

Effects of HRT on biological nitrogen removal in single-stage autotrophic process

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Abstract: An external circulation Sequencing Batch Reactor (ecSBR) was used to study the efficiency of nitrogen removal by autotrophic microbe. With gradually reducing the dissolved oxygen (DO) concentration from 1.2 mg/L to 0.04 mg/L, the single-stage autotrophic biological nitrogen removal (sABNR) process could be operated stably. After removing the aeration, the process could still stay sABNR stably, and the concentration of $\text{NH}_4^+\text{-N}$ was 0.9 mg/L in effluent, the rate of nitrate (produced)/ $\text{NH}_4^+\text{-N}$ (removed) was in the range of 0.12–0.40. The results showed that the concentration of $\text{NH}_4^+\text{-N}$ in effluent was 0.8, 0.8 and 9.9 mg/L with the hydraulic retention time (HRT) at 8 h, 6 h and 4 h respectively, the removal efficiency of ammonia were 98.2%, 98.1% and 73.6% respectively. The rate of nitrate (produced)/ $\text{NH}_4^+\text{-N}$ (consumed) was 0.05 at HRT 6 h, and the nitrogen loading rate (NLR) and nitrogen removal rate (NRR) were 169.7 and 129.7 $\text{g/m}^3\text{/d}$, the removal efficiency of total nitrogen (TN) was 77.5%. In conclusion the optimal HRT was 6 h instead of 8 h or 4 h enough for ammonia removal without causing energy wastage.

Keywords: hydraulic retention time, autotrophic biological nitrogen removal, deammonification, wastewater treatment

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1. Introduction

Deammonification process is considered as a sustainable wastewater treatment. Compared with conventional nitrification–denitrification process, deammonification process could save 62.5% oxygen and 50% alkali without organic carbon consumption^[1,2]. The deammonification process mainly consists of single-stage and two-stage autotrophic biological nitrogen removal processes: the single-stage process include completely autotrophic nitrogen removal over nitrite (CANON) and oxygen limited autotrophic nitrification and denitrification (OLAND); the typical two-stage process is a single reactor for high ammonium removal over nitrite-anaerobic ammonium oxidation (Sharon-Anammox)^[3,4]. The CANON process is the combination of partial nitrification and

Anammox process within a single reactor^[5]. Compared with two-stage process, single-stage has a higher nitrogen removal rate (NRR) with small occupation area and lower operating cost^[6–8]. Currently the study of sABNR mainly focus on high concentration ammonia removal such as sludge digestion liquid and landfill leachate^[9–11], while it is rarely applied in municipal wastewater with low ammonia removal. With the rapid increase of municipal wastewater in China, sABNR is a promising process; this method not only improves the efficiency of nitrogen removal but also lowers the cost of wastewater treatment.

Studies of CANON have shown that DO would affect the performance of nitrogen removal^[12]. Since the Anammox is an anoxic bacterium, the inhibition of low DO to Anammox is reversible, however, the high DO is irreversible^[13,14]. At the same time, low DO

could inhibit the activity of nitrite oxidizing bacteria (NOB)^[15]. Previous research showed that 4.0–4.5 mg/L of DO in influent would be sufficient to oxidize 29–200 mg/L ammonia without extra aeration, and the oxygen consumption was 0.06 O/mg N/day^[16]. The municipal wastewater has low nitrogen concentration, the application of the sABNR process without aeration has practical significance due to its low cost.

This study was to investigate the effect of HRT on sABNR. In order to achieve sABNR without aeration, the dissolved oxygen was gradually reduced. The efficiency of nitrogen removal and nitrogen transforming were discussed. Furthermore, the effect of HRT on the efficiency and stoichiometric ratio of nitrogen removal was investigated.

2. Materials and Methods

2.1 ecSBR Reactor Setup

An ecSBR reactor was used in this study (Figure 1). External circulation was added to enhance the circulation of the mixture and improve the efficiency of mass transfer. The reactor was first run under aeration with DO at 1.2 mg/L. In order to reduce the concentration of effluent nitrite, the aeration was decreased gradually from the Day 47, and completely removed at the Day 55. As a result, the concentration of DO began to decrease from 1.2 mg/L to 0.04 mg/L. Nitrosation was only relied on the supplement of DO in influent (1.5–2.0 mg/L) from external atmosphere circulation. The reactor consisted of plexiglass with a total effective volume of 500 mL, 80 cm height and the ratio of

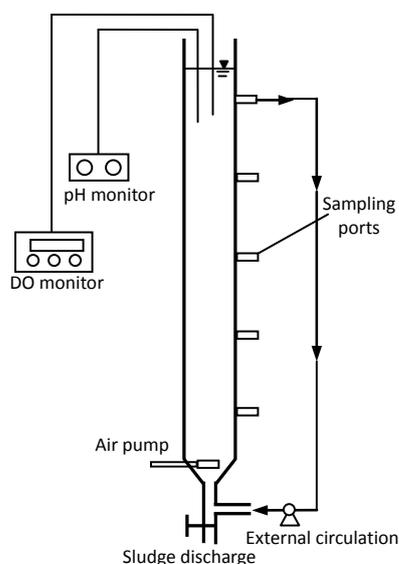


Figure 1. Diagram of the experimental process.

height and diameter of 20:1. There was an electric insulation wire wrapped outside, which maintained the temperature at $30.0 \pm 0.5^\circ\text{C}$.

2.2 Experimental Wastewater

Raw sewage was collected from the campus community at Northeast Forestry University, and then goes through an anaerobic reactor for COD removal. The effluent of the anaerobic reactor was used as the experimental wastewater: COD < 30 mg/L, $\text{NH}_4^+\text{-N}$ = 10–55 mg/L, $\text{NO}_2^-\text{-N}$ < 0.2 mg/L, $\text{NO}_3^-\text{-N}$ < 1 mg/L, pH = 7.0–8.5.

2.3 Analytical Methods

COD, $\text{NH}_4^+\text{-N}$, $\text{NO}_2^-\text{-N}$, $\text{NO}_3^-\text{-N}$, MLSS, MLVSS were measured according to the standard methods^[17]. DO and pH were measured by WTW (pH/Oxi 340i, Germany).

3. Results and Discussion

3.1 The Performance of sABNR Process

Process Start-up

The aeration was gradually reduced to achieve an anoxic condition. A well nitrogen removal performance was achieved after 31 days. From day 44 the concentration of DO was 1.2 mg/L, nitrate levels in the effluent suddenly increased, and reached 18.6 mg/L at day 46 (Figure 2). This was because the process had a relatively high concentration of DO, and the growth of aerobic bacteria far exceeded Anammox^[18]. The rate of aerobic ammonia-oxidizing bacteria (AOB) exceeded that of Anammox and finally the accumulation of nitrite took place. In order to control the concentration of nitrite in the effluent, aeration was gradually reduced from the Day 47; the aeration device was completely dismantled at the Day 55, then DO

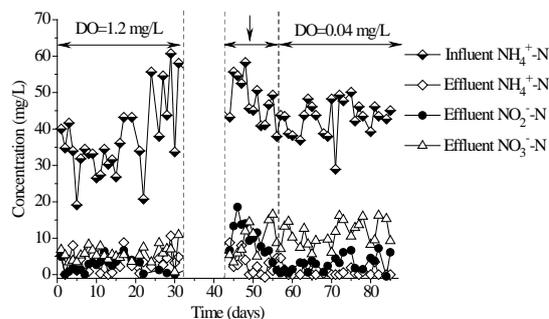


Figure 2. $\text{NH}_4^+\text{-N}$, $\text{NO}_2^-\text{-N}$ and $\text{NO}_3^-\text{-N}$ concentrations under aerobic and micro-aerobic conditions.

gradually decreased from 1.2 mg/L to 0.04 mg/L. Later the process operated in no aeration, and nitrosation was only relied on atmospheric reoxygenation by the external circulation (DO in influent was 1.5~2.0 mg/L). It was observed that the rate of aerobic ammonia oxidation decreased corresponding the increase rate of Anammox while the decrease of DO (Figure 2). At the Day 55, partial nitrification and anaerobic ammonia oxidation reaction rebalanced in the process, the concentration of ammonia and nitrite in effluent was 0.9 mg/L and 2.9 mg/L. Compared with the added aeration, the reactor without aeration achieved anoxic sABNR. The effluent of the reactor achieved better water quality with lower concentration of nitrogen below 1 mg/L and the low energy cost was achieved. If this anoxic sABNR could be applied to the current municipal plant, it would bring huge economic benefits.

The Conversion of Nitrogen under Aerobic and Anoxic Conditions

The proportion of effluent $\text{NH}_4^+\text{-N}$ to influent under aerobic and anoxic conditions was 10.0% and 2.2% respectively, which indicated a higher efficiency of ammonia removal under anoxic conditions (Figure 3). The amount of effluent of $\text{NO}_2^-\text{-N}$ without being used was 7.0% and 8.0% of influent. The amount of nitrate ($\text{NO}_3^-\text{-N}_{\text{ANAMMOX}}$) produced by Anammox indicated the activity of Anammox was slightly different during the two conditions. Accordingly the ratio of $\text{N}_2/\text{NH}_4^+\text{-N}(\text{influent})$ was 65% and 62%. Due to the low COD concentration in influent, the denitrification was not noticeable in the process and this part of N_2 should be produced by Anammox^[2]. In the anoxic conditions, the concentration of $\text{NO}_3^-\text{-N}$ produced by the NOB was higher, which might relate to the previous accumulation

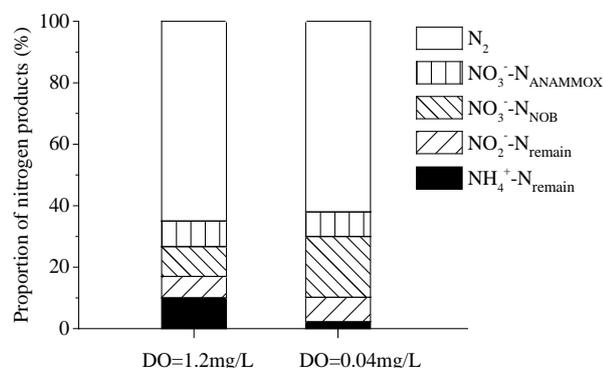


Figure 3. The conversion of nitrogen under aerobic and anoxic conditions.

of nitrite that promoted NOB growth^[15]. The conversion of nitrogen under aerobic or anoxic conditions was marginally different; overall anoxic conditions had better ammonia removal efficiency.

3.2 Effect of HRT on Efficiency of sABNR Process

Effect of HRT on Nitrogen Removal

Ammonia concentration in the influent was 17.4–52.1 mg/L. The ammonia concentration in effluent was 0.8 mg/L in both HRT 8 h and 6 h, thus the ammonia removal efficiency was about 98% (Figure 4). When HRT was dropped down to 4 h, the ammonia concentration in effluent was 9.9 mg/L, and the removal efficiency was 73.6%. Thus the results showed that the ammonia removal efficiency was better at HRT 8 h and 6 h, while ammonia was not able to be consumed by AOB or Anammox at HRT 4 h^[19]. When the HRT was down to 4 h, the concentrations of nitrite and nitrate in effluent were 1.6 and 0.9 mg/L. When HRT was 6 h or 8 h, the concentrations of nitrite and nitrate in effluent were 6.0 mg/L, 2.5 mg/L and 2.9 mg/L, 11.2 mg/L respectively. With increasing HRT, the concentration of nitrate in effluent was becoming higher, and the nitrite was lower. When HRT was 6 h, the ammonia in the reactor had been consumed completely, and the continuously operation of the reactor would make excess nitrite convert to nitrate due to the atmospheric reoxygenation from the influent.

More ammonia remained under HRT 4 h, while wasted energy was at HRT 8 h due to a long running time, resulting in increased activity of NOB. HRT 6 h was the optimal process suitable for the conversion of ammonia and proper inhabitation of NOB. The only

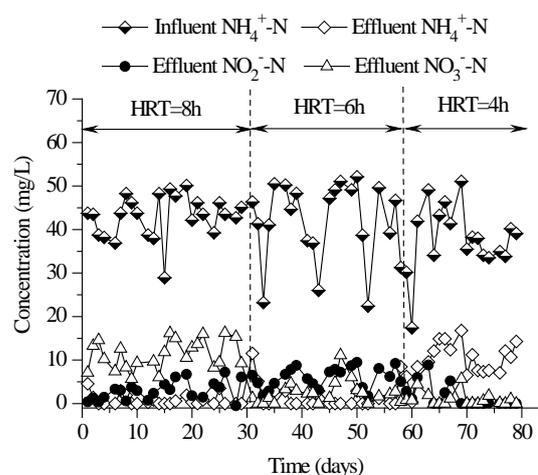


Figure 4. Nitrogen removal under different HRT conditions.

problem at HRT 6 h was the nitrite concentration in the effluent was higher than HRT 4 h and 8 h. Further research may be needed to reduce nitrite concentration in the effluent by adjusting the external circulation flow to lower the atmospheric reoxygenation effect and the AOB activity. Identifying a new equilibrium point of nitrification and Anammox process with low ammonia and nitrite concentration in the effluent will be the next stage in this research.

Effect of HRT on Stoichiometric Ratio

Under HRT 8 h, the ratio of $\text{NO}_3^- \text{-N}_{\text{production}} / \text{NH}_4^+ \text{-N}_{\text{consumption}}$ was significantly higher (0.26) than the theoretical value of 0.13^[20,21]. $\text{NO}_3^- \text{-N}_{\text{production}} / \text{NH}_4^+ \text{-N}_{\text{consumption}}$ significantly reduced to 0.05 at HRT 6 h (Figure 5). At the point of HRT dropped to 4 h, the ammonia of the reactor was not completely consumed, due to the lower production of nitrate, the ratio of $\text{NO}_3^- \text{-N}_{\text{production}} / \text{NH}_4^+ \text{-N}_{\text{consumption}}$ dropped to 0.03.

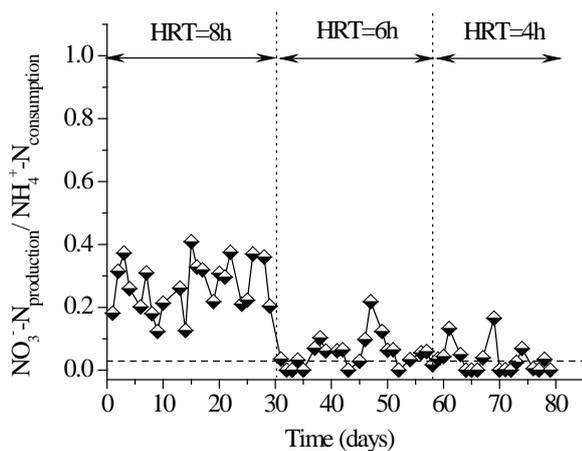


Figure 5. Stoichiometry of sABNR with different HRT.

The longer the HRT the process had, the more excessive atmospheric re-oxygenation took place. With an increase in the activity of NOB, the nitrate concentration in the effluent kept on increasing. However, under HRT 6 h and 4 h, the nitrate production was lower than the theoretical value of single-stage deammonification, probably due to other microorganism consumption. Some other points are as follows:-

(i) Although the influent COD was under the limitation of the test, the denitrifying bacteria could still survive and denitrify using nitrate generated by Anammox and COD from the decayed bacteria^[22].

(ii) Some desulfurization bacteria such as *Thiobacillus denitrificans* might exist in the reactor. Under aerobic conditions *Thiobacillus denitrificans* could

oxidize sulfide to elemental sulfur, meanwhile consuming dissolved oxygen. Under anaerobic conditions sulfide can be used as an electron donor, and the nitrate as an electron acceptor for denitrification and nitrogen removal^[23]. When the experiment continued, the activity of *Thiobacillus denitrificans* will be further improved, and the part of nitrate produced by Anammox process was converted to nitrogen gas.

(iii) Sulfate-reducing Anammox process used sulfate as an electron acceptor and ammonia as electron donor^[24], the final product was sulfur and nitrogen gas, but not nitrate. Due to the long-term sulfide adding, the sulfide could generate sulfates which would promote the occurrence of sulfate-reducing Anammox process.

Effect of HRT on Nitrogen Removal Rate

With decreasing HRT, the nitrogen loading rate (NLR) and nitrogen removal rate (NRR) kept increasing (Figure 6). When HRT was 8 h, the NLR and NRR were the lowest (129.0 and 86.1 $\text{g}/\text{m}^3/\text{d}$), and when HRT was shortened to 6 h, NLR and NRR were 169.7 and 129.7 $\text{g}/\text{m}^3/\text{d}$, and the TN removal was 77.5%. When HRT was 4 h, although NLR increased to 225.2 $\text{g}/\text{m}^3/\text{d}$, NRR was only 149.7 $\text{g}/\text{m}^3/\text{d}$. Due to the short HRT which resulted in incomplete nitrogen removal, the NRR was only slightly increased, and TN removal was 67.6% far below the TN removal at HRT 6 h.

Other sABNR processes run worldwide is shown in Table 1. This study achieved a higher and more stable NLR and NRR by using 6 h HRT. Previous studies showed that the type of seed sludge and operating conditions after inoculation played a key role at the startup of sABNR process. Other studies had obtained a relatively high NRR^[25,26], but it was operated using

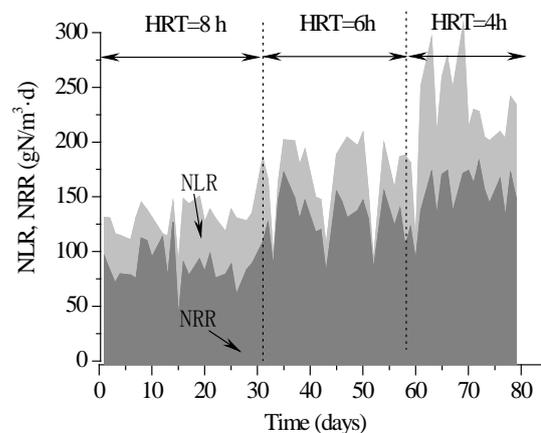


Figure 6. NLR and NRR with different HRT.

Table 1. The sABNR process worldwide

Reference	Reactor	Water Type	Seed sludge	Later adding sludge	HRT(h)	Removal (%)	NRR(gN/m ³ /d)	Influent ammonia concentration (mg/L)
[20]	SBR	synthetic water	Anammox	NOB	24	36-92	40–120	–
[27]	SBR	synthetic water	Anammox	–	24	42	60	131
[6]	SBR	synthetic water	Anammox	NOB	–	–	80	–
[25]	SBR	sludge digestion	Nitrification	Anammox	12	78	250	180–330
[26]	RBC	sludge digestion	OLAND ^a	–	32	50–83	400–1100	1215±54
[28]	Up-flow	sludge digestion	Mix ^b	–	120	76	70	438±26
[29]	Up-flow	synthetic water	Nitrification	–	5	22.1	230	130–300
[30]	RBC	synthetic water	OLAND ^a	–	1.81–1.97	40	25–58	70
[31]	MBBR	synthetic water	Nitrification	Anammox	32.16–45.84	–	25.2–38.6	100
This study	ecSBR	municipal wastewater	Anammox	NOB	6	78	130	22–52

a OLAND sludge (aerobic ammonium-oxidizing bacteria + Anammox bacteria)

b Mix sludge (anaerobic sludge + aerobic activated sludge)

high ammonia wastewater and longer HRT. This study was based on real municipal wastewater, and the NLR was controlled at low concentration of the substrate (influent $\text{NH}_4^+\text{-N}=22\text{--}52$ mg/L), and still achieved a desirable NRR. In addition, Table 1 showed most ammonia removal efficiency was lower, but the ammonia removal efficiency obtained in this study was relatively higher than other studies.

Effect of HRT on Nitrogen Conversion

When HRT was at 8 h, 6 h and 4 h, the ammonia concentration in effluent ($\text{NH}_4^+\text{-N}_{\text{remain}}$) were 1.7%, 3.2% and 25.5% of influent ammonia, which showed ammonia removal was not complete at short HRT (Figure 7). Optimal HRT was at 6 h with higher ammonia removal efficiency, but excessive unused nitrite remained in the effluent and accounted for 13.0% of the influent nitrogen. This was because external circulation enhanced atmospheric reoxygenation, and AOB and Anammox bacteria could not reach the balance

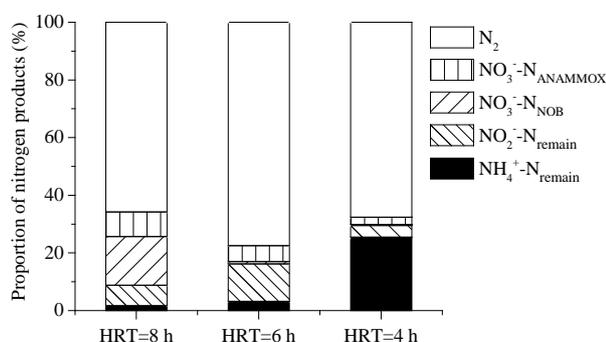


Figure 7. Nitrogen conversions under different HRT.

of substrate utilization. At HRT 8 h, nitrate production by the NOB ($\text{NO}_3^-\text{-N}_{\text{NOB}}$) was higher (16.9%) due to increasing NOB activity. TN removal under the three conditions was 65.8%, 77.5% and 67.6%.

3.3 Typical Cycle of sABNR with Different HRT

In a typical cycle, at HRT 8 h the first 6 h of the reaction had similar nitrogen removal trends with HRT 6 h (Figure 8). As the reaction proceeded, nitrate gradually accumulated. Since the substrates concentrations were too low, the Anammox bacteria could not effectively consume nitrite which is produced by AOB. Because Ammonia was depleted at the first 6 h, AOB and Anammox bacteria were not involved during the next 2 h. During the last 2 h, only NOB oxidized the accumulative nitrite to nitrate, which would promote NOB activity. Therefore, HRT 6 h should be selected to avoid further oxidation of nitrite.

When the HRT was shorter than 4 h, the reaction was not completed, at the end of the reaction 10 mg/L ammonia remained. Figure 8 showed the concentration of nitrate was not significantly increased with the continued reaction. Because the sABNR reactor was conducted for nearly 200 d, long-term constant sulfide addition, the ability of nitrate consumption from the sulfate-reducing Anammox bacteria or *Thiobacillus denitrificans* in the process had gradually increased; this might be the reason that nitrate production was less than the theoretical value.

4. Conclusion

The conclusions are as follows:-

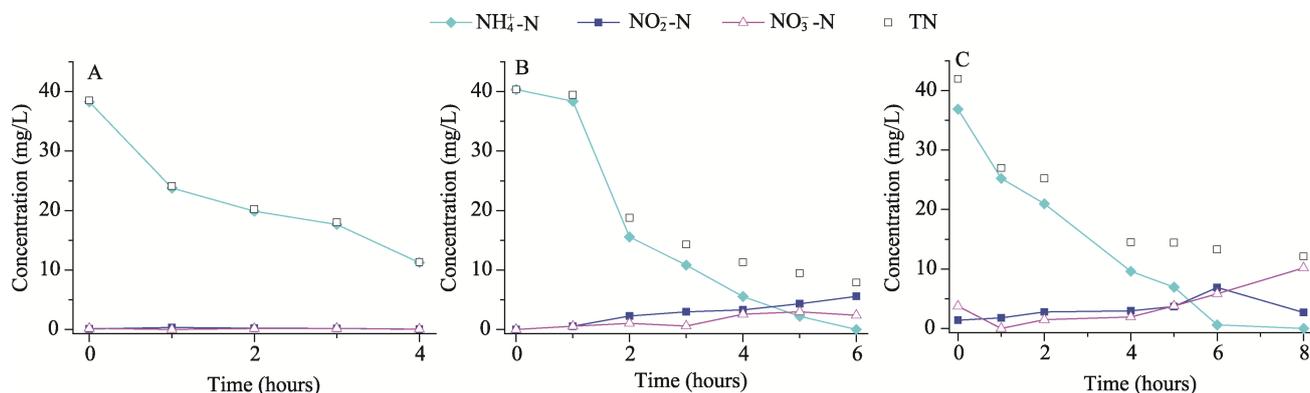


Figure 8. The variation of ammonia, nitrite and nitrate in the typical cycle under different HRT; (A): 4 h HRT, (B): 6 h HRT, (C): 8 h HRT.

(i) In the aerobic sABNR process, we gradually reduced DO in the reactor and eventually achieved the anoxic sABNR. The concentration of $\text{NH}_4^+\text{-N}$ and $\text{NO}_2^-\text{-N}$ in the effluent were 0.9 mg/L and 2.9 mg/L.

(ii) HRT had significant impact on the performance of sABNR process. When HRT was shortened from 8 h to 4 h, the concentration of $\text{NH}_4^+\text{-N}$ in the effluent increased from 0.8 mg/L to 9.9 mg/L. The optimal HRT was 6 h which resulted in the complete consumption of ammonia in the reactor, and the ratio of $\text{NO}_3^-\text{-N}_{\text{production}}/\text{NH}_4^+\text{-N}_{\text{consumption}}$ was 0.05. Ammonia removal efficiency at 6 h HRT was relatively higher than in previous studies.

(iii) The low ratio of $\text{NO}_3^-\text{-N}_{\text{production}}/\text{NH}_4^+\text{-N}_{\text{consumption}}$ could be due to nitrate consumption by the sulfate-reducing Anammox bacteria or *T. denitrificans* in the process since there was continuous sulfide addition in the reactor.

Conflict of Interest and Funding

No conflict of interest was reported by all authors.

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