

# *Biodegradation of BTXS and substrate interactions in a Bioactive Foam Reactor*

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## **ABSTRACT**

A bioactive foam reactor (BFR), using surfactant-driven bubbles and suspended microorganisms, has emerged as a potential alternative to packed-bed biofiltration systems for the treatment of volatile organic compounds (VOCs). The study presented herein was designed to investigate the effects of VOC mixtures (benzene, toluene, p-xylene, and styrene) on biodegradation efficiencies and substrate interactions in the BFR. Benzene, toluene p-xylene, and styrene were applied individually to the toluene-acclimated BFR at the same inlet concentration ( $0.78 \text{ g/m}^3$ ), and then paired BTXS mixtures (BT, BX, BS, TX, TS, XS, and BTXS in the same ratio by volume) were applied but the total inlet concentration were maintained constant. The overall removal rates of each of the four VOCs were in the following order: toluene, styrene, benzene, and p-xylene in the inlet concentration range tested. However, styrene biodegradation was the highest in the presence of other VOC compounds. The removal efficiency for toluene as a single substrate was 82%, but toluene removal efficiencies dropped when the paired mixtures were applied. The removal efficiency for benzene also decreased in the presence of other TXS compounds. In contrast, the removal efficiency for p-xylene as a single substrate was only 21% in the BFR, but p-xylene removal efficiencies ranged 35 – 41% in the presence of other BTS compounds. As a result, the biodegradation of benzene and toluene was inhibited by the other carbon sources, whereas the biodegradation of styrene and p-xylene was enhanced by the others. Consequently, a careful attention needs to be given when BFR performance and biodegradation rates of mixed VOCs are utilized for system design and operational purposes.

## 1 INTRODUCTION

Packed-bed bioreactors including biofilters and biotrickling filters have drawn increased interests for the treatment of volatile organic compounds (VOCs). Many studies have shown that these bioreactors can successfully treat a wide range of VOCs including benzene, toluene and xylenes (van Groenestijn and Hesselink, 1993; Devinny *et al.*, 1999). However, operational problems such as excess biomass accumulation and biodegradation activity loss make these treatment methods less attractive (Song and Kinney, 2000), especially when subjected to high concentrations of VOC mixtures.

In order to overcome those problems, several new approaches have been made using suspended microorganisms instead of the fixed biofilm (Kennes and Veiga, 2001). One of them is a bioactive foam reactor (BFR) that is operated with a surfactant bubble solution containing pollutant-degrading microorganisms (Phipps, 1998). In the BFR, a VOC-laden air stream is sparged into the surfactant solution and forms biologically active foams. The fine foams can provide a large surface area for the mass transfer of VOCs as well as enhance microbial activity without a significant accumulation of biomass over time. Kan and Deshusses (2003, 2005) demonstrated that a foamed bioreactor using an organic-phase emulsion and active microorganisms could achieve a high elimination capacity for toluene. Recently we have modified the defoamer of the BFR system from its patented prototype to make it simple, and an organic emulsion such as oleyl alcohol was not used in the liquid phase.

Since BFR performance mainly relies on the mass transfer of VOCs and the subsequent microbial degradation, substrate interactions between the VOCs present in inlet streams are another important factor that must be considered to achieve successful BFR operation when it is subjected to different chemical mixtures. Emissions from various industrial sources often consist of a mixture of compounds with different chemical characteristics and biodegradability. Therefore, several bioreactor studies have examined the biodegradation of binary mixtures of VOCs, such as BTEX (Collins and Daugulis, 1999; Strauss *et al.*, 2004), and methanol and  $\alpha$ -pinene (Mohseni and Allen, 2000). These studies have consistently indicated that the presence of a readily degradable compound can inhibit the removal of the other recalcitrant compound. However, few attempts have been made to investigate the effects of substrate inhibition between complex VOC mixtures in bioreactors using suspended microbial cultures such as the BFR. The study presented herein was therefore designed to investigate the effects of a VOC mixture (benzene, toluene, p-xylene, and styrene) on biodegradation efficiencies and substrate interactions in the BFR.

## 2 MATERIALS AND METHODS

### 2.1 MICROBIAL CULTURE AND SURFACTANT

In order to obtain the toluene-degrading culture used in this study, a mixed microbial culture was initially collected from a wastewater treatment plant in Seoul, Korea. *Pseudomonas putida* TDB4 was isolated from the mixed culture and cultivated in our laboratory using gaseous toluene as a sole carbon and energy source. The mineral medium used for cultivating the mixed culture and *P. putida* TDB4 was slightly modified from the composition described by Song and Kinney (2000), which contained 1.36 g/L  $\text{KH}_2\text{PO}_4$ , 1.42 g/L  $\text{Na}_2\text{HPO}_4$ , 3.03 g/L  $\text{KNO}_3$  and trace metals per liter of distilled water.

The surfactant used in the BFR was TritonX-100 (Sigma-Aldrich, USA), which was selected based on a bottle test that showed no adverse effects on toluene degradation of *P. putida* TDB4. The surfactant concentration in the liquid phase was maintained at 0.013% (v/v) that was slightly lower than its critical micelle concentration (0.014%).

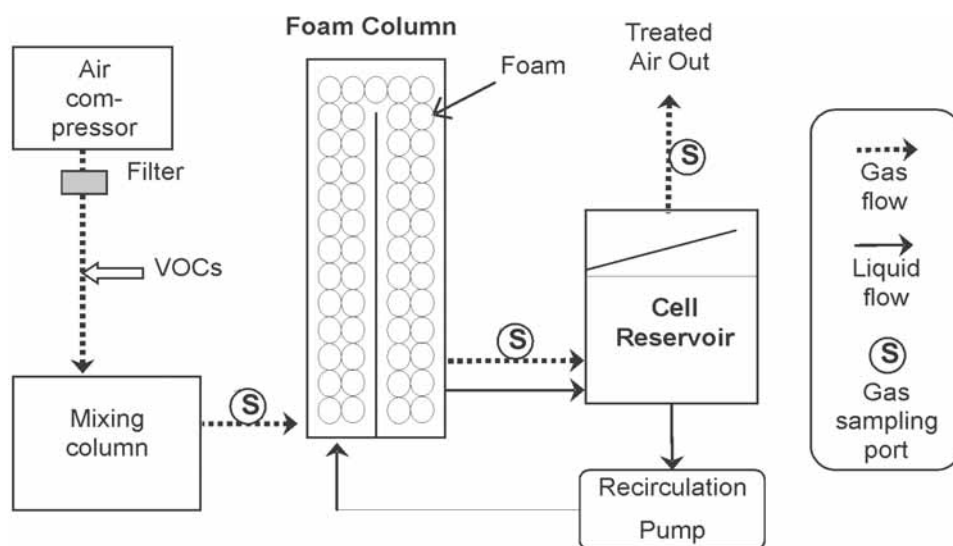


Figure 1. Schematic of the bioactive foam reactor (BFR) tested in this study.

### 2.2 BFR CONFIGURATION

As shown in Figure 1, the lab-scale bioreactor used in this study consisted of a foam column (volume 1.8 L), where the mass transfer of VOCs between the gas and foams took place, and a cell reservoir (volume 2.5 L), where biologically active microorganisms degraded the VOCs in the liquid phase. An air stream, generated by a

compressor and controlled by a flow meter, was contaminated with VOC vapour by the slow injection of research-grade, pure compounds using a syringe pump (KD Scientific, USA). The VOC-contaminated air stream was introduced to the bottom of the foam column through an aeration stone. As the air stream was sparged into the nutrient solution containing the surfactant and the microbial culture, fine foams were generated and moved along with the air stream in the foam column. The foam itself broke down at the top of the cell reservoir, returned to the liquid phase, and then the air trapped in the moving foam was released and exited the reactor. The liquid phase was continuously recirculated from the cell reservoir to the foam column in a closed-loop, and the total liquid volume in the entire system was 1.8 L.

### 2.3 BFR OPERATION AND SUBSTRATE INTERACTIONS

Two sets of BFR experiments were conducted to determine the effect of various combinations and concentrations of the VOCs (BTXS) on removal efficiencies at a constant gas retention time of 40 seconds. Prior to each BFR start-up, a microbial solution consisted of 1 L of pre-grown *P. putida* TDB4 was mixed with 1 L of the nutrient medium containing the surfactant. And then, the BFR was acclimated to toluene biodegradation at an inlet toluene concentration of 0.38 g/m<sup>3</sup> (i.e., 100 ppm<sub>v</sub>) until the steady state condition was obtained.

First, in order to determine short-term responses of the bioreactor system at the inlet concentration of 200 ppm<sub>v</sub>, benzene, toluene, p-xylene, and styrene were applied individually to the toluene-acclimated BFR for five hours. And the short-term experiments were repeated for various VOC mixtures (BT, BX, BS, TX, TS, XS, and BTXS) at the same total concentration of 200 ppm<sub>v</sub>. Between each short-term experiment, the acclimation condition was restored to maintain the BFR at steady state.

The second set of BFR experiments was conducted to observe the performance of the BFR operated continuously using BTXS that were mixed in a 1:1:1:1 ratio by volume. Throughout the 13-day operational period, the inlet VOC concentrations were changed twice. Initially the VOC mixture at the total concentration of 100 ppm<sub>v</sub> was introduced to the toluene-acclimated BFR (referred to as «Phase M1»). On day 6, the inlet concentrations of the VOC mixture were increased stepwise to 200 ppm<sub>v</sub> («Phase M2»).

As a measure of substrate interaction between the compounds in the mixtures, a substrate interaction index (SII) was defined as a ratio of the changes in removal efficiency due to the other coexisting carbon source to the removal efficiency determined when a single carbon source was supplied. The SIIs were calculated for each compound in the presence of other compounds at the different concentrations tested in this study.

$$\text{Substrate Interaction Index (SII)} = (\text{VOC}_{\text{mix}} - \text{VOC}_{\text{single}}) / \text{VOC}_{\text{single}} \quad (1)$$

Where  $\text{VOC}_{\text{mix}}$  is the removal efficiency of the target compound in mixture, and  $\text{VOC}_{\text{single}}$  is the removal efficiency of the compound as a single substrate at the given concentration.

## 2.4 ANALYTICAL METHODS

To determine VOC removals, gas samples were collected from three ports located at the inlet, at the middle of the connecting line between the foam and the microbial columns, and at the outlet of the BFR. The samples were collected with 0.5-mL gas-tight syringes and immediately analyzed using a gas chromatograph (HP 6890, Agilent, USA) equipped with a flame ionization detector.

## 3 RESULTS AND DISCUSSION

### 3.1 SUBSTRATE INTERACTIONS IN THE SHORT-TERM RESPONSES

The short-term changes in VOC removal efficiencies were determined when various combinations of different VOCs were applied to the toluene-acclimated BFR. The biodegradation of toluene in the absence of other compounds (BXS) by the toluene-degrading pure culture (*P. putida* TDB4) showed a high and stable removal efficiency. The removal efficiencies dropped when BXS compounds were applied individually to the BFR during the short-term period.

The overall removal efficiencies of the four VOCs were in the following order: toluene, styrene, benzene, and p-xylene. Styrene is the most soluble compound among the VOCs tested, but its removal efficiency was lower than that of toluene in the toluene-acclimated microbial system. In addition, the biodegradation efficiency of p-xylene was the lowest indicating that p-xylene was the most recalcitrant compound, and this finding was similar to other results reported in the biofiltration literature (Collins and Daugulis, 1999; Deshusses and Johnson, 2000; Strauss *et al.*, 2004).

The removal efficiencies of a target compound in the presence of the other compounds were monitored in the toluene-acclimated BFR. Figure 2 illustrated the SIIs calculated using the experimental data obtained in the short-term BFR operation. For instance, the removal efficiency of toluene as a single substrate was 82% at the inlet concentration of 200 ppm<sub>v</sub>, and it dropped to 55% when both toluene (100 ppm<sub>v</sub>) and styrene (100 ppm<sub>v</sub>) was applied. Therefore, the SII for toluene in the presence of styrene («Ts» in Figure 2) was -0.32, indicating that the toluene biodegradation was inhibited by the styrene addition. In comparison, the styrene removal efficiency was found to be 77% when styrene was individually supplied to the BFR, but it increased to 91% when toluene and styrene were applied together in the paired mixture, yielding

the SII of 0.18 for styrene in the presence of toluene («St» in Figure 2). These findings indicate that the styrene biodegradation was enhanced by the presence of toluene.

Overall, as shown in Figure 2, the SIIs for both benzene and toluene were negative values, implying that the biodegradation of these compounds was always inhibited by the presence of the other compounds. Strauss *et al.* (2004) reported the results obtained from a packed-bed biofilter system that the toluene biodegradation was inhibited by the other compounds (benzene, p-xylene and ethylbenzene), and toluene had an enhancing effect on the removal efficiency of the other compounds when paired. However, in this study, the presence of toluene and benzene in the paired mixtures resulted in the mutual deterioration of the removal efficiencies of both compounds.

The biodegradation of both p-xylene and styrene in the mixtures was enhanced by the presence of other compounds. The improved removal efficiencies of p-xylene and styrene occurred at the expense of toluene removal efficiency, presumably due to the similarities of the enzymatic systems used in the metabolic pathways for the aromatic compounds. Therefore, p-xylene, the most recalcitrant compound, had a greatest enhancing effect by the presence of other structurally-related aromatics. In addition, the presence of p-xylene and styrene in the paired mixture resulted in the mutual enhancement of the removal efficiencies of both compounds.

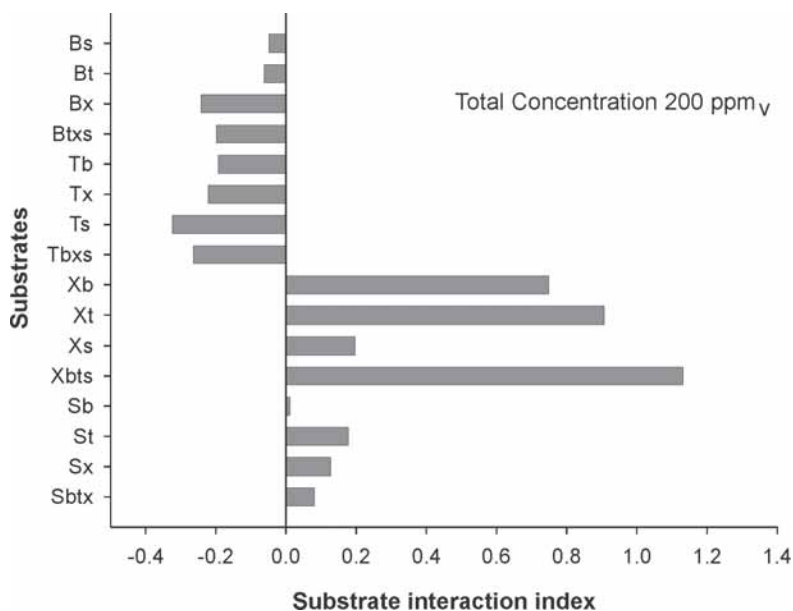


Figure 2. Substrate interaction indices for target compounds in the presence of the other compounds in paired mixtures.

### 3.2 SUBSTRATE INTERACTIONS IN THE CONTINUOUS BFR OPERATION

The response of the BFR operated continuously using BTXS in the paired mixture of a 1:1:1:1 ratio by volume was monitored throughout the 13-day period. Figure 3 illustrates that the inlet concentration of each compound and its outlet concentration during the operational period. In the continuous experiment when subjected to the mixture of the four compounds, the removal efficiency was ranked in the following order: styrene, toluene, benzene, and p-xylene. The ranking of removal efficiencies for styrene differed from that of the short-term experiment. This finding implies that the toluene-acclimated microbial culture could become adapted to the biodegradation of styrene as the BFR operation continued, since styrene is a more soluble and readily biodegradable compound than the others.

An averaged removal efficiency of each compound at pseudo-steady-state during Phase M2 was used to calculate SII values. The SII values were -0.01, -0.10, 2.02, 0.10 for Btxs, Tbxs, Xbts, Sbtx, respectively. Similar to the short-term BFR experiment, the SII values showed that the biodegradation of benzene and toluene was inhibited, but the biodegradation of p-xylene and styrene was enhanced by the presence of other compounds in the continuous BFR experiment.

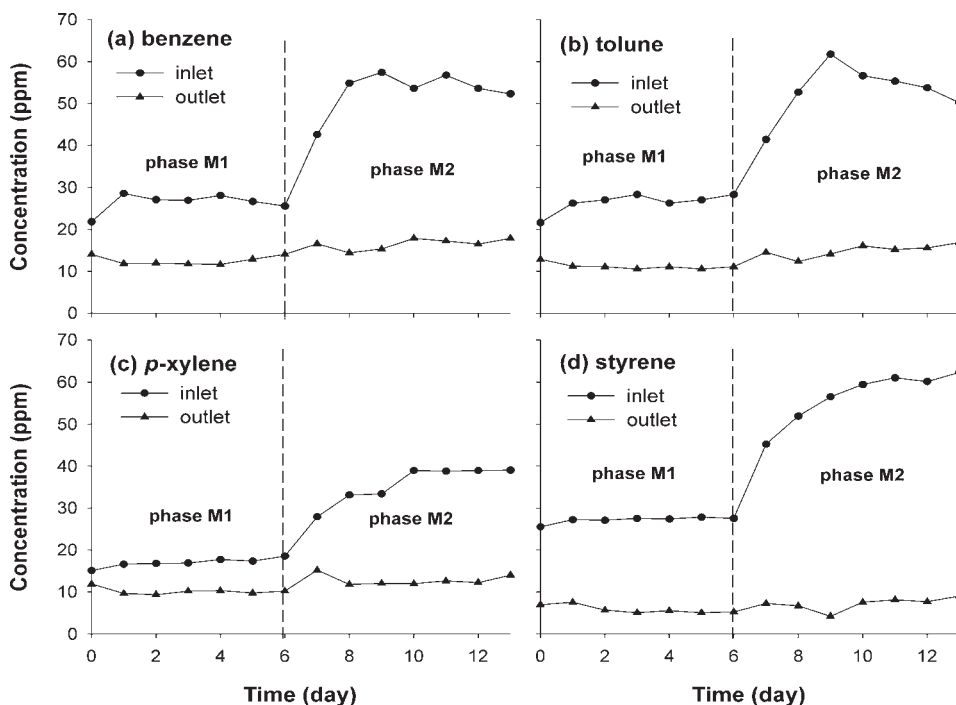


Figure 3. Changes of inlet and outlet concentrations of (a) benzene, (b) toluene, (c) p-xylene, and (d) styrene in the VOC mixture during the continuous BFR operation.

It is interesting to note that all the SII values obtained in the continuous operation shifted to the positive direction from its value obtained from the previous short-term experiment (e.g., -0.20  $\rightarrow$  -0.01 for Btxs). The enhancement of substrate interaction in the paired mixture was presumably due to an increase in microbial density in the cell reservoir as well as gradual adaptation of the microbial strain to the biodegradation of the BTXS mixture over time. Consequently, a careful attention needs to be given when BFR performance and biodegradation rates of mixed VOCs are utilized for system design and operational purposes.

#### 4 ACKNOWLEDGEMENTS

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#### REFERENCES

- Collins, L.D. and Daugulis, A.J. (1999) Benzene/toluene/p-xylene degradation: solvent selection and toluene degradation in a two-phase partitioning bioreactor. *Appl. Microbiol. Biotechnol.* 52: 359-365.
- Deshusses, M.A. and Johnson, C.T. (2000) Development and validation of a simple protocol to rapidly determine the performance of biofilters for VOC treatment. *Environ. Sci. Technol.* 34: 461-467.
- Devinsky, J.S., Deshusses, M.A. and Webster, T.S. (1999) Biofiltration for air pollution control. Boca Raton: Lewis Publishers.
- Kan, E. and Deshusses, M.A. (2003) Development of foamed emulsion bioreactor for air pollution control. *Biotechnol. Bioeng.* 84: 204-244.
- Kan, E. and Deshusses, M.A. (2005) Continuous operation of foamed emulsion bioreactors treating toluene vapors. *Biotechnol. Bioeng.* 92: 364-371.
- Kennes, C. and Veiga, M.C. (2001) Bioreactors for waste gas treatment. Kluwer Academic Publishers.
- Mohseni, M. and Allen, D.G. (2000) Biofiltration of mixtures of hydrophilic and hydrophobic volatile organic compounds. *Chem. Eng. Sci.* 55: 1545-1558.
- Phipps, D.W. (1998) Biodegradation of volatile organic contaminants from air using biologically activated foam. US Patent No.5,714,379.
- Song, J. and Kinney, K.A. (2000) Effect of Vapor-Phase Bioreactor operation on biomass accumulation, distribution, and activity. *Biotechnol. Bioeng.* 68: 508-516.
- Strauss, J.M., Riedel, K.J. and du Plessis, C.A. (2004) Mesophilic and thermophilic BTEX substrate interactions for a toluene-acclimated biofilter. *Appl. Microbiol. Biotechnol.* 64: 855-861.
- van Groenestijn J.W. and Hesselink P.G.M. (1993) Biotechniques for air pollution control. *Biodegradation.* 4: 283-301.