(35) REDUCTIVE DECHLORINATION OF TETRACHLOROETHYLENE IN A BIO-ELECTRO REACTOR SYSTEM BY METHANE FERMENTATIVE MICROORGANISMS

メタン発酵微生物を用いたバイオエレクトロによるPCEの還元的脱塩素に関する研究

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Abstract; Reductive dechlorination of tetrachloroethylene (PCE) was investigated in a bio-electro reactor system. It consisted of cathodic electrodes on which methane fermentative microorganisms were immobilized and anodic carbon electrodes. Methane fermentative microorganisms used in this study were cultured from a mixture of lake sediment and anaerobic treatment sludge as inoculum source. PCE dechlorination, supported either by hydrogen gas or by organic compounds fed as primary energy source and electron donor, was also investigated at 35°C in small vials used as bioreactors. In the bio-electro reactor system, hydrogen generated by electrolysis of water supports PCE dechlorination. PCE was transformed mainly into ethylene (ETH) and trans-1,2-dichloroethylene (*trans* -DCE). On the other hand, when PCE dechlorination was supported by hydrogen gas or organic compounds, PCE was dechlorinated mainly up to cis-1,2 dichloroethylene (*cis*-DCE) and vinyl chloride (VC) respectively. The fact that PCE was dechlorinated into harmless metabolites such as ETH in the bio-electro reactor system, suggested that such process investigated would be applied for PCE contaminated groundwater remediation.

Keywords; Bio-electro reactor, Chlorinated hydrocarbons, Groundwater remediation, Methane fermentative mixed culture, PCE dechlorination.

1. Introduction

An urgent need exists for innovative technologies to treat various sites that have been contaminated with hazardous chlorinated chemicals. Stripping and vacuuming methods are often used for reclaiming soil and groundwater pollution (Mackay *et al.*, 1989). However, interest has been growing in biological processes because they have the prospect of converting contaminants to harmless products. Chlorinated hydrocarbons tend to resist degradation in conventional biological waste water treatment process and in natural ecosystems. Many researches have reported that under anaerobic conditions, PCE is dechlorinated via sequential reductive dechlorination steps to replace the chlorine atoms with hydrogen atoms (Bagley *et al.*, 1990; Carter *et al.*, 1993; De Bruin *et al.*, 1992; DiStefano *et al.*, 1991). PCE can be partially mineralized to CO_2 (Vogel *et al.*, 1985). However, most bio-transformations result in the accumulation of less-chlorinated ethenes such as TCE (Cabirol *et al.*, 1996; Fathepure, et al., 1987),

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DCE isomers (Bagley *et al.*, 1990; Fathepure *et al.*, 1994; Gibson *et al.*, 1992) and the most frequently observed in large amounts, VC (Carter *et al.*, 1993), which is a metabolite even more toxic than its parent compounds (Infante *et al.*, 1982). Among the three possible DCE isomers, 1,1-dichloroethylene (1,1-DCE) is the least significant intermediate, and several studies have reported that *cis*- DCE predominates over *trans*- DCE (Chu *et al.*, 1994). Moreover, PCE dechlorination to ethylene (DiStefano *et al.*, 1991) and ethane (De Bruin *et al.*, 1992; Komatsu, 1997) have been also reported.

In previous studies, it has been reported that the electron donor for the PCE reductive dechlorination is derived from organic compounds and organic acids (DiStefano *et al.*, 1991; Freedman *et al.*, 1989; Gibson *et al.*, 1992; Komatsu *et al.*, 1994; Komatsu, 1997; Wu *et al.*, 1998). Moreover, dechlorination of PCE supported by hydrogen gas has been also reported (DiStefano *et al.*, 1992). Hydrogen gas may be more favorable as electron donor than organic materials, but the poor solubility of hydrogen gas in water causes low removal efficiency. Systems being generated hydrogen would be selected as an alternative process. A bio-electro reactor system (BER) may be an efficient process for utilization of hydrogen (Kuroda *et.al.*, 1994; Sakakibara *et.al.*, 1993).

The BER consists of anodic and cathodic immobilized bacteria electrodes. Hydrogen gas is produced on the surface of the cathodic electrodes through electrolysis of water according to Eq. 1, when electric current is applied.

$$2\,H_2O + 2e^- \rightarrow H_2 + 2OH^- \qquad (1)$$

It diffuses into the biofilm adhered on the surface of the cathode and it is utilized effectively by the microorganisms (Kuroda *et.al.*, 1994; Sakakibara *et.al.*, 1993). When a carbon electrode is used as anode, the following electrochemical reaction of CO_2 formation occurs.

$$C + 2\,H_2O \rightarrow CO_2 + 4H^+ + 4e^- \qquad (2)$$

It is considered that oxidation of carbon to CO_2 and subsequent dissociation to carbonate and bicarbonate ions are favorable for developing anoxic conditions.

This paper reports dechlorination performance of PCE in a bio-electro reactor system (BER) in which methane fermentative microorganisms were adhered on the cathode surface. The specific objectives of our research were: (1) To accumulate a methane fermentative mixed culture able to dechlorinate PCE. (2) To investigate PCE dechlorination pathway when it is supported by hydrogen generated on the surface of the cathodic electrode on which methane fermentative microorganisms are immobilized. (3) To compare the PCE biotransformation pathway observed in the BER with those observed when dechlorination is supported by organic matter or hydrogen gas as hydrogen donor.

2. Materials and Methods

2.1 Chemicals

PCE and TCE were purchased in liquid form. Analytical standards of PCE and TCE were obtained as liquid solution (1 mg/ml and 4 mg/ml respectively in hexane). *Trans*- DCE, *cis*- DCE, and 1,1 DCE

analytical standards were also purchased as liquid solution (1 mg/ml in methanol). VC and ETH were purchased as gas (4% and 1%, respectively in N_2 gas).

2.2 Culture and enrichment procedure

A mixture of anaerobic sludge taken from a municipal wastewater treatment facility and anaerobic sediment from a lake near Ashikaga city, (Tochigi prefecture Japan) were used as inoculum source for the culture. 200 ml of the inoculum source was mixed with substrate solution containing, $C_6H_{12}O_6$, HCOONa and CH_3COOH (nominal initial concentration of 2.5 mM each) as well as mineral solution containing trace nutrients and vitamins in a 5-liters bottle. PCE and TCE were added at initial concentration of 300 μg/liter each in order to allow the adaptation for the microorganisms to the toxic chemicals . Suspension was stirred by magnetic stir bar for sufficient mixing.

The mineral solution contained (per liter of distilled water) 200 mg of K_2HPO_4, 100 mg of KH_2PO_4, 100 mg of NH_4Cl, 200 mg of NaCl, 47 mg of $MgSO_4 \cdot 7H_2O$, 45 mg of $CaCl_2 \cdot 2H_2O$, 3.5 mg of $FeCl_3 \cdot 6H_2O$, 50 mg of $Na_2S \cdot 9H_2O$, 2 mg of resazurin, 1 ml of trace nutrients solution and 1 ml of vitamin solution. The micronutrients solution contained (per liter of distilled water) 500 mg of H_3BO_3, 215 mg of $CuSO_4$, 1200 mg of $CoCl_2 \cdot 6H_2O$, 120 mg of $(NH_4) \cdot 6Mo_7O_2$, 330 mg of $ZnCl_2$, 150 mg of $Ni(NO_3)_2$. The vitamin solution contained (per liter of distilled water) 0.1016 mg of pyridoxine-HCl , 0.05 mg of thiamine-HCl, 0.05 mg of riboflavin, 0.05 mg of nicotic acid, 0.05 mg of biotin, 0.02 mg of folic acid, 0.008 mg of cobalamin and 0.05 mg of *p*-aminobenzoic acid.

When the complete consumption of added organic substrate was verified, part of the supernatant was withdrawn and equal volume of mineral solution containing $C_6H_{12}O_6$, HCOONa and CH_3COOH were fed into the reactor after the sedimentation of microorganisms. Prior to PCE and TCE addition, the culture solution was stripped thoroughly with N_2 gas. PCE and TCE were added in order to attain a given chlorinated compounds initial concentration. PCE and TCE initial doses accompanying wasting and feeding operation were increased gradually up to 5000 μg/liter. The pH value was kept in the range of 6.8 to 7.8 and the ORP value was kept lower than -160 mV.

2.3 Experimental apparatus

PCE dechlorination pathways by the enriched bacteria have been investigated under batch operation using three different systems. For PCE dechlorination in a BER, a 1L-vessel set the immobilized bacteria electrode as cathode was used. The schematic diagram of the apparatus was shown in Fig. 1. The total effective surface area of the anodic and the cathodic electrodes was 22.6 cm^2 each. The distance between cathode and anode was 1 cm apart from each other. PCE dechlorination supported by organic hydrogen donor and hydrogen gas was investigated in 32-ml vials sealed with teflon rubber. The temperature for all the experiments was maintained at 35° ± 1°C by a

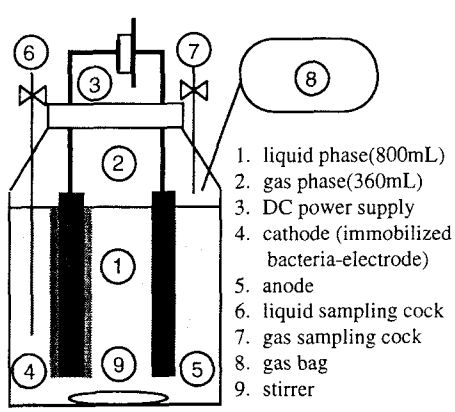

Fig. 1 Schematic diagram of a bio-electro reactor (BER)

water bath controlled. The bio-electro reactor setup was performed by the following procedure: 400 ml of mixed culture cultivated as described previously and 400 ml of anaerobic mineral medium were pumped in the reactor and the culture was allowed to develop the anaerobic biofilm on the electrodes for 1 month. In order to increase the microorganisms population adhered on the surface of the cathodic electrode, substrate solution containing acetate, formate, glucose and trace nutrients was added periodically. PCE concentration was 5000μg/L. After 1 month, 1 mA of electric current was also applied to acclimatize the microorganisms. The anaerobic biofilm was also acclimatized to PCE and hydrogen gas generated on the surface of the cathodic electrode for 4 weeks prior to testing its PCE dechlorination ability.

2.4 Procedure

PCE dechlorination by the BER: After the cultivation and acclimatization steps, reactor liquid with suspension was replaced to solution containing 5000μg/L of PCE without organic hydrogen donor. The applied electric current was of 1 mA with DC power supply. Control experiment by using an abiotic electro reactor without immobilization of microorganisms on the electrodes (ER) was also conducted.

PCE dechlorination supported by organic matter (BR1) or hydrogen gas (BR2): Experimental conditions are shown in Table 1. Reductive conditions were maintained by the presence of $Na_2S \cdot 9H_2O$. Nonsterile samples were compared with sterile controls in order to differentiate between losses due to adsorption or other abiotic processes and biodegradation. For obtaining reproducibility in the results, each bioreactor was set up in duplicate.

Table 1. Experimental conditions

Reactor No.	Temperature [°C]	MLVSS [mg/L]	PCE initial conc. [μg/L]	Hydrogen donor	Org. matter [mg-COD/L]	H_2 gas [mg-COD/L]
BR1	35	2000	3500	Glucose, acetate and formate	68	0
BR2	35	2000	3500	H_2 gas (90 ml/L-liquid phase)	0	65

2.5 Analytical methods

PCE and its metabolites concentrations were determined by headspace gas chromatography. The concentrations of PCE, TCE and DCE isomers considered as liquid bases were determined by a method involving a single 0.1-ml headspace gas injection into a gas chromatograph equipped with ECD and FID. Separation was effected in a 25 m x 0.22-mm I.D. Shimadzu CBP5-M25-025 fused silica capillary column (slight polarity, 5% phenyl silicone- methyl silicone liquid phase). Standards were prepared in 32 ml-reactors sealed with teflon curtain rubber containing 22 ml of standard solution of chlorinated compounds and 10 ml of headspace volume. Before analysis, the vials were maintained at 35° ± 1°C for at least 1 hour to adjust the equilibrium between gas and liquid phase. ETH and VC concentrations were measured by the injection of a 0.1 ml headspace sample into a gas chromatograph equipped with FID. VC and ETH concentration were measured as gas bases and adjusted to liquid bases. Redox potentials (ORP), MLVSS and pH were measured by standard methods (APHA, 1995).

3. Results

PCE dechlorination in the BER

Experimental results of PCE dechlorination in the bio-electro reactor (BER) is shown in Fig. 2(a). In the

1st day, only *trans*-DCE was detected as metabolite. TCE, *cis*-DCE, 1,1-DCE, VC and ETH were not detected. In the 3rd day, ETH was identified as metabolite, indicating that the methane fermentative mixed culture immobilized on the cathodic electrode has the ability to dechlorinate PCE to ETH. From the 4th to the 8th day, the PCE concentration diminished at the same time during which the *trans*-DCE concentration increased almost linearly. Although *cis*-DCE was detected as metabolite, *trans*-DCE concentration remained at high concentration. From the 8th day to the 14th day, the *trans*-DCE concentration remained almost constant, whereas, *cis*-DCE concentration gradually decreased. On the other hand, during the tested period, the ETH concentration increased linearly, reaching its maximum value on the 14th day.

PCE dechlorination in the ER

Experimental results of PCE dechlorination in the abiotic-electro reactor (ER) is shown in Fig. 2(b). In the 3rd day, *cis*-DCE was identified. During the tested period, *trans*-DCE was detected at traces level. TCE, VC and ETH were never detected.

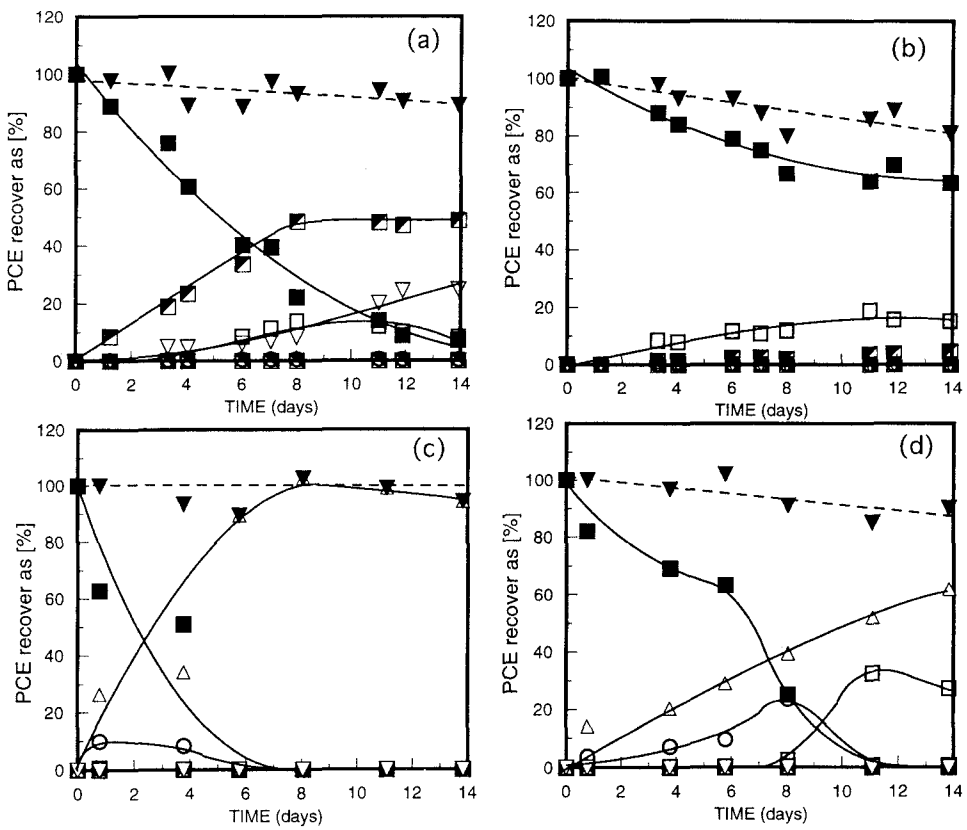

Fig. 2 ■ PCE,○ TCE, □ *cis*-DCE, ◩ *trans*-DCE, △ VC, ▼ ETH and▼ mass balance as percent of PCE recovered in(a) the bio-electro reactor BER, (b) abiotic-electro reactor ER, (c) bioreactor BR1, (d)bioreactor BR2.

PCE dechlorination in the BR1

Experimental results of PCE dechlorination supported by organic hydrogen donors is shown in Fig.2(c). TCE, *cis*- DCE as well as VC were detected as metabolites within 1 day. From the 1st to the 4th day, a slight decrement on the PCE and TCE concentrations was observed, being more significant in the case of PCE. Consumption of PCE was the highest among all the experiments. On the other hand, the VC concentration increased during the same period of time. Moreover, *trans*- DCE was not detected. It seems that PCE and TCE transformation into DCE were slower than that for DCE to VC. In the 6th day, neither TCE nor PCE were more detected in the analysis. PCE initial dose was recovered as VC. After the 6th day and for a period of 1 week, VC concentration was constant and almost equivalent to the PCE initial dose. The mass balance could be achieved and the initial dose of PCE was recovered as VC having an efficiency of 94.2%.

PCE dechlorination in the BR2

PCE dechlorination pathway when it is supported by hydrogen gas fed as electron donor is shown in Fig. 2(d). In the first day, small amount of TCE and VC were detected as metabolites but DCE isomers

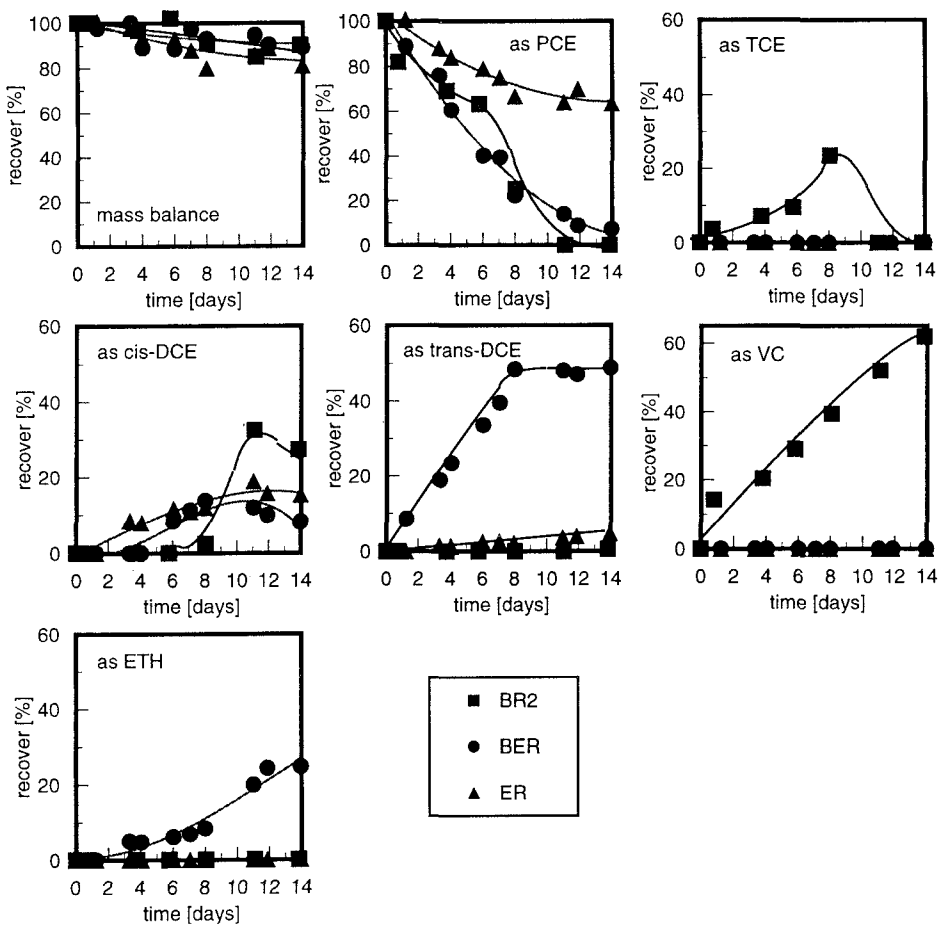

Fig. 3 PCE initial dose recovered as PCE, TCE, *cis*-DCE, *trans*-DCE, VC and ETH.

were not detected. From the 1st to the 4th day, a decrease in the PCE concentration was observed at the same time in which the TCE and VC concentrations increased. In the 8th day, the TCE concentration reached its maximum value and *cis*-DCE was detected as metabolite. In the day 11th, neither PCE, nor TCE were detected in the analysis and the *cis*-DCE concentration reached its maximum value. In the day 14th, traces of *trans*- DCE and 1,1-DCE were detected. As can be seen in Fig. 2(d), during the experimental period, 27.3% and 61.8% of the PCE initial dose was recovered as *cis*-DCE and VC respectively. ETH was never detected during the tested period.

Comparison of conversion percent during dechlorination

Figure 3 shows the percent of PCE recovered as PCE, TCE, *cis*-DCE, *trans*-DCE, VC and ETH in the experiment by the BR2, BER and ER. As can be seen, in the ER, during the tested period, the 15.2% and 3.8% of the PCE initial dose was recovered as *cis*-DCE and *trans*-DCE respectively. In the BR2, in which the PCE dechlorination was supported by hydrogen gas, from the 11th day to the end of the experiment, PCE was not detected any more. During the tested period, 27.3% and 61.8% of the PCE initial dose was recovered as cis-DCE and VC respectively. In the BER, 8.1%, 48.8% and 24.7% of the PCE initial dose was recover as *cis*- DCE, *trans*- DCE and ETH respectively.

4. Discussion

The dechlorination of PCE to ETH, *cis*-DCE and *trans*-DCE has been demonstrated to occur readily in the BER. This is the first report of a reduction of PCE to ETH in such systems. In the BER in which PCE dechlorination was supported by hydrogen generated on the surface of the cathodic electrode, *trans*-DCE, ETH and *cis*-DCE were detected as metabolites. *Trans*-DCE concentration remained almost constant after the 8th day while ETH concentration increased. This result suggests that PCE may be transformed to ETH via *cis*-DCE rather than via *trans*-DCE. However the conversion of PCE via *trans*-DCE will be also possible since *trans*-DCE, ETH and *cis*-DCE were detected as metabolites in the initial experimental period. Freedman *et.al.* (1989) has reported the dechlorination of PCE and TCE to ETH via *trans*-DCE and VC. In their experiment, the culture was enriched by addition of glucose, methanol, acetic acid or sodium formate.

TCE was never detected during the tested period. It could indicate either PCE is transformed without being dechlorinated to TCE, or TCE is transformed into lesser-chlorinated compounds as soon as it is generated.

When organic hydrogen donors supported dechlorination, PCE was mainly transformed to VC via sequential reduction of chlorine. Vogel *et al.* (1985) reported that PCE can be transformed by reductive dehalogenation to TCE, DCE isomers and VC under anaerobic conditions feeding organic hydrogen donors such as acetic acid, acetone and isopropanol. Carter *et al.* (1993) reported that dechlorination of PCE to VC and ETH (low conversion) by methanogenic bacteria was supported by sucrose.

When PCE dechlorination was supported by hydrogen gas in the bioreactors, ETH was not detected and dechlorination of PCE could not be supported at the same efficiency that in the case of dechlorination supported by organic hydrogen donors. These results accord with those reported by

previous researchers. DiStefano *et. al.* (1992) reported that hydrogen gas served as an electron donor in the reductive dechlorination of PCE to VC and ETH; however, sustained dechlorination for extended periods required the addition of filtered supernatant from a methanol-fed culture.

Table 2. Characteristics of hydrogen donor (HD) depending on the tested process

Process	Hydrogen donor	PCE conc. (mg/L).	Organic matter as mg-COD/L	Ratio HD fed/HD req.	% of HD used for dechlorination	% of PCE recovered as ETH
BER	H_2 generated by electrolysis (175 ml/L-liquid phase)*	5	0	56.89	0.945	34.75
BR1	Organic matter (Glucose, acetate and formate)	3.5	68	50.34	1.403	0
BR2	H_2 gas (90 ml/L-liquid phase)	3.2	0	52.58	1.15	0

* Calculated by Faraday's law (day 14th)

As shown in Table 2, PCE was dechlorinated to ETH only in the BER. In order to explain why PCE could not be transformed completely into ETH and why ETH was only detected in the BER, the amount of hydrogen donor in each tested conditions were compared. In our research even the amount of hydrogen donor was theoretically enough to dechlorinate completely PCE into ETH in all the reactors and the ratio of hydrogen donor fed to hydrogen donor necessary to support complete dechlorination of PCE was equivalent at all experiments. However PCE dechlorination was not complete. Therefore it was deduced that PCE dechlorination might not be supported completely due to the microorganisms and their environment and no due to insufficient hydrogen donors. Komatsu (1997) has reported the reductive transformation of PCE to ETH and ethane by an anaerobic filter. Low concentrations of PCE (2.8 mg/L) could be completely dechlorinated at short HRT by high-rate anaerobic reactors if a proper inoculum source and a suitable electron donor were used. The author also reported PCE concentrations as high as 80 mg/L (never reported before) could be dechlorinated to ETH with a relatively low supply (200-mg COD/L) of ethanol.

In the BER, the main DCE isomer detected was *trans*-DCE and not *cis*-DCE while in the BR1 and BR2, the main DCE isomer detected was *cis*-DCE as reported by Fathepure *et.al.* (1991).

Some electrochemical reactions might transform *cis*-DCE to *trans*-DCE in the BER as one of the reasons. Therefore, we investigated the electrochemical transformation of *cis*-DCE in the ER. In the reactor, an aqueous solution containing *cis*-DCE at an initial concentration of 1000 μg/L was set up and an electric current of 1 mA was applied during two weeks. At the end of the experiment, the 94% of the *cis*- DCE initial dose were recovered as *cis*-DCE and *trans*-DCE converted from *cis*-DCE was only 2%. The fact that *cis*-DCE was scarcely transformed by the applied electric current indicates that PCE was not transformed into *trans*-DCE by electrochemical reactions alone. Therefore, more research is needed on the mechanism of dechlorination in order to explain: (a) what kinds of microorganisms are involved on the dechlorination process, (b) how the dechlorination is affected by the electric current. (c) how intermediate products such as *cis*-DCE, *trans*-DCE and VC affect dechlorination of PCE in the BER, (d) what kinds of reactions , except for hydrogen and CO_2 formation, occur on the anodic and cathodic electrodes.

5. Conclusions

In this study, PCE could be transformed by the methane fermentative mixed culture enriched in our laboratory. In the BER, PCE could be transformed to ETH and the main DCE isomer detected was *trans*-DCE and no *cis*-DCE. Since PCE was recovered as ETH only in the BER, we conclude that (1) hydrogen gas generated by electrolysis of water might play an important role in the dechlorination of PCE and, (2) the BER developed in our laboratory could be an alternative technology for solving the contamination problem of groundwater pollution due to PCE.

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