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ENHANCED DECHLORINATION OF 2,4,6-TRICHLOROPHENOL BY ANAEROBIC MICROBIAL POPULATIONS IN THE PRESENCE OF ETHANOL

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SUMMARY

A consortium of anaerobic microorganisms was grown on acetate, ethanol, glucose or methanol and dechlorinated 50 umol 2,4,6-trichlorophenol, through 2,4-dichlorophenol, to 4-chlorophenol. The highest rate of dechlorination of 2,4,6-trichlorophenol was observed when ethanol was used as a growth substrate.

INTRODUCTION

Chlorinated phenols are produced in large amounts as solvent and precusors of herbicides, insecticides, etc. These compounds are often resistant to both biotic and abiotic degradation. Recent research findings indicate that anaerobic processes that remove chlorine from these compounds produce dechlorinated compounds that are generally less toxic and susceptable to further microbial attack, especially by aerobic microorganisms utilizing oxidative biodegradative processes (Armanate et al., 1992; Zhang et al., 1990; Krumme et al., 1988).

Anaerobic dechlorination for chlorinated phenols have been observed to proceed via reduction (Krumme et al. 1992). Reductive dechlorination is occurred preferably under methanogenic condition in which typical redox potential is -300mV, preferred electron acceptor is carbon dioxide, and the product is methane. Recent studies on reductive dechlorination under methanogenic condition have been conducted in sediments or bioreactor fed with chlorinated phenols and a growth substrate (Armenante et al., 1992; Krumme et al., 1988; Zhang et al., 1990). Without a growth substrate, dechlorination, especially of highly chlorinated phenols, did not occur(Krumme et al., 1988).

From the literature review, growth substrate may be very important to the dechlorinating anaerobic microbial populations. In the process of dechlorination, chlorinated phenols do not appear to support cell growth and, therefore, cells need a growth substrate to obtain energy for their growth and maintenance. Furthermore the dechlorination usually occurs under highly reducing environment like methanogenic condition. Anaerobic microbial populations, thus, need a source of reducing power for dehalogenation.

In this study, batch tests were conducted to dechlorinate 2,4,6-

trichlorophenol using unacclimated anaerobic microbial population. Anaerobic microbial population was fed with acetate, ethanol, glucose or methanol. It was hypothesized that dechlorination rate of 2,4,6-trichlorophenol may be dependent on the growth substrate. When each growth substrate was fed to the anaerobic microbial population, dechlorination rate of 2,4,6-TCP was compared each other. Intermediate produced in the process of dechlorination was analyzed.

MATERIALS AND METHODS

Microbiological methods

Medium and stock solutions were prepared anaerobically under an N_2 gas atmosphere. The medium contained the following components (per liter): KH_2PO_4, 0.27g; K_2HPO_4, 0.35g; NH_4Cl, 0.53g; $CaCl_2 2H_2O$, 0.1g; $MgCl_2 6H_2O$, 0.02g; $FeCl_2 4H_2O$, 0.2g;$NaHCO_3$, 1.2g. All chemicals (analytical grade) were obtained from Sigma Chemical Co.. The final pH of the medium was 7.2 to 7.5.

Batch tests were conducted in 500 ml serum bottles sealed with Teflon-septa which were held in place with aluminum crimp seals. Anaerobic cultures were obtained from the anaerobic digester in municipal treatment plant of Seoul, Korea. At the start of each test the defined mineral medium containing a growth substrate and 2,4,6-trichlorophenol was added to the serum bottles. The serum bottles were inoculated with anaerobic culture, incubated at 35 °C and shaken at 150 rpm using shaking incubator.

Substrate and intermediate analysis

The degradation of 2,4,6-trichlorophenol and the appearance of metabolic intermediates were monitored by gas chromatograph. For gas chromatographic analyses of 2,4,6-trichlorophenol, the samples were acidified with HCl to a pH of < 2 and extracted with diethyl ether. Diethyl acetate fraction was dried by using $MgSO_4$. 20 ul of the dried fraction was injected into a gas chromatograph (Hewlett Packard 5890) equipped with HP-5 capillary column and a flame ionization detector. The column temperature was maintained at 60°C for 2 minutes, increased to 150 °C at 12 °C min^{-1} and maintained at 150°C for 1 minute. Temperatures for injector and detector were 200 and 250 °C, respectively. Helium was used as a carrier gas. The metabolic intermediate was identified by using GC/MSD.

RESULTS AND DISCUSSION

2,4,6-TCP dechlorination enhanced in the presence of a growth substrate

Figure 1 illustrates that the rate of dechlorination of 2,4,6-TCP by anaerobic microbial populations can be markedly increased when a growth substrate is added. The dechlorination of 2,4,6-TCP was also occurred in the control. A growth substrate was not added to the control but some carbon source could be present in the anaerobic sludge from the anaerobic digeter. Therefore this carbon source was utilized in supporting cell growth and maintenance and providing reducing power for dechlorination. In the control it took more than 400 hours for 50uM of 2,4,6-TCP to be dechlorinated. When ethanol was added to the anaerobic microbial populations, it took only about 137 hours. The dechlorination rate of 2,4,6-TCP was increased markedly with addition of a growth substrate.

Instead of ethanol, glucose, acetate or methanol was used as a growth substrate to know if it can enhance the rate of the dechlorination of 2,4,6-TCP. Figure 2 shows how long it takes for 50 uM of 2,4,6-TCP to be dechlorinated to 2,4-DCP when different growth substrate is added. It took about 230 hours when acetate or glucose was used as a growth

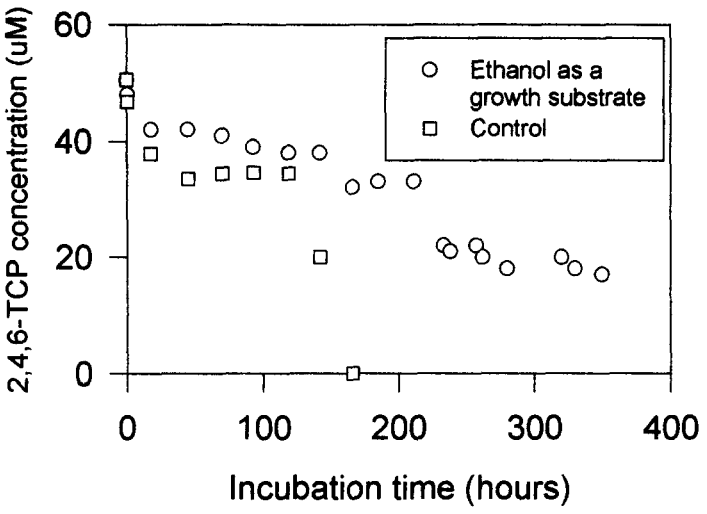

Figure 1. Enhanced dechlorination rate of 2,4,6-TCP by anaerobic microbial population in the presence of ethanol as a growth substrate

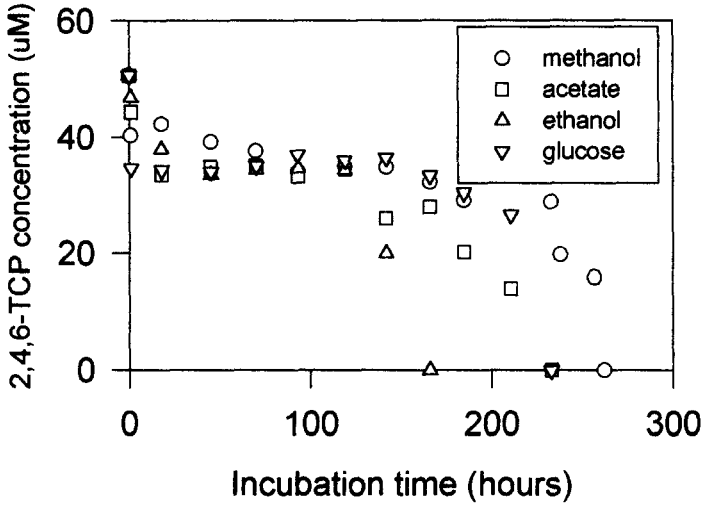

Figure 2 Dechlorination of 2,4,6-TCP by anaerobic microbial population in the batch reactor where different growth substrate was used.

substrate. With methanol it took about 280 hours. The dechlorination rate of 2,4,6-TCP was highest when ethanol was used as a growth substrate.

The metabolic pathway of dechlorination of 2,4,6-TCP

The metabolic pathways in dechlorination of 2,4,6-TCP were identified in several batch experiments. Disappearance of 2,4,6-TCP and accumulation of the metabolic intermediates are shown in Figure 3. Figure 3 indicates that the dechlorination of 2,4,6-TCP takes place at ortho position. Therefore 2,4,6-TCP was dechlorinated to 2,4-DCP and then 2,4-DCP was dechlorinated to 4-CP. 4-CP was not dechlorinated further and, therefore, accumulated. This high specificity for the removal of chlorine in the ortho position in comparison with the para position has also been observed in experiments done by other researchers (Zhang et al., 1990; Armenante et al., 1992). This metabolic pathway was not changed when different growth substrate such as glucose, acetate or methanol was used. Accumulation of 4-CP has also been observed by Zhang et al. (1990). They reported that the enrichment culture for the conversion of 4-CP to phenol was most difficult one to obtain. The reason for this fact may be that 4-CP is more toxic compound than 2,4-DCP and 2,4,6-TCP.

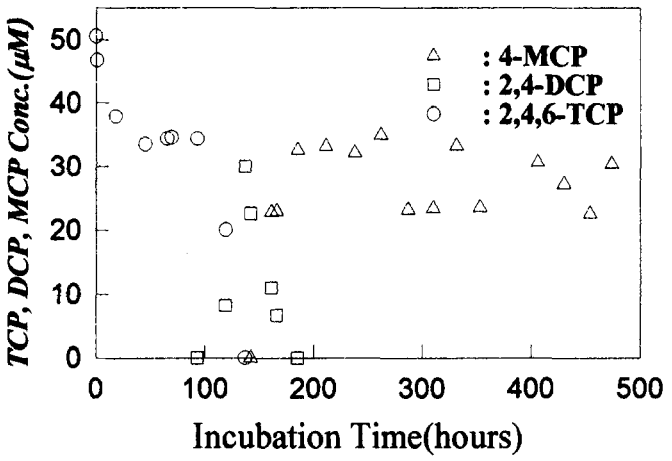

Figure 3. Dechlorination pathway of 2,4,6-TCP by anaerobic microbial populations in the presence of ethanol as a growth substrate

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