J. Microbiol. Biotechnol. (2006), **16**(11), 1734–1739

JOURNAL OF MICROBIOLOGY AND BIOTECHNOLOGY

Serial Degradation of Perchloroethylene by *Delftia* sp. N6 after Dechlorination Using Fenton's Reagent

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Received: April 19, 2006
Accepted: July 31, 2006

Abstract The degradation of perchloroethylene (PCE) was investigated with the serial treatment of biological reaction after dechlorination using Fenton's reagent. The dechlorination of PCE was expressed using D_m (dechlorination value), calculated from ΔCl^- mol/ΔPCE mol, and was 2.58 with 5 mM of H_2O_2 and Fe^{3+}. The 150 μM of PCE was transformed to 37 μM of dichloroacetic acid (DCAA). Biological treatment with *Delftia* sp. N6 was applied after degradation of PCE by the Fenton reaction. The optical densities indicating cell growth were 0.53/0.10 with/without the Fenton reaction after one day, respectively. The N6 strain degraded 95% of the DCAA produced from PCE by the Fenton reaction within one day. Consequently, it seemed that the serial treatment of a Fenton reaction and biological reaction was effective in the removal of not only PCE, but also DCAA, one of the major metabolites of PCE.

Key words: Dechlorination, *Delftia* sp., Fenton's reagent, perchloroethylene, serial treatment

Perchloroethylene (PCE) is a volatile aliphatic chlorinated compound that has been introduced into the environment from a variety of sources, including the improper disposal of degreasers and solvents [1, 3, 8]. PCE maintains a persistent presence in the environment owing to its resistance to microbial degradation and its toxicity to microorganisms [4]. Although it is difficult for PCE to be degraded by microorganisms under aerobic conditions, field studies and laboratory evidence of dechlorination in the absence of oxygen have been widely reported [5, 6, 20].

Since the Fenton reaction is a well-known potent oxidative reaction, many researchers have investigated the application of the Fenton reaction to degradation of various organic compounds [14, 22, 23]. Several studies have also reported that PCE was degraded by a Fenton reaction [7, 18]. In spite of the strong oxidation ability of Fenton's reagent, the Fenton reaction could not mineralize organic compounds to carbon dioxide [18]. The dechlorination of PCE by a Fenton reaction could achieve partial mineralization, lower toxicity, and increased susceptibility to biodegradation [2, 7]. Therefore, it was studied as a pre-oxidant of biodegradation [7, 16]. Larking *et al.* [10] report that the pretreatment with Fenton's reagent enhanced degradation of polyvinyl alcohol (PVA) by *Pycnoporus cinnabarinus*.

Recently, there have been several reports on the stimulated degradation of hazardous compounds as follows: the simultaneous chemical and biological mineralization of PCE [2], increased biodegradation of phenanthrene by salicylate [11], and increased degradation of phenanthrene by bacterial strains [9]. Considering the toxicity of hydrogen peroxide on the bacterium, *Xanthobacter flavus* FB71, a H_2O_2-resistant strain, was used in the simultaneous system. Although *X. flavus* FB71 is a hydrogen peroxide-resistant strain, it was inhibited by a high concentration of hydrogen peroxide and ferrous ion. The degradation efficiency of the microorganism in the system was lower than that of the microorganism without Fenton's reagent. Therefore, we designed a serial treatment system, which consists of a Fenton reactor and bioreactor to avoid inhibition to microorganism by Fenton's reagent.

Accordingly, the current study investigated the serial treatment of a Fenton reaction and biological reaction for degrading PCE in wastewater. First, the dechlorination of PCE by the Fenton reaction was examined. Second, the biodegradation of the metabolite of PCE produced by the Fenton reaction was investigated with *Delftia* sp. N6. Finally, a serial reactor consisting of a Fenton reactor and bioreactor was used to determine the removal efficiencies of PCE and total organic carbon (TOC) from the artificial wastewater.

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MATERIALS AND METHODS

Chemicals

The PCE, dichloroacetic acid (DCAA), and $Fe(NO_3)_3 \cdot 9H_2O$ were all of analytical grade and purchased from Sigma Chemical Co. (Milwaukee, WI, U.S.A.). The H_2O_2 was obtained from Wako (Tokyo, Japan).

Isolation of Microorganisms

Samples of various soils and wastewaters in the vicinity of Daegu and Gumi industrial complex that have a history of contamination with chlorinated alkenes were collected and screened to isolate PCE-degrading microorganisms. Ten strains were initially isolated and then one strain capable of degrading PCE effectively was finally selected. The isolate, redesignated as *Delftia* sp. N6, which was originally named as *Alcaligenes odorans* N6 in a previous study [12], was enriched and developed by adding the soil and wastewater to a basal salt medium [BSM: K_2HPO_4 (4.35 g), KH_2PO_4 (1.7 g), NH_4Cl (2.1 g), $MgSO_4$ (0.2 g), $MnSO_4 \cdot 7H_2O$ (0.05 g), $FeSO_4 \cdot 7H_2O$ (0.01 g), $CaCl_2 \cdot 2H_2O$ (0.03 g), and distilled water (1,000 ml)] containing PCE. The liquid portion of the enrichment was transferred to a fresh medium to obtain a pure strain. After one week of incubation, the culture was subcultured in a fresh BSM and subsequently diluted with the same medium. Further isolation and confirmation of purity were performed using BSM along with a 2% agar. The well-isolated colonies on the agar plate were selected and restreaked to attain pure cultures. For biological degradation, the isolate was cultured on a Luria-Bertani (LB) medium or BSM containing 0.2 mM PCE. The pH level of the medium was adjusted to 7.0.

Dechlorination of PCE by Fenton Reaction

For the Fenton reaction, deionized water (5 ml) containing H_2O_2 and $Fe(NO_3)_3 \cdot 9H_2O$ was dispensed into 60-ml headspace vials. PCE was added as an aqueous stock using a 5-μl syringe through the teflon-faced neoprene rubber septa (Wheaton, Millville, NJ, U.S.A.) of each vial, resulting in a final concentration of 10 μM. The pH was adjusted to 6.0 and the vials incubated in a shaker (120 rpm) for 90 min at 30°C.

Biodegradation of the Metabolites of PCE

The *Delftia* sp. N6 was incubated in an LB medium for 24 h. The culture was then centrifuged at 14,500 ×*g* and washed twice with a phosphate buffer. The diluted culture was mixed with BSM to create a mixture of the isolate and the medium. Aliquots (5 ml) of the mixture were pre-incubated with PCE and Fenton's reagent in 60-ml headspace vials that were sealed with septa secured with aluminum hole caps to allow access using a syringe. The culture was inoculated to the vials and incubated at 30°C.

Analysis of PCE, Chloride Ions, and TOC

The PCE degradation was quantitatively investigated based on the reduction of PCE using a gas chromatograph (Varian 3400 CX, CA, U.S.A.). The samples were injected using a 250-μl gas-tight syringe into a gas chromatograph equipped with an electron capture detector and SPB-5 (Supelco Dark, Bellefonte, PA, U.S.A.) on a 30 m×250 μm capillary column (column temperature, 70°C; injector temperature, 150°C; detector temperature, 250°C; flow rate, 30 ml/min; N_2 as carrier gas). The chloride ions were analyzed by ion chromatography (IC). The IC system (Waters, MA, U.S.A.) consisted of a Model 526 HPLC Pump, 530 column heater, and 550 conductivity detector. The recently developed ERIS 1000HP autosuppressor (Alltech, Nicholasville, KY, U.S.A.) was used in the suppressor analyses. The analytical columns used for the anion separation were Ion Pac AG 14, AS 14 (Dionex, San Francisco, CA, U.S.A.). The TOC was analyzed using a TOC analyzer (Shimadzu 5000A, Kyoto, Japan). All measurements were carried out in triplicate.

Serial Wastewater Treatment System

The serial treatment system was composed of a Fenton reactor and bioreactor (Fig. 1). The volume of the Fenton reactor and sub-tank was 1,000 ml. The sub-tank was installed to store the wastewater flowing through the reactor during the Fenton reaction and precipitation of Fe salt. The bioreactor used in the current study was previously designed by Oh *et al.* [17]. The cell number for the *Delftia* sp. N6 culture was within a range of 3.2×10^6–2.1×10^7 CFU/ml. This mixture was then used as the inoculum in the bioreactor.

The serial reactor experiment was carried out using artificial wastewater containing PCE. Before reaching the bioreactor, the wastewater was pretreated in the Fenton reactor using a continuous culture. The artificial wastewater included several organic compounds, as indicated by Lee *et al.* [12], and dry cleaning wastewater taken from a local laundry. The TOC in the wastewater was about 5,000 mg/l. The operating conditions of the reactor were as follows: DO controlled by an air blower, 2–3 mg/l;

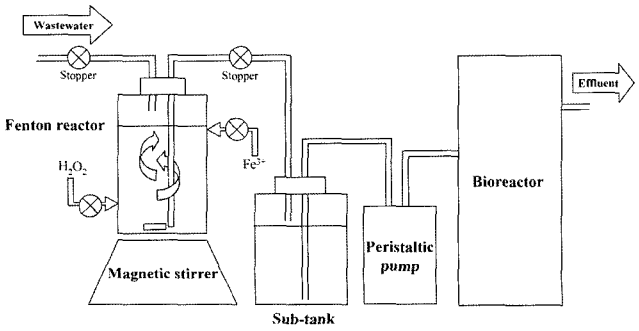

Fig. 1. Schematic diagram of serial wastewater treatment system.

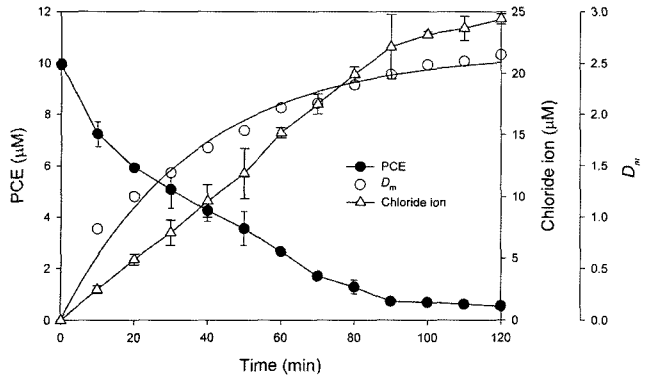

Fig. 2. Degradation of perchloroethylene (PCE) and increase in dechlorination value (D_m) due to the Fenton reaction.
Fenton's reagent was composed of 5 mM of H_2O_2 and Fe^{3+}.

hydraulic retention time (HRT), 24 h; temperature, 25±2°C. Activated sludge from the wastewater treatment plant of a pharmaceutical company was also added to the bioreactor and the concentration of the mixed liquor suspended solids (MLSS) was maintained at 2,000–3,000 mg/l by recycling the sludge in a settling tank.

RESULTS

Dechlorination of PCE with Fenton's Reagent

Fig. 2 shows the removal of PCE and production of chloride ions by a Fenton reaction when using 5 mM of H_2O_2 and Fe^{3+} for 120 min. The initial 10.0 μM of PCE decreased to 0.7 μM (93% reduction) after 90 min and 0.6 μM (94% reduction) after 120 min. At the same time, 22.1 and 24.4 μM of chloride ions were released from the PCE after 90 and 120 min, respectively. As such, 24.4 μM of chloride ions was produced during the removal of 9.4 mM of PCE. The dechlorination values (D_ms), calculated based on ΔCl^- mol/ΔPCE mol, were 2.39 and 2.58 after 90 and 120 min, respectively.

DCAA was one of the metabolites of PCE produced by the Fenton reaction (Fig. 3). PCE was transformed into DCAA after 50 min. The metabolite, DCAA, was identified by mass spectrometry (data not shown). The maximum concentration of DCAA produced was 37 μM, while 150 μM of PCE decreased to 24 μM owing to the Fenton reaction. After 90 min, the removal rate of PCE and DCAA was significantly reduced, yet after 120 min, no further PCE was removed and its concentration remained at 13 μM.

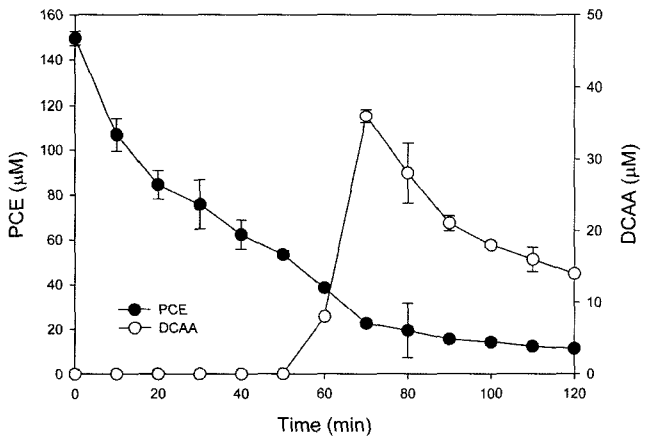

Fig. 3. Degradation of perchloroethylene (PCE) and dichloroacetic acid (DCAA) as metabolite due to the Fenton reaction.
Fenton's reagent was composed of 5 mM of H_2O_2 and Fe^{3+}.

Biodegradation of Dechlorination Products of PCE

For the biological treatment, *Delftia* sp. N6 was inoculated into the medium after incubation with Fenton's reagent and PCE. The initial optical density of the Fenton's reagent-treated medium that was incubated for 90 min was 0.12, while the final optical density (after 36 h) was 0.53 (Fig. 4). Meanwhile, no *Delftia* sp. N6 cell growth was observed in the medium that was not treated by the Fenton reaction. As such, *Delftia* sp. N6 was unable to use PCE as a carbon source under aerobic conditions, yet could use the metabolites of PCE produced by the Fenton reaction.

The level of degradation of DCAA, one of the metabolites of PCE produced by the Fenton reaction, was investigated with *Delftia* sp. N6. The strain degraded 105 μM of DCAA to 16 μM within one day; *i.e.*, a degradation efficiency of 85% (Fig. 5).

Degradation of PCE by a Serial Treatment System

The serial treatment system was tested using a simulated wastewater containing PCE. Fig. 6 shows the changes in the pH, cell number, TOC, PCE, and DCAA when treated with sludge only, sludge+*Delftia* sp. N6, a Fenton reaction,

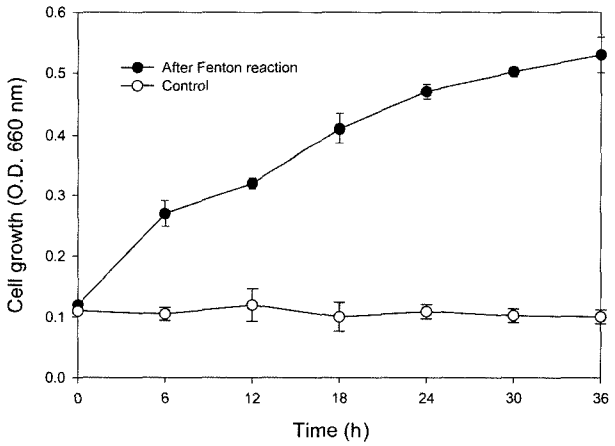

Fig. 4. Effect of the duration of the Fenton reaction on cell growth of *Delftia* sp. N6.
Fenton's reagent was composed of 20 μM of perchloroethylene (PCE) and 1 mM of H_2O_2 and Fe^{3+} and applied before inoculation of the strain.

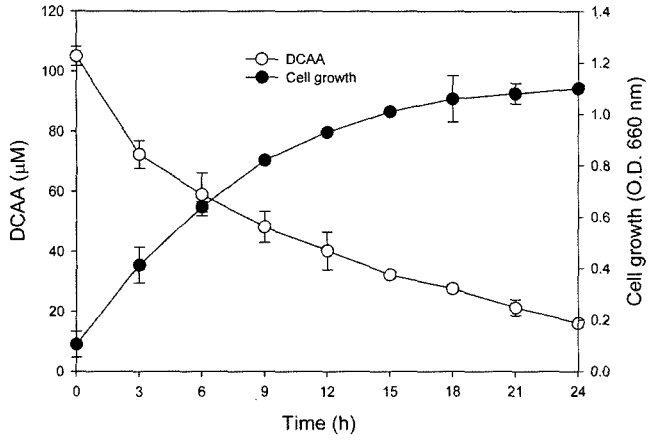

Fig. 5. Degradation of dichloroacetic acid (DCAA) by *Delftia* sp. N6, and cell growth with DCAA as a sole carbon source.

and Fenton reaction+biological reaction. The pH varied from 6.0 to 6.8 with the various treatments. The cell numbers with a Fenton reaction and Fenton reaction+biological reaction remained above 2.0×10^7 CFU/ml, whereas those with sludge only and sludge+isolate recovered to 1.8×10^7 CFU/ml after decreasing to 1.0×10^7 CFU/ml.

The removal efficiencies of TOC when treated with sludge only, sludge+isolate, a Fenton reaction, and Fenton reaction+biological reaction were 87, 88, 91, and 93%, respectively. The concentration of PCE in the wastewater decreased from 100 μM to 22 and 24 μM after treatment with a Fenton reaction and Fenton reaction+biological reaction, respectively. The PCE remaining in the bioreactor was completely removed after 18 h. As a result, the Fenton reaction was very effective in the removal of PCE.

DCAA was found in the bioreactor after treating with a Fenton reaction and Fenton reaction+biological reaction at concentrations of 41 and 37 μM, respectively. The degradation efficiencies of DCAA were 85 and 99% when treated with a Fenton reaction and Fenton reaction+biological reaction, respectively. The DCAA completely disappeared in the treatments of sludge or isolate, indicating the importance of the biological treatment.

The first-order degradation rate constants for TOC, PCE, and DCAA were calculated (Table 1). The constants

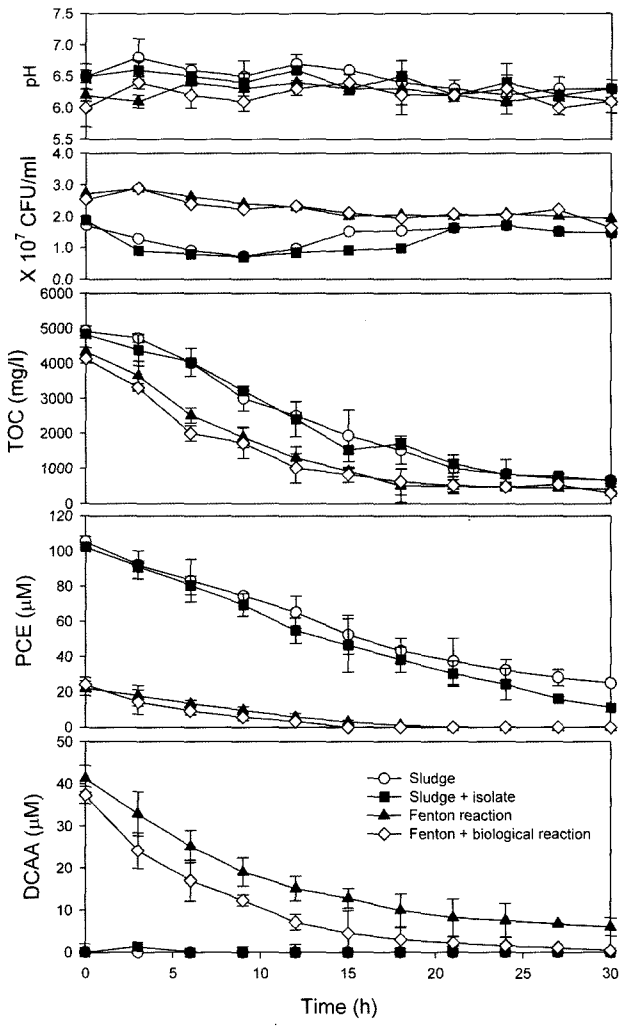

Fig. 6. Changes in pH, cell number, total organic carbon (TOC), perchloroethylene (PCE), and dichloroacetic acid (DCAA) with various artificial wastewater treatments.
A *Delftia* sp. N6 culture was added to the sludge and Fenton reaction before the sludge in the biological reaction. The cell number was counted in the bioreactor, while the other factors were analyzed in the effluent.

for TOC with sludge only and sludge+isolate were as low as 0.046 and 0.041, respectively, whereas with a Fenton reaction and Fenton reaction+biological reaction, the constants for TOC were as high as 0.120 and 0.124,

Table 1. First-order degradation rate constants with various types of treatment.

Treatment	TOC		PCE		DCAA	
	k_m[b]	r^2	k_m	r^2	k_m	r^2
Sludge	0.046	0.984	0.066	0.998	-	-
Sludge+isolate	0.041	0.982	0.998	0.998	-	-
Fenton reaction	0.120	0.995	0.042	0.985	0.098	0.999
Fenton+biological reaction[a]	0.124	0.994	0.991	0.991	0.131	0.998

[a]Fenton reaction and addition of isolate.
[b]First-order degradation rate constant relative to time.

respectively. The degradation constants for PCE were similar with the various treatments, yet the constants for DCAA with a Fenton reaction and Fenton reaction+ biological reaction were 0.098 and 0.131, respectively.

DISCUSSION

Chloride ions were concomitantly produced during the removal of PCE by the Fenton reaction (Fig. 2). The dechlorination of PCE was expressed by D_m. Since the value was 2.39, about 2.39 chloride ions were released from one molecule of PCE after 90 min. In other words, the PCE was not transformed into non-chlorinated intermediates by the Fenton reaction. Pignatello *et al.* [18] reported that PCE yields products such as mono-, di-, and trichloroacetic acids, and dichloroaldehyde. Therefore, we could predict the dechlorination of one molecular of PCE with D_m in the Fenton reaction.

For the biological treatment, *Delftia* sp. N6 that could degrade trichloroethylene (TCE) was inoculated into the medium after incubation with Fenton's reagent and PCE [12]. The strain was unable to use PCE as a carbon source under aerobic condition, yet could use it after pretreatment with Fenton's reagent (Fig. 4). The strain could degrade DCAA, the main metabolite of PCE produced by a Fenton reaction (Fig. 5). Since DCAA was ordinarily produced as the one of the intermediates to mineralize to carbon dioxide for degrading by bacterium, it could be easily degraded by TCE-degrading bacterium such as *Delftia* sp. N6. Lontoh *et al.* [15] reported that DCAA was produced from TCE and degraded by *Methylomicrobium album* BG8. Therefore, it would seem that PCE was first transformed into DCAA by the Fenton reaction and then DCAA was degraded by *Delftia* sp. N6, which was a TCE-degrading bacterium. Therefore, PCE could be degraded by the TCE-degrading bacterium after the Fenton reaction.

The degradation of PCE was tested in an artificial wastewater using the proposed serial treatment system under several conditions (Fig. 6). The pHs were lower when treated with a Fenton reaction than without. It would seem that the pH was decreased because of the HCl and DCAA produced from the PCE by the Fenton reaction. Leung *et al.* [13] previously reported that PCE was transformed into DCAA and released chloride ions, resulting in the formation of HCl.

Since the initial concentration of PCE including compounds toxic to the microorganism was high, there was a momentary decrease in the cell number without Fenton's reagent. Without Fenton's reagent, 100 mM of PCE flowed into the bioreactor; however, the cell number did recover its initial level after 21 h, because the concentration of PCE decreased to 40 μM, at which concentration the growth of microorganism was not inhibited. Sharma and McCarty [21] also reported that PCE had a high toxicity on microorganisms.

The degradation rate constants of TOC when treated with a Fenton reaction and Fenton reaction+biological reaction were higher than when treated with sludge only and sludge+isolate (Table 1). As such, Fenton treatment enhanced the degradation of TOC. It would seem that the various organic compounds in the wastewater were transformed into more biodegradable compounds by the Fenton reaction. Lin and Lo [14] also reported that Fenton's reagent could desize wastewater, resulting in the removal of TOC.

The initial concentration of PCE in the bioreactor was lower after treatment with a Fenton reaction and Fenton reaction+biological reaction than after treatment with sludge only and sludge+isolate, as the Fenton reaction and Fenton reaction+biological reaction degraded the PCE in the Fenton reactor before it entered the bioreactor. However, the degradation rate constants of the PCE that remained in the bioreactor were similar for all the treatments. In other words, PCE was not degraded by treatment with sludge only or sludge+isolate, as PCE is rarely degraded by aerobic microorganisms [19].

DCAA was produced after treatment with a Fenton reaction and Fenton reaction+biological reaction, yet not produced after treatment with sludge only and sludge+ isolate. However, the DCAA was degraded by the sludge+ isolate treatment at a higher degradation rate constant than with sludge only. Consequently, the addition of *Delftia* sp. N6 in the bioreactor enhanced the degradation of DCAA, a metabolite of PCE, produced by the Fenton reaction.

Büyüksönmez *et al.* [2] and Howsawkeng *et al.* [7] reported on the chemical and biological mineralization of PCE based on a simultaneous treatment system. In their study, *Xanthobacter flavu*, which is a H_2O_2-resistant strain, was inoculated into the reactor to degrade PCE and Fe-NTA. The efficiency of the mineralization was low (54%) compared with the current results (85%), and it is possible that the microorganism was still inhibited by H_2O_2 despite it being a H_2O_2-resistant strain. Therefore, the serial treatment system could be more effective on the degradation of PCE than the simultaneous system. It seems that the different efficiency was caused by the constitution of the bioreactor, which was not inhibited by Fenton's reagent in the serial treatment system.

In summary, PCE in wastewater was degraded by a Fenton reaction, while its metabolite, DCAA, was degraded by *Delftia* sp. N6. In addition, the Fenton reaction was found to decrease the concentration of TOC in wastewater. Therefore, the proposed serial treatment system composed of a Fenton reactor and bioreactor can be effectively applied to the degradation of PCE in wastewater.

Acknowledgments

This research was supported by grants from the Korea Research Institute of Bioscience and Biotechnology and the MOST/KOSEF to the Environmental Biotechnology National Core Research Center (R15-2003-012-02001-0).

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