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Removal of *o*-xylene using biofilter inoculated with *Rhodococcus* sp. BTO62

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Abstract

Rhodococcus sp. BTO62 was isolated from activated sludge from a wastewater treatment plant as an *o*-xylene-degrading microorganism. BOT62 degraded not only *o*-xylene, but also benzene, toluene, ethylbenzene, *m*- and *p*-xylenes and styrene (BTEXS). A laboratory scale biofilter packed with Biosol as packing material, which is made from foamed waste glass mixed with corrugated cardboard, was inoculated with strain BTO62 and operated to remove relatively high loading of *o*-xylene at different space velocities under non-sterile and sterile conditions. The *o*-xylene elimination capacity to maintain more than 90% removal efficiency was 41 g/m^3/h under sterile condition, but it enhanced to 160 g/m^3/h under non-sterile condition. This indicates possibilities of the role of other contaminants for degradation of *o*-xylene and the degradation of intermediate products of *o*-xylene by contaminants. Quick recovery of *o*-xylene degradation was observed after shutdown of *o*-xylene gas supply and mineral medium circulation for 10–30 days.

Keywords: *o*-Xylene; *Rhodococcus* sp.; Biofilter; Elimination capacity; BTEXS

1. Introduction

The atmospheric emission of volatile organic compounds (VOCs) from industrial processes causes the air quality deterioration, environmental pollution, and potential health risks. Representative conventional treatments of exhaust gases contaminated with VOCs include activated carbon adsorption, absorption, incineration, ozonation, condensation, and catalytic oxidation [1,2]. Biofiltration as a method of treating VOCs provides significant advantages over conventional techniques in terms of large air volumes and low gaseous pollutant concentrations if proper operational conditions are maintained [3]. Biofiltration requires also low capital and operating costs [4,5], is convenient for treating discontinuous emissions [6], and can be used to eliminate various VOCs simultaneously [7,8].

Among VOCs, xylene (or dimethylbenzene), including *o*-, *m*- and *p*-xylene isomers is used as solvent in the printing, rubber, synthetic fiber, plastics, insecticide, pesticide and leather industries and as a cleaner and paint thinner. Xylene is toxic to the liver, kidneys and the central nervous system when it enters the body by skin contact or inhalation [9]. The industrial emission of xylene in the atmosphere is estimated to be second to that of toluene at about 48,000 metric tons per year in Japan [10]. Thus, several biofilters and biotrickling filters using microbial consortia have been used in xylene removal [1,6,11–20] and their maximum elimination capacity range is 60–78 g/m^3/h in relatively low loading range of 10–195 g/m^3/h. Among the three xylene isomers, *o*-xylene is considered to be biologically the least degradable. *Rhodococcus opacus* R7 [21], *Rhodococcus* sp. B3 [22], non-identified strain TRM1 and OX39 [23] and *Rhodococcus* sp. strain DK-1 [24,25], have been reported as *o*-xylene degrading bacteria. However, no application of these isolated strains to the biofiltration of *o*-xylene has been investigated.

In this study, we report removal characteristics of an *o*-xylene-degrading bacterium, *Rhodococcus* sp. strain BTO62 for *o*-xylene and other mono-ring aromatic hydrocarbons, such as benzene, toluene, ethylbenzene, *m*- and *p*-xylenes, and styrene, in a shaken flask experiment. Then, BTO62 was applied to a biofilter to evaluate *o*-xylene removal efficiency under a relatively high loading of *o*-xylene under non-sterile and sterile conditions.

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2. Materials and methods

2.1. Culture media

Nutrient broth (NB) medium which was used for preculture was composed of 5 g of meat extract (Wako Pure Chemical Industries, Ltd., Japan) 10 g of Polypeptone (Wako Pure Chemical Industries, Ltd., Japan) and 5 g of NaCl in 1 L of distilled water. Nutrient agar (NA) added to 15 g of agar in 1 L NB medium was used for counting cell number. Basal mineral (BM) medium consisted of the following in 1 L of distilled water: 1.55 g K_2HPO_4, 0.85 g $NaH_2PO_4{\cdot}2H_2O$, 2.0 g $(NH_4)_2SO_4$, 0.1 g $MgCl_2{\cdot}6H_2O$, and a trace mineral solution (TMS) 1 mL. TMS was composed of the following in 1 L distilled water: 10 g EDTA, 2.0 g $ZnSO_4{\cdot}7H_2O$, 1.0 g $CaCl_2{\cdot}2H_2O$, 5.0 g $FeSO_4{\cdot}7H_2O$, 0.2 g $Na_2MoO_4{\cdot}2H_2O$, 0.2 g $CuSO_4{\cdot}5H_2O$, 0.4 g $CoCl_2{\cdot}6H_2O$, and 1.0 g $MnCl_2{\cdot}4H_2O$. BM medium containing *o*-xylene as sole carbon and energy sources was used for screening *o*-xylene-degrading bacteria in batch experiments. The pH values of all media were adjusted to 7.0 ± 0.2.

2.2. Biofilter setup for enrichment of o-xylene degraders and isolation

One biofilter shown in Fig. 1 was used to enrich *o*-xylene-degrading bacteria. The packing material used was Biosol® (Nishinihon Engineering Co., Japan), which is made from foamed waste glass mixed with corrugated cardboard. Property of Biosol is shown in Table 1. Before packing Biosol into the biofilter, Biosol (180 g) was sterilized with an autoclave four times at 120 °C for 60 min and mixed with 40 mL of an activated sludge (1.5% (w/v)) from a wastewater treatment plant (Naruse, Tokyo, Japan) in a beaker. After standing overnight, Biosol was packed to a glass column (5 cm in diameter × 30 cm long). *o*-Xylene vapor was generated by flowing air from a compressor into a generating bottle containing *o*-xylene solution and was diluted with a secondary air stream from a compressor to an appropriate concentration. *o*-Xylene vapor was supplied to the biofilter with down flow until 100% removal efficiency was achieved by controlling both *o*-xylene concentration and flow rate using a needle valve on the flow meter. The medium was supplied at several velocities by peristaltic pump and optimum flow rate was determined, as 200 mL/min because that was a minimum velocity at which non-drying at the upper of bed was observed. Under this condition, the moisture content of the biofilter was ~45%. To keep this moisture content of the biofilter and to supply mineral nutrients to the biofilter, 500 mL of BM medium was recycled from the top of the column using a peristaltic pump at a flow rate of 200 mL/min and a fresh medium was replaced every 2–3 days.

Table 1
Properties of Biosol used as a packing material

	Biosol
Bulk density	460 g-dry/L
Water holding capacity	35–50%
Composition	Glass and cardboard
Specific surface area	6.0×10^3 m^2/m^3
Average diameter	4–6 mm

One gram of the packing material was taken out of the biofilter and incubated in 30 mL of BM medium in a 100-mL conical flask containing 400 μL of *o*-xylene in a glass tube (5 mm in diameter × 20 mm long) at 30 °C at 120 strokes/min (spm). When the culture was turbid, the culture broth was transferred to fresh BM medium at a 1% inoculation ratio and incubated under the same conditions. This procedure was repeated three times, and the resulting culture was spread on NA plate and incubated at 30 °C. Colonies that grew on the plates were selected on the basis of their morphology. Each isolate was incubated in a 100-mL shaken flask containing 30 mL of BM medium supplemented with 400 μL of *o*-xylene in a glass tube in a similar way previously reported [41] at 30 °C at 120 spm. The strain BTO62 was selected as the best candidate strain of *o*-xylene degraders, because it grew fastest and had the highest *o*-xylene degradation rate. The identification of the strain BTO62 was carried out by the National Collection of Industrial and Marine Bacte-

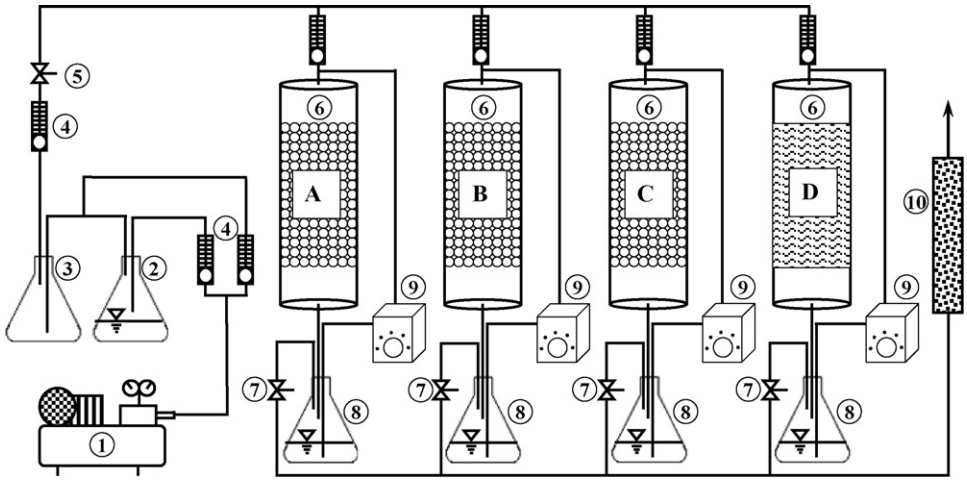

Fig. 1. Schematic diagram of biofilter: (1) air compressor, (2) xylene generator, (3) mixing chamber, (4) flow meter with needle valves, (5) inlet sampling port, (6) column, (7) outlet sampling port, (8) leachate and nutrient, (9) peristaltic pump and (10) activated carbon tower.

ria (NCIMB), Japan Co., Ltd. BTO62 was preserved in a 15% glycerol solution at −80 °C.

2.3. Cultivation of strain BTO62 in o-xylene

Stocked BTO62 100 μL glycerol solution was inoculated into 10 mL of NB medium in a test tube (16 mm in diameter × 18 cm long) containing 1 μL of *o*-xylene and incubated at 30 °C at 120 spm for 3 days. Cultured broth was collected by centrifugation at 10,000 rpm for 30 min, and the cells were washed two times with sterile deionized water. The washed cells were suspended in 1 mL of deionized water, and the suspension was inoculated into 30 mL of the BM medium in a 100-mL shaken flask with a glass tube containing 400 μL of *o*-xylene and incubated at 120 spm at 30 °C. One milliliter of the culture was sampled periodically and optical density and pH were measured using a spectrophotometer (UV-1200 UV Spectrophotometer, Shimadzu, Japan) at 660 nm (OD_{660}) and a pH meter (Φ300 pH Meter, Beckman, USA), respectively. A portion of the culture was serially diluted in deionized water and spread on NA plates, which were incubated at 30 °C for 72 h to count the number of viable cell.

2.4. Determination of degradation rate of o-xylene and other mono-ring aromatic hydrocarbons by BTO62

The washed cell suspension of BTO62 obtained by the same procedure described above was inoculated into 30 mL BM medium in a 100-mL-sealed shaken flask containing 6 μL of *o*-xylene and incubated at 30 °C at 120 spm. BTO62 was cultured in a BM medium containing other xylene isomers, *m*- and *p*-xylenes, or four mono-ring aromatic hydrocarbons such as ethylbenzene, benzene, toluene and styrene under the same culture conditions. The mixtures of each BTEXS with *o*-xylenes were also used as substrates for BTO62. The substrate degradation rate was determined from the maximum slope divided by the final cell number in the plot of substrate concentration versus time. Control experiments were carried out without BTO62 under the same conditions.

2.5. Biofilter operation for o-xylene removal by strain BTO62

The strain BTO62 was precultured in 200 mL of NB medium in a 500-mL shaken flask at 30 °C at 120 spm. The washed cell suspension obtained by the same method described above was suspended in 100 mL of BM medium. Then, the cell suspension was mixed with the sterile 180 g of Biosol in a beaker and incubated at room temperature for 1 day. Three laboratory-scale biofilters (A–C) packed with Biosol as shown in Fig. 1 were operated under non-sterile conditions at different flow rates and SV of 153, 76 and 30 h^{-1}. The initial operating conditions of each column are shown in Table 2. *o*-Xylene concentration and flow rate were controlled by the same method of enriching *o*-xylene-degrading bacteria as described above. Inlet *o*-xylene loading was changed in the range of 8–335 $g/m^3/h$ by adjusting *o*-xylene concentrations (0.3–11.8 g/m^3). One column (D) was operated under sterile condition in which air was filtered through a 0.2 μm filter (Tokyo Roshi Kaisha, Ltd., Japan) under the same condition as column B (Table 2).

Table 2
Initial operating condition of each biofilter

	A	B	C	D
Packing material	Biosol	Biosol	Biosol	Biosol (sterile)
Viable cell number (cfu/g dry material)	7.3×10^6	7.3×10^6	7.3×10^6	2.2×10^7
Flow rate (mL/min)	1000	500	200	500
SV[a] (h^{-1})	153	76	30	76
LV[b] (m/h)	31	15	6	15
EBRT[c] (s)	24	47	118	47

[a] Space velocity.
[b] Linear velocity.
[c] Empty bed residence time.

2.6. Analytical methods

Headspace gas samples of all the VOCs were obtained using gas-tight syringes from shaken flasks. Their concentrations in the flasks were calculated using Henry's partition coefficient (HC) based on headspace of gas concentrations determined by gas chromatography (GC) (GC-14A, Shimazu, Japan). Values of HCs at 30 °C for toluene, benzene, ethylbenzene, *o*-, *m*-, and *p*-xylenes, and styrene are 0.15, 0.16, 0.12, 0.19, 0.13, 0.13 [26], and 0.37 [27], respectively. In the biofilter experiment, 0.2–5 mL samples were taken from sampling ports at the inlet and outlet of the biofilter using 0.5, 1.0, 2.5 and 5 mL gas-tight syringes. *o*-Xylene and VOC concentrations were determined using the GC equipped with a flame ionization detector (FID) and a 30 m URBON HR-1 capillary column (0.53 mm i.d., 3 μm phase thickness). The GC conditions used were as follows: 45 mL of He per min as a carrier gas, injector and detector temperatures of 150 °C, an oven temperature of 50 °C, a thermal gradient of 10 °C/min to 230 °C, and finally the temperature was maintained at 150 °C for 9 min.

Viable cell number in the Biosol biofilter was determined by the following procedure. About 3 g-wet weight of the packing material was homogenized in a homogenizer (EX-3, Nihon Seiki LTD., Japan) in 10 mL of sterilized distilled water at 10,000 rpm for 3 min, and the homogenized suspension was serially diluted with sterilized distilled water and spread on NA plates. After a 72-h incubation at 30 °C, total cell number was calculated from colonies that grew on the plates and was expressed as colony forming units (cfu) per gram dry packing material. The strain BTO62 was distinguished from other contaminants by the difference in morphology and color and the cell number of the strain BTO62 was also calculated on the same plates.

3. Results

3.1. Identification and characteristics of BTO62

One isolate of BTO62 was an irregular gram-positive nonmotile bacillus. The morphology of the colonies was regular,

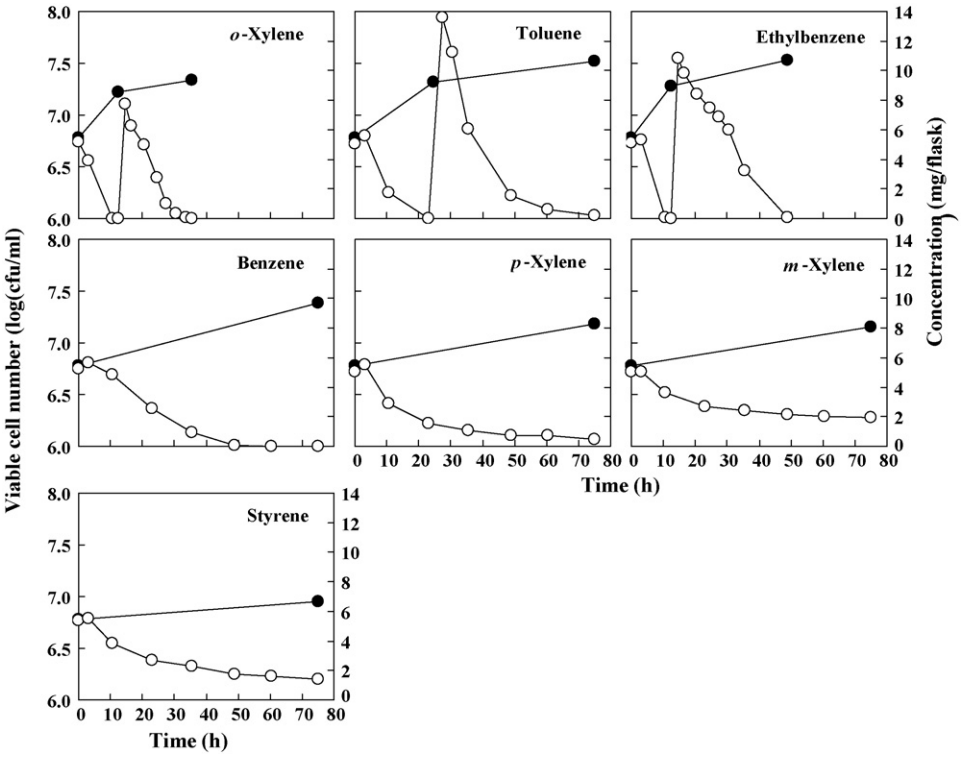

Fig. 2. Changes in viable cell number (closed circles) and ethylbenzene, *o*-, *m*-, and *p*-xylenes, benzene, toluene, and styrene concentrations (open circles) in BM medium inoculated with BTO62 in sealed flask experiments.

pinkish, non-light, hemispheric, opaque, and the surface was rough. The isolate was rod-shaped (0.8–1.0 μm × 1.5–2.0 μm and 1.0 μm × 1.0–1.2 μm after 24 and 72 h incubations, respectively), catalase test-positive, oxidase test-negative, alkaliphosphatase test-positive, and β-glucosidase test-positive. BTO62 was classified as *Rhodococcus* from these results and the 16S rDNA analyses using MicroSeq and GenBank/DDBJ/EMBL.

Fig. 2 shows the time courses of toluene, benzene, ethylbenzene, *o*-, *m*- and *p*-xylenes and styrene (BTEXS) concentrations and the viable cell number of strain BTO62 when BTO62 was cultured with each BTEXS in a sealed flask. *o*-Xylene, benzene, ethylbenzene and toluene were completely degraded after 10, 50, 10 and 22 h, respectively. Although *p*- and *m*-xylenes and styrene remained even after 75 h, the increase in viable cell number indicates that BTO62 can degrade and assimilate those substrates. A mixture of *o*-xylene and each BTEXS was supplied to sealed flasks and specific degradation rates with those substrates added to *o*-xylene are shown in Table 3. The degradation rates were not significantly different from those in single supply. On the other hand, the specific degradation rate of *o*-xylene decreased slightly with the addition of BTEXS substrates except for toluene as shown in Table 4. The specific degradation rate of *o*-xylene mixed with toluene was 10-fold higher than that of pure *o*-xylene.

Table 3
Specific degradation rates of each compound either in single or mixed supplies with *o*-xylene in sealed flask batch experiment

Substrates	Specific degradation rate (μmol/h/cell)	Specific degradation rate (μmol/h/cell)
	Single supply	Mixed supply with *o*-xylene
m-Xylene	1.3×10^{-7}	1.1×10^{-7}
p-Xylene	2.4×10^{-7}	1.3×10^{-7}
Benzene	1.1×10^{-7}	1.3×10^{-7}
Toluene	3.8×10^{-7}	2.1×10^{-7}
Ethylbenzene	3.9×10^{-7}	2.0×10^{-7}
Styrene	2.7×10^{-7}	2.0×10^{-7}

3.2. Performance of o-xylene removing biofilter inoculated with BTO62 under non-sterile condition

o-Xylene was supplied under non-sterile condition to the biofilters with a constant gas flow rates of 0.06 m^3/h (SV of 153 h^{-1}), 0.03 m^3/h (SV of 76 h^{-1}) and 0.012 m^3/h (SV of 30 h^{-1}). *o*-Xylene removal efficiency in the biofilter at SV of 76 h^{-1} for 86 days is shown in Fig. 3. The inlet concentrations of *o*-xylene supplied were in the range of 0.3–4.4 g/m^3. More than 90% *o*-xylene removal efficiency was achieved after 7 days at an average inlet loading of 21 g/m^3/h. After 11 days, the inlet loading was increased to 148 g/m^3/h, and the removal efficiency decreased to 55%, but increased to 90% 3 days later. After that, the average inlet loadings were increased to 224 and 281 g/m^3/h, and removal efficiencies decreased to 76% and 53%, respectively. During the period from 32nd to 41st day, *o*-xylene gas supply and mineral medium circulation were stopped for 9 days to investigate the effect of shutdown of the biofilter. The removal efficiency of the biofilter returned to the same state as

Table 4
Specific degradation rates of *o*-xylene in single supply or a mixed supply with other VOCs in sealed flask batch experiment

Substrate	Specific removal rate (μmol/h/cell)	Substrate	Specific removal rate (μmol/h/cell)
o-Xyl (5.17 g)[a]	3.7×10^{-7}	*o*-Xyl (5.17 g) + Bz (5.25 g)	3.1×10^{-7}
o-Xyl (5.17 g) + Tol (5.06g)	3.3×10^{-6}	*o*-Xyl (5.17 g) + Eb (5.11 g)	1.9×10^{-7}
o-Xyl (5.17 g) + *m*-Xyl (5.08 g)	2.6×10^{-7}	*o*-Xyl (5.17 g) + *p*-Xyl (5.06 g)	2.8×10^{-7}
o-Xyl (5.17 g) + Sty (5.39 g)	2.1×10^{-7}		

o-Xyl:*o*-xylene, Tol: toluene, *m*-Xyl: *m*-xylene. Sty: styrene, *p*-Xyl: *p*-xylene, Eb: ethylbenzene.
[a] Concentration of each substrate in flask.

before shutdown within 2 days after re-supplying *o*-xylene and mineral medium. After 41st days, inlet loading was changed arbitrarily to evaluate maximum elimination capacity for more than 90% removal efficiency. The results are shown in Fig. 5 together with the data obtained from Fig. 4.

The *o*-xylene removals conducted under non-sterile condition at a SV of 153 h^{-1} for 41 days and at SVs of 30 h^{-1} for 140 days are shown in Fig. 4A and B, respectively. In the biofilter with a SV of 153 h^{-1} (Fig. 4A), the inlet concentrations of *o*-xylene supplied were in the range of 0.3–1.4 g/m^3. After 17 days, more than 90% xylene removal efficiency was achieved at the inlet loading of 42 g/m^3/h. After 25 days, inlet loading was increased to 150 g/m^3/h, but removal efficiency decreased to 50%. Then, the inlet loading was gradually decreased to 75 g/m^3/h and 100% removal efficiency was obtained, and then the biofilter was stopped after 41 days of operation. In the biofilter set at a SV of 30 h^{-1} (Fig. 4B), the inlet concentration of *o*-xylene was supplied in the range of 0.3–11.8 g/m^3 for 140 days. After 6 days, the removal efficiency increased to 99% and the inlet loading of *o*-xylene gradually increased to 335 g/m^3/h until 97th day. Whenever the inlet loadings were increased, the removal efficiencies decreased temporarily, but they were recovered to 99% soon. During the periods from 32nd to 41st day and from 97th to 134th day, *o*-xylene gas supply and mineral medium circulation were stopped to investigate the effect of shutdown of the biofilter. Removal efficiencies in the two shutdown experiments were recovered to the same state as before shutdown within 1 day after re-supply of *o*-xylene and mineral medium.

The relationship between elimination capacity and inlet loading of *o*-xylene at the three SVs is shown in Fig. 5. Under non-sterile condition, the elimination capacities for more than 90% removal efficiency are 80, 160 and 180 g/m^3/h at SVs of 153, 76 and 30 h^{-1}, respectively. Compared to the values of *o*-xylene removal capacities for more than 90% removal efficiency at SVs of 76 and 30 h^{-1}, the value was considerably lower at a SV of 153 h^{-1}. Elimination capacities reported previously were 60–78 g/m^3/h as shown in Table 5. These researches used a microbial consortium as seed and supplied a mixture of xylene isomers. In this study, higher elimination capacity was obtained using an isolated strain and supply of pure *o*-xylene under non-sterile conditions, suggesting that the strain BTO62 has potential to effectively remove *o*-xylene by biofiltration.

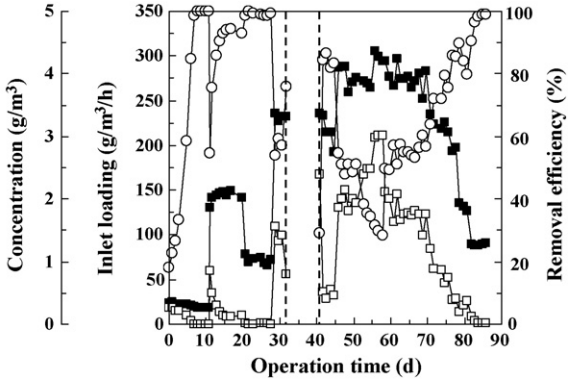

Fig. 3. Biofilter performances using strain BTO62 for *o*-xylene removal at a SV of 76 h^{-1}. Closed squares, inlet *o*-xylene concentration and load; open squares, outlet *o*-xylene concentration; open circles, *o*-xylene removal efficiency; dotted line, shutdown period.

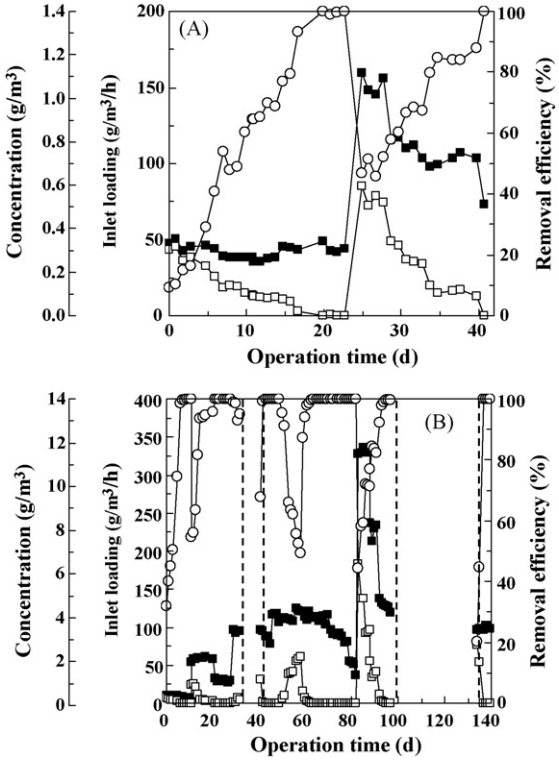

Fig. 4. Biofilter performances using strain BTO62 for *o*-xylene removal at SVs of 153 h^{-1} (A) and 30 h^{-1} (B). Closed squares, inlet *o*-xylene concentration and load; open squares, outlet *o*-xylene concentration; open circles, *o*-xylene removal efficiency.

Table 5
Data of xylene removal reported previously in biofilters

EBRT[a] (s)	Packing materials (diameter)	Seed	Inlet loading (g/m^3/h)	EC[b] (g/m^3/h)	Refs.
67	Peat balls (5–10 mm)	Microbial consortium	25–195	60 (Maximum)	[11]
56–150	Peat	Microbial consortium	34–95	61 (93% of RE[c])	[6]
Not Shown	Pall rings	Microbial consortium	20–120	78 (67% of RE)	[14]
63–157	Peat	Microbial consortium	10–110	67 (Maximum)	[1,20]

[a] EBRT: empty bed retention time.
[b] EC: elimination capacity.
[c] RE: removal efficiency.

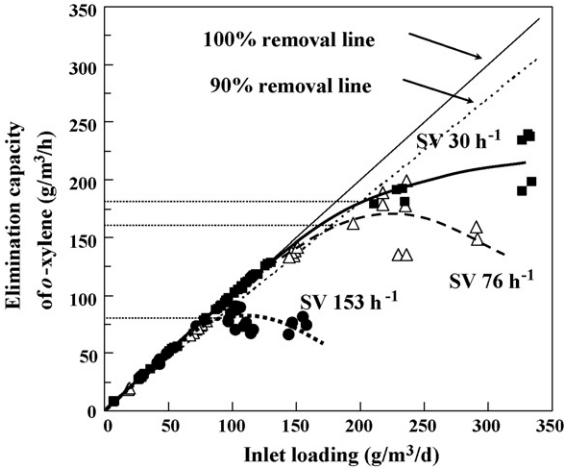

Fig. 5. Relationship between elimination capacity and inlet loading of *o*-xylene at SVs of 153 (closed circles), 76 (open triangles) and 30 h^{-1} (closed squares) in non-sterile Biosol packed biofilter.

3.3. Comparison of non-sterile and sterile biofilters

Under sterile condition, a biofilter inoculated with strain BTO62 was operated at a SV of 76 h^{-1} as shown in Fig. 6. Removal efficiency of the biofilter under sterile condition could not attain 90% even for a lower *o*-xylene loading of 49 g/m^3/h, whereas 100% removal efficiency attained in the non-sterile biofilter. After 9 days, the SV was decreased to 30 h^{-1} and the removal efficiency reached almost 100%. However, as contamination with other bacteria was observed on day 27, the experiment was stopped. The relationship between elimination capacity and inlet loading in the sterile biofilter is shown in Fig. 7. The *o*-xylene elimination capacity under sterile condition for more than 90% removal efficiency was 41 g/m^3/h, which is significantly lower than that of 160 g/m^3/h at a SV of 76 h^{-1} under non-sterile condition.

4. Discussion

Rhodococcus sp. BTO62 isolated from activated sludge from a wastewater treatment plant was able to effectively degrade *o*-xylene, which is the isomer of xylene most recalcitrant to microbial degradation. BTO62 was also able to degrade benzene, toluene, ethylbenzene, *m*-, *p*-xylenes and styrene (BTEXS). The specific degradation rates of *o*-xylene, toluene and ethylbenzene were similar and higher than those of *m*-, *p*-xylenes, benzene and styrene (Tables 3 and 4). However, these degradation rates of BTEXS seemed to decrease when BTEXS were mixed with *o*-xylene. The specific degradation rates of *o*-xylene mixed with other substances tested in this study were lower than that of pure *o*-xylene except for toluene (Table 4). The specific degradation rate of *o*-xylene was 10-fold higher in the mixture of toluene. The exact reason of the higher specific degradation rate of *o*-xylene in a mixture of toluene is not clear, but we assume that BTO62 co-oxidization of *o*-xylene with toluene enhanced the degradation of *o*-xylene, as reported previously [42]. The genus *Rhodococcus* exhibits a diverse range of metabolic activi-

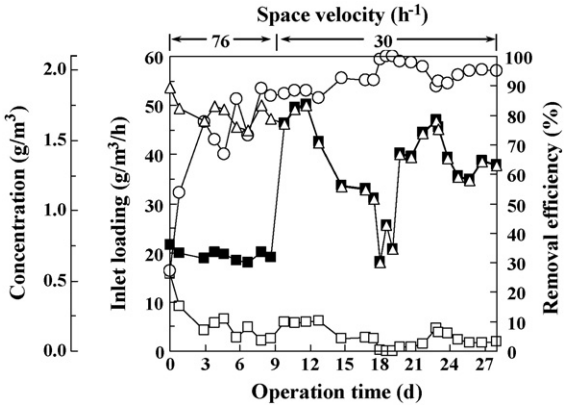

Fig. 6. Biofilter performances using strain BTO62 for *o*-xylene removal ata SV of 78 h^{-1} under sterile condition. Closed squares, inlet *o*-xylene concentration and load; open squares, outlet *o*-xylene concentration; open circles, *o*-xylene removal efficiency.

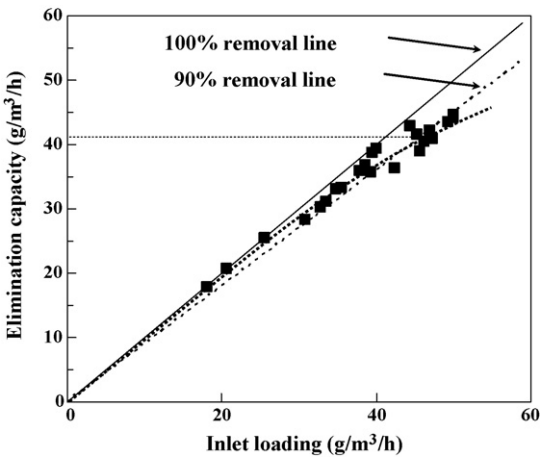

Fig. 7. Relationship between elimination capacity and inlet loading of *o*-xylene in sterile Biosol packed biofilter.

ties, including the degradation of various aromatic hydrocarbons [24,28,29]. The substrate specificity of strain BTO62 was similar to that of *Rhodococcus* sp. DK17, which can utilize mono-cyclic aromatic hydrocarbon such as benzene, toluene, phenol, ethylbenzene and isopropylbenzene, but not *m*- and *p*-xylenes [24], suggesting involvement of the mono-oxygenases and dioxygenases in *o*-xylene degradation [25,30–33].Laboratory scale biofilters inoculated with strain BTO62 were run to remove *o*-xylene at different space velocities under non-sterile condition. At each time when the inlet loading was increased, removal efficiency was remarkably decreased, indicating that inducing time was necessity to accommodate for increased *o*-xylene loading. After that, the removal efficiency tended to increase to a stable state. When inlet loading was increased artificially after 41st day to evaluate maximum elimination capacity, removal efficiency became unstable, but it was gradually recovered by decreasing the inlet loading (Fig. 3). The maximum elimination capacities of the non-sterile three biofilters at more than 90% removal efficiency were 80, 160 and 180 g/m^3/h at space velocities of 153, 76, and 30 h^{-1}, respectively. The chemical and physical adsorption to packing material was estimated without inoculation of BTO62 under gas flow conditions at 100 mL/min and with *o*-xylene inlet load of 2.3 g/m^3/h. At equilibrated time, when the outlet concentration of *o*-xylene became constant, the removal rate was 0.09 g/m^3/h, only 4% of the inlet load. Therefore, the *o*-xylene removal in the biofilter inoculated with BTO62 was judged to be mostly biological.

When BTO62 was applied to biofilter at SV 76 h^{-1} under sterile condition, the removal efficiency was about 80%. When the SV was reduced to 30 h^{-1}, the removal efficiency increased to 90–100%, indicating high load gave an adverse effect on activity of BTO62 (Fig. 6). The elimination capacities of *o*-xylene to maintain greater than 90% removal efficiency were 41 g/m^3/h and 160 g/m^3/h at a SV of 76 h^{-1} under sterile and non-sterile conditions, respectively. The enhanced removal efficiency under non-sterile condition presumably was due to existence of other microorganisms including fungi capable of *o*-xylene degradation as reported previously [43,44], although fungal degradation of *o*-xylene has been studied to a limited extent.

Another possibility is that the products degraded by *o*-xylene accumulated in biofilter were removed by contaminants.

In this study, the inoculated strain, BTO62 was contaminated with airborne microbes but still the activity of BTO62 was maintained during experimental periods. The viable cell number of BTO62 was estimated to be 5.8×10^7 cfu/g in the sterile biofilter and was 3.6×10^7 cfu/g in non-sterile biofilter which was 2.3% of the total cell number in the non-sterile biofilter. The cell number of BTO62 was almost the same in both biofilters. The apparent specific *o*-xylene degradation rates under sterile condition was estimated to be 1.5×10^{-8} μmol/h/cell which was one-tenth of the specific *o*-xylene degradation rate of BTO62 that in sealed flask batch experiment (3.7×10^{-7} μmol/h/cell) using a pure culture of BTO62. This indicates that the accumulated intermediates derived from *o*-xylene degradation in the biofilter may give adverse effect of the activity of BTO62. Thus, it is speculated that under non-sterile condition the enhanced *o*-xylene degradation may be partly due to degradation of intermediates by other contaminants. When the circulation medium of sterile biofilters was extracted with ethyl acetate, and analyzed by GC [41], several peaks were detected in the extract. However, no peak was detected in the non-sterile biofilter (data not shown). This similar phenomenon was observed in the previous paper [41].

Under the higher space velocity, the contact time of *o*-xylene with its degrader was shortened, which resulted in the lower *o*-xylene removal in the biofilter. These results indicate that the diffusion of *o*-xylene into water is a limiting step in biofilters when operated in high flow rates [2,6,7,20,34]. Thus, gas flow rate is an important parameter in optimum operation of biofiltration.

The packing material in biofilters is an important factor to maintain microbial activities. Packing materials must fulfill following requirements for efficient removal of gases: (a) enough area to sustain growth and the activity of microorganisms; (b) particle size not to provoke high pressure drop in the packed bed; (c) capable of resisting compaction to avoid high bed pressure drop and to allow uniform air flow distribution through the bed.

Packing materials used in biofilter beds can be categorized as either “organic” or “inorganic”. Organic packing materials include natural products such as soil, peat, bark, and compost which have advantage of containing microorganisms indigenous to each material and some nutrients available for microbial growth. However, loss of weight due to their degradation requires replacement of fresh ones. On the other hand, the inorganic packing materials such as perlite, vermiculite, rashig rings, and others have the advantage of having a uniform structure and size which reduces compaction and allows better air flow distribution [35]. High elimination capacities of *o*-xylene for Biosol reflects these advantages of this material, and especially a specific surface area in Biosol is approximately 10-fold larger than the surface areas of most of inorganic materials [35–40].

Microbial xylene breakdown is complex, consisting of oxidation of methyl group and ring cleavage, and these intermediates compounds formed also result in toxic and inhibitory to the cells [22,25,30–33]. Therefore, it is speculated that the acclimation time to reach steady state will take longer time in xylene removal biofilter. The acclimation time in most of previous researches on xylene degradation biofilters using microbial consortium was varied from 1 week to 30 days under relatively lower loading and lower SV conditions. In this study, the acclimation times were varied from 7 to 20 days in the range of SVs 30–153 h^{-1} under relatively higher loading conditions. One advantage of the inoculation of specific microorganisms is to be able to impose relatively high load from the start of experiments and to shorten the acclimation period.

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