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Distinct bacterial community dynamics during the start-up of sequencing batch reactors treating pharmaceutical wastewater with different inocula

Lianpeng Sun[a,b], Yuji Lin[a], Guofang Xu[a,c,d,*], Yuhong Liao[a], Lu Wang[a]

[a]*School of Environmental Science and Engineering, Sun Yat-Sen University, Guangzhou 510275, China, Tel. +86-20-39332690; emails: 1291847869@qq.com (G.F. Xu), 354460953@qq.com (Y. Lin), 351442863@qq.com (Y.H. Liao), 1025380325@qq.com (L. Wang)*
[b]*Guangdong Provincial Key Laboratory of Environmental Pollution Control and Remediation Technology, Guangzhou 510275, China*
[c]*Department of Civil and Environmental Engineering, National University of Singapore, Singapore 117576*
[d]*NUS Graduate School for Integrative Sciences and Engineering, National University of Singapore, Singapore 119077*

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ABSTRACT

Inoculum plays an important role in establishing a bacterial community in biological treatment reactors. Two identical anaerobic-aerobic-anoxic sequencing batch reactors (SBRs) were inoculated with sludge from a municipal (*R*1) or pharmaceutical (*R*2) wastewater treatment plant (WWTP) to investigate the influence of inoculum on the start-up of SBRs by comparing the pollutants removal efficiencies and bacterial community. Both SBRs were successfully started up within 30 d. Comparably high chemical oxygen demand (97% ± 2%), total nitrogen (89% ± 8%) and ammonia (96% ± 6%) removals were achieved in both SBRs. But *R*1 performed much better in total phosphorus removal (95% ± 6% vs. 69% ± 7%) than *R*2 due to a higher relative abundance of phosphorous-accumulating organisms (5.5% vs. 0.4%) in *R*1 at the end of the experiment. The bacterial community structures were distinctly different in the inoculating sludge, and no trend of becoming similar was observed all the time. *Candidatus Competibacter* was enriched to be the most dominant bacteria in both *R*1 (27.3%) and *R*2 (19.8%) during the start-up. However, *Candidatus Moranbacteria* (16.2%) and *Roseiflexus* (4.2%) were only dominant in *R*1, whereas *Zoogloea* (10.5%) and *Anaerolineaceae* (9.7%) were only dominant in *R*2 finally. These results indicated that inoculating sludge is one deciding factor shaping the microbiomes in bioreactors, and activated sludge from municipal WWTPs was a potential inoculum to start-up industrial wastewater biotreatment reactors with desirable performances.

Keywords: Pharmaceutical wastewater; Bioreactor start-up; Microbiome; Inoculum; Fluorescent dissolved organic matter

1. Introduction

The pharmaceutical industry is booming with rapid economic development and increasing demand for health care. The production of crude chemical drugs was more than 2.89 million tons in China in 2011, simultaneously generating a large quantity of wastewater [1]. The properties of pharmaceutical wastewater varied greatly which depend on the products and related manufacturing processes, with the chemical oxygen demand (COD) varying from 180 to 32,500 mg L^{-1} [2]. Moreover, pharmaceuticals are typically produced in batch processes, making pharmaceutical wastewater changed dramatically [3]. Physical and chemical treatment approaches like coagulation and chemical oxidation have been used to treat the pharmaceutical wastewater, but they were generally used as a pretreatment or advanced treatment approaches coupled with the biological treatment [4–6]. Many biological technologies have been studied and applied in pharmaceutical wastewater treatment including

* Corresponding author.

up-flow anaerobic stage reactor, membrane-aerated biofilm reactor (MABR) and sequencing batch reactor (SBR) [7], among which the SBR has been gaining popularity due to its single-tank design, easy automatic operation and flexibility [8]. The batch operation of SBR makes it compatible to be applied in treating the pharmaceutical wastewater which is also generated in batch processes. By designing the SBR with anaerobic, aerobic and anoxic reaction stages, organics, phosphorous and nitrogen could be simultaneously and efficiently removed.

Microorganisms play a key role in wastewater biotreatment systems and understanding the microbial community structure is of vital importance to improve reactor performance. Start-up is an important step to establish functional microbiomes in all biotreatment reactors. By analyzing the reactor performance and microbial community during the start-up period, the correlation between pollutants removal and microbial community could be elucidated [9]. During the start-up of biotreatment systems, the microbial community generally changed due to different operating conditions and properties of influent wastewater [2,10–12]. During the start-up of aerobic granulation SBR treating piggery wastewater, the relative abundance of *Proteobacteria* and *Bacteroidetes* increased from 36.7% and 18.5% to 46.0% and 34.6%, respectively, whereas the *Chloroflexi* decreased from 19.7% to 2.8% [8]. However, the relative abundance of *Proteobacteria* decreased and *Chloroflexi* increased during the start-up of an anaerobic ammonium oxidation SBR. The dominant genera in the inoculum were identified as *Flavobacterium*, *Perlucidibaca* and *Thiobacillus*, which all decreased finally [13]. Ren et al. [10] found that the microbial populations changed greatly during the start-up which was in line with the reactor performance. The presence of *Nitrospira* and *Dechloromonas* was associated with high nitrogen removal during the start-up of an integrated A/O reactor treating diluted livestock wastewater [10].

Inoculum is the source to establish the microbial community in wastewater biotreatment reactors. Terada et al. [14] found that the predominant ammonia oxidation bacteria (AOB) was *Nitrosospira* (48.9%–61.2%) in MABR inoculated with the activated sludge from an autotrophic nitrifying bioreactor, whereas it was *Nitrosomonas* (54.8%–63.7%) in the reactor with the activated sludge from a municipal wastewater treatment plant (WWTP) as the inoculum which exhibited higher nitrification efficiency [14]. The SBR inoculated with sludge from the municipal WWTP showed faster 4-chlorophenol degradation compared with that inoculated with sludge from industrial WWTP, with *Alcaligenes*, *Acinetobacter*, *Brevibacillus* and *Pseudomonas* dominant. Different microbial community compositions were also identified in the reactors inoculated with different inocula in other studies [15–17]. Pharmaceutical wastewater usually contains various antimicrobial pollutants (e.g., antibiotics and pharmaceuticals) that may cause alternations of the microbiomes and thus affect the performances of bioreactors [18,19]. For example, the addition of antibiotics decreased the total nitrogen (TN) removal from 87%–89% to 15%–20% in a bioreactor treating synthetic wastewater [18]. The feasibility to use sewage sludge from municipal WWTPs as inoculum to start up bioreactor treating pharmaceutical wastewater remains understudied.

Although lots of literature had reported the performance of SBRs treating pharmaceutical wastewater [17], and some studies had shown that inoculum influenced the microbial community structure in SBR fed with synthetic wastewater [19,20], there is a lack of study about the influence of inoculum on the bacterial community dynamics in SBRs treating real industrial pharmaceutical wastewater. The objectives of this work were to investigate the performance and microbial community change during the start-up of SBRs treating pharmaceutical wastewater inoculated with the sludge from a municipal or pharmaceutical WWTP, as well as evaluate the feasibility to use a three-dimensional excitation-emission matrix (3D-EEM) to predict the quality of treated pharmaceutical wastewater effluent. The pollutant removal (i.e., COD, TN, total phosphorus (TP) and ammonia) and fluorescent dissolved organic matters were monitored continuously, and the microbial community of the sludge during the start-up period was sequenced. The results should be important for us to understand the start-up of SBRs treating real wastewater and give us helpful suggestions to choose suitable inoculum when starting up the industrial wastewater biotreatment reactors.

2. Materials and methods

2.1. Pharmaceutical wastewater

The pharmaceutical wastewater used in experiments was supplied by a local pharmaceutical company (Dongguan, China), which was mainly generated from product manufacturing containing intermediates, solvents, reactants and catalysts. The average physicochemical parameters of pharmaceutical wastewater were as following: total COD, 2,270 ± 708 mg L^{-1}; TN, 14 ± 5 mg L^{-1}; TP, 4 ± 1 mg L^{-1}; turbidity, 50 ± 23 NTU; and pH, 5.4 ± 0.4. Due to nutrients deficiency, nitrogen and phosphorous were added into the raw wastewater to adjust the COD:N:P ratio to ~100:5:1. And the raw wastewater was diluted to 785 ± 81, 1,462 ± 97 and 2,274 ± 131 mg L^{-1} COD in stage 1 (day 1–10), stage 2 (day 11–21) and stage 3 (day 22–30), respectively.

2.2. Seed sludge and reactors operation

The SBRs were two identical cylindrical vessels with an internal diameter of 10 cm and a height of 40 cm, having a working volume of 2 L. The operation parameters of two reactors were the same except that reactor1 (*R*1) and reactor2 (*R*2) were inoculated with sludge from the aeration tank of a municipal WWTP (Guangzhou, China) and the biological contact oxidation tank of a pharmaceutical WWTP (Dongguan, China), respectively. The sludge volume index (SVI) was approximately 67 and 22 mL g^{-1} for the sludge from the municipal and pharmaceutical WWTPs, respectively. The sludge was inoculated into two reactors with a similar final mixed liquor suspended solids (MLSS) concentration, namely 4,590 mg L^{-1} in *R*1 and 4,970 mg L^{-1} in *R*2. One cycle was operated one day, composed of feeding (1 h), anaerobic phase (10 h, dissolved oxygen: <0.1 mg L^{-1}), aerobic phase (6 h, dissolved oxygen: 2–3 mg L^{-1}), anoxic phase (4 h, dissolved oxygen: 0.2–0.4 mg L^{-1}), settling (2 h) and decant phase (1 h). The concentration of dissolved

oxygen was monitored with a portable DO meter and controlled by adjusting the air-flow of the air pump. A stirrer (120 rpm) was used to mix the wastewater and sludge except the settling and decant phase. The volume discharge ratio was 50%. During the start-up period, no excess sludge was intentionally discharged from the reactors except sludge sampling. The concentration of influent increased step by step with 785 ± 81, 1,462 ± 97 and 2,274 ± 131 mg L^{-1} COD for stage 1 (day 1–10), stage 2 (day 11–20), and stage 3 (day 21–30), respectively. 1 M HCl or NaOH solution was used to adjust the pH of influent to 8.0 ± 0.2, and the experiments were carried out in a room with the ambient temperature controlled 30°C ± 2°C.

2.3. Analytical methods

The influent and effluent were collected for analysis. The COD, TN, ammonia, TP, turbidity, MLSS, mixed liquor volatile suspended solids (MLVSS), and sludge volume index at 30 min (SVI_{30}) were measured as described in the standard methods [21]. The pH was measured using a pHS-25 meter. A luminescence spectrometry (F-4500 FL spectrophotometer, Hitachi, Japan) was used to carry out the 3D-EEM spectra with scanning parameters as excitation wavelength, 240–450 nm; emission wavelength, 280–580 nm; increments, 5 nm; scanning speed, 1,200 nm/min; and excitation and emission slits, 10 nm [22]. All samples were put into a quartz cuvette (10 mm × 10 mm) when scanning. The deionized water was scanned and regarded as the blank.

2.4. Microbial community analysis

The sludge samples were collected on days 0, 10, and 30 for the bacterial community analysis. Extraction, purification, and quantification of the genomic DNA carried out at 0.5 g biomass, using a DNA kit (12888-50, MOBIO, USA). The quality of the extracted DNA was examined by 1% agarose gel electrophoresis. The V3-V4 region of the 16S rRNA gene was amplified with the 338F (ACTCCTACGGGAGGCAGCA) and 806R (GGACTACVSGGGTATCTAAT) for the bacterial community analysis [9]. Polymerase chain reaction (PCR) was conducted using a PCR amplifier (ABI GeneAmp® 9700). All amplifications were performed in 20 μL reactions. Each reaction volume contained 4 μL 5 × FastPfu Buffer, 2 μL dNTPs (2.5 mM), 0.8 μL Forward Primer (5 μM), 0.8 μL Reverse Primer (5 μM), 0.4 μL Fast Pfu Polymerase, 0.2 μL bull serum albumin, 2 μL Template DNA and 9.8 μL H_2O. The following cycle parameters were used for bacterial PCR: initial denaturation for 3 min at 95°C; 27 cycles of the 30s at 95°C, 30s at 55°C, and 45 s at 72°C; and final extension for 10 min at 72°C. The PCR products were sequenced on the Illumina Miseq platform. The modified pipelines of mother and UPARSE were used to process and analyze the sequencing data as previously described [23]. Sequences were clustered into operational taxonomic units (OTUs) at a 97% similarity level. OTUs were assigned taxonomy with the RDP classifier with a confidence cut-off of 80% (Green Gene database). Shannon index and principal components analysis (PCoA) based on Bray–Curtis dissimilarity matrix were performed at the OTU level to reveal the community difference using QIIME.

3. Results

3.1. Performance in pollutants removal

To start up the SBRs, the COD concentration of influent pharmaceutical wastewater was adjusted to 785 ± 81, 1,462 ± 97 and 2,274 ± 131 mg L^{-1} in stage 1 (day 1–10), stage 2 (day 11–21) and stage 3 (day 22–30), respectively. Similar COD removals were observed in both reactors inoculated with different sludge (Fig. 1a). In stage 1, the COD removal increased steadily from 92.6% and 92.8% to 98.8% and 98.2% in *R*1 and *R*2, respectively, indicating the sludge microbiomes adapted gradually to the pharmaceutical wastewater. High COD removal was maintained in stage 2 and stage 3 in both *R*1 (98.8% ± 0.5%) and *R*2 (98.1% ± 0.6%). Similar and high ammonia and TN removals were achieved in both *R*1 and *R*2 (Figs. 1c and d). However, *R*1 performed better than *R*2 in TP removal. In *R*1, the TP removal increased from 73.2% to 97.4% in stage 1 which was kept above 80% in the following days. The TP removal in *R*2 also increased from 88.3% to 93.5% in stage 1, but it gradually dropped to 56.3% at the end of the operation (Fig. 1b). The turbidity removal was 95.1% ± 2.5% and 85.7% ± 6.9% in *R*1 and *R*2, respectively. Overall, both reactors succeeded in starting up within 30 d. Similar and high COD removal could be achieved in both reactors regardless of different inocula. But higher TP and turbidity removals were observed in *R*1 inoculated with activated sludge from the municipal WWTP.

3.2. Removal of fluorescent dissolved organic matter

To further get more insights into the degradation of specific groups of dissolved organic compounds in the pharmaceutical wastewater, 3D-EEM fluorescence was performed (Fig. 2). And Table 1 summarized the detailed fluorescence spectra parameters. Three fluorescence peaks were identified in the so-called soluble microbial by-product-like, fulvic acid-like and humic acid-like regions: peak *A* (tryptophan-like) at E_x/E_m = (275–280 nm)/(298–379 nm), peak *B* (fulvic acid-like) at 240 nm/(419–430 nm) and peak *C* (humic acid-like) at (305–335 nm)/(402–430 nm) [26]. The intensity of peak *A* was much higher than that of peak *B* and peak *C* in the influents (Table 1). In *R*1, the intensity of peak *A*, peak *B* and peak *C* decreased by 85%, 42% and 43% in the effluents compared with that in the influents on the day 1, respectively, the value of which decreased by 76%, 14% and 7% in *R*2. The removal of peak *A* was maintained above 71% and 60% in the following operation in *R*1 and *R*2, respectively. But the removal of peak *B* and peak *C* decreased significantly. On day 30, the intensity of peak *B* and peak *C* only decreased by 15% and 2% in the effluents of *R*1 compared with that of influents, respectively, which increased by 11% and 35% in *R*2. These results indicated that the tryptophan-like organic compounds were efficiently degraded by the microorganisms in both SBRs, whereas, humic acid and fulvic acid-like compounds could be more efficiently degraded in SBR inoculated with activated sludge from municipal WWTP. Additionally, peak locations of the effluents showed differences compared with that of influents, of which peak *A* in effluents demonstrated a red-shift, while peak *B* and *C* were blue-shifted. The carbonyl, hydroxyl, alkoxyl, amino, and carboxyl groups

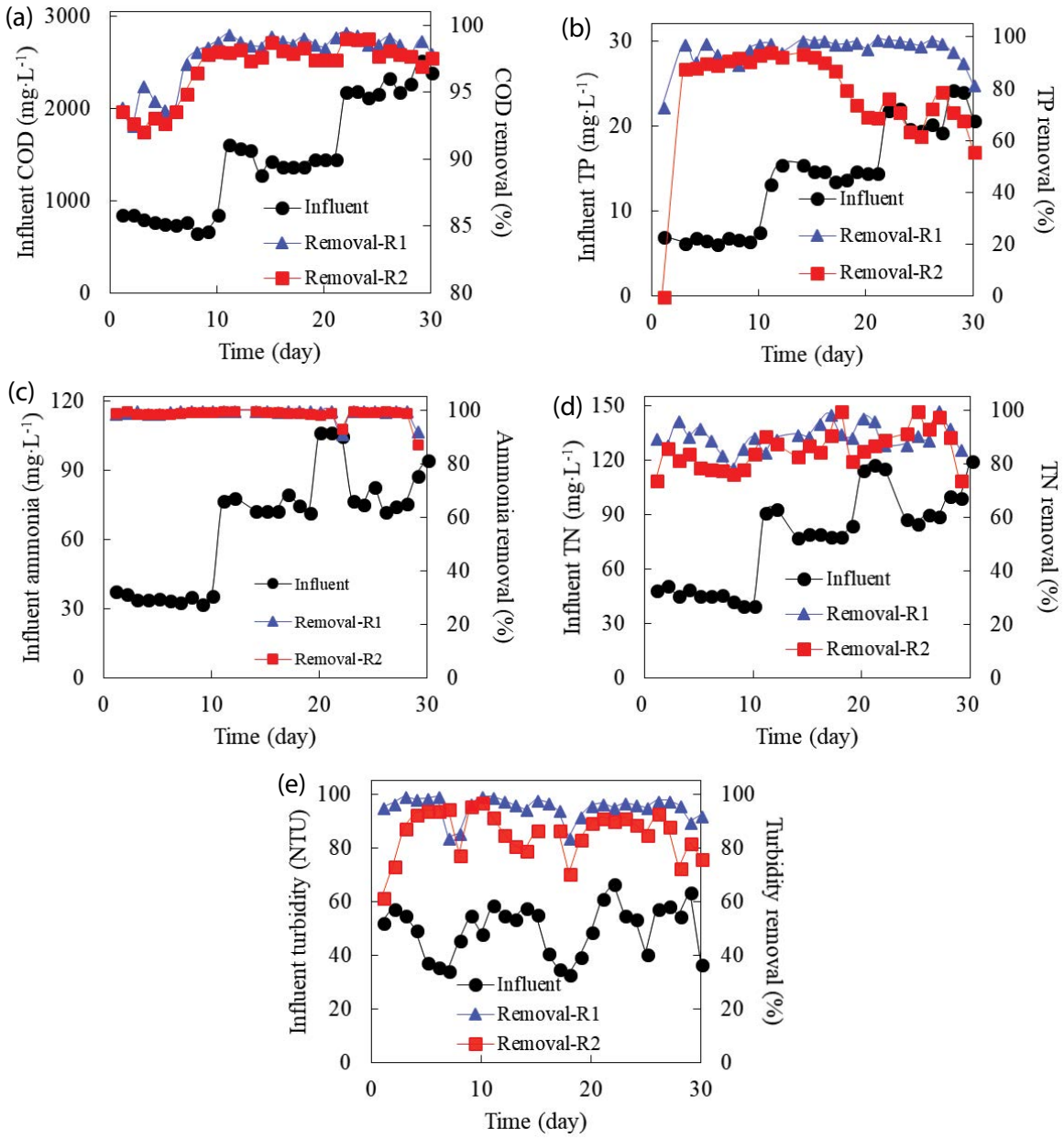

Fig. 1. Pollutants removal during start-up of SBRs: (a) COD, (b) TP, (c) ammonia, (d) TN, and (e) turbidity.

are supposed to increase if a red-shift happened, while a blue shift usually means a reduction of specific functional groups including carbonyl, hydroxyl, amine, and a number of aromatic rings [27]. 3D-EEM had been applied to predict the degradation of industrial wastewater and emerging contaminants in municipal WWTPs [24,25]. To verify the feasibility to use 3D-EEM as a predictor to determine the quality of treated pharmaceutical wastewater effluent, correlation analysis was performed between the COD and fluorescence intensities in the treated effluent in both reactors. Surprisingly, the fluorescence intensities of all peaks, particularly for peak *A*, were well correlated with COD, implying that 3D-EEM was a potential predictor to assess the quality of the treated pharmaceutical wastewater, which could be measured more timely than conventional COD measurement and easier for online monitoring in WWTPs (a few mins from sample preparation to data analysis).

3.3. Change of sludge properties

The properties of sludge in *R*1 and *R*2 were also measured. The MLVSS/MLSS ratio didn't change much in the beginning 8 d with an average of 0.58 and 0.40 in *R*1 and *R*2, respectively, which increased gradually to 0.81 and 0.77 (Fig. 3a). The increased MLVSS/MLSS ratio indicated that the activity of sludge increased during the start-up period. And the higher MLVSS/MLSS ratio in *R*1 could partly account for the higher pollutants removal in *R*1.

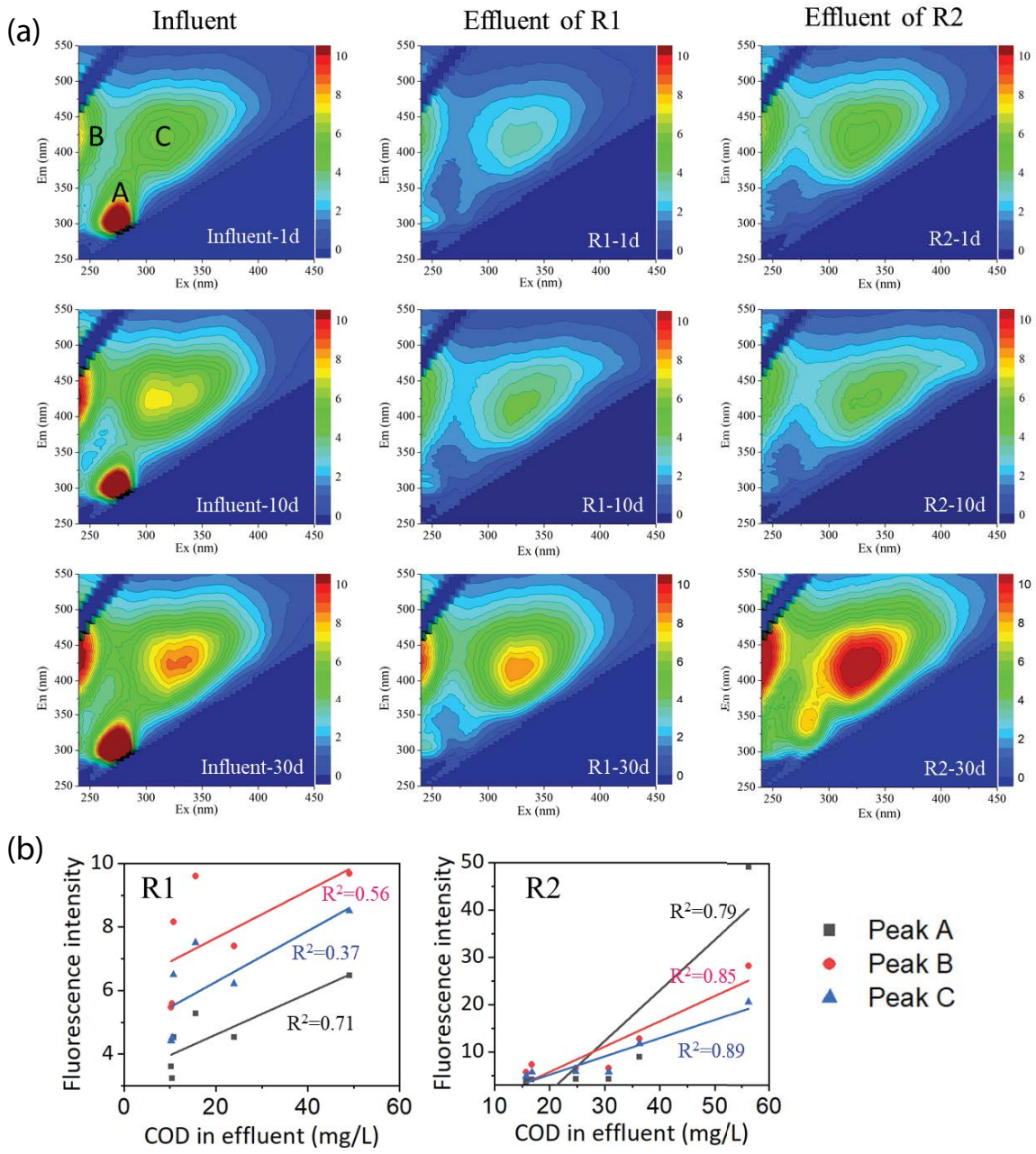

Fig. 2. (a) Three-dimensional excitation-emission matrix (3D-EEM) spectra of the influents and effluents in different operation time and (b) correlation analysis between fluorescence intensity and COD in the effluent of SBRs.

The sludge settling ability was crucial for operation, which is usually reflected by SVI_{30}. Stable SVI_{30} (47~80 mL g^{-1}) was maintained in *R*1, but the SVI_{30} in *R*2 firstly increased from 22.1 mL g^{-1} on day 1 to 117.1 mL g^{-1} on day 15 and ending up with 70.7 mL g^{-1} (Fig. 3b), suggesting a better and more stable sludge settling ability in *R*1.

3.4. Change of microbial community diversity during the start-up of SBRs

To identify the influence of inoculum on bacterial community dynamics during the start-up period, the sludge samples were collected and sequenced on day 0, day 10 and day 30. The number of OTUs (97% similarity) decreased from 818 and 1,028 in the inocula to 662 and 906 on day 30 in *R*1 and *R*2, respectively. The change of the Shannon index was consistent with that of OTUs, which implied that the diversity of the bacterial community decreased during the start-up period (Table 2). The PCoA (Fig. 4) showed that the microbial community of the same reactor was clustered together, but the microbial community in *R*1 separated with that in *R*2. And the microbial community on the day 30 separated with that on day 0 and day 10 in both reactors. The Bray–Curtis analysis also showed that the

Table 1
Fluorescence intensities of peaks *A*, *B* and *C* in influents and effluents during start-up of *R*1 and *R*2

Samples	Peak *A*		Peak *B*		Peak *C*	
	E_x/E_m	Intensity	E_x/E_m	Intensity	E_x/E_m	Intensity
Influent-1 d	275/303	17.1	240/426	8.0	320/419	5.9
Influent-10 d	275/298	18.0	240/428	10.8	320/430	7.5
Influent-30 d	275/301	22.5	240/430	11.4	335/426	8.7
*R*1-1 d	280/326	1.7	240/421	4.6	320/415	3.4
*R*1-10 d	280/379	2.1	240/419	5.5	320/402	4.4
*R*1-30 d	280/377	3.0	240/428	9.7	320/416	8.5
*R*2-1 d	280/379	2.2	240/419	6.9	320/416	5.5
*R*2-10 d	280/377	2.0	240/421	5.6	320/419	4.6
*R*2-30 d	280/337	8.1	240/423	12.8	320/412	11.8

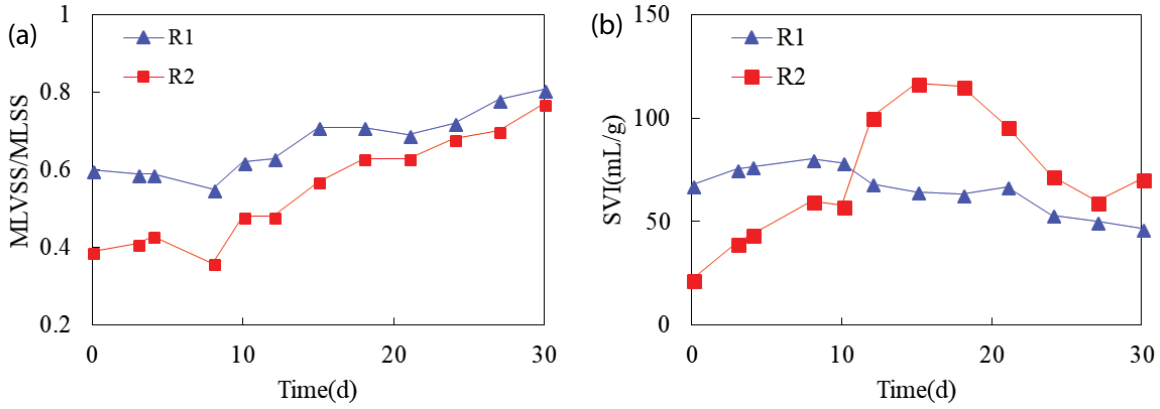

Fig. 3. Properties of sludge in *R*1 and *R*2 during the start-up period: (a) MLVSS/MLSS and (b) SVI.

bacterial community in *R*1 differed from that in *R*2 (Table 3). The results showed that the inoculum had a significant influence on the bacterial community structure during the start-up of SBRs.

3.5. Bacterial community dynamics during the start-up of SBRs

The bacterial community compositions and relative abundance at the phylum and genus levels are shown in Fig. 5. The most dominant phylum was *Proteobacteria* in all samples, whose relative abundance increased from 23.8% and 38.2% on day 0 to 41.3% and 62.0% on day 30 in *R*1 and *R*2, respectively. In contrast, the relative abundance of *Chloroflexi* decreased from 20.7% to 12.3% in *R*1 and 23.2% to 13.9% in *R*2. A similar abundance of *Parcubacteria* was observed in *R*1 (2.7%) and *R*2 (2.2%) on day 0, which finally rose to 16.6% in *R*1 but decreased to 1.0% in *R*2. *Saccharibacteria* took up 3%–6% in *R*1, but its relative abundance in *R*2 was less than 1%. The relative abundance of *Bacteroidetes* and *Acidobacteria* was also higher in *R*1.

At the genus level, the most dominant genus was *Saprospiraceae*_norank (6.3%) in *R*1 on day 0, followed by *Caldilineaceae*_norank (6.0%), *Saccharibacteria*_norank (5.5%), *Nitrospira* (5.3%) and *Blastocatellaceae*_Subgroup4_norank (5.0%). But as for *R*2, *Anaerolineaceae*_norank (13.7%) was the most dominant genus on day 0, with *Dechloromonas*, *Xanthomonadales*_norank, *VadinHA17*_norank and *SC-I-84*_norank taking up 4.2%, 4.0%, 3.1% and 2.5%, respectively. After 30 d operations, the *Candidatus Competibacter* became the most dominant genus in both reactors with a relative abundance of 27.3% in *R*1 and 19.8% in *R*2. *Candidatus Moranbacteria*_norank (16.2%) and *Roseiflexus* (4.2%) were also dominated in *R*1 finally, whose relative abundance was less than 0.1% in *R*2. However, *Zoogloea* ended up with a higher abundance in *R*1 (10.5%) than *R*2 (0.3%). These results indicated that the inoculum was a deciding factor shaping the microbial community during the start-up of SBRs.

Table 2
Diversity of the bacterial community in *R*1 and *R*2 during the start-up period

Samples	*R*1			*R*2		
	0 d	10 d	30 d	0 d	10 d	30 d
OTU	818	813	602	1,028	920	906
Shannon	5.35	4.92	4.08	5.78	5.43	5.13
Coverage (%)	99.1	99.2	99.1	99.5	99.1	99.2

To further compare the influence of inoculum on the microbial community functions, rough taxonomic groups were carried out according to functions (i.e., AOB; nitrite oxidation bacteria, NOB; anaerobic ammonia oxidation bacteria, Anammox; denitrification bacteria, DNB; phosphorous-accumulating organisms, PAO; organics removal bacteria, ORB) (Fig. 6). With increasing concentration of organics in the influents, the bacteria capable of degrading organic compounds increased as operation time progressed, the relative abundance of which increased from 17.5% and 13.6% to 36.1% and 50.1% in *R*1 and *R*2, respectively. The relative abundance of AOB and NOB decreased from 1.4% and 5.3% to 0.4% and 1.3% in *R*1, respectively, which was finally similar in two SBRs. The DNB accounted for 4.0% ± 0.9% in both reactors. 5.5% ± 0.1% of the bacteria were identified as phosphorous accumulation bacteria in *R*1 during the start-up period, whose relative abundance was 0.5% ± 0.2% in *R*2, which could explain higher TP removal in *R*1. The dynamics of bacterial community functions indicated that the ORB played an increasing role in both reactors as the concentration of the organic of influent increased during the start-up period of two SBRs, but a higher relative abundance of phosphorous accumulation bacteria could be kept in *R*1.

4. Discussions

In this study, SBRs were successfully started up with activated sludge from municipal WWTP or sludge from a pharmaceutical WWTP as the inoculum. The COD, TN, ammonia and turbidity removal were over 98%, 88%, 95% and 86% (average value in the final stage) in both reactors, respectively. And the fluorescence intensity of dissolved organic matter in the treated effluent was a promising indicator to assess the quality of the treated effluent. It was striking that high organics removal could be achieved in *R*1 inoculated with the activated sludge from the municipal WWTP, which was initially fed with the low concentration municipal wastewater. Contrary to previously reported severe inhibition of pharmaceuticals and antibiotics on bioreactor performance, the real pharmaceutical wastewater did not show obvious inhibition on bioreactor performance in this study [18,19]. The rapid adaption of activated sludge microbiomes from municipal WWTP to the high concentration pharmaceutical wastewater was possibly due to the ubiquitous occurrence of pharmaceuticals in the sewage influent of municipal WWTP [11,20,28,29], leading to the development of resistance genes to pharmaceuticals in the activated sludge microbiomes. Various pharmaceuticals as well as antibiotics had been detected in the sewage in municipal WWTPs with the concentrations up to hundreds of micrograms per liter, which could be further concentrated in the activated sludge [20,28,29]. The efficient removal of pharmaceuticals and antibiotics had been reported in WWTPs, implying a high capacity of the activated sludge microbiomes to degrade some pharmaceuticals and antibiotics [30]. Members of γ-*Proteobacteria* (including *Pseudomonas* and *Stenotrophomonas*) were reported to have potential degradation capacity for pharmaceuticals [29,30], which was enriched during the start-up of both reactors in this study (Fig. 5b). The presence of these bacteria in both reactors facilitates the degradation of complex organics and pharmaceuticals in pharmaceutical wastewater. In both reactors, *Candidatus Competibacter* was enriched during the start-up, with a relative abundance of 27.3% and 19.8% in *R*1 and *R*2 at the end of the experiment (Fig. 5c), respectively, which was a typical glycogen-accumulating organism (GAOs) identified in wastewater biotreatment reactors [31,32]. *Candidatus Competibacter* possessed a high ability to degrade simple organic compounds, like acetate, pyruvate, propionate and some amino acids. The high abundance of GAOs in reactors enables further clean-up of the fermentation products. Although pharmaceutical wastewater was efficiently treated in both SBRs, the compositions and removal efficiency of pharmaceuticals in the present study were not investigated due to the challenges to identify the pharmaceuticals in the complex real wastewater, which deserves further study. As expected, the SBR inoculated

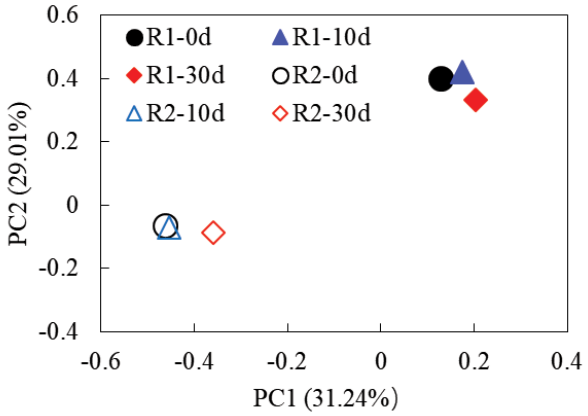

Fig. 4. The principal components analysis (PCoA) of the bacterial community during the start-up period.

Table 3
Bray–Curtis analysis of the bacterial community in *R*1 and *R*2 during the start-up period

	*R*1-0 d	*R*1-10 d	*R*1-30 d	*R*2-0 d	*R*2-10 d	*R*2-30 d
*R*1-0 d	0	0.40457	0.59517	0.82503	0.83366	0.83718
*R*1-10 d	0.40457	0	0.37286	0.86019	0.85383	0.84673
*R*1-30 d	0.59517	0.37286	0	0.90032	0.88614	0.86633
*R*2-0 d	0.82503	0.86019	0.90032	0	0.41336	0.56471
*R*2-10 d	0.83366	0.85383	0.88614	0.41336	0	0.45001
*R*2-30 d	0.83718	0.84673	0.86633	0.56471	0.45001	0

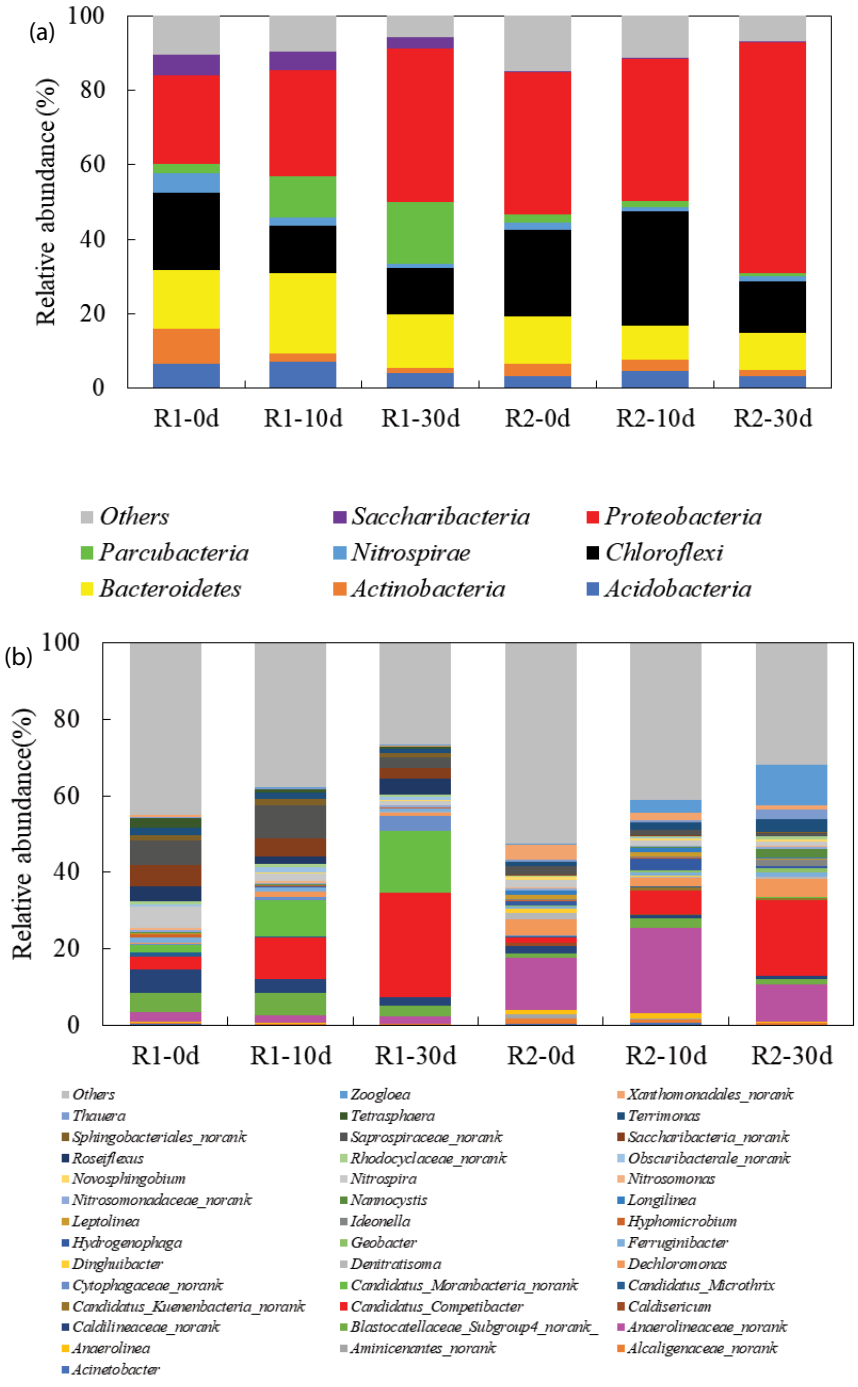

Fig. 5. Bacterial community composition and relative abundance at the (a) phylum and (b) genus level during the start-up period of SBRs.

with activated sludge from municipal WWTPs had more abundant PAOs [33], consistent with higher TP removal in *R*1. The sludge from bioreactors fed with similar wastewater was generally considered as the best inoculum to start up new industrial wastewater biotreatment reactors, believing more efficient start-up and better pollutant removal could be achieved. But in some cases, there is a lack of access to such inoculum. A huge amount of activated sludge was generated in the municipal WWTPs every year, which was proved as a potential inoculum to start up the industrial wastewater biotreatment reactors in this study.

Moreover, we found that inoculum sludge was a deciding factor shaping the microbial community in bioreactors. And the whole microbial community structure decoupled with the functions of bioreactors, as supported by similar pollutants removals (except for TP) but the distinct microbial community. The decoupling between whole bacterial community structures and functions were also found

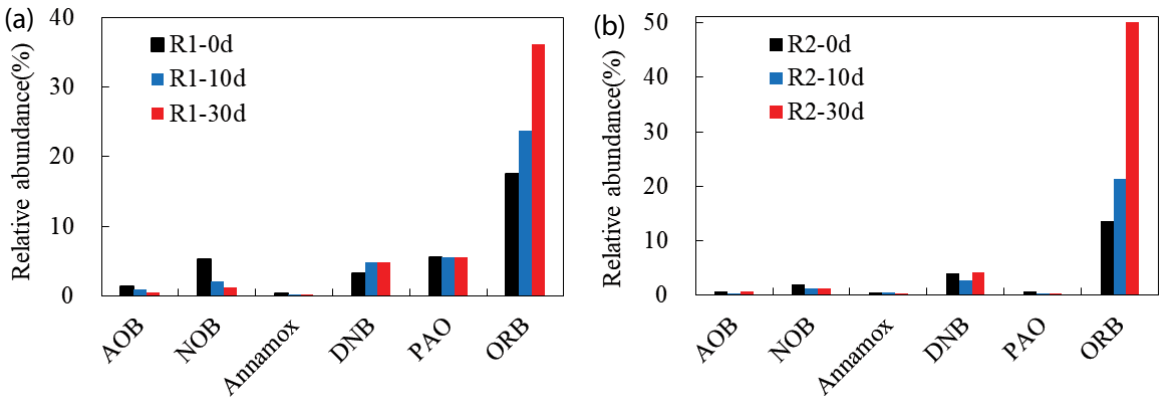

Fig. 6. The relative abundance of microbes with different functions in (a) *R*1 and (b) *R*2. AOB, ammonia oxidation bacteria; NOB, nitrite oxidation bacteria; Annamox, anaerobic ammonia oxidation bacteria; DNB, denitrification bacteria; PAO, phosphorous accumulation organism; ORB, organics removal bacteria. The compositions of each functional group are listed in Table S1.

in methanogenic bioreactors and other environmental matrices (e.g., marine and soil) [34]. Here, we found that the performance of bioreactors was more related to specific functional groups of bacteria, but the compositions of which may differ in bioreactors even with similar performance. For example, the taxonomic compositions within the functional bacterial group related to denitrification were distinct in two SBRs although the nitrogen removal was similar. *Rhodocyclaceae*_norank was the dominant DNB in *R*1, whereas, *Thauera* and *Anaeromyxobacter* were responsible for denitrification in *R*2. The compositions of PAO were also different in two SBRs. In the beginning, *Tetrasphaera*, *Candidatus Microthrix* and *Rhodocyclaceae*_norank were the dominant bacteria capable of removing phosphorous in *R*1, but only the denitrifying PAO-*Rhodocyclaceae*_norank was maintained during the start-up [35,36]. The conventional PAO-*Candidatus Accumulibacter* was slightly enriched from 0.1% to 0.6% in *R*1. The main PAOs in the inoculum of *R*2 were *Candidatus Microthrix* and *Pseudomonas*, whose abundance decreased as the GAOs accumulated during the start-up. The conventional PAO, *Candidatus Accumulibacter*, was rare in *R*2 [36].

Collectively, our results proved that the activated sludge from a municipal WWTP could be used as the inoculum to start up the SBR treating pharmaceutical wastewater, and comparable pollutants could be achieved compared with SBR inoculated with sludge from the pharmaceutical WWTP in this study. The higher relative abundance of bacterial related to phosphorous removal in the activated sludge from municipal WWTP provided additional advantages to be used as inoculum to start up bioreactors treating pharmaceutical wastewater. However, whether the activated sludge from municipal WWTP could be used to start-up bioreactors treating other organic industrial wastewaters deserves further investigations.

5. Conclusions

This study showed that the SBRs, treating real pharmaceutical wastewater, could be successfully started up within 30 d with the sludge from a municipal (*R*1) or pharmaceutical (*R*2) WWTPs as the inocula. Comparable and high COD, TN and ammonia removals were achieved in *R*1 and *R*2. But *R*1 performed much better in TP removal than *R*2 due to a higher relative abundance of PAOs in *R*1. The bacterial community structures were distinct in the inocula, and no trend of becoming phylogenetically similar was observed during the start-up period. Three fluorescence peaks were observed in the pharmaceutical wastewater which was identified as tryptophan-like, fulvic acid-like and humic acid-like compounds, and the fluorescence compounds could be degraded in SBRs. Moreover, the fluorescence intensity of dissolved organic matter was found as a promising indicator to assess the quality of treated pharmaceutical wastewater.

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Supporting information

Table S1
Compositions and relative abundance of each functional group bacteria

Functional group	Genus	Relative abundance (%)					
		*R*1-0 d	*R*1-10 d	*R*1-30 d	*R*2-0 d	*R*2-10 d	*R*2-30 d
Denitrification bacteria	g_*Pseudomonas*	0.20	0.08	0.29	0.37	0.26	0.12
	g_*Bacillus*	0.00	0.00	0.00	0.08	0.02	0.01
	g_*Acidovorax*	0.05	0.06	0.03	0.08	0.04	0.02
	g_*Comamonas*	0.03	0.01	0.04	0.03	0.03	0.00
	g_*Hyphomicrobium*	0.90	0.30	0.11	0.29	0.22	0.13
	g_*Rhodobacter*	0.28	0.13	0.03	0.32	0.78	0.18
	g_*Rhodocyclaceae*_g_norank_f_*Rhodocyclaceae*	0.95	1.07	0.57	0.11	0.00	0.00
	g_*Rhodocyclaceae*_g_unclassified_f_*Rhodocyclaceae*	0.62	3.03	3.51	0.03	0.03	0.17
	g_*Thauera*	0.06	0.10	0.19	0.44	0.47	2.45
	g_*Denitratisoma*	0.08	0.10	0.06	1.89	0.22	0.37
	g_*Anaeromyxobacter*	0.00	0.00	0.00	0.31	0.66	0.88
Anaerobic ammonia oxidation bacteria	g_*Planctomycetaceae*_g_norank_f_*Planctomycetaceae*	0.19	0.06	0.04	0.53	0.49	0.36
	g_*Planctomycetaceae*_g_unclassified_f_*Planctomycetaceae*	0.08	0.02	0.00	0.02	0.05	0.03
	g_*Planctomycetes*_g_unclassified_p_*Planctomycetes*	0.07	0.20	0.05	0.04	0.03	0.02
	g_*Candidatus Brocadia*	0.00	0.00	0.00	0.02	0.01	0.00
Ammonia oxidation bacteria	g_*Nitrosomonadaceae*_g_norank_f_*Nitrosomonadaceae*	0.67	0.47	0.34	0.40	0.33	0.64
	g_*Nitrosomonas*	0.69	0.41	0.09	0.30	0.11	0.16
Nitrite oxidation bacteria	g_*Nitrospira*	5.32	2.11	1.25	1.99	1.24	1.33
Phosphorous accumulating organism	g_*Tetrasphaera*	2.51	0.92	0.54	0.00	0.01	0.02
	g_*Rhodocyclaceae*_g_norank_f_*Rhodocyclaceae*	0.95	1.07	0.57	0.11	0.00	0.00
	g_*Rhodocyclaceae*_g_unclassified_f_*Rhodocyclaceae*	0.62	3.03	3.51	0.03	0.03	0.17
	g_*Candidatus Microthrix*	1.21	0.11	0.02	0.22	0.12	0.05
	g_*Pseudomonas*	0.20	0.08	0.29	0.37	0.26	0.12
	g_*Candidatus Accumulibacter*	0.13	0.31	0.56	0.00	0.00	0.04
Organics removal bacteria	g_*Arthrobacter*	0.00	0.01	0.00	0.01	0.05	0.00
	g_*Comamonas*	0.03	0.01	0.04	0.03	0.03	0.00
	g_*Candidatus Competibacter*	3.33	11.01	27.28	1.43	6.25	19.79
	g_*Exiguobacterium*	0.00	0.00	0.00	0.12	0.03	0.00
	g_*Pseudomonas*	0.20	0.08	0.29	0.37	0.26	0.12
	g_*Rhodococcus*	0.00	0.00	0.00	0.22	0.09	0.07
	g_*Sediminibacterium*	0.00	0.00	0.00	0.00	0.00	0.00
	g_*Sphingomonas*	0.01	0.01	0.00	0.17	0.06	0.11
	g_*Anaerolinea*	0.12	0.07	0.04	1.18	1.47	0.34
	g_*Acinetobacter*	0.27	0.05	0.03	0.29	0.57	0.07
	g_*Dechloromonas*	0.23	1.25	0.94	4.16	2.10	4.90
	g_*Ferruginibacter*	1.31	1.18	1.03	0.66	0.92	1.18
	g_*Geobacter*	0.00	0.04	0.02	0.26	0.53	1.02
	g_*Leptolinea*	0.09	0.02	0.02	1.23	1.17	0.32
	g_*Nannocystis*	0.09	0.19	0.04	0.10	0.30	2.29
	g_*Novosphingobium*	0.18	0.07	0.08	0.79	0.70	0.35
	g_*Saccharibacteria*_g_norank_p_*Saccharibacteria*	5.53	4.92	2.87	0.23	0.26	0.17
	g_*Sphingobacteriaceae*_g_norank_f_*Sphingobacteriaceae*	0.00	0.00	0.00	0.00	0.00	0.00
	g_*Sphingobacteriales*_g_norank_o_*Sphingobacteriales*	1.34	1.71	1.24	0.08	0.09	0.37
	g_*Sphingobacteriales*_g_unclassified_o_*Sphingobacteriales*	0.00	0.00	0.00	0.03	0.10	0.10
	g_*Terrimonas*	2.11	1.65	1.03	1.20	2.06	3.40
	g_*Thauera*	0.06	0.10	0.19	0.44	0.47	2.45
	g_*Tetrasphaera*	2.51	0.92	0.54	0.00	0.01	0.02
	g_*Zoogloea*	0.05	0.32	0.30	0.19	3.29	10.54