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Specific microbial diversity and functional gene (AOB amoA) analysis of a sponge-based aerobic nitrifying moving bed biofilm reactor exposed to typical pharmaceuticals

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Abstract

Four bench-scale sponge-based aerobic nitrifying moving bed biofilm reactors (MBBRs) were used to treat municipal wastewater containing typical pharmaceuticals (1 mg/L, 2 mg/L and 5 mg/L). This preliminary research aims to investigate the effects of sulfadiazine (SDZ), ibuprofen (IBU) and carbamazepine (CBZ) on nitrification performance and explore specific microbial diversity and functional gene (Ammonia-oxidizing bacteria (AOB), amoA) of MBBRs. After 90 days of operation, the MBBR without pharmaceuticals could remove up to 97.4 ± 1.5% of NH_4^+-N while the removals of NH_4^+-N by the MBBRs with SDZ, IBU and CBZ were all suppressed to varying degrees. Based on the Shannon and Chao 1 index, the specific microbial diversity and richness in biofilm samples increased at a range of 1 mg/L to 2 mg/L pharmaceuticals (SDZ, IBU or CBZ) and started decreasing after the pharmaceutical concentration was higher than 2 mg/L. The determination of functional gene (AOB amoA) showed that *Proteobacteria* was the most dominant bacteria within all biofilms with the relative abundance ranging from 24.81% to 55.32%. Furthermore, *Nitrosomonas* was the most numerous genus in AOB, followed by *Campylobacter* and *Thauera*, whose relative abundance shifted under the pressure of different pharmaceuticals.

Keywords: Moving bed biofilm reactor; Ammonia-oxidizing bacteria; Pharmaceuticals; Microbial diversity

1. Introduction

Moving bed biofilm reactor (MBBR) is an efficient sewage treatment process developed on the basis of activated sludge and biofilm processes (Song et al., 2019a). By adding a

certain number of suspended carriers, the biomass and biological species of the reactors can be increased, which helps to improve the treatment efficiencies (Huang et al., 2016). Many studies have confirmed that MBBR performs excellently in removing nitrogen. Three aerobic MBBR reactors with different carriers filling rates (10%, 20% and 30%) were operated by Zhang et al. (2016), and all reactors exhibited high NH_4^+-N removal efficiencies (>95%) at an HRT of 12 h. Young et al. (2016) investigated the effects of two surface area loading rates on nitrogen removal in an aerobic MBBR system, and high NH_4^+-N removal was observed at both high loading and normal loading states with average efficiencies of 93.6 ± 3.0% and 97.6 ± 1.0%, respectively.

MBBRs are also considered an alternative technology for treating pharmaceutical wastewater because they have certain advantages over the conventional activated sludge process (Ooi et al., 2018). Being contaminants that are most closely related to people's daily lives, pharmaceuticals have generated much concern because of their adverse effects on the environment (Song et al., 2019b). Their long-term discharge will not only do harm to the majority of aquatic organisms and destroy the ecological balance of water bodies, they also create problems for wastewater treatment plants (Praveena et al., 2019). To date, the research on the effect of high concentrations of pharmaceutical wastewater on MBBRs' nitrogen removal performance has not been reported.

Microorganisms play an important biochemical role in nutrient recycling in the ecosystem, and their communities often exhibit high microbial diversity and respond to changes in environmental conditions (Li et al., 2016). Pollutants in biological wastewater treatment systems are mainly removed by biodegradation, thus microbial communities have

been regarded as the ultimate factor responsible for pollutants' removal (Fang et al., 2012). Some functional genes play a significant role in nitrogen removal's processes, mainly including genes for ammonia oxidation (AOBamoA), nitrate reduction (narG) and nitrite reduction (nirK and nirS). The starting and pivotal step of nitrification process is the stage of ammonia nitrogen conversion to nitrate, which is completed by the functional gene (AOB amoA) (Fan et al., 2019).

Nitrogen removal efficiency can be affected indirectly by changing the abundance and distribution of functional genes according to environmental factors (Deng et al., 2019). Thus, the influence of pharmaceuticals on the nitrification performance of MBBR can be reflected through their effects on the functional genes (AOB amoA). In-depth understanding the microbial community changes within functional genes under different concentrations pharmaceuticals' stress are therefore essential to understand the inhibitory effect of different pharmaceuticals on the removal of nitrogen and the impact resistance of MBBR process on pharmaceuticals. However, it still remains unclear and relevant studies have not been reported. Therefore, three kinds of pharmaceuticals (sulfadiazine (SDZ), ibuprofen (IBU) and carbamazepine (CBZ)) with large daily use but having different physical and chemical characteristics were selected to explore the changes occurring in specific microbial diversity and functional gene (AOB amoA) when exposed to them.

2. Materials and methods

2.1 MBBR system and operation

Four bench-scale MBBR reactors with a working volume of 12 L were operated in parallel (Fig. 1), and they were named $MBBR_1$ (no pharmaceuticals), $MBBR_2$ (SDZ), $MBBR_3$

(IBU) and $MBBR_4$ (CBZ), respectively. As the pharmaceuticals concentration increased, the whole experimental process was divided into three periods (Day 1-30: 1 mg/L, Day 30-60: 2 mg/L and Day 30-90: 5 mg/L). Polyurethane sponges (Joyce Foam Pty, Australia) with a 28 kg/m^3 density were selected as the biocarriers, which were cut into cubes with a diameter of 15mm and added into the MBBR reactors at a 20% filling fraction. The activated sludge came from a local wastewater treatment plant (Tianjin, China). After acclimatization, the activated sludge seeded to the MBBR reactors with 2.8 g/L mixed liquor suspended solids (MLSS). The airflow was kept at around 0.09 m^3/h in all MBBRs, and in this case the dissolved oxygen concentration was 5~6.5 mg/L. Throughout the entire experiment, the flow rate was kept at 16.7 mL/min and the hydraulic retention time (HRT) was 12 h.

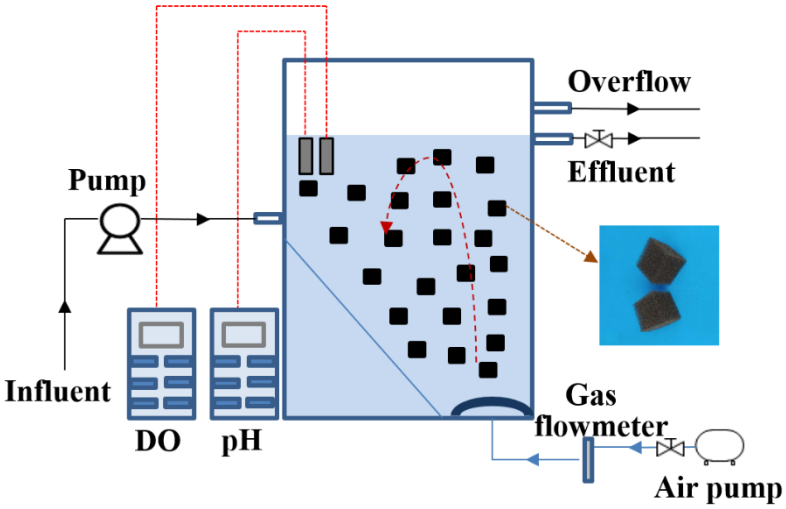

Fig. 1 Schematic of experimental set up for continuous flow MBBR

2.2 Synthetic wastewater

In this study, glucose, $(NH_4)_2SO_4$ and KH_2PO_4 were used as carbon, nitrogen and phosphorus sources, respectively. The influent contained approximately 115 mg/L total organic carbon (TOC), 30 mg/L NH_4^+-N and 3 mg/L total phosphorus. The trace nutrient

solution contained: $MgSO_4 \cdot 7H_2O$, 5.07 mg/L; $CaCl_2 \cdot 2H_2O$, 0.37 mg/L; $FeCl_3$, 1.45 mg/L; $MnCl_2 \cdot 7H_2O$, 0.28 mg/L; $ZnSO_4 \cdot 7H_2O$, 0.44 mg/L; $CuSO_4 \cdot 5H_2O$, 0.39 mg/L; and $CoCl_2 \cdot 6H_2O$, 0.42 mg/L. All chemicals and supplies were obtained from Tianjin, China. The pharmaceuticals (SDZ, IBU and CBZ) were purchased from Shanghai Dibai Biotechnology Co., Ltd.. Among them, SDZ belongs to sulfonamide antibiotics, and is frequently used as antimicrobials and bacterial infections. IBU is a non-steroidal anti-inflammatory drug and contains a strong electron donor (-OH). The antispasmodic drug CBZ contains an electron acceptor (amide: $CONH_2$).

2.3 Chemical analyses

The standard methods (APHA, 2005) was used to examine the concentrations of MLSS, mixed liquor volatile solids (MLVSS), attached-growth biomass (AGBS) and volatile attached-growth biomass (VAGBS). NH_4^+-N was measured using an ultraviolet spectrophotometer (UV- 2700, Shimadzu, Japan). The TOC analyzer (TOC-VWP, Shimadzu, Japan) was used to measure TOC. The pharmaceuticals analysis refers to the study previously published by Zhang et al. (2020). In summary, the samples were pretreated by solid phase extraction (SPE) with Oasis (HLB) extraction cartridges (500 mg, 6 cc, Waters, USA). A high performance liquid chromatography-triple quadrupole mass spectrometer (Agilent 1200 series) equipped with an Agilent Eclipse Plus C18 column (the diameter, length and pore size are 2.1 mm, 150 mm, 3.5 μm, respectively) served the quantitative analysis of pharmaceuticals.

The biofilm of biocarriers was extracted into 20 ml ultrapure water by hand extrusion and ultrasound, and the ultrasound was operated continously at 40 W power to destroy the adhesion structure of biofilm and form a uniform and stable state. Extracellular polymeric

substances (EPS) was obtained by the heat method reported by Liu et al. (2018). The total amount of EPS was reflected by the biofilm's TOC content. The concentration of polysaccharide (PS) was determined by the anthrone-sulfuric acid method (Dubois et al., 1956) while the protein (PN) was found with the Coomassie brilliant blue method (Frølund et al., 1995).

2.4 Microbial community diversity and functional gene analysis

According to the different concentration of pharmaceuticals in different operation periods, the microbial samples obtained from the biocarriers in $MBBR_1$, $MBBR_2$, $MBBR_3$ and $MBBR_4$ on the 20^{th} day (1 mg/L pharmaceuticals) were named A1, A2, A3 and A4, respectively. Similarly, the samples obtained on the 50^{th} day (2 mg/L pharmaceuticals) were named B1, B2, B3 and B4, respectively, and obtained on 80^{th} day (5 mg/L pharmaceuticals) were named C1, C2, C3 and C4, respectively. The functional gene (AOB amoA) associated with nitrification was investigated by the real-time fluorescent quantitative polymerase chain reaction (qPCR). After the DNA was extracted by a Qubit ssDNA Assay Kit, PCR amplification was carried out. The amplification region was AOB amoA, and the amplification sequence was GGGGTTTCTACTGGTGGT and CCCCTCKGSAAAGCCTTCTTC. PCR amplification performed the following sequence in a thermal cycle: initial denaturation at 94 ℃ for 5 minutes, followed by 35 cycles of denaturation at 94 ℃ for 30 s, annealing at 50 ℃ for 30 s, and elongation at 72 ℃ for 60 s, finally 72 ℃ for 7 mins and 4 ℃ until the user stopped. The Illumina MiSeq sequencing were carried out by the Novogene (Beijing, China) (Yang et al., 2019b).

The software Bcl2fastq (V2.17.1.14) was used for image base calling to obtain the original sequencing data, which was stored in FASTQ file format. OTU clustering to unique sequence (repeat number >1) according to the 97% similarity were carried out, and the chimera sequence in clustering process were removed further. Alpha diversity is applied in analyzing community richness and species diversity, and the indices were calculated with QIIME (Version1.7.0) and displayed with R software (Version 2.15.3) (Yang et al., 2019a). Based on the classification data, the relative abundance of microorganisms at the level of phylum and genus was calculated.

3. Results and discussion

3.1 Effect of pharmaceuticals on nitrification performance

From the experimental results, the removal efficiency of NH_4^+-N in $MBBR_1$ without adding any pharmaceuticals could reach 97.4 ± 1.5%. However, the nitrification performance in the MBBRs with different pharmaceuticals was inhibited to some extent, and the removal efficiency of NH_4^+-N in $MBBR_4$ with CBZ was the lowest, only 74.1 ± 6.7%. This was caused by a large amount of residual CBZ in $MBBR_4$ which had the toxic effect on microorganisms and inhibited the NH_4^+-N removal. About 83.1 ± 6.1% of NH_4^+-N was removed from $MBBR_3$ with IBU. This phenomenon was contrary to $MBBR_4$ reactor because IBU is an easily biodegradable organic matter with a removal efficiency of 74.9 ± 8.8% (Table 1). The residual was relatively small, which has a relatively moderate inhibitory effect on functional gene (AOB amoA). Meanwhile the inhibition of SDZ on nitrification performance was the worst with a relatively high NH_4^+-N removal efficiency (89.4 ± 5.2%). The removal efficiency of SDZ in $MBBR_2$ was 61.1 ± 8.8% (Table 1), but it had little

inhibitory effect on NH_4^+-N removal, which may be related to its almost non-hydrophilic property. As a whole, the removal efficiency of NH_4^+-N in $MBBR_2$, $MBBR_3$ and $MBBR_4$ declined as the concentration of added pharmaceuticals increased, indicating that the more pharmaceuticals contained in sewage, the greater the negative impact on the wastewater treatment plant.

Table 1

Removal efficiencies of SDZ, IBU and CBZ at different periods.

Period	SDZ ($MBBR_1$)	IBU ($MBBR_2$)	CBZ ($MBBR_3$)
1-30d (1mg/L pharmaceuticals)	71.05 ± 0.48%	84.83 ± 0.34%	37.46 ± 1.64%
31-60d (2mg/L pharmaceuticals)	62.65 ± 0.68%	76.37 ± 0.30%	27.66 ± 0.67%
61-90d (5mg/L pharmaceuticals)	49.64 ± 0.47%	63.48 ± 0.62%	19.69 ± 1.22%
Average	61.11 ± 8.82%	74.89 ± 8.79%	28.27 ± 7.37%

Note: sulfadiazine (SDZ), ibuprofen (IBU), carbamazepine (CBZ)

3.2 Effect of pharmaceuticals on biofilm properties

The production of EPS is an important factor affecting the physiological characteristics of microorganisms. Fig. 2 shows the effect of different kinds of pharmaceuticals on the change in EPS while the reactor is in operation, including the changes in PN, PS and humic acid. The total amount of EPS in $MBBR_1$ without any pharmaceuticals was the highest, while that of the EPS in $MBBR_4$ with CBZ was the smallest, which was related to the total biomass in the reactors (More et al., 2014). As shown in Table 2, the order of attached biomass in four parallel MBBR systems was as follows: $MBBR_1$ (no phamaceuticals) > $MBBR_2$ (SDZ) >

$MBBR_3$ (IBU) > $MBBR_4$ (CBZ).

While with the rising pharmaceuticals concentration, the content of EPS in the four reactors also increased. This may be due to the environment in the MBBR system becoming worse after adding more pharmaceuticals. In this case, the self-protection mechanisms employed by microorganisms gradually improved, and more EPS was released to absorb nutrition and store energy (Liu et al., 2018). It should be noted here that those microorganisms not able to adapt to the hostile environment and die became part of the organic matter and contributed to the EPS content.

Referring to the content of PS, compared with $MBBR_1$, the PS in the reactors with pharmaceuticals all decreased, which indicated that pharmaceuticals could inhibit the secretion of PS by microorganisms, thus affecting the latter's polymerization; the content of PN in the four MBBR reactors was slightly different, which meant that PN was virtually unaffected by the pharmaceuticals. However, the value of PN/PS was quite different in the four reactors, among which, the value of PN/PS in $MBBR_4$ with CBZ was the largest, followed by $MBBR_3$ (IBU) and $MBBR_2$ (SDZ), and the value in $MBBR_1$ was the smallest, which was very different to the total content of EPS. The value of PN/PS is closely related to the stability of the reactor. The higher the value of PN/PS is, the worse the stability of the reactor is (Tan et al., 2014). These results showed that the toxic effect of pharmaceuticals on the MBBR system in this study was CBZ > IBU > SDZ, which was also consistent with the nitrification effect in the four MBBR reactors.

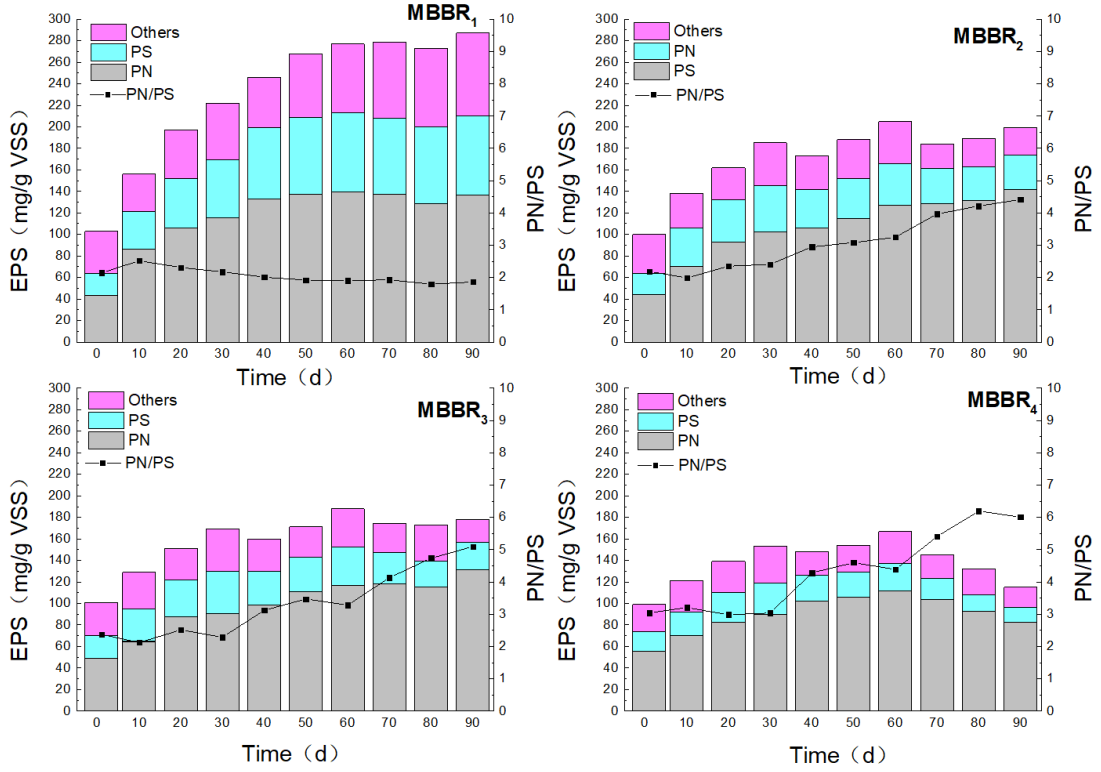

Fig. 2 Variations of EPS, PS, PN concentrations in MBBRs with adding different pharmaceuticals ($MBBR_1$: no pharmaceuticals; $MBBR_2$: SDZ; $MBBR_3$: IBU; $MBBR_4$: CBZ)

Table 2

AGBS and VAGBS in the four parallel MBBRs

	$MBBR_1$	$MBBR_2$	$MBBR_3$	$MBBR_4$
aAGBS(g/g carrier)	0.405±0.094	0.349±0.078	0.308±0.060	0.298±0.054
aVAGBS(g/g carrier)	0.320±0.085	0.258±0.070	0.215±0.054	0.192±0.042

Note: $MBBR_1$: no pharmaceuticals, $MBBR_2$: SDZ, $MBBR_3$: IBU, $MBBR_4$: CBZ

3.3 Effect of pharmaceuticals on specific microbial diversity

The alpha diversity of functional amoA genes in the MBBR system exposed to typical pharmaceuticals was further analyzed. Shannon index is a common community diversity index. The larger its value is, the more obvious the biodiversity is (Zhang et al., 2018). This

species diversity index which takes the number of species as well as their relative abundance into account was calculated at equal sequencing depths for all the biofilm communities (Table 3). On the whole, the values of Shannon index were between 3.15 and 4.82. For the three MBBR reactors in which the pharmaceuticals were added, the greatest value was all found in biofilm samples of group B (2 mg/L pharmaceuticals) compared to the samples of group A (1mg/L pharmaceuticals) and group C (5 mg/L pharmaceuticals). Previous studies have also explained this phenomenon, which indicated that microbial communities may have specific adaptive changes and emerge stronger resistance to pharmaceuticals' effects when exposed to various loads of pharmaceuticals (Chonova et al., 2016; Vasiliadou et al., 2018). However, compared to other reactors, $MBBR_4$ with CBZ has the lowest biodiversity. As the average removal efficiency of CBZ was only 27.67 ± 0.67%, when the influent concentration was 2mg/L, a large number of untreated pollutants remained in the reactor, which has a negative impact on biodiversity. The biofilm samples in group C under 5 mg/L pharmaceuticals had the lowest diversity from the Shannon index value. This is due to the toxicity of pharmaceuticals far exceeding the self-protection abilities of microorganisms at excessively high concentrations. In this stage, the average removal efficiencies of SDZ, IBU and CBZ were 49.6 ± 0.47%, 63.48 ± 0.62% and 19.69 ± 1.22%, respectively (Table 1). The excessive residues caused many microorganisms to lose their vitality, and led to the decline of biodiversity.

Comparable differences in biodiversity were also observed when using species richness instead of the Shannon index. While the index of Chao 1 was relevant to microbial richness, and revealed a tendency to increase from 1 mg/L to 2 mg/L pharmaceuticals and a decreasing

tendency from 2 mg/L to 5 mg/L pharmaceuticals. The results exhibiting the greatest richness were observed in biofilm samples of group B and not groups A and C.

Table 3

Alpha diversity analysis of AOB amoA

Sample	OTU number	Shannon index	Chao1 index	Coverage
A1	642	4.632	842.98	0.996
A2	772	4.174	787.26	0.997
A3	737	4.054	655.11	0.996
A4	722	3.857	603.29	0.995
B1	767	4.818	1091.16	0.997
B2	784	4.654	872.12	0.997
B3	767	4.318	810.93	0.996
B4	787	4.073	708.05	0.996
C1	756	4.786	944.59	0.996
C2	725	3.492	547.77	0.995
C3	645	3.264	480.02	0.995
C4	676	3.149	374.13	0.995

Note: A1, A2, A3 and A4 represent the samples obtained from the biocarriers in $MBBR_1$, $MBBR_2$, $MBBR_3$ and $MBBR_4$ on the 20^{th} day (1 mg/L pharmaceuticals), respectively. Similarly, B1, B2, B3 and B4 represent the samples obtained on the 50^{th} day (2 mg/L pharmaceuticals) and C1, C2, C3 and C4 represent the samples obtained on 80^{th} day (5 mg/L pharmaceuticals), respectively.

3.4 Effects of pharmaceuticals on microbial community in AOB amoA

3.4.1 Changes in microbial community structure at the phylum level

Microbial community structures and functions were analyzed in detail in this study to

assess the effects of pharmaceuticals on the MBBR system. At the phylum level, among all biofilm samples, *Proteobacteria* was the most dominant phylum with a relative abundance of 24.81% to 55.32%, which has been documented in other studies (Zhang et al., 2018). *Actinobacteria*, the second dominant phylum, could withstand the biological toxicity of pharmaceuticals and always appeared under each pharmaceutical concentration. The lowest relative abundance (1.07% on average) of *Actinobacteria* was found in the biofilm samples of $MBBR_1$ without any pharmaceuticals, indicating that the presence of pharmaceuticals stimulated the proliferation of *Actinobacteria*. In contrast, the relative abundance of *Actinobacteria* in $MBBR_4$ with CBZ was the highest (2.78% on average), followed by $MBBR_2$ with SDZ (1.57% on average), and then $MBBR_3$ with IBU (1.11% on average). A similar conclusion was also observed in another recent study, where *Actinobacteria* contributed to the degradation of carbamazepine, but cannot be present in the wastewater containing high concentrations of ibuprofen (Zhou et al., 2019).

Bacteroidetes, *Firmicutes* and *Ascomycota* were the three species with high relative abundance in the tested samples. After adding pharmaceuticals in $MBBR_2$, $MBBR_3$ and $MBBR_4$, *Firmicutes* increased gradually (from 0.01% to 1.14% on average) while *Ascomycota* had the opposite trend (from 0.81% to 0.07% on average), which suggested that *Firmicutes* adapted better to the pressure of pharmaceuticals compared to *Ascomycota*. Meanwhile, *Firmicutes* may also make a big difference in pharmaceuticals degradation as found elsewhere (Wan et al., 2018). Ng et al. (2016) also reported that the phylum *Firmicutes* is able to tolerate unfavorable environments.

3.4.2 Changes in microbial community structure at the genus level

The microbial community at the genus level displayed a considerable difference between the four MBBR reactors with or without different pharmaceuticals. The relative abundance of the 20 largest genera of functional genes (AOB) in all samples is shown in Fig. 3.

For the control reactor $MBBR_1$ without any pharmaceuticals, *Nitrosomonas* showed an increasing trend from Day 20 (13.06%) to Day 80 (22.75%), so did *Nitrosospira* (A1: 0.003%, B1: 0.58%, C1: 1.12%), which were the main ammonia oxidizers and nitrite oxidizers, respectively. The results indicated that the microorganisms related to nitrogen removal accumulated continuously, and the nitrogen removal performance retained a high efficiency and stability in continuous operation mode. *Campylobacter* was the second most dominant bacteria after *Nitrosomonas* in $MBBR_1$, which increased continuously throughout the operation from Day 20 (0.29%) to Day 50 (21.38%), indicating that this genus could adapt to the current environment. However, some genera, mainly including *Delftia* (from 3.28% to 0.08%), *Thauera* (from 2.64% to 0.50%), *Acidovorax* (from 3.32% to 0.10%), *Polymorphum* (from 4.47% to 0.57%) and *Lysobacter* (from 1.32% to 0.14%) showed a downward trend.

For $MBBR_2$ with SDZ, *Campylobacter* was the most abundant genus (A2: 18.99%, B2: 23.97%, C2: 9.55%), and studies had reported a significant increase in antibiotic-resistant *Campylobacter* strains when the antibiotic concentration increases (Teh et al., 2019). Additionally, *Nitrosomonas* which plays an important role in the nitrification process came second in terms of relative abundance (A2: 16.85%, B2: 13.05%, C2: 11.46%) and it kept going down gradually with the increase of SDZ concentration, which was consistent with the nitrification effect of $MBBR_2$. Results achieved here agreed with those reported by Wan et al. (2018). The next three most abundant species were *Capronia*, *Streptomyces* and *Pseudomonas*,

of which the last could metabolize diverse chemicals (Ma et al., 2015).

For $MBBR_3$ with IBU, the five largest genera were *Nitrosomonas*, *Campylobacter*, *Thauera*, *Acidovorax* and *Streptomyces*, of which the first three increased the concentration of IBU. This observation was very different to that of Zhou et al. (2019), who reported that with the increase of ibuprofen concentration (0-5 mg/L), *Nitrospira* and *Nitrosomonas* decreased but *Acidovorax* increased. These differences could be explained by the different forms of biological existence (one was biofilm and the other was activated sludge). There were also some microorganisms that were insensitive to IBU, such as *Pseudoxanthomonas* (always around 0.30%) and *Lysobacter* (always around 0.15%), because they remained stable throughout the whole process.

For $MBBR_4$ with CBZ, and the same as $MBBR_1$, *Nitrosomonas* was the most abundant, but the difference was that *Delftia* became the second most abundant (A4: 17.19%). The third most abundant was *Campylobacter* (from 0.90% to 10.06% and then 10.81%), which was associated with the degradation of CBZ, and it was significantly enriched in the samples at higher CBZ concentration (2 mg/L and 5 mg/L). Together with *Campylobacter*, it emerged that *Mitsuaria* in the biofilm samples at 5 mg/L CBZ concentration (4.01%) were larger than those at 1 mg/L CBZ (0.16%). This was thought to be related to the biodegradation of CBZ because they seem to develop a specific adaptation to the CBZ's effects. Compared with the biofilm sample at 1 mg/L CBZ concentrations, some common genera tended to decline, mainly including *Delftia*, *Acetobacter*, *Lysobacter* and *Acidovorax*, which were generally considered to be related to nitrogen removal and not be resistant to the harmful environment.

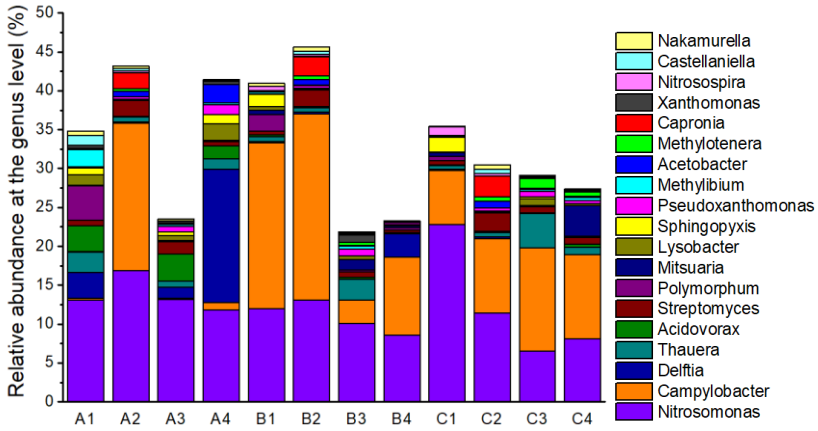

Fig. 3 The relative abundance of the 20 largest genera of functional genes (AOB)

4. Conclusions

The key findings from this study were drawn below:

1. Sponge-based aerobic nitrifying MBBR reactors had a certain tolerance to the high concentrations of pharmaceuticals;
2. Different types of pharmaceuticals could affect the MBBR system's nitrogen removal by changing the community structure of functional gene (AOB amoA);
3. Among the pharmaceuticals, CBZ performed the best in inhibiting nitrification, followed by IBU and SDZ.
4. The presence and degradation of typical pharmaceuticals resulted in changes of EPS, and PS was more sensitive to SDZ, IBU and CBZ than PN.
5. Within the limited concentration range (< 2 mg/L), an increase in the concentration of pharmaceuticals could improve the diversity and richness of functional genes (AOB amoA) in the MBBR system.

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References

APHA, AWWA, WEF, 2005. Standard Methods for the Examination of Water and Wastewater. 20th ed. American Public Health Association, Washington, D.C.

Chonova, T., Keck, F., Labanowski, J., Montuelle, B., Rimet, F., Bouchez, A. 2016. Separate treatment of hospital and urban wastewaters: A real scale comparison of effluents and their effect on microbial communities. *Science of the Total Environment*, **542**(Pt A), 965-975.

Deng, Z., Wang, Z., Zhang, P., Xia, P., Ma, K., Zhang, D., Wang, L., Yang, Y., Wang, Y., Chen, S., Deng, S. 2019. Effects of divalent copper on microbial community, enzymatic activity and functional genes associated with nitrification and denitrification at tetracycline stress. *Enzyme and Microbial Technology*, **126**, 62-68.

Dubois, M., Gilles, K.A., Hamilton, J.K., Rebers, P.A., Smith, F. 1956. Colorimetric method for determination of sugars and related substances. *Analytical Chemistry*, **28**, 350-356.

Fan, Y., Li, B., Yang, Z., Cheng, Y., Liu, D., Yu, H. 2019. Mediation of functional gene and bacterial community profiles in the sediments of eutrophic Chaohu Lake by total nitrogen and season. *Environmental Pollution*, **250**, 233-240.

Fang, H., Cai, L., Yu, Y., Zhang, T. 2012. Metagenomic analysis reveals the prevalence of biodegradation genes for organic pollutants in activated sludge. *Bioresource Technology*, **129c**(2), 209-218.

Frølund, B., Griebe, T., Nielsen, P.H. 1995. Enzymatic activity in the activated-sludge floc matrix. *Applied Microbiology and Biotechnology*, **43**(4), 755–761.

Huang, C., Shi, Y., Xue, J., Zhang, Y., El-Din, M.G., Liu, Y. 2016. Comparison of biomass from integrated fixed-film activated sludge (IFAS), moving bed biofilm reactor (MBBR) and membrane bioreactor (MBR) treating recalcitrant organics: importance of attached biomass. *Journal of Hazardous Materials*, **326**, 120-129.

Li, C.Y., Mu, B.Z., Hu, H., Gu, J.D., Liu, J.F., Feng, J.Y., Mbadinga, S.M., Yang, S.Z. 2016. Diversity and abundance of ammonia-oxidizing bacteria (AOB) revealed by PCR amplification of amoA gene in a polyacrylamide transportation system of an oilfield. *International Biodeterioration & Biodegradation*, **115**, 110-118.

Liu, Y., Guo, J., Lian, J., Chen, Z., Li, Y., Xing, Y., Wang, T. 2018. Effects of extracellular polymeric substances (EPS) and N-acyl-L-homoserine lactones (AHLs) on the activity of anammox biomass. *International Biodeterioration & Biodegradation*, **129**, 141-147.

Ma, Q., Qu, Y., Zhang, X., Shen, W., Liu, Z., Wang, J., Zhang, Z., Zhou, J. 2015. Identification of the microbial community composition and structure of coal-mine wastewater treatment plants. *Microbiological Research*, **175**, 1-5.

More, T.T., Yadav, J.S.S., Yan, S., Tyagi, R.D., Surampalli, R.Y. 2014. Extracellular polymeric substances of bacteria and their potential environmental applications. *Journal of Environmental Management*, **144**, 1-25.

Ng, K.K., Shi, X., Ong, S.L., Ng, H.Y. 2016. Pyrosequencing reveals microbial community profile in anaerobic bio-entrapped membrane reactor for pharmaceutical wastewater

treatment. *Bioresource Technology*, **200**, 1076-1079.

Ooi, G.T.H., Tang, K., Chhetri, R.K., Kaarsholm, K.M.S., Sundmark, K., Kragelund, C., Litty, K., Christensen, A., Lindholst, S., Sund, C., Christensson, M., Bester, K., Andersen, H.R. 2018. Biological removal of pharmaceuticals from hospital wastewater in a pilot-scale staged moving bed biofilm reactor (MBBR) utilising nitrifying and denitrifying processes. *Bioresource Technology*, **267**, 677-687.

Praveena, S.M., Rashid, M.Z., Nasir, F.A., Yee, W., Aris, A.Z. 2019. Occurrence and potential human health risk of pharmaceutical residues in drinking water from Putrajaya (Malaysia). *Ecotoxicology and Environmental Safety*, **180**, 549-556.

Song, Z., Zhang, X., Ngo, H.H., Guo, W., Song, P., Zhang, Y., Wen, H., Guo, J. 2019a. Zeolite powder based polyurethane sponges as biocarriers in moving bed biofilm reactor for improving nitrogen removal of municipal wastewater. *Science of The Total Environment*, **651**, 1078-1086.

Song, Z., Zhang, X., Ngo, H.H., Guo, W., Wen, H., Li, C. 2019b. Occurrence, fate and health risk assessment of 10 common antibiotics in two drinking water plants with different treatment processes. *Science of The Total Environment*, **674**, 316-326.

Tan, C.H., Koh, K.S., Xie, C., Tay, M., Zhou, Y., Williams, R., Ng, W.J., Rice, S.A., Kjelleberg, S. 2014. The role of quorum sensing signalling in EPS production and the assembly of a sludge community into aerobic granules. *The ISME Journal*, **8**, 1186-1197.

Teh, A.H.T., Lee, S.M., Dykes, G.A. 2019. Growth in the presence of specific antibiotics induces biofilm formation by a Campylobacter jejuni strain sensitive to them but not

in resistant strains. *Journal of Global Antimicrobial Resistance*, **18**, 55-58.

Vasiliadou, I.A., Molina, R., Martinez, F., Melero, J.A., Stathopoulou, P.M., Tsiamis, G. 2018. Toxicity assessment of pharmaceutical compounds on mixed culture from activated sludge using respirometric technique: The role of microbial community structure. *Science of the Total Environment*, **630**, 809-819.

Wan, X., Gao, M., Ye, M., Wang, Y., Xu, H. 2018. Formation, characteristics and microbial community of aerobic granular sludge in the presence of sulfadiazine at environmentally relevant concentrations. *Bioresource Technology*, **250**, 486-494.

Yang, G., Fang, H., Wang, J., Jia, H., Zhang, H. 2019a. Enhanced anaerobic digestion of up-flow anaerobic sludge blanket (UASB) by blast furnace dust (BFD): Feasibility and mechanism. *International journal of hydrogen energy*, **44**, 17709-17719.

Yang, G., Wang, J., Zhang, H., Jia, H., Zhang, Y., Gao, F. 2019b. Applying bio-electric field of microbial fuel cell-upflow anaerobi sludge blanket reactor catalyzed blast furnace dusting ash for promoting anaerobic digestion. *Water Research*, **149**, 215-224.

Young, B., Banihashemi, B., Forrest, D., Kennedy, K., Delatolla, R. 2016. Meso and micro-scale response of post carbon removal nitrifying MBBR biofilm across carrier type and loading. *Water Research*, **91**, 235-243.

Zhang, S., Sophie, C., Stephen, G., F., R.R., E., D.J., Mengyan, L., Lisa, A. 2018. Microbial community analysis in biologically active filters exhibiting efficient removal of emerging contaminants and impact of operational conditions. *Science of the Total Environment*, **640-641**, 1455-1464.

Zhang, X., Chen, X., Zhang, C., Wen, H., Guo, W., Ngo, H.H. 2016. Effect of filling fraction

on the performance of sponge-based moving bed biofilm reactor. *Bioresource Technology*, **219**, 762-767.

Zhang, X., Song, Z., Ngo, H.H., Guo, W., Zhang, Z., Liu, Y., Zhang, D., Long, Z. 2020. Impacts of typical pharmaceuticals and personal care products on the performance and microbial community of a sponge-based moving bed biofilm reactor. *Bioresource Technology*, **295**, 122298.

Zhou, G., Li, N., Rene, E.R., Liu, Q., Dai, M., Kong, Q. 2019. Chemical composition of extracellular polymeric substances and evolution of microbial community in activated sludge exposed to ibuprofen. *Journal of Environmental Management*, **246**, 267-274.