







Study on Microbial Population Difference for the Treatment of Domestic Sewage between Micro-Pressure Swirl Reactor (MPSR) and SBR

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Abstract: The comparative study on the microbial community structure of activated sludge from micro-pressure swirl reactor (MPSR) and SBR under stable operation was conducted by the 16S rRNA third-generation high-throughput sequencing, which was sampled from simulated domestic sewage. The experimental results indicated that the two reactors had great differences in the microbial community structure of activated sludge due to the different circulating flow patterns under the same water quality and intermittent operation mode. Compared with SBR, MPSR had more dominant bacteria phyla and some functional bacteria of higher relative abundance such as *Flavobacterium* and *Thiotrix*, and simultaneously strictly anaerobic, strictly aerobic and facultative anaerobic microbial species existed so as to produce higher species diversity and population richness, which is accord with the polyphase theory of the reactor.


1 INTRODUCTION


In the urban sewage treatment system, activated sludge is very important for the removal of pollutants, and the microbial community structure will directly affect the stability and treatment efficiency of sewage biological treatment. Therefore, the diversity of community structure and dominant bacteria are one of the main indicators to evaluate the structure and function of sewage treatment system (Tian 2020). Many scholars pointed out that the activated sludge of sewage treatment plant had high species diversity and community richness, and the influent quality, process composition and operating conditions have a certain impact on the microbial community structure


of activated sludge (Ma 2021, Chang 2021, Li 2021). However, there are few comparative studies on the microbial community structure of activated sludge with different process types.


MPSR is a new sewage treatment device with anaerobic, anoxic and aerobic environments coexisted, which has good organic matter removal, nitrogen and phosphorus removal effects (Bian 2020, Bian 2020). In this study, high-throughput sequencing technology was used to study the microbial community structure of MPSR and SBR in order to provide a theoretical basis for the optimization of Activated Sludge Method wastewater treatment process performance and provide reference for subsequent research.


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2 MATERIAL AND METHOD

2.1 Test Devices

The test device was shown in Figure 1. The effective volumes of reactors were both 36L. The size of the SBR was 300mm long, 300mm wide and 500mm high. MPSR included two parts, the main reaction zone and micro-pressure zone. The main reaction zone was a diameter of 900mm and 90mm wide, and the micro pressure zone was 130mm long, 90mm wide and 400mm high. The flow rate of MPSR gradually decreased from outside to inside, and the concentration of DO also showed an obvious gradient change from outside (2.02mg/L) to inside (lower than 0.05mg/L) (Bian 2020). SBR sludge was sampled about 200mm below the liquid level, and MPSR sludge was sampled from the mixed liquid at the nine points in Figure 1. The inoculated sludge was taken from an aeration tank of a sewage treatment plant at Changchun, and the initial sludge concentration of the mixed liquid was 2000mg/L. After aeration culture, the sludge was evenly divided into two reactors (abbreviated as R).

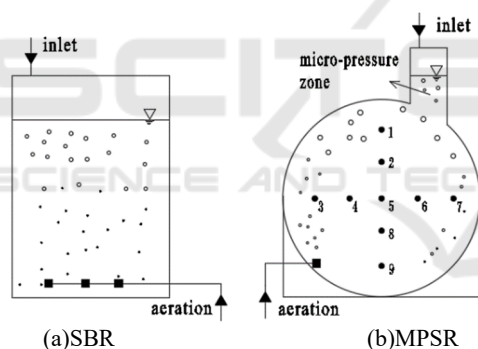


Figure 1: Test devices

2.2 Experiment Water Quality

The synthetic wastewater was used to simulate domestic sewage, which including nutrients and trace elements required by microorganisms from beef extract, peptone, starch and etc. The concentration of main water quality indexes was shown in Table 1.

Table 1: Influent water quality.

Index	Range	Average
COD/mg·L ⁻¹	305.2~386.0	338.1
NH ₄ ⁺ -N/mg·L ⁻¹	29.8~36.4	31.4
TN/mg·L ⁻¹	32.1~38.6	34.2
TP/mg·L ⁻¹	2.5~4.5	3.2

2.3 Operating Condition

The two reactors were operated for two cycles every day, with cycle time of 12h including 8h of aeration (initial 5min of feeding), 3h of sedimentation, 10min of drainage and 50min of free time. The aeration capacity was 1.5L/min, and the operating temperature was (20±1) °C. The sludge residence time (SRT) was 22d, and the drainage ratio was 0.5. The two systems operated stably for 30 days. During the operation, the average removal rates of COD, NH₄⁺-N, TN and TP of SBR were 92.0%, 98.8%, 66.3% and 95.8% respectively, while 94.0%, 98.8%, 75.6% and 98.6% in MPSR.

2.4 High-throughput Sequencing

Total genomic DNA samples were extracted using the OMEGA Soil DNA Kit (M5635-02) (Omega Bio-Tek, Norcross, GA, USA), following the manufacturer's instructions, and stored at -20°C prior to further analysis. The quantity and quality of extracted DNAs were measured using a NanoDrop NC2000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA) and agarose gel electrophoresis, respectively.

The extracted DNA was amplified with two-step PCR, with sample-specific 16-bp barcodes were incorporated into the forward and reverse primers for multiplex sequencing in the second PCR step. A total of PCR amplicons were purified with Agencourt AMPure Beads (Beckman Coulter, Indianapolis, IN) and quantified using the PicoGreen dsDNA Assay Kit (Invitrogen, Carlsbad, CA, USA). After the individual quantification step, amplicons were pooled in equal amounts, and Single Molecule Real Time (SMRT) sequencing technology was performed using the PacBio Sequel platform at Shanghai Personal Biotechnology Co., Ltd (Shanghai, China).

3 RESULTS AND DISCUSSION

3.1 Microbial Alpha Diversity Analysis

The statistical results of the samples were shown in Table 2. It showed Chao1 of MPSR was the largest, followed by R and SBR, indicating that the population abundance in MPSR is the highest. The order of Shannon and Simpson value was R > MPSR > SBR, indicating that the community diversity of MPSR was better than SBR. The good's coverage values of the three samples all reached 0.94, which

indicated that the high-throughput sequencing results of the samples were in good agreement with the real situation and could represent the real situation of the samples.

Table 2: Statistics of activated sludge population abundance and diversity index.

Index	Sample		
	R	MPSR	SBR
Chao1	458.43	484.48	418.42
Simpson	0.98	0.92	0.85
Shannon	7.20	6.02	4.91
Good's coverage	0.94	0.93	0.94

3.2 Species Community Differences

The number of OTU clusters of R, MPSR and SBR samples were represented by Venn diagram. As shown in Figure 2, the OTU numbers of R, MPSR and SBR were 387, 316 and 263 respectively, and the species numbers of both reactors decreased after 30 days. The number of unique species in the three samples was 159, 86 and 92, respectively, and owned number of OTU was 79 (12.91%), which meant quite different species communities. After the activated sludge was cultured in their respective reactors, the microbial environmental conditions changed, and different dominant bacteria was formed in the system due to the different internal structures of MPSR and SBR.

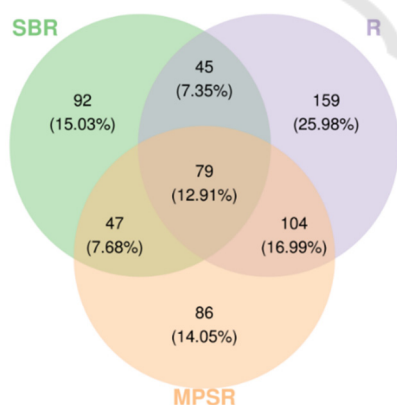


Figure 2: Wayne diagram of OTU distribution of sludge sample.

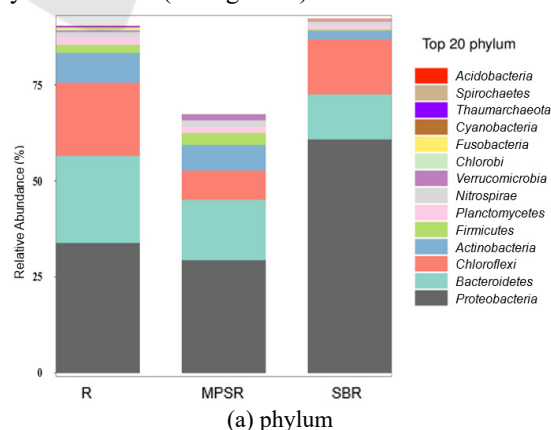
Species number and relative abundance were counted from top20 phylum levels of the average abundance of the sample. The results showed that there were 2114, 3708 and 4712 species inside R, MPSR and SBR, respectively. The number of species in MPSR and SBR increased by 75.4% and 122.9%

respectively, indicating that the two processes provided a good living environment for microorganisms and improved species diversity. Proteobacteria were major group in all the sludge systems, followed by Bacteroidetes, Chloroflexi and Actinobacteria. However, the abundance of sludge samples varied significantly (Figure 3a).

3.3 Species Abundance Composition

As can be seen from Figure 3a, the dominant bacteria phyla in SBR were Proteobacteria (60.2%), Chloroflexi (14.7%) and Bacteroidetes (11.9%), of which Proteobacteria was the most dominant phyla involved in nitrogen and phosphorus removal and organic matter degradation (Zhang 2015). The dominant phyla of MPSR were Proteobacteria (29.7%), Bacteroidetes (15.0%), Chloroflexi (7.6%), Actinobacteria (6.9%), Firmicutes (3.4%) and other phyla greater than 1% including Nitrospirae, Planctomycetes and Verrucomicrobia, while another 32% of the species were not clear. Compared with SBR, MPSR had more dominant bacteria phyla and richer species.

As can be seen from Figure 3b, Thauera was the main dominant bacteria of R, while its abundances were reduced in both reactors, substitute for more suitable for their own environment. Thiotrix occupied a relatively high abundance in SBR. It belongs to chemoautotrophic flora and plays a major role in nitrification of the denitrification process. In MPSR, nitrification and denitrification were in progress simultaneously, and the dominant flora were various functional flora such as Flavobacterium dominated by heterotrophic denitrification and Thiotrix dominated by nitrification (Zhang 2019).



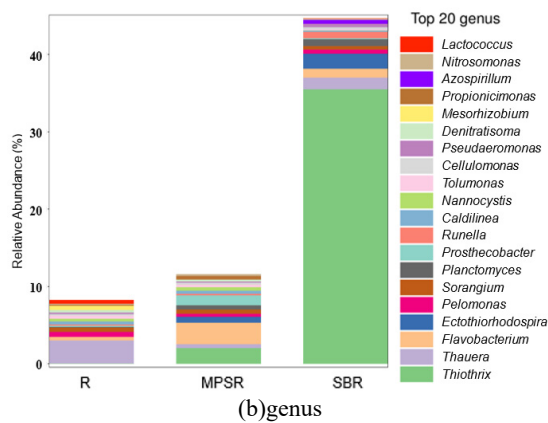


Figure 3: Species abundance of different sludge samples.

3.4 Species Composition Heat Map

In order to further compare the species composition differences between samples, the abundance data of top50 species in the average abundance were used to draw a heat map for species composition analysis. In the Figure 4, the red color block indicates that the abundance of this species in this sample is higher than that in other samples, and the blue color block indicates that the abundance of this species in this sample is lower than that in other samples.

The bacterial clustering results of three sludge samples R, MPSR and SBR showed that there were significant differences in microbial abundance

between the two reactors, which was due to the effects of different circulating flow patterns and oxygen environment on microbial flora. In MPSR samples, the abundances of Propionificimonas paludicola, Nitrospira sp. and Prevotella Copri were larger, and in SBR samples, the dominant bacteria were Thiiothrix eikelboomii, Haliscomenobacter hydrossis and etc.

Propionificimonas paludicola was a Gram-positive bacterium, belonging to Actinobacteria. It was facultative anaerobic and chemotrophic heterotrophic, which could ferment and metabolize glucose and other carbohydrates into a large amount of acetic acid and propionic acid. Nitrospira sp. was a gram-negative bacterium, belonging to Nitrospirae. It was strictly aerobic and could oxidize nitrite into nitrate to obtain energy which was the main nitrite bacteria in the sewage treatment system (Siripong 2007). Prevotella Copri was a polymorphous bacterium and did not produce spores. It was strictly anaerobic and chemotrophic heterotrophic which could use organic matter for anaerobic fermentation. Therefore, compared with aerobic bacteria in SBR, there were dominant microbial species of strict anaerobic, strict aerobic and facultative anaerobic inside MPSR. From the perspective of microorganisms, the multiphase theory of MPSR was proved and the function of simultaneous nitrification and denitrification in multi-oxygen environment was realized.

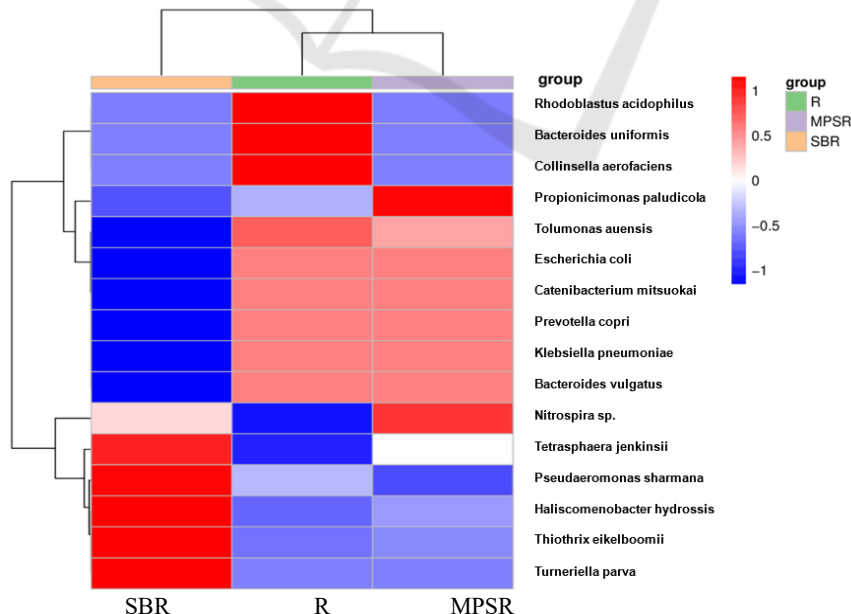


Figure 4: Species level species composition heat map based on double clustering.

4 CONCLUSIONS

Although water quality and intermittent operation mode were the same, the two reactors had significant differences in microbial community structure due to different circulating flow patterns. Compared with SBR, MPSR had more dominant bacteria phyla and some functional bacteria of higher relative abundance such as *Flavobacterium* and *Thiotrix*, and there existed strictly anaerobic, strictly aerobic and facultative anaerobic microbial species simultaneously so as to produce higher species diversity and population richness, which is accord with the polyphase theory of the reactor.

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