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Thermophilic Biofiltration of Benzene and Toluene

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Abstract In the current studies, we characterized the degradation of a hot mixture of benzene and toluene (BT) gases by a thermophilic biofilter using polyurethane as a packing material and high-temperature compost as a microbial source. We also examined the effect of supplementing the biofilter with yeast extract (YE). We found that YE substantially enhanced microbial activity in the thermophilic biofilter. The degrading activity of the biofilter supplied with YE was stable during long-term operation (approximately 100 d) without accumulating excess biomass. The maximum elimination capacity (1,650 $g \cdot m^{-3} \cdot h^{-1}$) in the biofilter supplemented with YE was 3.5 times higher than that in the biofilter without YE (470 $g \cdot m^{-3} \cdot h^{-1}$). At similar retention times, the capacity to eliminate BT for the YE-supplemented biofilter was higher than for previously reported mesophilic biofilters. Thus, thermophilic biofiltration can be used to degrade hydrophobic compounds such as a BT mixture. Finally, 16S rDNA polymerase chain reaction-DGGE (PCR-DGGE) fingerprinting revealed that the thermophilic bacteria in the biofilter included *Rubrobacter* sp. and *Mycobacterium* sp.

Keywords: Thermophilic biofilter, mesophilic biofilter, thermophilic bacteria, benzene, toluene, yeast extract

Biofiltration has been successfully used for the cost-effective and environmentally friendly treatment of waste gases containing odorous and volatile organic compounds (VOCs). Despite its advantages, the application of biofiltration has been limited to the treatment of waste gases at temperatures in the mesophilic range (15°C to 40°C) [10, 13, 16–18, 22]. This has prevented its use for high-temperature industrial waste gases; for example, gases released during the processing of tobacco, pulp, paper, food, fodder, and rubber (*i.e.*, curing process) and from coating processes (*i.e.*, from spray booths and bake ovens), chemical manufacturing, and waste treatment (*i.e.*, drying of activated sludge cake and food wastes). Cooling these gases to below 40°C prior to biological treatment is very costly because the gas must be saturated with water [10, 13]. Biofilters using thermophilic microorganisms should greatly reduce the cost of degrading contaminants at temperatures over 40°C and should extend the use of biofilters [10, 13].

Only a few studies have focused on the biotreatment of waste gases at temperatures between 40°C and 70°C [10, 13, 20, 26, 33, 34]. Most of these have examined the treatment of gases containing hydrophilic compounds such as methanol and ethanol [10, 13, 20, 34]. Three of these studies investigated the biotreatment of gases containing toluene and α-pinene, which have high Henry's Law coefficients [20, 26, 33, 34].

Few studies have investigated the thermophilic treatment of BTEX (benzene, toluene, ethylbenzene, and xylene), which is a major component of hot waste gases from many industrial processes. Two thermophilic microorganisms, *Thermus aquaticus* and *Thermus* sp., have been shown to be able to degrade BTEX [7]. In addition, high-temperature compost has been shown to degrade toluene [26]. Generally, for commercial and industrial applications, typical empty-bed residence times (EBRTs) of a biofilter range from 25 s for the removal of odors and the treatment of low VOC concentrations, to over 1 min for high concentrations of VOCs [13, 25]. The EBRTs needed for effective performance of thermophilic biofilters (57–120 s) [10, 20, 26, 33] are longer than those needed for ambient biofilters [21, 23, 32]. Further studies on thermophilic microbial resources and degradation of hydrophobic VOCs with high Henry's Law coefficients are necessary to reduce EBRTs close to those needed for ambient biofilters.

In the current study, we examined the feasibility of thermophilic biofiltration of pollutants that have high Henry's Law coefficients. We used a mixture of benzene and toluene (BT) as the target pollutant, and polyurethane (PU) was included as a support in the biofilter because it has a high moisture holding capacity and therefore may be suitable for high-temperature operation [21]. To obtain a satisfactory performance at high temperature (60°C) and

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reasonable EBRTs, we added yeast extract (YE) to enhance the biofilter performance, because some research had reported that YE could improve the biodegradability of pollutants [4, 5, 19]. In addition, we examined the structure of the microbial community by 16S rDNA polymerase chain reaction-denaturing gradient gel electrophoresis (PCR-DGGE).

MATERIALS AND METHODS

Packing Material and Inoculum

The filter material consisted of cubic PU foam (Seilsponge, Seoul, Korea) with a dimension of 2.0 cm×2.0 cm×2.0 cm. The bulk density, water holding capacity, porosity, average pore size, and surface area of the material were 0.015 $g \cdot cm^{-3}$, 57 $g\text{-}H_2O \cdot g^{-1}$, 98.8%, 0.8 mm, and 76.81 $m^2 \cdot g^{-1}$, respectively. For an inoculum source, high-temperature compost was suspended in mineral salts (MS; 1.5 $g \cdot l^{-1}$, KH_2PO_4, 9.0 $g \cdot l^{-1}$, $Na_2HPO_4 \cdot 12H_2O$, 3 $g \cdot l^{-1}$, $(NH_4)_2SO_4$, 0.01 $g \cdot l^{-1}$, $CaCl_2 \cdot 12H_2O$, and 0.15 $g \cdot l^{-1}$, $MgSO_4$), and the large particles were removed by sieving (200 mesh).

Biofilter Setup and Experimental Conditions

The laboratory-scale biofilter used in this study was made of a square acrylic resin column (0.15 m×0.15 m×0.83 m), and consisted of a drain storage tank (0.15 m×0.15 m× 0.40 m), two biofilter beds (0.15 m×0.15 m×0.35 m), a liquid distributor, and a circulating pump. Each biofilter bed was packed with 0.3 m of the filter material, and the volume of the packing medium at each biofilter bed was 6.75 l. Waste gas containing BT flowed through a humidifier and a volatilization chamber before entering the biofilter [21]. To maintain the BT gases at 60°C, the gas tube supplying the biofilter was heated using a heating band. The humidifier, volatilization chamber, and biofilter were placed in an incubator to maintain them at 60°C.

To establish steady-state conditions, the biofilter was acclimated for one week with an inlet BT concentration of 1 $g \cdot m^{-3}$ and a space velocity (SV) of 30 h^{-1}. The BT removal experiments examined changes in the inlet concentrations and the SV. The biofilter was operated for 170 d. To supply essential nutrients for growing the microorganisms and to avoid drying the packing materials, during the initial 70 d of operation, 4 l of tap water supplemented with MS was sprayed on the top of the biofilter for 2 min every 6 h using a circulating pump. After 70 d, 4 l of tap water supplemented with MS and 5 $g \cdot l^{-1}$ of YE was supplied in the same manner. The circulating water in the tank was replaced every 2 d. Once the outlet concentration was constant, the inlet BT concentration was increased stepwise. The concentrations of benzene and toluene in the inlet gas varied from 0.2 to 12 $g \cdot m^{-3}$, and the SV of the inlet gas in the biofilter varied from 30 to 100 h^{-1} when it was supplied with MS and from 80 to 400 h^{-1} when it was supplied with a combination of MS and YE.

Analytical Methods

The BT gases were collected from the biofilter using 1-ml gastight syringes equipped with Teflon Minnert fittings. BT concentrations were measured using a gas chromatograph [21]. The cell mass immobilized in the biofilter was measured as previously described [21].

Analysis of the Microbial Community Structure

PCR-DGGE was used to analyze the bacterial community structure in the biofilter. At the termination of the biofiltration experiment, 0.3 g of bacterial cells was obtained from the PU sampled in the middle of the filter bed at 100th d (SV=100 h^{-1}), and genomic DNA was extracted using the BIO101 FastDNA SPIN KIT for soil (Q-BioGene, Solon, Ohio, U.S.A.) [29]. A 177-base pair portion of the 16S rDNA was amplified by PCR using primers 341FGC and 518R [2] under previously reported conditions [9, 28]. DGGE [2] and sequencing [9] were performed as previously described. The obtained sequences were compared using the BLAST algorithm and deposited in the National Center for Biotechnology Information database under accession numbers EF117235-EF117245.

RESULTS

Effect of YE on Performance of the Biofilter

We started the biofilter with a suspension (1 $g \cdot m^{-3}$) of high-temperature compost and a low gas flow rate (SV= 30 h^{-1}) and then gradually increased the SV and inlet concentration of the compost suspension. After the biofilter was started, we found that it could degrade BT (data not shown), indicating that the microbial source contained thermophilic BT-degrading microorganisms. Fig. 1A shows the efficiency of BT elimination as a function of the inlet concentration at three different gas flow rates (SV=30, 50, and 100 h^{-1}) using only MS to supplement the biofilter. The elimination efficiency strongly correlated with the inverse of the gas flow rate. The removal efficiency at 30, 50, and 100 h^{-1} remained above 90% up to inlet concentrations of approximately 6, 3, 0.2 $g \cdot m^{-3}$ for benzene and 4, 1.0, and 0 $g \cdot m^{-3}$ for toluene, respectively.

To enhance the removal efficiency, 5 $g \cdot l^{-1}$ YE was supplied to the biofilter along with the MS. Addition of YE substantially improved the elimination efficiency (Fig. 1B). Effective efficiency could be achieved at high gas flow rates and high inlet concentrations. The removal efficiency at 80, 100, 150, 200, and 300 h^{-1} remained above 90% up to inlet concentrations of approximately 8.9, 8.1, 3.3, 3.3, and 1.6 $g \cdot m^{-3}$ for benzene and 3.0, 1.5, 0.8, and 0.2 $g \cdot m^{-3}$ for toluene, respectively. This indicates that the activity of the thermophilic microbial consortia in the thermophilic biofilter was enhanced by the addition of YE. In addition, our results show that thermophilic biofiltration can be achieved for volatile BT compounds at a high temperature (60°C).

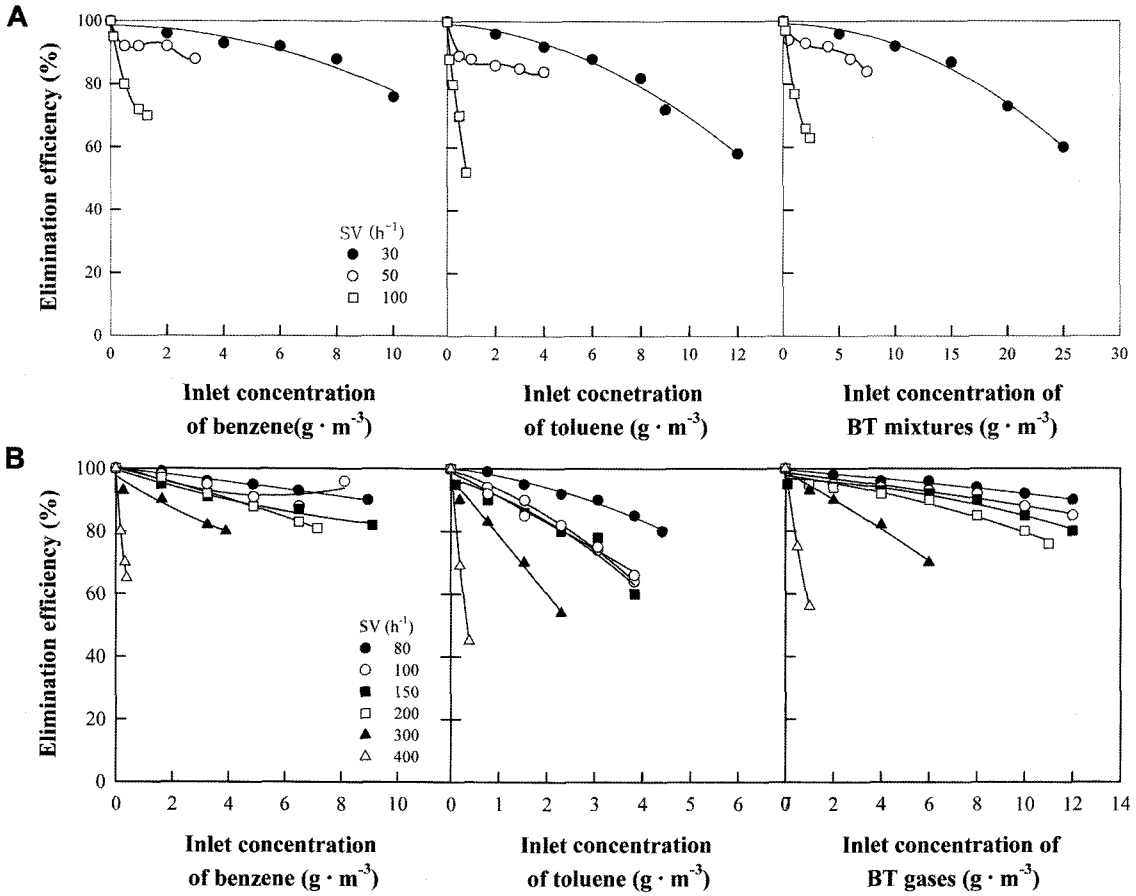

Fig. 1. Elimination efficiency of the biofilter as a function of the inlet concentration (**A**) without and (**B**) with added YE.

We also monitored the biomass concentration, pH of the drainage fluid, and the pressure drop in the biofilter during long-term (170 d) operation. The biomass accumulation was 0.39±0.08 g-cell·g-PU^{-1} during first 30 d of operation. It increased to 0.54±0.12 g-cell·g-PU^{-1} after 90 d of operation and then remained constant (data not shown). The pH of the drainage from the biofilter ranged from 6 to 7, and the pressure drop in the filter bed remained below 5 mm H_2O (data not shown).

Elimination Capacities for Benzene and Toluene

We examined the inlet load and elimination capacities to investigate the relationship between the SV and the inlet gas concentration and to identify the main factors

Table 1. Elimination capacities for the degradation of benzene and toluene in the BT mixture by the thermophilic biofilter.

Addition of YE	SV (h^{-1})	Benzene (g·m^{-3}·h^{-1})				Toluene (g·m^{-3}·h^{-1})				Total (g·m^{-3}·h^{-1})			
		Load	EC_{80}	EC_{90}	EC_{max}	Load	EC_{80}	EC_{90}	EC_{max}	Load	EC_{80}	EC_{90}	EC_{max}
No addition	30	~330	240	210	240	~360	215	200	230	~670	455	410	470
	50	~185	170	155	170	~230	200	200	200	~415	370	355	370
	100	~110	60	20	75	~80	35	15	40	~190	95	35	115
Addition	80	~770	740	740	740	~170	160	160	160	~920	800	900	900
	100	~810	720	660	720	~390	240	180	280	~1,200	960	840	1,000
	150	~1,200	1,000	840	1,000	~600	390	160	390	~1,800	1,300	1,000	1,390
	200	~1,200	1,000	840	1,130	~800	495	240	520	~2,000	1,495	1,080	1,650
	300	~1,200	960	600	960	~600	280	70	350	~1,600	1,340	670	1,310
	400	~160	36	10	110	~160	12	12	100	~320	48	22	210

Load, inlet loading; EC_{max}, maximum elimination capacity; EC_{80}, elimination capacity guaranteeing more than 80% elimination efficiency; EC_{90}, elimination capacity guaranteeing more than 90% elimination efficiency.

influencing the efficiency of benzene and toluene removal from the BT mixture. The load and the elimination capacities were calculated according to the method described by Shim *et al.* [32]. Table 1 summarizes the elimination capacities of the biofilter, including the maximum elimination capacity (EC_{max}) and the mean elimination capacities for guaranteeing more than 80% and 90% elimination (EC_{80} and EC_{90}, respectively). When the biofilter was supplemented with only MS, the highest elimination capacity was obtained at SV=30 h^{-1}, and the EC_{80}, EC_{90}, and EC_{max} values decreased substantially as the SV was increased. When the biofilter was supplemented with both MS and YE, the highest elimination capacity was obtained at SV=200 h^{-1}, and the EC_{80}, EC_{90}, and EC_{max} values decreased substantially at SV>400 h^{-1}. The highest EC_{max} (1,650 $g \cdot m^{-3} \cdot h^{-1}$) in the presence of YE was 3.5 times higher than that in the absence of YE (470 $g \cdot m^{-3} \cdot h^{-1}$). Comparison of elimination capacities in the biofilter revealed that elimination capacities in the biofilter supplied with MS were higher for benzene than for toluene, except when the SV was 50 h^{-1} and the inlet load was relatively low.

Kinetic Analysis

We next performed a kinetic analysis to determine the maximum rate of benzene and toluene removal by the biofilter. The following equation can be applied, assuming the plug flow of gas in the biofilter [8, 21].

$$\frac{C_{ln}}{R}=\frac{K_s}{V_m}+\frac{C_{ln}}{V_m},$$

where R is the removal rate and is equal to $SV(C_o-C_e)$, C_{ln} is the log of the mean concentration and is equal to $(C_o-C_e)/\ln(C_o/C_e)$, C_o is the inlet benzene concentration ($g \cdot m^{-3}$), C_e is the outlet benzene concentration ($g \cdot m^{-3}$), the V_m is the maximum removal rate ($g \cdot m^{-3} \cdot h^{-1}$), and K_s is the saturation constant ($g \cdot m^{-3}$). Based on the linear relationship between C_{ln}/R and C_{ln}, V_m of benzene and toluene in the absence of YE ranged from 134 to 340 and 80 to 310 $g \cdot m^{-3} \cdot h^{-1}$, respectively, with the highest values for both at an SV of 30 h^{-1} (Table 2). In the presence of YE, the V_m of benzene and toluene ranged from 350 to 3,770 and from 200 to 770 $g \cdot m^{-3} \cdot h^{-1}$, respectively, and were maintained at high levels, even at SVs as high as 300 h^{-1}. In the absence of YE, the V_m for benzene was 1.1- to 1.7-fold higher than the V_m for toluene, whereas in the presence of YE, it was 1.8- to 5.8-fold higher for benzene than for toluene. These results indicate that YE enhances the degradation of both benzene and toluene, although the enhancement is greater for benzene, and that the V_m of benzene and toluene depend on the EBRT.

Table 2. Kinetic analysis of the degradation of benzene and toluene in the BT mixture by the thermophilic biofilter.

SV (h^{-1})	No addition of yeast extract				Addition of yeast extract			
	Benzene		Toluene		Benzene		Toluene	
	V_m[a]	K_s[b]	V_m	K_s	V_m	K_s	V_m	K_s
30	340	2.5	310	2.6				
50	330	2.4	220	1.9	-	-	-	-
80	-	-	-	-	1,910	6.6	330	1.0
100	134	0.55	80	35	2,110	6.8	440	1.7
150	-	-	-	-	2,330	4.5	590	1.5
200					3,770	6.5	770	1.2
300					2,120	2.4	550	0.7
400					350	0.5	200	0.5

[a]Maximum elimination rate ($g \cdot m^{-3} \cdot h^{-1}$).
[b]Saturation constant ($g \cdot m^{-3}$).

Analysis of the Microbial Community

We used 16S rDNA PCR-DGGE to examine the structure of the microbial community present the PU medium in the thermophilic biofilter (Fig. 2A). The phylogenetic relationship of the clones according to the DGGE fingerprint is shown in Fig. 2B. Clone BT1 was highly related to *Adelie penguin-guano* bacterium 253 (AY377478), which belongs to the *Moraxellaceae/Pseudomonadaceae* [37], and *Psychrobacter arenosus* (AJ609273), which was isolated from coastal sea ice and sediments [30]. Clone BT2 was closely related (94% sequence similarity) to (AB231786) isolated from a compost pile (unpublished data). Clones BT3 and BT8 were very similar to an uncultured bacterium (AB246713; 100% and 99% sequence similarity, respectively) present in bark compost during petroleum degradation (unpublished data). The closest neighbor to clones BT4 and BT6 was an uncultured bacterium, F2-70 (AY096183), that was detected in a biofilter for treating hydrogen sulfide and methanol (unpublished data). Clones BT5 and BT7 were most similar to an uncultured bacterium (AJ853611) associated with the leachate of a closed municipal solid waste landfill [14]. Clone BT9 was similar to an unidentified bacterium (AJ518494) in sediments from reservoirs of different trophic states [35]. Clone BT10 was closely related to *Rubrobacter xylanophilus* (X87135; 97% sequence similarity), which is a new thermophilic species isolated from thermally polluted effluent [3]. Clone BT was the most similar to *Mycobacterium hassiacum* (U49401; 94% sequence similarity), which is a newly identified, rapidly growing thermophilic mycobacterium [31].

Discussion

This study demonstrated that hot mixed gases of benzene and toluene could be degraded in the thermophilic biofilter. The degradation of hot mixed gases was enhanced by the addition of YE (Fig. 1, Tables 1 and 2). Microbial growth and their enzyme activity in the thermophilic biofilter

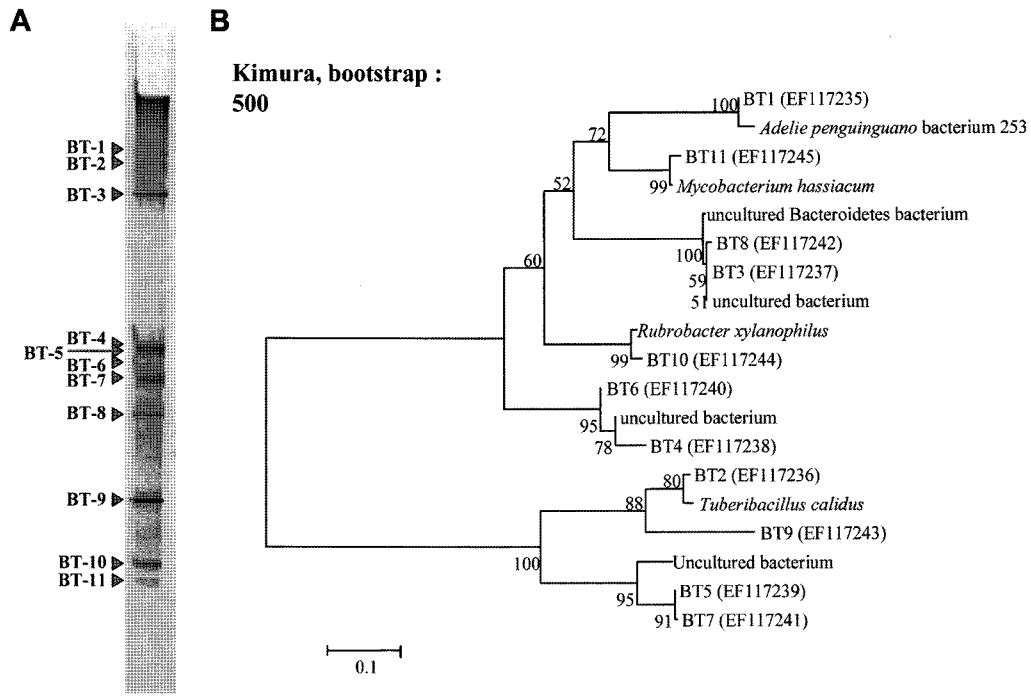

Fig. 2. DGGE fingerprint of the bacterial community in the thermophilic biofilter (**A**) and phylogenetic relationship among the clones obtained from the DGGE bands (**B**).

might be enhanced by the addition of YE [4, 5]. The enhancement of biodegradation efficiency by the addition of YE had been reported by some researchers [4, 5, 18].

Several studies have shown that benzene inhibits the degradation of toluene [1, 23, 32] or that benzene and toluene competitively inhibit each other's degradation [7]. The microbial consortia described here, however, was able to efficiently degrade benzene and, to a lesser extent, toluene. This may be because the thermophilic consortia consisted of different types of bacteria with different degradative pathways. In fact, for some of the thermophilic bacteria isolated from this biofilter, the degradation rate was higher for benzene than for toluene (data not shown).

To evaluate the capacity of the biofilter used in this study, we compared the elimination capacities with those of previously reported biofilters. Previous studies on thermophilic biofilters revealed that the maximum elimination capacities for α-pinene, methanol, and ethanol, which are degraded more rapidly than BT by thermophilic biofilters, are approximately 60 (EBRT=120 s), 100 (EBRT=60 s), 200 (EBRT=57 s) $g \cdot m^{-3} \cdot h^{-1}$, respectively [2, 3]. In this study, the maximum capacities of the biofilter in the absence of YE were similar to previously reported values, whereas in the presence of YE, the capacities were much higher than in the previous reports.

In a previous study, the elimination capacity of a mesophilic biofilter was 3 to 130 $g \cdot m^{-3} \cdot h^{-1}$ for BTX [11, 12, 15, 27, 29, 36, 38]. The elimination capacities for the BT mixture overall and for benzene and toluene alone were similar for the current biofilter in the absence of YE and for our previous mesophilic PU biofilter. These elimination capacities were also higher than for other mesophilic biofilters. To obtain similar elimination capacities and removal efficiencies (at least 80% to 90%), however, a longer EBRT is required for thermophilic biofilters than for mesophilic biofilters (12–72 s) [21, 32]. On the other hand, for the thermophilic biofilter supplemented with YE, the elimination capacities for BT were higher than those achieved by other mesophilic biofilters at similar EBRTs. Thus, thermophilic biofiltration can be used to degrade not only highly biodegradable and water-soluble compounds but also hydrophobic compounds such as BT.

The effect of temperature on biofilter performance depends on both the sensitivity of the biodegradation process and mass transfer. The physicochemical effects of higher temperatures are usually unfavorable. In our previous and current experiments [21], the packing material was PU, and the EBRTs and operating concentrations of BT were similar, which allows direct comparison of the effects of the operating temperature. In an ambient PU biofilter inoculated with activated sludge, the elimination capacities of benzene and toluene at an EBRT of 72 s were 165 and 204 $g \cdot m^{-3} \cdot h^{-1}$, respectively [32], and in a PU biofilter inoculated with *Stenotrophomonas maltophilia* T3-c, the elimination capacity of benzene at an EBRT of 12 s was 110 $g \cdot m^{-3} \cdot h^{-1}$ [21]. This indicates that the rate of BT

removal was identical at ambient temperature and 60°C. Other reports found similar rates of ethanol removal at ambient and high (50°C) temperatures [10]. Thus, a high temperature may not negatively affect the ability to degrade pollutants. In general, the rates of enzyme reaction and cell growth double every 10°C, so that the cellular activities of thermophilic organisms are higher than for mesophilic organisms. As the temperature increases, the increase in the activities of the thermophilic consortia may be counteracted by lower mass transfer. This agrees with previous findings that the optimum temperature for the treatment of methanol, ethanol, and α-pinene by thermophilic biofilters is between 50°C and 60°C [10, 20]. In addition, this study clearly showed that the activities of the thermophilic biofilter at 60°C were greatly enhanced by the addition of YE. Furthermore, the results indicate that the most important factor affecting the performance of the thermophilic biofilter is the bioavailability of pollutants to the microbial consortia.

Although biofilters can effectively treat waste gases containing VOCs and organic compounds, they accumulate biomass, which can cause clogging and therefore a loss of efficiency [11, 12, 15, 27, 29, 36]. In our previous study, we found that removal of benzene at ambient temperature by a PU biofilter leads to the accumulation of 1.5 to 3.0 g-DCW (dry cell weight)·g-PU^{-1} (average of 2.25 g-DCW·g-PU^{-1} during operation), which can cause substantial clogging [21]. In contrast, biomass accumulation by the thermophilic biofilter described in the current study remained below 0.56±0.12 g-DCW·g-PU^{-1}, and the pressure drop through the filter bed was negligible during the long-term (170 d) operation, despite a high inlet loading of benzene and toluene (~1,240 g·m^{-3}·h^{-1}). Others have also reported lower biomass accumulation at higher temperatures [10, 20]. Thus, accumulation of biomass in a biofilter depends on the operating temperature. Low growth yields at high temperatures may be due to high energy requirements for cell maintenance under conditions of high temperature and low moisture.

Analysis of the microbial community in the thermophilic biofilter by 16S rDNA-DGGE fingerprinting indicated that *Rubrobacter xylanophilus* and *Mycobacterium hassiacum* were the dominant thermophilic bacteria (Fig. 2), although mesophilic bacteria were also found.

In summary, we report for the first time a thermophilic biofilter that can degrade BT, and the enhancement of the degradation by YE. This thermophilic biofilter was stable during 170 d of operation and could treat hot waste gases containing organic compounds without biomass accumulation. We found that YE greatly enhanced the activity of microbial consortia in the thermophilic biofilter. Additional studies are needed to further characterize the thermophilic bacteria that can degrade BT in the biofilter, and the effects of fluctuating temperatures on the stability of the thermophilic biofilter.

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