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RESEARCH PAPER

Bacterial Community Diversity and Functional Gene Abundance of Structured Mixed Packing and Inert Packing Materials Based Biotrickling Filters

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Abstract Packing is the most important factor in biofilter design. A structured mixed packing (SMP) material, combined with various organic and inorganic materials (mineral matter is 80.18 ± 0.48%, w : w), was constructed by urea-formaldehyde resin in order to minimize the disadvantages of these materials when used as stand-alone components. The performance of the toluene biotrickling filter (BTF) packed with SMP was compared with the other BTFs packed with a ceramic raschig ring, ceramic pall ring, and lava rock, respectively, for 217 day under various operating conditions. Real-time PCR and DGGE techniques were applied to reveal the gene coding for the toluene-degrading enzymes and the bacterial community structure in the BTFs. The toluene-degradation gene copies exponentially increased, and bacterial diversity significantly decreased with the improving elimination capacities of the BTFs. The overload and shutdown operations resulted in insignificant fluctuations in the toluene-degradation gene copies at equal levels as well as a slight variation in the bacterial community structures in the BTFs. Various putative toluene-degrading bacteria were found using sequencing bands from the DGGE gels; some bacteria, such as *Burkholderia* spp., were further confirmed by real-time PCR; other bacteria, such as *Alcaligenes* spp., might not have been reported. The packing properties of SMP material supported more toluene-degradation gene copies in the biofilm, and higher toluene-degrading bacterial diversity of the BTF, than did inert packing. Thus, the BTF with SMP demonstrated excellent performance, suggesting the suitability of SMP for real applications, whereas the capabilities of inert packing materials are more suited to the treatment of steady low VOC loads.

Keywords: toluene biotrickling filtration, structured mixed packing, inert packing material, real-time polymerase chain reaction, denaturing gradient gel electrophoresis

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1. Introduction

Packing materials play a key role in waste gas treatment. Packing media used for waste gas biofiltration are typically divided into three categories: organic, inert, and mixed packing materials [1,2]. The early biofilters are usually packed with natural organic packing, such as soil, compost, peat, and wood chips. These allow the biofilter a quick startup, high removal efficiency, high elimination capacity, and short recover period on waste gas treatment [1-4]. Bed compaction often occurs in organic packing media after a period of operation, limiting the application of organic

packing materials [5]. Inert packing materials, such as perlite, ceramic, granular activated carbon, polyurethane foam, and lava rocks, have been used by workers, with varying degrees of success [6,7]. The advantages of inert packing media include physical intensivity, chemical stability, and minimum bed compaction. However, most synthetic inert packing materials are more expensive than natural organic packing materials [5]. To minimize bed compaction and avoid clogging, several researchers have obtained mixed packing by mixing with organic packing materials and the inert packing materials of large particle size, such as glass beads, polystyrene spheres, and lava rocks [8-11].

Packing properties significantly characterize microorganism community structures in biofilters [2]. Several molecular techniques that do not depend on cell culture have been widely used to analyze microbial community structures during biofiltration. The PCR-DGGE technique of 16S rDNA fragments has been used to determine total bacterial community composition. Thus, it can be used to explore bacterial diversity in various environmental samples and population shifts in response to environmental changes in the operation course of bioreactors [12-16].

The expression and activity of the degrading enzymes of VOCs in biofilm on the surface of packing determine the *RE* and *EC* of these compounds in biofiltration [5,20]. Real-time PCR offers an accurate, sensitive, and non-culture-based method of quantifying biogeochemical, ecological, and environmental processes [17-19]. Toluene biofiltration consists of both aerobic and anaerobic degradation. The initial attack of toluene degradation contains toluene/benzene/chlorobenzenemonooxygenase (TMO), toluene/benzene/chlorobenzene dioxygenase (TOD) [20-22], or benzoyl-coenzyme A (benzoyl-CoA) [23,24].

Despite several published works on the *RE* and *EC* of biofilters packed with inert and organic packing, respectively, few reports have examined bacterial community diversity and the functioning gene abundance of structured mixed packing in biofitration. Previous studieshave evaluated the long-term performance of four toluene biotrickling filters (BTFs) packed with structured mixed packing (SMP) materials (namely, ceramic raschig ring, ceramic pall ring, and lava rock for 217 day) under various operating conditions; these data were obtained by reference [25]). The objective of the present study is to apply real-time PCR and PCR-DGGE in order to investigate functional gene abundance and bacterial community structure in toluene biotrickling filtration, which is used to characterize the variations in the *RE* and *EC* of the BTFs packed with varied packing materials.

2. Materials and Methods

2.1. Packing materials

The SMP material contained coral rock, bark, ceramisite, charcoal, and compost (160 : 120 : 100 : 60 : 15, w : w). The urea-formaldehyde resin (2 ~ 3%, w: w) was dissolved to the same weight as water and then added to the mixed materials. The mixture was fully stirred, parched dry, and formed into an SMP structure. Detail composition and packing properties of SMP were shown in the reference [25]. The ceramic pall ring, ceramic raschig ring, and lava rock are commercially available in China (Table 1).

2.2. System configuration and operating conditions of the BTFs

Experiments were carried out in four laboratory-scale BTFs. The upper PVC column (20.00 cm *ID*; 50.00 cm *H*) was loaded, with four types of packing materials, to a height of 31.85 cm and a total bed volume of 10 L. The lower PVC column (20.00 cm *ID*; 40.00 cm *H*) contained a nutrient solution of about 31.85 cm in height. The total liquid volume was 10 L. Inlet gas sampling port was set to a height of 35.00 cm in the under column, and an outlet gas sampling port was set to a height of 45.00 cm in the upper column.

Compressed air was humidified by water, and pure liquid toluene (Panreac, 99.5%) was stripped by compressed air. Both compressed air and toluene was mixed together into one primary gas flow; the resulting gas mixture was fed to

Table 1. Initial characteristics of the four packing materials*

Parameter	Raschig ring (r)	Pall ring (p)	Lava rock (L)	SMP (s)
Natural organic matter (%, w:w)	0	0	0	19.82 ± 0.48
Size (mm)	$\Phi(25) \times H(25) \times (3)$	$\Phi(25) \times H(25) \times (3)$	$25 < S\Phi < 30$	30 × 30 × 30
Water-holding capacity (g/g)	$3.55 \pm 0.17 \times 10^{-3}$	$6.63 \pm 0.32 \times 10^{-3}$	0.166 ± 0.011	0.510 ± 0.024
Wet maximum sorption (mg/g)	-	-	-	20.34 ± 1.11
Conductivity (S)	22.0 ± 1.0	21.6 ± 1.1	30.9 ± 2.2	83.8 ± 4.2
Buffer capacity (mmol SO_4^{2-}/L)	20.13 ± 1.43	25.33 ± 1.78	30.15 ± 0.52	65.95 ± 3.29
pH	6.030 ± 0.031	5.839 ± 0.027	7.211 ± 0.145	6.480 ± 0.373

*These data was shown in reference [25].

Table 2. The schedule of experiment

Section	Sequence days (day)	C (mg/m^3)	*EBRT* (s)	*IL* (g/m^3/h)
Progressive *IL*	1 ~ 105	24.68 ~ 5,330.38	60	1.48 ~ 319.82
Overload	106 ~ 181	5,330.38/5,330.38*	10/60*	1,918.94/319.82*
Shutdown	182 ~ 217	0/5,330.38*	0/60*	0/319.82*

*Conditions for convalescence: 5,330.38 mg m^{-3} C_{in} and 60 s *EBRT*.

the bottom of each bioreactor in an up-flow mode. The nutrient solution was replenished every week and continuously sprinkled onto the packing materials at 0.1 L/h using a peristaltic pump. The nutrient solution contained $NaNO_3$ (5 g/L), NaH_2PO_4 (1 g/L), KH_2PO_4 (1 g/L), NaCl (0.1 g/L), $MgSO_4$ (0.2 g/L), $CaCl_2$ (0.02 g/L), and a trace element solution (1 mL/L); its pH was 7.5. The trace element solution was sourced from a nonprescription drug named "Gold Theragran" (China drug approval no. H10930015). One Gold Theragran tablet was pounded and dissolved in 100 mL water as the trace element solution.

The schedule of experiments comprised progressive *IL*, lethal overload, and temporary shutdown (Table 2). In the Progressively *IL* phase, the BTF progressively contained 24.68, 148.07, and 888.40 mg toluene/m^3. Each toluene concentration section contained the decreasing residence times of 60, 30, 20, and 10 sec. The lethal overload phase involved 5330.38 mg/m^3 C_{in} and a decreasing residence time of 60, 30, 20, and 10 sec. At temporary shutdown, the medium was cycled for 1, 4, and 7 day while the waste gas was turned off. Conditions for convalescence (5330.38 mg/m^3 C_{in} and 60 sec empty bed residence time (*EBRT*)) were programmed between operation steps in the overload and shutdown phases, until the *EC* of BTFs was restored to EC_{max}.

2.3. Inoculum

Each BTF was inoculated with 1 L of activated sludge, containing 5 g TSS/L, from an urban wastewater treatment plant. This sludge was repeatedly trickled over the bed for 24 h.

2.4. Toluene concentration in air

Gas samples were collected from inlet and outlet gas sampling ports using a sample bag (Tedlar, USA). Toluene concentration was measured using gas chromatography

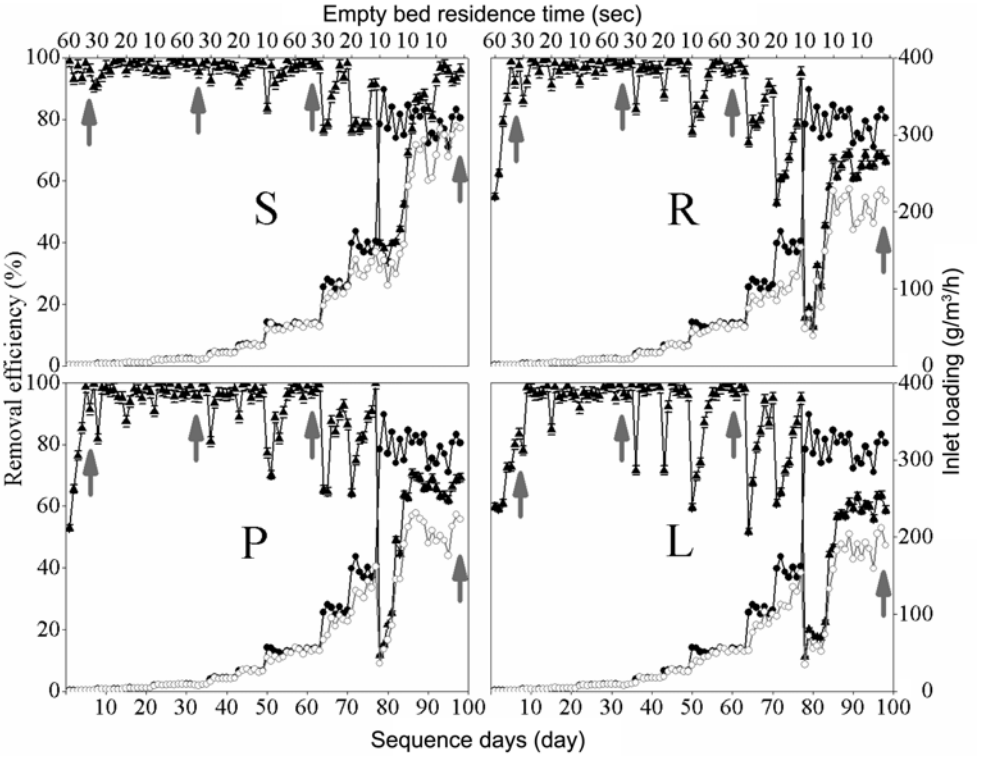

Fig. 1. Evolution of the *RE* and *EC* in the BTFs under progressive *IL*. S: *S*-filter, R: *R*-filter, P: *P*-filter, L: *L*-filter, (▲) RE, (●) *IL*, (○) *EC*, (↑) biofilms' sampling time (These data was shown in reference [25].).

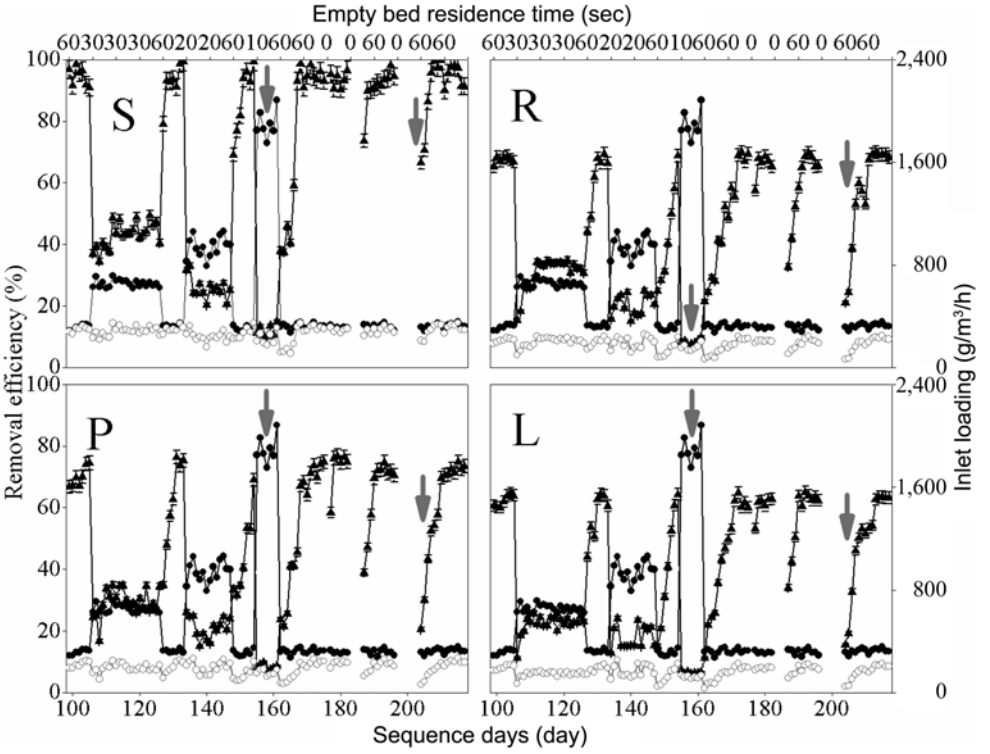

Fig. 2. Evolution of the *RE* and *EC* in the BTFs under overload and shutdown. S: *S*-filter, R: *R*-filter, P: *P*-filter, L: *L*-filter, (▲) RE, (●) *IL*, (○) *EC*, (↑) biofilms' sampling time (These data were shown in reference [25].).

(GC 5890, HP) equipped with a flame ionization detector and an HP-5 chromatographic column (25 mm × 0.32 mm, 0.52 μm). The column temperature was 180°C and the oven temperature was 60°C. Nitrogen was used as the carrier gas, injection volume was 50 μL, and the linear range of the method was 1 ~ 2,000 ppm (r^2 = 0.9985).

2.5. Extraction of total DNA from the biofilm

Biofilms were sampled from different packing materials after operating 7, 35, 63, 98, 161, and 203 days. The biofilms' sampling times involved three phases: progressive IL, overload, and shutdown. The sampling times loaded 1.48, 8.88, 53.30, 319.82, 1918.94, and 0 g toluene/m^3 packing material/h, whereas the *RE* and *EC* remained at a steady state for at least 7 days (Figs. 1 and 2). The sampling port was set to a biofilter bed height of 5 ~ 15 cm, which possessed the major bacterial populations in the biofiters. Each sampling bulk volume was 50 mL of every packing material sampled, which minimized the sampling's influence on biofilter performance Biofilm samples were isolated from packing materials according to the method [26]. Extraction, purification, and quantification of the genomic DNA in the samples were carried out at 0.5 g of biomass, using a EZgene™ Soil gDNA Kit (Biomiga, USA) as per the manufacturer's instruction. Genomic DNA preparation was determined and stored as a reference [27].

2.6. DGGE and phylogenic analysis of the bacterial community structures

PCR-DGGE was used to investigate the bacterial community structures in the biotrickling filtrations. Prior to DGGE analysis, the V6 region of 16S rDNA was amplified with primer set of 968f and 1401r, using a GC clamp. DGGE analysis of the PCR products was conducted as a reference [14]. PCR products were loaded onto 10% (w/v) polyacrylamide gels, with an inner denaturing gradient of 30 ~ 70%, and run in 1× TAE buffer (pH 7.4) at 60°C for 14 h at 80 V. After electrophoresis, the gels were stained, the bands were excised, and the 16S rDNA V6 region was used as a reference [14]. The sequences have been added to the Genbank™ database under accession numbers JF284270 to JF284349. The profiled alignment technique of the ClustalW method in Mega 5.0 was used to align the sequences [28]. The chimeric sequences were eliminated from the alignment according to the reference [29]. Phylogenetic trees were constructed with parsimony. The positions and signal intensities of bands in the gel track were determined with a Gel Doc 2000 gel documentation system and analyzed using Quantity One 4.6.2 (Bio-Rad, USA) in order to evaluate the bacterial community in the biotrickling filtrations at different stages. The Shannon index (H') of bacterial diversity was estimated from the intensity and number of bands and using the following

Table 3. Primers of aromatic oxygenase genes and expected sizes of real-time PCR products

Primer pair	Sequence (5′–3′)	Amplicon size (bp)	Reference
TMOA-F	CGAAACCGGCTTYACCAAYATG	505	[21]
TMOA-R	ACCGGGATATTTYTCTTCSAGCCA		
TODC1-F	CAGTGCCGCCAYCGTGGYATG	510	[21]
TODC1-R	GCCACTTCCATGYCCRCCCCA		
*bzA*Q4-F	GTGGGCACCGGNTAYGGNMG	485	[23]
*bzA*Q4-R	GGTTCTTGGCGAYNCCNCCNGT		
341F	CCTACGGGAGGCAGCAG	178	[40]
518R	ATTACCGCGGCTGCTGG		
968F*	AACGCGAAGAACCTTAC	433	[14]
1401R	CGGTGTGTACAAGACCC		

*GC-clamp: CGCCCGGGGCGCGCCCCGGGCGGGGCGGGGGCACGGGGGG.

equation [15,30]:

$$\text{Shannon index}(H') = \sum_{i=0}^{n} \frac{\frac{n_i}{N}}{\ln\frac{n_i}{N}}$$

where n_i is the peak height of each band i, i is the number of bands in each DGGE gel profile, and N is the sum of the peak heights in a given DGGE gel profile. Based on the individual bands and environmental factors [31], representational difference analysis (RDA) was performed using Canoco 4.5 (PRI Inc., USA).

2.7. Real-time PCR with SYBR Green I for the toluene-degrading enzyme genes

The initial attack of toluene includes TMO, TOD [20-22], or benzoyl-CoA [23,24]. The *tmo*A, *tod*C1, and *bzA*Q4 primer set were used to detect the gene coding for subunits of TMO, TOD, and benzoyl-CoA [20-23]. The 16s rDNA V3 region primer set was used to estimate the total bacteria in the BTFs (Table 3). Real-time PCR was performed as a reference [20] (linear range, 10 ~ 10^8 copies/μL; $r^2 > 0.99$). Experiments were repeated twice, and each sample was run in triplicate. The specificity of the PCR products was verified (apart from the dissociation curve analysis) using electrophoresis (data not shown).

3. Results and Discussion

3.1. Bacterial diversity and abundance of the initial stage of biotrickling filtration

The *RE* of the BTFs steadily increased to the elevated levels on the 7th day (*IL* was 1.48 g/m^3/h, Fig. 1). Bacterial community diversity in the BTFs characterized by the H' indices showed significant distinction among the different packing materials (Table 4; Fig. 3). The toluene-degradation gene copies and total biomass in the BTFs, detected by real-time PCR, reached identical magnitudes among the different packing materials (Fig. 4).

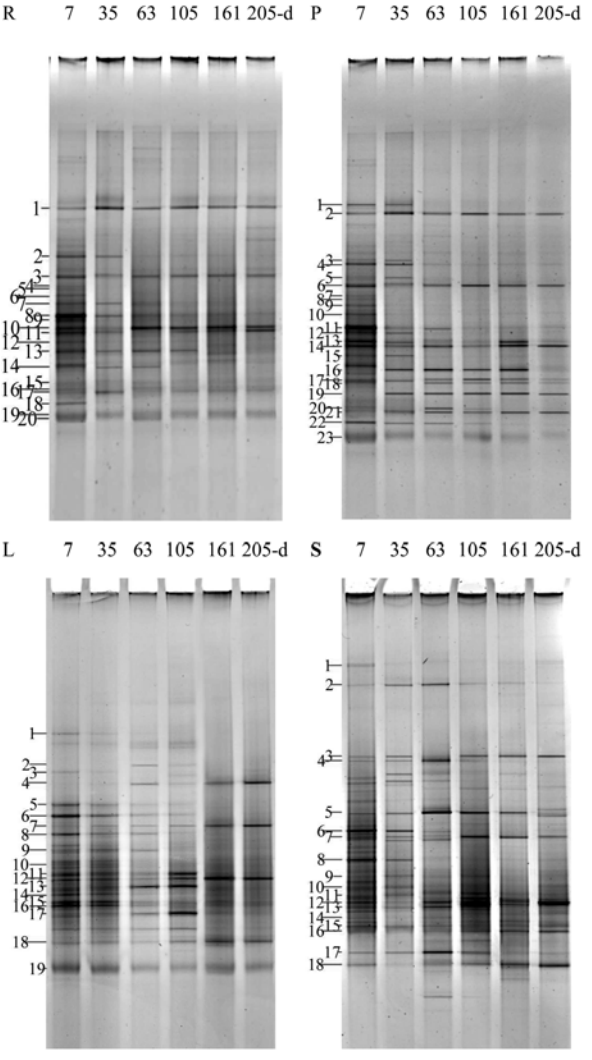

Fig. 3. DGGE profiles of the PCR-amplified 16S rDNA V6 region of the bacterial community structures in the BTFs. R: *R*-filter; P: *P*-filter; L: *L*-filter; S: *S*-filter.

The difference in packing properties determined the

Table 4. *H′* indices of the BTFs

Biofilm sampling time	*S*-filter	*R*-filter	*P*-filter	*L*-filter
7th day	2.98	2.56	2.54	2.55
35th day	2.76	2.33	2.43	2.37
63rd day	2.62	2.28	2.31	2.24
98th day	2.60	2.04	2.04	1.96
161st day	2.48	1.98	2.04	1.91
203rd day	2.54	1.94	2.05	1.95

major directive distinctions of the bacterial communities of the initial stage (Table 1). The positive evolution-based enormous microbes of activated sludge resulted in minor random distinctions in the bacterial communities [15,32]. SMP showed complex packing properties distinct from those of the other packing materials (Table 1). The *RE* at the initial stage of the *S*-filter was significantly higher than that of the other filters (F, $P < 0.05$) as evidenced by the 98.91% toluene removal on the first day and the 95% stabilization efficiency rate, mentioned above, that was achieved (Fig. 1). The *S*-filter also exhibited a higher *H′* index (Table 4) and showed a greater total bacterial biomass (Fig. 4) than those of the other filters (F, $P < 0.05$). The results suggest that the packing properties of SMP materials provided higher bacterial population density and diversity at the initial stage of biotrickling filtration, resulting in the shorter start-up time in the *S*-filter compared with the other filters.

SMP consists of 80.18 ± 0.48% inert packing materials and 19.82 ± 0.48% organic packing materials (Table 1), such as bark and compost. Organic materials can provide the abundant nutrients necessary to accelerate the growth of bacteria that can shorten the start-up time of the biofilters [1,2,33,34].

The high water- and toluene- holding/sorption capacities of SMP materials result from the combination of such materials as charcoal and compost (Table 1). Pollutants in the air stream are dissolved in the wet biofilm layer before degradation by microorganisms [5]. The sorption capacity can accelerate the acclimation of bacteria [12].

The SMP materials demonstrated a higher pH buffering capacity and leachate conductivity than the other packing materials (Table 1). The variations in these capacities can improve VOC biodegradation into consumable nutrients and can positively impact the generation of metabolites, such as organic acids [33,35,36].

3.2. Variation in the bacterial diversity and abundance in BTFs

Considering that the *IL* progressively increased to 319.82 g/m^3/h, the EC_{max} value of the *S*, *R*, *P*, and *L*-filters reached

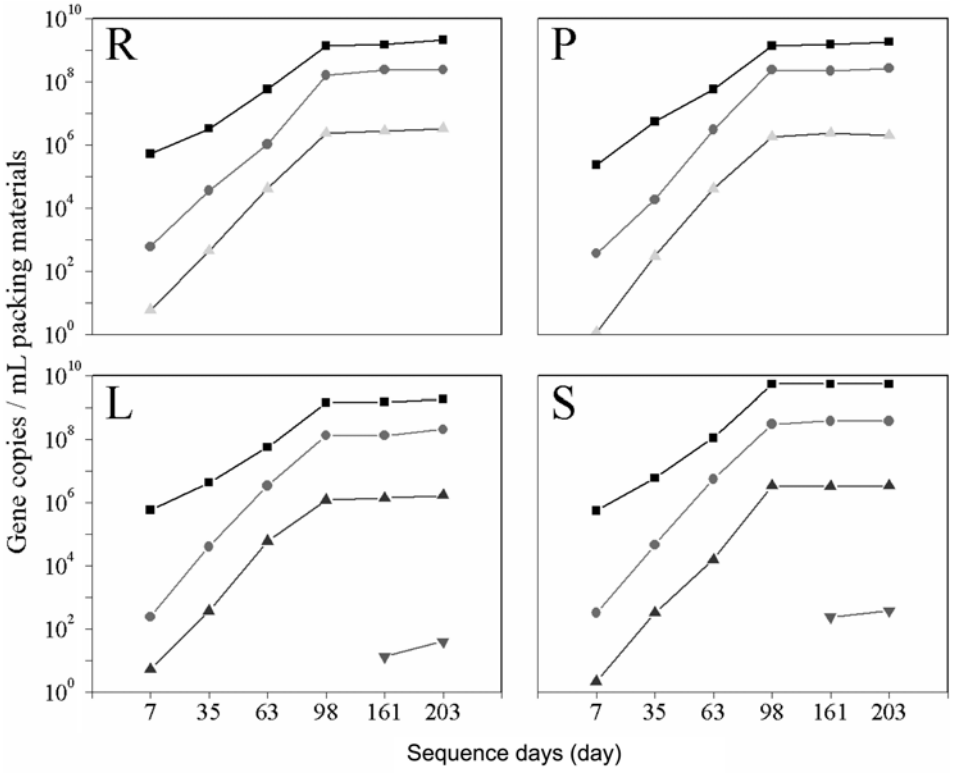

Fig. 4. Gene copies of various toluene-degrading enzymes and the 16s rDNA V3 region of bacteria in the BTFs. R: *R*-filter, P: *P*-filter, L: *L*-filter; S: *S*-filter, (■) V3, (●) *tmo*A, (▲) *tod*C1, (▼) *bzA*Q4.

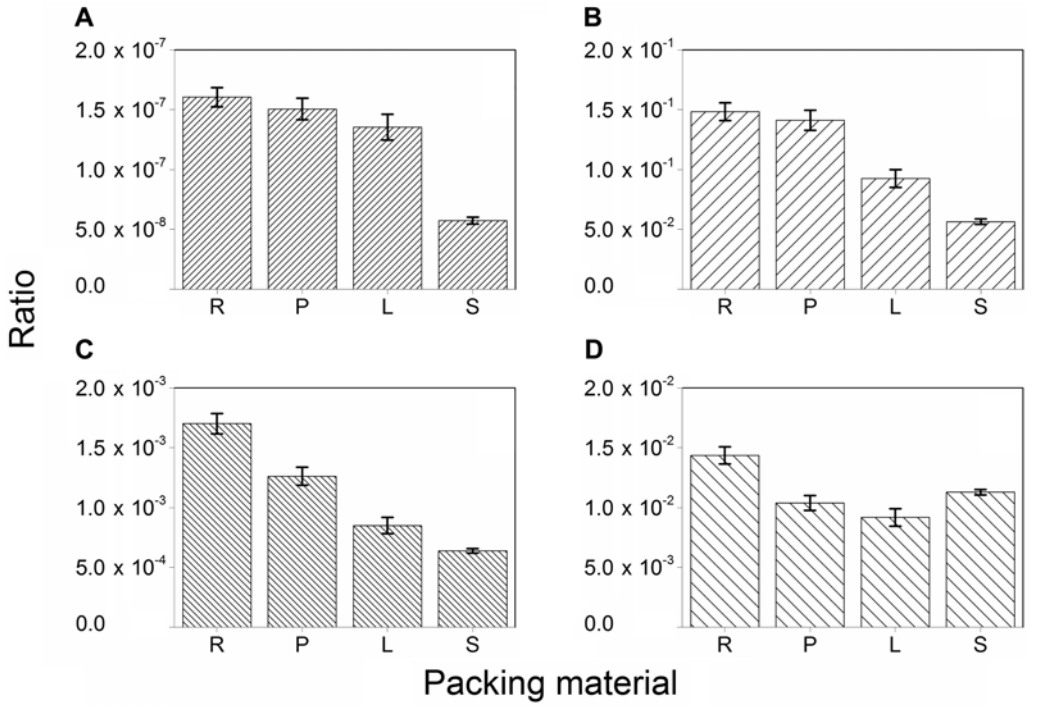

Fig. 5. Gene copies ratio of EC/V3 region, *tmo*A/V3 region, *tod*C1/V3, and *tod*C1/*tmo*A region at EC_{max} on the 98th d. A, EC/V3 region; B, *tmo*A/V3 region; C, *tod*C1/V3; D, *tod*C1/*tmo*A; R, raschig ring; P, pall ring; L, lava rock; S, SMP material.

306.20 ± 7.90, 208.91 ± 7.84, 219.78 ± 10.59, and 191.60 ± 12.70 g/m^3/h on 98th day, respectively (Fig. 1). A detailed comparison of *EC* in the BTFs with other biofilters was showed in reference [25]. The bacterial diversity in the BTFs, characterized by the *H′* indices, continually decreased during the 98 day of operation (Table 4). Similar results have been reported previously, by several scholars [12-16]. The toluene-degradation gene copies and total biomass in the BTFs detected, by real-time, that the PCR exponentially increased after 98 day of operation (Fig. 4). These findings suggest that the major toluene-degradation genes in the BTFs may be *tmo*A of TMO and *tod*C1 of TOD, although the latter gene copies were significantly lower than the former (Figs. 4 and 5). A few *bzA*Q4 gene copies in the *S*-, and *L*-filter were detected, implying that the properties of SMP and lava rock can provide bacteria with various habitats that will induce various aromatic pathways in biotrickling filtration (Table 1).

Considering that the BTFs had similar inocula and operating conditions, Multivariate tests demonstrated that the V3, *tmo*A, and *tod*C1 gene copies, as well as the *H′* index of the *S*-filter, were significantly higher than those of the other filters when the *IL* was a covariate variable (Table 4 and Fig. 4). This result suggests that the *S*-filter might possess a higher biomass than the other filters. However, the EC/V3 region, *tmo*A/V3 region, and *tod*C1/V3 region of the *S*-filter demonstrated significantly lower levels than did the other filters (F, $P < 0.05$; Fig. 5). These results indicated that the extra nutrients and complex materials (Table 1) in SMP would be favorable to VOCs degradation for microorganisms and supported more toluene degraders and co-degraders than the other packing (Figs. 4 and 5). Abundant toluene degraders and co-degraders, sustained by SMP materials, showed the advantage of high *RE*, *EC*, and process robustness under high VOCs concentration, as well as the disadvantages of high biomass and pressure drop when VOCs concentration is low [25]. The high *EC* is useful not only in enhancing biofilter performance under a given operating condition but also in improving design criteria, including biofilter size and *EBRT* [37]. The excellent process robustness can improve the design and operation of the bioreactor by identifying the critical operational variables, leading to a greater acceptance of biological air pollution control reactors [12]. However, the pressure drop of the *S*-filter enhanced to near 250 Pa after prolonged operation (data was shown in reference [25]) was significantly higher than the pressure drop in the other filters. This disadvantage may shorten the estimated durability of the SMP in biofiltration, whereas the normal pressure drop range has not been exceeded in the *S*-filter [38].

3.3. Detection of putative toluene-degrading bacteria in BTFs

The BTFs were dominated by putative toluene-degrading bacteria until the EC_{max} were achieved, after 98 day of

Table 5. The relative abundance of the putative toluene-degrading bacteria at EC_{max} on the 98th day

R-filter			*P*-filter		
Band[a]	Relative Qty.	Closest genera[b]	Band[a]	Relative Qty.	Closest genera[b]
1	7.41%	*Burkholderia*	2	8.90%	*Burkholderia*
3	15.44%	*Acidocella*	6	12.63%	*Acidocella*
10	23.43%	*Phyllobacterium*	14	5.54%	*Legionella*
11	5.71%	*Legionella*	16	8.41%	*Burkholderia*
13	8.57%	*Burkholderia*	19	7.75%	*Myxococcales*
16	8.65%	*Burkholderia*	21	6.99%	*Burkholderia*
20	14.47%	*Burkholderia*	23	11.49%	*Burkholderia*
L-filter			*S*-filter		
Band[a]	Relative Qty.	Closest genera[b]	Band[a]	Relative Qty.	Closest genera[b]
4	6.70%	*Acidocella*	3	5.44%	*Burkholderia*
7	8.09%	*Burkholderia*	5	15.15%	*Pseudomonas*
12	13.97%	*Burkholderia*	7	13.21%	*Burkholderia*
18	11.46%	*Frateuria*	12	16.19%	*Alcaligenes*
19	20.57%	*Burkholderia*	15	2.42%	*Citrobacter*
			16	4.45%	*Dokdonella*
			18	10.90%	*Burkholderia*

[a]The bands are designated as shown in Fig. 3.
[b]The closest genus were obtained with both the sequence match in Blastn and the phylogenetic analysis in ESM 1.

operation (Fig. 1). The relative abundance of these bacteria was quantified using Quantity One v4.5.0, in order to analyze their bands in the DGGE profiles. These bands were excised, re-amplified, purified, sequenced, and evaluated using phylogenesis in order to identify the putative toluene-degrading bacteria in the BTFs [Electronic Supplementary Material (ESM)]. The major putative toluene-degrading bacteria in the *S*-filter were distinct from those in the other filters (Table 5).

The VOC-degrading bacteria determined the performance of biofilters under steady-state operating conditions [7,8]. The phylogenetic analysis (ESM 1) and relative abundance of bacteria (Table 5) showed that *Burkholderia* spp. was active in all BTFs, whereas *Pseudomonas* spp. was active only in the *S*-filter. The *tmo*A gene copies detected by real-time PCR increased with increasing *EC* of the BTFs (Fig. 4). Several scholars have verified the aromatic degrading capacity and the genes involved in aromatic degrading enzymes in some species of these genera, such as the *tmo*A gene of some species of the *Burkholderia* and *Pseudomonas* genera [20-22]. Thus, some of the identified phylotypes, such as *Burkholderia* spp. and *Pseudomonas* spp., may be the toluene-degrading bacteria that significantly participated in the toluene biotrickling filtration.

Findings for the other putative toluene-degrading bacteria in the BTFs, such as *Phyllobacterium* spp. and *Alcaligenes* spp., might not have been reported (Table 5). These bacteria showed a relative abundance in the BTFs (Fig. 3). The putative toluene-degradation of various bacteria may originate from the horizontal gene transfer in biotrickling filtration. Some researchers have reported that most of the genes involved in aromatic degrading enzymes are carried over to various plasmids [20-23]. Another possible explanation is that the experimental primer sets could not detect all toluene-degradation genes in the known toluene-degrading bacteria. Other researchers have concluded that the genes involved in aromatic degrading enzymes might have various mutations that go undetected by their experimental primer sets [20,22].

3.4. Overload and shutdown operations in BTFs

The buffering effect of fluctuant loading is one of the key parameters used in evaluating the appropriate packing for biofilter [12]. The *RE* and *EC* of the BTFs significantly decreased during the overload and shutdown periods (Fig. 2). The overload and shutdown operations resulted in insignificant fluctuations in the V3 region and the toluene-degradation gene copies at equal levels (Fig. 4). These operations similarly resulted in slight variations in the bacterial community structure, which were confirmed by the smooth shift of band patterns (Fig. 3) and insignificant variations in the H' index in the DGGE profiles (Table 4).

The abovementioned changes in the BTFs suggest that the diffusion of the VOCs, through the biofilm, rather than the properties of packing materials, served as the limiting step under overloading operations (Fig. 2). Similar results have been reported by several scholars [12] who stated that the most superficial active layers of the biofilm are not sufficient to transform the nearly-complete substrate at low residence time and that the enzymes' systems of micro-

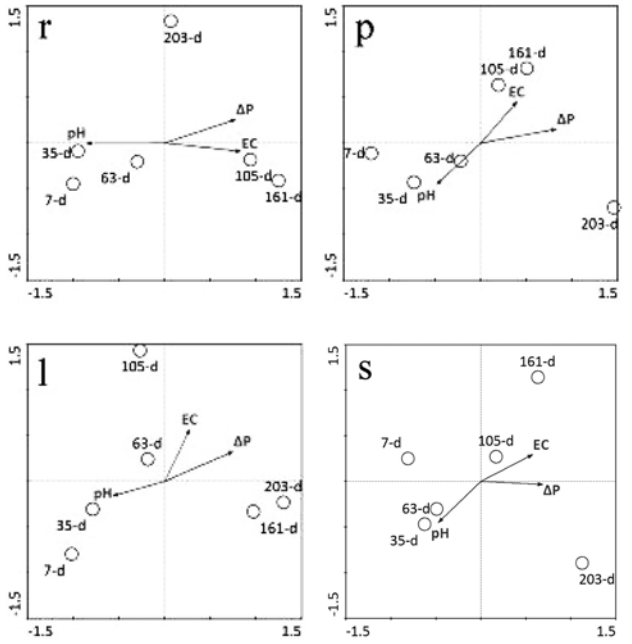

Fig. 6. RDA plots of the biotrickling filtrations. r, *R*-filter; p, *P*-filter; l, *L*-filter; s, *S*-filter. ΔP indicates pressure drop.

organisms are unable to process the substrate at overloading conditions [7,12].

The rapid recovery of *RE* and *EC* in the BTFs, after shutdown, suggested that bacteria in the biofilm were in a resting state (Figs. 2 and 4). The *S*-filter showed faster *RE* and *EC* recovery rates under convalescence conditions, after the overload and shutdown periods, compared with the other filters (Fig. 2). Based on these results, the extra nutrients in the SMP materials (Table 1) can contribute to sustaining the survival of the bacteria (Fig. 4). Additional toluene-degradation gene copies of the biofilm in SMP (Fig. 4) may also support the rapid *RE* and *EC* recovery rates (Fig. 2). Previous researchers have similarly indicated that the resumption of shutdown in biofilters depend on the rate and number of recuperation of metabolic and enzymatic activities of microorganisms after moderately prolonged starvation periods [39].

3.5. RDA of the BTFs

RDA was applied in the presence and absence of DGGE profile bands as well as the relative environmental factors in the BTFs. Such factors as the *IL* and temperature, among others, were removed because they were deemed insignificant by the Monte Carlo permutation test of RDA, using Canoco 4.5. The RDA plots showed clear distinctions among the BTFs packed with different packing materials under various parallel operating conditions (Fig. 6).

Though the *EC* of the BTFs increased, the Day 7, 35, 63, and 98 samples trended the *EC* and pressure drop arrows in a positive direction. These samples in RDA plots indicated that continuous changes in the bacterial community structure, related decreasing pH value and increasing *EC* and pressure drop, characterized the performance of the BTFs. Similar results were confirmed by several scholars [14,15,26].

4. Conclusion

The toluene-degradation gene copies in the biofilm exponentially increased, and the *H′* indices in the BTFs continually decreased with increasing *EC*. The overloading and shutdown operations resulted in insignificant fluctuations in the toluene-degradation gene copies at equal levels and a slight variation in the bacterial community structures in the BTFs. Various putative toluene-degrading bacteria were found by sequencing bands from the DGGE gels; some bacteria were further confirmed by real-time PCR, and other bacteria might not have been reported.

The packing properties of SMP material supported more toluene-degradation gene copies in the biofilm, and higher toluene-degrading bacterial diversity of *S*-filter, than those of the other filters. Thus, *S*-filter demonstrated excellent performance, suggesting the suitability of SMP to real applications, whereas the capability of inert packing materials is more suited to the treatment of steady low VOC loads.

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Nomenclature

BTF : Biotrickling filter
C : Toluene inlet concentration in the air (Mg/m^3)
EBRT : Empty bed residence time (sec)
EC : Toluene elimination capacity ($g/m^3/h$)
H : Height of PVC column (mm)
ID : Inside diameter of PVC column (mm)
IL : Inlet loading rate in biotrickling filter ($g/m^3/h$)
L-filter : Biotrickling filter packed with ceramic lava rock
P-filter : Biotrickling filter packed with ceramic pall ring
RE : Toluene removal efficiency of biotrickling filter (%)

R-filter : Biotrickling filter packed with ceramic raschig ring
SMP : Structured mixed packing
S-filter : Biotrickling filter packed with structured mixed packing
max : Maximum value
key : Steady removal efficiency > 95%

Appendix

Electronic Supplementary Material associated with this article can be found in the online version.

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