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Original Research

Regulation of Partial Nitritation in Constructed Rapid Infiltration System and Analysis of Microbial Community Structure

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Abstract

Partial nitritation (PN) and the anaerobic ammonium oxidation (ANAMMOX) process provides a novel method to solve the problem of low TN removal efficiency in a constructed rapid infiltration (CRI) system, and PN is the prerequisite to realize the process. In this study, the feasibility of achieving PN in a CRI system by synergistic regulation of free chlorine, filter height and influent pH was investigated. The characteristics of microbial community structure during the stable operation of the PN process were also discussed. The results showed that after adding 3 mg/L of free chlorine continuously for 23 days, adjusting the effluent filter height to 75 cm and the influent pH to 8.2~8.5, the PN process successfully started with the NO_2^--N accumulation rate staying stable at about 90% and effluent NO_2^--N/NH_4^+-N ratio between 1.23 and 1.35, thus providing suitable influent conditions for the subsequent ANAMMOX. Based on 16S rRNA high-throughput sequencing, a total of 41 phyla, 144 classes and 310 genera were detected from the stable running PN-CRI system. The detected ammonia-oxidizing microorganisms mainly included *Nitrosomonas*, *Nitrosovibrio* and *Candidatus Nitrososphaera*; while only *Nitrospira* was detected in nitrite-oxidizing bacteria genera with its relative abundance at only 6.7~10.0% of the total abundance of ammonia-oxidizing microorganisms. This indicated that the synergistic regulation strategy could selectively eliminate nitrite-oxidizing bacteria, thus creating favorable conditions for the occurrence of PN. These findings could provide a theoretical basis and scientific reference for efficient and economical nitrogen removal in a CRI system.

Keywords: synergistic regulation, CRI system; partial nitritation, microbial community structure

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Introduction

Constructed rapid infiltration (CRI) is a new ecological wastewater treatment technology developed in recent years with the advantages of less construction investment, simple operation and low operating cost [1]. It uses material such as natural river sand, zeolite sand and marble sand with good percolation performance as filter media, and the hydraulic loading is higher than the traditional soil infiltration and constructed wetland system, which made it particularly suitable for the treatment of wastewater in small towns or rural areas that are not yet covered by a municipal pipe network [2, 3]. However, the concentration of organic matter in wastewater decreased gradually with the increase of filter depth, and the C/N ratio of wastewater entering the lower part of the CRI system was low. As a result, the total nitrogen (TN) of the CRI system could not be removed effectively due to the lack of carbon sources for the denitrification process. The TN removal rate of the CRI system was only 10~30% [4], which limited the further application and promotion of the technology. Therefore, it is of great scientific and practical significance to explore an efficient and economical nitrogen removal method for a CRI system.

Recently, partial nitritation (PN) and the anaerobic ammonium oxidation (ANAMMOX) process has been considered to be the most efficient and economical biological nitrogen removal method [5]. Compared with conventional nitrification and denitrification, the PN-ANAMMOX process can reduce oxygen consumption by 62.5% without additional carbon sources. The process not only saves cost, but also avoids secondary pollution, and can effectively solve the problem of high energy consumption and insufficient carbon source in traditional biological nitrogen removal, which made it a focus of low C/N ratio wastewater treatment research [6, 7]. ANAMMOX is a biological oxidation process that uses NH_4^+-N to replace the organic carbon source as an electron donor and NO_2^--N to replace O_2 as an electron acceptor to translate them into N_2 in an anoxic or anaerobic environment [8]. The stoichiometric equation for the process is as follows:

$$NH_4^+ + 1.32NO_2^- + 0.066HCO_3^- + 0.13H^+ \rightarrow 1.02N_2 + 0.26NO_3^- + 0.066CH_2O_{0.15}N_{0.15} + 2.03H_2O \quad (1)$$

According to Equation (1), the theoretical influent NO_2^--N/NH_4^+-N ratio of ANAMMOX is 1.32:1 [9]. The efficient removal of nitrogen pollutants can be achieved when the influent meets the condition. However, the concentration of NO_2^--N in general domestic sewage is low, which cannot meet the influent requirement of ANAMMOX [10]. Thus, control of the nitrification reaction stop at the partial nitritation stage, that is, control 50~60% of NH_4^+-N oxidized to NO_2^--N, and let the concentration ratio of NO_2^--N and NH_4^+-N in effluent be close to 1.32, which is the prerequisite and necessary condition for the successful realization of efficient nitrogen removal by ANAMMOX [11].

Previous studies have investigated a series of regulation methods for the start-up of partial nitritation. Ge et al. [12] studied the effect of free chlorine on the nitritation process of a biological aerated filter. The results showed that the accumulation rate of NO_2^--N reached 60~70% when the free chlorine concentration in influent was 4 mg/L after 20 days. Song et al. [13] added 0.2 mg/L chlorine into an SBR reactor 1 hour after the start of the reaction. It was found that NO_2^--N started to accumulate when the reactor had been running for 10~17 days, and the transformation from NO_2^--N to NO_3^--N was almost completely inhibited on the 20th day. According to Li et al. [14], control of the reaction endpoint pH to 8.17~8.19 in the process of treating landfill leachate with SBR, the effluent NO_2^--N/NH_4^+-N ratio value stabilized to 1.1~1.5. Gu et al. [15] reported that partial nitritation could be achieved in the SBR system at 11~16ºC within 40 days and be stably running for up to 140 days via applying a real-time control method over pH and blower frequency. Among all these methods, the long-term addition of free chlorine would not only increase costs but also lead to secondary pollution. Additionally, most studies on pH effect have been aimed at the activated sludge system, while the studies on the start-up strategy and microbial structure of partial nitritation in the CRI system have not been reported. Unlike the activated sludge system, the substrates in the CRI system are in a non-flowing state. The influent condition changes with the increase of the filter depth, and it is difficult to achieve efficient and stable accumulation of NO_2^--N just by adding free chlorine or changing pH alone. The distribution of pollutants and dissolved oxygen in different filter depth varies, so filter depth is also an important factor affecting nitrification efficiency, which should be taken into account in the start-up of partial nitritation in a CRI system. Therefore, it is of great necessity to explore the synergistic regulation strategy for the start-up of partial nitritation in a CRI system.

In view of this, this study investigated the effect of synergistic regulation of free chlorine, filter height and influent pH on the transformation of nitrogen pollutants in a CRI system, and explored the feasibility of this regulation strategy to achieve partial nitritation in a CRI system. Furthermore, the mechanism of partial nitritation was revealed via analyzing the characteristics of microbial community structure in a stable operation period. Such information will provide technical reference for the regulation of partial nitritation in a CRI system and create suitable influent conditions for the subsequent ANAMMOX.

Materials and Methods

Experimental Equipment

As shown in Fig. 1, the CRI reactor was made of polyvinyl chloride with an internal diameter of 7 cm and a filter height of 120 cm. Water sampling ports were set at 60, 75, 90, 105 and 120 cm of filter height. On the other side, sand sampling ports were set at each 25 cm filter layer and numbered from top to bottom by PN1 to PN5. Filter material used natural river sand (particle size 0.1~0.4 mm), shell sand (particle size 0.5~1.2 mm) and zeolite sand (particle size 0.8~1.5 mm) that mixed well with a mass ratio of 7:2:1. The top and bottom of the filter were each laid with a layer of gravel (particle size 5~18 mm), respectively acting as a buffer and a supporting role. Annular pipe was adopted to distribute water evenly, influent was adjusted by peristaltic pump and rotor flowmeter, and the temperature during the experiment was maintained at 25±1ºC.

Influent and Seeding Sludge

To reduce the disturbance of water quality fluctuation, $C_6H_{12}O_6$, NH_4Cl, $NaNO_2$, KNO_3 and KH_2PO_4 were added to tap water to simulate domestic sewage for the experiment. The theoretical concentration of influent COD, NH_4^+-N, NO_x^--N and TP were 120~160, 45~50, 0.05~0.37 and 2.1~4.5 mg/L, respectively. Add 0.1 mol/L HCl and NaOH to adjust the influent pH value, and add an appropriate amount of $NaHCO_3$ to supplement alkalinity. Meanwhile, 0.1 mL of nutrient solution was added to every 10 L of influent, and its components were as follows: KCl 2100 mg/L, NaCl 1500 mg/L, $CaCl_2{\cdot}2H_2O$ 2800 mg/L, $MgSO_4{\cdot}7H_2O$ 2000 mg/L, $FeSO_4{\cdot}7H_2O$ 150 mg/L, $ZnSO_4{\cdot}5H_2O$ 50 mg/L, $MnCl_4{\cdot}4H_2O$ 50 mg/L, and $CuSO_4{\cdot}5H_2O$ 30 mg/L. The seeding sludge was collected from the returned sludge (MLSS = 7600 mg/L) of a sewage treatment plant in Chengdu, China. The CRI system was started by gradually lifting the hydraulic loading, and it ran for 2 cycles per day, 12 hours per cycle. The ratio of flooding and draining time was 1:2. The biofilm culturing completed when the hydraulic loading reached 1.0 m/d and the removal rates of COD and NH_4^+-N were stable at about 80%.

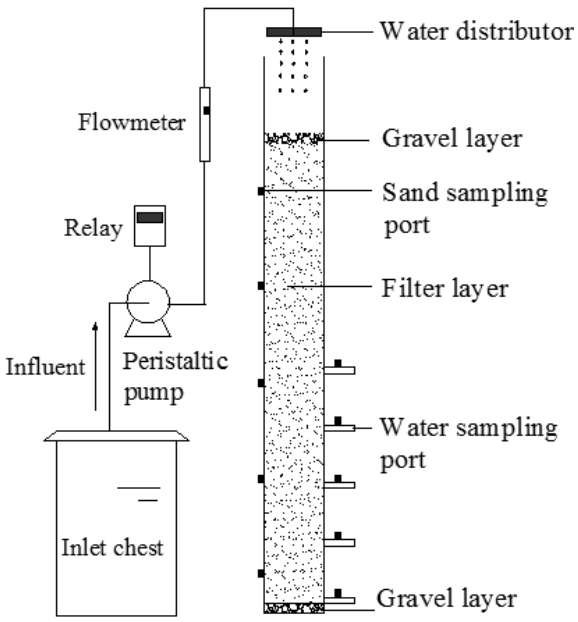

Fig. 1. Schematic of experimental equipment.

Experiments Performed

Stage I (free chlorine regulation): During the second cycle of daily experiment, influent was changed to tap water to which NaClO was added to make the concentration of free chlorine in water 3 mg/L. Water samples were taken from the sampling port at 105 cm after the end of daily operation. When NH_4^+-N removal and NO_2^--N accumulation became stable, the chlorine supply was stopped and the original influent state was restored.

Stage II (filter height regulation): The effluent quality at the filter height of 60, 75, 90, 105 and 120 cm was respectively investigated during the stable operation of the CRI system, and the optimum effluent filter height for partial nitritation was explored.

Stage III (influent pH regulation): Adjust the influent pH value to 7.3, 7.6, 7.9, 8.2, 8.5 and 8.8 respectively, and analyze the impact of influent pH on nitrogen pollutant transformation. When NH_4^+-N removal rate and NO_2^--N accumulation rate were stable at around 60% and 90% respectively, and the effluent NO_2^--N/NH_4^+-N ratio was close to 1.32, it marked the successful realization of partial nitritation in the CRI system. On this basis, sand samples were taken from different filter heights for analysis of microbial community structure, which provided the basis for analyzing the mechanism of partial nitritation in the CRI system. It should be noted that different CRI systems may have different treatment performances even under the same start-up conditions. In order to avoid the experimental errors caused by the differences among different CRI systems, each regulation experiment in this study was carried out in one CRI system. The data obtained from the regulation experiments were compared with those from the initial experiment lest the errors resulting from setting separate parallel groups occur.

Analytical Methods

Analysis of Water Quality

The concentrations of NH_4^+-N, NO_2^--N, NO_3^--N and COD in water samples were measured by standard methods (APHA, 1998). The values of pH and DO were detected by a pH meter (PHS-3C$^+$, INESA, China) and

a DO meter (DO200, YSI, USA), respectively. The content of free chlorine was determined by the iodometry method. The calculation methods of NH_4^+-N removal rate (ARR), NO_2^--N accumulation rate (NAR) and effluent NO_2^--N/NH_4^+-N ratio (NNR) were as follows:

$$ARR(\%) = \frac{inf.NH_4^+ - N - eff.NH_4^+ - N}{inf.NH_4^+ - N} \times 100\% \quad (2)$$

$$NAR(\%) = \frac{eff.NO_2^- - N}{eff.NO_2^- - N + eff.NO_3^- - N} \times 100\% \quad (3)$$

$$NNR = \frac{eff.NO_2^- - N}{eff.NH_4^+ - N} \quad (4)$$

…where inf.NH_4^+-N, eff.NH_4^+-N, eff.NO_2^--N and eff. NO_3^--N respectively represents the concentration of influent NH_4^+-N, effluent NH_4^+-N, NO_2^--N and NO_3^--N, mg/L.

Analysis of Microbial Community

DNA extraction: after the partial nitritation of the CRI system was stable, appropriate filter materials were taken out from the sand sampling ports PN1, PN2 and PN3. In the conical bottle with particle size of 2~3 mm glass beads on the bottom, 100 mL phosphate buffer solution was added, and the vortex oscillated for 60 min to fully peel the biofilm on the filter surface. Then the mixture was removed and centrifuged at 10000 rpm for 10 min at 4ºC, the supernatant was discarded, the precipitate was collected, and DNA was extracted using a DNA extraction kit (MO BIO Laboratories, USA).

16S rRNA high-throughput sequencing: the extracted DNA samples were sent to Sangon Biotech (Shanghai, China) Co., Ltd. for Miseq high-throughput sequencing. The corresponding primers were 515F(5'-GTGCCAGCMGCCGCGGTAA-3') and 909R(5'-CCCCGYCAATTCMTTTRAGT-3') targeting the V4 region of bacterial 16S rRNA. The PCR reaction conditions were as follows: pre-denaturation at 94ºC for 3 min, 30 cycles of pre-denaturating for 40 s at 94ºC, annealing for 60 s at 56ºC and extension for 60 s at 72ºC, and finally extension for 10 min at 72ºC.

Results and Discussion

Effects of Free Chlorine on Nitrogen Transformation

The transformation of nitrogen pollutants in the CRI system before and after chlorine addition is shown in Fig. 2. Before chlorine addition (phase I), ARRs were stable at around 80%, with a higher concentration of NO_3^--N in the effluent and less than 2% of NO_2^--N accumulation rate, indicating that the transformation

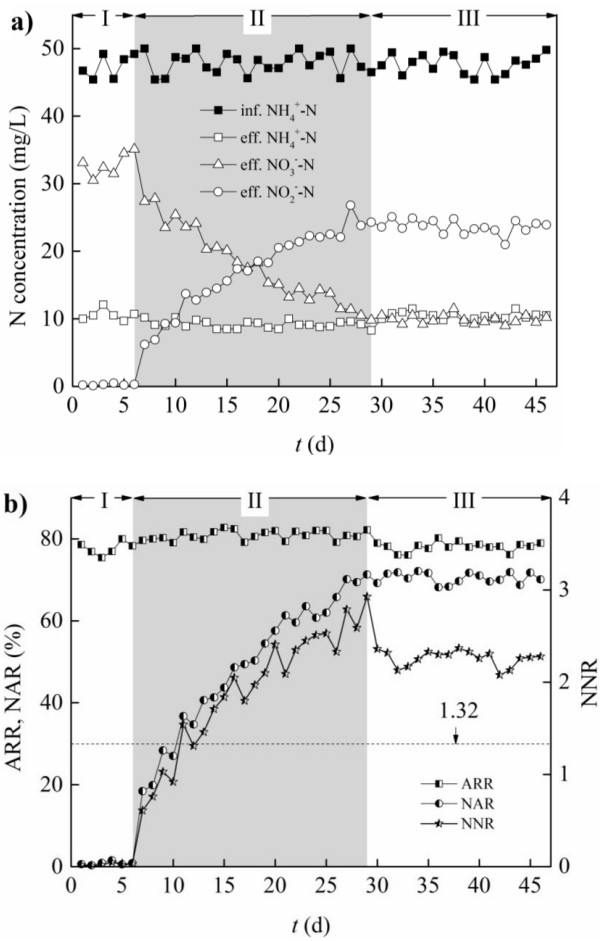

Fig. 2. Nitrogen transformation before and after chlorine addition: a) variations of NH_4^+-N, NO_3^--N and NO_3^--N concentrations; b) variations of ARR, NAR and NNR.

of nitrogen pollutants in the CRI system during the stable operation period was dominated by complete nitrification, that is, most of NH_4^+-N was finally converted into NO_3^--N.

After 3 mg/L free chlorine was added (phase II), the removal rate of NH_4^+-N was slightly increased compared with phase I. As the days of chlorine addition increased, the effluent concentration of NO_3^--N gradually decreased and the concentration of NO_2^--N gradually increased. After 23 days of continuous chlorine addition, the NAR reached 71.3%, and began to change from complete nitrification to partial nitrification. After the stable operation of phase II, it entered phase III (no free chlorine supply), and the NAR remained above 70% by the 17th day, and the NNR was greater than 2.0. It can be seen that free chlorine can effectively inhibit the transformation from NO_2^--N to NO_3^--N, and such an inhibitory effect was difficult to recover from in the short term. According to previous studies, some chemicals – such as chlorate [16], hydroxylamine [17] and methanoic acid [18] – may have different inhibitory effects on the activities of ammonia-oxidizing bacteria (AOB) and nitrite-oxidizing bacteria (NOB) involved in nitrification.

By controlling the appropriate concentration of inhibitor, NOB could be selectively eliminated while having little impact on AOB. Therefore, the further transformation of NO_2^--N to NO_3^--N could be inhibited without affecting the oxidation of NH_4^+-N to NO_2^--N, and the efficient accumulation of NO_2^--N could be achieved, which is similar to the results obtained in this study.

Effect of Filter Height on Nitrogen Transformation

The hydraulic residence time and DO distribution of sewage in the CRI system were different at different filter heights, which would affect the conversion efficiency of pollutants. Fig. 3 shows the effect of different filter heights on the transformation of nitrogen in the CRI system. As can be seen, NH_4^+-N concentration in the range of 60~105 cm decreased with the increase of filter height, and the ARRs gradually increased. When the filter height was 120 cm, the effluent NH_4^+-N concentration increased instead, and the average removal rate decreased to 74.7%. The concentration of NO_3^--N did not change much when the filter height was 60~90 cm, then increased slightly when the filter height was 105 cm, and began to decrease when it was over 105 cm. The concentration of NO_2^--N increased first and then tended to be stable within the range of 60~105 cm. When the filter height was 120 cm, the concentration of NO_2^--N and the corresponding NARs decreased significantly.

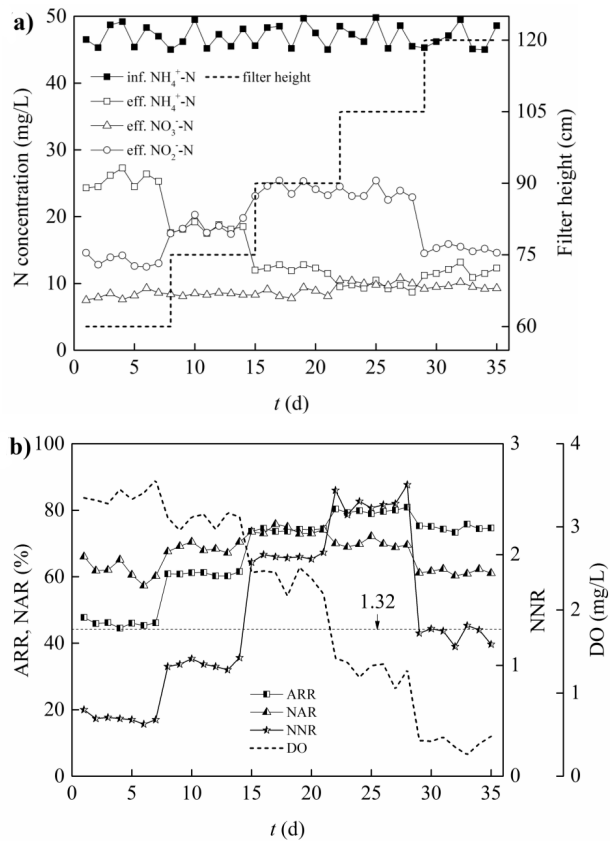

Fig. 3. Nitrogen transformation under different filter depths: a) variations of NH_4^+-N, NO_3^--N and NO_3^--N concentrations; b) variations of ARR, NAR and NNR.

The analysis showed that the CRI system was reoxygenated by alternately running between flooding and draining. When the filter height was 120 cm, less oxygen could enter the bottom filter section, and the reoxygenation effect was ineffective, the DO concentration was only 0.26~0.48 mg/L. As a result, an anoxic or anaerobic environment could be formed within the pores or biofilms of the filter material, and the process of dissimilatory nitrate reduction to ammonium (DNRA) could easily occur, that is, microorganisms used NO_2^--N or NO_3^--N as an electron acceptor to produce NH_4^+-N under the action of nitrite reductase (NiR) or nitrate reductase (NaR) [19]. It has been reported that the DNRA process widely existed in the paddy soil [19], intertidal sediments [20], wetland [21] and other habitats. Schmidt et al. [22] found that filling sandy soil was beneficial to the occurrence of DNRA. Zheng et al. [23] reported that increased sand depth in the biological filter can create favorable conditions for DNRA. Similarly, the CRI system in this study adopted a variety of sand mixture filling as the filter material, and the increase of the filter height led to a significant reduction in the amount of DO, so the possibility of DNRA action was higher, which led to the reduction of NO_2^--N and NO_3^--N concentration in effluent and the increase of NH_4^+-N concentration.

On the whole, when the filter height was 60 cm and the effluent NNR was only 0.47~0.60, it cannot meet the requirement of ANAMMOX for influent. When the filter height was 90~105 cm, the effluent NNR was 1.93~2.63. As the influent of ANAMMOX, it was not only easy to cause the high concentration of NO_2^--N in effluent, but also possible to inhibit the ANAMMOX activity [24]. When the filter depth was 120 cm, the effluent NNR was 1.17~1.36, which was close to 1.32, but the average NAR was only 61.4% as the concentration of NO_2^--N decreased significantly. In comparison, when the filter height was 75 cm, the average ARR, NAR and NNR were 60.9%, 68.7% and 0.96~1.07, respectively. Only by further selectively inhibiting the activity of NOB to further improving the NO_2^--N accumulation rate can influent requirements of ANAMMOX be satisfied. Therefore, 75 cm was selected as the suitable effluent filter height for the subsequent experiment.

Effect of Influent pH on Nitrogen Transformation

AOB and NOB are very sensitive to the acidity and alkalinity of the environment, and the influent pH value is a key factor affecting nitrification performance. Fig. 4 reflects the transformation of nitrogen pollutants under different influent pH conditions. It can be seen that the NAR increased slowly with the increase of influent pH value. The NAR jumped to 91.4% when the influent pH rose to 8.2. It indicated that under this condition,

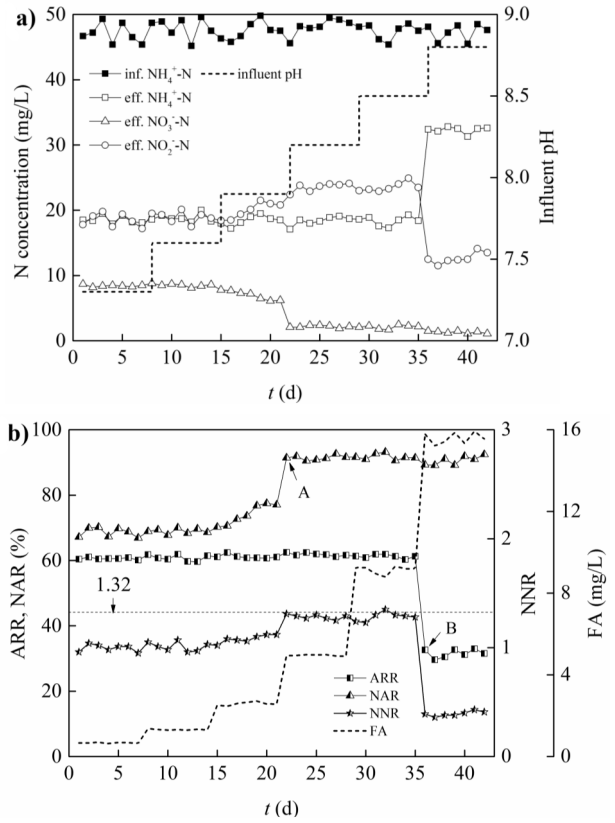

Fig. 4. Nitrogen transformation under different influent pH values: a) variations of NH_4^+-N, NO_3^--N and NO_3^--N concentrations; b) variations of ARR, NAR and NNR.

NOB activity was seriously inhibited, and NO_2^--N cannot be successfully converted into NO_3^--N, resulting in increased NO_2^--N concentration in the effluent and significant reduction of NO_3^--N concentration. The ARRs remained at around 60% when the influent pH was 7.3~8.5, while the effluent NH_4^+-N concentration significantly increased when the influent pH value increased to 8.8, and the ARR reached a drop point (B), which dropped to 32.6%. This indicated that under this condition, AOB activity started to be inhibited, and the conversion process of NH_4^+-N to NO_2^--N was both blocked, leading to a significant reduction in NH_4^+-N removal rate. To sum up, on the basis of chlorine addition and filter height regulation, the ARRs, NARs and NNRs were respectively stable at around 60%, 90% and 1.23~1.35 when the influent pH value was adjusted to 8.2~8.5. At this point, the partial nitritation in the CRI system was successfully achieved, and the influent requirements of ANAMMOX could be well matched.

According to the equation $NH_3+H_2O \Leftrightarrow NH_4^+ + OH^-$, the pH value would affect the concentration of free ammonia (FA) in the solution. As the real substrate used by AOB was NH_3 instead of NH_4^+, the change of pH would affect the process of nitrification and the composition of nitrifying bacteria. The calculation formula of FA is as follows [25]:

$$FA\ (NH_3/mg\cdot L^{-1}) = \frac{17}{14} \times \frac{[NH_4^+ - N] \times 10^{pH}}{e^{6334/(273+T)} + 10^{pH}} \quad (5)$$

When the FA concentration was appropriate, it could be used as the ammonia oxidation matrix of AOB, but when the FA concentration reached a certain threshold value, the activity of AOB and NOB would be inhibited to different degrees. Rongsayamanont et al. [26] determined that the initial concentration range of inhibition of FA on AOB and NOB was 60~120 and 0.6~60 mg/L, respectively. Fux et al. [27] found that when the concentration of FA exceeded 5 mg/L, it can inhibit AOB, while the concentration of FA that can completely inhibit NOB was only 0.6 mg/L. Until now, there have been differences in the research conclusions about the inhibition concentration of FA on AOB and NOB, but all reflected that the sensitivity of NOB to FA was higher than that of AOB.

In this study, the average FA concentration corresponding to each pH value was 0.67, 1.32, 2.59, 4.95, 9.14, and 15.59 mg/L, respectively. NO_2^--N accumulation appeared at a jump point (A) when FA concentration was 4.95 mg/L, indicating that NOB activity was severely inhibited at this point. NAR was still higher when FA concentration increased to 9.14 mg/L, implying that AOB had stronger adaptability when FA concentration was 4.95~9.14 mg/L. The removal rate of NH_4^+-N appeared a drop point (B) when FA concentration increased to 15.59 mg/L, indicating that when FA concentration was too high, it would also significantly inhibit AOB activity, leading to increased NH_4^+-N concentrations in effluent and decreased NO_2^--N concentrations.

Microbial Community Structure

Microbial Richness and Diversity

A total of 130687 effective sequences were obtained by 16S rRNA high-throughput sequencing, and the effective sequence ratio was higher than 90%. In order to analyze the microbial richness and diversity of the filler samples, the effective sequences were analyzed by cluster analysis under the similarity level of 97%, and the obtained numbers of OUT from PN1, PN2 and PN3 were 1714, 1940 and 1859, respectively. A certain number of sequences were randomly selected to count the number of species they represent. The rarefaction curves constructed are shown in Fig. 5. It can be seen that the rarefaction curves all tended to be flat at a similarity level of 97%, indicating that the sequencing depth was reasonable and the sequencing results could reflect the microbial community structure accurately [28].

Alpha diversity index analysis results are shown in Table 1. The Chao1 index reflected the microbial richness, and the larger the value, the higher the microbial richness. The Shannon index and Simpson index both reflected the microbial diversity. The larger

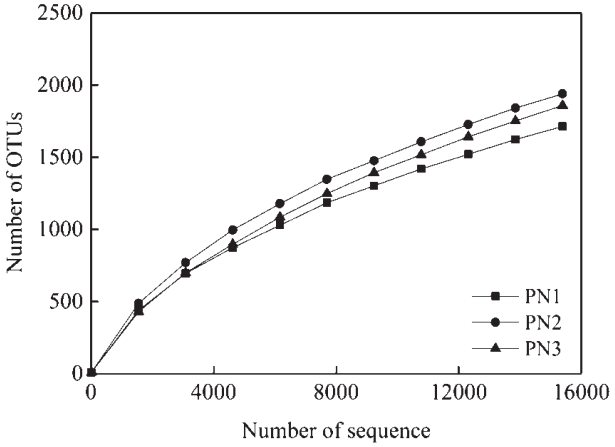

Fig. 5. Rarefaction curves of each sample.

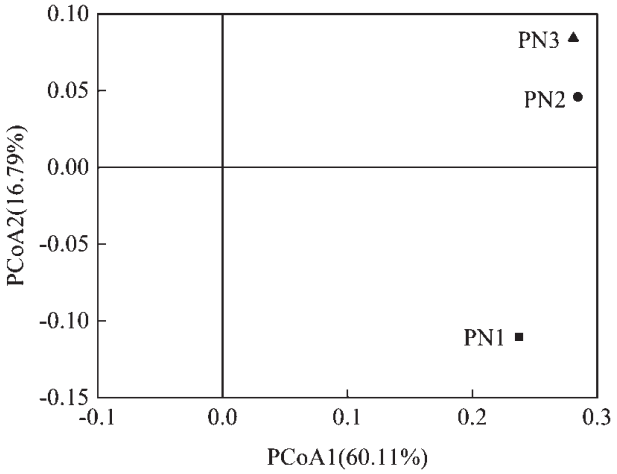

Fig. 6. PCoA graph based on weighted Unifrac distance.

the Shannon index, the higher the microbial diversity; while the larger the Simpson index, the lower the microbial diversity [29]. According to Table 1, the Shannon indexes of the 3 samples were successively PN2>PN1>PN3, while the order of the Simpson index was the opposite. This indicated that the microbial diversity in the middle layer (PN2) of filter segment was the highest, which was because the environmental conditions of the middle layer of filter segment were relatively stable, which was conducive to the growth of microorganisms in different populations, and therefore the distribution uniformity of each microbial community was also higher. The Chao1 indexes were successively PN3>PN2>PN1, indicating that the microbial richness decreased with the increase of filter depth. This was because the nutrients in the sewage were degraded step by step in the CRI system with the increase of filter depth, and thus the microbial community richness decreased accordingly.

PCoA analysis was performed on the OTU composition of 3 samples, and the result is shown in Fig. 6. The contribution rate of principal component 1 (PCoA1) and principal component 2 (PCoA2) in Fig. 6 were respectively 60.11% and 16.79%, and the distance between points indicated the difference degree of OTU composition [30]. As seen, the distance between point PN1 with point PN2 and PN3 was far, indicating that the OUT composition of the upper filter and the middle and lower filter was quite different, thus the microbial community structure was obviously different. However, the distance between point PN2 and PN3 was relatively close, indicating that the OTU composition of the middle filter and the lower filter was relatively similar, therefore the microbial community structure difference was relatively small.

Table 1. Alpha diversity indexes of each sample.

Sample ID	Shannon	Chao1	Simpson
PN1	7.30	3270.67	0.95
PN2	7.79	3374.69	0.94
PN3	7.13	3380.08	0.96

Community Structure and Distribution

Phylum Level

Fig. 7 shows the main microbial structure and distribution at phylum level in each sample. Of the detected 41 known phyla, the relative abundance higher than 1% in each sample were mainly Proteobacteria (66.89%~81.54%), Acidobacteria (4.41%~13.19%), Crenarchaeota (2.38%~6.33%), Bacteroidetes (2.39%~4.25%) and Actinobacteria (1.73%~2.68%). The relative abundance of Proteobacteria in 3 samples was successively PN3>PN1>PN2. The relative abundance of Acidobacteria and Chloroflexi in the middle of the filter was the highest, while the relative abundance of Crenarchaeota, Bacteroidetes and Actinobacteria decreased with the increase of filter depth. It can be seen that the microbial abundance of different filter heights was quite different, and the microbial community changed with the migration and transformation of pollutants in the CRI system.

Proteobacteria had the highest relative abundance in 3 samples, and it was a common dominant phylum in the sewage treatment system. The bacteria of this phylum were diversified and of various species, covering a wide range of physiological metabolic types such as aerobic, anaerobic, autotrophic and heterotrophic, which were closely related to the metabolism of C, N, and S, and provided a basis for efficient removal or transformation of pollutants in the CRI system [31]. Acidobacteria was widely distributed in soil, lake sediments and activated sludge, which was vital for the ecological construction and material circulation of the CRI system. Its distribution was affected by various factors such as pH, C/N and DO [32]. Crenarchaeota was a major branch of archaea, and usually lived in extreme environments such as low pH, high temperature and high salt.

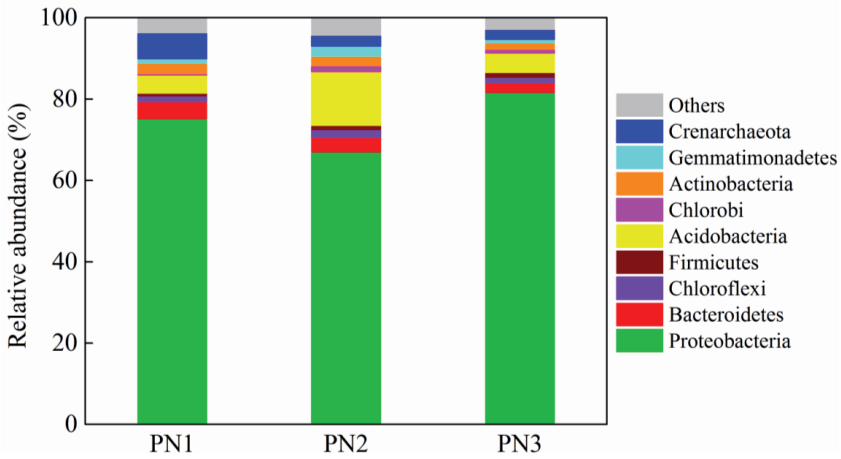

Fig. 7. Microbial community distribution at the phylum level in each sample.

With the in-depth study of environmental biotechnology in the field of microbiology, researchers have successively found a large number of Crenarchaeota in non-extreme environments, such as the soil, lake, grassland, freshwater and sewage treatment system, which played an important role in the material cycle of C and N [33, 34]. Both Bacteroidetes and Actinobacteria can degrade organic matter in sewage, which existed widely in an environment with rich organic matter content [35, 36]. As organic matter was degraded step by step in the CRI system, their relative abundance in the lower part of the filter was the lowest. In addition, the phyla such as Chloroflexi, Gemmatimonadetes, Firmicutes and Chlorobi were also detected. Although their relative abundance was not as high as the above phyla, they all played an indispensable role in the sewage treatment of the CRI system.

Class Level

A total of 144 known classes were identified and the distribution of dominant classes is shown in Fig. 8. The relative abundance higher than 1% in each filter layer were mainly β-proteobacteria (15.02~45.54%), δ-proteobacteria (18.22~40.12%), γ-proteobacteria (4.23~23.59%), α-proteobacteria (4.51~6.93%), iii1-8(1.11~8.79%), Thaumarchaeota (2.36~6.33%) and Saprospirae (1.06~2.12%).

β-proteobacteria had the highest relative abundance in Proteobacteria. Hu et al. [37] collected activated sludge from 12 sewage plants of different types for analysis and found that the relative abundance of β-proteobacteria was higher at class level. β-proteobacteria included various aerobic and facultative bacteria, such as *Nitrosomonas*, *Nitrosovibrio* and *Thauera*, which were related to nitrogen removal and provided conditions for the transformation of nitrogen pollutants in the CRI system. In addition, Thaumarchaeota had the highest relative abundance in Crenarchaeota, and its proportion decreased with the increase of filter depth. In the genome of Thaumarchaeota, there was the gene *amoA* sequence that can encode ammonia monooxygenase (AMO) [38], so it can carry out ammoxidation. As a type of ammonia-oxidizing archaea (AOA), Thaumarchaeota was an important participant in the nitrogen cycle and played an active role in the biological nitrogen removal of sewage.

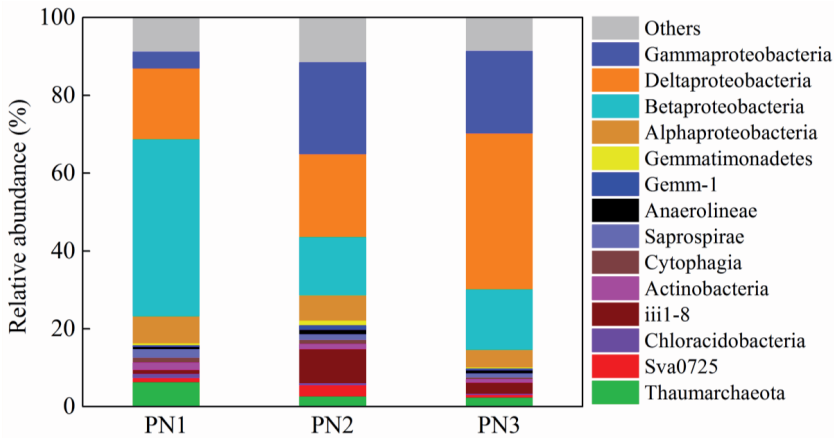

Fig. 8. Microbial community distribution at the class level in each sample.

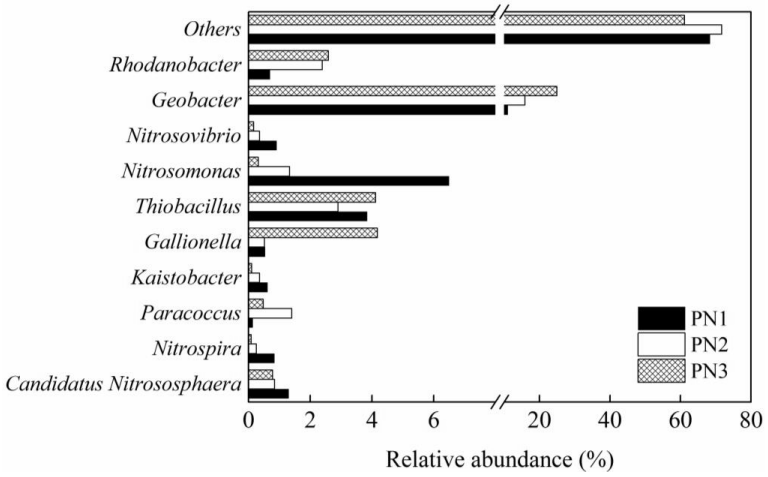

Fig. 9. Microbial community distribution at the genus level in each sample.

Genus Level

Fig. 9 shows the main microbial structure and distribution at genus level in each sample. Of the detected 310 known genera, *Nitrosomonas* and *Nitrosovibrio* belonging to β-proteobacteria, *Candidatus Nitrososphaera* belonging to Thaumarchaeota and *Nitrospira* belonging to Nitrospira were found to be associated with nitrogen transformation.

Nitrosomonas and *Nitrosovibrio* were the most common types of AOB in wastewater biological treatment while *Candidatus Nitrososphaera* was typical AOA with ammonia oxidation function, and all of them can convert NH_4^+-N in sewage to NO_2^--N. Limpiyakorn et al. [39] investigated the ammonia-oxidizing bacteria in 12 sewage treatment systems and found that the dominant bacteria in AOB were all *Nitrosomonas*. Tietz et al. [40] found that the major species of ammonia-oxidizing bacteria in vertical flow artificial wetland (VFCWs) were *Nitrosomonas* and *Nitrosospira*. Wang et al. [41] used a biological filter to treat simulated high ammonia livestock breeding wastewater, the results showed that the main AOB in the biological filter were *Nitrosomonas* and *Nitrosovibrio*. Compared with the above reports, the dominant species of ammonia-oxidizing microorganisms in this study were more abundant, which was related to the unique operation mode (flooding-draining alternative operation) of the CRI system and the filter material structure (mix sand with different particle sizes) providing a rich ecological niche for different types of microorganisms. The relative abundance of *Nitrosomonas*, *Nitrosovibrio* and *Candidantus Nitrososphaera* were respectively 0.31~6.49%, 0.17~0.91% and 0.78~0.84%, indicating that *Nitrosomonas* had an absolute advantage in ammonia-oxidizing microorganisms. As the filter depth increased, NH_4^+-N and DO concentration decreased step by step, so the relative abundance of the above ammonia-oxidizing microorganisms showed a decreasing trend as filter depth increased.

As the most common type of NOB in wastewater biological treatment, *Nitrospira* can further convert NO_2^--N into NO_3^--N, but its relative abundance was only 0.08~0.83%, which is equivalent to only 6.7~10.0% of the total abundance of *Nitrosomonas*, *Nitrosovibrio* and *Candidantus Nitrososphaera*. This indicated that through the synergistic regulation of free chlorine, filter height and influent pH, the NOB activity in the CRI system could be selectively inhibited, which was reduced significantly due to the low metabolic activity, thus achieving the enrichment of ammonia-oxidizing microorganisms. As the content of AOB and AOA in the CRI system was much higher than that of NOB, NH_4^+-N could be transformed into NO_2^--N, while NO_2^--N cannot be oxidized into NO_3^--N smoothly and accumulated efficiently, resulting in the occurrence of partial nitritation in the CRI system.

Conclusions

The partial nitritation of the CRI system could be achieved successfully through the synergistic regulation of free chlorine, filter height and influent pH. After continuously adding 3 mg/L of free chlorine for 23 days, adjusting the effluent height to 75 cm and the influent pH to 8.2~8.5, the NARs and NNRs were respectively stable at around 90% and 1.23~1.35, which well matched the influent requirement of subsequent ANAMMOX. The results of high-throughput sequencing indicated that the main species of microorganisms associated with nitrogen transformation at genus level were *Nitrosomonas*, *Nitrosovibrio*, *Candidatus Nitrososphaera* and *Nitrospira*. Among them, AOB mainly included *Nitrosomonas* and *Nitrosovibrio.* AOA mainly included *Candidatus Nitrososphaera*. NOB mainly included *Nitrospira*, and its relative abundance was only equivalent to 6.7~10.0% of AOB and AOA. As a result, NH_4^+-N could be successfully transformed into NO_2^--N, while NO_2^--N cannot be further transformed

into NO_3^--N, providing favorable conditions for the occurrence of partial nitritation in the CRI system. These results provide a novel method for the regulation of partial nitritation in the CRI system and promote the development of CRI technology in treating low C/N domestic wastewater.

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Conflict of Interest

The authors declare no conflict of interest.

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