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Effects of temperature on nutrient removal performance of a pilot-scale ABR/MBR combined process for raw wastewater treatment

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ABSTRACT

For the purpose of achieving relatively high efficiency, low energy demands, and easy maintenance for nutrient removal, the performance of a pilot-scale biological nutrient removal process consisting of anaerobic baffled reactor and membrane bioreactor has been evaluated for 301 d in treating two kinds of raw wastewaters. The results showed that the process enabled a relatively stable and high performance in both organics and nutrient removals, and high quality effluent was achieved under temperature of 25 ± 5˚C. When the ambient temperature were 10 ± 5 and 35 ± 5˚C, average COD, NH_4^+-N, TN, and TP removal efficiencies of both kinds wastewaters were more than 88, 87, 70, and 75%, respectively. Analysis of the results by fluorescence *in situ* hybridization showed that ammonia-oxidizing bacteria, nitrite-oxidizing bacteria, and phosphorus-accumulating organisms were always the enriched micro-organisms in the process during the change of temperature, ensuring the efficient nutrient removal under ambient environment with low energy exhaustion.

Keywords: ABR; Community analysis; MBR; Temperature; Domestic sewage; Nutrient removal

1. Introduction

Nowadays, more and more developing countries face a great challenge in treating increasing amounts of municipal wastewater and decentralized domestic sewage. The proportions of domestic sewage treated are as low as 4.9% for rural villages and 18.1% for county towns in China by 2009 [1]. The discharge of such untreated wastewater containing excess nutrient compounds, leads to the eutrophication of receiving waters and potentially threatens the safety of drinking water resources. It follows that, nutrient removal from rural domestic sewage is a real social concern. Eutrophication, mainly caused by nitrogen and phosphorus, is one of the serious environmental problems in LakeTaihu [2], which is the third largest fresh water lake in China. It is known that controlling nutrient

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input is the most effective way of reducing the risk of blooms [3], and significant efforts have been made to reduce the external nutrient loads in Taihu [4,5].

Anaerobic baffled reactor (ABR) is known as a high-rate bioreactor and has the ability to partially separate the various phases of anaerobic catabolism [6]. Results obtained at lab-scale are promising [7], but more information about the stability and their performance at large scale is needed in order to establish whether the process could be a feasible treatment to remove nutrients from real domestic sewage in the ambient environment. In this study, a pilot-scale ABR/MBR combined process was purposely developed to achieve the simultaneous removal of nitrogen and phosphorus from municipal wastewater and domestic sewage based on the previous study [7]. However, seasonal temperature change is a factor that greatly affects the nutrient removal efficiency of biological nutrient removal process. The objective of this work is to achieve relatively high efficiency, low energy demands, and easy maintenance for nutrient removal at ambient environment. Therefore, aeration gas which was substituted for a pumped source was gathered to provide the intermixture reflux. It not only can save pump energy and investment, but also is easy to operate. The process performance has been monitored for 301 sequential days. The evaluations are focused on the following operational parameters: (1) COD, N, and P removals, (2) temperature, and (3) microbial community.

2. Materials and methods

2.1. Wastewater

The influent was a mix of raw and synthetic wastewater (1:1) that had an average COD concentration of approximately 400 mg L^{-1} and ammonium-nitrogen concentration of 35 mg L^{-1}, total nitrogen concentration of 50 mg L^{-1}, and total phosphorus concentration of 4 mg L^{-1}. The selected synthetic wastewater contained glucose, ammonium chloride, dipotassium hydrogen phosphate, and potassium dihydrogen phosphate, which were used as the primary organic, nitrogenous, and phosphorous components. A solution of the trace elements [8,9] was added to sustain the microbial growth (see Table 1). Raw domestic sewage from a campus housing facility's sewer line was pumped into a storage tank for sedimentation, and then mixed into the synthetic wastewater. The composition of the raw domestic sewage can be seen in Table 2, and the composition of the wastewater from a wastewater treatment plant (WWTP) (Suzhou, China) can be seen in Table 3.

2.2. The configuration

The diagram of the process used in these experiments is shown in Fig. 1. The reactor was made of perspex with a total effective volume of 120 L. It consisted of three parts: an ABR of 60 L, an aerobic tank 1 of 20 L, and an aerobic tank 2 of 40 L. In order to save power, aeration gas, which was substituted for a pumped source, was gathered to provide the intermixture reflux. The ABR was inoculated with anaerobic sludge. The aerobic tanks were inoculated with aerobic sludge collected from the Municipal WWTP (Suzhou, China). A hollow fiber membrane module (PVDF, hydrophilic, pore size: 0.1 μm, area of membrane: 1.2 m^2) was immersed in the aerobic tank 2 (MBR). For all experiments, the mixed liquor, with recycle ratio 1 of 200%, was introduced to the first compartment of the ABR, while the mixed liquor, that kept at a constant flow rate of 50% influent (recycle 2), was introduced to the third compartment of the ABR. The recycle ratio could be controlled by raising or lowering the position of the outlet. The sludge retention time was controlled about 20 d and the hydraulic retention time was controlled about 7.5 h. The temperature of the reactor was controlled by water bath. A peristaltic pump was connected to the membrane module. The suction pump was operated in a timing

Table 1
Composition of trance elements solution

Substrates	Concentration (mg L^{-1})	Trace elements	Concentration (mg L^{-1})
H_3BO_3	30	$Na_2SeO_3·5H_2O$	25
$ZnCl_2$	25	$K_2HPO_4·3H_2O$	25
$CuCl_2$	25	$FeCl_3·6H_2O$	25
$AlCl_3$	25	$MgSO_4·7H_2O$	30
$NiCl_2$	25	$CoC_{12}·6H_2O$	25
EDTA	40	$MnSO_4·H_2O$	25
$CaCl_2$	30	$NaMoO_4·2H_2O$	25

Table 2
Characteristics of domestic wastewater in a university (mg L^{-1})

	Minimum	Maximum	Average
COD	199	384	288
NH_4^+-N	20	33	25
TN	24	46	35
NO_3^--N	0	0.4	0.1
TP	1	6	3
SS	69	307	166

Table 3
Characteristics of the wastewater from a wastewater treatment plant

	Minimum	Maximum	Average
COD	276	448	369
NH_4^+-N	22	39	30
TN	36	51	44
NO_3^--N	0	0.4	0.1
TP	1	7	3.5
SS	47	254	138

sequence consisting of 10-min switched on, and 2-min backwashing.

2.3. Experimental procedure

This experiment consisted of two phases. For the first phase, the process was operated over a 91 d period under temperature of 25 ± 5°C. For the second phase, different operation modes, shown in Table 4, were tested to examine the operation performance of the process at different ambient temperature during day 92–301 d. In this study, a steady-state condition was considered to be reached when the variation of the measurements was less than 10%. The average values of the data obtained under the steady-state condition were used for further calculations.

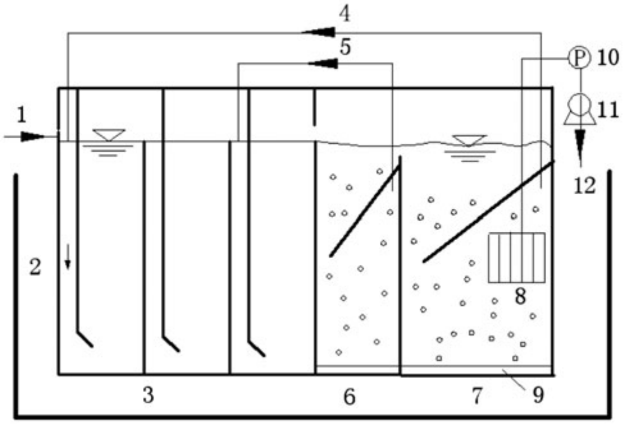

Fig. 1. Schematic diagram of the process. Diagram of the process (1) influent; (2) water bath; (3) ABR; (4) recycle 1; (5) recycle 2; (6) aerobic tank 1; (7) aerobic tank 2 (MBR); (8) membrane model; (9) air diffuser; (10) pressure meter; (11) pump; (12) effluent.

2.4. Chemical analysis

COD, NH_4^+-N, TN, and TP concentration were measured regularly according to standard methods, as set out by the American Public Health Association/American Water Works Association/Water Environment Federation [10]. DO was continuously monitored by WTW, pH/oxi340i meter with DO and ORP probes (WTW Company, Germany). The pH and temperature were measured online using WTW level 2 pH meters (WTW Company, Germany).

2.5. Fluorescence *in situ* hybridization (FISH) analysis

For *in situ* hybridization, the eight samples were taken from the MBR tank and treated as described by Roske et al. [11]. All *in situ* hybridizations were performed according to the standard hybridization protocol [12]. All oligonucleotide probes were obtained from TaKaRa (Dalian, China): the domain-specific EUB338 labeled with the fluorescence dye Cy3, and other oligonucleotide probes labeled with fluorescein isothiocyanate. After hybridization the slides were rinsed with distilled water and air dried. Finally the slides were immediately analyzed under a fluorescence microscope. The information of oligonucleotide probes EUB338 (5´-GCTGCCTCCCGTAGGAGT-3´), Non 338 (5´-ACTCCTACGGGAGGCAGC-3´), Nso190 (5´-CGATCCCTGCTTTTCTCC-3´), Nit3 (5´-CCTGTGCTCCATGCTCCG-3´), CNit3 (5´-CCTGTGCTCCAGGCTCCG-3´), Ntspa 662 (5´- GGAATTCCGCGCTCCTCT-3´), and PAOMIX (PAO462 (5´-CCGTCATCTACWCAGGGTATTAAC-3´), PAO651 (5´-CCCTCTGCCAAACTCCAG-3´), PAO 846 (5´-GTTAGCTACGGCACTAAAAGG-3´) along with different concentration of NaCl and formamide correspondingly referred to others [13–15]. Competitor probes were not labeled. Both *Nitrospira*-like nitrite-oxidizing cells stained with FITC-Ntspa662 and *Nitrobacter*-like nitrite-oxidizing cells stained with FITC-Nit3 were used together to target NOB. Microscopy was performed using an epifluorescence microscope (OlympusCX41, Japan) together with the standard software package delivered with the instrument (version 6.0). The mean values of cells were calculated by

Table 4
Operation mode of the combined process

Periods	Time (d)	Temperature (°C)	Wastewater
Run 1	1–91	Middle (25 ± 5)	Synthetic wastewater
Run 2	92–121	Middle (25 ± 5)	Municipal wastewater
Run 3	122–154	Middle (25 ± 5)	Domestic sewage
Run 4	155–184	Low (10 ± 5)	Municipal wastewater
Run 5	185–223	Low (10 ± 5)	Domestic sewage
Run 6	224–262	High (35 ± 5)	Municipal wastewater
Run 7	263–301	High (35 ± 5)	Domestic sewage

examining at least 10 visual fields. The measurements were performed in triplicate for each probe.

2.6. Statistical analysis

T test was performed using Microsoft Excel 2007, and significance level (*p*) was determined.

3. Results and discussion

3.1. COD removals in the process

COD removal efficiencies of the process are shown in Fig. 2(a). The results showed that the process had a good performance on COD removal at the end of each mode. The ABR effluent COD significantly fluctuated in each mode, while the effluent COD slightly fluctuated. The effluent COD removal efficiencies of both wastewaters were similar under the same temperature mode. The average ABR effluent COD generally decreased during the startup, finally, the effluent average COD was 27 mg L^{-1} with the removal efficiency of 93%. The average ABR effluent COD was 90 mg L^{-1} with the removal efficiency of 63% under the middle temperature, and the average effluent COD was 22 mg L^{-1} with the removal efficiency of 91%. The ABR effluent average COD significantly ($p < 0.05$) increased with temperature decreased to low-temperature mode. However, the ABR effluent average COD quickly decreased from 134 to 105 mg L^{-1}. The ABR effluent average COD significantly ($p < 0.05$) increased with the change of influent from municipal wastewater to university sewage. However, the ABR effluent average COD quickly decreased from 154 to 102 mg L^{-1}. It indicated that the ABR has a good ability to adapt to decrease in temperature and change of influent. The average ABR effluent COD was 102 mg L^{-1} with the removal efficiency of 62% under the low temperature, and the average effluent COD was 31 mg L^{-1} with the removal efficiency of 88%. While the temperature significantly increased from low temperature to high temperature, the ABR effluent average COD significantly ($p < 0.05$) increased. Then, the ABR effluent average COD quickly decreased from 161 to 103 mg L^{-1}. The ABR effluent average COD also significantly ($p < 0.05$) increased with the change of influent from municipal wastewater to university sewage. However, the ABR effluent average COD quickly decreased from 150 to 102 mg L^{-1}. It indicated that the ABR has a good ability to adapt to significant increase in temperature and change of influent. The average ABR effluent COD was 102 mg L^{-1} with the removal efficiency of 70% under the high temperature, and the average effluent COD was 27 mg L^{-1} with the removal efficiency of 91%.

3.2. NH_4^+-N removals in the process

Fig. 2(b) presents NH_4^+-N removal efficiencies of the process. As can be seen in the figure, the process had a good performance on NH_4^+-N removal at the end of each mode. The effluent NH_4^+-N generally decreased in each mode. The effluent NH_4^+-N removal efficiencies of both wastewaters were similar under the same temperature mode. The average effluent NH_4^+-N gradually decreased during the startup, finally, the effluent average NH_4^+-N was 0.3 mg L^{-1} with the removal efficiency of 99%. The average effluent NH_4^+-N was 0.4 mg L^{-1} with the removal efficiency of 98% under the middle temperature. The effluent average NH_4^+-N significantly ($p < 0.05$) increased with temperature decreased to low-temperature mode. However, the effluent average NH_4^+-N quickly decreased from 5.8 to 3.5 mg L^{-1}. The effluent average NH_4^+-N significantly ($p < 0.05$) increased with the change of influent from municipal wastewater to university sewage. However, the effluent average NH_4^+-N quickly decreased from 4.9 to 2.4 mg L^{-1}. The average effluent NH_4^+-N removal efficiency was 88% under the low temperature. While the temperature significantly increased from low temperature to high temperature, the effluent average NH_4^+-N significantly

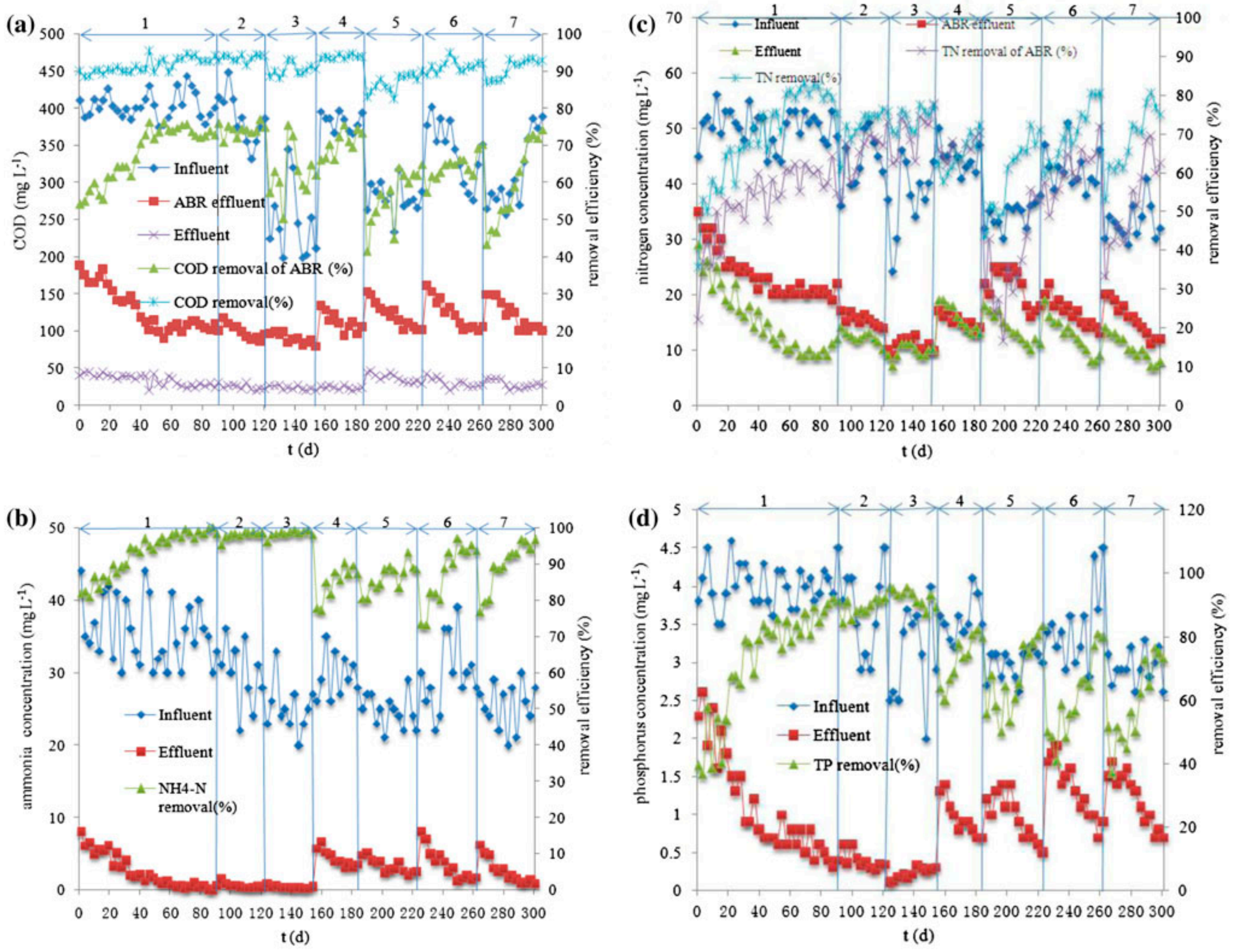

Fig. 2. Variations of: (a) COD, (b) NH_4^+-N, (c) TN, (d) TP, as well as removal efficiencies in the process.

($p < 0.05$) increased. Then, the effluent average NH_4^+-N quickly decreased from 8 to 1.8 mg L^{-1}. The effluent average NH_4^+-N also significantly ($p < 0.05$) increased with the change of influent from municipal wastewater to university sewage. However, the effluent average NH_4^+-N quickly decreased from 6.3 to 1.1 mg L^{-1}. It indicated that the process has a good ability to adapt to the change of temperature and the NH_4^+-N sudden drop. The average effluent NH_4^+-N was 1.8 mg L^{-1} with the removal efficiency of 94%.

3.3. TN removals in the process

The NO_x^--N produced in the MBR via the nitrification process was recycled into the ABR, where it was then converted to nitrogen gas leading to the removal of TN in the process. Fig. 2(c) shows the TN concentration in both influent and effluent as well as removal efficiencies of the process. The results showed that the process had a good performance on TN removal at the end of each mode. The ABR effluent TN, and the effluent TN significantly fluctuated in each mode and then stabilized. There were no significant differences found between these two effluents under the same temperature mode. The average ABR effluent TN generally decreased during the startup, finally, the effluent average TN was 21 mg L^{-1} with the removal efficiency of 56%. The average ABR effluent TN was 14.5 mg L^{-1} with the removal efficiency of 67% under the middle temperature, and the average effluent TN was 11.6 mg L^{-1} with the removal efficiency of 74%, revealing that about 7% of TN reduced simultaneously through nitrification and denitrification (SND). The ABR effluent average TN significantly ($p < 0.05$) increased with temperature decreased to low-temperature mode. However, the ABR effluent average TN quickly decreased from 17 to 14 mg L^{-1}. The ABR effluent average TN significantly ($p < 0.05$) increased with the change of influent from municipal wastewater to university sewage. However, the ABR effluent average TN quickly decreased from 22 to 17 mg L^{-1}. It indicated that the ABR has a good ability to adapt to the decrease in temperature and change of influent. The average ABR effluent TN was 17 mg L^{-1} with the removal efficiency of 54% under the low temperature, and the average effluent TN was 14 mg L^{-1} with the removal efficiency of 70%, revealing that about 14% of TN reduced through SND. While the temperature significantly increased from low temperature to high temperature, the ABR effluent average TN significantly ($p < 0.05$) increased. Then, the ABR effluent average TN quickly decreased from 20 to 14 mg L^{-1}. This indicates that the ABR has a high efficiency in denitrification. The ABR effluent average TN also significantly ($p < 0.05$) increased with the change of influent from municipal wastewater to university sewage. However, the ABR effluent average TN quickly decreased from 20 to 12 mg L^{-1}. It indicated that the ABR has a good ability to adapt to significant increase in temperature and the TN sudden drop. The average ABR effluent TN was 14 mg L^{-1} with the removal efficiency of 63% under the high temperature, and the average effluent TN was 9 mg L^{-1} with the removal efficiency of 77%, revealing that about 14% of TN reduced through SND. It was consistent with the results of Kim et al. [16] and Chen et al. [17]. Thus, the process has a stable performance on TN removal.

3.4. TP removals in the process

Total phosphorus concentration increased to a maximum in the anaerobic zone by the PAOs P-release by the PAOs, and then decreased in the anoxic zones due to the dilution by the recycling stream and P-uptake by the DPAOs. Then, phosphorus was further taken up by the PAOs and complete biological phosphorus removal was achieved in the aerobic zones [17]. The influent and effluent TP as well as removal efficiencies of the process are shown in Fig. 2(d). The results showed that the process have a stable performance on TP removal at the end of each mode. The TP in the municipal wastewater was significantly higher than that in the university sewage, but also no significant differences were found between these two effluents under the same temperature mode. The effluent average TP was 0.4 mg L^{-1} with the removal efficiency of 91% at the end of the startup. The average effluent TP of the middle temperature was similar with that of startup. The effluent average TP significantly ($p < 0.05$) increased with temperature decreased to low-temperature mode. However, the effluent average TP quickly decreased from 1.3 to 0.7 mg L^{-1}. The effluent average TP significantly ($p < 0.05$) increased with the change of influent from municipal wastewater to university sewage. However, the effluent average TP quickly decreased from 1.2 to 0.6 mg L^{-1}. It indicated that the process has a good ability to adapt to decrease in temperature and change of influent. The average effluent TP was 0.7 mg L^{-1} with the removal efficiency of 80% under the low temperature. While the temperature significantly increased from low temperature to high temperature, the effluent average TP significantly ($p < 0.05$) increased. Then, the effluent average TP quickly decreased from 1.9 to 0.9 mg L^{-1}. The effluent average TP also significantly ($p < 0.05$) increased with the change of influent from municipal wastewater to university sewage. However, the effluent average TP quickly decreased from 1.5 to 0.8 mg L^{-1}. It indicated that the process has a good ability to adapt to significant increase in temperature and the sudden drop on the influent TP. The average effluent TP was 0.9 mg L^{-1} with the removal efficiency of 75% under the high temperature. Generally, the effluent TP was similar with the results obtained by Zeng et al. [18], Kim et al. [16] and Ge et al. [19].

3.5. Analysis of electric energy consumption

The specific electric energy consumption of the lab-scale process was 0.85 kWh m^{-3} [7], which was consistent with other study for traditional wastewater treatment using the oxidation ditch/extended aeration system (0.48–1.04 kWh m^{-3}) [20]. While aeration gas was substituted for a pumped source in the study, electric energy consumption was significantly decreased to 0.54 kWh m^{-3}. However, it was still higher than that 0.39 kWh m^{-3} of electric energy consumption was obtained based on a single-stage biological process for municipal sewage treatment by Iaconi et al. [21]. Nevertheless, the pilot-scale ABR/MBR combined process has better performance on nutrient removal than the single-stage biological process (Fig. 3).

3.6. Analysis of fluorescence *in situ* hybridization

To assess the composition of the sludge in steady state of every mode, FISH was performed with the 16S rRNA targeting oligonucleotide probes NSO190, Nit3, and EUB338. NSO190 targeted AOB, while Nit3 and Ntspa662 targeted NOB, and PAOMIX targeted PAOs, respectively; EUB338 targeted the eubacteria

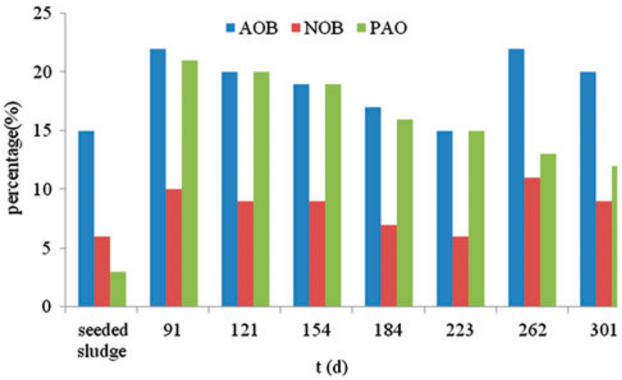

Fig. 3. Bacterial composition of the activated sludge in the process.

cluster. It can be seen that AOB, NOB, and PAOs were enriched after startup with the proportion of 22, 10, and 21%, respectively, while the proportion of AOB, NOB, and PAOs were 15, 6, and 3% in seed sludge, respectively. The proportions of AOB, NOB, and PAOs in treating municipal wastewater were similar to that of university sewage under the same temperature. In addition, the proportions of AOB and NOB under temperature of 25 ± 5˚C were similar to that of temperature of 35 ± 5˚C. The proportions of AOB and NOB under temperature of 10 ± 5˚C were lowest in the three modes of different temperature; The cause may be that the specific growth rates of both AOB and NOB were inhibited under low temperature [22]. Furthermore, the proportions of AOB and NOB were significantly ($p < 0.05$) increased with an increase in temperature. However, the proportion of PAOs was significantly ($p < 0.05$) decreased while the temperature increased from 10 ± 5 to 35 ± 5˚C, its properly the case that high-temperature environment was not beneficial for PAOs accumulating. After all, the high sum of the percents of the three kinds of enriched micro-organisms [7,23,24] was the underlying reason why the process was efficient for nutrient removal.

4. Conclusion

The process with low energy demands and easy maintenance achieved a high quality effluent under middle temperature. There were no significant differences found between these two effluents under the same temperature mode. Average COD, NH_4^+-N, TN, and TP removal efficiencies were more than 88, 87, 70, and 75%, respectively, with HRT 7.5 h at recycle ratio of 200% and DO of 3 mg L^{-1}. The existence of the enriched micro-organisms was the underlying reason why the process can quickly adapted to the change of temperature and influent and was efficient for nutrient removal. Thus, the process has proved to be efficient in nutrient removal under ambient environment and is suitable for application with low energy exhaustion.

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