

Technical Note

The Effect of Salinity on N₂O Emissions during Domestic Wastewater Partial Nitrification Treatment in a Sequencing Batch Reactor

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Abstract: Previous studies have highlighted the salinization caused by the use of seawater to flush toilets and industrial wastewater entering the urban wastewater systems in coastal areas. Thus, in this study, the effect of salinity on N₂O emissions during the partial nitrification process, as well as the emission mechanism, was investigated using a partial nitrification system of wastewater as the research object. The results showed that (1) the increase in salinity decreased the oxidation rate of NH₄⁺ and the formation rate of NO₂[−] during partial nitrification; (2) the increase in salinity increased the N₂O emissions during NH₄⁺ oxidation and NH₂OH oxidation and decreased the formation rate of NO₂[−]-N during hydroxylamine oxidation; (3) the total N₂O emissions during hydroxylamine oxidation were less than those during ammonia nitrogen oxidation, and a greater amount of NO₂[−] was reduced to N₂ instead of N₂O during hydroxylamine oxidation; and (4) a novel finding was that, during partial nitrification with the available organic matter, the N₂O emissions via heterotrophic denitrification by heterotrophic bacteria should not be ignored, and the increase in salinity can increase the N₂O emissions generated via heterotrophic denitrification. These results would provide a theoretical basis for reducing the N₂O emissions in the wastewater treatment process.

Keywords: N₂O; domestic wastewater; partial nitrification; mechanism; greenhouse gas reduction



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1. Introduction

Recent years have witnessed rapid economic development in the coastal areas of China, with an increased population density and greater demand for fresh water, along with a severe shortage of water. Thus, seawater utilization is considered as critical to alleviating the shortage of freshwater resources in coastal areas. Seawater flushing [1], as an important means of seawater utilization, has been paid more and more attention; however, the resultant salt-containing wastewater treatment needs more focus for urban wastewater treatment. In addition, this high-salt industrial wastewater has been shown to enter the municipal wastewater network due to its non-standard discharge, resulting in a change in the salinity of municipal wastewater, along with an increase in the salt load of the wastewater plant. This increase in salinity has the potential to affect the nitrogen removal efficiency of biological wastewater treatment, because salinity can cause changes in the metabolic enzymes and cell structure in microorganisms [2,3].

Currently, the studies on the influence of salinity on the wastewater treatment process at home and abroad have mostly been limited to the analysis of the influence of salinity on the traditional nitrification and denitrification of wastewater. In addition, the studies investigating the influence of salinity on the changes in nitrogen and phosphorus contents in the wastewater treatment process have mainly been focused on evaluating its impact on N₂O production and rarely on its mechanism in the nitrification and denitrification process.

N_2O is a super greenhouse gas, with a greenhouse effect of 300 times that of CO_2 [4]; it is also associated with the occurrence of the ozone hole [5]. N_2O can be produced in both the nitrification and denitrification processes of wastewater biological treatment. Currently, there are many studies on the influence of salinity on N_2O in the denitrification process, but there is a scarcity of research on the influence of salinity on N_2O in the nitrification process and its mechanism.

In the nitrification process of wastewater biological treatment, N_2O is produced mainly during the oxidation of NH_3 by Ammonia-Oxidizing Bacteria (AOB) to NO_2^- [5,6]. In this process, there are three possible production paths: (1) N_2O is the byproduct of the incomplete oxidation of hydroxylamine in the oxidation process [7–10]; (2) N_2O is the product of the autotrophic denitrification of AOB using NO_2^- as an electron acceptor [8,11]; and (3) the chemical reaction of NH_2OH with NO_2^- or O_2 can also produce partial N_2O [12,13]. Additionally, similar to the denitrification process, N_2O might also be produced during nitrification due to the denitrification of heterotrophic bacteria [14]. However, there have been no studies documenting the influence of salinity on the N_2O produced by heterotrophic denitrification during nitrification.

Partial nitrification is a novel process for wastewater treatment in practical engineering. Compared to whole-process nitrification, the partial biological nitrogen removal process offers several advantages, such as reducing oxygen supply by 25%, saving carbon sources by 40%, reducing sludge production by approximately 50%, shortening the reaction time, and reducing the reactor volume [15]. However, in the partial nitrification process of the AOB enrichment system, the yield of N_2O is higher than that of the whole-process nitrification system because the end product of NH_3 oxidation is NO_2^- rather than NO_3^- , and because NO_2^- is a receptor for autotrophic denitrification and reacts with NH_2OH [16]. This study investigated the effect and mechanism of salinity on the N_2O production in short-cut nitrification system sludge enriched with AOB. The results would provide a theoretical basis for the reduced emissions of N_2O in the wastewater treatment process under the background of “carbon neutrality”.

2. Methods

2.1. Sludge, Wastewater, and SBR (Sequencing Batch Reactor) Operations

A 12 L SBR was used for cultivating the partial nitrification sludge. Table 1 shows the index of domestic wastewater inflow. The partial nitrification operation mode was as follows: the average operational cycle (420 min), including feeding (30 min), aeration (240 min), anoxic denitrification (120 min), and settling (30 min), three cycles per day, with the DO at 1 mg/L and Mixed Liquid Suspended Solids (MLSS) at 3000 mg/L. The Sludge Retention Time (SRT) was 11 days, and the running temperature was 30 °C. The effluent's composition consisted of NH_4^+-N , NO_2^--N , and NO_3^--N , which were all <1 mg/L. The sludge taken from the SBR was aerated for 12 h, followed by repeated washing. The effluent's COD was less than 50 mg/L and could not be oxidized further.

Table 1. The quality of real domestic wastewater.

	COD (mg/L)	NH_4^+-N (mg/L)	NO_2^--N (mg/L)	NO_3^--N (mg/L)	TN (mg/L)	pH	Alkalinity
Minimum	88	39.6	0	0	56.4	6.9	262
Maximum	276	91.2	2.8	1.2	98.5	7.7	343
Average	182	65.4	1.4	0.6	77.4	7.3	303

2.2. Batch Test Rules

Figure 1 shows the experimental batch test reactor. The effective volume of the reactor was 3 L. At the beginning of each batch test, 1 L of concentrated sludge was added into the reactor, followed by 2 L of wastewater, and the Mixed Liquor Suspended Solids (MLSS) was controlled at 3000 mg/L. The nitrogen compounds, DO, and pH levels were then adjusted

for the batch set upon commencing the operation (Table 2). The running time for the batch tests was 180 min.

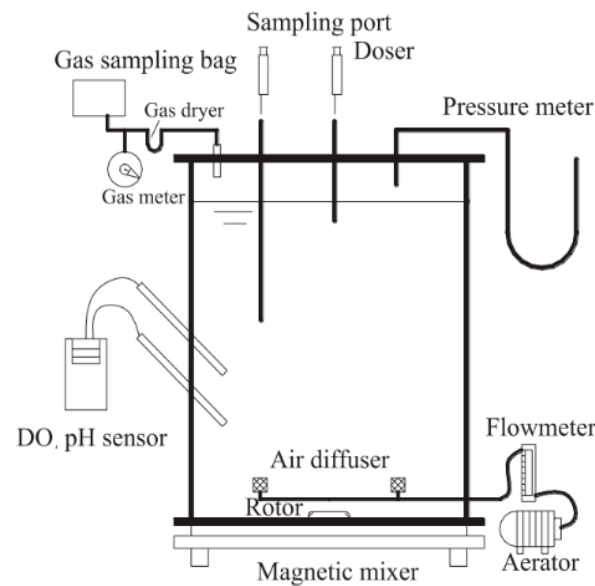


Figure 1. Schematic diagram of batch-mode SBR system.

Table 2. Batch test rules.

Batch Test Number	Salinity (mg/L NaCl)	Sludge-Water Mixture Type	Initial pH	ATU (mg/L)	NH ₄ ⁺ -N (mg/L)	NH ₂ OH-N (mg/L)	NO ₂ ⁻ -N (mg/L)	DO (mg/L)
1	0	Partial nitrification sludge/effluent	7.5		20			0.5
	10		7.5		20			0.5
	20		7.5		20			0.5
	30		7.5		20			0.5
2	0	Partial nitrification sludge/effluent	7.5			20		0.5
	10		7.5			20		0.5
	20		7.5			20		0.5
	30		7.5			20		0.5
3	0	Partial nitrification sludge/effluent	7.5		10		10	0.5
	10		7.5		10		10	0.5
	20		7.5		10		10	0.5
	30		7.5		10		10	0.5
4	0	Partial nitrification sludge/effluent	7.5			10	10	0.5
	10		7.5			10	10	0.5
	20		7.5			10	10	0.5
	30		7.5			10	10	0.5
5	0	Partial nitrification sludge/inflow	7.5	20		20		0.5
	10		7.5	20		20		0.5
	20		7.5	20		20		0.5
	30		7.5	20		20		0.5
6	0	Partial nitrification sludge/effluent	7.5	20		20		0.5
	10		7.5	20		20		0.5
	20		7.5	20		20		0.5
	30		7.5	20		20		0.5

Note(s): ATU: Allylthiourea, an ammonia nitrogen oxidation inhibitor.

2.3. Detection Method

The NH₄⁺-N, NO₂⁻-N, and NO₃⁻-N were measured according to methods described in previous studies [17]. The DO, pH, and T were measured using an oxygen, pH, and

temperature meter (WTW 340i, WTW Company, Munich, Germany), and the temperature and DO were controlled using an appropriate sensor and PLC, respectively. The Mixed Liquid Suspended Solids concentration was measured at the beginning and end of each test to obtain an average value, which was used for the calculation of the $\text{NH}_4^+\text{-N}$ oxidation rate, $\text{NO}_x^- \text{-N}$ production rate, and N_2O emission rate. The total N_2O production comprised the N_2O emitted in the gaseous phase (emission gas N_2O) and the N_2O dissolved in the mixed liquid phase (dissolved N_2O). The N_2O concentrations in the gas samples were analyzed in triplicate using a gas chromatograph (Agilent 6890N, Santa Clara, CA, USA). The overhead space method was used to analyze the dissolved N_2O . Water and N_2O samples were taken at 30 min intervals.

3. Results and Discussion

3.1. Effect of Salinity on N_2O Emissions in Ammonia Nitrogen Oxidation Process

Figure 2 shows the changes in the nitrogen levels (initial $\text{NH}_4^+\text{-N}$ = 20 mg/L) at salinity: 0 mg/L NaCl. The oxidation of NH_4^+ caused N_2O to reach its maximum value (0.26 mg/L) at 60 min. Next, with the gradual completion of NH_4^+ oxidation, even though there was still a high amount of $\text{NO}_2^-\text{-N}$ produced in the system, the output of N_2O gradually decreased and tended to 0. This indicated that, during the oxidation of NH_4^+ , the NH_4^+ oxidation process was the source of the electrons produced by the N_2O . When the oxidation of NH_4^+ ended, NO_2^- could not be denitrified due to the high concentration of no electron donor in the system. During the reaction time of 180 min, the total yield of N_2O was 0.75 mg/L.

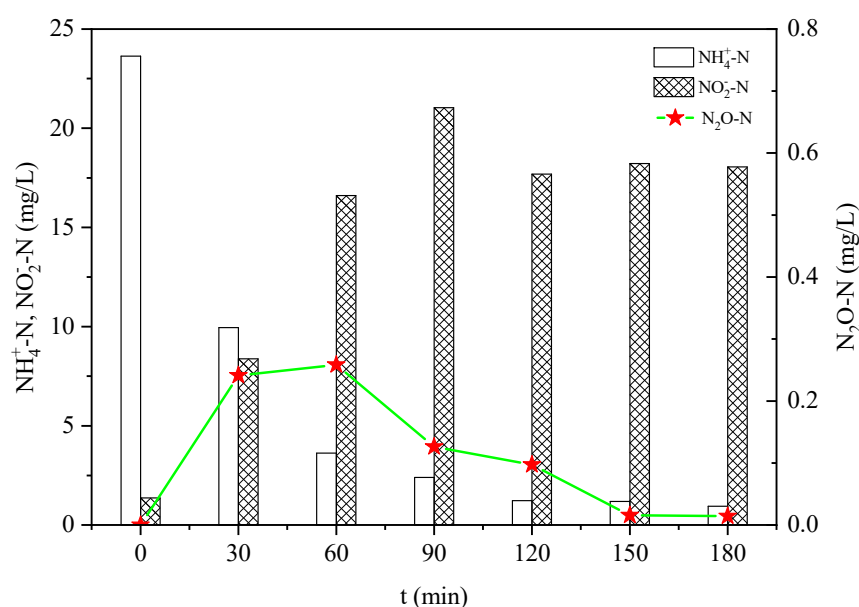


Figure 2. The variations in N_2O during NH_4^+ oxidation (0 mg/L NaCl), Batch Test Rules 1.

Figure 3 shows the rate of the nitrogen change in organic-free water (effluent +20 mg/L $\text{NH}_4^+\text{-N}$) under varying salinity gradients. With a gradual increase in the concentration of NaCl in the system from 0 mg/L to 30 mg/L, the oxidation rate of NH_4^+ and the formation rate of $\text{NO}_2^-\text{-N}$ showed downward trends. However, the rate of N_2O emissions increased from 0.08 mgN/(gMLSS·L·h) to 0.25 mgN/(gMLSS·L·h). This indicated that the increase in salinity increased the N_2O emissions in the NH_4^+ oxidation process of the AOB enrichment system.

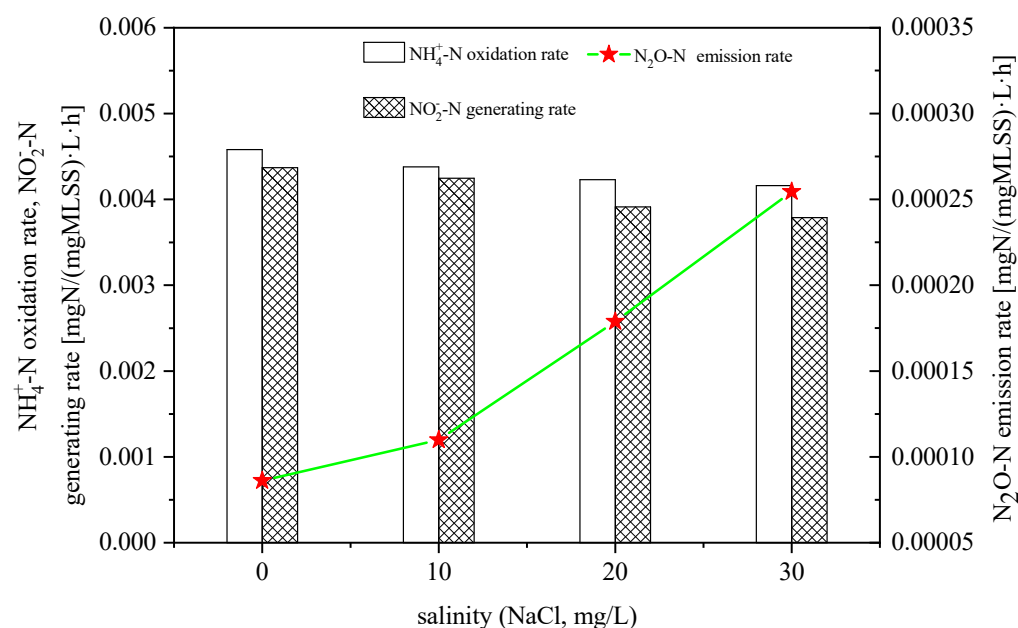


Figure 3. The variations in N conversion rates during NH_4^+ oxidation under different salinity gradients.

During the oxidation of NH_3 , NH_3 is first oxidized to NH_2OH under the action of AOB, and then NH_2OH is oxidized to NO_2^- [18]. The first step in this process is catalyzed by ammonia oxidase (AMO), where molecular oxygen acts as an electron acceptor [7]. The second step is performed under the action of hydroxylamine oxidase (HAO), where molecular oxygen is the primary electron acceptor [7]. The current literature has shown that the effect of N_2O on AOB might be caused by the following two ways: (1) N_2O as a product of the autotrophic denitrification of AOB using NO_2^- as an electron acceptor [8,11]; or (2) N_2O as a byproduct of the incomplete oxidation of hydroxylamine [7–10]. In the first pathway of N_2O production, NO_2^- is reduced to N_2O by NO in the presence of copper-containing enzymes, NO_2^- reductase and NO reductase. With an increase in salinity, the ammonia oxidation rate and NO_2^- formation rate both decreased, indicating that the increase in salinity inhibited the nitrification of AOB, which could have been due to the fact that salinity inhibited both AMO and HAO during the oxidation of NH_3 , resulting in incomplete NH_3 . The ammonia oxidation rate in the system was always found to be greater than the NO_2^- generation rate under different gradient salinification, indicating that a part of the generated NO_2^- was denitrified during NO_2^- generation (Figure 3). With an increase in salinity, the ammonia oxidation rate and NO_2^- production rate both decreased, but the N_2O emission rate increased, indicating that salinity had an inhibitory effect on the reduction in N_2O . The mechanism of action might involve the inhibitory effect of salinity on N_2O reductase.

3.2. Effect of Salinity on N_2O Emissions in the Hydroxylamine Oxidation Process

Figure 4 shows the change in N_2O during the initial addition of 20 mg/L of $\text{NH}_2\text{OH-N}$ when the salinity was 0. When 20 mg/L of $\text{NH}_2\text{OH-N}$ was initially added to the outflow water, the maximum yield of N_2O (0.20 mg/L) appeared at 30 min, and the speed of reaching the maximum yield was faster than that of NH_4^+ alone, which could have been due to the fact that, compared to NH_4^+ , the oxidation from NH_2OH was one step less than the oxidation from NH_4^+ . Furthermore, the NO_2^- levels continued to increase as the reaction progressed, which indicated that NH_2OH was being continuously oxidized. With the continuous oxidation of NH_2OH , the emissions of N_2O decreased in turn. During the reaction time of 180 min, the total yield of N_2O was 1.19 mg/L.

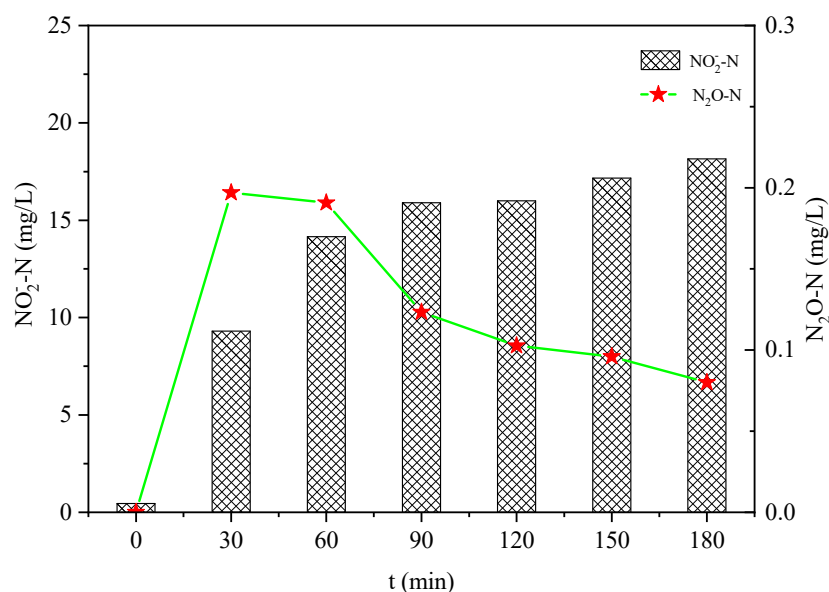


Figure 4. The variations in N₂O during NH₂OH oxidation (0 mg/L NaCl), Batch Test Rules 2.

Figure 5 shows the trend in the variation of N₂O during the oxidation of NH₂OH at salinity = 0. With a gradual increase in salinity from 0 mg/L to 30 mg/L, the rate of NO₂[−] generation showed a downward trend, i.e., 0.00197 mgN/(gMLSS·L·h) and 0.00172 mgN/(gMLSS·L·h) at 0 mg/L and 30 mg/L, respectively. The N₂O emission rate increased from 0.00013 mgN/(gMLSS·L·h) at a salinity of 0 mg/L to 0.00017 mgN/(gMLSS·L·h) at a salinity of 30 mg/L. This suggested that an increase in salinity increased the N₂O emissions in the hydroxylamine oxidation process with the salinity load.

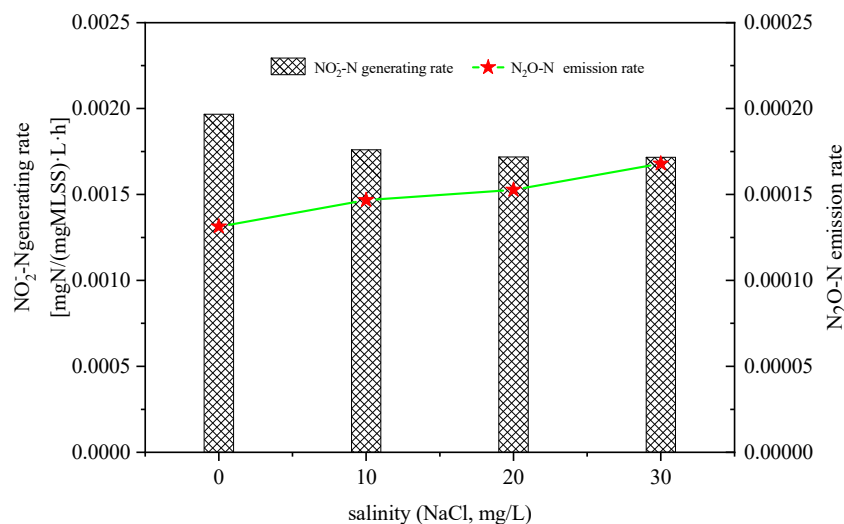


Figure 5. The variations in N conversion rates during NH₂OH oxidation under different salinity gradients.

Figures 2 and 4 show that the total amount of N₂O emissions with the initial addition of 20 mg/L of NH₂OH in the outflow water was more than that produced by the initial addition of 20 mg/L NH₄⁺, which was the same as that reported by Kim [11] and Wunderlin [19]. The study by Kim reported that four electrons were produced during the NO₂[−] oxidation by NH₂OH from HAO, of which two electrons were consumed during the NH₄⁺ oxidation to NH₂OH from AMO; thus, only two electrons were used for the autotrophic denitrification of NO₂[−].

However, when NH₂OH was used as a nitrification substrate, all four electrons were used for the autotrophic denitrification of NO₂[−], resulting in a greater N₂O production. The NO₂[−] production rate showed a downward trend under four salinity gradients, indicating

that the nitrification with NH_2OH as substrate was inhibited, which could have been due to the salinity-induced inhibition of hydroxylamine oxidase (HAO) (Figure 5). When NO_2^- decreased with an increase in salinity, the N_2O emissions increased with an increase in salinity, which could have been due to the inhibitory effect of salinity on N_2O reductase during the autotrophic denitrification of AOB using NO_2^- as an electron acceptor.

3.3. Effect of Salinity on N_2O Emissions in the Autotrophic Denitrification Process

Figure 6 shows the variation trend in N_2O during the autotrophic denitrification at salinity = 0. In this experiment, 10 mg/L of $\text{NH}_4^+\text{-N}$ and 10 mg/L of $\text{NO}_2^-\text{-N}$ were added to the mud–water mixture of the partial nitrification effluent, and then nitrification was performed at 0.5 mg/L DO. With the oxidation of $\text{NH}_4^+\text{-N}$, N_2O rapidly reached its maximum value (0.33 mg/L) at 30 min. In this reaction process, both the N_2O emissions due to the oxidation of $\text{NH}_4^+\text{-N}$ itself and the electrons provided by the oxidation of $\text{NH}_4^+\text{-N}$ were used to conduct denitrification, with the addition of NO_2^- as an electron acceptor. In the whole reaction process, the total N_2O emissions were 0.54 mg/L. The study by Kim [11] showed that the autotrophic denitrification of AOB in the nitrification process is the primary pathway for N_2O emissions. Therefore, in this experiment, the effect of salinity on the N_2O emissions during autotrophic denitrification was mainly affected.

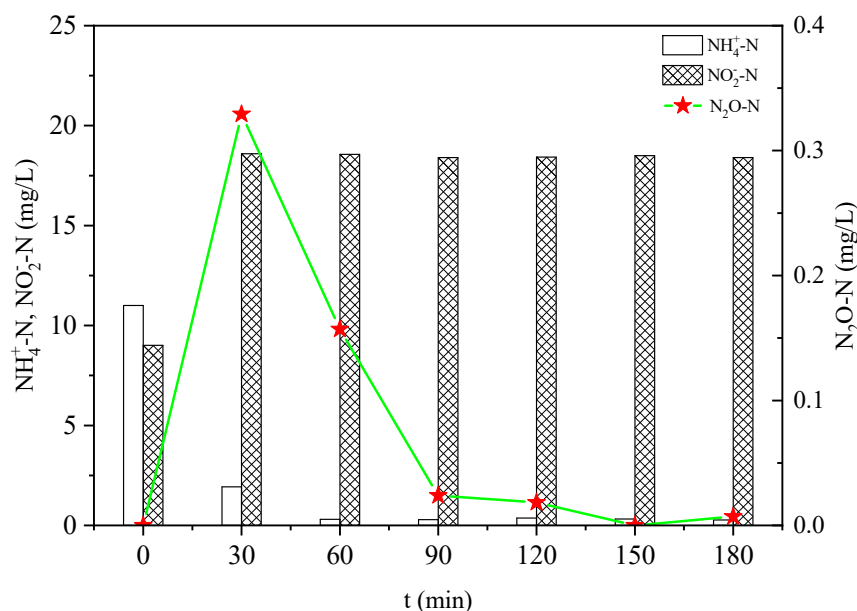


Figure 6. The variations in N_2O during autotrophic denitrification (10 mg/L $\text{NH}_4^+\text{-N}$, 10 mg/L $\text{NaNO}_2\text{-N}$ at initial reaction, 0 mg/L NaCl), Batch Test Rules 3.

Figure 7 shows the variation trend in N_2O in autotrophic denitrification under the influence of salinity. There was a decrease in the oxidation rate of $\text{NH}_4^+\text{-N}$ with an increase in salinity, and the formation rate of $\text{NO}_2^-\text{-N}$ also decreased with an increase in salinity. There was a successive increase in the emission rate of $\text{N}_2\text{O-N}$ with an increase in the salinity load. At salinity = 0 mg/L, 10 mg/L, 20 mg/L, and 30 mg/L, the values were 0.22 mgN/(gMLSS·L·h), 0.28 mgN/(gMLSS·L·h), 0.31 mgN/(gMLSS·L·h), and 0.63 mgN/(gMLSS·L·h). At salinity (0 mg/L to 20 mg/L), the rate of N_2O emissions increased relatively slowly. At salinity = 30 mg/L, the formation rate of $\text{N}_2\text{O-N}$ rapidly increased to 0.63 mgN/(gMLSS·L·h). This indicated that an increase in salinity resulted in an increase in N_2O emissions during partial nitrification.

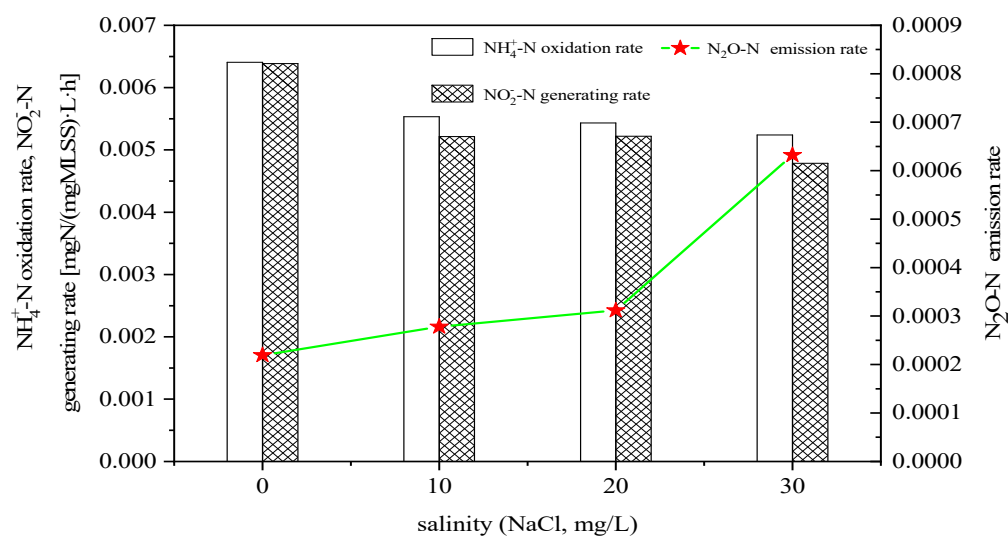


Figure 7. The variations in N conversion rates during autotrophic denitrification (10 mg/L $\text{NH}_4^+\text{-N}$, 10 mg/L $\text{NaNO}_2\text{-N}$ at initial reaction, 0 mg/L NaCl) under different salinity gradients.

Figure 8 shows the variation trend in N_2O during autotrophic denitrification (10 mg/L $\text{LNH}_2\text{OH-N}$ and 10 mg/L $\text{NaNO}_2\text{-N}$) under the influence of salinity. There was a rapid increase in $\text{N}_2\text{O-N}$ to 0.16 mg/L at 30 min. The NaNO_2 levels increased during the whole reaction process, which indicated that NH_2OH was continuously oxidized by microorganisms. As the reaction progressed, there was an insignificant increase in $\text{NO}_2^-\text{-N}$, and the N_2O emissions were stabilized and gradually declined, which indicated that the presence of NH_2OH was critical for the N_2O emissions. During the whole reaction process, the total N_2O emissions were 0.68 mg/L.

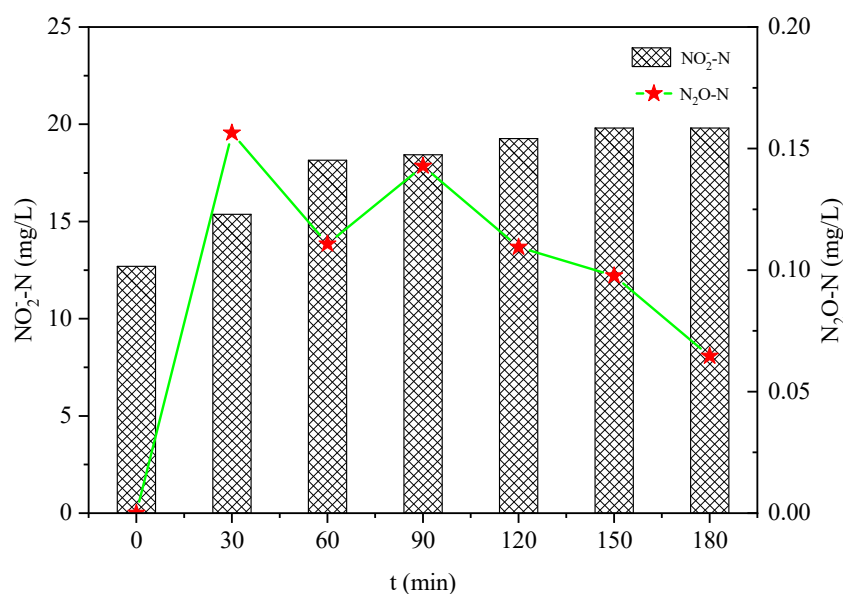


Figure 8. The variations in N_2O during autotrophic denitrification (10 mg/L $\text{NH}_2\text{OH-N}$, 10 mg/L $\text{NaNO}_2\text{-N}$ at initial reaction, 0 mg/L NaCl) under different salinity gradients, Batch Test Rules 4.

Figure 9 shows the variations in the nitrogen rate during autotrophic denitrification (10 mg/L $\text{LNH}_2\text{OH-N}$ and 10 mg/L $\text{NaNO}_2\text{-N}$) under the influence of salinity. The formation rate of $\text{NO}_2^-\text{-N}$ decreased with an increase in salinity. At salinity = 0 mg/L, 10 mg/L, 20 mg/L, and 30 mg/L NaCl, the formation rates of $\text{NO}_2^-\text{-N}$ were as follows: 0.79 mgN/(gMLSS·L·h), 0.75 mgN/(gMLSS·L·h), 0.71 mgN/(gMLSS·L·h), and 0.69 mgN/(gMLSS·L·h), respectively. However, the N_2O emission rate showed a gradual

upward trend, with N_2O production rates of $0.10 \text{ mgN}/(\text{gMLSS}\cdot\text{L}\cdot\text{h})$, $0.11 \text{ mgN}/(\text{gMLSS}\cdot\text{L}\cdot\text{h})$, $0.12 \text{ mgN}/(\text{gMLSS}\cdot\text{L}\cdot\text{h})$, and $0.18 \text{ mgN}/(\text{gMLSS}\cdot\text{L}\cdot\text{h})$, respectively. At salinity levels of $< 30 \text{ mg/L}$, the increasing trend of N_2O was not apparent, but at salinity = 30 mg/L , the N_2O rapidly increased to $0.18 \text{ mgN}/(\text{gMLSS}\cdot\text{L}\cdot\text{h})$.

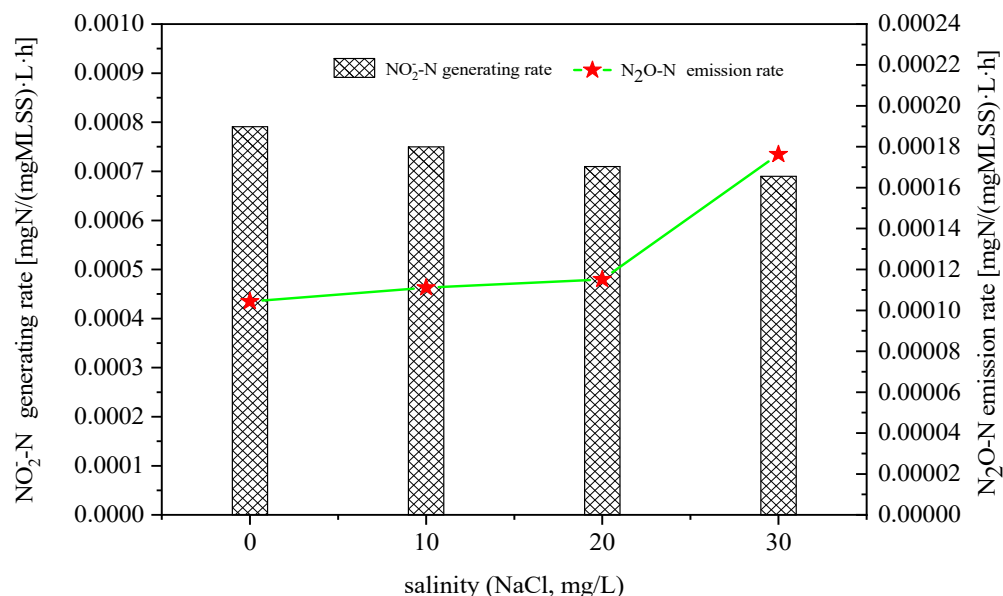


Figure 9. The variations in N conversion rates during autotrophic denitrification (10 mg/L $\text{NH}_2\text{OH-N}$, 10 mg/L $\text{NaNO}_2\text{-N}$ at initial reaction, 0 mg/L NaCl) under different salinity gradients.

For nitrification using NH_4^+ and NH_2OH as substrates, the formation rate of NO_2^- showed a downward trend, but the N_2O emissions increased with an increase in salinity. This indicated that high levels of salinity had an inhibitory effect on N_2O reductase; consequently, the N_2O emissions of AOB in autotrophic denitrification with NO_2^- as an electron acceptor could not be further reduced, in turn increasing N_2O emissions.

3.4. Effect of Salinity on N_2O Emissions of Heterotrophic Denitrification during the Nitrification Process

Figure 10 shows the variation in the N_2O emissions during heterotrophic denitrification under the influence of salinity. When 20 mg/L of $\text{NH}_2\text{OH-N}$ was added to the partial nitrification effluent, the N_2O of the nitrification process mainly resulted from the self-oxidation of NH_2OH and autotrophic denitrification of AOB. However, when 20 mg/L of $\text{NH}_2\text{OH-N}$ and ATU were added to the influent, the nitrification began with the oxidation of NH_2OH , because ATU could completely inhibit the oxidation of NH_4^+ . However, since there was a greater amount of biostable organic matter in the inlet than in the effluent, the available organic matter might have affected the N_2O emissions during nitrification. The total N_2O yield of the influent water used as test water was reduced from that of the effluent water. The N_2O emissions after the reduction were not 0, which could have been due to the denitrification of NO_2^- by heterotrophic bacteria using organic matter during the nitrification (Figure 10). When in the effluent water, the N_2O emissions of the NH_2OH oxidation and AOB autotrophic denitrification increased with an increase in salinity, and the values under four salinity gradients were 0.79 mg/L , 1.02 mg/L , 1.08 mg/L , and 1.19 mg/L , respectively. The N_2O emissions due to the denitrification of heterotrophic bacteria also increased with an increase in salinity, and the values were 0.017 mg/L , 0.024 mg/L , 0.044 mg/L , and 0.067 mg/L , respectively. The ratios of the N_2O emissions due to heterotrophic denitrification to the total N_2O emissions of the influent as the test object were: 2.1%, 2.3%, 3.9%, and 5.4%, respectively. During nitrification, the N_2O produced by heterotrophic denitrification could not be ignored, and the effect of the N_2O emissions due to heterotrophic denitrification was enhanced at a high salinity. This could have been due to the inhibitory effect of salinity on N_2O reductase.

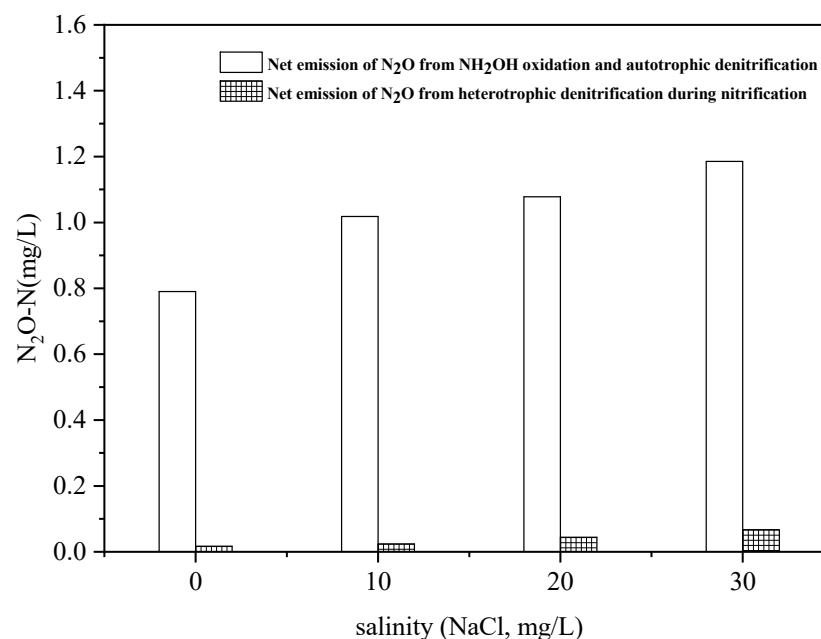


Figure 10. The variations in N₂O during heterotrophic denitrification under different salinity gradients, (Batch Test Rules 5, 6).

4. Conclusions

This study investigated the AOB enrichment system cultured in actual domestic wastewater along with the influence of salinity on N₂O emissions in a partial nitrification system and their mechanism. The following conclusions were drawn:

- (1) The increase in salinity decreased the oxidation rate of NH₄⁺ and the formation rate of NO₂[−] during the reaction.
- (2) The increase in salinity increased the N₂O emissions during the oxidation of NH₄⁺ and NH₂OH, and decreased the production rate of NO₂[−]-N during the oxidation of hydroxylamine.
- (3) The total amount of N₂O emissions in the hydroxylamine oxidation process was less than that during ammonia nitrogen oxidation, and more NO₂[−] might have been reduced to N₂ instead of N₂O during hydroxylamine oxidation.
- (4) During partial nitrification with the available organic matter, the N₂O emissions due to heterotrophic denitrification by heterotrophic bacteria could not be ignored, and the increase in salinity could increase the amount of N₂O emissions due to heterotrophic denitrification.

Author Contributions: P.L.: Investigation, Sampling, Data curation, Writing—original draft; Y.L. and Y.W.: review & editing; S.W.: Resources, Funding acquisition; Y.P.: Supervision, Visualization. S.W. and P.L.: Resources, Funding acquisition. All authors have read and agreed to the published version of the manuscript.

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Data Availability Statement: The original data is backed up on my computer, ready for investigation.

Conflicts of Interest: The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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