Desalination and Water Treatment
www.deswater.com
doi: 10.5004/dwt.2022.28482

260 (2022) 13–22
June

Effects of microbiological diversity in the anoxic-oxic-settling-anaerobic process strengthened by humic biological composite fillers

Lei Sang[a,b], Jukui Zhang[c], Xuefeng Wang[d], Jun Yin[c,*]

[a]*College of Environmental Science and Engineering, Jilin Normal University, Siping 136000, China, Tel. +86 13689855070; email: sanglei810330@126.com*
[b]*Key Laboratory of Environmental Materials and Pollution Control, The Education Department of Jilin Province, Jilin Normal University, Siping 136000, China*
[c]*Key Laboratory of Songliao Aquatic Environment Ministry of Education, Jilin Jianzhu University, Changchun 130118, China, Tel. +86 13689855070; email: 935158697@qq.com (J. Yin), Tel. +86 13596488969; email: zhangjukui@126.com (J. Zhang)*
[d]*Department of Municipal and Environmental Engineering, Hebei University of Architecture, Hebei, Zhangjiakou 075000, China, Tel. 15530303309; email: falcon5680@163.com*

Received 26 December 2021; Accepted 7 April 2022

ABSTRACT

Excess sludge of wastewater treatment plants can cause environmental threats; hence, it should be treated. This study placed the humic biological composite fillers in the anoxic-oxic-settling-anaerobic (A-OSA) process to reduce sludge production and improve nitrogen and phosphorus removal efficiency. The results indicated that the humic biological composite fillers could intensify the excess operational taxonomic units of the A-OSA process. The cumulative excess sludge production of humic biological composite fillers was reduced to 147.89 g (81.61% amount of A-OSA process) by strengthening the A-OSA process (A-O_{HA}SA). In addition, the average total nitrogen (TN) and total phosphorus (TP) removal efficiencies of A-O_{HA}SA were 79.23% and 91.74%, respectively. Miseq high-throughput sequencing technology was used to study the effects of composite humic biological filler on sludge reduction and TN-TP removal in the A-O_{HA}SA process. The microbiological diversity of the A-O_{HA}SA process was also studied. The humic biological composite fillers could affect the operating environment of the A-O_{HA}SA process. Compared with A-OSA, the microbial diversity and community structure were changed in A-O_{HA}SA. *Proteobacteria* and *Flavobacteria*, beneficial to nitrification, denitrification, and phosphorus-accumulating, became the dominant microflora. *Burkholderiales* and *Flavobacteriales* reproduced slowly, favoring sludge reduction.

Keywords: Humic biological composite fillers; Sludge reduction; Nitrogen and phosphorus removal; Microbial diversity; Microbial community structure

1. Introduction

The anoxic-oxic (A/O) process is a popular and efficient biological wastewater treatment process with mature operation management experience. However, massive quantities of sludge often result from the aerobic biological wastewater treatment stage of the A/O process, causing secondary pollution. Therefore, excess sludge must be discarded safely. Nevertheless, the disposal cost is high, reaching more than 50% of wastewater treatment costs [1,2]. Hence, reducing sludge production from the A/O process remains a problem.

In recent years, the anoxic-oxic-settling-anaerobic (A-OSA) process is proven effective for improving the A/O process [3,4]. An anaerobic sludge reaction tank is inserted into the return sludge system in the A-OSA process.

* Corresponding author.

The return activated sludge undergoes an anoxic-oxic-anaerobic circle. Some microbes disappear in this anaerobic environment due to insufficient nutrients in the anaerobic sludge reaction tank, causing a reduction in the microbial population. As a result, anaerobes (such as hydrolytic acidifying bacteria, methanogens, and denitrifying phosphorus-removing bacteria) are predominant in the tank. The environment changes when the sludge is recycled to the main reaction tanks (the anoxic and oxic tanks). Because the various nutritional substrates are adequate, the main reaction tanks' microbial community structure and metabolic pathway change.

The growth rate of anaerobes is reduced in the anoxic-oxic-anaerobic circle, which is suitable for sludge reduction. In addition, the pollutants removal rates are also affected. Li et al. [5] reported that the excess sludge production of the A-OSA process is 27% lower than that of the A/O process. Also, the chemical oxygen demand (COD) removal efficiency is 2% higher than the A/O process, while those of total nitrogen (TN) and total phosphorus (TP) are lower by 19% and 11%, respectively.

To improve the TN-TP removal efficiency [6–8], the humic biological composite fillers were placed in the oxic tank of the A-OSA process to build a strengthened A-OSA (A-O_{HA}SA) process. The humic biological composite fillers can then release humic acid, which changes the environment for microbial growth. Therefore, the microbial diversity could be affected too. In addition, some specific functional microbes, such as nitrobacterium, denitrifying bacteria, and polyphosphate accumulating bacteria, may-be enrich the process [9,10]. Hence, reducing excess sludge production and improving wastewater treatment efficiency would be gotten in the A-O_{HA}SA process.

In this study, the Miseq high-throughput sequencing technology was selected to reveal the changes in microbiological diversity that affect sludge reduction and nitrogen-phosphorus removal from the A-O_{HA}SA process. The assessment of molecular biology involved studying the microflora in the A-O_{HA}SA and A-OSA processes, and the respective dominant floras were verified and compared. Furthermore, the microbial properties of A-O_{HA}SA process were studied micro-cosmically. This study provides an excellent method and scientific exploration to use A-O_{HA}SA technique.

2. Materials and methods

2.1. Experimental devices

The experimental devices of A-O_{HA}SA and A-OSA processes were fabricated from acrylic plates. The components of these processes are shown in Figs. 1 and 2, respectively. The operations consisted of the anoxic, closed vessel, secondary settling, and anaerobic sludge reaction tanks. The main difference between the units was that the humic biological composite fillers were placed in a cage inside the oxic tank of the A-O_{HA}SA process. The size of experimental devices was the same for both processes. The working volume of the anoxic tank (20 cm × 20 cm × 35 cm) and oxic tank (60 cm × 20 cm × 35 cm) was 14 and 42 L, respectively. The working volume, diameter, and height of the closed vessel were 1.2 L, 10 cm, and 15 cm, respectively, while those of the secondary sedimentation tank were 28 L, 30 cm, and 40 cm.

The humic biological composite fillers used in this study are depicted in Fig. 3. The fillers (gray cylinders) have coarse surface and porous inner space. The main components of the fillers are residual activated sludge, humus, clay, and silicon compounds, with the physical characteristics listed in Table 1.

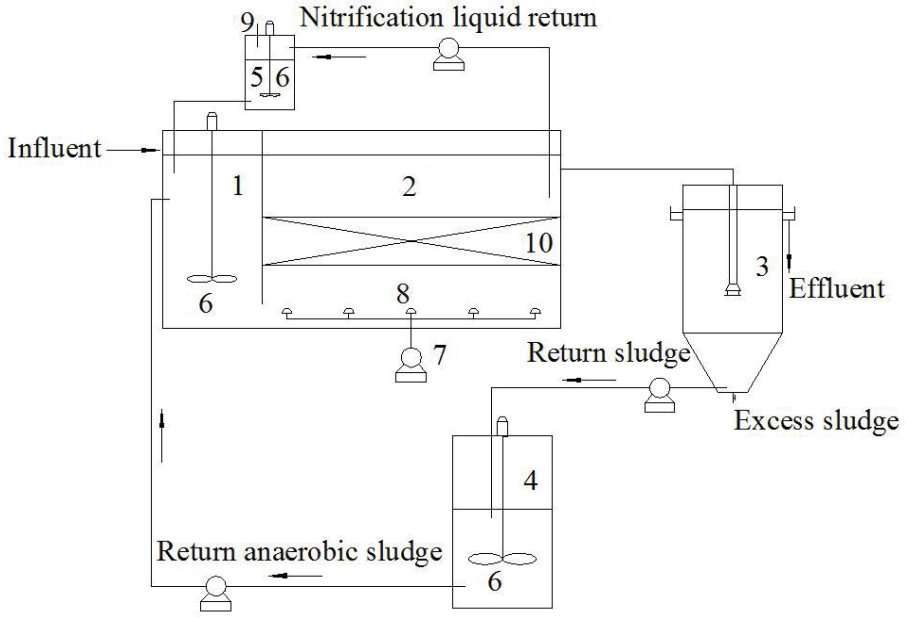

Fig. 1. The set-up of the A-O_{HA}SA process. 1–anoxic tank, 2–oxic tank, 3–secondary sedimentation tank, 4–anaerobic sludge reaction tank, 5–closed vessel, 6–the mechanical stirrer, 7–air blower, 8–aerator, 9–exhaust pipe, and 10–humic biological composite fillers.

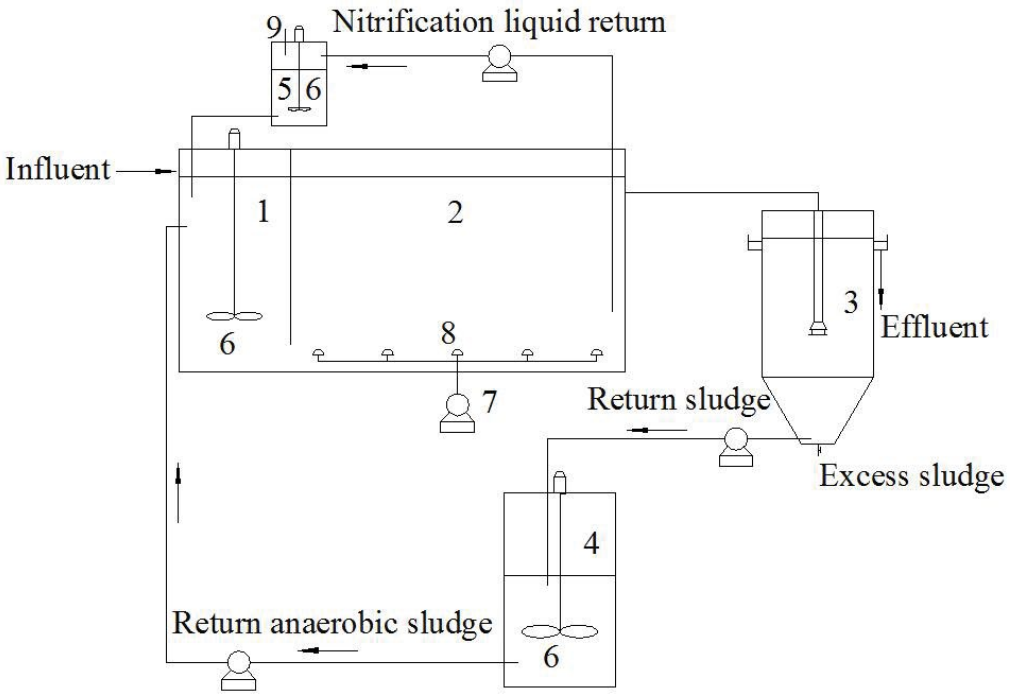

Fig. 2. The set-up of the A-OSA process. 1–anoxic tank, 2–oxic tank, 3–secondary sedimentation tank, 4–anaerobic sludge reaction tank, 5–closed vessel, 6–mechanical stirrer, 7–air blower, 8–aerator, and 9–exhaust pipe.

Fig. 3. Composite humic biological filler.

Table 1
Physical characteristics of humic biological composite fillers

Physical characteristics	Numerical value
Diameter (cm)	1.5
Length (cm)	4
Density (g/cm^3)	2
Surface area (m^2/g)	9.89
Total porous volume (cc/g)	0.04
Pore diameter (A)	18.8

Table 2
Quality indexes the experiment influent

Quality indexes	Numerical value
COD (mg/L)	400~420
TN (mg/L)	50~60
TP (mg/L)	6~7
pH	6.5~7.5
Temperature (°C)	25

2.2. Experimental conditions

The experimental sludge was taken from a domestic sewage treatment plant. First, the sludge was seeded for cultivation and domestication under the same plant conditions simulated in the lab. Then, when the sludge's mixed liquor suspended solids (MLSS) reached 3,000 mg/L, the excess sludge was discharged.

The influent in this study is the synthetic domestic wastewater, configured by 0.15 g/L peptone and 0.20g/L $C_6H_{12}O_6$ for COD, 0.25 g/L $(NH_4)_2SO_4$ for TN, and 0.03 g/L KH_2PO_4 for TP. In addition, $NaHCO_3$ was used to adjust the pH at 6.5~7.5. The quality indexes of the influents are listed in Table 2.

The inlet flow rate was 0.002 L/s. The air compressors and fine bubble aerators were used in the oxic tanks to keep the dissolved oxygen (DO) concentration at 2–3 mg/L. The hydraulic residence time (HRT) ratio between the anoxic and oxic tank was 1:3, 2.10 and 6.30 h, respectively.

The residence time of return sludge was set at 5.5 h in the anaerobic sludge reaction tank, while fixed sludge retention time was 10 d. The reflux ratio of nitrification liquid in the internal recycling from the oxic tank to the anoxic tank was 200%. The reflux ratio of return sludge internal recycling was set at 100% from the secondary sedimentation tank to the anaerobic sludge reaction and anoxic tank. The closed vessel between the oxic tank and anoxic tank had 5 min HRT of the nitrification liquid. A blender and exhaust pile were placed in the closed vessel, with the latter responsible (for degassing) to ensure that the nitrification liquid is returned in anoxic condition [11].

2.3. Calculation of excess sludge production

Three parallel sludge samples were extracted each time to compare A-O_{HA}SA and A-OSA processes. The daily excess sludge production (DSP_i) was used to calculate sludge wastage in both processes. The DSP_i was calculated based on Eq. (1) [12,13]. The other two samples were DSP_{i-1} and DSP_{i-2}.

Statistical analysis was performed on parallel samples according to Eq. (1):

$$DSP_i = \left(Q_{EAS} \times X_{EAS}\right) + \Delta X\left(Q_{eff} \times X_{eff}\right) \quad (1)$$

where Q_{EAS} is the flow velocity of the excess activated sludge in L/d, X_{EAS} is the MLSS concentration of excess activated sludge in g/L, ΔX is the net solid produced by the devices in g/L, Q_{eff} is the flow velocity of effluent in L/d, and X_{eff} is the MLSS concentration of effluent in g/L.

The daily excess sludge production (DSP_{day}) was used to calculate the mean of the parallel sludge samples in the A-O_{HA}SA and A-OSA processes. The DSP_i was calculated based on Eq. (2).

$$DSP_{day} = \frac{1}{3}\left(DSP_i + DSP_{i-1} + DSP_{i-2}\right) \quad (2)$$

The cumulative excess sludge production (DSP_{cum}), expressed in Eq. (3) [12,13], compares the sludge reduction efficiency in both processes.

$$DSP_{cum} = \sum_{day=1}^{i} DSP_{day} \quad (3)$$

2.4. Analytical methods

The influent and effluent's MLSS, TN, and TP were detected using the methods detailed in Monitor Analysis Methods of Water and Wastewater (4th ed.) [14]. Paired-samples *t*-test was used for identifying the significant difference of mean values the DSP_{day}, DSP_{cum}, TN and TP removal efficiency data in different processes with the SPSS. The believable probabilities of test data were 95%. The significance level was 0.05.

The microbial diversity in both experimental devices was detected using Miseq high-throughput sequencing [15–18]. The source of sludge samples used for testing the microbial diversity is presented in Table 3. Before analysis, the sludge samples were placed in the sterile centrifuge tubes and frozen at −80°C. After defrosting, DNA extraction kits (OMEGA E.Z.N.A Soil DNA Kit) were used to extract the sludge samples' DNA under aseptic conditions. The DNA was stored at −20°C. Then, DNA was used for polymerase chain reaction (PCR) amplification (TransGen AP 221-02: TransStart Fastpfu DNA Polymerase, 20 μL reaction system, Shanghai Biozeron). Each sample was repeated three times. PCR products of the same sample were mixed, and were detected by 2% AGE. According to the instructions of AxyPrep DNA gel extraction kit (AXYGEN), recovered PCR products. Tris-HCl was used for elution. According to the detection results of 2% AGE, PCR products of the sludge samples were quantitatively detected with the blue fluorescence quantitative system (Promega QuantiFluor™-ST). Then, according to the sequencing requirements, the samples were mixed to scale.

After, the Miseq library was built. Restriction enzymes was divided florescence groups from termination groups of DNA chains [16]. Then, the DNA was sequenced. According to the overlapping relationships, sequences were spliced with restriction enzymes, filtered, and underwent quality control. At the same time, the samples' sequences were determined. Finally, a set of related biological information (such as microbial abundance, diversity, and community structure) was analyzed. Diversity analysis for operational taxonomic unit (OTU) testing results of experimental samples reflected the microbial diversity and microbial abundance varieties.

Chao indexes assess the microbial abundance in the microbial ecology. The difference between microbial abundance and Chao indexes values is positive [19]. In addition, Shannon indexes assess the microbial diversity in the microbial ecology. Similarly, the relationship between microbial diversity and Shannon indexes values is positive [13].

Table 3
Sources of the various sludge samples

Sample number	Sources of the different sludge samples	Operational conditions of anaerobic sludge reaction tank
1	Anoxic tank of A-OSA process	HRT = 5.5 h
2	Oxic tank of A-OSA process	HRT = 5.5 h
3	Anaerobic sludge reaction tank of A-OSA process	HRT = 5.5 h
4	Anoxic tank of A-O_{HA}SA process	HRT = 5.5 h
5	Oxic tank of A-O_{HA}SA process	HRT = 5.5 h
6	Anaerobic sludge reaction tank of A-O_{HA}SA process	HRT = 5.5 h

3. Results and discussion

3.1. Sludge reduction and wastewater treatment efficiency

Fig. 4 illustrates the variation of DSP_{day} and linear fit results of the A-O_{HA}SA and A-OSA processes when the residence time of return sludge was 5.5 h in the anaerobic sludge reaction tank. DSP_{day} concomitant probability of *t*-test was 0.000. It was lower than 0.05. Significant differences existed between the DSP_{day} mean values of the A-O_{HA}SA and A-OSA processes.

The DSP_{cum} and linear fit results of the experimental devices of A-O_{HA}SA and A-OSA processes are shown in Fig. 5. DSP_{cum} concomitant probability of T-test was also 0.000. Significant differences also existed between the DSP_{cum} mean values of the A-O_{HA}SA and A-OSA processes, too. The DSP_{cum} of the A-O_{HA}SA and A-OSA processes was

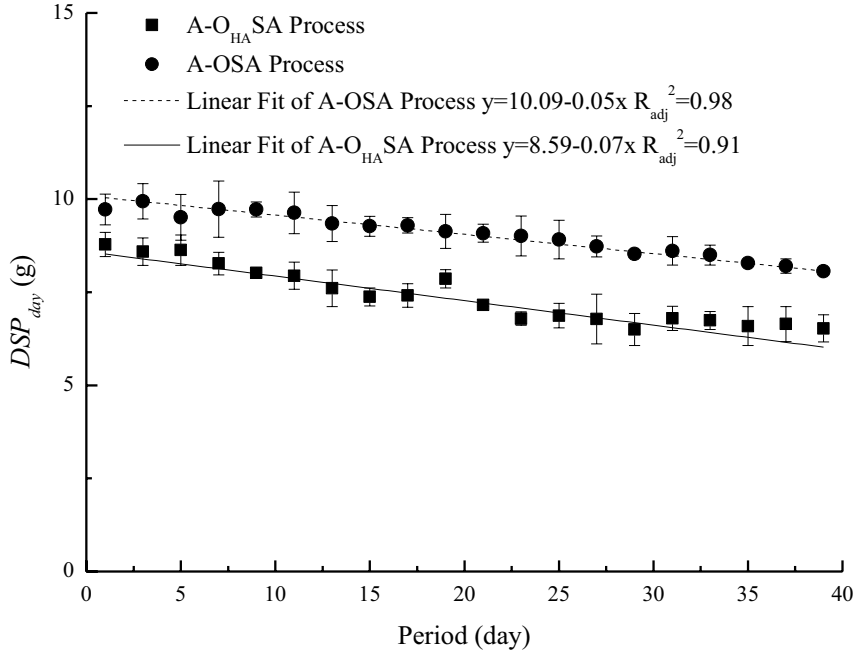

Fig. 4. The DSP_{day} in both processes.

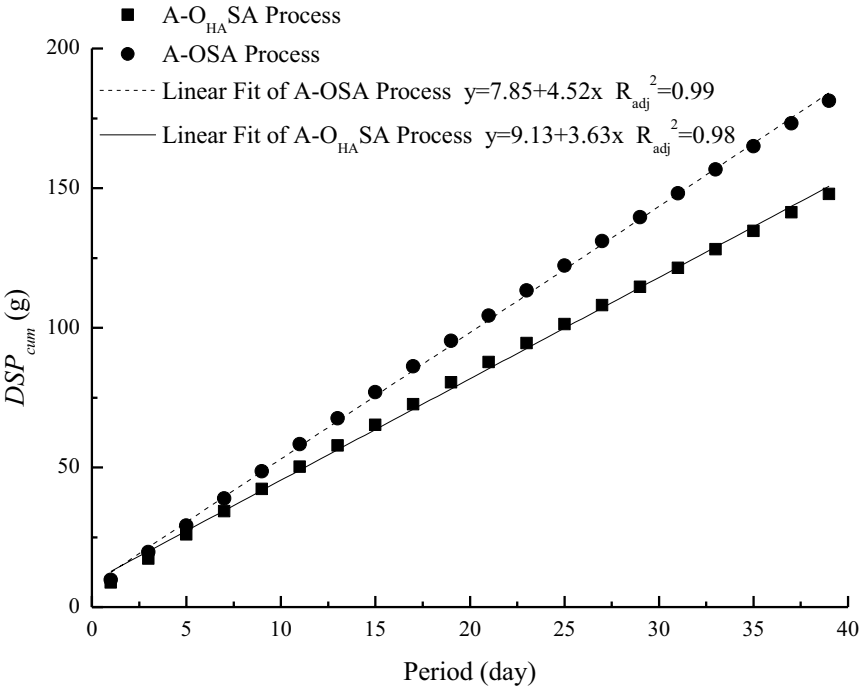

Fig. 5. The DSP_{cum} in both processes.

147.89 and 181.21 g, respectively, while the DSP_{cum} of the A-O_{HA}SA process was 18.39% lower than that of the A-OSA process. The sludge reduction efficiency of the A-O_{HA}SA process was superior to the A-OSA process. The difference in the efficiency would be attributed to the humic biological composite fillers placed inside the oxic tank of the A-O_{HA}SA process, which increased the humic acid concentration. The higher humic acid concentration changed the sludge environment and the microbial community structure of the activated sludge [9]. Moreover, slow-growing microbes were more concentrated in the systems. As a result, the proliferation rate of activated sludge was slowed down, reducing the excess sludge production.

The TN and TP removal efficiency and linear fit results in the experimental devices during the study period are shown in Figs. 6 and 7. The T-test concomitant probability of TN and TP efficiency both were 0.000. The results indicated a significant difference in the nitrogen and

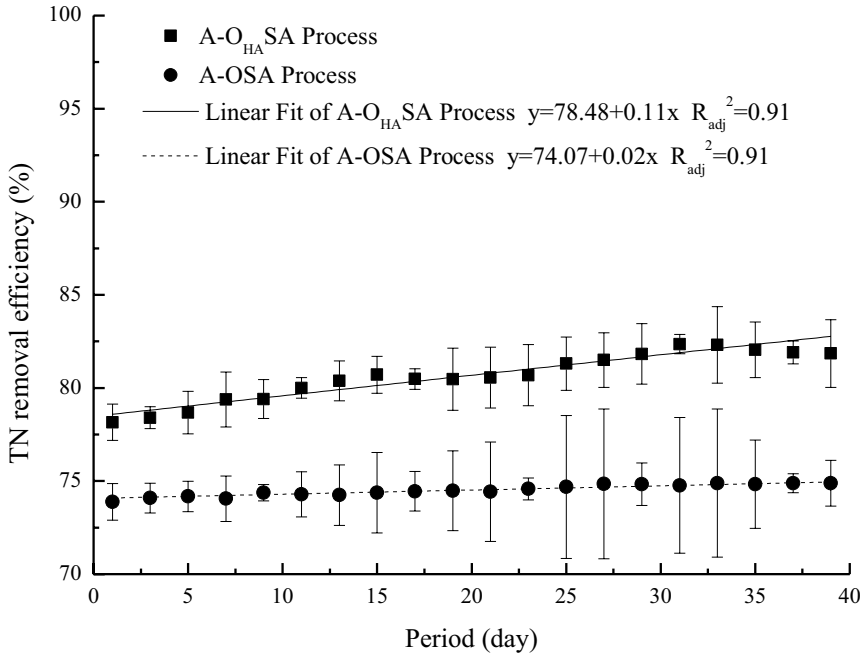

Fig. 6. TN removal efficiency in both processes.

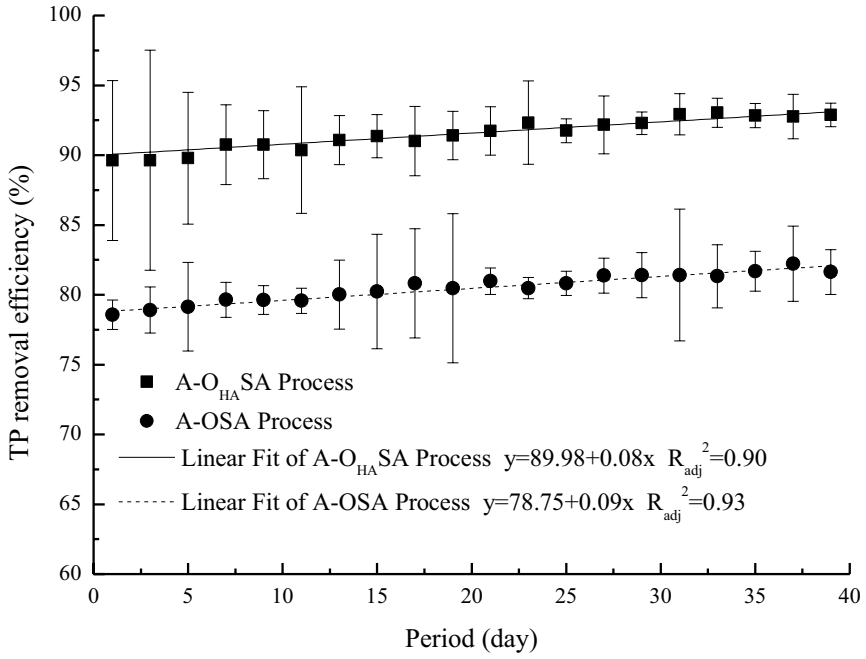

Fig. 7. TP removal efficiency in both processes.

phosphorus removal efficiencies of the A-O_{HA}SA and A-OSA processes. The average TN removal efficiency of the A-O_{HA}SA and A-OSA processes was 80.62% and 74.50%, respectively, whereas that of TP removal efficiency was 91.53% and 80.52%, respectively. The A-O_{HA}SA process was more efficient in removing nitrogen and phosphorus.

Because of the numerous Ca^{2+} and Mg^{2+} ions in the humic-activated sludge of the A-O_{HA}SA process, the concentration of flocculent sludge increased, forming large granular sludge. Also, a DO gradient was formed in the flocculent of the humic-activated sludge. Because the DO concentration was higher on the surface of the activated sludge, aerobic and nitrifying bacteria predominated. As the DO concentration gradient lessened, an anoxic zone was formed in the flocculent humic activated sludge, and denitrifying bacteria predominated. The simultaneous nitrification–denitrification (SND) ensued in the aerobic tank of the A-O_{HA}SA process [20]. Therefore, the nitrogen removal efficiency of A-O_{HA}SA process improved. In addition, the hydrolytic acidification of the humic activated sludge was better than the ordinary activated sludge, as more volatile fatty acid was produced in the anoxic tank of the A-O_{HA}SA process. The volatile fatty acid condition was excellent for phosphorus-accumulating bacteria. Therefore, the phosphorus removal efficiency of the A-O_{HA}SA process was superior to that of the A-OSA process.

3.2. Microbial diversity of the A-O_{HA}SA process

The electrophoretic results of total extracted DNA for sludge samples are depicted in Fig. 8. The extracted DNA fragments were complete with few impurities. Therefore, they are appropriate for subsequent PCR experiments.

The results of PCR are shown in Fig. 9. Here, target band sizes and concentrations of PCR products were within the expected range. Therefore, the Miseq library could be built to further study.

Table 4 lists the microbial abundance and diversity of the sludge samples. The OTU, Chao indexes, and Shannon indexes in each tank of the A-O_{HA}SA process were lower than those of the A-OSA process. These findings indicate that the humic biological composite fillers in the A-O_{HA}SA process changed the microbial abundance and diversity.

First, the humic biological composite fillers could release substances, such as humic acid, silicate, and trace metal ions in the A-O_{HA}SA process. Microbial community structure could be affected, and certain microbes could be enriched. Second, the humic activated sludge was in the anoxic-oxic-anaerobic alternate environment. Some environmentally sensitive microbes were eliminated in the anaerobic sludge reaction tank of the A-O_{HA}SA process. Then, a fermentative bacterium appeared, and humic-activated sludge decayed. When the decaying sludge returned was recycled into the anoxic tank of the A-O_{HA}SA process, the diversity of nutritional substrates changed, altering the microbial diversity.

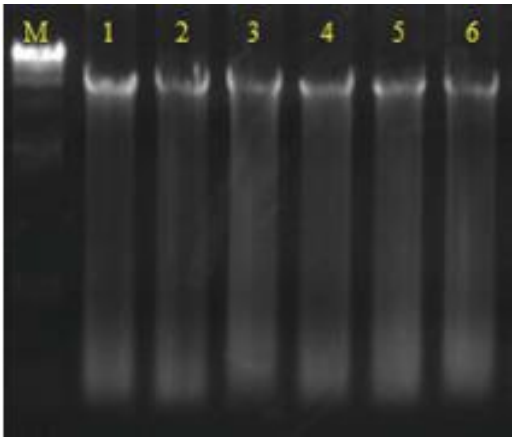

Fig. 8. Electrophoresis of extracted DNA.

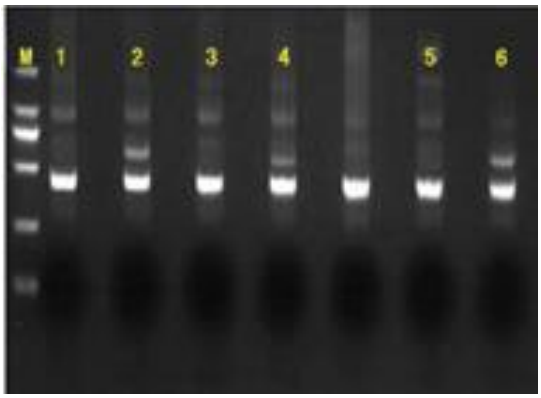

Fig. 9. Electrophoresis of PCR results.

Table 4
Microbial abundance and diversity of the sludge samples

Sample number	Majorized sequences	Sequence similarity = 0.97		
		OTU	Chao indexes	Shannon indexes
1	41975	845	967.28	4.85
2	36357	818	902.40	4.64
3	32887	802	833.14	4.55
4	35118	798	945.01	4.82
5	30560	553	668.81	4.48
6	35168	453	517.88	4.23

3.3. Microbial community structure of A-O_{HA}SA process

The phyla distribution of the microbial community in the sludge samples is depicted in Fig. 10. The proportion of phylum *Proteobacteria* was the predominant difference between the A-O_{HA}SA and A-OSA processes. Under the experimental conditions, the proportion of *Proteobacteria* was 63.4%, 66.8%, and 73.2% in the anoxic, oxic, and anaerobic sludge reaction tanks in the A-O_{HA}SA process, respectively. However, in the A-OSA process, the respective proportion of *Proteobacteria* were 52.1%, 55.1%, and 60.0%. respectively. *Proteobacteria* have strong adaptability for the wastewater treatment environment. Most of *Proteobacteria* are auxohetertrophs. Therefore, *Proteobacteria* could remove nitrogen and phosphorus during biological wastewater treatment through the SND and phosphorus-accumulating effect [21].

The class distribution of the microbial community in sludge samples is shown in Fig. 11. The proportions and classification of *Flavobacteria* showed the difference between the A-O_{HA}SA and A-OSA processes based on class. Under the experimental conditions, the proportion of *Flavobacteria* was 3.1%, 4.0%, and 4.4% in the anoxic, oxic, and anaerobic sludge reaction tanks of the A-O_{HA}SA process, respectively, while those of *Flavobacteria* were 0.9%, 1.5%, and 4.0% in the A-OSA process. *Flavobacteria* is a multiple biological functional auxohetertroph and denitrifying bacteria. In biological wastewater treatment, *Flavobacteria* is crucial in nitrogen removal through denitrification.

Based on order, the distribution of the microbial community structure in the sludge samples is illustrated in Fig. 12. The proportions of *Burkholderiales* and *Flavobacteriales* depict the major difference between the A-O_{HA}SA and A-OSA processes. Under the experimental conditions, the proportion of *Burkholderiales* was 19.8%, 22.1%, and 30.1% in the anoxic, oxic, and anaerobic sludge reaction tanks in the A-O_{HA}SA process, respectively, whereas the respective proportions in the A-OSA process were 6.8%, 5.3%, and 7.3%. *Burkholderiales* is also a multiple biological functional auxohetertroph and a denitrifying bacterium, capable of accelerating organic pollutant degradation and biological nitrogen fixation. Furthermore, *Burkholderiales* is selective towards the metabolism of organic substrates, exhibiting low energy metabolism efficiency. Therefore, *Burkholderiales* reproduced slowly [22].

Under the experimental conditions, the proportion of *Flavobacteriales* was 3.1%, 4.4%, and 4.4% in the anoxic, oxic, and anaerobic sludge reaction tanks in the A-O_{HA}SA process, respectively, whereas the respective values were 0.9%, 1.5%, and 4.0% in the A-OSA process. *Flavobacteriales* is a denitrifying, phosphorus-accumulating bacterium. It could effectively improve the phosphorus-removal efficiency of biological wastewater treatment processes. *Flavobacteriales* also has a slow growth cycle. A 40% decrease in its metabolizable energy and 20%–30% reduction in cell yield compared with other microbes was observed [9].

The proportions of *Proteobacteria*, *Flavobacteria*, *Burkholderiales*, and *Flavobacteriales* in the A-O_{HA}SA process were higher than those of the A-OSA process. The results indicate that several microbes had the stronger ability to adapt to the environment in the A-O_{HA}SA process, bearing the humic biological composite fillers. Hence, sludge production reduced under the influence of the microbes, while

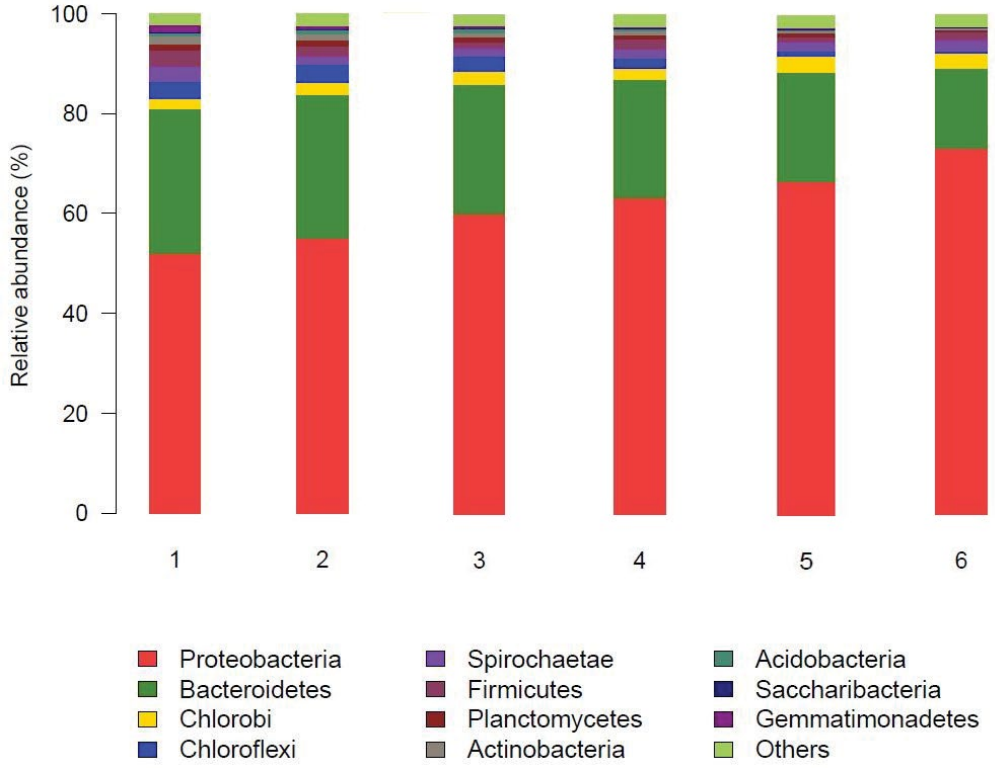

Fig. 10. Distribution on phyla of microbial community structure in the sludge samples.

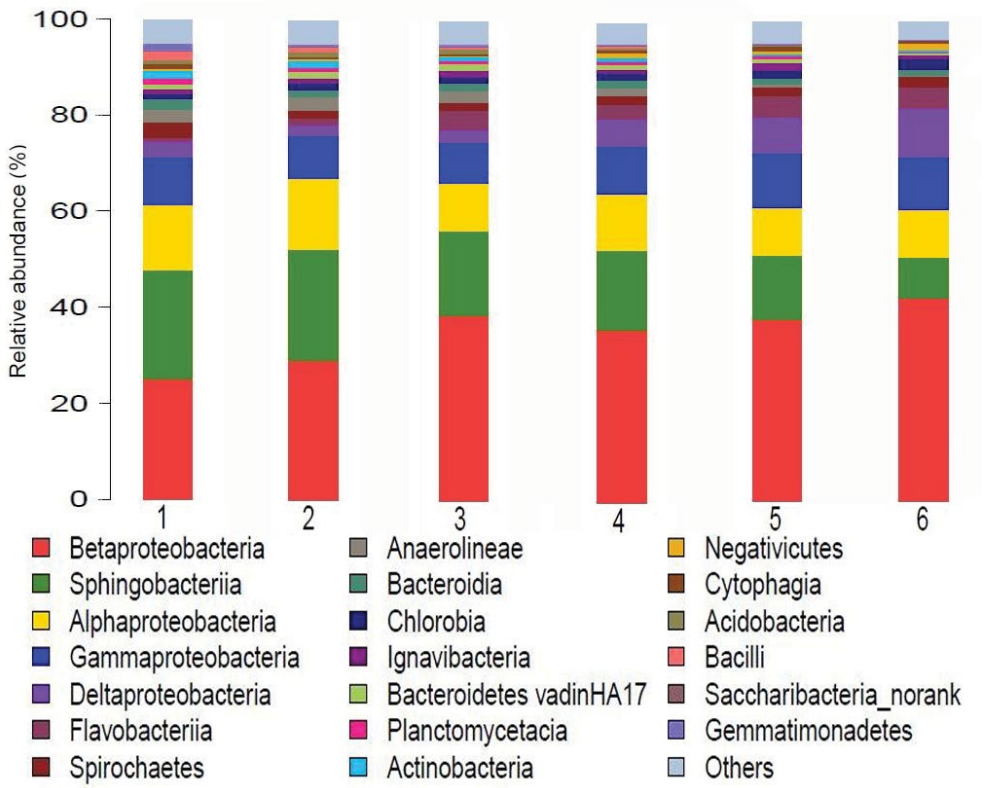

Fig. 11. Distribution of microbial community structure in sludge samples based on class.

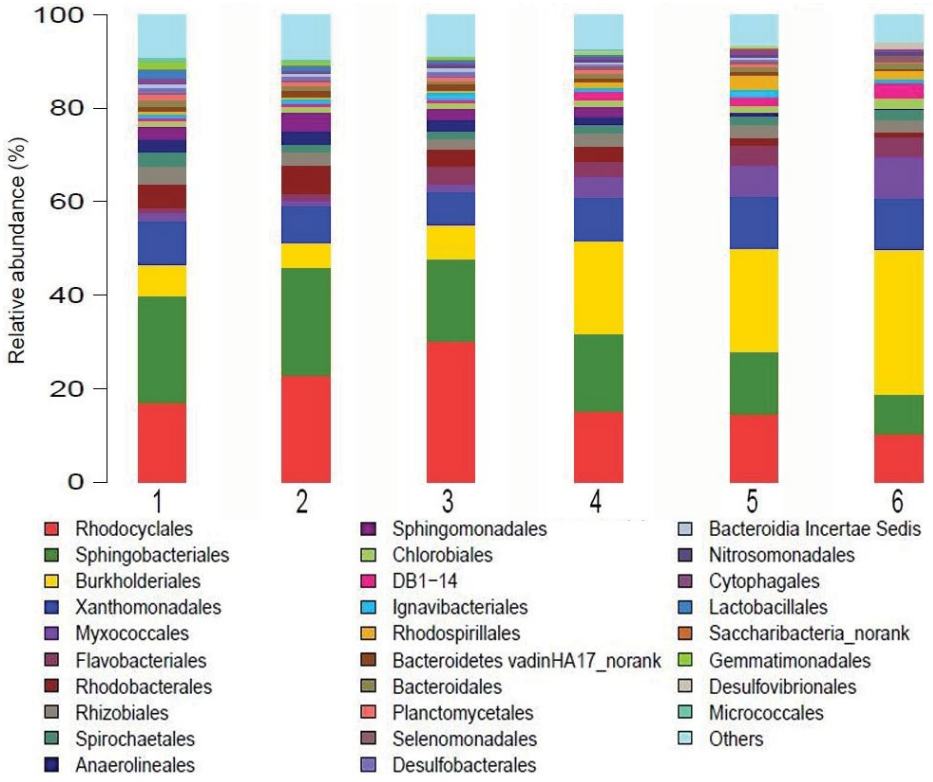

Fig. 12. Distribution microbial community structure in sludge samples based on order.

SND and phosphorus accumulation also was improved. Therefore, the A-O_{HA}SA process exhibited an outstanding performance on sludge reduction and nitrogen-phosphorus removal.

4. Conclusion

The A-O_{HA}SA process lowers DSP_{cum} effectively, exhibiting higher nitrogen and phosphorus removal efficiencies than the A-OSA process. Such superiority was attributed to the presence of the humic biological composite fillers in the former. When the reused sludge residence time was 5.5 h in the anaerobic sludge reaction tank, the DSP_{cum} of the A-O_{HA}SA process was 18.39% lower than the A-OSA process, with the TN and TP removal efficiency 6.12% and 11.01% higher, respectively. From a microbial perspective, such difference in efficiency could be attributed to the humic biological composite fillers, which affect the microbial living environment in the A-O_{HA}SA process. *Proteobacteria*, *Flavobacteria*, *Burkholderiales*, and *Flavobacteriales* were the predominant microflora, and they contributed to the slow growth rate of sludge. These bacteria ensure that the A-O_{HA}SA process evinced extraordinary performance on nitrogen and phosphorus removal through SND and phosphorus accumulation.

Acknowledgments

The authors gratefully acknowledge the financial support of the Science and Technology Planning Project of Ministry of Housing and Urban-Rural Development of the People's Republic of China (2019-K-134).

References

[1] G.-H. Chen, K.-J. An, S. Saby, E. Brois, M. Djafer, Possible cause of excess sludge reduction in an oxic-settling-anaerobic activated sludge process (OSA process), Water Res., 37 (2003) 3855–3866.
[2] Z. Zhou, W. Qiao, C. Xing, Y. An, X. Shen, W. Ren, L.M. Jiang, L. Wang, Microbial community structure of anoxic-oxic-settling-anaerobic sludge reduction process revealed by 454-pyrosequencing, Chem. Eng. J., 266 (2015) 249–257.
[3] S. Saby, M. Djafer, G.H. Chen, Effect of low ORP in anoxic sludge zone on excess sludge production in oxic-settling-anoxic activated sludge process, Water Res., 37 (2003) 11–20.
[4] X.M. Zhai, G. Xu, M.M. Zhang, J. Li, J.S. Guo, Analysis of carbon balance and study on mechanism in anoxic-oxic-settling-anaerobic sludge reduction process, Environ. Sci., 33 (2012) 2444–2450 (in Chinese).
[5] J. Li, G. Xu, P.C. You, X.M. Zhai, Y. Fan, Studies on excess sludge reduction and microbial community growing in a novel A+OSA reactor, Environ. Eng. Manage. J., 12 (2013) 2097–2105.
[6] K. Imai, N. Sasaki, H. Shinohara, M. Ishikawa, A study on decrease in excess sludge of the activated sludge process by the addition of humus or the extract of field horstail, Environ. Conserv. Eng., 28 (2010) 324–330.
[7] Z.X. Liu, B.L. Ma, J.L. Zuo, Improvement of wastewater treatment efficiency and deodorization by side-stream humus soil activated sludge process, Adv. Mater. Res., 183–185 (2011) 1517–1521.
[8] K. Zhao, G. Zhu, Humus activated sludge process applied in Japan and Korea, Appl. Mech. Mater., 209–211 (2012) 1973–1976.
[9] M. Wu, R. Zhu, H. Zhu, X. Dai, J. Yang, Phosphorus removal and simultaneous sludge reduction in humus soil sequencing batch reactor treating domestic wastewater, Chem. Eng. J., 215–216 (2013) 136–143.
[10] X. Yan, B. Li, F. Lei, X. Feng, B. Pang, Enhanced deodorization and sludge reduction in situ by a humus soil cooperated anaerobic/anoxic/oxic (A^2O) wastewater treatment system, Environ. Sci. Pollut. Res., 23 (2016) 15963–15969.
[11] K.U. Do, J.R. Banu, I.J. Chung, I.T. Yeom, Effect of thermo-chemical sludge pretreatment on sludge reduction and on performances of anoxic-aerobic membrane bioreactor treating low strength domestic wastewater, J. Chem. Technol. Biotechnol., 84 (2009) 1350–1355.
[12] E. Jami, A. Israel, A. Kotser, I. Mizrahi, Exploring the bovine rumen bacterial community from birth to adulthood, ISME J., 7 (2013) 1069–1079.
[13] Y. Wang, H.F. Sheng, Y. He, J.Y. Wu, Y.X. Jiang, F.Y. Tam, H.W. Zhou, Comparison of the levels of bacterial diversity in freshwater, intertidal wetland, and marine sediments by using millions of illumina tags, Appl. Environ. Microbiol., 78 (2012) 8264–8271.
[14] Ministry of Environmental Protection of the People's Republic of China, Monitor Analysis Methods of Water and Wastewater (4th ed.), China Environ Press, Beijing, 2009.
[15] N. Qin, D. Li, R. Yang, Next-generation sequencing technologies and the application in microbiology – a review, Acta Microbiol. Sin., 51 (2011) 445–457.
[16] Y.C. Pang, J.Y. Xi, Y. Xu, Z.Y. Huo, H.Y. Hu, Shifts of live bacterial community in secondary effluent by chlorine disinfection revealed by Miseq high-throughput sequencing combined with propidium monoazide treatment, Appl. Microbiol. Biotechnol., 100 (2016) 6435–6446.
[17] Q.L. Zhu, S. Liu, P. Gao, F.S. Luan, High-throughput sequencing technology and its application, J. Northeast Agric. Univ. (Engl. Ed.), 21 (2014) 84–96.
[18] L. Zhang, Z. Shen, W. Fang, G. Gao, Composition of bacterial communities in municipal wastewater treatment plant, Sci. Total Environ., 689 (2019) 1189–1191.
[19] K.R. Amato, C.J. Yeoman, A. Kent, N. Righini, F. Carbonero, A. Estrada, H. Rex Gaskins, R.M. Stumpf, S. Yildirim, M. Torralba, Habitat degradation impacts black howler monkey (*Alouatta pigra*) gastrointestinal microbiomes, ISME J., 7 (2013) 1344–1353.
[20] L. Wu, X.K. Han, Z.S. Liu, Z. Ma, Biological characterization and treatment efficiency of humic activated sludge process, J. Harbin Inst. Technol., 46 (2014) 31–35 (in Chinese).
[21] V. Bucci, N. Majed, F.L. Hellweger, A.Z. Gu, Heterogeneity of intracellular polymer storage states in enhanced biological phosphorus removal (EBPR)-observation and modeling, Environ. Sci. Technol., 46 (2012) 3244–3252.
[22] C. Tom, V. Peter, Diversity and significance of burkholderia species occupying diverse ecological niches, Environ. Microbiol., 5 (2003) 719–729.