

Dechlorination of High Concentrations of Tetrachloroethylene Using a Fixed-bed Reactor

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ABSTRACT

We evaluated the properties of a fixed-bed column reactor for high-concentration tetrachloroethylene (PCE) removal. The anaerobic bacterium *Clostridium bifermentans* DPH-1 was able to dechlorinate PCE to *cis*-1,2-dichloroethylene (cDCE) via trichloroethylene (TCE) at high rates in the monoculture biofilm of an upflow fixed-bed column reactor. The first-order reaction rate of *C. bifermentans* DPH-1 was relatively high at $0.006 \text{ mg protein}^{-1} \cdot \text{l} \cdot \text{h}^{-1}$, and comparable to rates obtained by others. When we gradually raised the influent PCE concentration from $30 \mu\text{M}$ to $905 \mu\text{M}$, the degree of PCE dechlorination rose to over 99% during the operation period of 2,000 h. In order to maintain efficiency of transformation of PCE in this reactor system, more than 6 h hydraulic retention time (HRT) is required. The maximum volumetric dechlorination rate of PCE was determined to be $1,100 \mu\text{mol} \cdot \text{d}^{-1}$ of reactor volume⁻¹, which is relatively high compared to rates reported previously. The results of this study indicate that the PCE removal performance of this fixed-bed reactor immobilized mono-culture is comparable to that of a fixed-bed reactor mixture culture system. Furthermore, our system has the major advantage of a rapid (5 days) start-up time for the reactor. The flow characteristics of this reactor are intermediate between those of the plug-flow and complete-mix systems. Biotransformation of PCE into innocuous compounds is desirable; however, unfortunately cDCE, which is itself toxic, was the main product of PCE dechlorination in this reactor system. In order to establish a system for complete detoxification of PCE, co-immobilization of *C. bifermentans* DPH-1 with other bacteria that degrade cDCE aerobically or anaerobically to ethene or ethane may be effective.

Keywords: *Clostridium bifermentans* DPH-1, dechlorination, tetrachloroethylene, monoculture, bioreactor

I. Introduction

Tetrachloroethylene (perchloroethylene, PCE), a chlorinated aliphatic solvent and metal degreaser, is one of the top five U.S. Department of Energy facility groundwater contaminants and the most common groundwater contaminant in Japan.^{24,30,58} PCE pose a serious public health problem, and the remediation of contaminated sites is an urgent issue worldwide.

Recently, it has been reported that high levels of PCE, trichloroethylene (TCE), *cis*-1,2-dichloroethylene (cDCE) and vinyl chloride (VC) were detected at an upstream segment of the Taishogawa River in Osaka, Japan.⁵⁸ At many of hazardous waste sites, as well as in groundwater plumes originating at a dry cleaning facilities, PCE is migrating by groundwater flow to stream and rivers.^{5,53} In addition, high levels of PCE contamination pose technologically difficult problems for bioremediation.⁵⁴

PCE is an important model for the study of biodegradation of chlorinated aliphatic compounds because of its high halogen content and toxicity.

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Although aerobic cometabolic degradation of PCE by toluene-*o*-xylene monooxygenase of *Pseudomonas stutzeri* OX1 has been recently reported,⁴⁷⁾ PCE is recalcitrant under aerobic condition because of its oxidation state.¹⁶⁾ PCE can be reductively dechlorinated to VC, ethylene or carbon dioxide under anaerobic conditions.^{7,12-14,17,20,30,55)}

In a fixed-bed column inoculated with a mixture of sediment and granular sludge, PCE has been sequentially dechlorinated to TCE, cDCE, and finally to VC and ethene.^{2,6,14,20,22,28,45)} Often, however, less chlorinated intermediates (cDCE and VC) are observed as the dominant products in the treated water. TCE and cDCE were the major intermediates in a PCE-degrading anaerobic fixed-film (fixed-bed) reactor as well as an upflow anaerobic sludge blanket (UASB) reactor, though DCE and VC were also detected.^{10,15,18,27,42-44,49,55)}

UASB reactors are commonly used in the anaerobic treatment of waste water.⁴⁸⁾ Dechlorination of PCE has been investigated in a UASB reactor after incorporation of the strictly anaerobic, reductively dechlorinating bacterium *Sulfurospirillum multivorans* into granular sludge to improve dechlorination of PCE.^{15,27)} With this system it was possible to study the behavior of the dechlorinating organism in a mixed culture biofilm and to obtain specific turnover rates for the dechlorination of PCE to cDCE in the biofilm. However, during the past year, the anaerobic reactors have been used to select, enrich and modify the physiological state of one or more of the many possible microbial consortia that can participate in the reductive dechlorination of PCE.^{2,6,14,15,20,22,26,28,35,45)}

Recently, multiple research groups have reported reactors for PCE removal. Most of them used a mixed culture (soils, sediments, and sludge) as PCE degrader in wastewater, and the application of mixed culture has proven effective for the complete degradation of PCE as well as for increasing of the degradation rate of PCE. However, from a commercial point of view, a reactor containing an immobilized monoculture seems to be more attractive than a mixed culture, from the standpoint of performance stability as well as simplicity of maintenance and operation. It has been reported that reactor performance is unstable even when the same TCE-contaminated aquifers

that provided source inoculums were used for PCE degradation.³⁵⁾ Indeed, the mechanism of reduction in the mixed culture is very complex. Many studies have indicated that methanogens play a critical role in the reduction of lesser-chlorinated breakdown products.^{14,17,20,55)} In contrast, DiStefano *et al.*¹³⁾ reported complete reduction of PCE by an acetogenic culture was able to be accomplished in the absence of methanogenesis. Furthermore, Maymó-Gatell *et al.*³⁷⁾ reported complete dechlorination could be performed by H₂-utilizing culture in the absence of methanogenesis and acetogenesis.

The bioreactor system is a good tool for understanding the dechlorination mechanisms of PCE in natural groundwater, as well as a good tool for increasing PCE removal rates. However, bioreactors using monoculture have not been reported, both because immobilization of monoculture to support material is not straightforward, and because of an extraordinary degradation efficiency with that of granular sludge or sediments was not expected.

In this study, we aimed at removal of PCE at high concentrations as a fundamental experiment while assuming the treatment of wastewater and groundwater originating at a dry cleaning facility. Using a small laboratory scale fixed-bed column reactor, we investigated and compared with previous reports the extent of PCE dechlorination by immobilized *C. bifermentans* DPH-1, the effect of hydraulic retention time (HRT), and the performances and characteristics of our fixed-bed reactor.

II. Materials and Methods

1. Chemicals

All chemicals used were of analytical grade and purchased from Wako Chemical (Kyoto, Japan). PCE was from Kanto (Tokyo, Japan) and other chlorinated chemicals were from GL Sciences (Tokyo, Japan).

2. Packing material and organism

C. bifermentans DPH-1 was kindly provided by Professor Takamizawa Kazuhiro, Gifu University, Japan. Ceramic beads (Kubota Co., Ltd., Tokyo) of the following characteristics have been used as

the carrier for immobilization of *C. bifermentans* DPH-1^{1,8}): diameter, 4-6 mm; composition, 90% Al₂O₃ and 10% SiO₂; distribution of pore size, 10-500 µm; average pore size, 80-90 µm; porosity, 52-55% (vol%); surface area, 300 cm g⁻¹; water absorption capacity, 30% (W%); average weight per granule, 104 mg; pore volume, 0.6-2.0 ml g⁻¹.

3. Immobilization of *C. bifermentans* DPH-1

Cells (0.0069 mg protein ml⁻¹) were transferred to a 26-ml serum bottle containing 10 ml of MY medium with different number of ceramic beads (20 to 100) and ethanol (0.61 mM) as an electron donor. The basal medium (MY) contained (g l⁻¹): K₂HPO₄, 7.0; KH₂PO₄, 2.0; MgSO₄·7H₂O, 0.1; (NH₄)₂SO₄, 1.0; yeast extract, 2.0; FeSO₄·7H₂O, 0.02; resazurin, 0.001, and 0.1 ml vitamin solution (containing 1 g of p-aminobenzoic acid and 1 mg of biotin per liter). The pH of the medium was 7.2. Headspace was flushed with N₂ gas (>99.9%), sealed with Teflon-lined rubber septa and aluminium crimp caps. PCE (6 µM) was added. Cultivation was conducted for 4 days at 30°C. After immobili-

zation, the culture was decanted from the bottles very carefully and fresh MY medium (10 ml) was added to the bottle containing immobilized cells with PCE (6 µM) and ethanol (0.61 mM). Headspace was flushed with N₂.

4. Fixed-bed reactor

A continuously fed up-flow anaerobic column reactor was developed using a pure organism. The reactor was made using a glass column of 22 cm × 4.5 cm i.d. (Beckman Instrument Inc.) filled with immobilized ceramic beads and MY medium (Fig. 1). The liquid volume was 230 ml. The reactor was maintained at 30°C in a thermostatically controlled water bath. The reactor was percolated in an upflow mode (ascending flow) under anaerobic conditions with MY medium containing 1.22 mM ethanol and 12 µM PCE. The operation was conducted at 0.29 ml min⁻¹ flow rate (13.1 h of column retention time). A Teflon bag was connected to the medium tank with nitrogen and the connecting part was equipped with a filter (0.22 µm, LABODISK-50CS). The

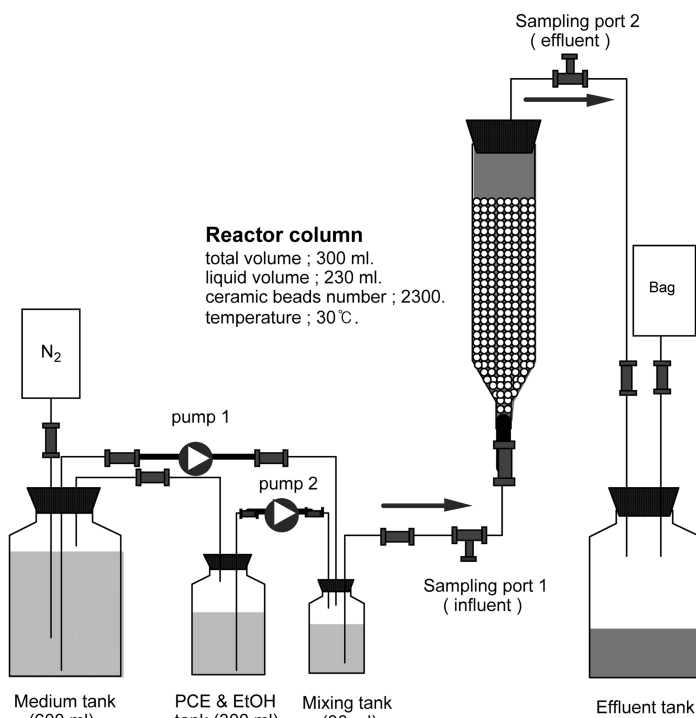


Fig. 1. Schematic diagram of an upflow fixed-bed reactor system.

flow rates of the pumps 1 and 2 were maintained at a 2:1 ratio. Apparent steady state is defined as the time when daily measurements of optical density and PCE degradation activity of the reactor effluent were stable. The measurement of optical density was done at 660 nm using a UV spectrophotometer (Shimadzu UV-160; Shimadzu Co., Japan). The reactor performance (or removal efficiency) was calculated as follows: (the amount of PCE-degraded/the amount of initial PCE loading) $\times 100$. Influent samples (2 ml) were collected with a 10-ml glass syringe via a port located near the entrance of the reactor as shown in Fig. 1. Effluent samples were collected at 20 h interval in a bottle; these bottles were closed using a Teflon-lined septa and aluminum crimp caps.

5. Batch culture kinetics

In order to determine K_B (velocity constant), an equation was proposed as follows; this reaction was also assumed as first-order reaction on batch culture.

$$dCe/dt = -K_B X C_e \quad (1)$$

Equation (1) can be rewritten as

$$\ln C_e = -X_B X t + \ln C_o \quad (2)$$

Equation (2) can be rewritten as

$$\ln(C_e/C_o) = -X_B X t \quad (3)$$

Where

when $t = 0$ ($C_e = C_o$)

C_o : initial concentration of PCE (μM)

C_e : final concentration of PCE (μM)

D : dilution rate (h^{-1})

K_B : the velocity constant of dechlorination rate of PCE ($\text{mg protein}^{-1} \cdot \text{h}^{-1}$)

t : cultivation time (h)

X : cell protein mass (mg) in a serum bottle (10 ml culture)

6. Determination of HRT

Dried ceramic beads of 230 grams were added to distilled water in order to remove air. The reactor column was filled with the beads, and the

column was percolated with distilled water. Distilled water is percolated in ascending mode flow at 60 ml h^{-1} after confirming the steady state of the effluent (about five times of column volume). The pump was once stopped, and 0.9 mg ml^{-1} of NaCl ($750 \mu\text{l}$) was injected rapidly, and distilled water was again added in descending flow mode at 60 ml h^{-1} .

7. Flow characteristics of the reactor

230 g of ceramic beads were added to the reactor column, and the column was percolated with distilled water. Distilled water is percolated in ascending flow at 60 ml h^{-1} after confirming the steady state of the effluent, and a solution of sodium chloride (1% w/v) was added to the column at 60 ml h^{-1} . The concentration of sodium chloride in effluent from the upper part of the reactor was determined by measuring electric conductivity (breakthrough curve) at 15 min intervals. After that, influent was exchanged from the sodium chloride solution to distilled water after confirming the steady state of the effluent (1%), and the concentration of sodium chloride was calculated by measuring the electric conductivity of effluent at 15 minutes intervals (curve of residual concentration). The flow characteristics of the reactor were examined by comparing the theoretical value of a complete-mix reactor (continuous-flow stirred-tank reactor: CFSTR) and the flow of a plug-flow reactor (PFR) with the observed value.

8. The concentrations of free cells and immobilized cells

The concentrations of free cells and immobilized cells were determined spectrophotometrically as protein according to the method of Lowry³³⁾ using bovine serum albumin as standard. For quantification of the immobilized cells, the cells were removed from the support material by ultrasonication. The removed cells were quantitated after washing with phosphate buffer to remove interfering substances originated from medium components (especially yeast extract), followed by repeated ultrasonication. After centrifugation (12,000 rpm, 20 min, 4°C), the suspension was collected and used for the measurement of cell protein.

9. Analyses

PCE, TCE, and cDCE were identified and quantified by static-headspace analysis using a gas chromatograph. PCE, TCE and cDCE in a 10- μ l headspace sample were determined using a model GC-14B gas chromatograph (Shimadzu Co., Japan) equipped with an electron capture detector (ECD) and a glass column (i.d. $3.2\phi \times 2.1$ m; Silicone DC-550 20% ChromosorbW [AWDMCS] 80/100). The gas samples were analyzed two to three times to check reproducibility. High concentrations of PCE were determined by a model GC-9A gas chromatograph (Shimadzu Co., Japan) equipped with a flame ionization detector (FID) and a glass column (i.d. $3.2\phi \times 2.1$ m; Silicone DC-550 20% ChromosorbW [AWDMCS] 80/100). The following

temperatures were applied: column, 70°C; injector, 200°C; detector, 250°C. The nitrogen carrier gas flow rate was 20 ml min⁻¹, and the headspace sample volume was 100- μ l.

III. Results and Discussion

1. Dechlorination of high concentrations of PCE on batch culture

High-concentration PCE dechlorination by free cells is shown in Fig. 2. When the initial concentration of PCE was 30-151 μ M, PCE dechlorination started after 16 h, and PCE was completely dechlorinated after 48 h. In the range of 302-905 μ M, a similar pattern was observed, and PCE was completely dechlorinated below the detection

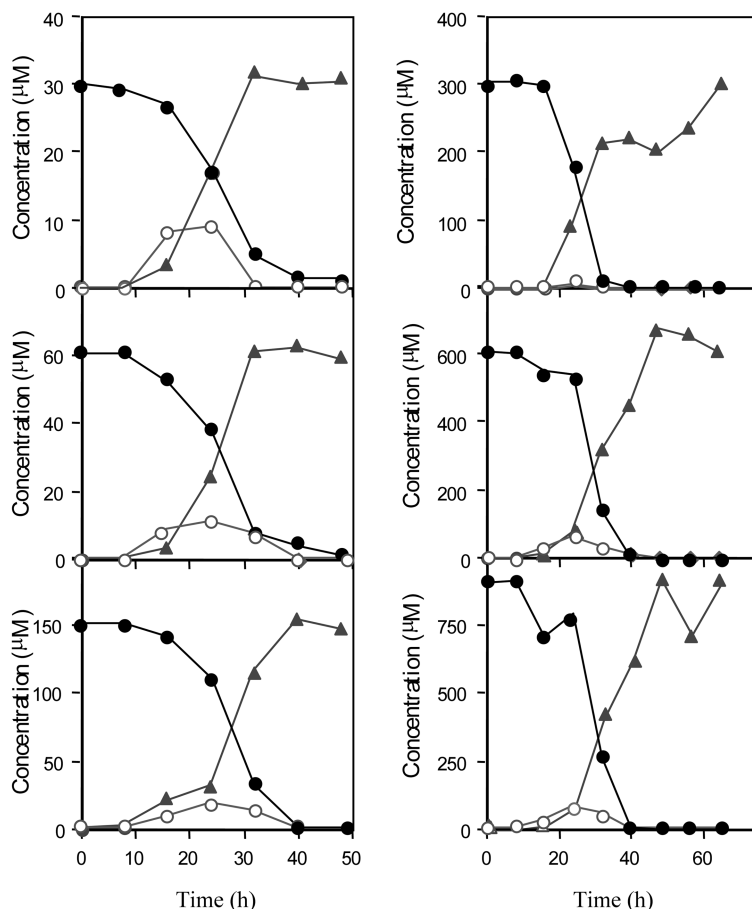


Fig. 2. Dechlorination of PCE on different initial PCE concentration by free cells. Initial concentrations of PCE were 30, 60, 151, 302, 603, and 905 μ M, respectively. Symbols: ●, PCE; ○, TCE; ▲, cDCE.

limit (0.03 μM) after 40 h of cultivation. PCE was dechlorinated to cDCE via TCE. We detected cDCE at levels that corresponded almost stoichiometrically

Table 1. Effect of initial concentration of PCE on cell growth

Initial concentration of PCE (μM)	Protein after incubation (mg/bottle)	
	Free cell	Fixed cell
0	0.16	0.19
30	0.18	0.32
60	0.25	0.28
151	0.28	0.25
302	0.22	0.28
603	0.19	0.27
905	0.22	0.26

Initial protein was 0.01 mg/bottle.

with the levels of added PCE.

The amount of cell protein, at the end of cultivation, was significantly increased when PCE was added compared with the case where PCE was not added in the cultures (Table 1). To date PCE dechlorination by the *C. bifermentans* DPH-1 has been thought as co-metabolism unrelated to cell growth. However, from our results (Table 1) it appears that PCE is utilized as an electron acceptor in energy metabolism in *C. bifermentans* DPH-1, and the dechlorination might be related to halogen respiration.^{8,46)}

The dechlorination of high concentrations of PCE by immobilized cells is shown in Fig. 3. When the initial concentration of PCE was 30-60 μM , PCE dechlorination occurred between 24 h and 48 h. At higher concentrations of PCE (151

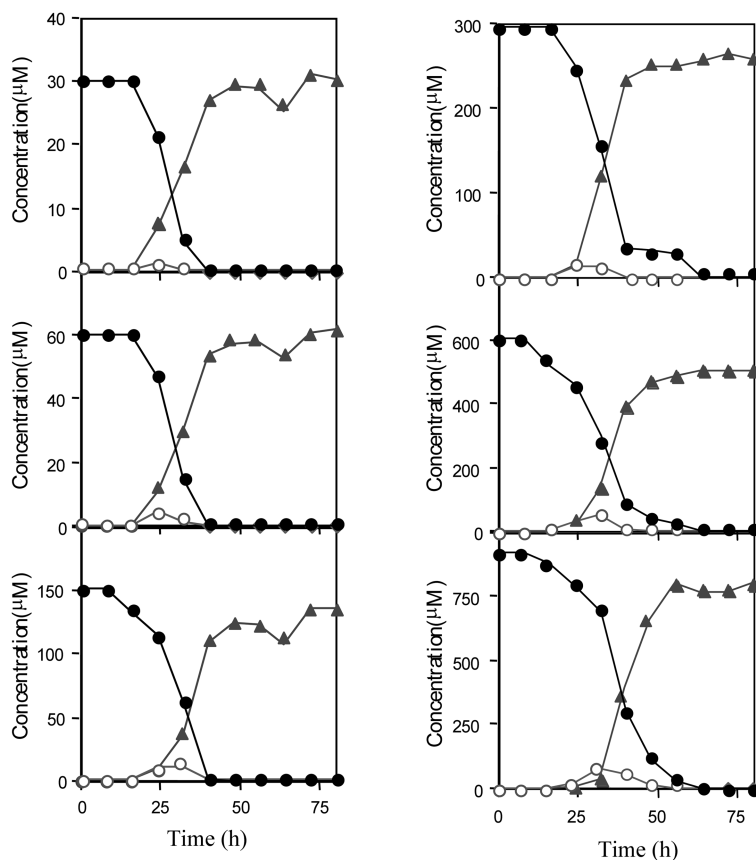


Fig. 3. Dechlorination of PCE on different initial PCE concentration by fixed cells. Initial concentrations of PCE were 30, 60, 151, 302, 603, and 905 μM , respectively. Symbols: ●, PCE; ○, TCE; ▲, cDCE.

μM), dechlorination started after 16 h, and PCE was completely dechlorinated after 48 h. In the range of 302-905 μM , PCE dechlorination was confirmed during 16-64 h cultivation. PCE was dechlorinated to cDCE via TCE, just as in free cell cultures. When ceramic beads with immobilized cells were used, the protein concentration was higher than when free cells were used (Table 1). The increase in cell number was more apparent in the high PCE levels than in low levels of PCE. This implies that the resistance of *C. bifermentans* DPH-1 to high concentrations of PCE and cDCE was increased by the immobilization to ceramic beads.

2. Batch culture kinetics

In order to determine the velocity constant of PCE, this reaction was assumed as first-order reaction and the all values were determined within 95% confidence intervals (Table 2). Because the inducement period with immobilized cells was longer than that of free cell culture during the PCE dechlorination, first-order reaction rate was determined after consideration of the inducement period (Table 3). Below 151 μM PCE, the first-order reaction rate of immobilized culture was similar to that of free cell culture. Above 151 μM PCE, the rate constant of immobilized culture was less than that for free cell culture. In general, the diffusion resistance of substrate to support material is relatively larger when immobilized biocatalyst is used than when free catalyst is used.³²⁾ Thus, when PCE concentration was increased, the

resistance would become larger than at low concentrations. Indeed, 905 μM of PCE is near the saturating concentration in water. Therefore, the delay of PCE diffusion in ceramic beads might have resulted in the decrease of first-order reaction rate observed in immobilized culture.

When we assumed the dechlorination by other PCE dechlorinating bacteria was also a first-order reaction, the first-order reaction rate constant could be determined at a significant level of 5% (Table 4).^{6,8,9,18,26,38,50-52)} The first-order reaction rate constant of *C. bifermentans* DPH-1 was equal to *Desulfomonile tiedje* and *Desulfitobacterium* sp. strain Y-51 as 0.006 $\text{mg protein}^{-1}\cdot\text{h}^{-1}$. However, the first-order reaction rate constant of *Sulfurospirillum multivorans* was higher about

Table 3. First-order reaction constants of PCE dechlorination by fixed cells (exception lag phase)

Initial concentration of PCE (μM)	First-order reaction constant ($l \cdot \text{mg protein}^{-1} \cdot \text{hr}^{-1}$)	Plot number	R ²
30	0.0041	3	0.95 ^a
60	0.0054	3	0.88 ^b
151	0.0068	5	0.79 ^a
302	0.0035	6	0.85 ^a
603	0.0036	7	0.86 ^a
905	0.0040	7	0.73 ^a

^aconfident >99%

^bconfident >95%

Table 2. First-order reaction constants of PCE dechlorination

Initial concentration of PCE (μM)	First-order reaction constant ($l \cdot \text{mg protein}^{-1} \cdot \text{hr}^{-1}$)		Plot number		R ²	
	Free cell	Fixed cell	Free cell	Fixed cell	Free cell	Fixed cell
30	0.0041	0.0026	6	5	0.82 ^a	0.67 ^b
60	0.0033	0.0030	7	5	0.89 ^a	0.69 ^b
151	0.0036	0.0053	7	6	0.67 ^b	0.72 ^a
302	0.0060	0.0026	6	8	0.77 ^a	0.89 ^a
603	0.0056	0.0031	6	8	0.60 ^b	0.82 ^a
905	0.0060	0.0033	6	8	0.75 ^a	0.74 ^a

^aconfident >99%

^bconfident >95%

Table 4. First-order reaction constant of PCE dechlorination

Organisms	Reaction	First-order reaction constant ($l \cdot \text{mg protein}^{-1} \cdot \text{hr}^{-1}$)	Electron donor
<i>Desulfomonile tiedje</i> DCB-1	PCE → TCE	0.00007	Pyruvate
	PCE → cDCE	0.0054*	Pyruvate
<i>Dehalobacter restrictus</i> PER-K23	PCE → cDCE	0.0005	H ₂ , methanol, butyrate
<i>Sulfurospirillum multivorans</i>	PCE → cDCE	0.0124	Pyruvate
<i>Dehalococcoides ethenogens</i>	PCE → VC	0.0562	H ₂ , methanol, butyrate
Strain 195	PCE → ethylene		
<i>Methanosarcina</i> sp. Strain FR	PCE → ?	0.00005	Methanol
<i>Desulfitobacterium hafniense</i>	PCE → cDCE	0.0064	Pyruvate
Strain Y-51			
Strain MS-1	PCE → cDCE	0.0001	Acetate
<i>Clostridium bifermentans</i> DPH-1	PCE → cDCE	0.006	Yeast extract, H ₂ , ethanol

*add 3-chlorobenzoate.

twice than that of the *C. bifermentans* DPH-1. *S. multivorans*, a dehalorespiring organism, has been reported that uses PCE as an electron acceptor in energy metabolism.⁵⁰ In contrast to co-metabolic processes, respiratory processes that utilize chlorinated compounds as an electron acceptor in energy metabolism are fast and generally accepted as major contributive processes to biological reductive dechlorination in anaerobic environments.^{19,40} *C. bifermentans* DPH-1 may also belong to halogen respiration organism since the number of cell was increased when PCE is present in the condition (Table 1). Moreover, in previous work, it was reported that hydrogen produced from yeast extract fermentation in cell free culture with PCE was utilized for the reductive dechlorination of PCE.⁸ Accordingly, cell proliferation and PCE dechlorination activity might be promoted by using a more appropriate electron donor and the acceptor in the culture of *C. bifermentans* DPH-1.

3. Dechlorination of high concentrations of PCE on continuous culture: Flow characteristics

The result of liquid HRT determination is shown in Fig. 4. The effluent peak of NaCl was detected at 195 min (3.25 h) slightly earlier than the set condition of HRT 213 min. As a result, it was indicated that the characteristics of this fixed-bed

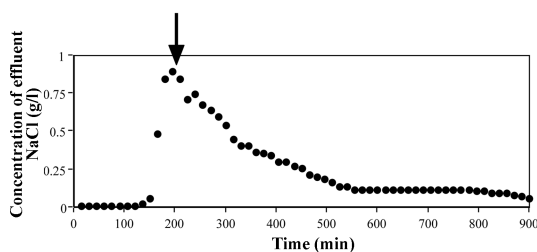


Fig. 4. Hydraulic characteristics of the bioreactor. ← Calculated HRT; 213 min.

reactor are similar to those of a plug-flow system. On the other hand, adsorption of NaCl to ceramic beads was not considered because total effluent amount of NaCl (0.212 g) was almost equal to the injected amount of NaCl (0.218 g). After appearance of the effluent peak, effluence of NaCl was delayed. It may be difficult to flush out NaCl, since it has a higher specific gravity than pure water, and mixing of pure water and NaCl occurred in the column.

The breakthrough curve and the curve of residual concentration are shown in Fig. 5 and Fig. 6. Relative density was 0.5 when liquid (equal to the liquid volume in the column) was added. The results indicate that adjusted HRT is slightly longer than the actual HRT. From the breakthrough curve,

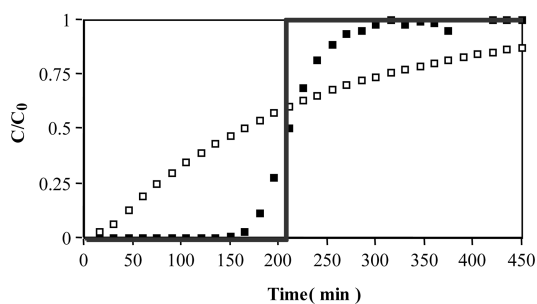


Fig. 5. Breakthrough curve.

Symbols: ■, Experimental value; □, Expected value in CSTR, —, Expected value in PFR.

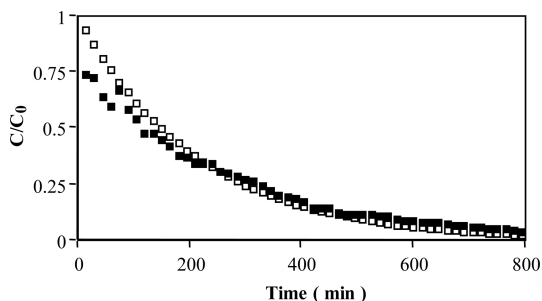


Fig. 6. Curve of residual concentration.

Symbols: ■, Experimental value; □, Expected value in CSTR.

it was also calculated that the flow characteristics of this reactor are close to those of a plug-flow though a small amount of decentralization was observed. The residual concentration curve corresponded relatively well to the theoretical value for a complete-mix reactor (Fig. 6). Although the breakthrough curve and the residual curve might seem to contradict each other, this is only due to the heavy specific gravity of NaCl, which we were not able to ignore (as discussed above). Thus, it could be concluded that flow characteristics of this reactor are intermediate between those of a plug-flow and a complete-mix system.

4. The influence of influent concentrations of PCE

At a HRT of 12 h, the result of dechlorination experiments in continuous culture with high concentrations of PCE is shown in Fig. 7. When we gradually changed the influent concentrations

of PCE from 30 μM to 905 μM , the PCE dechlorination rate was extended to over 99% during long periods ($\sim 2,000$ h). The main intermediate product of PCE was cDCE. When the influent concentration of PCE was 30–60 μM , the effluent concentration of PCE was stabilized at 0.25 μM , and the concentration of cell protein was below 10 mg l^{-1} . PCE was rapidly converted to cDCE without apparent accumulation of TCE. At 151 μM of PCE, effluent concentration of cell protein increased to an average of 17 mg l^{-1} as average whereas PCE was steadily dechlorinated. Above 302 μM of PCE was added, effluent concentration of PCE was temporarily exceeded 1.0 μM , and the maximum concentration of effluent was 41 μM . The effluent concentration of maximum cell protein was also increased up to 26 mg l^{-1} . A tendency was observed for the concentration of effluent protein to increase rapidly just before an increase in the effluent concentration of PCE. In batch culture, *C. bifermentans* DPH-1 was shown to proliferate enough on ceramic beads by immobilization of free cell, even under high concentrations of chlorinated ethylene. In contrast, in continuous culture with short HRT (12 h), cell growth and PCE dechlorination rate were decreased temporarily when high concentrations of PCE were added (Fig. 7). However, the decrease in PCE removal rate seemed to be temporary since stable reductive dechlorination of PCE was confirmed eventually during the operation.

Biotransformation of PCE into innocuous compounds is desirable; unfortunately, cDCE, which is toxic, was the main product of PCE dechlorination in the reactor system. Co-immobilization of *C. bifermentans* DPH-1 with other bacteria that degrade cDCE aerobically or anaerobically to ethene or ethane might be possible in order to establish a system for complete detoxification of PCE.¹⁵⁾

5. The influence of HRT on continuous culture

Continuous PCE dechlorination at various HRT of the reactor was carried out (Fig. 8). Influent concentration of PCE at each HRT was maintained at 905 μM . HRT was diminished from HRT 12 h to 6 h, 4.6 h, and 3.4 h gradually. At each HRT

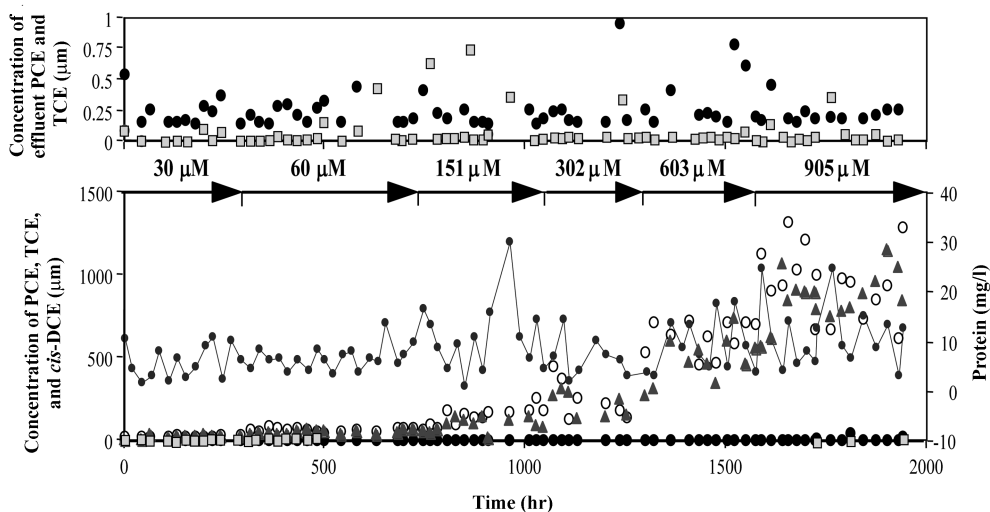


Fig. 7. The result of dechlorination experiments in continuous culture with high concentrations of PCE at HRT 12 h. Symbols: ●, effluent PCE; ○, influent PCE; ■, effluent TCE; ▲, effluent cDCE; —●—, effluent protein.

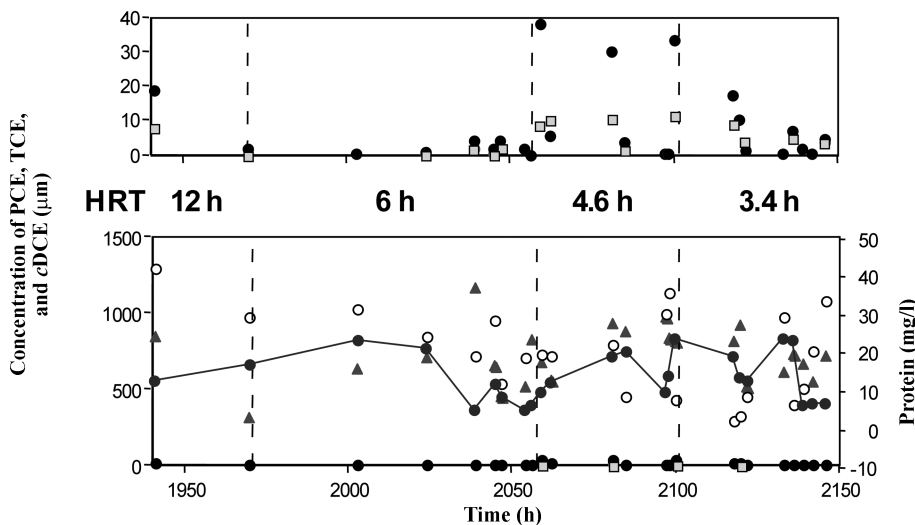


Fig. 8. Continuous PCE dechlorination at various HRT of the reactor.

Symbols: ●, effluent PCE; ○, influent PCE; ■, effluent TCE; ▲, effluent cDCE; —●—, effluent protein.

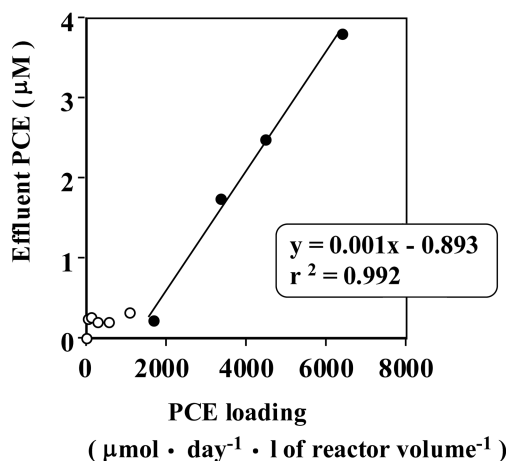
condition (12, 6, 4.6, and 3.4) TCE was always detected as an intermediate product, though cDCE was also detected. A sudden shift of effluent concentration of PCE and TCE occurred at the below HRT 6 h. This may be attributable to short HRT. At HRT 3.4 h, PCE dechlorination activity seemed to be recovered, but the PCE dechlorination was still unstable. At short HRT, immobilized

cells did not grow enough to dechlorinate PCE. Therefore, in order to maintain efficiency of transformation of PCE, more than 6 h HRT is required in this reactor system. In general, anaerobic reactors require more than 20 h HRT, because cell growth inside the reactor requires more time than in an aerobic system. If the reaction time is too short, transformation of PCE does not occur^{18,30,55-57}

Table 5. Examples of PCE dechlorination in various types of bioreactors

Reactor system	Efficiency	HRT	Q _{max} ($\mu\text{mol} \cdot \text{l}^{-1} \cdot \text{hr}^{-1}$)	Possible maximum PCE loading ($\mu\text{mol} \cdot \text{d}^{-1} \cdot \text{l}$ of reactor volume ⁻¹)	Reference
An/o (fixed)	>99%	47/55.6	0.069	7.4×10^{-3}	Gerritse <i>et al.</i> , 1995
An/o (chemostat)	88%	4.2/2.6	141	3.38×10^3	Gerritse <i>et al.</i> , 1997
An/o (fixed)	96%	37.5/2.24	0.017	0.5	Fathepure <i>et al.</i> , 1991
An (fluidized)	>98%	1.8	39.9	377	Carter <i>et al.</i> , 1993
An (fixed)	>99%	48	2.1	62	Timothy <i>et al.</i> , 1985
An (fixed)	>95%	2.4	3.7	89	de Bruin <i>et al.</i> , 1992
An (fixed)	>99%	17	7.1	171	Komatsu <i>et al.</i> , 1997
An (fixed)	98%	72	3.0	9.35	Nathalie <i>et al.</i> , 1998
An (fixed)	>99%	16.7	35.3	4.24×10^5	Mansour <i>et al.</i> , 1998
An (fluidized)	>99%	12	1.17	33.7	Eisenbeis <i>et al.</i> , 1997
An/o (fixed)	98%	72	3.0	720	Cabirol <i>et al.</i> , 1998
An (fixed)	>99%	49	15	131	Pampel & Livingston, 1998
An (fixed)	>99%	48	0.0074	23	Vogel & McCarty, 1985
An (fixed)	>99%	1.12	1.3	36.8	Christiansen <i>et al.</i> , 1998
An (fixed)	98.5%	12	3.58	6.87	Prakash & Gupta, 2000

($\mu\text{mol} \cdot \text{g VSS}^{-1} \cdot \text{hr}^{-1}$)

**Fig. 9.** Relationship between PCE loading rate and concentration of effluent PCE.

(Table 5).

We also examined the relationship between the PCE loading rate and the PCE effluent concentration was examined (Fig. 9). A linear relationship between the effluent concentration of PCE and the PCE loading rate was observed, and the maximum

volumetric dechlorination rate of PCE was calculated to be $1,100 \mu\text{mol} \cdot \text{d}^{-1} \cdot \text{l}$ of reactor volume⁻¹ when effluent concentration was set at $0.25 \mu\text{M}$. The maximum volumetric dechlorination rate was relatively high compared with some previously reported results (Table 5) though it was lower than two: $4.24 \times 10^5 \mu\text{mol} \cdot \text{d}^{-1} \cdot \text{l}$ of reactor volume⁻¹ (Gerritse *et al.* 1997)²³ and $3.38 \times 10^3 \mu\text{mol} \cdot \text{d}^{-1} \cdot \text{l}$ of reactor volume⁻¹.²⁸

IV. Conclusions

We have shown that the anaerobic bacterium *C. bifermentans* DPH-1 is able to dechlorinate PCE to cDCE at high rates in the monoculture biofilm of a fixed-bed reactor. The first-order reaction rate of *C. bifermentans* DPH-1 was relatively high and comparable to those obtained by others as $0.006 \text{ mg protein}^{-1} \cdot \text{l} \cdot \text{h}^{-1}$ (Table 4). Judging from the patterns of cell growth, DPH-1 seemed to behave as a halogen respiration organism when PCE is present, as has been suggested by previous reports.⁸⁾ When having gradually changed the influent concentrations of PCE from $30 \mu\text{M}$ to $905 \mu\text{M}$,

the PCE dechlorination rate was extended to over 99% during operation of the reactor. The maximum volumetric dechlorination rate of PCE was calculated as 1100 $\mu\text{mol}\cdot\text{d}^{-1}\cdot\text{l}$ of reactor volume⁻¹ and it was relatively high compared with previously reported results (Table 5). These results of this study suggest that the performance of this fixed-bed reactor immobilized mono-culture is enough to be comparable to that of fixed-bed reactor mixture culture systems for PCE removal. Furthermore, the rapid start-up of this reactor will be an advantage in bioremediation of PCE containing wastewater. For complete degradation of PCE, it might be possible either to combine this first dechlorination step with an aerobic process where cDCE is degraded to innocuous compounds^{31,36} or with a second anaerobic culture that would dechlorinate cDCE to ethene or ethane^{11,21,25,29,39,41} together with *C. bifermentans* DPH-1 in the same reactor unit. Further studies will determine which of the two possible reactor types is most suited for practical applications.

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