloroethenes appears to have been reached. The samples containing formate all exhibited a significant reduction in specific methane utilization, suggesting that they had essentially reached their full transformation capacities by the end of the 26-h incubation period.

1,1-DCE Transformation Product Toxicity. 1,1-DCE was transformed less than any of the other chloroethenes, and the methane utilization ability of the culture dropped low as well after exposure to 1,1-DCE. Tests with 1,1-DCE and the mixed methanotrophic culture were conducted to determine if the toxicity was from 1,1-DCE itself or due to TPT. Bottles were prepared as in the earlier experiment, but in addition, shaken cell controls and acetylene-treated cell controls were included as well. Shaken cell controls contained mineral media, some with and some without formate, and were shaken as were the 1,1-DCE-containing bottles. Since deactivation of MMO in resting cells when shaken in the presence of oxygen has been reported (7), these additional controls were felt necessary. Acetylene-treated cell controls in mineral media, some with and some

TABLE 2

Specific Rates of Methane Utilization for Cells after Exposure to 1,1-DCE

experimental conditions	formate added (mM)	1,1-DCE transformed (μg)	methane utilization rate (mg of CH_4 (mg TSS) $^{-1}$ day $^{-1}$)
cells alone	0	0	0.300
1,1-DCE exposed cells	0	15.5	0.014
1,1-DCE exposed cells	0	16.5	0.005
acetylene treated; 1,1-DCE exposed cells	0	~0	0.046
cells alone	10	0	0.210
1,1-DCE exposed cells	10	24.2	0.042
1,1-DCE exposed cells	10	23.3	0.033
acetylene treated; 1,1-DCE exposed cells	10	~0	0.063

without formate, were shaken for 30 min in a 25% acetylene atmosphere (99.6% purity, A.A. grade, Liquid Carbonics) to inhibit MMO activity (20) before the addition of 1,1-DCE.

Both the bottles with and without formate transformed significant amounts of 1,1-DCE within the first few hours before transformation ceased. The transformation capacities of 0.063 and 0.045 μmol of 1,1-DCE/mg of TSS with formate and without formate, respectively, are in general agreement with earlier tests. The acetylene-treated controls on the other hand transformed little or no 1,1-DCE, confirming that MMO was responsible for 1,1-DCE transformation. After 6 h, 1,1-DCE was purged from the test bottles, and either methane or formate was added and utilization was monitored (Table 2).

Both with and without formate, cells that had transformed 1,1-DCE exhibited less than 20% of the methane utilization rates found in the shaken cell controls. In both cases, less methanotrophic activity remained than with acetylene-treated controls, indicating a dramatic inactivation of the MMO system or whole-cell inactivation. Since MMO is not required to oxidize formate, a formate utilization test was conducted with the non-formate-fed bottles to determine whether the inactivation was specific to MMO or more general in nature. The acetylene treated cells, whether exposed to 1,1-DCE or not, exhibited formate utilization rates equal to that of shaken cell controls (Figure 3). However, a slower formate utilization rate was found for cells that had transformed 1,1-DCE. These results suggest that 1,1-DCE itself did not cause the inactivation, but rather the inactivation was caused by products of 1,1-DCE transformation. Reduction of the formate oxidation ability of the culture after 1,1-DCE transformation is indicative of possible cellular damage beyond the MMO system alone by transformation products.

VC and 1,1-DCE Transformation in Soil Microcosms.

Twelve microcosms containing aquifer material were used to evaluate the effect of 1,1-DCE on VC transformation. Pore water in eight of the microcosms was repeatedly exchanged with a feed solution containing DO and methane to enrich for methanotrophic activity. While the microcosms initially exhibited a spread in methane oxidation rates, after repeated exchanges, most of the microcosms had very similar rates with one microcosm obviously slower than the rest and one consistently faster. These two

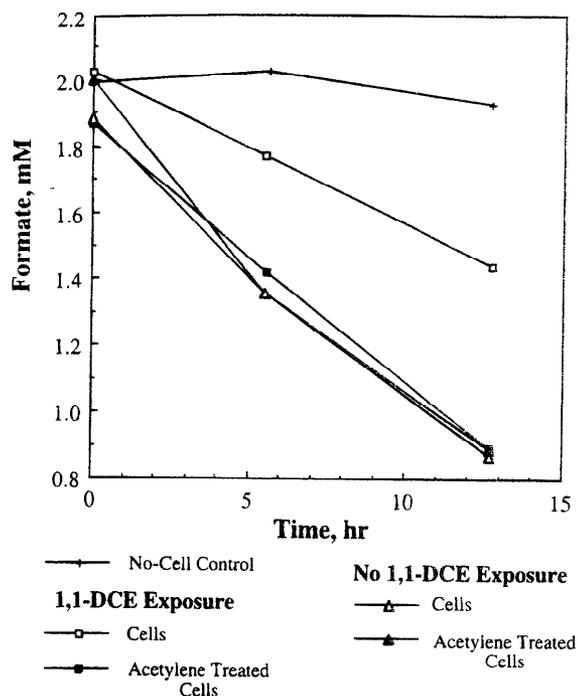


FIGURE 3. Formate utilization after 1,1-DCE exposure.

microcosms continued to be fed only methane and DO to act as the extreme cases of methanotrophic activity in the absence of chloroethene addition. Of the remaining six microcosms, two had about 5.3 mg/L VC added to their feed solution, another two had about 800 $\mu\text{g/L}$ 1,1-DCE, and the final two had both 5.3 mg/L VC and 800 $\mu\text{g/L}$ 1,1-DCE. In addition, four non-methane-fed control microcosms were all exchanged with water containing DO; also, one was exchanged with VC, one with 1,1-DCE, and two with both VC and 1,1-DCE in the same concentrations stated above.

Initially, the microcosms were exchanged as indicated above and allowed to incubate for only 6 h, whereupon they were exchanged again and incubated for 12 h, and similar exchanges were repeated but with increasing incubation times until a final incubation of 94 h was reached. The 1,1-DCE transformation that probably occurred was small and not measurable during the test period in any of the microcosms (Figure 4). An initial 12–24% decrease in 1,1-DCE was noted in all microcosms and was probably due to initial sorption to the aquifer matrix.

However, in VC-amended methane-fed microcosms, up to 90% VC transformation was obtained at longer detention times (Figure 5). The rate of methane oxidation was faster than the rate of VC transformation, with VC transformation leveling off after all of the methane was consumed. The rate of VC transformation was less with a 6-h incubation than it was with a 12-h incubation, perhaps due to competitive inhibition as methane concentration was higher during the 6-h incubation. Microcosms fed both VC and 1,1-DCE exhibited much less VC transformation than those fed only VC, with a maximum of only 20% removal achieved. With the initial 6-h incubation, these microcosms transformed VC at a rate similar to those fed only VC, but by the second exchange, VC transformation leveled off at about 20% removal. There was little change in VC concentration in the control microcosms over the test period.

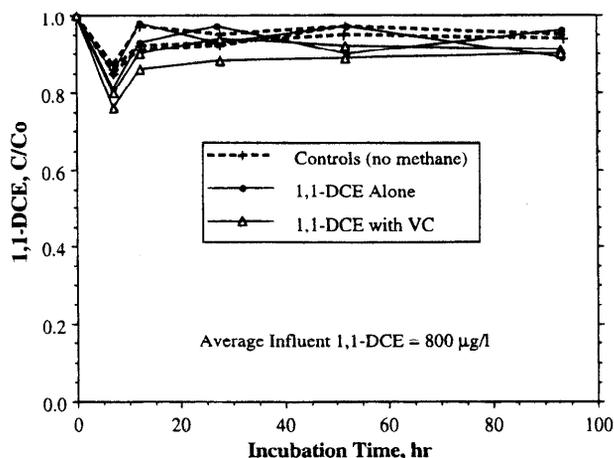


FIGURE 4. 1,1-DCE transformation in soil microcosms.

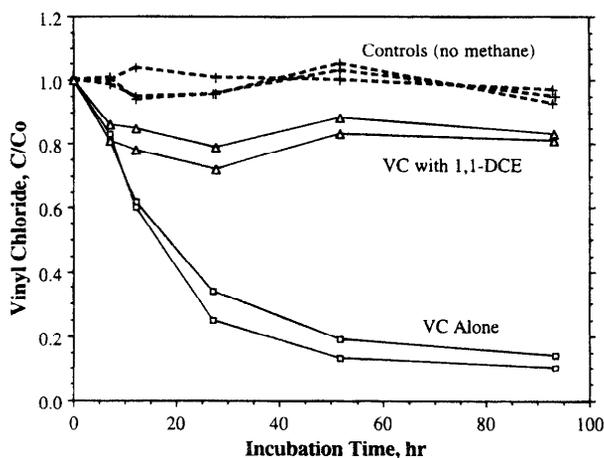


FIGURE 5. VC transformation in soil microcosms.

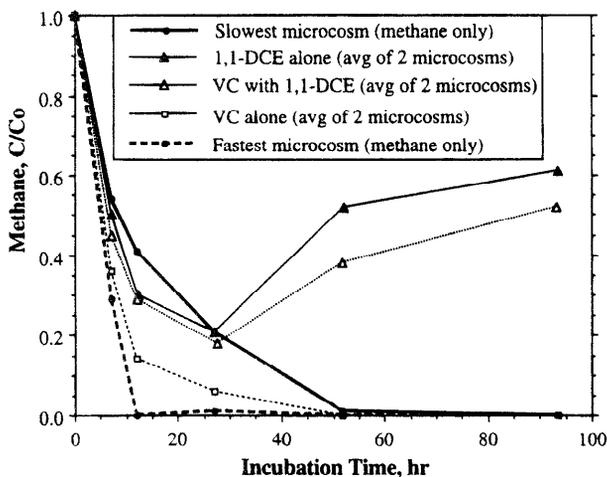


FIGURE 6. Methane utilization in soil microcosms.

Methane oxidation was also monitored in the eight active microcosms after each exchange (Figure 6). For the first two exchanges, methane oxidation in all six chloroethene-amended microcosms stayed within the envelope of the two microcosms fed only methane and DO. However by the third exchange, methane oxidation in all microcosms fed 1,1-DCE slowed, and at the end of the test less methane was being oxidized in 94 h than was oxidized in the first 6-h incubation period. Oxygen demand was greatest in the microcosms fed VC and methane, followed by the microcosms fed only methane. The four

microcosms fed 1,1-DCE exhibited the least oxygen demand, which was consistent with the decreased methane utilization results.

Discussion

A mixed methanotrophic culture fed formate but not methane had a finite transformation capacity (T_c), computed as the ultimate amount of chloroethene transformed per unit dry cell mass, for five chloroethenes in the following order: t-DCE > c-DCE > VC > TCE > 1,1-DCE. In the absence of formate, which served as an external source of reducing power, a finite transformation capacity was not achieved by the end of the 26-h test except for 1,1-DCE. From a previous study using a mixed methanotrophic culture from the same reactor, Alvarez-Cohen and McCarty (7) reported T_c values of 0.56 and 0.27 μmol of TCE/mg of TSS with and without formate, respectively. The higher T_c value of 0.85 μmol of TCE/mg of TSS for the formate-fed bottles in this study may be due to temporal variation in the activity of the culture.

Cell inactivation, as measured by a decrease in specific methane utilization rate, was most pronounced in the formate-fed bottles where greater chloroethene transformation had occurred. The toxic effect of transformation products with chloroethenes other than TCE appears similar to that found previously with TCE transformation (5, 7, 9). Methanotrophs are known to oxidize primary alkenes to their corresponding 1,2-epoxides (21–23), a reaction that has been proposed for chlorinated ethenes as well (5, 6, 24). Epoxides, and especially halogenated epoxides, are often unstable with short half-lives. The t-DCE epoxide is the most stable of the chlorinated ethene group with a reported half-life in aqueous solution of 30 h (24). The half-life of c-DCE is similar. However half-lives are much shorter for VC, TCE, and 1,1-DCE; 1.6 min, 12 s, and <1 s, respectively (25–27). The more asymmetrically substituted epoxides of 1,1-DCE and TCE thermally rearrange to form acyl chlorides (strong alkylating agents), whereas the epoxides of VC, c-DCE, and t-DCE rearrange to form less reactive chloroaldehydes (28, 29).

Isoprene degraders, reported to possess an epoxide-detoxifying enzyme, rapidly oxidized 1,1-DCE (16). In studies using whole-cell suspensions of *Methylosinus trichosporium* OB3b (9) and its purified sMMO (5), cell inactivation following TCE transformation was found to be caused by nonspecific covalent binding of TCE degradation products to cellular proteins. Fox et al. (5) further established that TCE epoxide itself was not likely to be responsible for the inactivation but rather a rearrangement product of the epoxide. In a study where radiolabeled TCE was degraded by toluene dioxygenase from *Pseudomonas putida* F1 (10), inactivation was caused by covalent modification of cellular molecules, specifically by alkylation of the protein fraction by glyoxylyl chloride and formyl chloride, breakdown products of TCE epoxide. Rasche et al. (13) reported that incubation of the soil bacterium *Nitrosomonas europaea* with [^{14}C]TCE destroyed the ammonia-oxidizing activity and TCE transformation ability of the organisms and resulted in numerous cellular proteins becoming covalently labeled with ^{14}C . The ammonia-oxidizing activity of the cells could be recovered through a process requiring de novo protein synthesis. A loss of oxygen uptake ability in *N. europaea* was also reported after transformation of 1,1-DCE, c-DCE, and to a lesser extent VC and t-DCE. It is plausible that the loss of

activity in methanotrophic cultures while oxidizing chloroethenes is due to similar toxic reactions.

Studies with pure methanotrophic cultures exposed to 1,1-DCE have produced divergent results. Both Tsien *et al.* (30), using whole cell suspensions of *M. trichosporium* OB3b, and Fox *et al.* (5), using purified sMMO from *M. trichosporium* OB3b, found 1,1-DCE to be readily degraded. However, Oldenhuis *et al.* (9) found 1,1-DCE to be a poor substrate and to be toxic at low concentrations when exposed to whole cell suspensions of *M. trichosporium* OB3b. Similarly, Green and Dalton (31) determined 1,1-DCE to quickly inactivate proteins of purified sMMO from *Methylococcus capsulatus* (Bath).

In this study, while 1,1-DCE itself was not toxic, its transformation products evidently were. Cell inactivation due to 1,1-DCE transformation was greater than for any of the other chloroethenes. 1,1-DCE was the only chloroethene for which the transformation capacity was achieved in the absence of formate during the 26-h test. Transformation product toxicity with 1,1-DCE was not restricted to MMO inactivation but also affected other cell components as demonstrated by the reduced formate oxidation ability of the culture after 1,1-DCE transformation.

When soil microcosms enriched for methanotrophic activity were exposed to 1,1-DCE, a similar product toxicity was observed. After three exchanges with 1,1-DCE, methane oxidation rates were significantly reduced, and after five exchanges with 1,1-DCE, they were about 15 times slower than with no exposure to 1,1-DCE. Methane-fed microcosms transformed up to 90% of the 5.3 mg/L influent VC with no apparent toxic effect when no 1,1-DCE was present. However, after several exchanges in microcosms fed both VC and 1,1-DCE, VC transformation decreased significantly from that found initially to only about 20% transformation of the influent VC. The toxicity of 1,1-DCE transformation products was readily apparent in this study, indicating that caution is needed in attempting to use an aerobic oxygenase for degrading other contaminants when 1,1-DCE is present. This is confirmed by the significant deactivation of TCE transformation found with phenol-fed cultures when only 67 $\mu\text{g/L}$ 1,1-DCE was present during in-situ groundwater treatment at the Moffett Federal Air Station field site (32).

By knowing the amount of methane required to grow a given mass of cells in the mixed methanotrophic growth reactor, the transformation capacity obtained from batch tests can be expressed as a transformation yield (T_y) or milligram of chloroethene transformed per milligram of methane utilized. T_y values from the aqueous batch tests with formate added were 0.05 mg/mg for VC and 0.004 mg/mg for 1,1-DCE. The above T_y value indicates that transformation in the soil microcosms would be less than 15 $\mu\text{g/L}$ 1,1-DCE, which is too low to be of statistical significance in the microcosms as was found to be the case, but was sufficient to cause much TPT.

Estimation of VC transformation in the soil microcosms from the above batch T_y values would suggest that less than 200 $\mu\text{g/L}$ VC would be transformed. However, a much higher VC transformation of 4.8 mg/L was achieved in the microcosms, similar to that found in previous microcosm studies with the same aquifer material (18), corresponding to a T_y of 1.25 mg of VC/mg of CII_4 or about 25 times that found in the aqueous batch tests. Even the microcosms with 1,1-DCE added produced a T_y of about 0.4 mg of VC/mg of CH_4 or almost 10 times that found in the aqueous

batch tests. The much different T_y values for one batch culture and one microcosm culture could be due to different methanotrophic species in the two systems or to the very different environmental conditions represented. Further study here would be of interest.

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