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Simultaneous removal of benzene, toluene and xylenes mixture by a constructed microbial consortium during biofiltration

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Abstract

A bacterial consortium with complementary metabolic capabilities was formulated and specific removal rates were 0.14, 0.35, 0.04, and 0.39 h^{-1} for benzene, toluene, *o*-xylene, and *m,p*-xylene, respectively. When immobilized on a porous peat moss biofilter, removal of all five (= BTX) components was observed with rates of 1.8–15.4 g m^{-3} filter bed h^{-1}. Elimination capacities with respect to the inlet gas concentrations of BTX and airflow rates showed diffusive regimes in the tested concentration range of (0.1–5.3 g m^{-3}) and airflow (0.55–1.82 m^3 m^{-2} h^{-1}) except for *o*-xylene which reached its critical gas concentration at 0.3 g m^{-3}.

Introduction

Benzene, toluene, and xylenes (BTX) are substantive constituents of gasoline (Prantera *et al.* 2002) and are also produced as industrial solvent and/or feedstocks for synthesis. Due to their wide usage, BTX have been commonly found in air, soils, and groundwaters worldwide. However, BTX have to be treated by gas cleaning techniques because BTX in contaminated soils or groundwaters are volatilized into air during the remediation process.

To date, a number of physical and chemical gas cleaning techniques have been developed which achieve highly efficient removal of various compounds from industrial waste gases. Chemical methods such as thermal and catalytic destruction, ozonization and chlorination usually are capable of removing a broad spectrum of compounds, but energy consumption and/or consumption of chemicals (oxidants or catalysts) are disadvantages of these methods. Physical methods, such as condensation, adsorption on solids or absorption in liquids have the disadvantage of the pollutants not being destroyed. The solid adsorbents or liquids have to be regenerated and often a new polluted material is created. As a cost-effective and environmentally safe alternative, biofiltration using soil beds (Prokop & Bohn 1985) or enclosed biofilters (Ottengraf 1986) has been successfully applied for the treatment of effluent gas stream containing low concentrations of air pollutants. However, there are only a few studies reported on the biofiltration of complex VOC mixtures such as BTX.

In this paper, we demonstrate simultaneous removal of all BTX components by a peat-biofilter inoculated with a microbial consortium consisting of members with complementary metabolic abilities.

Materials and methods

Microorganisms and growth conditions

Bacterial strains used in this study were isolated from wastewaters sampled in Kuro, Yeochon, Puchon, and Dongduchon industrial areas, Republic of Korea. The strains were cultured on a mineral salts basal (MSB) medium containing BTX as the sole source of carbon and energy, with rotary shaking (200 rpm) at 25 °C. The details of the isolation and identification processes

have been described in an earlier publication (Oh & Choi 1997).

Determination of BTX utilization

The BTX mixture consisted of equal parts of benzene, toluene, *o*-, *m*- and *p*-xylene isomers purchased from Sigma at the highest grades available. The strains were pre-grown on an MSB medium with substrate mixture as sole carbon and energy source. Cells were harvested by centrifugation (8000 × *g*, 20 min) and the resulting cell pellet was resuspended in fresh MSB medium at a cell concentration from 1.5 to 2 g dry biomass l^{-1}. Ten ml of the suspension were dispensed into 160 ml serum bottles (Wheaton Glass Co., USA) closed with Teflon-faced gray butyl rubber septa (Wheaton) and aluminum seals. Either each of the solvents or a mixture of them was added by a microsyringe, and incubated at 30 °C with rotary shaking (200 rpm). Identical bottles without bacteria served as negative controls. Any losses from these control bottles were subtracted from the values of substrate loss in the active bottles. Decrease of substrate in the headspace of the serum bottles was monitored by taking 100 μl gas samples from the headspace using a gas-tight syringe (Hamilton Co.), and the content was determined by gas chromatography equipped with a flame ionization detector and a 0.53 mm diameter × 30 m length capillary column (Hewlett-Packard HP-1). Operating conditions were: injector, 150 °C; oven, 70 °C; detector, 250 °C; and nitrogen carrier gas flow rate, 10 ml min^{-1}. Specific removal rates (h^{-1}) were calculated as g solvents removed per g dry cell biomass per h.

Operation of biofilter

Glass columns used for BTX vapor removal consisted of immobilized microbial cells on a stationary aqueous phase in peat moss. The arrangement shown in Figure 1 allowed adjustment of flow rates and solvent vapor concentrations independently by directing a greater or smaller part of an air stream through each solvent reservoir. Air streams generated by aquarium pumps were metered by low-capacity rotameters (Rate-Master, Dwyer Instruments, Inc.) and passed through water or BTX reservoirs. The separate flow meters allowed measurement of the total airflow and the portions passing through water and BTX. The air streams were then combined and passed through the column. The biofilter columns were installed in exhaust hoods and operated at ambient room temperature

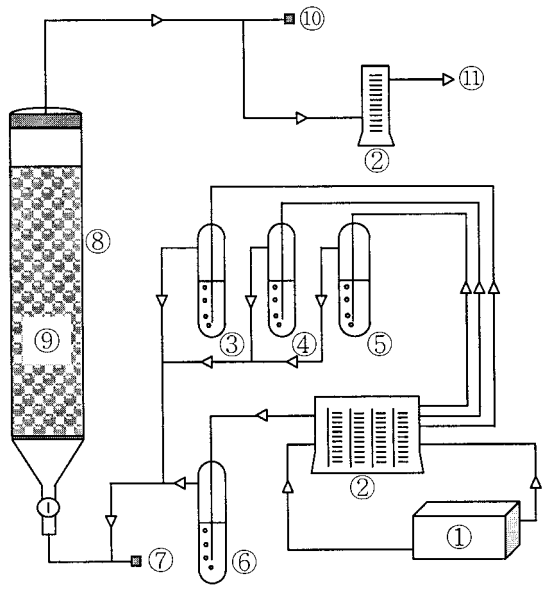

Fig. 1. Schematic diagram of a laboratory-scale peat moss biofilter for BTX removal. 1, air pump; 2, flow meter; 3, benzene; 4, toluene; 5, *o*-,*m*-,*p*-xylenes mixture; 6, water reservoir; 7, inlet gas sampling port; 8, glass column (6 cm diam. × 50 cm length); 9, peat moss with cells immobilized; 10, outlet gas sampling port; 11, gas outlet.

of 20–25 °C. For preparing the biofilter, a sufficient amount of dry peat moss was packed into a glass column (6 cm diam. × 50 cm length) at 367 kg m^{-3}. Each bacterial strain was grown on toluene, the cells harvested by centrifugation and suspended in fresh MSB medium. Peat moss, pre-sterilized by autoclaving for 30 min at 121 °C on three consecutive days, received an equal volume of the required microbial suspensions plus sufficient MSB medium to fill 50% of its water-holding capacity. BTX concentrations in the air streams were measured prior to and after exit from the biofilter columns using gas chromatography. From the concentration drop and the volume of air passing through the column, removal rates for the total and its individual BTX component could be calculated. Elimination capacities were expressed as g solvent removed per m^3 packing material per h.

Results and discussion

BTX degradation by constructed mixed culture

Individual bacterial strains were unable to degrade all the BTX components due to their limited metabolic capabilities. Benzene is degraded through direct ring attack, and methylbenzenes are oxidized by either

Table 1. Specific removal rates (h^{-1}) obtained form batch experiments using single[a] or mixed BTX solvents[b] with degrading individual strains or constructed consortium, respectively.

Strains	Specific removal rates[c] (h^{-1})			
	B	T	*o*-X	*m,p*-X
Pseudomonas pseudoalcaligenes BTXO2[a]	–[d]	0.10	–	0.12
Acinetobacter johnsonii BTXO3[a]	0.35	0.43	–	–
Pseudomonas alcaligenes BTXO12[a]	0.16	0.17	0.05	–
(BTXO2 + BTXO3 + BTXO12) consortium[b]	0.14	0.35	0.04	0.39

[c]B, benzene; T, toluene; *o*-X, *o*-xylene; *m,p*-X, *m*- and *p*-xylene.
[d]Substrate could not be utilized.

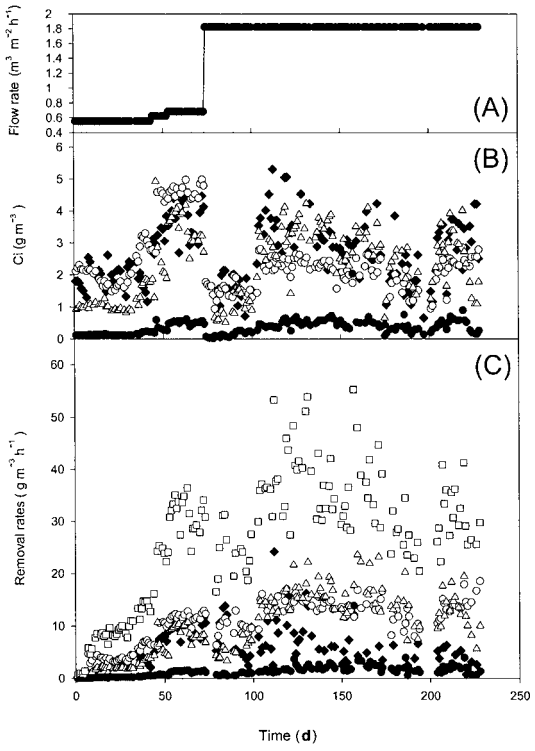

Fig. 2. Performance of the biofilter during 8-months operation. Profiles of (A) surface loading, (B) inlet BTX concentrations, and (C) BTX removal rates were presented (♦, benzene; ○, toluene; ●, *o*-xylene; △, *m,p*-xylene; □, total).

ring attack or methyl group hydroxylation (Smith 1994). Therefore, there should be several types of bacterial strains, one of which oxidizes benzene or toluene through initial ring attack, another which causes methyl group hydroxylation, and a third pathway for *o*-xylene degradation. *o*-Xylene is probably not degraded through the progressive oxidation of a methyl group as xylene monooxygenase, which is responsible for the conversion of *m*- and *p*-xylenes into the corresponding alcohols, but is ineffective towards the *ortho* isomer (Wubbolts *et al.* 1994). Lee *et al.* (1995) constructed a hybrid strain that had the *tod* and the *tol* pathways for degradation of BTX using a single bacterial strain. However, the hybrid strain was unable to degrade *o*-xylene and it is doubtful that such a hydrid strain could be constructed that possess the third pathway for degradation of *o*-xylene since the pathways either could not be expressed in the same cells (Barbieri *et al.* 1993) or would produce toxic metabolic intermediates (Di Lecce *et al.* 1997). Furthermore, there is still some controversy regarding the release of genetically engineered microorganisms into the environment.

Using various strains each responsible for degradation of one of the BTX components could be another way of approaching this problem. For that purpose, a mixed culture, composed of *Pseudomonas pseudoalcaligenes* BTXO2, *Acinetobacter johnsonii* BTXO3, and *Pseudomonas alcaligenes* BTXO12 in equal proportions was constructed for the complete degradation of the BTX mixture. Earlier analysis of substrate utilization kinetics (Oh & Choi 1997) showed that none of the three strains utilized all of BTX compounds as a sole source of carbon and energy. The metabolic capabilities of the three strains with regard to the BTX metabolism are summarized in Table 1. Each strain has different pathways for BTX degradation and combining these three strains can provide obvious advantages in terms of efficient removal of all five BTX components. Kinetic parameters such as specific removal rates obtained with single or mixed compounds have a significant impact on the successful operation of the overall treatment processes. Nevertheless, a few studies have evaluated the biodegradation kinetics of BTX chemicals with varying results. Findlay & Nirmalakhandan (1999) summarized the kinetic values

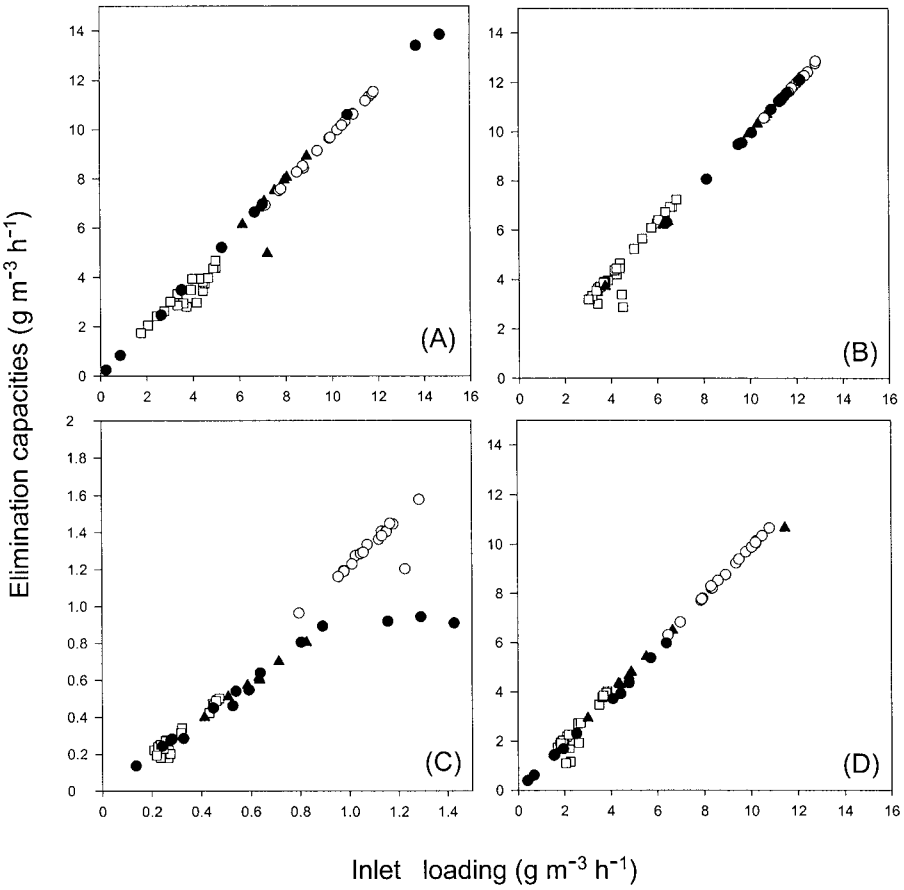

Fig. 3. Variation of elimination capacity versus the inlet loading of (A) benzene, (B) toluene, (C) *o*-xylene, and (D) *m,p*-xylene. Superficial airflow rates (m^3 m^{-2} h^{-1}) were 0.55 (□), 0.62 (▲), 0.68 (○), and 1.82 (●).

of BTX degradation and reported maximum removal rates (g m^{-3} h^{-1}) of benzene and toluene in the range of 0.108 to 0.160 and 0.021 to 0.493, respectively.

When the three strains were mixed in equal proportions, in our study, they efficiently removed all five BTX components without any lag period (data not shown). Moreover, specific removal rates of BTX components by the consortium were as high as those obtained by single strains, which indicated minimum substrate interaction (Table 1). The combined removal rate was higher than that reported by Lee *et al.* (1995) using a hybrid strain of *Pseudomonas putida* for BTX degradation. These results suggested that combining several strains with different metabolic capabilities could be a useful tool for complete degradation of mixed substrates.

BTX biofiltration

In previous experiments, peat moss was the best support for the biofiltration of toluene, *m*- and *p*-xylene vapors using *Pseudomonas pseudoalcaligenes* BTXO2 (Oh & Choi 2000). Based on the results, peat moss was chosen as solid support for the biofilter used in this study. Various physicochemical parameters including pH, porosity, pressure drop (per meter bed at 12.5 m^3 m^{-2} h^{-1}), filter media dry density (g ml^{-1}), and filter media water-holding capacity (ml g^{-1}) of the peat moss were 6.7, 0.74, 0–5 mm H_2O, 0.21, and 1.55, respectively. These parameters were in the range of the recommended operational values for most biofiltration systems.

When the three strains were grown separately on toluene and then equal amounts of biomass were immobilized on peat moss biofilter, BTX removal stabilized after 10 days of acclimation and did not decline in activity during 8 months operation (Figure 2). Ini-

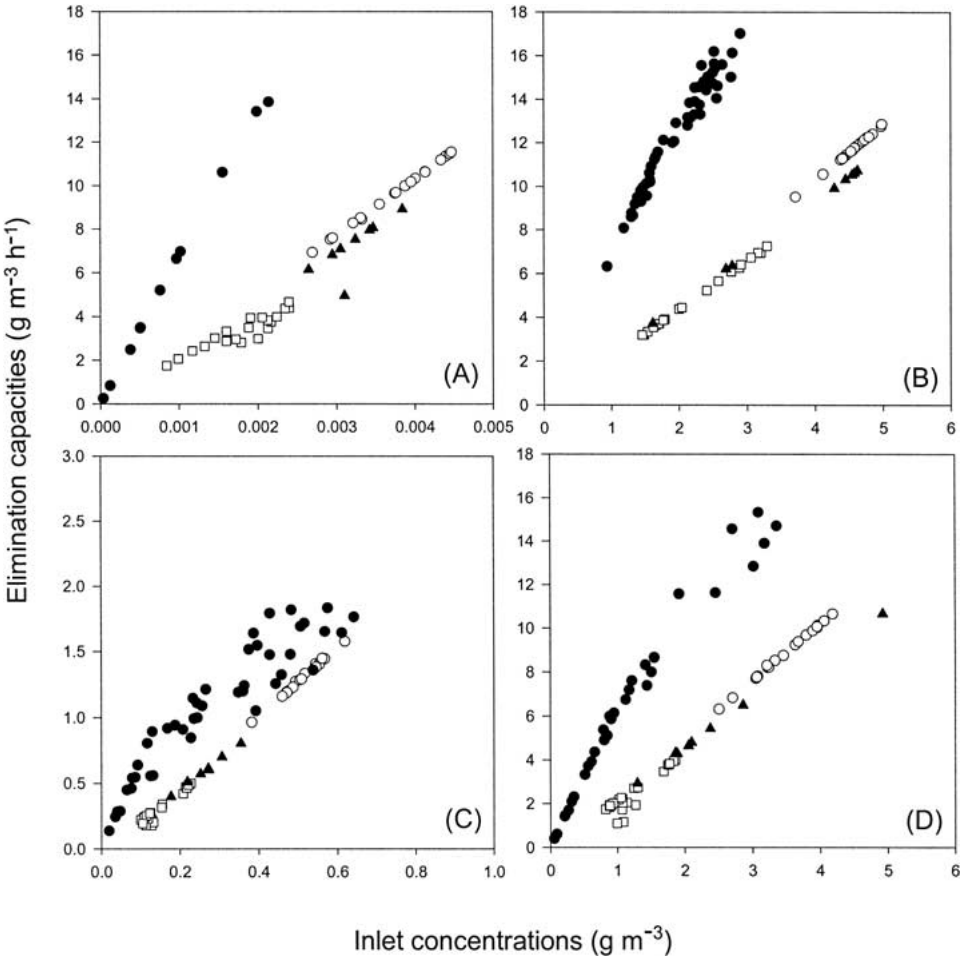

Fig. 4. Variation of elimination capacity versus the inlet concentrations of (A) benzene, (B) toluene, (C) *o*-xylene, and (D) *m,p*-xylene. Superficial airflow rates (m^3 m^{-2} h^{-1}) were 0.55 (□), 0.62 (■), 0.68 (○), and 1.82 (●).

tially, the surface loading was maintained at 0.55 m^3 m^{-2} h^{-1} and gradually increased to 1.82 m^3 m^{-2} h^{-1}at 74 days after the start-up. The same surface loading was maintained up to the end of the experiment and the retention time was 4.4 min during this period. With an assumption that the biofilter reached a quasi-steady state (Day 120), the average removal rates of benzene, toluene, *o*-xylene, and *m,p*-xylene vapors were 10.8 ± 2.9, 14.2 ± 1.5, 1.8 ± 0.8, and 15.4 ± 3.2 g m^{-3} h^{-1}, respectively. During the operation, airflow rates were in the range of 0.55 to 1.82 m^3 m^{-2} h^{-1}, and BTX concentrations were varied from 0.1 to 5.3 g m^{-3}.

One of the most important parameters used for designing biofilters is the elimination capacity (EC) since it relates the inlet load of the pollutant to its removal efficiency. The maximum EC of a biofilter depends on the microbial population and the activities of BTX-oxidizing bacteria existing in the filter media. Both the EC and microbial activity are related to the operating conditions of the system, such as temperature, water content, concentration of nutrients and inhibitory substances (Bibeau *et al.* 1997). Among the several representations of EC reported in the literature (Diks & Ottengraf 1991, Ottengraf 1986), the most widespread representation is the one which presents the variation of EC versus the inlet loading of the pollutant, as shown in Figure 3. The EC value was directly proportional to the inlet loading.

The results can be interpreted by diffusive limitations that occur in the biolayer and tend to make the biofilm not fully active; hence the conversion rate was mainly controlled by the diffusion rate. However, upon the high inlet load (beyond 1 g m^{-3} h^{-1}) of *o*-

xylene, EC reached its maximum value and remained independent of the inlet loading. Similarly, EC with respect to the inlet gas concentrations of BTX showed diffusive regimes in the tested range of concentration and airflow except for *o*-xylene (Figure 4). At the highest airflow rate (1.82 m^3 m^{-2} h^{-1}), the EC of *o*-xylene was roughly constant above 0.3 g m^{-3}, which is often called as the critical value of *o*-xylene gas concentration (Bibeau *et al.* 1997). Beyond the critical value, the limitations of the reaction rate govern the behavior of the biofilter and the overall degradation rate is determined by the micro-kinetics of the biomass exist in the biolayer. The different behavior of *o*-xylene degradation from other BTX components was consistent with the low specific removal rate of *o*-xylene (0.04–0.05 h^{-1}) by the consortium or BTXO12 strain compared to those of other BTX components (Table 1). During the operation of biofilters, superficial airflow rate has been thought to be an operational parameter as important as the pollutant concentration (Shareefdeen *et al.* 1993). At fixed inlet concentration, EC increased as the superficial airflow rate increased (Figure 4). Since the EC was dependent to the airflow rate, a high superficial airflow rate is recommended in the biofilter operation. Similar results were obtained during the biofiltration of both methanol by Shareefdeen *et al.* (1993) and benzene by Yeom & Yoo (1999).

Many researchers have monitored degradation of single compounds in biofiltration systems. Due to the limited reports on the biofiltration of BTX mixtures, it is difficult to compare the EC of BTX in the present study with those found in other studies. In at least four other cases of evaluating a complex mixture of VOCs, the investigators chose to monitor total VOCs and not individual compounds (Devinny *et al.* 1994, Saberiyan *et al.* 1994) or just reported the removal efficiencies of each component (Mallakin & Ward 1996, Kleinheinz & Bagley 1998). Thus, comparing the EC of a single compound in this type of mixture to a system that was designed for the removal of only a single compound would be difficult and possibly erroneous. Monitoring the removal efficiency of each component often cannot present comparable data to other studies because the removal efficiencies are highly dependent on the operating conditions such as the inlet concentration, airflow rate, and the scale of biofilter. To our knowledge, the present study is the first biofiltration laboratory study incorporating the monitoring and comparable quantification of specific compounds removal within a complex VOC mixture.

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