Optimization of Operation Performance and Bacterial Characteristics in SBR Processes for Short-cut Nitrification and Denitrification

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Keywords: SBR Process, Shortcut Nitrification and Denitrification, Denitrification, Microbial Community Structure.

Abstract: In order to explore the operation performance and bacterial characteristics in short-cut nitrification and denitrification (SND) processes, three laboratory scale sequencing batch reactors (SBRs) R_1, R_2 and R_3 were established using activated sludge as seeding sludge. Experimental results showed that three SND systems could operate effectively and stably in sludge preculture stage with the average NH_4^+-N removal efficiency of 88.6±1.7%, 93.5±1.2% and 91.4±1.5% in R_1, R_2 and R_3, respectively. Changing the aeration mode from continuous aeration to intermittent aeration with intermittent time of 2h, the removal efficiency of NH_4^+-N and total nitrogen (TN) were 66.3±2.4% and 85.3±1.4%, respectively. The nitrification performance was obviously inhibited, but intermittent aeration improved the TN removal performance. Decreasing aeration rate, the removal efficiency of NH_4^+-N decreased to 54.3±2.1%, while the removal efficiency of TN increased by 73.0±2.8%. When the C/N ratio changed from 1.5:1 to 2:1, the removal efficiency of NH_4^+-N and TN were 71.2±3.3% and 85.4±2.0%, respectively. In the SND-SBR system, Proteobacteria (47.0%), Bacteroidetes (25.9%), Actinobacteria (17.8%) were dominant at phylum level. At the same time, denitrification related genus *Thauera* with relative abundance of 10.6% was dominant in SND systems.

1 INTRODUCTION

The emissions of nitrogen in wastewater have caused the pollution of different water bodies in the whole world, especially in developing countries. The overloaded nitrogen in water environment may cause various risks to the water ecology and human health (Liu, Daigger, Liu, Zhao, Liu, 2020). Therefore, efficient and economical technologies for nitrogen removal were useful to ensure the water quality and safety. Up to date, many researchers have contributed a great deal of intellectual and financial resources to the development of novel biological processes for nitrogen removal (Kuypers, Sliekers, Lavik, Schmid, Jørgensen, Kuenen, Jetten, 2003). The short-cut nitrification and denitrification (SND) process proposed and developed by Delft University of Technology in Netherlands in 1997 was a promising biological technology for nitrogen removal (Yao, Chen, Guan, Zhang, Tian, Wang, Li, 2017). Although SBR technology has many successful practices in wastewater treatment, it is still the focus of researchers to affect the stability and system performance of SBR operation (Zhao, Wang, Li, Jia, Wang, Peng, 2019).

SND process simplified the nitrification process and controlled the conversion of NH_4^+-N in wastewater to NO_2^--N rather than NO_3^--N (Adav, Lee, Show, Tay, 2008); (Hou, Xia, Ma, Zhang, Zhou, He, 2017); (Li, Liu, Ma, Zheng, Ni, 2019); (Wu., Zhang, Yan, 2016). Comparing with widely used nitrification and denitrification process, 25% oxygen demand and approximately 40% organic carbon source for denitrification were saved. In addition, the hydraulic retention time (HRT) could reduce by approximately 50% with enhanced nitrogen removal performance (Yao, Chen, Guan, Zhang, Tian, Wang, Li, 2017); (Wu., Zhang, Yan, 2016); (Aslan, Miller, Dahab, 2009); (Ma, Han, Ma, Han, Zhu, Xu, Wang, 2017); (Zhang, Peng, Wang, Zheng, Jin, 2007).

Up to date, sequential batch reactor (SBR) has been widely used in many wastewater treatment processes in the worldwide due to its simple operation mode, higher separation effects of sludge and water. The SBR process has been often used for short-cut nitrification and denitrification (Zheng, Zhou, Wan, Luo., Su, Huang, Chen, 2018). Published

Yin, K., Wei, M., Long, G., Liu, Y. and Yang, G.
Optimization of Operation Performance and Bacterial Characteristics in SBR Processes for Short-cut Nitrification and Denitrification.
DOI: 10.5220/0011207000003443
In *Proceedings of the 4th International Conference on Biomedical Engineering and Bioinformatics (ICBEB 2022)*, pages 342-348
ISBN: 978-989-758-595-1

literature has proved that many factors including the influent NH_4^+-N concentration and loading rate, temperature, pH, operating mode, DO concentration and nitrite level affected the operation performance in SBR-SND processes (Choi, Cho, Jung, 2019); (Hellinga, Schellen, Mulder, van Loosdrecht, Heijnen, 1998); (Gilbert, Agrawal, Brunner, Schwartz, Horn, Lackner, 2014); (Kartal, Kuenen, Van Loosdrecht, 2010). For example, some studies have proved that the unconventional nitrogen removal pathway has been successfully explored in the sequencing batch biofilm reactor (SBBR) system under the condition of low C/N ratio (Chai, Xiang, Chen, Shao, Gu, Li, He, 2019). At the same time, some studies have shown that in the simultaneous nitrification and denitrification system, the DO concentration has an important impact on microbial metabolism, microbial community distribution and pollutant removal, especially under the condition of low DO (Ferrentino, Ferraro, Mattei, Esposito, Andreottola, 2018); (Layer, Villodres, Hernandez, Reynaert, Morgenroth, Derlon, 2020). In this study, three lab-scale SBR reactors were established and parallelly operated to study the optimization of operation performance and bacterial characteristics in SBR reactors for short-cut nitrification and denitrification.

2 MATERIALS AND METHODS

2.1 Experimental Setup

In this experiment, three lab-scale SBRs named R_1, R_2 and R_3 were constructed with the total volume and effective volume of 15.0L and 10.0L, respectively. The seeding sludge was activated sludge obtained from the secondary sedimentation tank of a municipal sewage plant located in Zhoushan, Zhejiang Province, China. Each reactor was inoculated with 6.0L activated sludge with sludge volume index (SVI) and mixed liquid suspended solids (MLSS) of 39.7±1.2 mL/g and 47.2±1.9g/L, respectively.

2.2 Synthetic Wastewater

Synthetic wastewater was prepared as the influent of each reactor. The synthetic wastewater of each reactor consisted of nitrogen source, carbon source, inorganic solution and trace elements solution (TES). The nitrogen source in influent of reactors R_1, R_2 and R_3 was NH_4^+-N, which was provided by $(NH_4)_2SO_4$ (1.875×10^{-5}mol/L). $NaHCO_3$ (1×10^{-4}mol/L) and glucose (1.45×10^{-5}mol/L) were used as inorganic and organic carbon source, respectively. The component of inorganic solution included KH_2PO_4 (0.01mol/L), $MgSO_4\cdot7H_2O$ (0.06mol/L) and $CaCl_2$ (0.06mol/L). The trace element solution included $FeSO_4\cdot7H_2O$ (0.3mol/L), $ZnSO_4\cdot7H_2O$ (1.5×10^{-3}mol/L), $CoCl_2\cdot6H_2O$ (1.0×10^{-3}mol/L), $MnCl_2\cdot4H_2O$ (0.005mol/L), $CuSO_4\cdot5H_2O$ (0.001mol/L), $NaMoO_4\cdot2H_2O$ (0.001mol/L), $NiCl_2\cdot6H_2O$ (8.8×10^{-4}mol/L) and H_3BO_3 (2.3×10^{-4}mol/L).

2.3 Experimental Setup and Procedures

2.3.1 Culture of Inoculated Sludge (P_1)

Three lab-scale SBR reactors including R_1, R_2 and R_3 were operated in parallel for the sludge acclimatization for SND-SBR process. The experimental period of SBR reactors was set at 24h, including influent 0.2h, reaction 23h, precipitation 0.5h, and drainage 0.3h. The influent NH_4^+-N and $C_6H_{12}O_6$-C were set at 56mg/L and 280mg/L, respectively. Corresponding NH_4^+-N loading rate (ALR) was 0.112kg/L/d. A mechanical aeration device with an aerator was used for aeration, and the aeration flow rate was 800mL/min. The temperature of each reactor was set at 25±1℃.

2.3.2 Effects of Different Operation Factors on the Operation Performance of SND-SBR Process (P_2)

On Day 44, the effects of changes in aeration mode, DO concentration, and C/N ratio on the performance of SND-SBR system were investigated. Reactor R_1 was changed from continuous aeration to intermittent aeration with an interval of 2 hours; the influent carbon to nitrogen ratio (C/N) of reactor R_2 was changed from 1.5:1 to 2:1; the aeration rate of reactor R_3 was changed from 800mL/min to 600mL /min, the remaining operation conditions remain unchanged.

2.4 Analytical Methods

2.4.1 Water Quality Analysis Methods

Influent and effluent samples of each reactor were regularly obtained and analyzed. The main water quality indicators, including chemical oxygen demand-chromium (COD_{Cr}), NO_3^--N, NO_2^--N, NH_4^+-N were measured according to the Standard Methods (SEPA, 2002).

2.4.2 Bacterial Structure Analysis

The seeding sludge (Day 1) (S1) and the SND sludge (Day 43) (S2) of reactor R_2 were obtained from each reactor for the analysis of bacterial community structure. The genomic DNA of each sample was extracted using the soil DNA extraction kit (OMEGA), and V3-V4 variable regions of 16S rRNA genes was analyzed by Illumina Miseq sequencing technology in OE biotech Co. Ltd (Shanghai, China). 16S rRNA genes in V3-V4 regions were amplified using bacterium-specific primers 343F (5'-TACGGRAGGCAGCAG -3') and 798R (5'-AGGGTATCTAATCCT-3'). PCR amplification was conducted in 25 μL reaction system, the detail determination information was same to that reported by Feng et al. (2017).

Illumina Miseq sequencing analysis was used after PCR amplification. Data preprocess was conducted to obtain the high-quality sequences. Vsearch software was used to classify the sequences according to the similarity of the sequences. And the sequences with similarity of greater than 97% were classified as an operational taxonomic unit (OTU). The subsequent analysis of bacterial information was based on the OTU. The α-diversity and bacterial structure at phylum and genus levels were further analyzed.

3 RESULTS AND DISCUSSION

3.1 Short-term Operation of Nitrification and Denitrification Process (P_1)

The operation performance of reactors R_1, R_2 and R_3 is shown in Fig. 1. In the initial 5 days, the NH_4^+-N removal performance of each reactor was unstable and the NO_2^--N accumulation phenomenon was observed. After 5 days' operation, nearly no NO_2^--N accumulation was observed in the effluent. The NH_4^+-N removal efficiencies were stable at 86.5±2.1%, 90.2±1.6% and 89.4±1.8%, and COD_{Cr} removal efficiencies were stable at 80.1±3.7%, 82.3±2.5% and 87.5±1.9%, respectively. There was almost no NO_2^--N accumulation in each reactor with the average NO_2^--N concentration of less than 0.23mg/L.

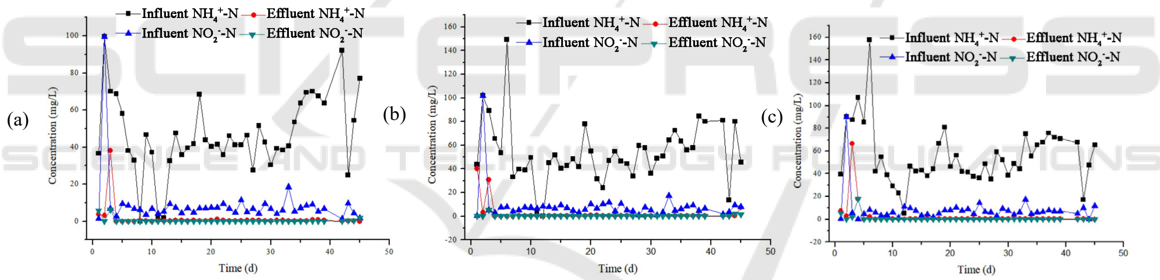

Figure 1: The performance of each reactor in stage P_1: (a) R_1, (b) R_2 and (c) R_3.

3.2 Operation Performance of SND-SBR System in P_2

3.2.1 Overall Nitrogen Removal Performance in P_2

Fig. 2 shows the operation performance of the reactors R_1, R_2 and R_3 in period P_2. In reactor R_1, the average ARE was 66.5% during the initial 7 days' operation, but quickly increased to 91.4% on Day 8. The effluent average NH_4^+-N concentration decreased from 15.3mg/L to 3.7mg/L. The NH_4^+-N removal efficiency was not obviously affected by the changes of aeration mode. The average DO levels in the effluent of aeration and non-aeration period were 3.6 mg/L and 0.8 mg/L, respectively. Compared with continuous aeration, intermittent aeration can slightly improve the nitrogen removal (Ma, Li, Bao, Li, Cui, 2020); (Niu, Feng, Wang, Liu, Liang, Liu, He, 2021).

After changing the reaction conditions, the effluent NH_4^+-N concentration of R_2 and R_3 decreased to approximately 3.0mg/L, but quickly decreased to a lower level of approximately 0.5 mg/L. Compared with the performance in startup period, the ammonia nitrogen removal performance of reactor R_2 was significantly improved. At the beginning of operation, the effluent nitrite concentrations of R_2 and R_3 were 72.5 mg/L and 79.3 mg/L, respectively. The C/N ratio of reactor R_2 was increased from 1.5:1 to 2:1 to promote the denitrification performance. For R_3, the reduction of aeration rate from 800 mL/min to 600 mL/min also promoted the denitrification performance. The TN removal efficiencies of R_2 and R_3 were 86.0% and 90.4%, respectively. There was almost no accumulation of nitrite nitrogen in the effluent of the two reactors. These results showed that the nitrite almost completely removed. Obviously, reactors R_2 and R_3 had good nitrogen removal performance.

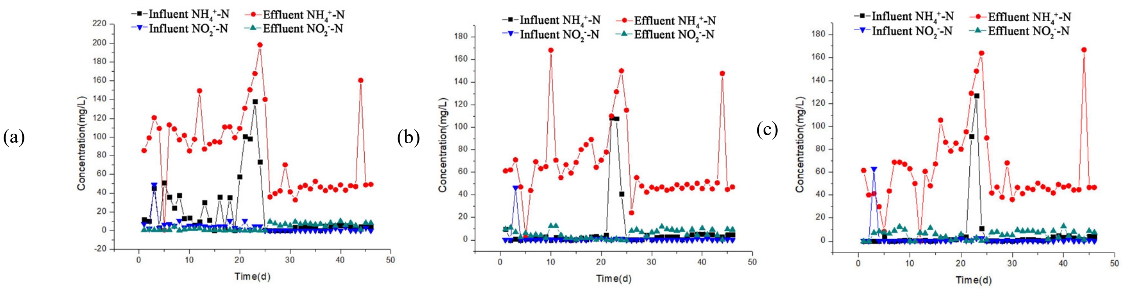

Figure 2: Operation performance of each reactor in the short-term nitrification and denitrification sludge cultivation stage: (a) R_1, (b) R_2 and (c) R_3.

3.2.2 Cod$_{cr}$ Removal Performance Analysis

Fig. 3 shows the COD_{Cr} removal performance of the reactors R_1, R_2 and R_3 during period P_2. Before changing operation conditions, the average COD_{Cr} removal efficiencies of reactor R_1, R_2 and R_3 were 93.4%, 92.0% and 95.8%, respectively. At the end of the experiment, corresponding values increased to 94.2%, 96.5% and 96.8%, respectively. Obviously, the COD_{Cr} removal efficiency of R_3 was higher than that of R_1 and R_2. This result showed that the aeration rate of reactor R_3 changed from 800mL/min to 600mL/min increased the organics removal due to the enhancement of denitrification effects.

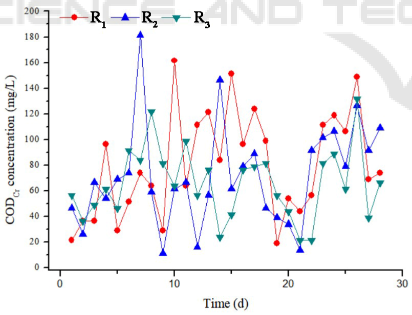

Fig. 3 COD_{Cr} removal performance of each reactor

3.2.3 Sludge Sedimentation and Biomass Analysis

At the beginning of the operation, the SVI level of seeding activated sludge of reactor R_1 was 40.6mL/g with MLSS of 48.9g/L. In reactor R_2, SVI and MLSS values were 41.0mL/g and 45.1g/L, respectively. In R_3, SVI and MLSS values were 37.5mL/g and 47.6g/L, respectively. In P_2, the SV_{30} of the reactors R_1, R_2 and R_3 were 85.0%, 91.0% and 93.0%, respectively. After long-term operation, SVI of reactors R_1, R_2 and R_3 were increased to 56.1 mL/g, 60.3 mL/g and 51.5 mL/g, respectively. Corresponding MLSS values were increased to 51.8 g/L, 50.3 g/L and 54.2, respectively.

3.3 Microbial Structure Analysis

3.3.1 Microbial Diversity Analysis

Table 1 shows the alpha diversity index of different sludge samples. The measured Goods coverage of S1 and S2 were 0.9959 and 0.9943, respectively. These index values were close to 1, which means that the sequencing depth has basically covered all species in the sample. The Shannon index and Simpson index of 7.29-7.57 and 0.9802-0.9873 indicated a higher biodiversity level. After long-term acclimatization, the bacterial diversity of shortcut denitrifying bacteria increased due to the directional selection of bacterial community (Niu, Feng, Wang, Liu, Liang, Liu, He, 2021); (Chen, Wei, Yang, Wang, Lu, Wang, Wang, 2021); (Mao, Zhang, Xia, Zhong, Zhao, 2010); (Ren, Ngo, Guo, Wang, Peng, Ni, Liu, 2020).

Table 1: Alpha diversity index of different sludge samples.

Index	Chao 1	Goods coverage	Observed species	Shannon	Simpson
S1	1131.0	0.9959	1054.3	7.57	0.9873
S2	1288.3	0.9943	1166	7.29	0.9802

3.3.2 Bacterial Structure Characteristics

Fig. 4a shows the distribution characteristics of the bacterial structure of each sludge sample at phylum level. There were 5 phyla with relative abundance (RA) of greater than 1.0% in the sample S1, including Proteobacteria (47.0%), Bacteroidetes (25.9%), Actinobacteria (17.8%), Gemmatimonadetes (1.5%) and Chlorobi (1.1%). There were 6 phyla in sample S2, including Proteobacteria (55.9%), Bacteroidetes (25.6%), Actinobacteria (9.2%), Gemmatimonadetes (2.45%), Nitrospirae (2.1%), Acidobacteria (1.5%) and Chlorobi (1.5%). In the two sludge samples, the dominant bacteria belonged to Proteobacteria, Bacteroidetes and Actinobacteria. After approximately 43 days' acclimatization, the RA of Proteobacteria increased by 19.0%, while Actinobacteria decreased by 4.9%. There were some reports shown that Bacteroidetes phylum was closely related to the removal of nitrogen (Shourjeh, Kowal, Drewnowski, Szeląg, Szaja, Łagód, 2020); (Winkler, Straka, 2019); (Yan, Liu, Liu, Zhang, Liu, Wen, Yang, 2019), and many microorganisms in the Proteobacteria were involved in the nitrogen cycle and related with nitrification and denitrification (Yang, Hou, Wang, Shi, Xu, Han, Li, 2018); (Zhang, Zhang, Chen, 2020); (Zhao, Feng, Yang, Dai, Mu, 2017). In this experiment, short-cut nitrification and denitrification to remove nitrogen and organic matter, Proteobacteria and Bacteroidetes microorganisms may play a key role.

At genus level, the structures of each sample are shown in Fig. 4b. The top 15 genera were *Ferruginibacter*, *Thauera*, *Dokdonella*, *Nitrospira*, *Paracocccus*, *Parvularcula*, *Defluviicoccus*, *Nitrosomonas*, *Terrimonas*, *Denitratisoma*, *Woodsholea*, *Amaricoccus*, *Candidatus Competibacter*, *Phaeodactylibacter* and *Haliang*. The main genera were *Ferruginibacter* (6.1%), *Defluvicoccus* (5.0%) and *Paracocccus* (2.1%) in sample S1, and *Thauera* (10.6%), *Ferruginibacter* (4.5%) and *Dokdonella* (4.1%) in S2. It was clearly showed that the main genera had changed significantly at the genus level after 43 days' operation. *Thauera* has the highest relative abundance, and a variety of bacteria belonging to the genus *Thauera* were related to denitrification in biological wastewater treatment systems (Feng, Xu, Xu, Zhu, Xu, Ding, Luan, 2012); (Lei, Yao, Li, 2021); (Li, Zhang, Xu, Shan, Zheng, 2021).

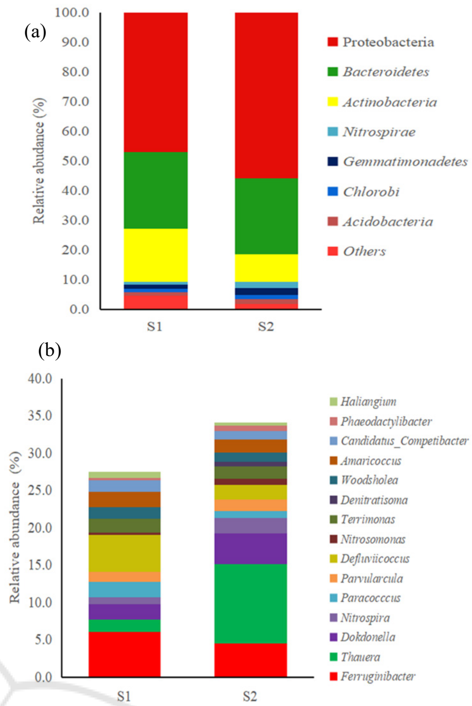

Figure 4: Distribution of main bacterial at different sludge samples: (a) phylum level and (b) genus level.

4 CONCLUSION

During the SND-SBR system sludge cultivation stage, the system could quickly reach stability, the average NH_4^+-N removal efficiencies of R_1, R_2 and R_3 were stable at 86.5±2.1%, 90.2±1.6% and 89.4±1.8%, respectively. The NH_4^+-N and TN removal efficiencies with intermittent aeration of every 2h were 66.3±2.4% and 85.3±1.4%, respectively, which enhanced the reactor performance. When the C/N ratio increased from 1.5:1 to 2:1, denitrification performance was also promoted. The effluent ammonia nitrogen concentration was 7.76mg/L with the TN removal efficiency of 86.0%. After the aeration rate changed from 800mL/min to 600mL/min, the removal efficiencies of NH_4^+-N and TN were 54.3±2.1% and 73.0±2.8%, respectively. In the SND-SBR system, the denitrification related phylums including Proteobacteria, Bacteroidetes and Actinobacteria were found in system after long-term operation, while the denitrification genus *Thauera* had the relative abundance of 10.6%.

ACKNOWLEDGMENTS

This study was funded by the National Natural Science Foundation of China (51808498), Natural Science Foundation of Zhejiang Province of China (No. Q17E090015), Fundamental Research Funds for the Provisional Universities (2019J00050) and Open Foundation from Marine Sciences in the First-Class Subjects of Zhejiang (No. 20190009).

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