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Free ammonia affects the nitrification performance and nitrifying community structure in the suspended activated system

JIANG Tingting[1], WANG Guangjie[2], YANG Hao[1], WU Changfeng[3], SUN Hongwei[1], CHEN Tongsheng[4]

(1. School of Environmental and Municipal Engineering, Lanzhou Jiaotong University, Lanzhou 730070, P. R. China; 2. Shandong Tongji Testing Technology Co., Ltd, Yantai 264000, Shandong, P. R. China; 3. Lanzhou Urban Water Supply (Group) Co. LTD, Lanzhou 730070, P. R. China; 4. South China Institute of Enviromental Sciencce, MEE, Guangzhou 510655, P. R. China)

Abstract: Four parallel SBRs were established to treat synthetic wastewater with preset concentrations of free ammonia (FA) (0.5, 5, 10 and 15 mg/L), including $S_{0.5}$, S_5, S_{10} and S_{15}. The four systems removed ammonia well throughout the experiment (average value of 98.7%). The inhibition of FA by nitrite-oxidizing bacteria (NOB) combined with process control was used to achieve a nitrite pathway in S_{10} and S_{15}. During the initiation of the nitrite pathway, the accumulation rate (NAR) increased dramatically to 90.3% on day 79 in S_{10} and to 90.5% on day 139 in S_{15}. For S_{10} on day 80～250 and S_{15} on day 140～250, the average NARs were steady at approximately 98.8% and 98.2%, respectively. High-throughput sequencing of the 16S rRNA gene played an ever-increasing role in analyzing the relative abundance and structure of the nitrifying bacteria in these samples. The results showed that the changes in the abundance of AOB and NOB were consistent with our experimental results. FA affected not only the relative abundance of AOB and NOB, but also the activity of NOB. Although AOB and NOB coexisted in the four systems, AOB was still the main nitrifying bacteria. We found that a lower abundance of AOB had a higher microbial utilization capacity of ammonia substrate at 15 mgFA/L.

Keywords: free ammonia (FA); partial nitrification; high-throughput sequencing; ammonia-oxidizing bacteria (AOB); nitrite-oxidizing bacteria (NOB)

游离氨对活性污泥系统中硝化性能和硝化群落结构的影响

姜婷婷[1],王光杰[2],杨浩[1],吴常峰[3],孙洪伟[1],陈桐生[4]

(1.兰州交通大学 环境与市政工程学院,兰州 730070;2.山东同济测试科技股份有限公司,山东 烟台 264000;3.兰州城市供水(集团)有限公司,兰州 730070;4.生态环境部华南环境科学研究所,广州 510655)

摘 要:建立了 4 个平行的 SBR 处理合成废水,游离氨(FA)浓度分别为 0.5、5、10、15 mg/L,命名为 $S_{0.5}$、S_5、S_{10}和 S_{15},4 个系统的脱氮性能在整个实验过程中均很好(平均值为 98.7%),利用 FA 对亚硝酸氧化细菌(NOB)的抑制作用,结合过程控制,成功在 S_{10}和 S_{15}系统中实现短程硝化。在建立短程硝化途径的过程中,S_{10}的 NAR 在第 79 天迅速达到 90.3%,S_{15}的 NAR 在 139 天迅速达到 90.5%。在 S_{10}的 80～250 d 和 S_{15}的 140～250 d 中,平均 NAR 分别稳定在 98.8%和 98.2%左右。

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Author brief:JIANG Tingting (1996-), main research interest: water pollution control, E-mail: 2278849150@qq.com.
CHEN Tongsheng (corresponding author), senior engineer, E-mail:chentongsheng@scies.org.

用 16S rRNA 基因的高通量测序技术分析样本中硝化细菌的相对丰度和结构，结果表明，AOB 和 NOB 丰度的变化与试验结果一致。FA 不仅可以显著影响 AOB 和 NOB 的相对丰度，而且还可以抑制 NOB 活性。此外，还发现较低的 AOB 含量在 FA 浓度为 15 mg/L 时具有较高的氨底物微生物利用能力。

关键词：游离氨；短程硝化；高通量测序；氨氧化细菌；亚硝酸氧化细菌

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Free ammonia (FA), a form of ammonia nitrogen (NH_4^+—N), is widely present in municipal/industrial wastewater. Commonly, Eq. (1) is used to calculate the concentration of FA[1].

$$FA = \frac{17}{14}\frac{[NH_4^+—N]\times 10^{pH}}{\exp\left(\frac{6\ 334}{273+T}\right)+10^{pH}} \quad (1)$$

There are positive relationships between the concentration of FA and NH_4^+—N, and the pH and T (temperature) based on Eq. (1).

FA has a significant effect on the nitrogen removal performance in the biological nutrient removal (BNR) pathway, because FA can affect the activity of nitrifying bacteria (including ammonia- oxidizing bacteria (AOB) and nitrite-oxidizing bacteria (NOB))[1-4]. The earliest inhibition threshold levels were reported by Anthonisen et al.[1], who demonstrated that AOB and NOB are inhibited by 10～150 mgFA/L and 0.1～1.0 mgFA/L, respectively. Considerable research has focused on the inhibition of AOB and NOB activity[5-8]. These studies mainly explored how to achieve effective partial nitrification during aerobic nitrification using the gap in the inhibitory concentration of FA between AOB and NOB. Nitrification, a two-step microbial process, is the oxidation of ammonia to nitrite by AOB and of nitrite to nitrate by NOB[9-12]. Partial nitrification is usually achieved by using the difference in activity between AOB and NOB, so ammonia is only oxidized to nitrite[13-15]. Over several decades, much research has been performed on stabilizing partial nitrification in BNR systems to remove nitrogen from high ammonia nitrogen wastewater by adjusting the concentration of FA to a specific level that inhibits NOB rather than AOB[16-18].

It is widely acknowledged that the nitrogen removal performance in BNR systems is affected by the activity of nitrifying bacteria[19-20]. From the perspective of the biological inhibition mechanism, the relative abundance and structure of nitrifying bacteria in the sewage treatment system is significantly influenced by the concentration of FA. Recently, Illumina high-throughput sequencing (HTS) technology was used to gain an in-depth understanding of characteristics of the microbial community in the water treatment system. During past years, much literature focused on the relative abundance and the structure of the nitrifying bacteria caused by FA in the BNR process[21-23]. These studies revealed that the relative abundance and structure of nitrifying bacteria are significantly affected at 2.9～50.1 mgFA/L.

Although exploration of the effects of FA on nitrifying bacteria during aerobic nitrification is increasingly successful, there is still a technical problem in that there is no consistent threshold level at which FA inhibits AOB and NOB. Thus, it is essential and urgent to investigate a consistent level at which FA inhibits AOB and NOB in biological systems. Most reported studies focused on the nitrifying bacteria in wastewater treatment bioreactors with a random FA concentration range (the concentration of FA depends on the quality of the wastewater). Few studies investigated nitrifying bacteria in the bioreactor under the precisely controlled concentration of FA. In addition, although previous studies revealed that FA has adverse effects on the microbial activity and stability of sludge, detailed information on the

mechanisms by which it accomplished this was limited to various sludge characteristics and the structure of the microbial community caused by the concentration of FA. To eliminate these drawbacks, it is indispensable to systematically assess the variety and structure of nitrifying bacteria of relative abundance under different concentrations of FA during aerobic nitrification.

During our experiment, we filled four parallel sequencing batch reactors (SBRs) with precisely controlled FA concentrations of 0.5, 5, 10 and 15 mg/L. Activated sludge (AS) was used to tame microorganisms exposed to a specific concentration of FA in the SBRs. First, the long-term nitrogen removal performance of the SBRs was investigated. A method was explored to successfully achieve rapid and stable partial nitrification in the SBR at a high concentration of FA. The variety and structure of AOB and NOB of relative abundance in the SBRs with different concentrations of FA and the related mechanisms were examined in detail using high-throughput sequencing of the 16S rRNA gene. The information obtained has implications for the biological mechanism of nitrogen removal and the mechanism by which FA inhibits nitrifying bacteria.

1 Material and methods

1.1 Batch experiments design and operation

Four 4 L parallel SBRs with 0.5, 5, 10 and 15 mg/L, concentrations of FA were operated to enrich the microbial community. The concentration of ammonia, the temperature and the pH were adjusted to obtain different concentrations of FA. Every cycle of the four SBRs consisted of 5 min filling, aerobic reaction, anoxic reaction, settling, 5 min decanting, and an idling period. The aerobic reaction, anoxic reaction, settling and idling period were flexible because of the different initial concentrations of FA.

The nitrification and denitrification were performed by adjusting the ORP, pH and DO. The pH was adjusted by the addition of 0.1 mol/L HCl and 0.4 mol/L NaOH. The temperature control system was used to control the temperature in the SBR. The DO concentrations in the four SBRs were maintained at 1.0～2.5 mg/L by an air compressor during aeration and by continuous stirring at 150 r/min by a mechanical stirrer rotating during the anoxic reaction. Table 1 summarizes the reactor operation.

Table 1 Operational conditions with variable concentrations of FA in four SBRs

FA/ (mg·L^{-1})	NH_4^+—N/ (mg·L^{-1})	The running time of each stage of SBR/min						Operational parameters			
		Cycle	Filling	Aeration	Hypoxia	Setting	Decantation	MLSS/ (mg·L^{-1})	Temperature/℃	pH	DO/ (mg·L^{-1})
0.5	40	620	5	270	300	40	5	3 900	20±2.0	7.5±0.2	1.0～2.5
5	90	710	5	300	360	40	5	4 400	25±2.0	8.0±0.2	1.0～2.5
10	130	810	5	360	420	20	5	4 500	30±2.0	8.0±0.2	1.0～2.5
15	55	570	5	240	300	20	5	4 400	35±2.0	8.5±0.2	1.0～2.5

Activated sludge with a mixed liquor suspended solid (MLSS) of 3 000 mg/L was collected at a local domestic sewage treatment plant (WWTP) in Lanzhou, Gansu to start up the batch reactors.

The four SBRs were operated for 250 days under the above mentioned concentrations of FA. After sustained long-term steady treatment by the reactors, four acclimated activated sludge samples, $S_{0.5}$, S_5, S_{10} and S_{15}, were obtained. On day 240, 12 samples were collected from the four SBRs (3 samples from each SBR). The collected samples were immediately mixed with absolute ethanol at a 1∶1 volume ratio, and placed in a refrigerator,

where they were maintained at −20 ℃ for DNA extraction.

1.2 Synthetic media

Nutrients and trace elements in the synthetic media support microbial growth in the reactors. The synthetic media contains (adapted from Kuai et al.[24]). 115 mg/L NH_4Cl, 385 mg/L CH_3COONa, 26 mg/L of K_2HPO_4 and KH_2PO_4, and 2 mL of trace elements solution. 5.07 mg $MgSO_4 \cdot 7H_2O$, 1.26 mg $Na_2MoO \cdot 2H_2O$, 2.49 mg $FeSO_4 \cdot 7H_2O$, 0.41 mg $CoCl_2 \cdot 6H_2O$, 0.44 mg $ZnSO_4 \cdot 7H_2O$, 0.31 mg $MnSO_4 \cdot 4H_2O$, 0.43 mg $CaSO_4 \cdot 2H_2O$, 0.25 mg $CuSO_4$, 1.88 mg EDTA and 0.25 mg NaCl were contained per liter in the trace elements solution.

1.3 Analytical measurements

The concentrations of NH_4^+—N, NO_2^-—N, NO_3^-—N and COD were measured every 30 minutes to determine the performance of the reactor. These parameters were monitored simultaneously in both influent and effluent throughout the experiment. Standard methods (APHA[25]) were used to measure NH_4^+—N, NO_3^-—N, NO_2^-—N, COD and MLVSS. The pH/oxi 340 analyzer (WTW, Germany) was used to measure the pH, DO and temperature.

1.4 Calculations

The concentration of FA was calculated by Eq. (1).

The ammonia oxidation rate (AOR) was calculated by Eq. (2).

$$\text{AOR (mgN/gVSS} \cdot \text{min)} = \frac{[NH_4^+—N_{inf}] - [NH_4^+—N_{eff}]}{\text{MLVSS} \times t} \quad (2)$$

The nitrite oxidation rate (NOR) was calculated by Eq. (3).

$$\text{NOR (mgN/gVSS} \cdot \text{min)} = \frac{[NO_2^- - N_{eff}] - [NO_2^- - N_{inf}]}{\text{MLVSS} \times t} \quad (3)$$

The nitrite accumulation rate (NAR) was calculated by Eq. (4).

$$\text{NAR}(\%) = \frac{NO_2^-—N_{eff}}{[NO_2^- - N_{eff}] + [NO_3^- - N_{eff}]} \times 100\% \quad (4)$$

Where $NH_4^+—N_{inf}$ and $NH_4^+—N_{eff}$ are the ammonia concentrations (mg/L) in the influent and the effluent, respectively; $NO_2^-—N_{inf}$ and $NO_2^-—N_{eff}$ are the nitrite concentrations (mg/L) in the influent and the effluent, respectively; $NO_3^-—N_{eff}$ is the nitrate concentration in the effluent (mg/L); T is the temperature (℃); t is the running time (min); and MLVSS is the mixed liquid volatile suspended solids (mg/L).

1.5 DNA extraction steps, PCR quantification and high-throughput sequencing

First, a 10 mL AS sample fixed with absolute ethanol was centrifuged at 12 000 r/min for 10 min. The obtained sediment was used for subsequent DNA extraction. We followed the manual steps to extract DNA with FastDNA™ Spin Kit for Soil (MP Biomedicals, USA). Agarose gel electrophoresis was used to assess the quality of the DNA and a NanoDrop spectrophotometer (Thermo Fisher Scientific, USA) was used to measure the concentration of DNA. The extracted DNA samples were stored in the refrigerator at −80 ℃. The V3-V4 hypervariable region of the 16S rRNA gene was targeted for PCR using the 338F (5′-ACTCCTACGGGCAGCA-3′) forward primer and the 806R (5′-GGACTACHVGGGTWTCTAAT-3′) reverse primer. The thermocycler was operated with an initial denaturation at 98 ℃ and 2 min, followed by 25 cycles of denaturation at 98 ℃ for 15 s, annealing at 55 ℃ for 30 s, extension at 72 ℃ for 30 s, and a final elongation at 72 ℃ for 5 min.

Agencourt AMPure Beads (Beckman Coulter, Indianapolis, IN) and PicoGreen dsDNA Assay Kit (Invitrogen, Carlsbad, CA, USA) were used to purify and quantify the PCR amplicons, respectively. The amplicons were combined in equal amounts and double-ended 2×300 bp sequencing was performed using the Illumina MiSeq platform and MiSeq kit V3 from the Shanghai Personal Biotechnology Co., Ltd. (Shanghai, China). QIMME software was used to

classify and quantify the effective sequences and the sequence with a similarity threshold higher than 97% was classified as the same operational taxonomic unit (OTU).

2 Result and discussion

2.1 Nitrogen removal performance in SBRs

We launched a study to explore the long-term nitrogen removal performance caused by variable concentrations of FA in SBRs. Fig. 1 shows the NH_4^+—N concentrations in the influent and effluent and the NH_4^+—N removal efficiency in the four SBRs throughout the experiment. $S_{0.5}$～S_{15} was fed in the influent with average NH_4^+—N concentrations of 40, 90, 130 and 55 mg/L, respectively. Low NH_4^+—N concentrations of 0.7, 1.1, 1.2 and 0.7 mg/L in $S_{0.5}$～S_{15}, respectively, were found in the effluent in the four SBRs throughout the experiment. A high ammonia removal efficiency of 98.1%, 98.8%, 99.0% and 98.8% in $S_{0.5}$～S_{15} (averaging 98.7%), respectively, was obtained at the variable FA concentrations. It is clear that the variable concentration of FA in the SBRs had an insignificant impact on the NH_4^+—N removal efficiency. The result confirmed the discovery of Vadivelu et al.[2] that the activity of ammonia-oxidizing bacteria (AOB) is minimally affected when the concentration of FA is lower than 16 mg/L. However, our findings directly contradicted those of Cao et al.[22], who found a downward trend in the removal efficiency of NH_4^+—N (99.5% to 38.8%) with the increased concentration of FA (2.9～19.5 mg/L).

The biological reaction rate is strongly affected by the concentration of FA. Past studies proved that AOR and NOR show a sharp downward trend when the concentration of FA is higher than 25 mg/L[26-28]. In our study, we also found that variation in the concentration of FA significantly affects the AOR and NOR during nitrification throughout the experiments. A considerable increase in AOR occurred from 0.038 mgN/gVSS

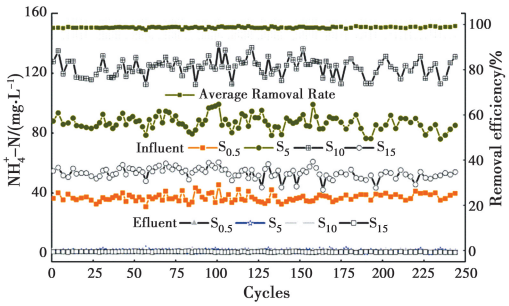


Fig. 1 The influent and effluent concentration of NH_4^+—N and NH_4^+—N removal efficiency in SBRs during the operational period

·min at 0.5 mg/L ($S_{0.5}$) to a maximum of 0.093 mgN/gVSS·min at 10 mg/L (S_{10}), and then slightly declined to 0.073 mgN/gVSS·min at 15 mgFA/L (S_{15}). However, the NOR was maintained at 0.038±0.009 in $S_{0.5}$ and S_5 and 0.012±0.004 in S_{10} and S_{15} (Fig. 2). The AOR was still maintained at a high level of 0.073-0.093 mgN/gVSS·min even though the concentration of FA was higher than 10 mg/L, indicating that stable and efficient NH_4^+—N removal was achieved.

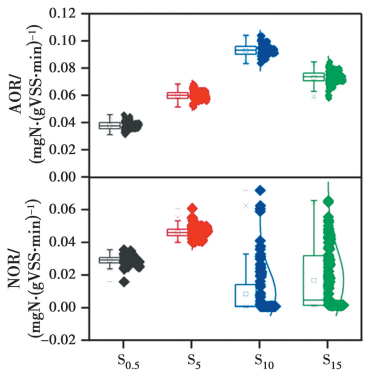


Fig. 2 Effect of concentration of FA on AOR and NAR

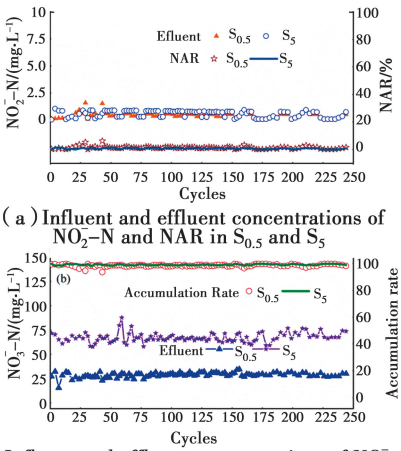
A high level of NH_4^+—N removal efficiency (above 98%) was observed in the four SBRs, demonstrating that NH_4^+—N is removed, to a great extent, during nitrification and denitrification in the SBR. Therefore, the SBR is a useful reactor to remove NH_4^+—N from high nitrogen wastewater.

2.2 Achieving a nitrate pathway in $S_{0.5}$ and S_5 and a nitrate pathway in S_{10} and S_{15} for nitrogen removal

2.2.1 Achieving a nitrate pathway in $S_{0.5}$ and S_5

at a lower concentration of FA

In $S_{0.5}$ and S_5, the SBRs were operated for 250 days by adjusting the pH, DO and temperature to switch between aerobic and anaerobic. In the nitrified effluent, the nitrite concentrations were 0.38 mg/L and 0.51 mg/L, the nitrate concentrations were 28.2 mg/L and 66.5 mg/L, and NAR and the accumulation rate of nitrate were 0.57% and 0.50%, 98.8% and 99.1% in the $S_{0.5}$ and S_5, respectively (Fig. 3). Extremely low NAR and nitrate accumulation indicated that complete nitrification was obtained in $S_{0.5}$ and S_5. Simultaneously, the results indicated that the NH_4^+—N removal efficiency was not affected by the rise of FA concentration (0.5 to 5 mg/L) and full nitrification was achieved in both $S_{0.5}$ and S_5. The results showed that nitrifying bacteria could quickly adapt to specific concentrations of FA.



(a) Influent and effluent concentrations of NO_2^-–N and NAR in $S_{0.5}$ and S_5

(b) Influent and effluent concentrations of NO_3^-–N and the accumulation rate of NO_3^-–N in $S_{0.5}$ and S_5

Fig. 3 Nitrogen removal performance in SBRs along the operational period

The concentrations of FA in $S_{0.5}$ and S_5 were 0.5 and 5 mg/L, that were both within and also much higher than the inhibition threshold of FA on NOB (begin at 0.1 ~ 1.0 mg/L) reported by Anthonisen et al.[1]. However, obvious nitrite accumulation was not found in $S_{0.5}$ and S_5 (Fig. 3 (a)), showing that NOB activity could not be inhibited significantly when the FA is lower than 5 mg/L. Our research fully confirmed the conclusion of Vadivivelu et al.[2], who also found no significant decrease of NOB activity from 0 to 4 mgFA/L, indicating that a low concentration of FA has a small inhibitory effect on NOB.

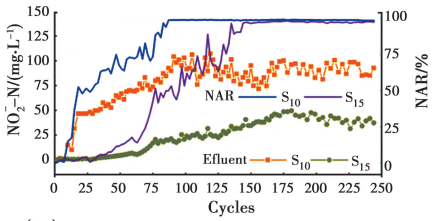
In addition, nitrite accumulation did not occur in $S_{0.5}$ and S_5 even though we strictly controlled the process in each SBR cycle within 250 days. Our finding was consistent with Cao et al.[22]. There was no obvious nitrite accumulation by applying process control after 29 cycles at 2.9 and 5.6 mgFA/L, but they also achieved high total nitrogen removal efficiency averaging over 99%. However, Vlaeminck et al.[13] obtained stable nitrite accumulation and high nitrogen removal efficiency when the concentration of FA was higher than 3 mg/L, meaning that NOB suffered from strong suppression.

2.2.2 Initiating the nitrite pathway at a high concentration of FA in S_{10} and S_{15}

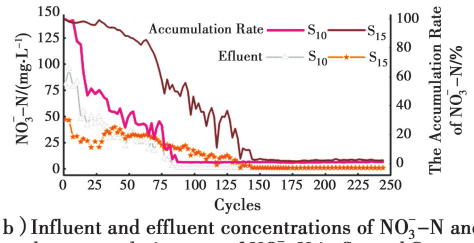
In the nitrified effluent, a remarkable decline took place in the NO_3^-—N concentration from 80.8 to 1.0 mg/L and 47.2 to 1.1 mg/L in S_{10} and S_{15}, respectively (Fig. 4). However, the NO_2^-—N concentration increased considerably, from 0.07 to 81.2 mg/L and 0.18 to 48.3 mg/L in S_{10} and S_{15}, respectively, indicating the accumulation of nitrite in S_{10} and S_{15}. The NAR dramatically went up to 90.3% on day 79 in S_{10} and 90.5% on day 139 in S_{15}, and the nitrate accumulation rate decreased to 1.1% on day 79 in S_{10} and 2.3% on day 139 in S_{15}, respectively (Fig. 4). The increase of NAR with the decline of nitrate accumulation showed that NOB activity was greatly inhibited and partial nitrification was successfully achieved. AOB gradually became the dominant bacteria, because NOB was inhibited, forming a new nitrifying bacteria structure. S_{10} and S_{15} successfully achieved partial nitrification at a high concentration of FA.

The nitrite pathway was successfully established at 10 mgFA/L and 15 mgFA/L, and S_{10} achieved the nitrite pathway faster than S_{15} (Fig. 4). NOB activity was strongly inhibited in S_{15} and S_{10}, while AOB activity was reduced more

in S_{15} than S_{10}, leading to the decline of the oxidation rate of the ammonia, and increasing the realization time of the nitrite pathway. Both the continuous ammonia oxidation process and the nitrite accumulation indicated that AOB activity wasn't greatly affected by 10 and 15 mgFA/L, which confirmed that AOB is more tolerant of FA than NOB.



(a) Influent and effluent concentrations of NO_2^--N and NAR in S_{10} and S_{15}



(b) Influent and effluent concentrations of NO_3^--N and the accumulation rate of NO_3^--N in S_{10} and S_{15}

Fig. 4 Nitrogen removal performance in SBRs along the operational period

Although several authors analyzed the inhibitory thresholds of FA on AOB and NOB, a consistent inhibitory threshold was not obtained. For example, the first inhibition threshold level of FA on AOB and NOB reported by Anthonisen et al.[1] was 10～15 mg/L and 0.1～1.0 mg/L, respectively. Kim et al.[4] suggested that only NOB was inhibited, while AOB could still oxidize ammonium to nitrite at 14～17 mgFA/L. Van Hulle et al.[27] reported that AOB activity was not inhibited in SHARON reactors at 70-300 mgFA/L.

Vadivelu et al.[2] found that the inhibitory effect of FA on NOB started from 1 mg/L and stopped growing when it was higher than 6 mg/L. In comparison, Vadivelu et al.[3] revealed that the inhibitory effect of FA on AOB started at 16 mg/L. Our research and previous studies showed that FA concentrations at S_{10} and S_{15} can strongly inhibit NOB activity, but have little effect on AOB activity.

2.2.3 Maintaining the nitrite pathway at a high concentration of FA in S_{10} and S_{15}

During the period the nitrite pathway was maintained (S_{10} on day 80～250 and S_{15} on day 140～250), the main product in the nitrification was nitrite (81.2 and 48.3 mg/L), the nitrate concentration was still maintained at the bottom level (1.0 and 1.1 mg/L) and the NARs remained stable at 98.8% for 170 days and 98.2% for 110 days in S_{10} and S_{15}, respectively (Fig. 4). Therefore, we can conclude that 10 mgFA/L and 15 mgFA/L can continue to inhibit NOB activity, and the nitrite conversion process was strongly inhibited.

In this study, the selective inhibition at high concentration of FA combined with process control is a basic method to achieve and stabilize the nitrite pathway in the treatment of synthetic wastewater in the SBR.

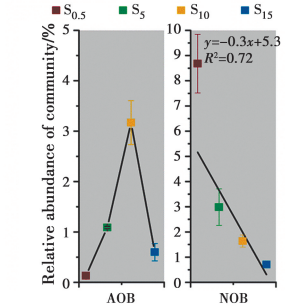
2.3 Confirmation of the dominant nitrifying bacterial population

Nitrifying bacteria plays a crucial role in nitrogen removal in the SBR. Therefore, we analyzed the relative abundance and structure of the nitrifying bacteria to better understand the microbial role in nitrification using high-throughput sequencing. Considerable studies have reported that five kinds of AOB (*Nitrosomonas*, *Nitrosococcus*, *Nitrosospira*, *Nitrosolobus* and *Nitrosovibrio*) and four kinds of NOB (*Nitrococcus*, *Nitrospira*, *Nitrobacter* and *Nitrospira*) were widely found in the sewage treatment system[29-32]. In this work, we found two types of nitrifying bacteria (*Nitrosomonas* (AOB) and *Nitrospira* (NOB)), which were regarded as the dominant nitrifying bacteria in WWTPs, as reported. A widespread and reliable consensus that NOB is even more sensitive to FA inhibition than AOB was reached and applied in numerous studies[6, 13, 23].

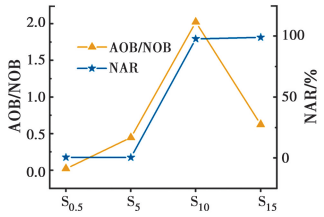
As depicted in Fig. 5 (a), the relative abundance of AOB and NOB suffered from a significant change by the variation in the

concentration of FA, influencing the nitrogen removal. Surprisingly, we found that AOB activity was not inhibited, but enhanced at S_{10} with the increasing concentration of FA. AOB first increased sharply from 0.13% in $S_{0.5}$ to 3.17% in S_{10} and then decreased to 0.60 in S_{15}, but NOB linearly decreased from 6.14% in $S_{0.5}$ to 0.96% in S_{15} and had a significant negative relationship with concentrations of FA ($y=-0.3x+5.3$, $R^2=0.72$). The reason for the entirely different trend could be that FA can serve as a matrix for AOB to increase its relative abundance when the concentration of FA is lower than 10 mg/L, but FA reached the threshold for inhibiting AOB and made its abundance decrease when the concentration of FA was higher than 10 mg/L. However, the inhibition threshold of NOB was 0.5 mgFA/L. Thus, there was a gradual decline in the abundance of NOB with the increasing concentration of FA. The results were in agreement with AOR and NAR under the four FA treatments and confirmed that NOB was even more sensitive to FA inhibition than AOB.

Furthermore, it was clear that that NOB *Nitrospira* was the dominant nitrifier (AOB/NOB <1) at FA below 5 mg/L, and led to complete oxidation of ammonia to nitrate. But AOB *Nitrosomonas* was the predominant nitrifier (AOB/NOB=2.03) at 10 mg FA/L, and resulted in sufficient oxidation of ammonia to nitrite. A stable nitrite pathway was maintained at 15 mg FA/L with higher AOR (0.073 mgN/gVSS·min) than NOR (0.017 mgN/gVSS·min) although the relative abundance of NOB *Nitrospira* (0.95%) was higher than AOB *Nitrosomonas* (0.6%) (AOB/NOB=0.63) (Fig. 5(a) and (b)). In other words, a higher abundance of NOB *Nitrospira* had lower activity of utilizing the nitrite substrate, indicating that the NOB activity was strongly inhibited in S_{15}. However, AOB *Nitrosomonas* exhibited the opposite trend. For example, the lower abundance had a higher microbial utilization capacity of the ammonia substrate. Thus, it was still able to make the AOR higher than the NOR when the AOB abundance was greater than that of the NOB and remain stable during the ammoxidation process. Similar inhibitory threshold levels of FA on NOB were reported by Sun et al.[32], who obtained a nitrite pathway of over 90% at 16.3 mgFA/L in the UASB-SBR system treating landfill leachate and intense oppression occurred of FA on NOB activity.



(a) Change of average relative abundances of AOB and NOB in SBRs



(b) Relationship between AOB/NOB and NAR

Fig. 5 Distribution and relative abundance of AOB and NOB, AOB/NOB and NAR in SBRs

Based on the results, we can draw the conclusion that FA can affect not only the relative abundance of AOB and NOB, but also the NOB activity. However, AOR decreases with AOB abundance. Thus, we cannot conclude that AOB activity is inhibited at 15 mgFA/L. Moreover, by adjusting the concentration of FA, promoting AOB but suppressing NOB, stable short-range nitrification was successfully achieved.

3 Conclusions

The SBR is an efficient and stable reactor to remove ammonia from synthetic wastewater. Stable partial nitrification was successfully achieved in the SBR at high concentrations of FA (10 and 15 mgFA/L). Although AOB and NOB coexist in the

four systems, AOB is still the main nitrifying bacteria. This finding emphasizes the importance of cultivating the appropriate bacteria to achieve short-range nitrification. FA can affect not only the relative abundance of AOB and NOB but also the activity of NOB. Furthermore, we found that a lower abundance of AOB had a higher microbial utilization capacity of ammonia substrate at 15 mgFA/L.

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