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Influences of hydraulic loading rate on SVOC removal and microbial community structure in drinking water treatment biofilters

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ABSTRACT

Six biofilters were used for advanced treatment of Yangtze River source water to investigate the effects of hydraulic loading rate (HLR) on pollutant removal and microbial community. HLR was found to exert significant influences on the removal efficiency of the conventional pollutants and 24 detectable semivolatile organic compounds (SVOCs). More than 85% of chemical oxygen demand and assimilable organic carbon was removed at the optimal HLR of 3.0 m h^{-1}. With the increase of HLR, SVOC removal showed a decreasing trend. Di-*n*-butyl phthalate and bis(2-ethylhexyl)phthalate, two main SVOCs in the source water, had the highest removals of 71.2% and 84.4%, respectively. Nearly 65% of 2,6-dinitrotoluene and 80% of isophorone were removed at the lowest HLR. Phylogenetic analysis showed that *Escherichia coli*, *Shigella* sp., *E. fergusonii* and Firmicutes bacteria predominated in the bioreactors. The dominance of *E. coli* in the low-HLR biofilters might contribute greatly to the high SVOC removal.

1. Introduction

A thriving and prosperous economy has resulted in severe environmental pollution in Yangtze River Basin of China. Most of the available resources in Nanjing region are not suitable for drinking water supply, with the result that people seek possibilities for exploiting Yangtze River resources. However, various semivolatile organic compounds (SVOCs), including polycyclic aromatic hydrocarbons, polychlorinated biphenyls and environmental endocrine disruptors, have been detected in the water of Yangtze River at Nanjing section [1,2]. These pollutants with high molecular toxicities may potentially endanger public health of the water-consuming population, although most of the compounds are present at low levels [3]. Therefore, Yangtze River source water needs advanced treatment before going into drinking water distribution systems.

Compared with other biological processes used for drinking water treatment, biofiltration enhances substrate removal by providing a surface for indigenous water microorganisms to attach and grow [4]. As a cost-effective and environmentally friendly method, biofiltration has been successfully applied to remove the pollutants of organic carbon [5] and nitrogen [6] in source water. The interactions among design, operation, and substrate availability in biofilters control the composition and activity of microorganisms in the filters [7]. In turn, the microbial activity in the filters governs the substrate utilization performance of the filters [8]. Current researches are mainly centered on the regulation and optimization of operational conditions in order to improve pollutant removal efficiency in the wastewater [9] or drinking water treatment biofilters [10]. It has been found that hydraulic loading rate (HLR) had the greatest impact on biofilter biomass and substrate removal performance [4], as well as the microbial community structure [8]. However, little information is availabe conerning the impacts of HLR on removal performance of the SVOCs in biological drinking water treatment system based on the assessment of microbial community structure.

The objective of this study is to investigate the influence of HLR on the microbial community structure in biofilters and the removal efficiency of the pollutants, including some SVOCs in source water. This study are expected to provide some baseline information about the regulation and optimization on biofilter operation, which is vital for effective removal of toxic organic pollutants in drinking water.

2. Materials and methods

2.1. Operation of biofilters

Six biofilters, numbered from C1 to C6, were employed to remove SVOCs in source water at Beihekou tap water plant (Nanjing, China) during June to August of 2008. The main part of each biofilter was a transparent polyvinyl chloride column with an inner diameter of 10 cm and an effective height of 100 cm. Each filter was packed with 2.3 l granular activated carbon (GAC) supported by 1.1 l sand. The parameters related to biofilter design were

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Table 1
Components in influent water of the biofilters.

Component	Concentration	Component	Concentration
COD_{Mn}, mg l^{-1}	3.01 ± 0.46	Benzo(*k*)fluoranthene, ng l^{-1}	14.6 ± 6.4
AOC, mg l^{-1}	0.46 ± 0.18	Benzo(*b*)fluoranthene, ng l^{-1}	14.9 ± 2.1
NH_3-N, mg l^{-1}	0.22 ± 0.07	Benzo(*a*)anthracene, ng l^{-1}	15.5 ± 7.7
TN, mg l^{-1}	0.84 ± 0.21	Indeno(1,2,3-*cd*)pyrene, ng l^{-1}	9.7 ± 5.3
TP, mg l^{-1}	0.13 ± 0.03	Dibenz(*a*,*h*)anthracene, ng l^{-1}	8.5 ± 3.9
pH	7.9 ± 0.1	Dimethyl phthalate, ng l^{-1}	103.1 ± 67.6
Ca, mg l^{-1}	38.1 ± 7.6	Diethyl phthalate, ng l^{-1}	16.5 ± 11.4
K, mg l^{-1}	2.8 ± 0.6	Di-*n*-butyl phthalate, ng l^{-1}	4446 ± 2083
Mg, mg l^{-1}	6.7 ± 1.8	Butyl benzyl phthalate, ng l^{-1}	15.4 ± 7.6
Na, mg l^{-1}	10.3 ± 3.2	Bis(2-ethylhexyl)adipate, ng l^{-1}	149.7 ± 21.7
Fluorene, ng l^{-1}	10.0 ± 4.2	Bis(2-ethylhexyl)phthalate, ng l^{-1}	1699 ± 403
Phenanthrene, ng l^{-1}	20.4 ± 8.9	Isophorone, ng l^{-1}	6.8 ± 3.2
Anthracene, ng l^{-1}	36.2 ± 12.4	Hexachlorocyclopentadiene, ng l^{-1}	7.1 ± 3.9
Pyrene, ng l^{-1}	8.2 ± 3.3	2,6-Dinitrotoluene, ng l^{-1}	3076 ± 2150
Chrysene, ng l^{-1}	7.9 ± 1.4	2,4-Dinitrotoluene, ng l^{-1}	16.2 ± 8.8
Acenaphthylene, ng l^{-1}	6.7 ± 2.0	Hexachlorobenzene, ng l^{-1}	4.4 ± 1.9
Benzo(*a*)pyrene, ng l^{-1}	10.1 ± 4.0	Pentachlorophenol, ng l^{-1}	9.4 ± 13.3

COD_{Mn}: chemical oxygen demand (Mn); AOC: assimilable organic carbon; NH_3-N: ammonia nitrogen; TN: total nitrogen; TP: total phosphorus.

previously described in detail [11]. The influent water of each biofilter was collected from the effluent of a pilot-scale sand filtration tank in Beihekou water plant. Table 1 shows the concentration of each component in the influent water of the biofilters. During the start-up period, the filters were operated continuously and stably at an influent HLR of 1.0 m h^{-1} for more than 35 days, with the removals of chemical oxygen demand (COD_{Mn}), assimilable organic carbon (AOC), ammonia nitrogen (NH_3-N), total nitrogen (TN) and total phosphorus (TP) at 74.4% ± 0.6%, 84.5% ± 0.5%, 73.6% ± 0.7%, 55.2% ± 1.1% and 76.9% ± 0.5%, respectively. No significant difference was found in operational performance among the six biofilters during this period. In order to investigate the effects of hydraulic loading on removal efficiency of SVOCs and microbial community structure, HLR was increased after the start-up period and arrived at 1.5, 2.0, 3.0, 5.0 and 8.0 m h^{-1} in the biofilters C2, C3, C4, C5 and C6, respectively. After HLR regulation, the treatment systems ran stably for 24 days at a backwashing interval of 7 d and an intensity of 20 l m^{-2} s^{-1}. Dissolved oxygen in each biofilter was maintained at 3.1 ± 0.2 mg l^{-1}, and water temperature varied between 24.8 and 30.2 °C.

2.2. Analyses of water quality and biomass

After the HLR regulations, water samples were collected from both influent and effluent of the six biofilters every 24 h for the measurement of COD_{Mn}, AOC, NH_3-N, TN and TP. COD_{Mn} was determined using the acid-potassium permanganate method [12]. NH_3-N, TN and TP were measured according to APHA [13], and AOC was analyzed according to Li et al. [14]. GAC samples collected every 24 h at a sampling site of each column (30.0 cm above bed bottom) were used for biomass measurement according to Yu et al. [15].

2.3. Measurement of SVOCs

For the measurement of the SVOCs, influent and effluent waters were sampled for three times at day 18, 21 and 24 within the post-regulation period (24 d). Water samples (10 l) were collected in brown glass bottles previously washed with detergent, followed by deionized water, 2 M nitric acid, then deionized water again and finally influent or effluent water, and then placed in an ice bath. The water samples were filtered onto cellulose ester filters (0.45-μm-pore-size) and kept at 4 °C until analyses of the 24 SVOCs (Table 1). Extraction of SVOCs from water sample were performed according to EMC [16], and the concentrations were determined by a DSQ II Single Quadrupole GC/MS (ThermoQuest, San Jose, USA) with selected ion monitoring mode according to Wu et al. [2].

2.4. DNA extraction

For analysis of microbial community structure, total DNA was extracted from each GAC sample collected from the six biofilters at the 24th day after HLR regulation. The carbon samples were vortexed for 10 min to separate out bacterial cells. 50 ml of the liquid phase was centrifuged and the pellet was used for DNA extraction with Fast Soil Genome DNA Isolation Kit (Bioteke, China). Integrity of DNA was confirmed after electrophoresis on 0.8% agarose gels by comparison with a known standard (marker: Lambda DNA/HindIII, Takara, Japan). Purity of the DNA was spectrophotometrically determined by the ratio of absorbance at 260 and 280 nm.

2.5. 16S rDNA PCR

Primers BSF63 and BSR534 were used to amplify a 472 base-pair hypervariable region of the 16S rRNA gene. The nucleotide sequences were 5′-CGCCCGCCGCGCCCCGCGCCCGTCCCGCCGCCCCCGCCCGAGGCCTAACACATGCAAGTC-3′ for BSF63 and 5′-ATTACCGCGGCTGCTGGC-3′ for BSR534. The conserved 16S rDNA was amplified in a 25-μl reaction system, containing 1× PCR buffer, 1.5 mM $MgCl_2$, 0.2 mM dNTP, 0.25 μM each primer, 1.25 U of Taq DNA polymerase (Takara, Japan), 100 ng of template DNA. Conditions of these PCR reactions were initial denaturation at 94 °C for 5 min, followed by 30 cycles of 94 °C for 40 s, 52 °C for 40 s and 72 °C for 1 min, with a final extension at 72 °C for 8 min. Duplicate PCR reactions were performed for each sample, and sterile water was used as a negative control with each primer set. PCR products were analyzed by electrophoresis on 1% agarose gel with ethidium bromide in 1× TAE buffer at 120 V for 15 min.

2.6. DGGE

Denatured gradient gel electrophoresis (DGGE) was performed using Bio-Rad Dcode™ Gene Mutation Detection System (Bio-Rad, USA) consisting of a waterbath maintained at 60 °C and a set-up for running vertical denaturing gradient gels using an 8% (w/v) acrylamide gel in 0.5× TAE buffer. Gradients of 40–60% denaturants and a running time of 5 h at 200 V were selected as the optimal conditions for the separation of the maximal number of bands. All gels were stained with ethidium bromide (0.5 mg ml^{-1}) for 30 min and band patterns were analyzed using Quantity One Software 4.5.2

(Bio-Rad, USA). After electrophoresis, the desired DNA bands were cut from the DGGE gel and DNA was recovered using Poly-Gel DNA Extraction Kit (Omega Co., USA). PCR product of each DNA was purified with PCRquick spin™ PCR Product Purification Kit (iNtRon Biotechnology Co., South Korea). Purified PCR fragments were cloned directly into pUCm-T vector using a TA cloning kit (Promega Co., USA) and the plasmids were digested with EcoRI to confirm the target DNA insertion. DNA sequencing was performed by automated means at Sangon (Shanghai, China). The 16S rDNA homology searches were performed using the BLAST program at the National Centers for Biotechnology Information (National Institutes of Health, Bethesda, USA).

2.7. Statistical analysis

Data obtained in this study were analyzed by computer assisted statistics using Excel 2003 (Microsoft Co., USA). The least significant differences test was used to measure the differences of each water quality parameter and the SVOC level among the six biofilters. An analysis of variance (ANOVA) test was carried out to assess the homogeneity of variance with significance level of 5% ($P < 0.05$).

2.8. Nucleotide sequence accession numbers

The 16S rDNA sequences of the eight predominant microbial species in the biofilters (eight bands on the DGGE gel) were submitted to GenBank, and the accession numbers were provided as GQ485634 to GQ485641 for bands A to H, respectively.

3. Results and discussion

3.1. Effects of HLR on removal of conventional pollutants

During the initial baseline operation with an HLR at 1.0 m h^{-1} for 35 d, the removals in COD_{Mn}, AOC, NH_3-N, TN and TP kept stable in the six biofilters and showed no significant difference among the biofilters. However, when the HLR was increased to 1.5, 2.0, 3.0, 5.0 and 8.0 m h^{-1} for the biofilters C2, C3, C4, C5 and C6, respectively, a sharp decrease in the removal of each pollutant took place in these biofilters, followed by a complete or incomplete recovery within 7 d (Fig. 1). Pollutant removal efficiency in each of the five biofilters kept stable for more than 2 weeks after the recovery. No change was observed for the performance of the biofilter C1 since HLR was maintained at 1 m h^{-1} during the whole operational time. Thus, the HLR regulations exerted a significant influence on the removal efficiency of the conventional pollutants.

Comparatively, the highest removals of both AOC and COD_{Mn} were achieved in the biofilter C4, which were averagely 93.5% and 85.2%, respectively (Fig. 1A and B), suggesting that the optimal HLR was 3.0 m h^{-1} for the removals of AOC and COD_{Mn}. Among the six biofilters, C3 had the highest removals of NH_3-N (84.4%) and TP

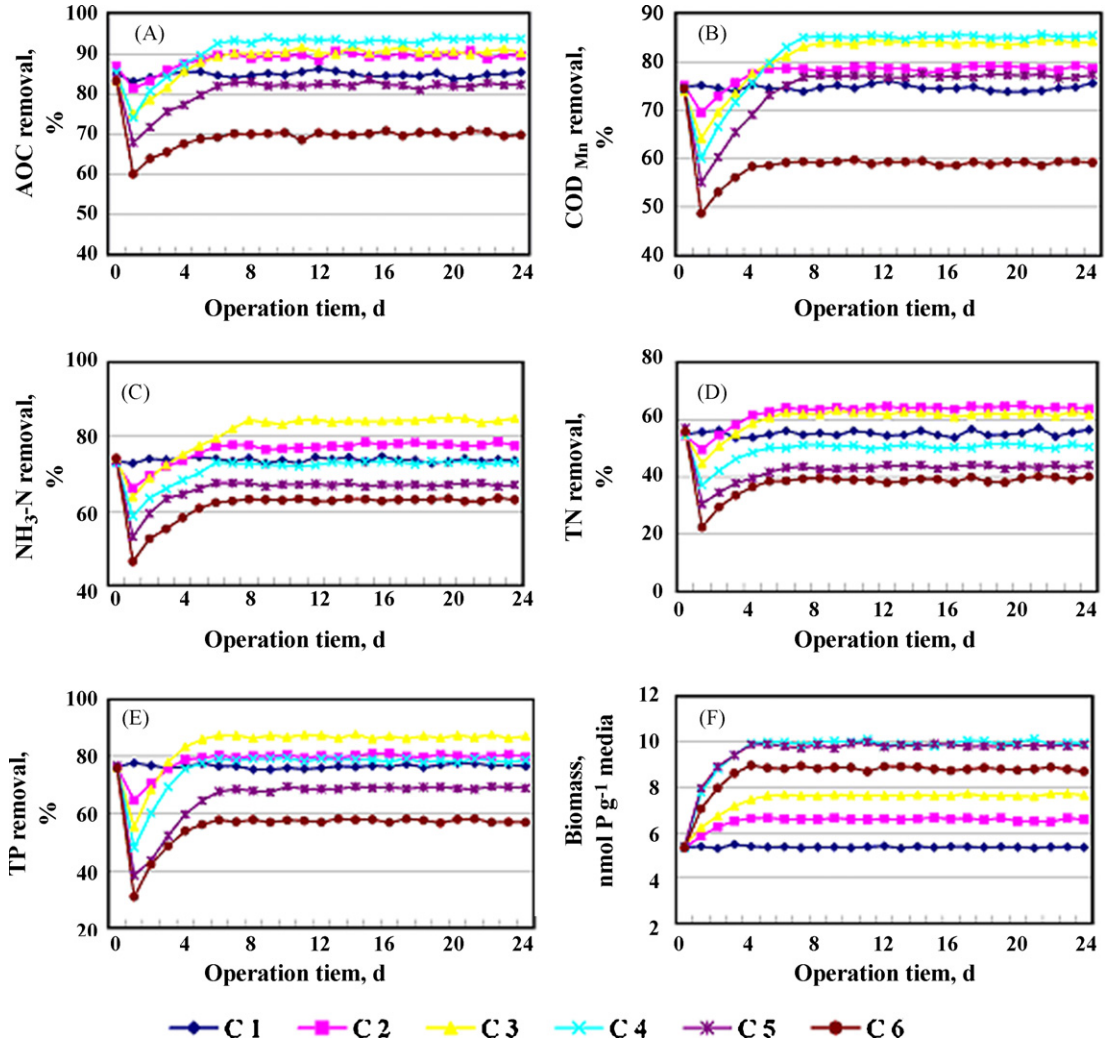

Fig. 1. Removal percentages of AOC (assimilable organic carbon), COD_{Mn} (chemical oxygen demand [Mn]), NH_3-N (ammonia nitrogen), TN (total nitrogen) and TP (total phosphorus) in the six biofilters during the operation period (24 d) after the regulation of hydraulic loading rate.

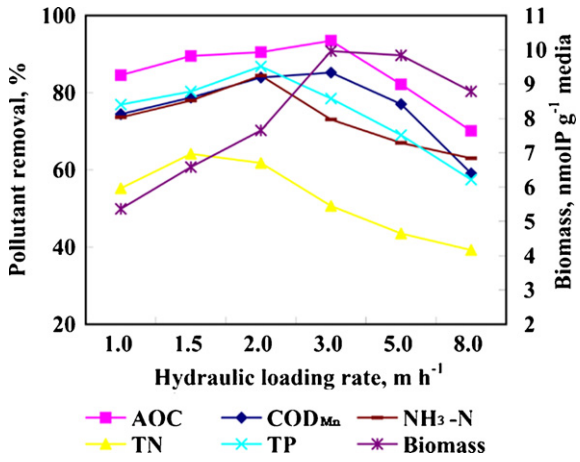

Fig. 2. Effects of hydraulic loading rate on removal efficiency of AOC (assimilable organic carbon), COD_{Mn} (chemical oxygen demand [Mn]), NH_3-N (ammonia nitrogen), TN (total nitrogen), TP (total phosphorus), and biomass in the six biofilters. The data represent the average value of each parameter obtained in the last 10 d of the post-regulation period (24 d).

(87.1%), and C2 was most efficient in reduction of TN (Fig. 1C–E). Yu et al. [17] found that more than 70% NH_3-N was removed in the biofilters at a filtration velocity of 8 m h^{-1}. Fig. 1F shows that biomass in filters C2 to C6 was enhanced significantly with the increase of HLR, but the highest biomass concentration was found in C4 and C5, not in C6. Although phosphate can be incorporated into the cell by microbial assimilation [17], in this study the increase of biomass was not found to contribute to phosphorus removal, since there was no statistically significant correlation between biofilm growth and TP removal in the six biofilters (Fig. 2). The reason could be that high water flow can remove loosely attached large microbial aggregates [18], and some phosphorus was released back into water with the decomposition of dead cells. Low-HLR constricts microbial growth in the biofilters, resulting into the low efficiency of pollutant biodegradation [8]. However, high HLR makes the pollutants, especially the compounds with complex structure, not available for microbial assimilation and degradation within a short contact time. This result is supported by Halle et al. [19], demonstrating that the biofilter with the longer contact time led to greater reductions in organic carbon. Thus, HLR regulation and optimization are necessary and crucial in improving bifiltration performance.

GAC biofilters can remove organic carbon more efficiently than conventional sand filtration process. Kim [20] found that an average of 26.5% total organic carbon in 30 conventional water treatment plants was removed with sand filtration, and Halle et al. [19] showed that only 11% of dissolved organic carbon in source water was removed in this process. However, more than 80% AOC removal was obtained in a pilot-scale GAC filtration process [21]. The higher organic carbon removal in the GAC biofilters could be due to effective bioadsorption and biodegradation taking place simultaneously in these biofilters since dissolved oxygen is sufficient for the microorganisms [22]. At the same time, temperature is another most influencing factor for the microbial growth and carbon metabolism in biofilters [19]. In this study, the biofilters were operated in summer with water temperature at 24.8–30.2 °C, which may contribute to the high removal efficiency. Additionally, hydrophobic portion can be more easily removed through biofiltration than the hydrophilic portion since raw water during rainy season in summer is predominantly hydrophobic in nature [23].

3.2. Effects of HLR on removal of SVOCs

Table 1 shows the concentration of the 24 individual SVOCs in the inlet water of the six biofilters. The total concentration of SVOCs

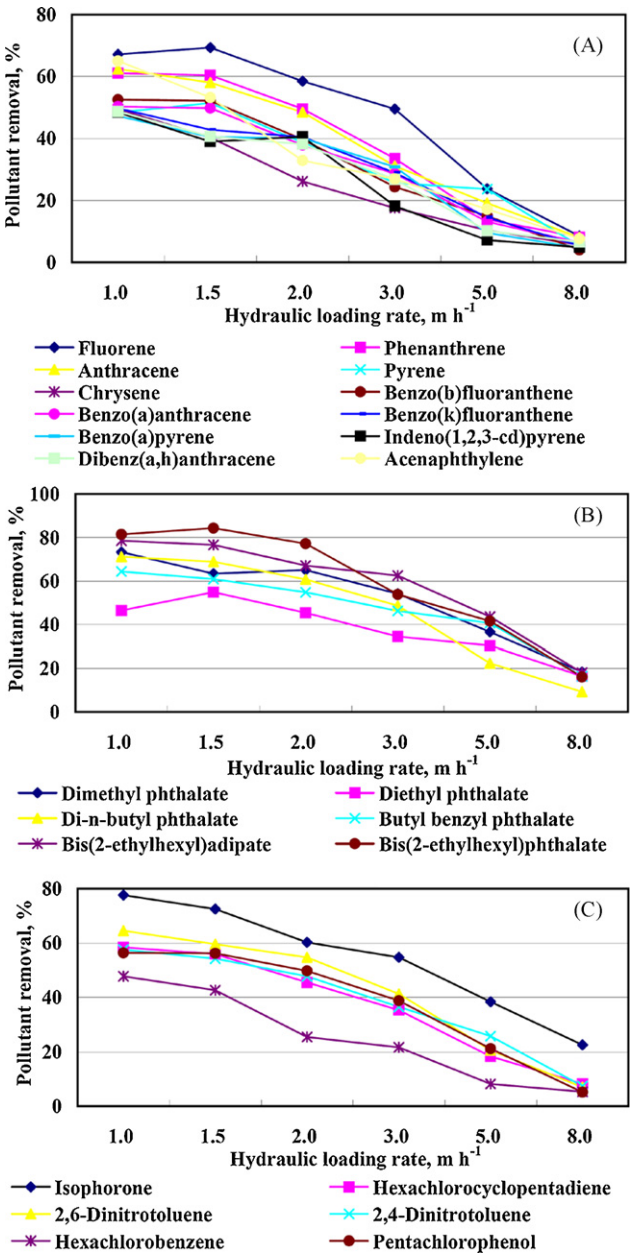

Fig. 3. Effects of hydraulic loading rate on removal performance of 24 semivolatile organic compounds in the biofilters, including 12 polycyclic aromatic hydrocarbons (A), 6 phthalates (B), and 6 benzene derivatives or heterocycle compounds (C).

was determined to be 9012 ± 4837 ng l^{-1}. Di-*n*-butyl phthalate, bis(2-ethylhexyl)phthalate and 2,6-dinitrotoluene contributed the most to this total concentration, of which the average concentrations were 4446, 1699 and 3076 ng l^{-1}, respectively. As shown in Fig. 3, the 24 SVOCs can be divided into three groups, including 12 polycyclic aromatic hydrocarbons (PAHs), 6 phthalates (PAEs), and 6 benzene derivatives or heterocycle compounds (BDHCs). The average concentrations for $\sum$PAHs, $\sum$PAEs and $\sum$BDHCs obtained in this study were 162, 6427 and 3119 ng l^{-1}, respectively.

With the increase of HLR from 1.0 to 8.0 m h^{-1}, the removal of each SVOC showed a decreasing trend, ranging from over 45% in C1 to below 20% in C6 (Fig. 3). The most removal efficiency of each compound was achieved in the biofilters with lower HLRs (1.0 or 1.5 m h^{-1}), demonstrating that biodegradation of the SVOCs needs a longer contact time in comparison with the removal of COD_{Mn} or AOC. Most of these SVOCs, especially PAHs, are composed of fused

aromatic rings whose biochemical persistence arises from dense clouds of π-electrons on both sides of the ring structures making them resistant to nucleophilic attack [24]. These properties stand against their ready microbial utilization and result into the need of a longer retention time for their biodegradation in bioreactors. In our previous study, more than 70% benzo(*a*)pyrene was removed in the biofilter cultured with functional microorganisms at an HLR of 3.0 m h^{-1} [11]. Recently, Cai et al. [25] has reported that a trickle-bed air biofilter had a high efficiency (over 90%) in removing methyl ethyl ketone, toluene, methyl isobutyl ketone and styrene, with empty bed retention time of no less than 2 min.

When HLR was maintained at 1.5 m h^{-1}, fluorene was more readily to be degraded than other PAHs (Fig. 3A), perhaps due to the compound comprising only two benzene rings, fewer than other PAHs. Another reason could be that the carbon atom linking the two benzene rings in fluorene molecule is often attacked by the oxygen free radical to form 9-fluorenone under the catalysis of naphthalene 1,2-dioxy-genase, and the two ring on the molecular structure of 9-fluorenone can be easily divided after subsequent oxidation reactions [24]. For the PAEs di-*n*-butyl phthalate and bis(2-ethylhexyl)phthalate, two of the main SVOCs in the influent water, the most highest removals were found in biofilters C1 (71.2%) and C2 (84.4%), respectively (Fig. 3B). Fig. 3C shows a significant correlation between the increase of HLR and the decrease of removal in each BDHC ($P < 0.05$). Among the six biofilters, C1 had the highest removal for 2,6-dinitrotoluene (64.5%), and nearly 80% of isophorone was also removed in this low-HLR biofilter. Isophorone was metabolized more readily because a carbonyl group is located on the ring of the compound, which makes ring-opening take place more easily [26].

3.3. *Effects of HLR on microbial community structure*

With PCR-DGGE, DNA fragments were separated, excised and sequenced to substantiate the presence of dominant bacteria in the six biofilters. A total of 14 bands were excised from the DGGE gel, out of which 8 yielded unanimous sequences for further analyses (Fig. 4). By BLAST search algorithm, high similarity values were found after DNA sequencing, and these results were confirmed by phylogenetic analysis (Fig. 5). Band A had 99% sequence similarity to *Shigella* sp. strains, and band B had 98% sequence similarity to *Escherichia fergusonii* strain. DNA sequencing showed that band C belonged to Firmicutes bacteria. The sequences of bands D, E, F and G were nicely grouped together and affiliated with *E. coli*, while the sequence of band H was more related to an uncultured bacterium strain.

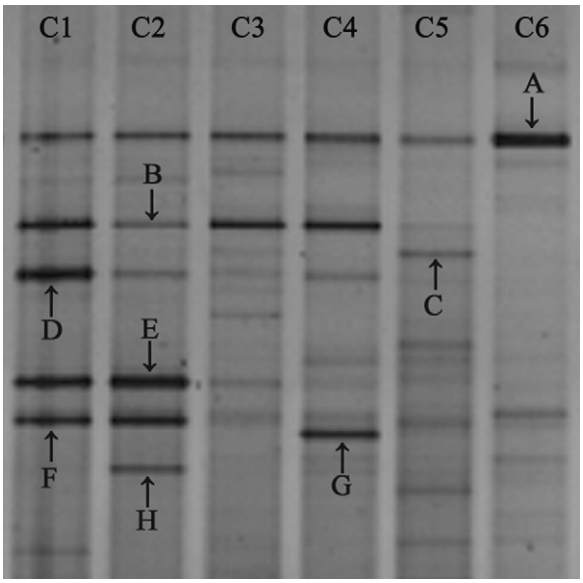

Fig. 4. Denaturing gradient gel electrophoresis (DGGE) analysis of PCR amplified 16S rDNA extracted from the media in the six biofilters (C1–C6).

DGGE pattern cannot reflect the actual diversity of, but only numerically dominant species in, these samples. DGGE analysis of the PCR products revealed that HLR exerted a significant impact on the microbial diversity in the biofilters. *Shigella* sp. (Band A) was dominant in each of the six bioreactors, while *E. fergusonii* occurred in the low-HLR biofilters (C1–C4) and disappeared in high-HLR ones (C5 and C6). *Shigella* sp. was frequently detected in drinking water, which caused public health surveillance for most infectious diseases [27,28]. Recently, *E. fergusonii* has also been reported to be isolated from potable water [29] or source water [30]. The bacterial pattern in biofilter C5 did not show many intense bands. Only a few weak bands were visible, of which band C could be sequenced and was found to be affiliated with Firmicutes bacteria, one type of Gram-positive bacteria frequently detected in drinking water systems [31].

As shown in Fig. 4, the bands of *E. coli* in biofilter C1 (band D) and C2 (bands E and F) were denser than those in the high-flow biofilters. It is well known that *E. coli* frequently occurs in drink-

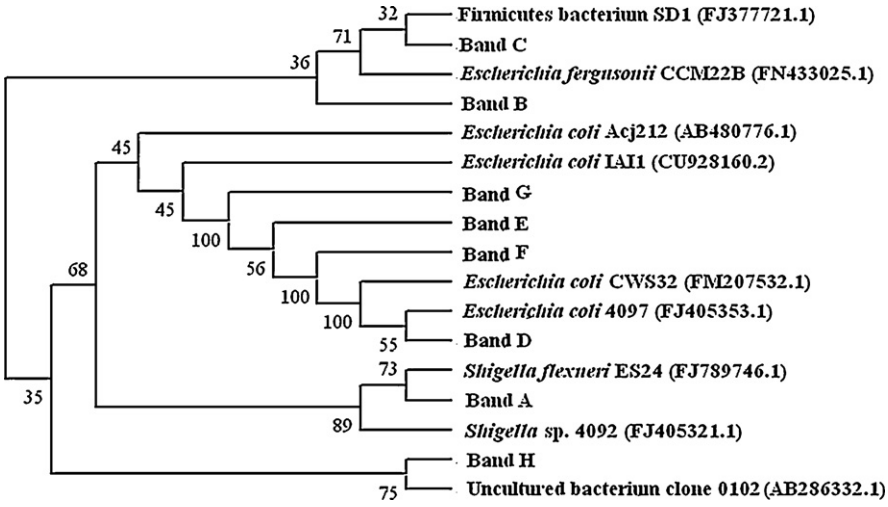

Fig. 5. 16S rDNA phylogenetic analysis of the dominant microbial species in the biofilters with the neighbor-jointing method of MEGA 3.1. GenBank accession numbers of sequences are given in parenthesis.

ing water distribution networks [32]. As listed in UM-BBD [26], *E. coli* can degrade a variety of organic compounds such as PAHs, phthalate, trinitrotoluene and heterocycle compounds. Catalytic effects of biochemical enzymes secreted by these dominant bacteria resulted into the higher removal of the SVOCs in the low-flow biofilters [33,34].

4. Conclusions

GAC biofiltration can be used as an efficient and feasible process for advanced drinking water treatment. Hydraulic loading potentially exerts significant influences on microbial community structure in biofilters, and SVOCs can be effectively removed at a low-HLR. Identifying microbial communities and assessing their changes under various operational conditions can provide some baseline information, which is crucial for the optimization of biofiltration process used to remove various toxic compounds. Further studies should be performed to investigate the relationship between the domiance of some specific microbial species and the removal of the toxic organic pollutants in the biofilters.

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