### COMETABOLIC BIODEGRADATION OF GASEOUS CHLORINATED COMPOUNDS IN A BIOFILTER

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#### Introduction

Remediation technologies, which involve gas transport, such as soil vapor extraction and air groundwater emerge chlorinated solvent-contaminated of gases. Specially, sparing trichloroethylene (TCE) is one of common chlorinated pollutants in contaminated groundwater. TCE mostly affects the nervous system of human. Exposure to very high concentration level of TCE for short times has caused unconsciousness and death. Many efforts have been made to treat the gas phase TCE using a biofiltration process, but it has not been so successful. In the case of TCE, aerobic degradation occurs almost exclusively by co-metabolic processes.<sup>1</sup> To achieve a high degradation efficiency of TCE, competitive inhibition and the toxicity effect of TCE to cell and enzyme should be minimized.<sup>1</sup> The goal of this research is to maximize TCE elimination capacity and efficiency by adopting appropriate feeding methods of toluene to a biofilter inoculated with *Pseudomonas putida*  $F_1$  that is widely known as one of TCE co-metabolic strains and utilizes toluene as a carbon and energy source. P. putida F<sub>1</sub> produces dioxygenase enzyme that oxidizes toluene and co-metabolizes TCE.<sup>2</sup>

#### **Material and Methods**

*P putida* was routinely cultivated in a modified Hunter Medium<sup>3</sup>. Toluene was supplied in the vapor phase into a glass bulb installed above the medium. Figure 1 shows the schematic diagram of a biofilter. The biofilter consists of three stage units made with glass and each unit is 30 cm in height and 11 cm in inner diameter. Each unit was packed with spherical diatomite-ceramic media that provided the physical habitat for the biofilm. Aqueous media flowed co-currently with flow rate of 1 ml/min from top to bottom to supplement with nutrients and moisture to the biofilm. The three flasks were filled with 99% TCE and toluene in liquid phase for generating contaminated sample gases. The influent concentrations of TCE and toluene were controlled by the flow rate of the gas stream regulated by mass flow controllers. TCE and toluene concentrations were determined by GAS Chromatography (Perkins-Elmer Autosystem XL, USA) equipped with a flame-ionization detector (FID).



Figure 1 Schematic diagram of a biofilter system.

#### **Results and discussion**

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TCE elimination capacity for absence of toluene feeding

Toluene was fed to the biofilter in conditions of 300 ppmv and 500 ml/min during 48 hrs to activate dioxygenase enzyme in the biofilter. After toluene feeding was ceased, only TCE was introduced at conditions of 500  $\mu$ g/l and 500 ml/min. Effluent gas samples were collected through each sampling port in the biofilter. Figure 2 shows the variation of TCE elimination efficiency with lapsed time after ceasing toluene feeding. The TCE degradation efficiency decreased exponentially along the course of each experiment, as would be expected, due to dioxygenase-enzyme inactivating byproducts.<sup>1</sup>

To understand the biokinetics of TCE co-metabolic oxidation in the biofilter, several runs of experiment were conducted under various influent TCE concentrations. The operating period for each run was 480 mins. Generally, a relationship between the TCE elimination capacity and the influent concentration of TCE is explained by using equation (1).<sup>4</sup> Figure 3 shows that without toluene feeding, TCE elimination capacity increased as a root function of TCE initial concentration. The maximum specific elimination capacity of TCE ( $k_{TCE}$ ) and the half saturation constant ( $K_{TCE}$ ) were found to be 2.91 µg(TCE)/g(VSS)min and 128.9 µg/l, respectively, by a non-linear regression method.

$$r_{TCE} = k_{TCE} \frac{C_o}{(K_{TCE} + C_o)}$$
(1)

Where 
$$C_o = Influent TCE$$
 concentration [µg/l]  
 $r_{TCE} = Specific TCE$  elimination capacity [µg(TCE)/g(VSS) min]  
 $k_{TCE} = Maximum$  specific TCE elimination capacity [µg(TCE)/g(VSS) min]  
 $K_{TCE} = Half$  saturation constant for TCE [µg(TCE)/l]

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#### TCE elimination capacity for toluene feeding

Figure 4 shows that normalized elimination capacity decreases exponentially with influent toluene concentrations under conditions of flow rate 500 ml/min and influent TCE concentration 500  $\mu$ g/l. These results may be due to competitive inhibition between both substrates, but the competitive inhibition appears to almost cease above about 10,000  $\mu$ g/l of toluene. Similar results about competitive inhibition of toluene occur at conditions of influent TCE concentration 500 and 40  $\mu$ g/l.



Figure 4 Normalized TCE elimination capacity for various concentration of toluene as a primary substrate

Enhancement of TCE elimination capacity by two different methods of feeding primary substrate.

Previous results revealed that the single feeding of toluene caused only  $20 \sim 50$  % of TCE elimination efficiency due to competitive inhibition of toluene. Therefore, the different methods for toluene feeding such as step feeding and intermittent feeding were applied to enhance TCE elimination capacity and efficiency. In the case of the step feeding, while TCE influent concentration increased gradually at fixed conditions of flow rate 500 ml/min and EBCT 1.72 min, toluene was introduced into each stage units at the loading rate of 112 µg/m<sup>3</sup> min to activate

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dioxygenase enzyme. Figure 5 shows the comparisons of TCE elimination capacity and efficiency for the step feeding and the single feeding of toluene. TCE elimination efficiency in the step feeding occurred  $10 \sim 30$  % better than in the single feeding.

Toluene was introduced intermittently to avoid or reduce the competitive inhibition and to enhance TCE elimination efficiency. Figure 6 shows the variation of TCE elimination efficiency with cyclic feedings of toluene. Under conditions of the flow rate 500 ml/min and TCE concentration 160  $\mu$ g/l, toluene with average concentration of 500  $\mu$ g/l was introduced for 1 hr to activate dioxygenase enzyme every 12 hr interval that regarded as one cycle. When toluene feeding ceased, TCE elimination efficiency increased rapidly, implying that dioxygenase enzyme was additionally produced by addition of toluene.



Figure 5 TCE elimination efficiency and capacity with its initial concentration for step and single feeding of toluene

Figure 6 TCE elimination efficiency during cyclic single feedings of toluene

#### Conclusion

A cometabolic oxidation process was used in a biofilter to eliminate gaseous TCE present in waste gases. The TCE elimination efficiency was limited by competitive inhibition and dioxygenase enzyme activity. In this research, to enhance the TCE elimination capacity and efficiency by avoiding competitive inhibition and activating enzyme activity, two different methods of feeding toluene were investigated in a biofilter inoculated with *Pseudomonas putida*  $F_1$ . Experimental data demonstrate that TCE elimination capacity and efficiency for the step feeding of toluene are enhanced compared to those for the single feeding. Also, intermittent feeding of toluene appeared to be a good way to enhance TCE elimination efficiency.

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