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Effect of temperature on simultaneous nitrogen and phosphorus removal and microbial community in anaerobic-aerobic-anoxic sequencing batch reactor

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ABSTRACT

In this study, we used simulated domestic wastewater which combines glucose and sodium acetate to serve as carbon sources and researched the effect of eight groups of temperatures on simultaneous nitrogen and phosphorus removal in an anaerobic-aerobic-anoxic sequencing batch reactor (AOA-SBR) to improve the efficiency of simultaneous nitrogen and phosphorus removal. The temperature used in this study were 13°C, 15°C, 17°C, 19°C, 21°C, 23°C, 25°C and 27°C, respectively. When the temperature was above 23°C, the average removal rates of NH_4^+–N, chemical oxygen demand, total nitrogen and total phosphorus in the effluent were higher than were 98.32%, 93.59%, 91.23% and 99.94%, respectively. The results showed that increasing the temperature was beneficial for simultaneous nitrogen and phosphorus removal. Microbial community analysis showed that bacteroidetes and proteobacteria were the dominant phylum-level bacteria for simultaneous nitrogen and phosphorus removal, which account for more than 80% of the total bacteria. *Sphingobacteriia*, about 30% of the total number of bacteria, were the dominant class-level bacteria for simultaneous nitrogen and phosphorus removal in the AOA-SBR bioreactor.

Keywords: Simultaneous nitrogen and phosphorus removal; Temperature; Anaerobic-aerobic-anoxic sequencing batch reactor; Microbial community analysis

1. Introduction

An appropriate amount of nitrogen and phosphorus can promote the growth of microorganisms and plants in the water and maintain ecological balance. However, excessive amounts of N and P will cause the rapid reproduction of algae and plankton, decrease the concentration of dissolved oxygen (DO) in the water and deteriorate the water quality, and threaten the safety of drinking water. Therefore, N and P are the key problems in wastewater treatment [1–4]. As the main pollutants of wastewater, N and P removal technologies have attracted a lot of attention worldwide, especially biological nutrient removal (BNR) technologies. Sequential batch reactor (SBR) and its modified process are applied widely in the treatment of industrial wastewater and municipal wastewater because of its operational flexibility, structural simplicity and high stability [5–9].

Although SBR has been applied successfully in various wastewater treatments, the important factors affecting its performance still need to be further studied. According to the traditional biological denitrification theory, denitrification consists of the aerobic stage and anoxic stage. Nitrification and denitrification processes were carried out by different aeration intervals or separately in different bioreactors. In the aerobic stage, NH_4^+–N in wastewater is oxidized by ammonia-oxidizing bacteria (AOB) to nitrite. Then, nitrite is being oxidized by nitrite-oxidizing bacteria (NOB) to nitrate. In the aerobic stage, denitrifying bacteria reduce nitrite nitrogen and nitrate nitrogen to gaseous nitrogen (N_2) [10]. However, phosphorus removal

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is accomplished by a specific metabolism of phosphorus-accumulating organisms (PAOs) in alternating anaerobic and aerobic phases. Therefore, different functional microorganisms have different requirements for the environment. They also have different requirements for temperature [11,12]. Since temperature affects the growth rate of microorganisms and the activity of microbial enzymes, it is an important factor for affecting SBR wastewater treatment [13]. Many researchers have investigated the effect of temperature changes on simultaneous N and P removal in SBR processes. The effect of temperature on the microbial communities in biological phosphorus removal systems was investigated by Panswad et al. [14]. As the temperature increased from 20.0°C to 35.5°C, the main microbial group changed from PAOs to glycogen-accumulating organisms (GAOs) and then to ordinary heterotrophs. In simultaneous denitrification and phosphorus removal systems, low temperature negatively affects microbial activity, so it is usually considered a serious problem, and has aroused a lot of research. Lower temperature will lead to reduced nitrification capacity. Nitrification capacity was significantly reduced when the temperature was below 15°C, and almost ceased when the temperature was below 5°C. Chai et al. [15] also studied the effect of temperature change on simultaneous nitrification and denitrification (SND) in the SBR process [15]. Liu and Yang used improved SBR reactors, resulting in improved nitrogen and phosphorus removal at about 15°C [16,17]. However, Liu only studied how to improve the efficiency of simultaneous denitrification and phosphorus removal at a single temperature (15°C), and did not study the effect of other temperatures.

Located in the central plains of China, Henan province has typical climatic conditions, with water temperatures ranging from 5°C to 25°C a year. The primary purpose of the study was to investigate the influence of different temperatures on the simultaneous denitrification and phosphorus removal capabilities of the anaerobic-aerobic-anoxic sequencing batch reactor (AOA-SBR) bioreactor in Henan province, and determined the best temperature to obtain high nitrogen and phosphorus removal efficiency simultaneously. The structure of the microbial community was described and the major species of bacteria at various levels in the bioreactor were analyzed. This study can provide some detailed information for the synchronous denitrification and dephosphorization of wastewater by controlling the optimal temperature in the SBR system.

2. Materials and methods

2.1. Bioreactor set-up and operating conditions

The SBR bioreactor used in the experiment was made of Plexiglass (Fig. 1). The bioreactor had a volume of 12 L, and the effective volume is 10 L. The operation process of the bioreactor system includes initial feed, anaerobic stage, aerobic stage, anoxic stage, sedimentation stage and ultimate discharge. Each stage was 10 min, 2 h, 2 h, 1 h, 40 min and 10 min, respectively. The reaction time and running state of AOA-SBR can be controlled through the control panel. Anaerobic, aerobic and anoxic stages were stirred by an electric stirrer at a speed of 30 rpm. The sludge was rotated in a bioreactor under the action of an agitator, and the aerobic phase was aerated with a microporous aerator. In the anaerobic, aerobic and anoxic stages, dissolved oxygen concentrations were kept at 0.5, 2.0–3.0 and 0.5–1.0 mg L^{-1}, respectively. To investigate the effect of temperature on the denitrification and dephosphorization capabilities and microbial community, the temperature

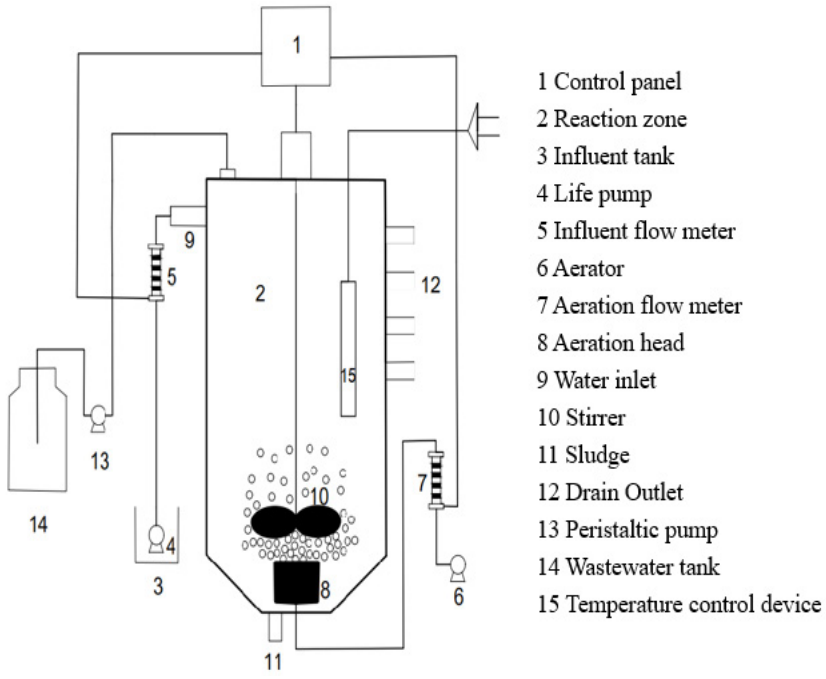

Fig. 1. SBR bioreactor schematic.

of the bioreactor was controlled by a constant temperature heating rod and measured by a mercury thermometer. Low temperature reduces the activity of denitrifying bacteria, which leads to the failure of complete removal of NO_3–N below 12°C [18]. In order to study the effect of common water temperature on simultaneous removal of nitrogen and phosphorus in Henan province, eight working conditions of T1, T2, T3, T4, T5, T6, T7 and T8 were established. Different temperatures were used in different operating conditions, including 13°C, 15°C, 17°C, 19°C, 21°C, 23°C, 25°C and 27°C, respectively. The sludge retention time for this experiment was 20 d. Sludge was obtained from the bioreactor at the end of each cycle and saved to be analyzed by high-throughput pyrosequencing analysis.

2.2. Wastewater and sludge

This AOA-SBR study used synthetic wastewater, and the concentration of chemical oxygen demand (COD), total nitrogen (TN), NH_4^+–N and total phosphorus (TP) were 290–310, 29–31, 26–30 and 2.9–3.1 mg L^{-1}, respectively. The composition of synthetic wastewater was (g L^{-1}): 0.08 CH_3COONa $3H_2O$, 0.4 glucose, 0.024 K_2HPO_4, 0.125 $NaHCO_3$, 0.115 NH_4Cl, 0.0275 $MgSO_4 \cdot 7H_2O$, 0.0025 $CaCl_2$, 0.004 KCl (Guangdong Guanghua Technology Co., Ltd, China.) and 0.12 mL L^{-1} trace nutrients solution. HCl and NaOH were used to adjust the pH of the inflowing wastewater to about 7.0. The seed sludge for the AOA-SBR system study was taken from the return sludge of a secondary sedimentation tank in a wastewater treatment plant in Henan Province, China. The conditions of sludge inoculation into AOA-SBR bioreactor were as follows: mixed liquid suspended matter (MLSS), mixed liquid volatile suspended matter (MLVSS) and settling velocity (SV) were 3.474 g L^{-1}, 2.082 g L^{-1} and 64%, respectively.

2.3. Analytical method

The water to be tested was collected daily from the outlet of the AOA-SBR bioreactor using a syringe. They were centrifuged at 4,000 rpm for 5 min and the supernatant was slowly poured out. The supernatant was analyzed for COD, TN, NH_4^+–N, TP and other major indicators according to standard methods. Instruments used include SBR bioreactor (QXG041, Kunshan Qingxu Environmental Technology Co., China), UV-Vis spectrophotometer (N5000, Shanghai Youke Instruments & Apparatus Co., China), Qubit® 3.0 Fluorescence quantification instrument (Q32866, Invitrogen), PCR instrument (T100™ Thermal Cyeler, BIO-RAD), Dissolved Oxygen Detector (JPB-607A, Shanghai Yidian Scientific Instruments Co., China) and Portable pH meter (PHBJ-260, Shanghai Yidian Scientific Instruments Co., China). Activated sludge samples were collected regularly and centrifuged to remove their supernatant. It was stored at –80°C for further analysis.

2.4. Microbial diversity analysis

The sludge samples to be tested were sent to Microbial Technology (Shanghai) Co., Ltd., China for DNA extraction, PCR amplification and DNA cloning and sequencing.

Eight samples were subjected to miseq library preparation (genomic DNA extraction, PCR amplification, recovery by AxyPrepDNA gel recovery kit, and real-time fluorescence quantification by FTC-3000TM real-time PCR instrument), followed by illumina miseq 2x300 bp high-throughput sequencing and bioinformatics analysis. Genomic DNA was extracted using MIO-BIO Power Soil DNA Isolation Kit. 3 μL of each sample was subjected to 1.2% agarose gel electrophoresis. According to illumina Miseq high-throughput sequencing requirements, bidirectional sequencing was performed to design target regions and fusion primers with "5′ Miseq splice-barcode-sequencing primer-specific primer-3′" fusion primers. The library was constructed by a two-step PCR amplification method. Table 1 shows the primer sequences for PCR system amplification.

The first PCR amplification system includes 10 μL of 5xBuffer, 1 μL of dNTP (10 mM), 1 U of Phusion ultrafidelity DNA polymerase, 1 μL of each F/R medial primer (10 uM) and 5 ng–50 ng of template, and finally ddH_2O is made up to 50 μL. Amplification was then performed using an ABI 9700 PCR instrument for 94°C for 2 min, followed by 23 cycles of 94°C for 30 s, 56°C for 30 s, 72°C for 30 s, followed by 72°C for 5 min, and finally maintained at 10°C. The second PCR amplification system includes 8 μL of 5xBuffer, 1 μL of dNTP (10 mM), 0.8 U of Phusion superfidelity DNA polymerase, 1 μL of each F/R outer primer (10 uM) and 5 μL of template, and finally ddH_2O is made up to 40 μL. Amplification was then performed for 94°C for 2 min, followed by 8 cycles of 94°C for 30 s, 56°C for 30 s, 72°C for 30 s followed by 72°C for 5 min, and finally maintained at 10°C.

All PCR products were recovered using the AxyPrepDNA Gel Recovery Kit and quantified using the FTC-3000™ real-time PCR instrument. The samples were mixed in equimolar ratios to complete library preparation, and the libraries were sequenced.

The PE reads obtained from sequencing are first distinguished from each sample according to barcode, and then quality-controlled and filtered for sequence quality. The overlap relationship is then used for splicing, and the spliced sequences are quality controlled and filtered again, and the optimized sequences are finally obtained. The optimized sequences were subjected to OTU clustering analysis and species taxonomy analysis. Based on the OTU clustering analysis results, a variety of diversity indices can be analyzed for OTU, as well as the detection of sequencing depth. A series of statistical analyses of community structure can be performed at various taxonomic levels.

3. Results and discussion

3.1. Effect of temperature on the COD and phosphorus removal of AOA-SBR system

AOA-SBR bioreactor was operated under 8 different temperature conditions. Fig. 2a shows the change of COD concentration and removal rate under the condition of 13°C–27°C. The removal rates of COD under eight temperature conditions were 87.97%, 90.76%, 93.80%, 94.97%, 94.88%, 94.78%, 95.24% and 95.40%, respectively. When the temperature is in the range of 13°C–17°C, the COD removal rate is less than 92%, and the COD concentration

of the effluent is above 20 mg L^{-1}. The COD removal rate is 93%~96% and the effluent concentration is less than 20 mg L^{-1} when the temperature is 19°C~27°C. This is because the removal of organic matter depends mainly on the metabolism of heterotrophic microorganisms. And low temperature reduces microbial activity and enzymatic reaction, resulting in lower COD removal [19]. The lower COD removal rate at a lower temperature in the results indicated that the organic matter utilization decreases at low temperatures. It can be inferred that low temperature can inhibit the activity of nitrifying bacteria, which is in agreement with the previous findings [20].

Fig. 2b shows the change of TP concentration and removal rate under the condition of 13°C–27°C. The removal rates of TP under 8 temperature conditions were 99.93%, 99.88%, 99.96%, 99.91%, 99.88%, 99.94%, 99.96% and 99.96%, respectively. As can be seen from the figure, the effect of different temperature conditions on TP removal was not significant in the range of 13°C–27°C. The TP removal rate was more than 99%, and the effluent of TP has a concentration less than 0.5 mg L^{-1}. Some researchers believe that PAOs have a competitive advantage at low temperatures, with the maximum rate of anaerobic phosphorus release at a temperature of 20°C. As the temperature increases, the ability of PAOs to utilize the substrate decreases and the growth rate becomes slower. The number of PAOs decreased, and GAOs replaced PAOs as the dominant group [14]. However, in this experiment, the removal of P was always maintained at a high level 99.92+0.4%. This may be due to the use of glucose as a carbon source for the simulated effluent. The use of glucose as a carbon source was able to obtain good phosphorus removal compared to acetic acid and propionic acid [21]. It is also possible that the low temperature favors the growth and enrichment of PAOs in the SBR reactor [22]. The reason could also be that the release rate and absorption rate of phosphorus increase with the increase of temperature. However, the amount of phosphorus released and absorbed did not change much [23].

Table 1
PCR system amplification primer sequences

Primer name	Sequence
515F	5′-GTGCCAGCMGCCGCGGTAA-3′
926R	5′-CCGTCAATTCMTTTGAGTTT-3′

3.2. *Effect of temperature on the denitrification performance of AOA-SBR system*

Fig. 2c shows the changes of NH_4^+–N concentrations and the removal rates at various temperatures. The NH_4^+–N removal rates were 97.14%, 97.30%, 98.18%, 98.31%, 98.38%, 98.32%, 99.39%, and 99.47% at eight temperature conditions, respectively. The removal rate of NH_4^+–N gradually increased with increasing temperature. When the temperature is

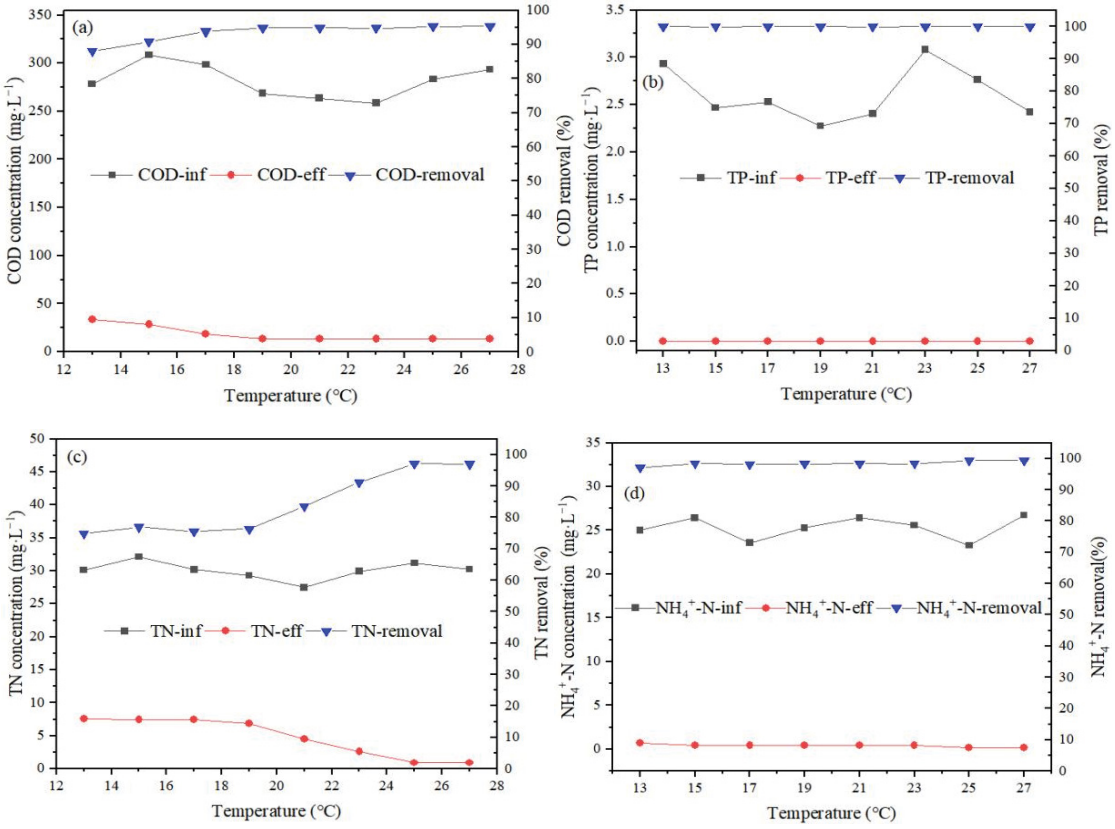

Fig. 2. The removal efficiency of AOA-SBR for carbon, nitrogen and phosphorus pollutants at different temperatures, expressed as changes in concentration and removal rate for COD (a), TP (b), TN (c), and NH_4^+–N (d) removal.

17°C~27°C, the removal rate of NH_4^+–N is more than 98%, and the effluent concentration of NH_4^+–N is lower than 0.5 mg L^{-1}. In the present experiments, high NH_4^+–N removal rates were achieved in the SBR bioreactor at eight different temperature conditions, which is different from the experimental results of Wang et al. [24]. It may be due to the fact that the sludge retention time is already longer at low temperatures and longer sludge retention time increases the capacity for biological nitrification [25]. Balmelle et al. [26] have suggested that nitrifying bacteria are most active at 10°C–20°C. The activity of nitrosobacteria gradually increased from 20°C to 25°C, and reached the maximum at 25°C. In the present experiment, this phenomenon did not occur and the NH_4^+–N removal rate did not decrease at 27°C.

The changes in TN concentration and removal rate under different temperature conditions are shown in Fig. 2d. The temperature had a remarkable effect on the TN removal rate. The removal rates of TN at different temperatures were 74.92%, 76.91%, 75.45%, 76.42%, 83.50%, 91.23%, 97.11% and 97.02%, respectively. It can be seen that the denitrification process was most sensitive to temperature change. Microbial activity enhanced with increasing temperature, and both nitrification and denitrification rates increased [27]. When the temperature was in the range of 23°C~27°C, The TN was removed by more than 90%, and the concentration of TN was less than 3 mg L^{-1}. It indicated that the sludge in the system had good nitrification and denitrification activities when the temperature was in the 23°C~27°C range. For the majority of heterotrophic nitrifying microorganisms, the optimum culture temperature was in the range of 20°C~37°C [28,29]. In the temperature range of 13°C~23°C, the removal rate of TN was less than 90% and the effluent concentration was higher than 3 mg L^{-1}, indicating that the bacterial activity was inhibited in this range of temperature, which was not conducive to denitrification, so the removal effect became poor.

3.3. Effect of temperature on microbial community structure in AOA-SBR

High-throughput sequencing technology and 16S rRNA gene amplification were used to obtain comprehensive information (Table 2) on microorganisms to understand the changes in microbial characteristics in temperature-controlled AOA-SBR systems.

The OTUs of the eight samples were 653, 718, 716, 728, 636, 708, 666 and 661, respectively. The OTUs number starts to increase from the temperature T1, and gradually stabilized T2, T3, and T4 during the period. But then decreased as the temperature increases. The trend of OTUs usually corresponded to the performance of the system. The appropriate bioreaction temperature conditions seemed to favor the stability of the microbial community. The too low or too high temperature will greatly affect the number of OTUs. The microbial communities of phylum, class, order and genus were distributed in the AOA-SBR bioreactor under different temperature conditions, as shown in Fig. 3. A total of 30 phyla were detected in activated sludge. Bacteroidetes and proteobacteria species accounted for more than 80% of the total bacteria, which was the same as the results of previous studies [30]. It is reported that they have the ability of organic matter degradation, denitrification and phosphorus removal, which can improve the nitrogen and phosphorus removal of AOA-SBR bioreactors. It shows that these two phylum-level bacteria represent the adaptation of bacterial communities in activated sludge after long-term domestication at different temperatures. Chloroflexi is also an important phylum in this system, mainly used for organic degradation and nitrate reduction. At the class level, Sphingobacteriia, Betaproteobacteria, Deltaproteobacteria and Gammaproteobacteria were the dominant colonies under these eight temperature conditions. We can see from the figure that the proportion of Sphingobacteriia, Betaproteobacteria and Deltaproteobacteria were lower in T2, T3, T4 temperature conditions, while the proportion of Sphingobacteriia, Betaproteobacteria and Deltaproteobacteria are greatly increased from T5 temperature conditions. This is consistent with the results of high nitrogen and phosphorus removal above 20°C. It shows that the Sphingobacteriia, Betaproteobacteria and Deltaproteobacteria played an important role in the process of nitrogen removal and phosphorus removal. This is in agreement with previous researchers that Betaproteobacteria were significant denitrifying bacteria that enhance nitrogen removal [31]. At the order level, sphingobacteriales were dominant, accounting for 35.76%, 31.7%, 35.42%, 34.97%, 38.93%, 34.68%, 37.35% and 37.65%, respectively. Sphingobacteriales were an important type reported to promote the accumulation of glycogen accumulating organisms (GAOs) [32]. At the genus level, unclassified species dominated.

Table 2
Estimators of species diversity and richness of microbial sequences

Samples	Temperature	Reads	Chaol	OTUs	ACE	Coverage
T1	13	47673	731.443	653	734.562	0.996
T2	15	46998	761.5	718	763.915	0.997
T3	17	45459	767.835	716	765.565	0.997
T4	19	48436	778.522	728	771.052	0.998
T5	21	42168	709.436	636	725.042	0.996
T6	23	45629	768.821	708	775.232	0.997
T7	25	43036	755.63	666	749.306	0.996
T8	27	49965	767.567	661	743.026	0.997

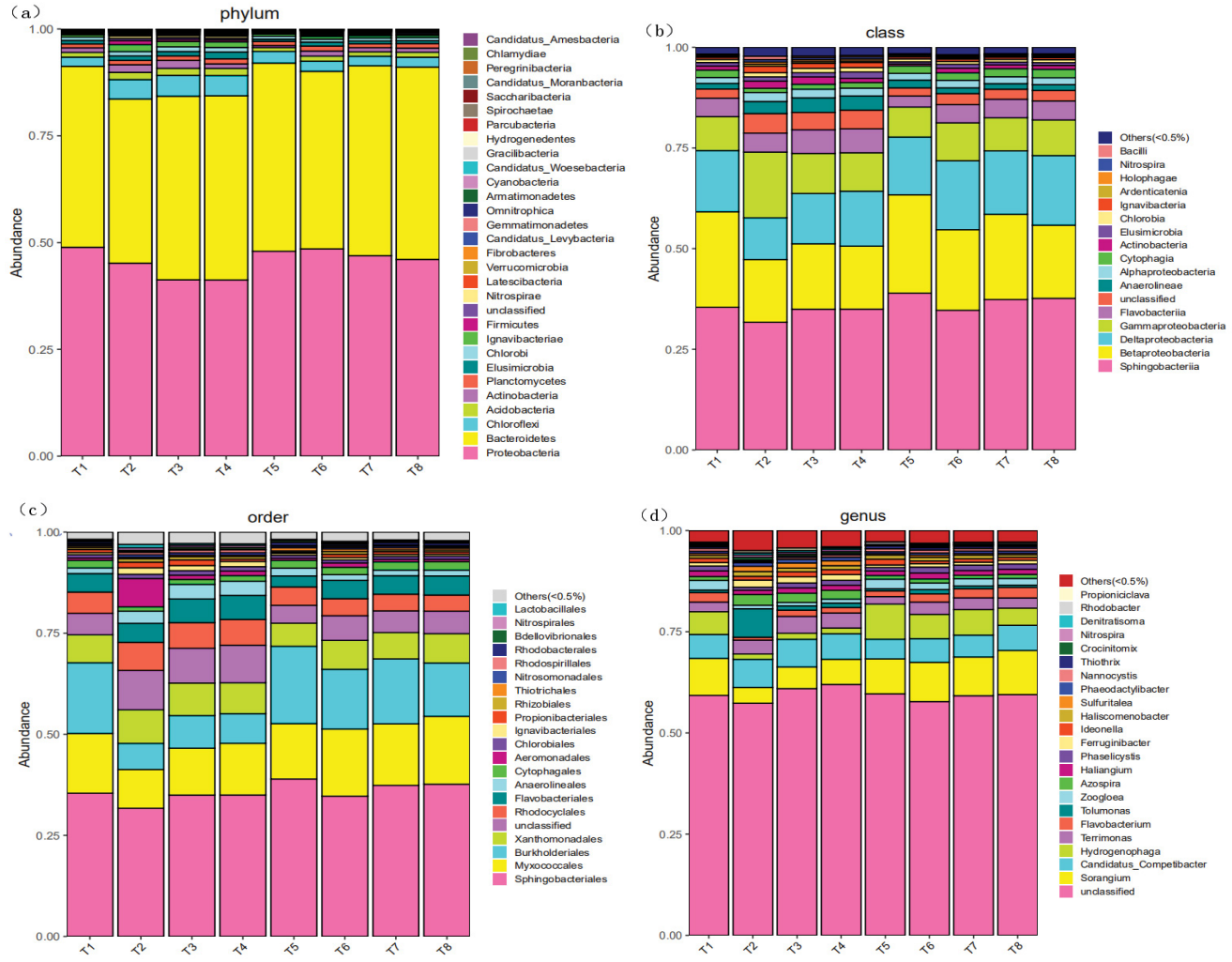

Fig. 3. Profiles of microbial communities under the eight conditions at the phylum level (a), class level (b), order level (c) and genus level (d).

Temperature affected the activity of microorganisms, the composition of the population, the proliferation of cells and the flocculation and sedimentation performance of activated sludge, and was a significant environmental factor. Different types of microorganisms have their specific growth temperature range, and the biological nitrification reaction can be carried out within 4°C~45°C. Hellinga et al. [33] believed that the optimal temperature for achieving and maintaining short-range nitrification is 30°C~35°C. While some studies have demonstrated that the temperature optimum for nitrite nitrification is 22°C~27°C, at least not lower than 15°C. In the past research, most researchers believed that low temperature is more conducive to PAOs in the EBPR system to occupy a dominant position in the competition with GAOs, but some scholars believed that high temperature is conducive to the competition of PAOs, phosphorus removal performance is closely related to PAOs and GAOs. The research proposed that the main phosphorus-absorbing microorganisms in the single-stage aerobic process were special phosphorus-accumulating bacteria. It may be that there were non-traditional PAOs in the system, such as Actinomyces and Acinetobacter, which are stored in the form of polyphosphate under aerobic conditions. Some scholars also pointed out that the performance of phosphorus removal is affected by the competition of PAOs and GAOs in the system [34,35]. Temperature can determine the stability of microorganisms and enzyme activity. At present, the research on the competition between temperature and PAOs and GAOs mainly focuses on the relationship between temperature change and treatment efficiency and the mutual transformation of flora [16]. There is no unified view on the competition between PAOs and GAOs under different temperature conditions, and the potential degradation mechanisms of biological phosphorus removal systems under different temperature conditions are not clear.

The carbon source directly affected the activities of the anaerobic section of PAOs and GAOs. Under anaerobic conditions, PAOs decomposed glycogen and polyphosphoric particles inside the cell to provide energy, and absorbed volatile organic acids (VFA) in the water body to convert into intracellular aggregation. Polyhydroxyalkanoate (PHA)

was hydrolyzed into polyphosphate particles in the cell, and orthophosphate was released into the body, resulting in a significant increase in the release of phosphorus in the anaerobic region. In anoxic or aerobic conditions, PAOs decomposed the anaerobic section of PHA accumulated in the cell body, excessively absorbed the orthophosphate in the water and converted it into polyphosphoric particles, which were discharged with the sludge to achieve the purpose of phosphorus removal [36].

The effect of different temperatures on the microorganisms in activated sludge is more obvious. If the temperature is too low, the microorganisms grow slowly and the enzyme activity decreases, the temperature is too high, the nitrifying bacteria multiply, the utilization ability of the PAOs of phosphorus accumulating bacteria to the substrate decreases, the growth rate becomes slower, and the number of PAOs cut back. The research found that when COD coexists with O_2 or NO_3^- in the anaerobic stage, PAOs will release phosphorus. Only when the COD is exhausted will excessive phosphorus absorption occur [37,38]. When the temperature was greater or equal to 23°C, the aerobic section had a higher nitrification rate, and NO_3^- generated by nitrification was fully utilized as an electron acceptor in the anoxic section, which was specifically reflected in that phosphorus was removed, and NO_3^- was also removed. And the two have the proper ratio, and there was not much left in the full response. When the temperature was greater or equal to 23°C, the concentrations of NH_4^+–N, TN and TP in the effluent were lower than 0.5, 3 and 0.5 mg L^{-1}. Therefore, the appropriate temperature can achieve the best effect of nitrogen and phosphorus removal.

It can be found from the changes in the distribution of microorganisms at eight different temperatures that temperature had a significant effect on the type of nitrification. It is generally considered that 20°C is the critical temperature for achieving short-range nitrification. However, some scholars have achieved short-range biological nitrogen removal at low temperatures (<20°C). For short-range nitrification, although there is no uniform temperature limit, there is a consensus that under high-temperature conditions, short-range nitrification can be achieved stably, and there are few reports on stability under low-temperature conditions. The principle lies in: under high-temperature conditions (>20°C), the proliferation rate of AOB is higher than that of NOB, AOB becomes the dominant nitrifying bacteria in the system, making the ammonia oxidation rate higher than nitrous acid, the rate of salt oxidation leads to the accumulation of nitrite. However, under low-temperature conditions (<20°C), the AOB proliferation rate was lower than the NOB proliferation rate, NOB was dominant in the system, the ammonia oxidation rate was lower than the nitrite oxidation rate, and nitrite accumulation cannot be formed [39].

4. Conclusion

The effect of temperature on simultaneous nitrogen and phosphorus removal in the AOA-SBR system was significant. When the temperature is greater then 23°C, the average removal rates of NH_4^+–N, COD, TN and TP were higher than 98.32%, 93.59%, 91.23% and 99.94%, respectively. Among them, the phosphorus removal effect did not vary significantly with temperature and remained at a high level, probably due to the use of glucose as a carbon source or the enrichment of PAOs at low temperatures. It showed that increasing the temperature in the range of 13°C~27°C was conducive to simultaneous denitrification and phosphorus removal. The microbial community analysis showed that the dominant phylum-level bacteria were Bacteroidetes and proteobacteria, and the dominant class-level bacteria were Sphingobacteriia in the AOA-SBR process, which contributed to the good nitrogen and phosphorus removal performance of the system. Raising the temperature can increase the proportion of dominant bacteria and improve their activities, so that increasing the efficiency of wastewater treatment. Therefore, the screening of low temperature tolerant strains needs to be carried out for further research. If the low temperature tolerant strain with good effect of nitrogen and phosphorus removal can be screened and isolated for enrichment culture. Then the reactor can have good treatment effect even at low temperature.

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