한국지하수토양환경학회 춘계학술발표회
2004년 4월 16~17일 국립환경연구원

Transformation of cis-1,2-Dichlororethylene and its Epoxide by a Butane-Grown Mixed Culture

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<요 약 문>

Aerobic cometabolism of cis-1,2-dichloroethylene (c-DCE) and c-DCE epoxide by a butane-grown mixed culture was evaluated. Transformation of c-DCE resulted in the concomitant generation of c-DCE epoxide. Chloride release studies showed nearly complete oxidative dechlorination of c-DCE (approximately 75%). Mass spectrometry confirmed the presence of a compound with mass-to-charge-fragment ratios of 112, 83, 48, and 35. The values are in agreement with the spectra of a chemically synthesized c-DCE epoxide. Some evidences indicating the involvement of the monooxygenase in the transformation of c-DCE epoxide are: 1) O_2 requirement for c-DCE transformation and butane degradation; 2) butane inhibition on c-DCE transformation and vice versa; 3) the inactivation of c-DCE and c-DCE epoxide transformations by acetylene (a known monooxygenase inactivator); and 4) the inhibition of c-DCE epoxide transformation by c-DCE.

Key words : a butane-grown mixed culture, aerobic cometabolism, cis-1,2-dichloroethylene, cis-1,2-dichloroethylene epoxide

1. INTRODUCTION

Microorganisms grown on a variety of substrates express oxygenase enzymes that are capable of transforming chlorinated aliphatic hydrocarbons (CAHs). Transformation of chlorinated ethenes by monooxygenases results in the formation of epoxides (Oldenhuis et al., 1991; Van Hylckama Vlieg et al., 1996). These electrophilic compounds are unstable in aqueous solutions. The reactivities of the epoxides and their degradation products often result in covalent modification of cellular components, causing transformation product toxicity (Oldenhuis et al., 1991, Van Hylckama Vlieg et al., 1997). Consequently, the amount of chlorinated ethene that can be transformed is limited due to

transformation product toxicity (Alvarez-Cohen and McCarty, 1991). Since the toxicity that is associated with cometabolic transformation products of chlorinated ethenes is the main limiting factor for the application of monooxygenase-expressing organisms, it is desirable to find ways to biologically detoxify transformation products.

c-DCE is an important groundwater pollutant that is generated from perchloroethylene (PCE) and trichloroethylene (TCE) by dechlorination under anaerobic conditions. However, fewer data about the aerobic degradation of c-DCE are available compared with the data about degradation of other chlorinated ethenes. van Hyclkama Vlieg et al. (1996, 1998) reported that c-DCE epoxide formed by transformation of c- DCE was actively transformed by *Methylosinus trichosporium* OB3b expressing soluble methane monooxygenase and *Rhodococcus* sp. strain AD45. However, rapid inactivation occurred during this transformation, indicating that even products are more toxic (van Hylckama Vlieg et al., 1996, 1997).

In long-term microcosm studies with aquifer core material from Hanford in Washington, butane was found to be an effective substrate for aerobic cometabolism of chloroform (CF) and 1,1,1-trichloroethane (1,1,1-TCA) (Kim et al. 1997). Resting-cell studies with a butane-oxidizing mixed culture enriched from the Hanford DOE site microcosms showed that butane-utilizers effectively transformed a broad range of CAHs, especially 1,1-dichloroethylene (1,1-DCE), 1,1,1-TCA, and 1,1-dichloroethane (1,1-DCA) (Kim et al., 2000 2002).

In this work, we evaluate: 1) how effectively a butane-grown enrichment culture can transform c-DCE; 2) if c-DCE epoxide is produced from c-DCE transformation; 3) if butane monooxygenase is involved in the transformations of c-DCE epoxide; and 4) how toxic transformations of c-DCE and c-DCE epoxide on cell activity. This study provides the first detailed evaluation of c-DCE and c-DCE epoxide by a butane-grown enrichment culture.

2. MATERIALS AND METHODS

Transformation of c-DCE. The transformation of c-DCE was monitored. Autoclaved phosphate buffer solution (58 mL) was added to autoclaved 125-mL amber serum bottles which were crimp sealed with Teflon -lined rubber septa (Kimble, Vineland, NJ). The c-DCE was added, and the initial c-DCE concentration was determined after 15 minutes of shaking. Washed and resuspended cells (4 to 6 mg on a TSS basis) were then added, and bottles were shaken at 180 rpm.

Cell Inactivation after Exposure to c-DCE. Cell inactivation was determined from butane uptake measurements after c-DCE exposure. After the 30-hr incubation, the bottles were purged with N_2 to remove the c-DCE, and air was then reintroduced. The bottles were recapped, and 2 mL of 10% butane in N_2 was added. Butane was also added to control bottles of cells incubated for 30 hours without any c-DCE exposure. The headspace butane concentration was monitored to determine rates of butane uptake. Butane uptake rates of cells exposed to c-DCE were compared to the control bottle of unexposed cells.

Chloride Release Study. To evaluate the degree of dechlorination of c-DCE, the amount of chloride released was measured. The observed chloride release was compared with stoichiometric release of

chloride required for the amount of c-DCE transformed. The dechlorination extent is presented on a percentage basis

3. RESULTS AND DISCUSSIONS

Transformation of c-DCE and the Effects of Its Transformation on Cell Inactivation. The effects of O_2, butane, or cell treatment with acetylene [35% (vol/vol) gas phase] on the transformation of c-DCE were examined. No transformation of c-DCE was observed in the absence of O_2. Acetylene-treated cells transformed less than 15% of the amount of c-DCE transformed by untreated cells. c-DCE transformation rates in the presence of butane [30% (vol/vol) gas phase] were about 13% of the rate in the absence of butane. The amount of c-DCE transformed decreased by 60%, respectively. However, 0.5% butane did not inhibit the transformation. Butane degradation was also inhibited by c-DCE (data not presented). A monooxygenase enzyme was likely involved in the transformation of the c-DCE, based on the lack of transformation in the absence of O_2 and the inactivation of c-DCE transformation by acetylene and the inhibition of c-DCE transformation by butane. The possible involvement of butane monooxygenase in the transformation of c-DCE is consistent with the results of Hamamura et al. (1997), with pure butane-utilizing cultures and an enrichment of the culture tested here.

Similar extents of cell inactivation (measured as a loss in butane uptake activity) occurred in the absence or presence of 0.5% butane, with less than 2% activity remaining. However, with 30% butane, 96% activity remained. These results indicate that toxicity resulting from c-DCE transformation could be greatly reduced in the presence of butane.

Transformations of c-DCE and c-DCE epoxide and chloride release. Transformation of c-DCE resulted in the concomitant generation of c-DCE epoxide (Figure 1). Mass spectrometry confirmed the presence of a compound with mass-to-charge-fragment ratios of 112, 83, 48, and 35. The values are in agreement with the spectra of a chemically synthesized c-DCE epoxide (Janssen et al. 1988). After transformation of approximately 70% of the c-DCE, c-DCE epoxide started to decrease rapidly. Chloride concentrations slowly increased during the initial transformation of c-DCE. Chloride concentrations more rapidly increased during transformation of c-DCE epoxide than c-DCE. Seventy five percentage Cl- release occurred after 30 h of incubation of c-DCE. The half-life of c-DCE epoxide is approximately 72 h (Janssen et al. 1988). The nearly complete oxidative dechlorination of c-DCE within 30 h of incubation suggests c-DCE epoxide transformation by the butane utilizers. The high degree inactivation of the butane utilizers after c-DCE transformation potentially resulted from the biotic transformation of the epoxide.

Effects of biological inactivators on transformations of c-DCE epoxide. To confirm if the c-DCE epoxide is biologically transformed, $HgCl_2$, a potent inhibitor of biological activity, in a parallel experiment $HgCl_2$ (~ 25 mg/L) was added 5.8 min after transformation of approximately 80% c-DCE (Figure 2A). This immediately stopped the transformations of both c-DCE and c-DCE epoxide, confirming that the epoxide may be biologically degraded.

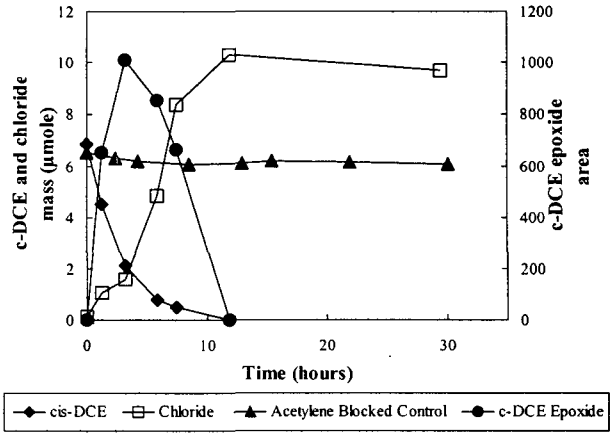

Figure 1. Formation and transformation of c-DCE epoxide during transformation of c-DCE by resting cell suspensions of the butane utilizers (4.3-mg TSS used).

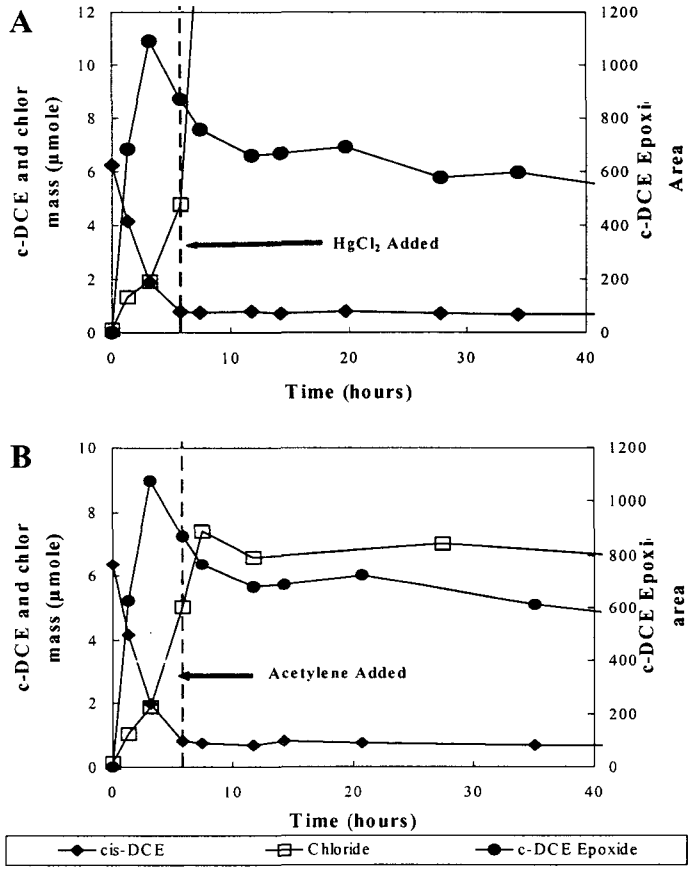

Figure 2. Effects of $HgCl_2$ [25 mg/L] (A) and acetylene[1%, vol./vol.] (B) additions on the transformations of c-DCE and c-DCE epoxide by resting cell suspensions of butane utilizers (4.5-mg TSS used). Chloride concentrations dramatically increased due to $HgCl_2$ addition.

Acetylene has been known to be an irreversible inactivator of methane monooxygenase (MMO) from *Methylococcus capsulatus* (Bath) (Prior and Dalton, 1985), butane monooxygenase (BMO) from butane-grown *Pseudomonas butanovora*, an environmental isolate, CF8 (Hamamura et al., 1999), and propane monooxygenase (PMO) from propane-grown *Mycobacterium vaccae* JOB5 (Vanderberg and Perry, 1994). This phenomenon has also been observed in studies with mixed cultures grown on

methane and propane (Alvarez-Cohen and McCarty, 1991). Acetylene was used to evaluate the involvement of a monooxygenase enzyme in the transformation of c-DCE epoxide. Acetylene addition also completely stopped the transformation both c-DCE and c-DCE epoxide (Figure 2B). This indicated that c-DCE epoxide was likely converted by a butane monooxygenase enzyme.

c-DCE inhibition on the transformation of c-DCE epoxide. A batch experiment was performed to evaluate if the same monooxygenase is involved in the transformations of both c-DCE and c-DCE epoxide (data not shown). c-DCE inhibition on the c-DCE epoxide was evaluated by adding c-DCE into a batch bottle when production and transformation of c-DCE epoxide were occurring. Just after spike c-DCE, c-DCE transformed and c-DCE epoxide started to be produced and accumulated. After transformation of approximately 76% c-DCE added, c-DCE epoxide concentrations started to decrease. When the c-DCE epoxide concentrations start to decrease, c-DCE was added into the bottle. After respike c-DCE, c-DCE epoxide accumulated again, and production rate of chloride decreased, indicating c-DCE epoxide transformation was likely inhibited by c-DCE. After respike c-DCE, rate of c-DCE transformation decreased, and the transformation rate of c-DCE epoxide also decreased. Both transformations essentially stopped after 40 hours of incubation. These results suggest that the same monooxygenase may be responsible for the transformations of both c-DCE and c-DCE epoxide.

4. CONCLUSIONS

We found several evidences indicating the involvement of a butane monooxygenase in the transformation of both c-DCE and c-DCE epoxide: 1) O_2 requirement for transformation of c-DCE; 2) the inactivation of c-DCE, and c-DCE epoxide transformation by acetylene; 3) the inhibition of c-DCE epoxide transformation by c-DCE; and the inhibition of c-DCE transformation by butane, and vice versa.

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