Biotechnology and Bioprocess Engineering **2005**, 10: 34-39

Recovery of Trichloroethylene Removal Efficiency through Short-term Toluene Feeding in a Biofilter Enriched with *Pseudomonas putida* F1

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Abstract Trichloroethylene (TCE) is an environmental contaminant provoking genetic mutation and damages to liver and central nerve system even at low concentrations. A practical scheme is reported using toluene as a primary substrate to revitalize the biofilter column for an extended period of TCE degradation. The rate of trichloroethylene (TCE) degradation by *Pseudomonas putida* F1 at 25°C decreased exponentially with time, without toluene feeding to a biofilter column (11 cm I.D. × 95 cm height). The rate of decrease was 2.5 times faster at a TCE concentration of 970 μg/L compared to a TCE concentration of 110 μg /L. The TCE itself was not toxic to the cells, but the metabolic intermediates of the TCE degradation were apparently responsible for the decrease in the TCE degradation rate. A short-term (2 h) supply of toluene (2,200 μg/L) at an empty bed residence time (EBRT) of 6.4 min recovered the relative column activity by 43% when the TCE removal efficiency at the time of toluene feeding was 58%. The recovery of the TCE removal efficiency increased at higher incoming toluene concentrations and longer toluene supply durations according to the Monod type of kinetic expressions. A longer duration (1.4~2.4 times) of toluene supply increased the recovery of the TCE removal efficiency by 20% for the same toluene load.

Keywords: trichloroethylene, toluene feeding, intermediates, recovery, *Pseudomonas putida* F1, toluene load

INTRODUCTION

Trichloroethylene (TCE) is widely used as a cleaning solvent in semi-conductor industry or as a coolant in the freezer industry [1]. TCE is a harmful environmental contaminant provoking genetic mutation, a suppression of central nerve system, and liver damage even at low concentration [2]. However, this chlorinated compound is difficult to remove from the environment because it is not utilized as a substrate for the microbes, and generates toxic compounds during degradation. Environmentally toxic compounds can be degraded biologically [3,4]. The biological degradation of TCE is generally accomplished under aerobic conditions by cometabolic oxidation, using methane, toluene, or ammonia as primary substrates. In anaerobic conditions, TCE is degraded into dichloroethylene and vinyl chloride, which are more toxic than TCE [5,6]. Using methane, toluene, or ammonia as cometabolic substrates, microorganisms degrade TCE by metabolic oxidation using mono-oxygenases or dioxygenases [7-10].

Due to its strong toxicity, TCE cannot be degraded continuously in a bioreactor. Therefore, several methods have been applied in order to maintain or enhance the degradation efficiency of TCE. These methods include the use of a two-stage reactor, which separates microbial growth and TCE degradation [11]; a hollow-fiber membrane reactor, which allows indirect contact of the substrate and the cells through the membrane [8,12]; and a compost filler rich in primary substrate [13].

A recent study showed that pulsed or cycled feeding, using propane as the primary substrate, could effectively recover the activity of the TCE-degrading enzyme [14]. In order for this type of feeding technique to be successful, the competitive inhibition of the primary substrate against the TCE degradation must be reduced, and the toxic effect of the metabolic intermediates on the bacterial growth and enzyme activity must be minimized [4]. The intermediates cause cell damage, which reduces the TCE degradation rate [10]. Consequently, the activity of the damaged cells has to be recovered. Since the primary substrate has to be supplied to recover the TCE removal rate, the correlation between the feeding of the primary substrate and the recovery of the TCE removal rate has to

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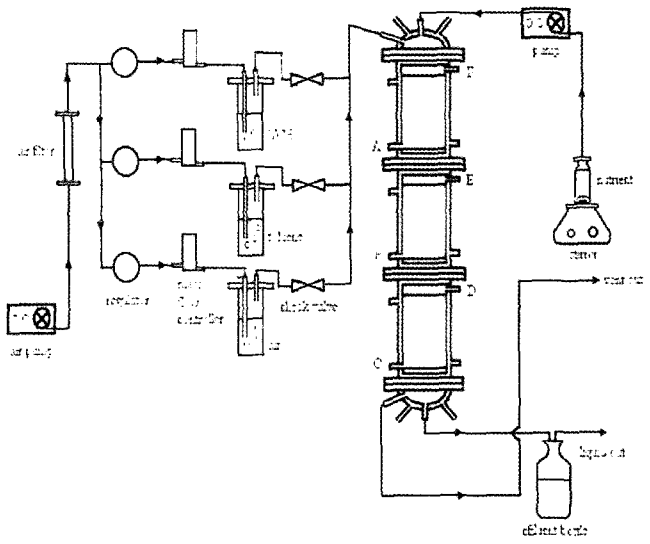

Fig. 1. Schematic diagram of a biofilter system for the removal of trichloroethylene (TCE). A, B, C, D, E, F, sampling ports.

be explored.

Previous studies were mainly focused on the recovery of cell activity in a lab-scale batch culture [10], and the application of a recovery term in the modeling of TCE recovery for TCE degradation [15-17]. On the other hand, this study examines the effect of the incoming TCE concentration on the TCE degradation rate by *Pseudomonas putida* F1 in a lab-scale biofilter. The effects of column deactivation, incoming toluene concentration, and toluene supply duration on the recovery of the TCE degradation rate in the biofilter were also investigated. The results suggest a possible way of maintaining the TCE removal capacity of a biofilter.

MATERIALS AND METHODS

Preparation of Inoculum and Biofilter Packing Material

Pseudomonas putida F1, obtained from David T. Gibson (University of Iowa), was routinely cultivated at 25°C in a 300-mL flask containing 50 mL of modified Hunters medium [18]. A phosphate buffer made of KH_2PO_4 (2.15 g/L) and K_2HPO_4 (5.3 g/L) was added to the medium, and the final pH was adjusted to 7.0. This modified Hunters medium plus phosphate buffer is called the medium or the fresh medium for the rest part of this report. Toluene was supplied in the gas phase from a glass bulb. The toluene vapor from the bulb was dissolved in the medium as a primary carbon source. The culture was incubated in a shaker (VS-5500N; Vision Co., Korea), and cells in the exponential phase (OD_{600} = 0.8~1.0) were inoculated into 500-mL flasks containing 100 mL of fresh medium and spherical ceramic particles (200 mL) with an average diameter of 1 cm and a specific surface area of 0.04 m^2/m^3 (SH VOC-01; Sam Whan Co., Ltd, Busan, Korea). After 3 days of cell growth in the shaker at 100 rpm, the particles in the flasks were placed into the column aseptically. Air containing 950 μg/L of toluene was supplied to the top of the column at 800 mL/min for 4 days.

Biofilter System Configuration and Operating Conditions

The biofilter consisted of a gas-mixture-generating system, a nutrient-supplying system, and a cylindrical, three-plate glass column (11 cm I.D. × 95 cm total height) (Fig. 1). In each plate, spherical ceramic particles were packed up to 18 cm in height. The whole system was set up in a room where the temperature was controlled at 25°C. The column temperature was maintained at 25°C by a water jacket. Mass flow controllers (5850E, Brooks, Hatfield, PA, USA) were used to control the concentrations of toluene and TCE, as well as the flow rate of the air-toluene-TCE mixture. The empty bed residence time (EBRT) was set at 6.4 min, while air containing 950 μg/L of toluene was supplied to the top of the column at 800 mL/min for approximately 3 weeks before the start of the experiments. The medium was supplied to the top of the column at 1 mL/min.

Analytical Methods

The concentrations of toluene and TCE in the gas sample were analyzed by using a gas chromatograph (Autosystem XL, Perkin-Elmer, Wellesley, MA, USA), which was equipped with a Flame Ionization Detector (FID) and a capillary column (DB-WAX; J&W Scientific, USA). The temperatures of the oven, the detector, and the injector were controlled at 100, 200, and 150°C, respectively. Gas samples (400 μL each) were taken in duplicate at the sampling ports using a 5-mL gas-tight syringe. The optical density (OD) of the culture was measured at 600 nm using a spectrophotometer (UV-1601, Shimadzu, Kyoto, Japan). To determine the amount of the attached biomass, two particles taken from each stage of the column were immersed for 1 h in a 20 mL solution of NaP_2O_7 (0.1%), and then sonicated for 3 sec three times at 30-sec intervals. The volatile suspended solid (VSS) in the obtained solution was determined as described in the literature [19].

RESULTS AND DISCUSSION

Decrease of the TCE Degradation Rate with Time

After the biofilter was stabilized for 3 weeks with toluene (950 μg/L) feeding, TCE (110 μg/L) was supplied to the biofilter without a toluene supply. The air flow rate was maintained at 800 mL/min throughout the experiment (EBRT 6.4 min). The specific TCE degradation rate decreased exponentially with time, and the dependence was as follows (Fig. 2).

$$v = v_0 \exp(-k_d \cdot t) \quad (1)$$

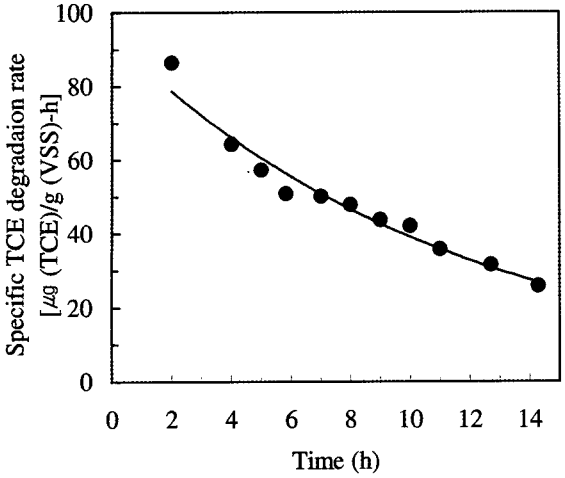

Fig. 2. Decrease in the specific TCE degradation rate with time after the toluene feeding was ceased. The TCE concentration was 110 μg/L, while the air flow rate was 800 mL/min (EBRT 6.4 min).

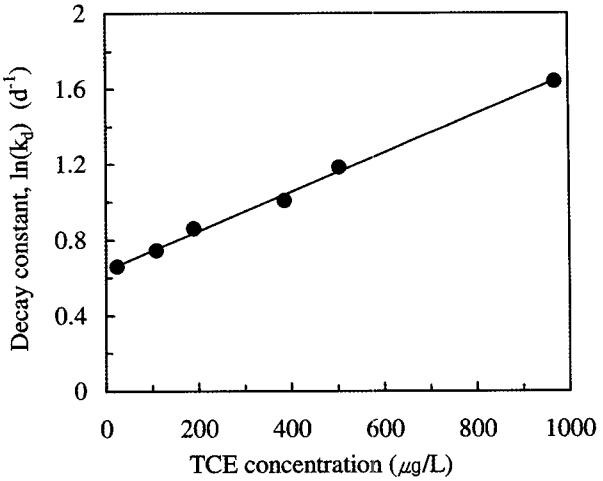

Fig. 3. Semi-log plot of decay constants of TCE degradation as a function of the incoming TCE concentration.

where v is specific TCE degradation rate after t hours of TCE supply [μg TCE/g VSS·h], v_0 is specific TCE degradation rate at the start of TCE supply [μg TCE/g VSS·h], and k_d is decay constant [h^{-1}].

The specific TCE degradation rate decreased to one half of its initial value after 7.9 h of exposure to TCE. The corresponding decay constant k_d was determined as 0.088 h^{-1}. The decay constant is inversely proportional to the time required to reach a 50% decrease in the degradation rate. The biomass in the column was 20.8 g VSS, and the average TCE load was 253.8 μg TCE/g VSS·h.

Effect of the TCE Concentration on the Decrease in the TCE Degradation Rate

The TCE degradation rate decreased more rapidly at higher incoming TCE concentrations as shown by an exponential increase in the decay constants at higher incoming TCE concentrations (Fig. 3). The dependence of the decay constants on the incoming TCE concentration was determined as follows.

$$\mathrm{Ln}(k_d) = 0.0010 \cdot C_{TCE} + 0.64$$

where k_d is the decay constant (d^{-1}), and C_{TCE} is the incoming TCE concentration (μg/L). The regression coefficient (R^2) was computed at 0.99. This experiment was performed at incoming TCE concentrations of 24, 110, 190, 390, 500, and 970 μg/L, and at a fixed air flow rate of 800 mL/min. Before switching from one TCE concentration to another, the column was fully activated for at least 48 h by toluene (950 μg/L) feeding at 800 mL/min. The decay constant at 970 μg/L was 5.2 (d^{-1}). This was 2.5 times larger than the decay constant at 110 μg/L.

The cause of the decrease in the TCE degradation rate was explored. The toxicity of TCE itself was determined bolic intermediates of TCE degradation, using succinate as a primary substrate instead of toluene. The cells of *P. putida* F1 were cultured on succinate (10 mM) with modified-Hunters medium for 15 h in a 40-mL sealed tube with 5 mL of culture volume at TCE concentrations of 0~5 × 10^4 μg/L. The final cell mass was measured at 160 ± 11 mg/L regardless of the TCE concentration (data not shown). Since the cells could not generate toluene dioxygenase on succinate, the metabolic intermediates of the TCE degradation were not produced. This result indicates that TCE was not toxic to the cells at the concentrations (less than 970 μg/L) applied to the experiments. Therefore, the decreased TCE degradation rate shown in Fig. 2 and Fig. 3 was attributable to the toxicity of the metabolic intermediates of the TCE degradation.

Toluene dioxygenase simultaneously oxidizes TCE when it oxidizes toluene to cis-toluene dihydrodiol. The metabolic intermediates of the TCE degradation, however, have not been well identified, except for the final products (formic acid, glyoxylic acid, and HCl) that are made from formyl chloride and glyoxylic chloride [10]. Previous studies also indicate that the toxic intermediates produced by toluene dioxygenase in *P. putida* F1 may be responsible for the decrease in the TCE degradation rate [21]. Moreover, growth inhibition was not observed in the presence of TCE for a *P. putida* F1 mutant, which was incapable of producing toluene dioxygenase [22].

Relative Recovery of the TCE Removal Efficiency through Short-term (2 h) Toluene Feeding

When TCE (110 μg/L) was supplied to the biofilter without a toluene supply, the TCE removal efficiency decreased with time similarly to Fig. 2. The removal efficiency was defined as follows

$$\text{Removal efficiency} = \frac{C_{in} - C_{out}}{C_{in}}$$

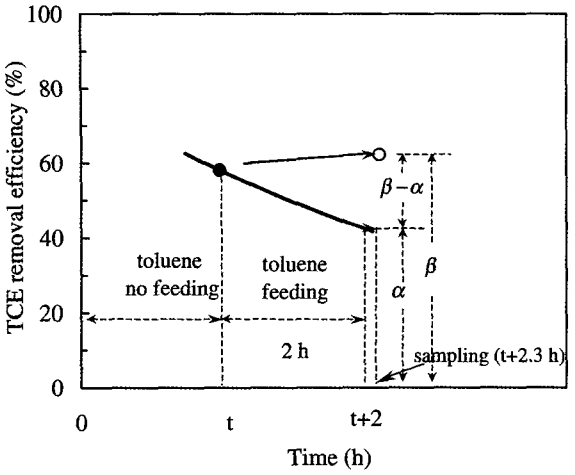

Fig. 4. Diagram showing the relative recovery of the TCE re moval efficiency (%). α, TCE removal efficiency estimated based on its dependence on the time exposed to TCE (refer to Fig. 2); β, TCE removal efficiency after 2 h of toluene feeding; $\frac{\beta-\alpha}{\alpha}\times 100(\%)$. Relative recovery of TCE removal efficiency (%); ● and ○, TCE removal efficiency before and after toluene feeding, respectively.

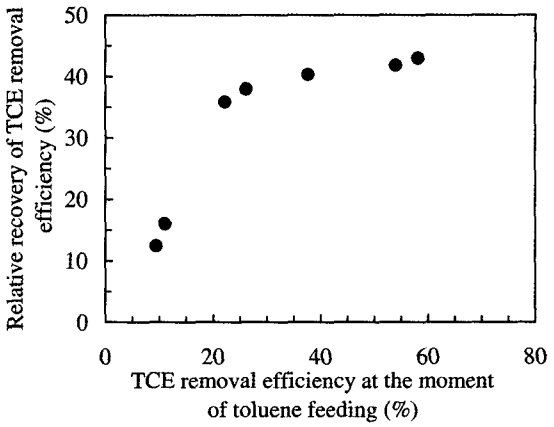

Fig. 5. Dependence of the relative recovery of the TCE removal efficiency upon the TCE removal efficiency at the moment of toluene feeding. The toluene supply duration was set at 2 h, the TCE concentration was fixed at 110 μg/L, and the air flow rate was controlled at 800 mL/min (EBRT 6.4 min).

where C_{in} refers to the incoming TCE concentration and C_{out} represents the outlet TCE concentration.

The toluene supply (2,200 μg/L) to the biofilter column for 2 h raised the TCE removal efficiency. The extent of recovery was dependent on the removal efficiency (column activity) at the moment of toluene feeding. The TCE removal efficiency was determined 20 min after the toluene supply was ceased. The air flow rate and TCE concentration in the feed were maintained at 800 mL/min (EBRT 6.4 min) and 110 μg/L, respectively. After one experiment was finished at a value of removal efficiency, the column activity was fully restored for at least 72 h by toluene (950 μg/L) feeding. This was done before the next experiment was carried out at another value of removal efficiency. Fig. 4 shows the details of the calculation of the relative recovery of the TCE removal efficiency defined as follows

$$\text{Relative recovery of TCE removal efficiency (\%)} = \frac{\beta-\alpha}{\alpha}\times 100$$

where α is the TCE removal efficiency estimated according to its dependence on the time exposed to TCE, and β is the TCE removal efficiency 2 h after the toluene supply.

The current experiment revealed that the relative recovery of the TCE removal efficiency (%) was dependent upon the TCE removal efficiency at the moment of toluene feeding. In particular, two distinct zones were observed, as shown in Fig. 5. When the TCE removal efficiency was less than 25% at the time of toluene feeding, the relative recovery of the TCE removal efficiency decreased rapidly from 38%. On the other hand, when the TCE removal efficiency at the time of toluene feeding was observed from 25 to 58%, the relative recovery of the TCE removal efficiency increased slightly from 38 to 43%.

The recovery of the TCE removal efficiency dropped suddenly when the TCE removal efficiency declined to less than 25% at the time of toluene feeding. This suggests that the toluene supply needs to be initiated before the removal efficiency drops to the 25~35% level, in order to recover the TCE removal efficiency in a biofilter. The relative recovery of the TCE removal efficiency, however, was limited to 45% after a 2 h duration of the toluene supply, probably due to the insufficient reactivation of toluene dioxygenase in *P. putida* F1. A longer period or larger amount of the toluene supply may have increased the recovery percentage.

In the case of *Nitrosomonas europaea* the time required to recover the ammonia-oxidizing activity in the TCE treated cells doubled from 2 to 4 h, when the deactivation of the ammonia-oxidizing activity increased from 80 to 90% [10]. In the case of *P. putida* F1, a higher deactivation due to a longer exposure to TCE decreased the cell growth rate [22].

Effect of Incoming Toluene Concentration and Toluene Supply Duration on the Relative Recovery of the TCE Removal Efficiency

The relative recovery of the TCE removal efficiency was improved when the incoming toluene concentration was increased. The incoming toluene concentrations were set at 48, 156, 361, 480, 875, and 1,221 μg/L, and more than 99% of incoming toluene was removed in the biofilter (Fig. 6). The toluene supply duration was fixed at 1 h. The relationship between the relative recovery of the TCE removal efficiency and the toluene concentration was modeled after the Monod type of kinetic expression, as follows

$$R = \frac{18.3\cdot C}{190+C}$$

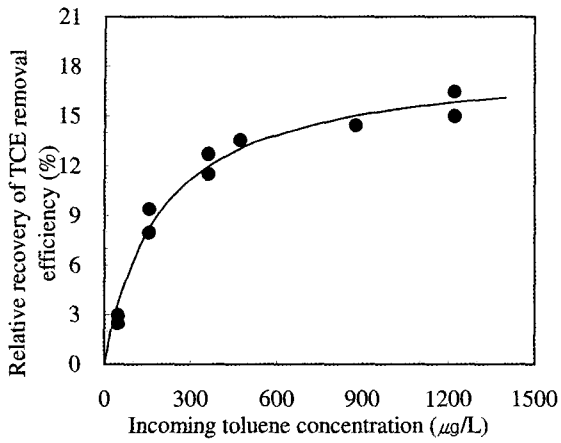

Fig. 6. Effect of the incoming toluene concentration on the relative recovery of the TCE removal efficiency. The toluene supply duration was 1 h, and the air flow rate was 800 mL/min (EBRT 6.4 min).

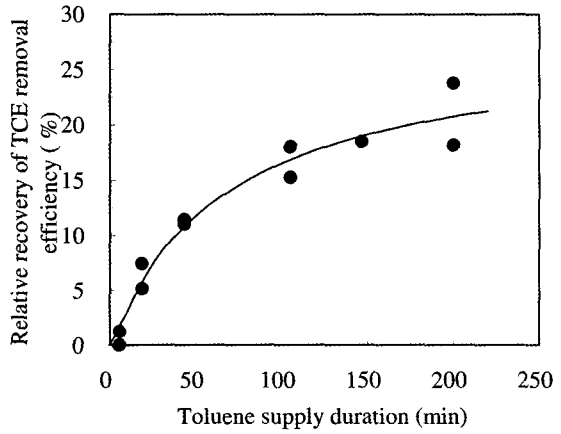

Fig. 7. Effect of the toluene supply duration on the relative recovery of the TCE removal efficiency. The incoming toluene concentration was 500 μg/L, while the air flow rate was 800 mL/min (EBRT 6.4 min).

where R is the relative recovery of the TCE removal efficiency (%), and C is the incoming toluene concentration (μg/L). The regression coefficient (R^2) was computed as 0.97. When an experiment was finished at one toluene concentration, the column activity was fully restored by toluene (950 μg/L) feeding for at least 48 h, and then, the activity was adjusted to 30% of removal efficiency by a TCE (110 μg/L) supply. The TCE removal efficiency was determined 20 min after the toluene supply was ceased, and the air flow rate was fixed at 800 mL/min (EBRT 6.4 min) throughout the experiment.

A longer duration of the toluene supply was better for the recovery of the TCE removal efficiency. For this experiment, the toluene supply duration was extended to 200 min, and the incoming toluene concentration was fixed at 500 μg/L (Fig. 7). The relationship between the

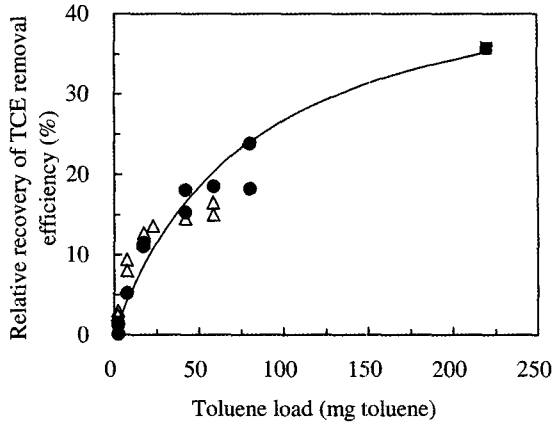

Fig. 8. Effect of the toluene load on the relative recovery of the TCE removal efficiency. The toluene load was defined as incoming toluene concentration × toluene supply duration × air flow rate. The air flow rate was 800 mL/min. The TCE removal efficiency at the time of toluene feeding was 30%. Symbols: △, the incoming toluene concentration was changed at a fixed toluene supply duration (1 h); ●, the toluene supply duration was changed at a fixed incoming toluene concentration (500 μg/L); ■, the incoming toluene concentration was 2,200 μg/L and the toluene supply duration was 2 h.

recovery of the TCE removal efficiency and the supply duration was modeled after the Monod type of kinetic expression, as follows

$$R = \frac{27.9 \cdot t}{68.7 + t}$$

where R is the relative recovery of the TCE removal efficiency (%), and t is the duration (min) of the toluene supply. The regression coefficient (R^2) was computed at 0.95. The air flow rate and other procedures, such as column activity restoration, activity adjustment to 30%, and the moment of efficiency determination, were the same as those applied to the previous experiment on the effect of the incoming toluene concentration.

The combined effects of the incoming toluene concentration and the toluene supply duration were tested using a toluene load, which was defined as the incoming toluene concentration × toluene supply duration × air flow rate. The relative recovery of the TCE removal efficiency was dependent on the toluene load as shown in Fig. 8. For the same toluene load, a longer toluene supply yielded 20% more relative recovery of the TCE removal efficiency compared to a higher toluene concentration.

CONCLUSION

Metabolic intermediates of TCE degradation were apparently responsible for the exponential decrease in TCE degradation rate by *Pseudomonas putida* F1 in a biofilter column, and the decrease was faster at higher TCE concentrations. The column activity could be partially recov-

ered by a short-term supply of a small amount of toluene as a primary substrate. At the same toluene load, duration of toluene supply played a more important role for the activity recovery compared with incoming toluene concentration. The results suggest a practical scheme of revitalizing the biofilter column for TCE degradation so that we can extend the period of column operation.

Acknowledgement This work was supported by the Korea Research Foundation (KRF-2003-D00014).

REFERENCES

[1] Oldenhuis, R., J. Y. Odedzes, J. J. Waarde, and D. B. Janssen (1991) Kinetics of chlorinated hydrocarbon degradation by *Methylosinus trichlorosporium* OB3b and toxicity of trichloroethylene. *Appl. Environ. Microbiol.* 57: 7-14.

[2] Cox, C. D., H. J. Woo, and K. G. Robinson (1998) Cometabolic biodegradation of trichloroethylene (TCE) in the gas phase. *Water Sci. Technol.* 37: 97-104.

[3] Nakamura, Y., M. Daidai, and F. Kobayashi (2004) Bioremediation of phenolic compounds having endocrine-disrupting activity using ozone oxidation and activated sludge treatment. *Biotechnol. Bioprocess Eng.* 9: 151-155.

[4] Kim, J.-H., W.-H. Jeong, T. B. Karegoudar, and C.-K. Kim (2002) Stable degradation of benzoate by *Klebsiella oxytoca* C302 immobilized in alginate and polyurethane. *Biotechnol. Bioprocess Eng.* 7: 347-351.

[5] Kleopfer, R. D., D. M. Easley, B. B. Hass, Jr. T. G. Deihl, D. E. Jackson, and F. P. Guengerich (1985) Anaerobic degradation of trichloroethylene in soil. *Environ. Sci. Technol.* 19: 277-280.

[6] Vogel, T. M. and P. McCarty (1986) Biotransformation of tetrachloroethylene to trichloroethylene, dichloroethylene, vinyl chloride, and carbon dioxide under methanogenic conditions. *Appl. Environ. Microbiol.* 49: 1080-1083.

[7] Speital, G. E. Jr. and D. S. McLay (1993) Biofilm reactors for treatment of gas stream containing chlorinated solvents. *J. Environ. Eng.-ASCE.* 119: 658-678.

[8] Dolasa, A. R. and S. J. Ergas (1999) Membrane bioreactor for cometabolism of trichloroethene air emissions. *J. Environ. Eng.-ASCE.* 126: 969-973.

[9] Arciero, D., T. Vannelli, M. Logan, and A. B. Hooper (1989) Degradation of trichloroethylene by the ammonia-oxidizing bacterium *Nitrosomonas europaea*. *Biochem. Bioph. Res. Co.* 159: 640-643.

[10] Hyman, M. R., S. A. Russell, R. L. Ely, K. J. Williamson, and D. J. Arp (1995) Inhibition, inactivation, and recovery of ammonia-oxidizing activity in cometabolism of trichloroethylene by *Nitrosomonas europaea*. *Appl. Environ. Microbiol.* 61: 1480-1487.

[11] Chang, H. L. and L. Alvarez-Cohen (1997) Two stage methanotrophic bioreactor for the treatment of chlorinated organic wastewater. *Water Res.* 31: 2026-2036.

[12] Pressman, J. G., G. Georgiou, and G. E. Speital (1999) Demonstration of efficient trichloroethylene biodegradation in a hollow fiber membrane bioreactor. *Biotechnol. Bioeng.* 62: 681-692.

[13] Sukesan, S. and M. E. Watwood (1997) Continuous vapor-phase trichloroethylene biofiltration using hydrocarbon-enriched compost as filtration matrix. *Appl. Microbiol. Biotechnol.* 48: 671-676.

[14] Lackey, L. W., J. R. Gamble, and J. L. Boles (2002) Bench-scale evaluation of a biofiltration system used to mitigate trichloroethylene contaminated air streams. *Adv. in Environ. Res.* 7: 97-104.

[15] Ely, R. L., K. J. Williamson, R. B. Guenther, M. R. Hyman, and D. J. Arp (1995) A cometabolic kinetics model incorporation enzyme inhibition, inactivation, and recovery: I. Model development, analysis, and testing. *Biotechnol. Bioeng.* 46: 218-231.

[16] Ely, R. L., M. R. Hyman, D. J. Arp, R. B. Guenther, and K. J. Williamson (1995) A cometabolic kinetics model incorporation enzyme inhibition, inactivation, and recovery: II. Trichloroethylene degradation experiments. *Biotechnol. Bioeng.* 46: 232-245.

[17] Zhang, X. H. and R. K. Bajpai (2000) A comprehensive model for the cometabolism of chlorinated solvents. *J. Environ. Sci. Health.* A35: 229-244.

[18] Stainer, R. Y., N. J. Palleroni, and M. Doudoroff (1966) The aerobic *Pseoudomonas*: a taxonomic study. *J. Gen. Appl. Microbiol.* 43: 159-171.

[19] Lenore, S. C., E. G. Arnold, and D. E. Andrew (1998) *Standard Methods for the Examination of Water and Wastewater*. 18th ed., American Public Health Association Press, Washington DC, USA.

[20] Li, S. and L. P. Wackett (1992). Trichloroethylene oxidation by toluene dioxygenase. *Biochem. Bioph. Res. Co.* 185: 443-451.

[21] Wackett, L. P. and D. Gibson (1988) Degradation of trichloroethylene by toluene dioxygenase in whole cell studies with *Pseudomonas putida* F1. *Appl. Environ. Microbiol.* 54; 1703-1708.

[22] Wackett, L. P. and S. R. Householder (1989) Toxicity of trichloroethylene to *Pseudomonas putida* F1 is mediated by toluene dioxygenase. *Appl. Environ. Microbiol.* 55: 2723-2725.

[Received September 14, 2004; accepted January 8, 2005]