Degradation of a Variety of Halogenated Aliphatic Compounds by an Anaerobic Mixed Culture

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= Abstract =

We investigated the biodegradation of tetrachloroethylene (perchloroethylene, PCE) and halogenated aliphatic compounds by using anaerobic mixed cultivation. The mixed culture degraded PCE at concentrations of up to 150 mg/ l in 40 d via trichloroethylene (TCE) to cis-1,2-dichloroethylene (cDCE). Small amounts of vinyl chloride (VC) and CO_2 were also detected. The same culture degraded various halogenated aliphatic compounds such as cDCE, VC, 1,2-dichloroethane (DE), 1,3-dichloropropene (DP), dichloromethane (DM), 1,1,2-trichloroethane (TE), and chloroform (CF). Acetate was the most effective electron donor for dechlorimation, although formate, glucose and lactate were also effective, but to a lesser extent. The mixed culture degraded PCE in the temperature range 25 to 43°C, with an optimum between 30 and 37°C, and the pH range of 6.2 to 11.0.

[**Key words** : reductive dechlorination, tetrachloroethylene (PCE), trichloroethylene (TCE), halogenated aliphatic compounds]

Tetrachloroethylene (perchloroethylene; PCE), a suspected carcinogen, is a pollutant of major concern among halogenated aliphatic compounds (1. 2) PCE is not degraded by microorganisms under aerobic conditions, but several inverstigators have observed biotransformation of PCE by microorganisms under strictly anaerobic conditions (3, 4).

To make anaerobic bioremediation useful, PCE and trichloroethylene (TCE) must be degraded to nonchlorenated, environmentally acceptable products. A few studies have demonstrated that this is possible using mixed cultures (5, 6). It was assumed that biodegradation of PCE at contaminated sites could be performed by several species of bacteria that are believed to be superior in treating the contaminat rather than by a single species of organism (6). However, high levels of PCE contamination pose technologically difficult problems for bioremediation (7. 8). Besides, in situ contamination is often a mixture of complex chemicals, i.e., halogenated aliphatic compounds containing PCE, and the concentration of such compounds at contaminated sites is very high (1).

Therefore, determining conditions allowing simultaneous degradation of several halogenated aliphatic compounds is desirable.

Culture enrichmint was performed using a sample which was aseptically collected from ditch slude (mixed with sewage) in Gifu (gifu Prefecture) contaminated with PCE and halogenated aliphatic compounds. Initial culture enrichment in 100-ml bottles was initiated by incoulation of 40 ml of medium containing 1.0 mg/l PCE with 4g of sample. The composition of the medium (MMY) was as follows (in g/l): K_2HPO_4, 7.0; KH_2PO_4, 2.0; MgSO4.7H_2O, 0.1; $(NH_4)_2SO_4$, 1.0; sodium citrate, 0.5; yeast extract, 2.0. The pH of the medium was 7.2. Bottles were purged with N_2 gas (>99.9%), and sealed with Teflon-lined rubber septa, and aluminum crimp caps. Incubation was 30° C for 30 d in the dark, after which, highly enriched in MM medium (based on MMY medium without yeast extract) containing PCE (1.0 mg/l). PCE was degraded in the presence of alternative electron donors such as acetate, lactate, glucose, formate, and yeast extract (data not shown). On the basis of these results, we chose acetate as the electron donor. Acetate (5mM) was added to the MM medium as an electron donor for PCE degradation, because in preliminary experiments acetate was found formate (9). The cultures in which TCE degradation was observed were subsequently transferred to fresh medium. After six successive cultivations, the subcultures were spread on thioglycolate medium with 1.5% agar (10). A sterile glass tube that contained cotton fiber soaked with PCE was attached inside the lid of a glass dish to select bacteria that contained cotton fiber soaked with PCE was attached inside the lid of a glass dish to select bacteria that had tolerance to, as well as the ability to degrade high concentrations of PCE. The gas inside the glass plate was replaced by nitrogen and the plate was then sealed with adhesive tape. The plate was incubated in an anaerobic jar at 30° C and colonies appeared after 7 d of incubation. PCE degradation was tested by inoculating liquid MM medium (acetate, 5 mM) with a single colony, however, this method did not result in PCE degradation. It was assumed that biodegradation of PCE degradation was determined by picking colonies randomly and mixing these together in liquid MM medium with acetate (5 mM) at 30° C for 2 weeks. By performing such selection, we obrained a mixed culture which was comprised mainly of two morphotypes, irregular cocci and long rods. The mixed culture was poured into a Plastic tube to give a final glycerol concentration of 50% and the tube was stored in a liquid nitrogen vessel until use.

PCE degradation experiments were carried out using MM medium containing 1.0mg/l PCE and acetate (5mM). The cells were pregrown on GPY medium (glucose 1.0g/l, Polypepton 10g/l) because PCE was degraded more rapidly at a high cell concentration. Precultures were harvested by centrifugation (10 min at 12,000 × g), then washed once and resuspended in MM medium. Two ml of cell suspension (cell protein concentration, 0.329mg/ml, determined by the Lowry method) was transferred to a 26-ml serum bottle containing 8.0 ml of fresh MM medium for degradation experimints of halogenated aliphatic compounds. Initial concentration of each compound was 1.0mg/l. The rates of degradation of PCE and halogenated aliphatic compounds were determined using MM medium to which cell suspension had not been added as a control.

PCE, TCE cis-1,2-dichloroethylene (cDCE), 1,2-dichloroethane (DE) and dichloromethane (DM) in a 10-$\mu\ell$ headspace sample were analyzed quantitatively using a model GC-14B gas chromatograph (Shimadzu Co., Kyoto) equipped with an electron capture detector (ECD). CO_2 was measured using a model GC-4C PTF gas chromatograph equipped with a thermal conductivity detector (TCD). High level PCE, vinyl chloride (VC) and chloroform (CF) were determined using a model GC-9A gas chromatograph (Shimadzu Co.) equipped with a flame ionization detector (FID).

The kinetics of dechlorination of PCE to cDCE were studied. The mixed culture dechlorimated PCE via TCE to cDCE within 2 d at 30° C as shown in Fig. 1.

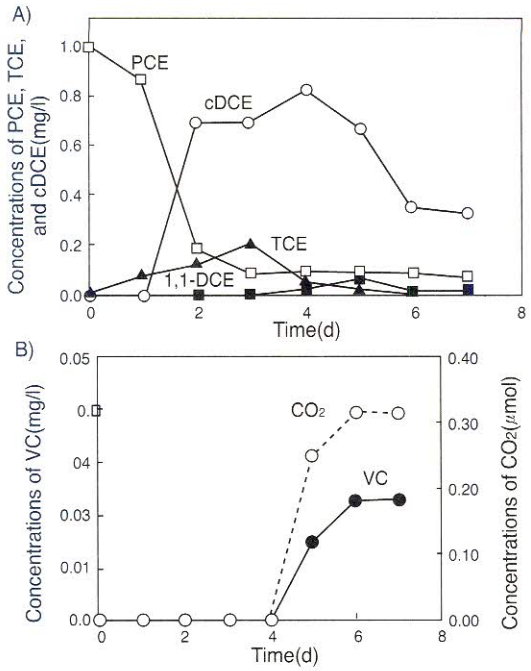

FIG. 1. Dechlorination of PCE to cDCE(A), and CO_2(B) by the mixed culture under anaerobic conditions. The gas atmosphere was replaced by nitrogen. Acetate was the external electron donor (5mM). Symbols: □, PCE; ▲ TCE; ○, cDCE; ■, 1,1-DCE; ●, VC; --○--, CO_2.

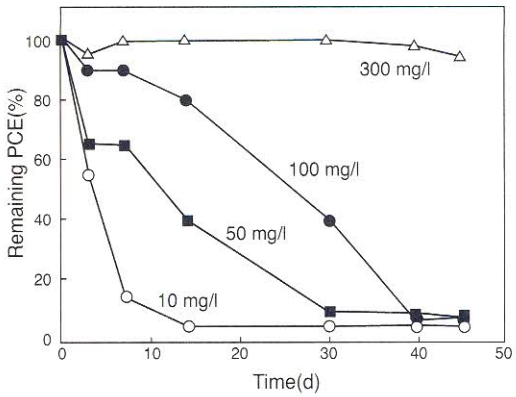

FIG. 2. Dechlorination of high concentrations of PCE. The mixed cultures were incubated at PH 7.2 and 30° C. The medium did not contain yeast extract(MM medium). Incubations were performed with acetate (5mM)as the electron donor. Symbols: ○, 10mg/l; ■, 50mg/l; ●, 150mg/l; △, 300mg/l.

Reductive dechlorination of PCE to cDCE began after 2d. TCE was identified as the intermediate product, indicating that sequential dechlorination occurred. Transformation via TCE to cDCE occurred within3 to 4 d of cultivation. cDCE was the main product of PCE, whereas a small amount of 1,1-dichloroethylene (0.07 mg/l) was also detected after 5d. We suspected that cDCE was partially dechlorinated because very small amounts of VC (0.0723 mg/l) and CO_2 (0.316 μmol) was detected with the decline of cDCE after 7 d. Though we did not conduct radiotracer experiment, there is a possibility that CO_2 was a product of PCDE, because CO_2 was not genarated under the same culture conditions without PCE.

To study the effect of temperature on the biological degradation of PCE, cultures containing 10 ml of MM medium with acetate (5 mM) as an electron donor were incubted in the dark at different temperatures (25, 30, 34, 37, and 43° C). The cultures degraded PCD in the temperature range 25 to 43° C, with an optimum between 30 and 37° C. To determine the optimum pH range for dechlorination of PCE, tests were conducted at different pH levels using 0.5N NaOH or HCI before autoclaving. PCE was degraded in the pH range 6.5 to 11.0, with an optimum between 7.2 and 9.0.

The effect of high concentrations of PCE on the degradation is shown in Fig. 2. The energy source used in this experiment was acetate (5 mM), and the initial concentration of PCE was 10, 50, 150, or 300 mg/l. At 10 mg/l PCE, both TCE and cDCE were detected whereas at 50 and 150 mg/l PCE, TCE was the only dechlorinated product detected. During the 7 d incubation, acetate (initially 5mM) had decreased to 2.4mM by day 5 and remained at this level until the end of experimint. Acetate, however, did not affect the growth of the mixed culture was able to degrade PCE at concentration of up to 150 mg/l, but the dechlorination was incomplete and the rates were very slow. Reductive dechlorination of 300mg/l PCE was not observed during 2 months of culture.

Table 1. Summary of the halogenated aliphatic compound degradative capactities of the enriched culture

Compund	Degradation(%)
Tetrachloroethylent (PCE)	94
Trichloroethylene (TCE)	93
cis-1, 2-Dichloroethylene (cDCE)	92
Vinyl chloride (VC)	39
1,2-Dichloroethane (DE)	90
Dichloromethane (DM)	77
Chloroform (CF)	89
1,3-Dichloropropene (DP)	90
1,1,2-Trichloroethane (TE)	60

Initial concentration of each compound: 1.0 mg/l.
Data represent the values after 7 d cultivation.

Using the mixed culture, the degradation percentage of PCE, TCE, cDCE, VC, DE, DM, CF, 1,3-dichloropropene (DP), and 1,1,2-trichloroethane (TE) were determined to be 94, 93, 92, 90, 77, 89, 90, and 60%, respectively after 7 d of incubation (Table 1). The results demonstrated that the enriched culture has the ability to degrade a variety of halogenated aliphatic compounds.

Transformation of PCE into innocuous compounds is desirable (11-13), but only a few studies have demonstrated this possibility (6, 7). Debruin et al. (6) reported that two different enriched cultures were obtained from a reactor in which PCE was completely converted to ethane. They hypothesized that several different microorganisms were required to achieve complete dechlorination of PCe (5). In the prsent study, we didi not detect ethene or ethane in the culture. Instead, we detected the more innocuous CO_2. We suspect that cDCE was dechlorinated, and decomposed to CO_2 via VC, however the scale of our experiment was too small to analyze PCE degradation stoichiometrically.

The heterotrophically enriched culture obtained in this study demonstrated the degradation of PCE at high concentrations as well as that of several other halogenated to the devolopment of biological remediation of halogenated aliphatic compounds.

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