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A reactor system combining reductive dechlorination with co-metabolic oxidation for complete degradation of tetrachloroentylene

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Abstract: A laboratory sequential anaerobic-aerobic bioreactor system, which consisted of an anaerobic fixed film reactor and two aerobic chemostats, was set up to degrade tetrachloroethylene (PCE) without accumulating highly toxic degradation intermediates. A soil enrichment culture, which could reductively dechlorinate 900 μM (ca. 150 mg/L) of PCE stoichiometrically into *cis*-1,2-dichloroethylene (*cis*-DCE), was attached to ceramic media in the anaerobic fixed film reactor. A phenol degrading strain, *Alcaligenes* sp. R5, which can efficiently degrade *cis*-DCE by co-metabolic oxidation, was used as inoculum for the aerobic chemostats consisted of a transformation reactor and a growth reactor. The anaerobic fixed film bioreactor showed more than 99 % of PCE transformation into *cis*-DCE in the range of influent PCE concentration from 5 μM to 35 μM at hydraulic retention time of 48h. On the other hand, efficient degradation of the resultant *cis*-DCE by strain R5 in the following aerobic system could not be achieved due to oxygen limitation. However, 54% of the maximum *cis*-DCE degradation was obtained when 10 μmol of hydrogen peroxide (H_2O_2) was supplemented to the transformation reactor as an additional oxygen source. Further studies are needed to achieve more efficient co-metabolic degradation of *cis*-DCE in the aerobic reactor.

Keywords: anaerobic-aerobic bioreactor; co-oxidation; reductive dechlorination; tetrachloroethylene

Introduction

Chlorinated ethylenes such as tetrachloroethylene (PCE) and trichloroethylene (TCE) are ubiquitous contaminants in soil and groundwater due to their widespread application in dry-cleaning, degreasing and chemical processes (Pankow, 1996). Because these compounds are toxic and suspected to be carcinogenic, techniques for eliminating these toxic solvents from contaminated sites have been developed. Recently, cost-effective bioremediation techniques that can completely mineralize chlorinated compounds have been receiving great attentions (Alexander, 1994).

It is generally accepted that PCE is extremely resistant to oxidative biodegradation, however, under anaerobic conditions PCE can be reductively dechlorinated into TCE, dichloroethylene (DCE) isomers, vinyl chloride (VC), and eventually ethene (ETH) or ethane (ETA; De Bruin, 1992; DiStefano, 1991; Freedman, 1989; Vogel, 1985).

However, the dechlorination rate declines remarkably as the number of chlorines decreases, which commonly causes the accumulation of partially dechlorinated intermediates like DCEs and VC. Particularly, VC, the highly toxic and apparent carcinogenic compound to human being, is often accumulated.

In contrast to the anaerobic biodegradation, the reductive metabolites of PCE, less-chlorinated ethylenes such as TCE and DCEs, are readily degraded into safe end products under aerobic conditions. The aerobic degradation is catalyzed by non-specific oxygenases produced by some groups of microorganisms including phenol-, toluene-, methane-degrading or ammonia-oxidizing bacteria, and is called co-metabolic oxidation (Semprini, 1997).

Based on these knowledges concerning the biodegradation of chlorinated ethylenes, several studies have recently suggested that the combination of the reductive dechlorination under anaerobic conditions and the co-metabolic oxidation under aerobic conditions would allow an efficient and complete degradation of PCE. Fogel *et al*. (Fogel, 1995) and Gerriste *et al*. (Gerriste, 1995) have experimentally demonstrated

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that it is possible to completely degrade PCE by such sequential anaerobic-aerobic systems using methanotrophs in the aerobic reactors, although the co-metabolic oxidation of less-chlorinated ethylenes by methanotrophs was not efficient. The reason for the inadequate degradation of less-chlorinated ethylenes in the aerobic reactors might be due to inactivation of methanotrophs by toxicity of metabolites generated during the co-metabolic oxidation of less-chlorinated ethylenes (Fennell, 1993). Since it was known that phenol-degrading bacteria appear to be more resistant to toxicity of co-oxidation metabolites of TCE (Landa, 1994) and to have higher ability to degrade TCE and *cis*-1,2-dichloroethylene (*cis*-DCE) than methanotrophs(Hopkins, 1993). Gerriste *et al*. (Gerriste, 1997) applied phenol-degrading bacteria to the aerobic reactor of the sequential anaerobic-aerobic system, resulted in more efficient PCE degradation. However such sequential systems still have common problems that VC tends to accumulated as an anaerobic PCE degradation product. Another problem is that the treatment efficiency often becomes unstable due to competitive inhibition of the co-metabolic oxidation caused by the remaining primary substrate, i.e. phenol, in the aerobic reactor.

In this study, a sequential anaerobic-aerobic reactor system combined an anaerobic fixed film reactor and two aerobic chemostats, which consisted of a growth reactor and a transformation reactor, was set up for complete degradation of PCE. To avoid the occurrence of VC, the anaerobic fixed film reactor was inoculated with the well-characterized anaerobic enrichment that can efficiently transform PCE into *cis*-DCE without accumulating VC (Lee, 1997). The following aerobic system was seeded with a selected phenol-degrading bacterium capable of efficiently degrading *cis*-DCE. Growth of the phenol-degrading bacterium on phenol and degradation of *cis*-DCE were performed separately in different chemostats so as to prevent the competitive inhibition of the co-metabolic oxidation.

1 Methodes

1.1 Bacterial cultures and media

For the reductive dechlorination of PCE in the anaerobic reactor, a soil enrichment culture was used. The enrichment culture was developed from a contaminated soil(depth 1m; TCE, 0.005 – 0.01 mg/L; *cis*-DCE, 0.007 – 0.021 mg/L) sampled in Chiba, Japan. The enrichment culture could dechlorinate high concentrations of PCE(150 mg/L) nearly stoichiometrically into *cis*-DCE via TCE at high rates(0.4 μmol of PCE transformed/mg volatile suspended solids per hr) using citrate as an electron donor. During the dechlorination, accumulation of VC has never been observed. The characteristics of the enrichment and the medium used(MMY medium) were reported in our previous publication(Lee, 1997).

Three different types of phenol-degrading bacteria; *Achromobacter* sp. E1, *Alcaligenes* sp. R5 (Watanabe, 1996), and *Pseudomonas putida* BH (Hashimoto, 1987), were used in a batch test of *cis*-DCE degradation. Of these, *Alcaligenes* sp. strain R5, which showed the most efficient *cis*-DCE degradation, was selected as the inoculum for the aerobic growth reactor. For the growth of phenol degrading bacteria, MP medium (Watanabe, 1996) was used.

1.2 Batch test of *cis*-DCE degradation by phenol degrading bacteria

Three phenol degrading bacteria were individually cultivated in a 100 ml of the MP medium containing phenol (0.1 – 1.0 mM) as a sole carbon source. When more than 90% of phenol was degraded, the cells were harvested by centrifugation (10000 × g for 10 min at 4℃), washed with phosphate-potassium buffer (pH 7.5), and resuspended in the same buffer at a turbidity of about 0.6 at 600 nm. Vials (120 ml) containing 20 ml of the washed cells suspension were sealed with Teflon septums and aluminum caps. The vials received 10 μmol/L (ca. 1.0 mg/L) of *cis*-DCE (ignoring equilibrium partition) and were incubated at 30℃ with reciprocal shaking at 100 r/min.

To investigate the competitive degradation of phenol and *cis*-DCE by the phenol degrading bacteria,

two sealed 120 ml vials containing 20 ml of cell suspension of strain R5 were prepared. One of the vials received additional phenol (0.5 mM) but the other one did not. And 70 μmol/L of *cis*-DCE was added to both vials and they were incubated at the same condition described above.

1.3 Sequential anaerobic-aerobic reactor system

The sequential reactor system consisted of an anaerobic fixed film reactor and two aerobic chemostats (Fig. 1). The up-flow, anaerobic stainless steel reactor (300 mm long × 100 mm i.d.) was filled with cubical-porous ceramic media (10 mm × 10 mm × 10 mm) to support biofilm. The superficial occupied volume of the media was about 1550 ml, consequently, the actual volume of anaerobic column was 1770 ml. In order to attach the enrichment culture to the porous ceramic media, it was cultivated in a 500 ml volume of chemostat which received MMY medium with 40 μM of PCE at 20.8 ml/h of flow rate, and the effluent from the chemostat was introduced into the fixed film reactor. After the attachment operation for 3 weeks, the anaerobic column showed apparent steady state, PCE concentration of the effluent was stably maintained below 0.3 μM. Then the cultivation chemostat was removed and PCE stock solution (300-600 μM) and MMY medium were directly supplied into the fixed film reactor with a syringe pump and a peristaltic pump (at rate 37 ml/h), respectively. MMY medium was purged with the filter sterilized N_2 gas before using and continuously stirred during feeding. After 35 days running, a part of the effluent of the anaerobic reactor was sent to the aerobic transformation reactor.

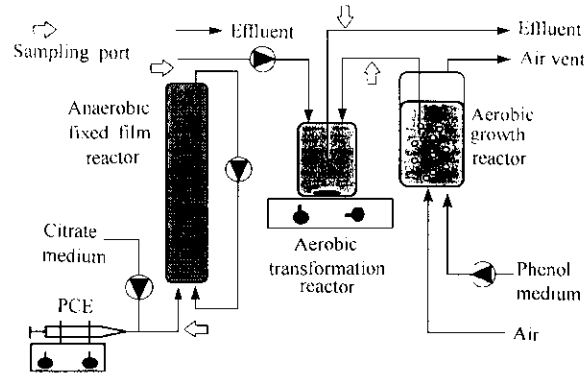

Fig. 1 Schematic diagram of the sequential anaerobic-aerobic bioreactor system. PCE is dechlorinated into *cis*-DCE in the anaerobic fixed film reactor. In the following transformation reactor, the *cis*-DCE is co-metabolically oxidized by strain R5 that was grew with phenol in the aerobic growth reactor

The aerobic system consisted of a growth reactor and a transformation reactor to avoid the competitive inhibition in use of phenol degrading bacteria. The MP medium containing phenol (0.5 – 1.0 mM) was supplied to the 2000 ml of growth reactor at 41.7 ml/min of influent flow rate. Strain R5 completely degraded phenol at 2 days of hydraulic retention time in the growth reactor. And the effluent of the growth reactor containing cells of strain R5 was then sent to the 500 ml volume of transformation reactor to degrade *cis*-DCE that was sent from the anaerobic reactor. In order to minimize the volatile loss of *cis*-DCE, the transformation reactor was not aerated but completely mixed with a magnetic stirrer. All connections were made of stainless steel, glass, Viton rubber, and Teflon to minimize abiotic losses of the chlorinated compounds. All reactors were operated at 30 ± 2℃. For an experiment to test the effect of oxygen supply to the transformation reactor on *cis*-DCE degradation, H_2O_2 (ca. 5 – 20 μmol) was directly introduced to the transformation reactor as a source of oxygen.

1.4 Analytical methods

Chlorinated ethylenes were measured by headspace analysis using a gas chromatography (GC-14A, Shimadzu, Tokyo, Japan) equipped with an FID and a gas chromatograph-mass spectrometer (GC/MS; GC-17A-QP-5000, Shimadzu). Phenol was analyzed with a high performance liquid chromatography (HPLC; Tosoh, Tokyo, Japan). Further details of the analyses followed our previous publication (Lee, 1997).

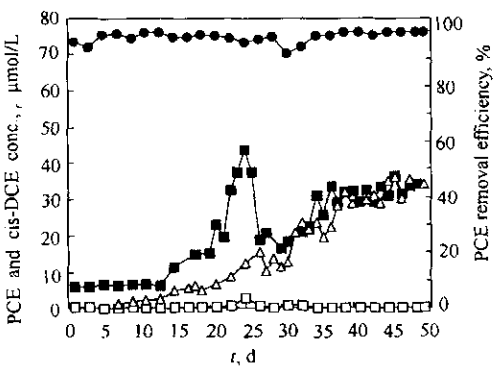

Fig.2 Reductive dechlorination of PCE in the anaerobic fixed film reactor

■ influent PCE concentration; □ effluent PCE concentration; △ effluent *cis*-DCE concentration; ● PCE removal efficiency = (inf. PCE-eff. PCE)/inf. PCE × 100

2 Results and discussion

2.1 Reductive dechlorination of PCE in the anaerobic fixed film reactor

The soil enrichment culture, which could transform 900 μM (ca. 150 mg/L) of PCE stoichiometrically into *cis*-DCE, was used as the inoculum for the anaerobic fixed film reactor (Lee, 1997). The PCE dechlorination in the anaerobic reactor is shown in Fig.2. Influent PCE concentration to the anaerobic column was increased stepwise from 5 μM to 35 μM (ca. 0.8 – 5.8 mg/L) during the 50-days operation. In the range of influent PCE concentration tested here, the reactor showed more than 99% of PCE removal efficiency. The major product was *cis*-DCE and other less-chlorinated ethylenes such as VC were not detected throughout the experiment. Although the amount of *cis*-DCE produced did not consist with that of PCE degraded during first 30 days of the operation, the extended operation showed good mass balance between PCE degradation and *cis*-DCE formation. The difference during the initial operating might be due to adsorption of chlorinated ethylenes to the porous ceramic media and biofilm. Production of methane was observed but its amount was negligible, suggesting that methanogens might not be concerned with PCE removal in the anaerobic reactor. Acetate (16.6 ± 2.9 mM) was produced as the major volatile organic acid in the effluent and lesser amounts of propionate (5.7 ± 1.2 mM) and butyrate (1.9 ± 0.2 mM) were also found (data not shown). After 35 days running, a part of the anaerobic reactor effluent was sent to the aerobic transformation reactor.

2.2 Batch co-oxidation of *cis*-DCE by phenol degrading bacteria

Three different types of phenol degrading bacteria, *Achromobacter* sp. E1, *Alcaligenes* sp. R5, and *Pseudomonas putida* BH, were used in a batch test of *cis*-DCE degradation. All of three phenol degrading bacteria could co-oxidize *cis*-DCE. Although strain BH left 10%-18% of added *cis*-DCE at the end of the incubation (5h), strains E1 and R5 completely removed 10 μM of *cis*-DCE (Fig.3). Among the three strains, *Alcaligenes* sp. R5 showed the most efficient *cis*-DCE. Therefore, strain R5 was selected as the inoculum to the aerobic reactor.

The effect of presence of phenol on *cis*-DCE degradation by strain R5 was investigated to evaluate the possible competitive reaction between phenol and *cis*-DCE (Fig.4). Cells of strain R5 without additional phenol started to degrade *cis*-DCE immediately and completely removed it within 3.5h, but the cells received additional phenol started *cis*-DCE co-oxidation after 1.5h when the added phenol was completely removed. From this result, it was confirmed that the presence of phenol completely inhibited the co-oxidation of *cis*-DCE by strain R5.

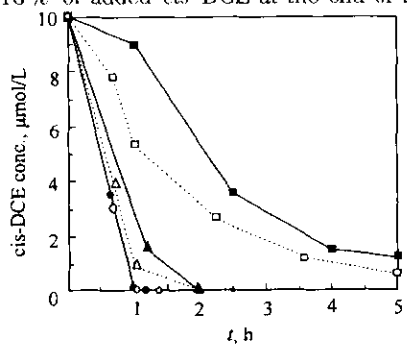

Fig.3 Co-metabolic degradation of *cis*-DCE in resting cells of tree different types of phenol degrading bacteria; *Pseudomonas putida* BH grown in phenol 10 ppm (■) and 100 ppm (□); *Achromobacter* sp. E1 grown in phenol 10 ppm (▲) and 100 ppm (△); *Alcaligenes* sp. R5 grown in phenol 10 ppm (●) and in 100ppm (○)

2.3 Co-metabolic degradation of *cis*-DCE in the aerobic reactor system

In order to avoid the competitive inhibition of *cis*-DCE degradation by phenol, cultivation of strain R5 on phenol and *cis*-DCE transformation were separately carried out in the growth reactor and the transformation reactor, respectively.

When effluent phenol concentration of the growth reactor became stable below 0.01 mM, the effluent was sent to the transformation reactor. The effluent from the anaerobic fixed film reactor containing *cis*-DCE was also supplied to the transformation reactor at 20 ml/h of flow rate; consequently, the influent *cis*-DCE concentration of the transformation reactor was about 11 μM. Degradation of *cis*-DCE in the aerobic system was not effective. Even though influent phenol concentration of the growth reactor was increased from 0.5 mM to 1.0 mM to induce more phenol hydroxylase, the degradation of *cis*-DCE was not improved (data not shown).

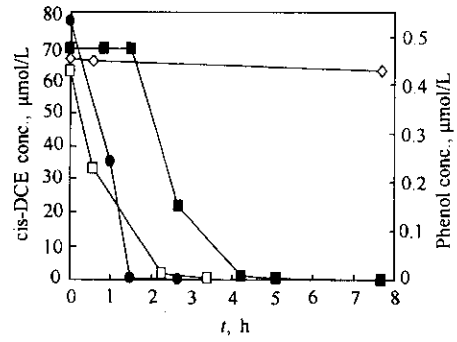

Fig.4 Effect of presence of phenol on co-metabolic degradation of *cis*-DCE in resting cells of strain R5. Degradation of *cis*-DCE in sterilized cells (◇), in resting cells without phenol (□), and in resting cells with phenol (■) and phenol degradation in resting cells with phenol (●)

Table 1 Effect of H_2O_2 on *cis*-DCE degradation in the transformation reactor

H_2O_2 dose, μmol	*cis*-DCE concentration, μM		Removal efficiency, %
	Influent	Effluent	
0	11.3	10.8	5
5	10.9	7.5	31
10	11.2	5.2	54
20	10.8	6.6	39
Control*	10.9	9.1	17

* 20 μmol H_2O_2 addition without resting cells of strain R5

Because the transformation reactor was not aerated in order to avoid volatile losses of *cis*-DCE, the oxygen deficiency in the transformation reactor was suspected as a reason of the low efficiency of *cis*-DCE degradation. To confirm this possibility, hydrogen peroxide (H_2O_2; 5 – 20 μmol) was directly added to the transformation reactor as a source of oxygen and concentration of *cis*-DCE in the effluent was monitored (Table 1). As a result of the increased addition of H_2O_2 up to 10 μmol, i.e. increased oxygen supply, the removal efficiency of *cis*-DCE was improved up to 54%. However, the addition of 2.0 μmol H_2O_2 showed lower removal efficiency of *cis*-DCE than that of 1.0 μmol H_2O_2 addition, suggesting that the high concentration of H_2O_2 inhibited the co-oxidation of *cis*-DCE maybe due to the toxic effects on strain R5. In a control experiment, in which only H_2O_2 (2.0 μmol) without cells of strain R5 was introduced, 17% of *cis*-DCE was chemically degraded. This indicated that the extended portion of *cis*-DCE degradation by the addition of H_2O_2 with cells of strain R5 depended on the biological co-oxidation. Therefore, we concluded that increasing oxygen concentration in the transformation reactor would improve biological degradation of *cis*-DCE in the two aerobic reactor system.

3 Conclusions

A sequential anaerobic-aerobic bioreactor system, which consisted of an anaerobic fixed film reactor and two aerobic chemostats, could degrade PCE without accumulating VC. The anaerobic fixed film bioreactor inoculated with a soil enrichment culture showed more than 99% of PCE transformation into *cis*-DCE in the PCE range from 5 to 35 μM. Although the degradation of *cis*-DCE in the aerobic system inoculated with a phenol degrading strain, *Alcaligenes* sp. R5, was not effective, the direct introduction of H_2O_2 to the transformation reactor as an oxygen source increased removal efficiency of *cis*-DCE up to 54%. Though further research is needed to improve the efficiency of *cis*-DCE degradation in the aerobic reactor

system, the experimental results suggest that the proposed system would be applicable for complete degradation of PCE without generation of highly toxic intermediate, VC.

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