Water Research X 27 (2025) 100311

Contents lists available at ScienceDirect

Water Research X

journal homepage: www.sciencedirect.com/journal/water-research-x

Mechanisms of N_2O production in salinity-adapted partial nitritation systems for high-ammonia wastewater treatment

Xiang Li [a], Yingxin Jin [a], Yanying He [a,*], Yufen Wang [a], Tingting Zhu [a], Yingxin Zhao [a], Bing-Jie Ni [b], Yiwen Liu [a,*]

[a] School of Environmental Science and Engineering, Tianjin University, Tianjin 300072, China
[b] School of Civil and Environmental Engineering, University of New South Wales, Sydney, NSW 2052, Australia

ARTICLE INFO

Keywords:
Partial nitritation
Nitrous oxide
Salinity
Ammonia-oxidizing bacteria
High-ammonia wastewater

ABSTRACT

Partial Nitritation/Anammox (PN/A) can achieve green, economical, and efficient biological nitrogen removal; however, the PN process contributes significantly to nitrous oxide (N_2O, the third most important greenhouse gas) emissions. Balancing the stability of PN systems while reducing N_2O emissions, particularly under varying salinity conditions, is a key challenge in applying PN/A for high-salinity and high-ammonia wastewater treatment. This study explored the long-term effects of salinity on PN performance and N_2O emissions in PN systems treating high-ammonia wastewater. The results showed that the specific ammonia oxidation rates of the control and two salinity-acclimated PN reactors were 78.84, 75.03, and 42.60 mg N/(g VSS·h), indicating that low salinity (2.5 g NaCl/L) had minimal effect, while high salinity (10 g NaCl/L) significantly inhibited ammonia-oxidating bacteria and associated nitritation processes. Moreover, N_2O emission factors increased from 0.08 ± 0.04% to 0.24 ± 0.03% as salinity rose from 0 to 10 g NaCl/L. Further analysis revealed that salinity stimulated N_2O production in both aerobic and anoxic stages. Particularly, the N_2O production increased by 2.84–11.14 times in the aerated stage and by 0.61–2.04 times in the nonaerated stage (i.e. anoxic and settling stages). Isotopic pathway analysis indicated that salinity enhanced N_2O production primarily by stimulating the nitrite reduction pathway. Additionally, the mechanism investigation examined the combined effects of salinity-induced changes in sludge properties and microbial community on N_2O emissions. These findings provide valuable insights for applying PN systems to treat high-strength wastewater and understanding the mechanisms of N_2O emissions.

1. Introduction

The alarming increase in global surface temperature due to greenhouse gas (GHG) emissions poses a critical threat of climate change and extreme weather (Dalpadado et al., 2024; Ghanbari et al., 2023). Reducing carbon footprints across industries is essential, particularly in wastewater treatment plants (WWTPs), which contribute ~1.6 % of global GHG emissions (Gruber et al., 2022; He et al., 2023; Liu et al., 2025). Notably, nitrous oxide (N_2O) released during biological nitrogen removal (BNR) processes at WWTPs is a potent GHG with a global warming potential 265 times that of carbon dioxide (Tong et al., 2024). This has sparked increased interest in the production of N_2O from BNR processes (Dai et al., 2021; Gruber et al., 2021).

Currently, partial nitritation (PN) process is widely studied as a very important BNR technology, namely controlling the nitrification process to stay in the stage of forming nitrite (NO_2^-), through oxidizing about 50 % of ammonia (NH_4^+) to NO_2^- solely by ammonia-oxidizing bacteria (AOB), which provides the substrates for subsequent anaerobic ammonia oxidation (Anammox) process (Hausherr et al., 2022; Zhou et al., 2024). This partial nitritation/anammox (PN/A) process can achieve a significant saving on energy demand and reduce excess sludge production due to the relatively slow growth rates of AOB (Laureni et al., 2016), thus being massively applied to side-stream high-ammonia wastewater treatment as a green, economic and efficient nitrogen removal channel (Lackner et al., 2014). However, the PN process is also a major contributor to N_2O emission, mainly originating from the AOB and heterotrophic denitrifying bacteria (HDN) (Wang et al., 2014). N_2O could be produced through AOB via the hydroxylamine (NH_2OH) oxidation pathway and nitrite reduction pathway. Even in the absence of organic carbon sources, HDN still contribute to N_2O production due to

* Corresponding authors.
E-mail addresses: yyhe@tju.edu.cn (Y. He), yiwen.liu@tju.edu.cn (Y. Liu).

https://doi.org/10.1016/j.wroa.2025.100311
Received 27 November 2024; Received in revised form 10 January 2025; Accepted 2 February 2025
Available online 3 February 2025

the soluble microbial products (SMPs) and extracellular polymeric substances (EPS) secretions unavoidably as possible carbon sources (Ni et al., 2011). How to maintain the stability of PN system while reducing N_2O emissions has become one of the hotspots in the field of wastewater treatment.

Notably, N_2O emission is stimulated by many factors (Chen et al., 2020), and salinity is one of the key factors affecting both NH_4^+ removal and NO_2^- accumulation during nitrification or PN processes (Aslan and Simsek, 2012; Kong et al., 2013; Wang et al., 2023a). Moreover, it was reported that salinity promoted N_2O production in mainstream BNR process. Zhao et al. (2014) investigated the mechanism of salinity stimulating N_2O production in nitrification process treating mainstream wastewater, and found that salinity increased N_2O production directly by altering the N_2O production pathways, and indirectly by causing reduced nitrite-oxidizing bacteria (NOB) activity and hence more NO_2^- accumulation. The shift in the N_2O production pathway under salinity gradient impact was also demonstrated using dual isotope labelling approach (Zhang et al., 2024a). However, the existing studies were limited to mainstream BNR processes with relatively low pollutant loading rates and specific operation conditions. The N_2O emission from side-stream autotrophic PN system treating high-strength wastewater, which had totally different dominant microorganisms, remains under-explored under salinity stress. Therefore, the key innovation of this study is that possible effects (i.e. NOB, NO_2^- concentration, organic carbon source, and etc.) on N_2O production in the PN system were reasonably excluded. Moreover, the overall N_2O emission, net N_2O production during aerobic and anoxic stages, and the response of N_2O production pathways to salinity were fully revealed.

This study aimed to reveal the effects of long-term acclimation with different salinity levels (0, 2.5, 10 g NaCl/L) on autotrophic PN system in three laboratory-scale sequencing batch reactors (SBR) treating high-ammonia wastewater. The mechanism of salinity acclimation increasing N_2O emission from PN system was illuminated in depth, in terms of the changes in BNR performance, N_2O production pathway, sludge physicochemical properties, and microbial community structure, so as to improve the existing theories on the effects of salinity on the PN system. In summary, this study could provide a deeper and wider understanding of the effects of salinity on N_2O emission from side-stream PN system.

2. Results and discussion

2.1. Comparisons of overall reactor performance and N_2O emission

During the initial feeding (Day 0–14), all three reactors had stable effluent with NH_4^+ concentrations at 269 ± 12 mg N/L, NO_2^- at 298 ± 15 mg N/L, and NO_3^- below 50 mg N/L (see in Fig. 1A and Fig. S1). After 14 days, when saline influent began to be pumped in PN reactors, adverse impacts on PN performance appeared in experimental reactors, with a slight increase of effluent NH_4^+ concentration in R2 (2.5 g NaCl/L), and a significant increase in R3 (10 g NaCl/L), compared to the control reactor (R1, 0 g NaCl/L). Namely, the increasing salinities gradually decreased PN activity, and the performance of R2 and R3 reactors became inferior sequentially, which was consistent with previous study (Moussa et al., 2006).

As shown in Fig. 1B, along with the increased salinity, the peak N_2O concentration of R1, R2 and R3 reached 49.52, 56.38 and 134.41 ppmv, respectively, at around 50 min. Throughout the whole cycle, gas-phase N_2O concentration maintained the trend of R3>R2>R1, basically suggesting the stimulation of salinity on N_2O emission from PN systems. As shown in Fig. 1C, the N_2O emission factors (EFs) were 0.08 ± 0.04%, 0.11 ± 0.01 %, and 0.24 ± 0.03 % for salinities of 0, 2.5, and 10 g NaCl/L, respectively, indicating that high salinity intensely enhanced N_2O emission by 2.95 times under long-term stimulation. N_2O production

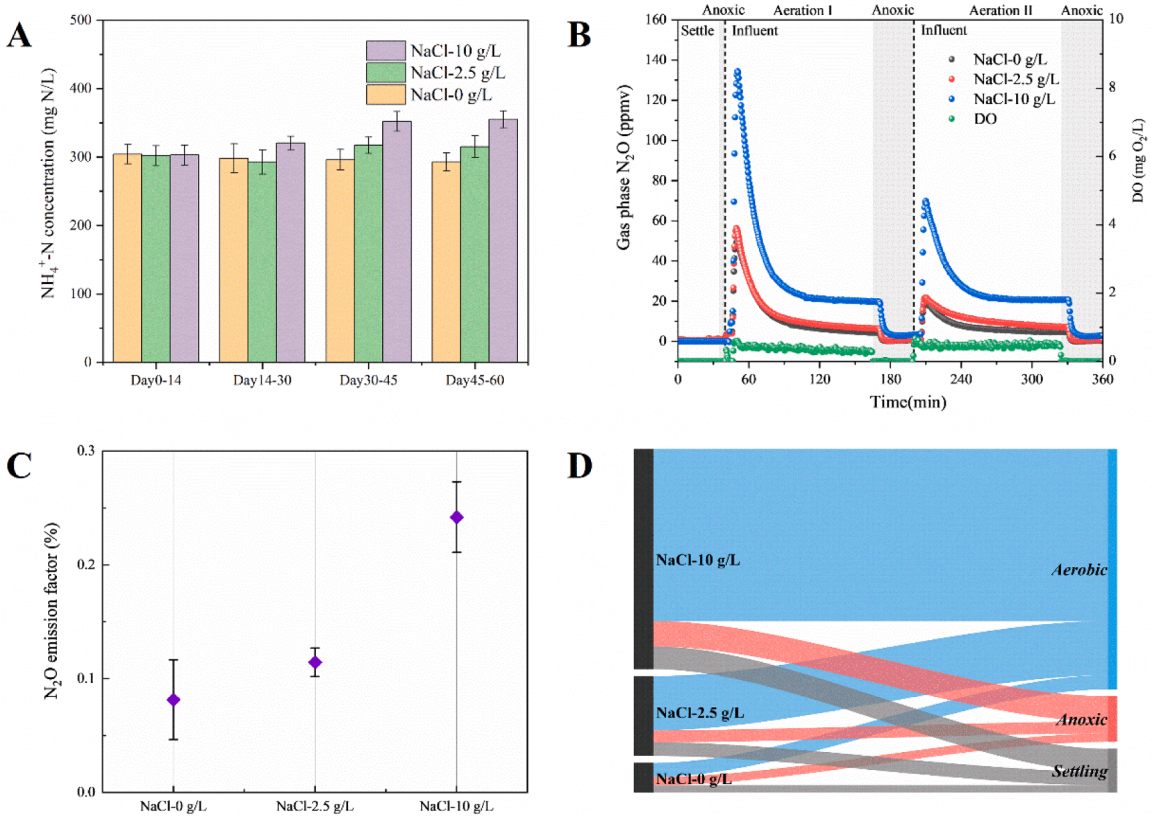


Fig. 1. Effects of long-term salinity acclimation on overall performance and N_2O emission: (A) effluent NH_4^+ concentrations; (B) Cyclic variations of gas phase N_2O concentrations; (C) N_2O emission factors under cyclic operations; (D) N_2O production within aerobic stage, anoxic stage and settling stage of a 6 h operation cycle.

during different operation stages was determined in Fig. 1D. Elevated salinity resulted in higher N_2O production, with the aerobic stage showing the highest increase, followed by the anoxic and settling stages. As the influent salinity increased to 2.5 g NaCl/L, N_2O production across all three stages exhibited an increasing trend. Notably, the proportion of the aerobic stage significantly elevated from 47.22 % in R1 to 68.03 % in R2 and 78.05 % in R3, making it the predominant contributor to total N_2O production in two experimental reactors, probably due to that AOB showed more tolerance than HDN under salinity conditions, and also associated with a significant increase in the N_2O EFs in the aerobic stage. In addition, the non-aeration stage (i.e., anoxic and settling stages) contributed significantly to N_2O production, accounting for 52.78 %, 31.97 % and 21.95 % of total N_2O production in R1, R2 and R3, respectively. As a whole, long-term exposure to saline conditions significantly increased N_2O emissions from PN system due to the stimulated N_2O production via both AOB and HDN. Salinity appeared to complicate the N_2O release pathway by inducing bacterial cell death, providing more SMPs as possible carbon sources in PN system (He et al., 2024; Wang et al., 2014; Ye et al., 2009).

2.2. *Effects of salinity on PN sludge activities and aerobic N_2O production*

Batch experiments were conducted to assess ammonia oxidation rate (AOR) at various DO concentrations to further investigate the impact of long-term salinity acclimation on ammonia oxidation activity (Fig. 2A). It was observed that AOR tended to decline with increasing salinities, in accordance with previous results (Wang et al., 2016). Specifically, the AOR exhibited a slight decline from 28.38 mg N/(g VSS·h) in the control reactor to 27.96 mg N/(g VSS·h) at a salinity of 2.5 g NaCl/L, further dropping to 19.74 mg N/(g VSS·h) at a salinity of 10 g NaCl/L, representing a 30.44 % decrease compared to the control reactor. Similar trends were noted under other DO conditions, indicating that low salinity (2.5 g NaCl/L) had a relatively minor impact on sludge activity, whereas high salinity conditions (10 g NaCl/L) resulted in a significant declined AOR following long-term acclimation.

Fig. 2B presented the gas phase N_2O concentration emitted from the reactor during 1 h batch experiments conducted at a salinity of 2.5 g NaCl/L. The PN activity was enhanced obviously by elevating DO values, consequently leading to a soar in the N_2O production. Fig. 2C displayed the numerical variation of N_2O emission factors under different conditions. A noticeable increasing trend in N_2O emission factors from 0, 2.5 to 10 g NaCl/L under varying DO conditions was observed. For instance, N_2O emission factors increased 0.63 to 0.71 times in the low-salinity condition compared to the control group, and more markedly by 1.23 to 1.31 times with high-salinity, indicating that salinity significantly boosted the N_2O emission potential of PN sludge. The relative contributions of different N_2O production pathways were quantified by measuring the SP values of N_2O produced in batch tests. At DO=0.5, 1 mg O_2/L, N_2O production via the NH_2OH oxidation pathway accounted for >67 % in both the control and the experimental group with a salinity of 2.5 g NaCl/L, representing the primary contributor to N_2O production of PN sludge. Under the same DO conditions, when salinity was increased to 10 g NaCl/L, it was observed that nitrite reduction pathway dominated over NH_2OH oxidation pathway to be the predominant contributor to N_2O production via the PN process. In addition, at DO = 1.5 mg O_2/L, although the NH_2OH oxidation pathway had been the main contributor to N_2O emissions in all reactors, the promotion of the nitrite reduction pathway was still observed with increasing salinity up to 10 g NaCl/L. In view of the fact that N_2O production was higher with elevated salinity, it could be concluded that

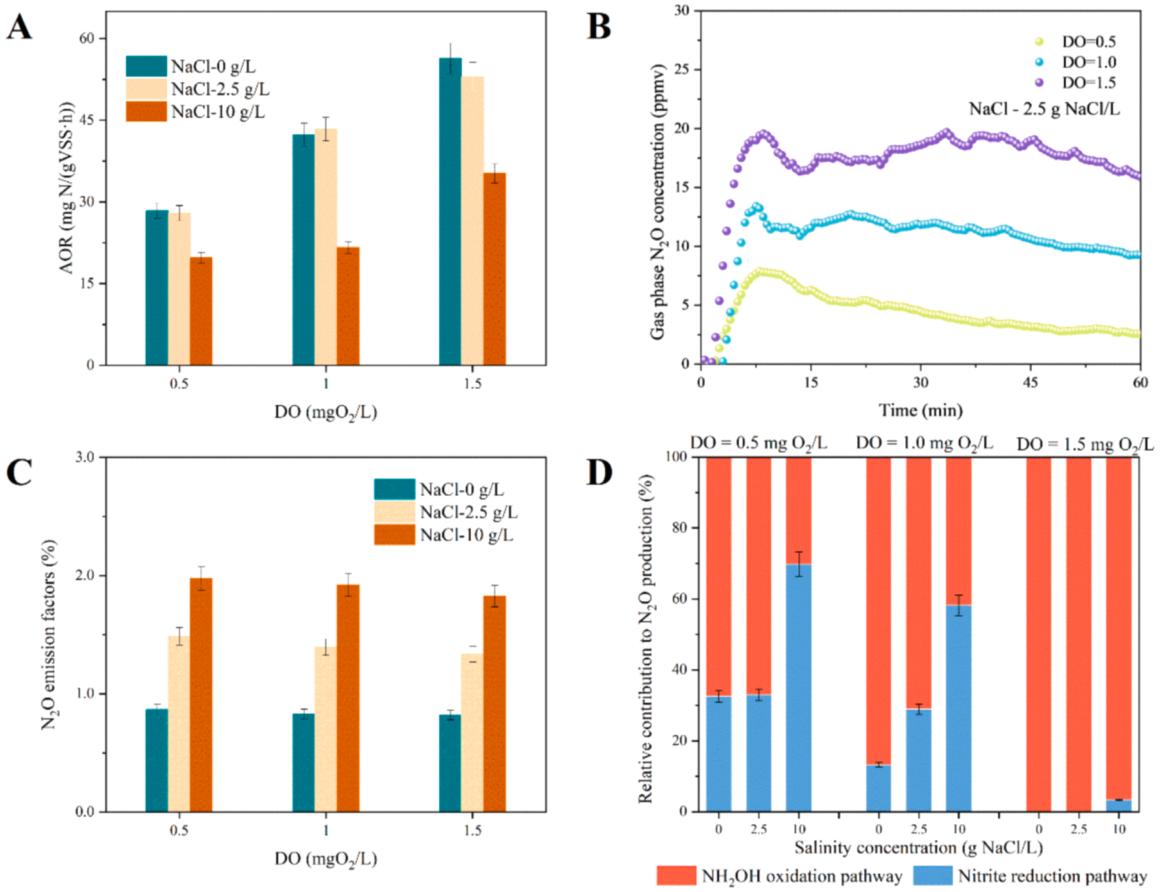
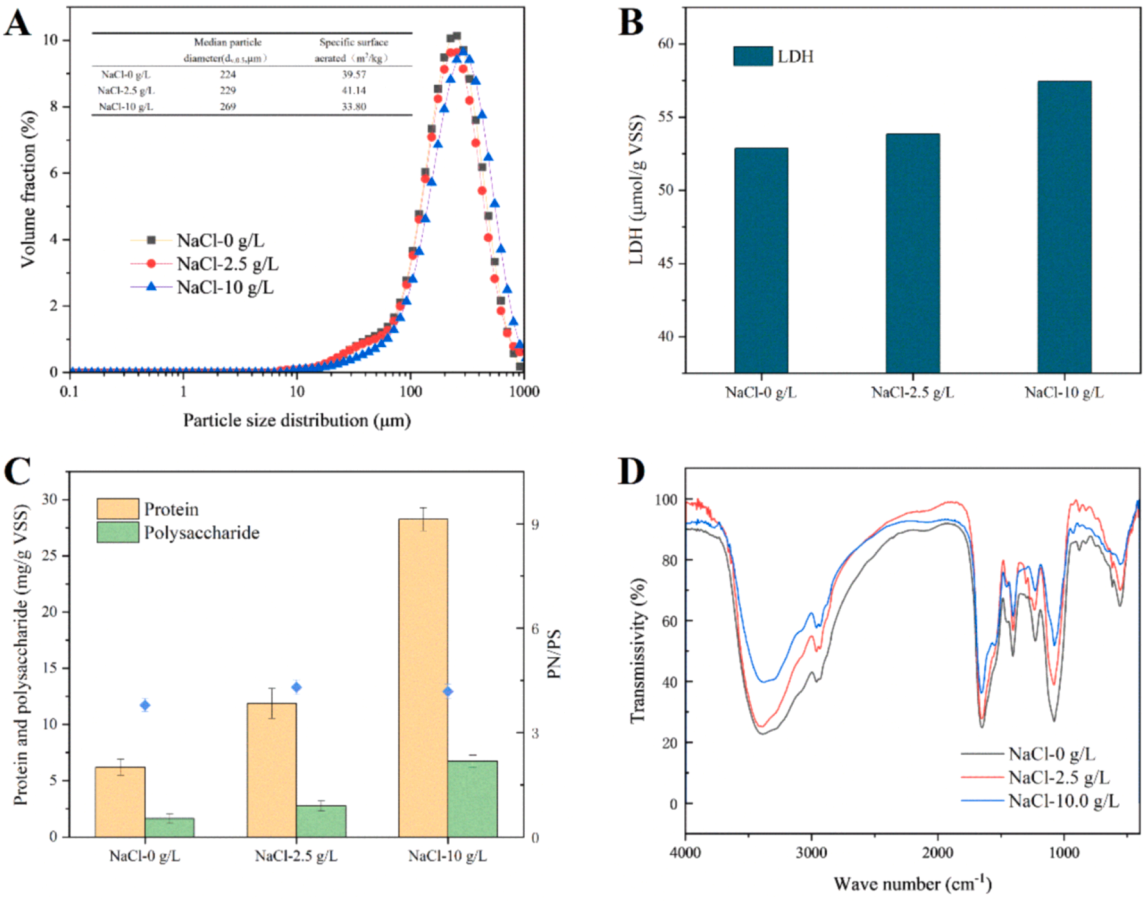


Fig. 2. Batch experiments under varying DO (0.5, 1.0, 1.5 mg O_2/L) and salinity conditions: (A) AOR variations, (B) Gas-phase N_2O concentration, (C) N_2O emission factors, and (D) relative contribution of different N_2O production pathways.

salinity enhanced N_2O emission mainly by stimulated nitrite reduction pathway. In summary, salinity had an inhibitory effect on AOR, but significantly increased the N_2O production capacity of PN sludge by promoting the nitrite reduction pathway.

2.3. *Effects of salinity on the PN sludge properties*

Changes in the physicochemical properties were investigated as following. As shown in Fig. 3A, a trend of enlarged sludge particle size could be observed with salinity increasing, and the medium particle



Samples	Secondary Structures (%)						
	Aggregated strands (Peak1, 1610-1625 cm^{-1})	β-sheet (Peak2, 1630-1640 cm^{-1})	Random coil (Peak3, 1640-1645 cm^{-1})	α-helix (Peak4, 1648-1657 cm^{-1})	3-Turn helix (Peak5, 1659-1672 cm^{-1})	Antiparallel sheet/aggregated strands (Peak6, 1680-1695 cm^{-1})	α-helix/(β-sheet + random coil)
R1 (NaCl-0 g/L)	6.96	14.70	19.73	22.77	21.32	14.52	0.66
R2 (NaCl-2.5 g/L)	6.56	14.28	19.97	23.25	21.40	14.53	0.68
R3 (NaCl-10 g/L)	4.03	10.44	22.14	25.96	21.56	15.87	0.80

Fig. 3. Physicochemical properties of activated sludge: (A) sludge particle size distribution, (B) LDH concentrations, (C) protein values and polysaccharide content in TB-EPS, (D) infrared spectra of sludge TB-EPS by FTIR spectroscopy, (E) the curve-fitted amide I region (1700−1600 cm^{-1}).

diameter increased by 1.2 times (from 224 μm to 269 μm). Besides, the specific surface areas of PN sludge decreased from 39.57 to 33.80 m^2/kg. The increased particle size and smaller specific surface area possibly resulted in more anoxic space within the sludge, favoring the production of more N_2O through the nitrite reduction pathway. As presented in Fig. 3B, after salinity acclimation at 2.5 g NaCl/L, the released lactate dehydrogenase (LDH) concentration increased slightly to 53.84 μmol/g VSS compared to 52.86 μmol/g VSS in the control group, while at 10 g NaCl/L, it further rose to 57.44 μmol/g VSS, suggesting that salinity adversely affected cell membrane to some extent. As a result, decreased AORs were detected in experimental reactors (Fig. 2A). Meanwhile, the broken membrane fragments might act as available organics, thus promoting N_2O production under anoxic conditions (Fig. 1D).

Fig. 3C showed the alternations in the composition of tightly bound extracellular polymeric substances (TB-EPS) secreted by the sludge after long-term salinity acclimation. Microbes tended to secrete more EPS to resist extreme environments (Wang et al., 2023b; 2024a), and the stimulatory effect of salinity on EPS secretion was significantly observed. As salinity increased, the proteins content of TB-EPS rose to 1.92 times (at 2.5 g NaCl/L) and 4.56 times (at 10 g NaCl/L) that of the control group, and polysaccharides content increased by 1.68 and 4.12 times, respectively, which could be considered as a mitigation response of bacteria to salinity (Corsino et al., 2017; Zhang et al., 2011). Under salinity conditions, the value of protein/ polysaccharides increased compared to the control, indicating that protein grew faster than polysaccharides, and larger ratio was favorable to promote sludge aggregation (Guo et al., 2023; Zhang et al., 2024b). The infrared spectra of TB-EPS at different influent salinities were similar (Fig. 3D), but the relative intensities showed almost decreasing trends, indicating that salinity resulted in reduction of major functional groups in the TB-EPS of PN sludge, e.g., N-H, C-O component of proteins and O-H, C-O components of polysaccharides, which might be associated with NaCl adsorption (Liu et al., 2023; Wang et al., 2024b). Additionally, analysis of the curve-fitted amide I region revealed a decrease in the proportion of β-sheet and an increase in the proportion of α-helix under salt conditions (Fig. 3E). This change resulted in a higher ratio of α-helix to (β-sheet + random coil), suggesting that prolonged salinity could lead to a more compact protein structure in EPS (Zhang et al., 2025). Such compactness might restrict oxygen diffusion, thereby altering the metabolic pathway (e.g., reducing AOR) and potentially favoring N_2O production via the nitrite reduction pathway. In summary, under salinity stimulation, bacteria tended to secret more EPS that could be accessed as available carbon by heterotrophic bacteria, thus promoting the growth of HDN and more N_2O emission from the nitrite reduction pathway.

2.4. Effects of salinity on the microbial community

Sludge samples were withdrawn from three reactors for high-throughput sequencing analysis. Table S1 presented a thorough assessment of the richness and diversities within the microbial communities of the three sequencing batch reactors (SBRs). The Coverage indexes were above 0.99 in all samples, validating the reliability of sequencing data. The Ace, Chao, Shannon and Sobs indexes were elevated, reflecting a significant increase in community richness under salinity acclimation conditions, probably caused by the emerging halophilic microorganisms or heterotrophic bacteria under long-term salinity acclimation (Yuan et al., 2021). Venn diagram (Fig. S6) also confirmed above results by visualizing the similarity and operational taxonomic units (OTUs) in different environmental samples. OTUs in the salinity group increased by 49.48 % in R2 and 68.86 % in R3, which further demonstrated that salinity acclimation increased the species diversity of PN sludge. This increased diversity, contrary to a few researches (He et al., 2017), might be due to the experimental salinity being within microbial tolerance ranges and the long acclimation period, which allowed the microbial community to adapt and evolve toward salinity tolerance.

Fig. 4A revealed compositions of the microbial community at phylum level. As salinity increased, the abundance of *Proteobacteria* (35.9~55.6 %), including microorganisms with nitrogen removal function in wastewater treatment, decreased significantly, indicating that salinity had an inhibitory effect on these microorganisms, explaining the deterioration of PN performance and a shift in the balance of N_2O production pathways. The relative abundance of *Deinococcota* (15.6~28.1 %) that are highly resistant to extreme environmental hazards (Tian and Hua, 2010), increased with elevated salinity, which was inferred to be the possible reason for the rise of microbial diversity. *Chloroflexi* (0.56~1.16 %) plays a role in degrading SMP in activated sludge systems, and the increase in its content implied a risk of filamentous bacteria growing under long-term salinity stress (Speirs et al., 2019). Microorganisms in the *Actinobacteriota* phylum (0.52~0.86 %) are supposed to decompose the organic matter of dead microorganisms. The abundances of these two phyla increased with the elevated salinity, suggesting more dead microorganisms or microbial secretion of organic products under salinity stress. Moreover, salinity might result in a gradual evolution of microbial structure towards salt-tolerant and heterotrophic bacteria, might alter the community structure in ways that indirectly influence N_2O production. Relative abundance of bacterial community at genus level was shown in Fig. 4B. The relative abundance of *Nitrosomonas* under salinity conditions was slightly elevated in comparison to the control, and an indication of enrichment was observed, possibly due to that *Nitrosomonas* generally had a favorable adaptation to salinity in PN process (Zhao et al., 2022). In addition, the relative abundance of NOB (e.g., *Nitrospira, Nitrobacter*) in all three reactors was <0.001 %, further suggesting the strongly suppressed NOB activities in these PN systems. Some types of HDN might be negatively affected by salinity. For example, *Arenimonas* is regarded as a typical salt-intolerant bacteria (Xing et al., 2018), exhibiting a significant decrease in the relative abundance even a low salinity of 2.5 g/L. *Unclassified_f_Comamonadaceae* (Chen et al., 2022) also had a decreased activity at 10 g NaCl/L. Nevertheless, other types of HDN showed strong adaptability to salinity conditions. For example, the relative abundance of *Truepera*, a salt-tolerant denitrifying bacteria (Shi et al., 2022), increased from 16 % (in the control reactor) to 21.33 % in R2, and 28.48 % in R3, which might be responsible for increased N_2O production under anoxic conditions.

3. Conclusion

The following conclusions were obtained:

(1) Long-term salinity acclimation significantly deteriorated the PN performance. Cycle experiments showed that salinity promoted N_2O emissions from the PN sludge, and the stimulatory effect was more pronounced with higher salinity concentrations.
(2) Even within the autotrophic system, heterotrophic denitrification contributed to N_2O production owing to the presence of SMPs and EPS as possible carbon sources. Since the salinity had a greatly stronger stimulating effect on the N_2O production in aerobic nitrification process than that under anoxic conditions, aerobic stage became the dominant contributor to N_2O production under the salinity conditions.
(3) High salinity (10 g NaCl/L) acclimation decreased activity of PN sludge (AOR decreasing from 28.38 to 19.74 mg N/(g VSS·h). Analysis of isotope analyses indicated that salinity increased N_2O emission by promoting the nitrite reduction pathway.
(4) Increased salinity stimulated the secretion of EPS, which could provide more available carbon sources for microorganisms. Other physicochemical properties (e.g., particle size, SVI, protein structure in EPS, and LDH release), as well as microbial community, also changed under prolonged adaptation. These provided potential conditions for N_2O emissions via nitrite reduction pathway.

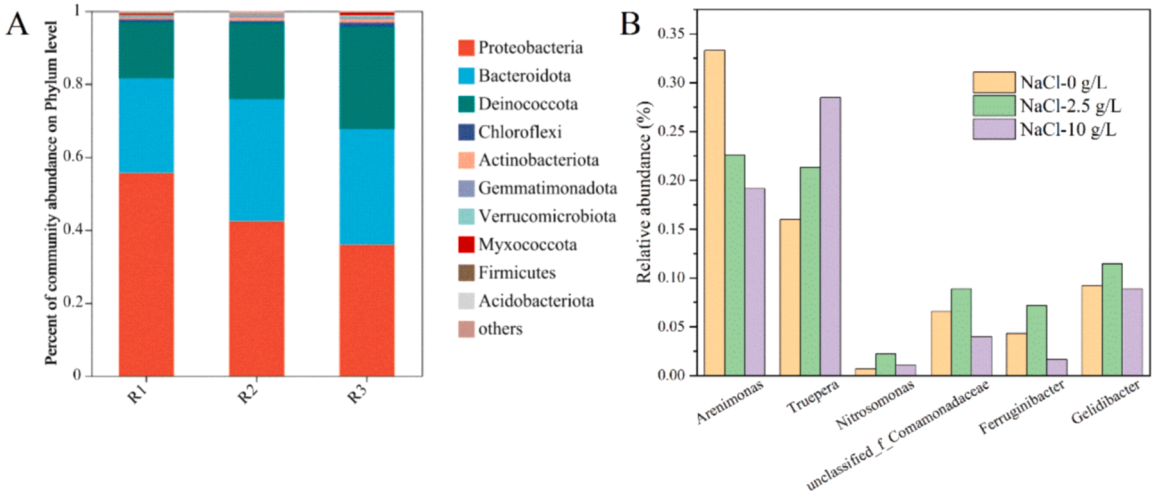


Fig. 4. Relative abundance of bacterial community (A) at phylum level and (B) at genus level in control and experimental reactors after long-term salinity acclimation at different concentrations.

In summary, this study highlights the importance of controlling salinity to optimize PN system performance and reduce N_2O emissions. Future researchers may explore effective N_2O abatement strategies, such as modification of operating parameters or enhancement of microbial adaptations, to further reduce N_2O emissions in full-scale applications.

4. Materials and methods

4.1. Reactor setup and operation

Three replicate SBRs with a valid volume of 4 L were conducted under controlled temperature (25±1 °C) conditions. Each reactor had a cycle time of 360 min, consisted of 25 min settling, 10 min decanting, 5 min anoxic stage I, 5 min feeding (aeration on), 120 min aerobic stage I, 35 min anoxic stage II, 5 min feeding (aeration on), 120 aerobic stage II, 35 min anoxic stage III (Wang et al., 2014). 0.5 L synthetic wastewater was pumped into every reactor during each feeding phase, achieving a hydraulic retention time of 24 h. The PN sludge was obtained from a parent reactor which has been stably operated for one year, with a mixed liquor suspended solids (MLSS) of 1300±150 mg/L in each SBR. Mini CHEM-DO2 and pH probes were used for on-line continuous monitoring in each reactor. The DO was maintained at 0.4~0.6 mg O_2/L through the aerobic stage and pH ranged from 6.7~7.3 during a whole cycle. The components per liter of synthetic influent were as follows: 2292.43 mg NH_4Cl, 3800 mg $NaHCO_3$, 73.6 mg $K_2HPO_4{\cdot}3H_2O$, 15 mg $MgSO_4$, 10 mg $CaCl_2$ and 0.6 mL of trace element solution (see details in Text S1).

After 14 days of operation, the effluent concentrations of NH_4^+, NO_2^- and NO_3^- at the end of reaction were stable and similar in each SBR. In the next period, synthetic wastewater with salinities of 0, 2.5 and 10 g NaCl/L was added into the three parallel reactors (named as R1, R2, and R3), respectively. According to previous studies (Aslan and Simsek, 2012; Xu et al., 2024; Yuan et al., 2021), the salinity of 2.5 g NaCl/L was used as a gradient transition concentration; 10 g NaCl/L had a significant effect on reactor performance, which did not exceed the tolerance limit of AOB. And the two concentrations were therefore selected as the salinity acclimation condition. Apart from the salinity levels, all other conditions and operations remained the same, and the reactors were operated continuously for over 60 days.

4.2. Experiment design

4.2.1. Cycle tests

In order to investigate the long-term effect of different salinities on the PN performance and N_2O emission of the PN sludge, three duplicate cycle tests were implemented on day 60, 65 and 70 during one 6-h cycle. Samples were taken at specific intervals for measuring NH_4^+, NO_2^- and NO_3^-. The whole cycle experiment was conducted in parent reactors, same as the daily operation (see Section 4.1). The gas phase N_2O emissions from each reactor were continuously monitored using an on-line N_2O analyzer. At points of the beginning and end of the anoxic stage I, II, III and settling stage, liquid samples were collected to measure dissolved N_2O concentrations at different stages with a gas chromatograph after processing and stabilizing (see details in Text S2), thereby calculating the N_2O production during nonaerated stages.

4.2.2. Batch tests

To further analyze the long-term effect of different salinities on N_2O emission in PN sludge, a series of batch tests were performed after the reactor had been operating for 60 days and reached a relatively stable state. The operation was similar to previous studies (He et al., 2024). For each batch experiment, 800 mL of sludge mixture liquid was withdrawn from the reactor, centrifuged at 6000 r/min for 10 min to remove the supernatant, and washed three times with tap water before the sludge was re-suspended with 800 mL configured simulated wastewater (with the same initial NH_4^+ and NO_2^- concentrations), which was transferred to a 1.2 L reactor for subsequent experiments at room temperature. In the batch reactor, on-line DO and pH sensors were used to continuously monitor DO and pH throughout the reaction. The gas flow rates of air and argon (Ar) were adjusted by two flow controllers (Liu et al., 2025), and the total sum was always at 1 L/min in all batches. DO value was controlled at 0.5, 1.0, 1.5 mg O_2/L, respectively, by adjusting the flow rate ratio of the air and Ar gases. The pH was maintained at 7.0 during experiments by selectively adding $NaHCO_3$ (0.5 M) or HCl (1 M) solution. The N_2O concentration in the reactor headspace was determined by an on-line N_2O analyzer.

4.3. Calculations

The N_2O collected during the batch experiment was measured in ppmv, requiring unit conversion. The total N_2O emissions were calculated using the following formula:

$$m_{N_2O} = \frac{Q \cdot M_{N_2O} \cdot P}{RT} \sum_2^n \left[\frac{C_{n-1} + C_n}{2} \cdot \Delta t \right]$$

Where Q is the headspace pumping gas flow rate of the reactor used to detect N_2O concentration, L/min; P is atmospheric pressure, 1 atm; R is the gas constant, 0.082 L atm K^{-1} mol^{-1}; T is the temperature, K; C_n is the N_2O concentration of the collected sample, ppmv; Δt is the time interval

at which the data is recorded, 0.5 min.

At the end of each batch test, a 200 mL gas sample was collected from the analyzer inlet for isotopic analysis. Isotopic techniques are essential in identifying N_2O production pathways in wastewater treatment research (Duan et al., 2024), and site preference (SP) was used to calculate isotopic variations. Based on the measured SP value, the contributions of the NH_2OH pathway or nitrite reduction pathway were calculated with the following formula:

$$F_{ND} = (1 - F_{NO}) = \frac{SP_{tot} - 39\%}{-14\% - 39\%}$$

Where F_{ND} is the contribution of nitrite reduction pathway, %; F_{NO} is the contribution of the NH_2OH pathway, %; SP_{tot} is the SP value measured in each set of experiments, ‰; 39 ‰ is the SP signature value of the NH_2OH pathway to produce N_2O; 14 ‰ is the SP signature value of N_2O produced by the nitrite reduction pathway, with reference to actual measured SP value (Duan et al., 2017).

4.4. Other analytical methods

NH_4^+, NO_2^-, NO_3^- concentrations were determined using a UV spectrophotometer. Sludge volume index (SVI), MLSS, and mixed liquor volatile suspended solids (MLVSS) were measured following standard methods (Washington and Association, 1995). The maximum specific ammonia oxidation rate and N_2O emission factor was further calculated based on MLVSS, NH_4^+ concentrations and total N_2O emission. EPS were categorized into soluble EPS (S-EPS), loosely bound EPS (LB-EPS), and TB-EPS. These fractions were obtained using the heat extraction method, and contents (proteins and polysaccharides) were quantified using the phenol-sulfuric method and Lowry-Folin method, respectively (Wang et al., 2022a). The three-dimensional excitation-emission (3D-EEM) fluorescence spectra of different EPS fractions were investigated using a fluorescence spectrometer (Hitachi F-2710, Japan) (Wang et al., 2024c). And further analysis of TB-EPS was conducted using FTIR spectroscopy (Dai et al., 2024). The particle size distribution of activated PN sludge was determined by using a particle size analyzer (ZS3000, Malvern Instruments, UK). The LDH release can be used to reflect sludge cell integrity (Wang et al., 2022b), and methods for determining LDH concentrations are listed in the Supplementary Material (Text S3). SP measurements were performed using a N_2O isotope analyzer (LGR914–0060). High-throughput sequencing was employed to characterize bacterial community variations under different salinity acclimations. Sludge samples were obtained from three SBRs on day 60, and real-time PCR assays targeting the V3-V4 region of bacterial 16S DNA genes were conducted to measure microbial community compositions. Illumina Novaseq6000 was utilized for sequencing analysis.

CRediT authorship contribution statement

Xiang Li: Writing – original draft, Visualization, Investigation, Formal analysis, Data curation, Conceptualization. **Yingxin Jin:** Writing – review & editing, Methodology, Data curation, Conceptualization. **Yanying He:** Writing – review & editing, Supervision, Resources, Methodology, Funding acquisition, Conceptualization. **Yufen Wang:** Writing – review & editing, Supervision, Methodology, Investigation, Formal analysis. **Tingting Zhu:** Validation, Supervision, Resources, Methodology, Conceptualization. **Yingxin Zhao:** Supervision, Resources, Investigation, Conceptualization. **Bing-Jie Ni:** Supervision, Resources, Methodology, Investigation, Conceptualization. **Yiwen Liu:** Writing – review & editing, Supervision, Resources, Methodology, Funding acquisition, Conceptualization.

Declaration of competing 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.

Acknowledgments

Corresponding author Yanying He is funded by the Shanghai Tongji Gao Tingyao Environmental Science & Technology Development Foundation.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.wroa.2025.100311.

Data availability

Data will be made available on request.

References

Aslan, S., Simsek, E., 2012. Influence of salinity on partial nitrification in a submerged biofilter. Bioresour. Technol. 118, 24–29.
Chen, H., Zeng, L., Wang, D., Zhou, Y., Yang, X., 2020. Recent advances in nitrous oxide production and mitigation in wastewater treatment. Water Res. 184, 116168.
Chen, S., Yang, D., Wang, Q., Huang, X., Ren, H., Xu, K., 2022. Study on the advanced nitrogen removal under low temperature by biofilm on weak magnetic carriers. Bioresour. Technol. 360, 127580.
Corsino, S.F., Capodici, M., Torregrossa, M., Viviani, G., 2017. Physical properties and extracellular Polymeric substances pattern of aerobic granular sludge treating hypersaline wastewater. Bioresour. Technol. 229, 152–159.
Dai, H., Han, T., Sun, T., Zhu, H., Wang, X., Lu, X., 2021. Nitrous oxide emission during denitrifying phosphorus removal process: a review on the mechanisms and influencing factors. J. Environ. Manag. 278, 111561.
Dai, S., Guo, H., Li, Y., Hou, J., Wang, Y., Zhu, T., Ni, B.J., Liu, Y., 2024. Application of organic silicon quaternary ammonium salt (QSA) to reduce carbon footprint of sewers: long-term inhibition on sulfidogenesis and methanogenesis. Water Res. X 25, 100275.
Dalpadado, P., Roxy, M.K., Arrigo, K.R., van Dijken, G.L., Chierici, M., Ostrowski, M., Skern-Mauritzen, R., Bakke, G., Richardson, A.J., Sperfeld, E., 2024. Rapid climate change alters the environment and biological production of the Indian Ocean. Sci. Total Environ. 906, 167342.
Duan, H., Watt, S., Erler, D., Li, H., Wang, Z., Zheng, M., Hu, S., Ye, L., Yuan, Z., 2024. Coarse bubble mixing in anoxic zone greatly stimulates nitrous oxide emissions from biological nitrogen removal process. Water Res. X 25, 100263.
Duan, H., Ye, L., Erler, D., Ni, B.J., Yuan, Z., 2017. Quantifying nitrous oxide production pathways in wastewater treatment systems using isotope technology – A critical review. Water Res. 122, 96–113.
Ghanbari, M., Arabi, M., Georgescu, M., Broadbent, A.M., 2023. The role of climate change and urban development on compound dry-hot extremes across US cities. Nat. Commun. 14 (1), 3509.
Gruber, W., Magyar, P.M., Mitrovic, I., Zeyer, K., Vogel, M., von Känel, L., Biolley, L., Werner, R.A., Morgenroth, E., Lehmann, M.F., Braun, D., Joss, A., Mohn, J., 2022. Tracing N_2O formation in full-scale wastewater treatment with natural abundance isotopes indicates control by organic substrate and process settings. Water Res. X 15, 100130.
Gruber, W., von Känel, L., Vogt, L., Luck, M., Biolley, L., Feller, K., Moosmann, A., Krähenbühl, N., Kipf, M., Loosli, R., Vogel, M., Morgenroth, E., Braun, D., Joss, A., 2021. Estimation of countrywide N_2O emissions from wastewater treatment in Switzerland using long-term monitoring data. Water Res. X 13, 100122.
Guo, H., Tian, L., Liu, S., Wang, Y., Hou, J., Zhu, T., Liu, Y., 2023. The potent effects of polyoxometalates (POMs) on controlling sulfide and methane production from sewers. Chem. Eng. J. 453, 139955.
Hausherr, D., Niederdorfer, R., Bürgmann, H., Lehmann, M.F., Magyar, P., Mohn, J., Morgenroth, E., Joss, A., 2022. Successful year-round mainstream partial nitritation anammox: assessment of effluent quality, performance and N_2O emissions. Water Res. X 16, 100145.
He, H., Chen, Y., Li, X., Cheng, Y., Yang, C., Zeng, G., 2017. Influence of salinity on microorganisms in activated sludge processes: a review. Int. Biodeterior. Biodegrad. 119, 520–527.
He, Y., Li, Y., Li, X., Liu, Y., Wang, Y., Guo, H., Hou, J., Zhu, T., Liu, Y., 2023. Net-zero greenhouse gas emission from wastewater treatment: mechanisms, opportunities and perspectives. Renew. Sustain. Energy Rev. 184, 113547.
He, Y., Liu, Y., Li, X., Guo, H., Zhu, T., Liu, Y., 2024. Polyvinyl chloride microplastics facilitate nitrous oxide production in partial nitritation systems. Environ. Sci. Technol. 58 (4), 1954–1965.
Kong, Q., Liang, S., Zhang, J., Xie, H., Miao, M., Tian, L., 2013. N2O emission in a partial nitrification system: dynamic emission characteristics and the ammonium-oxidizing bacteria community. Bioresour. Technol. 127, 400–406.

Lackner, S., Gilbert, E.M., Vlaeminck, S.E., Joss, A., Horn, H., van Loosdrecht, M.C.M., 2014. Full-scale partial nitritation/anammox experiences – An application survey. Water Res. 55, 292–303.
Laureni, M., Falås, P., Robin, O., Wick, A., Weissbrodt, D.G., Nielsen, J.L., Ternes, T.A., Morgenroth, E., Joss, A., 2016. Mainstream partial nitritation and anammox: long-term process stability and effluent quality at low temperatures. Water Res. 101, 628–639.
Liu, X., Yao, T., Chai, J., Han, J., 2023. Adsorption of sodium ions by exopolysaccharides from Pseudomonas simiae MHR6 and its improvement of Na+/K+ homeostasis in Maize under salt stress. J. Agric. Food Chem. 71 (50), 19949–19957.
Liu, Y., Chen, F., He, Y., Wang, Y., Zhu, T., Tong, Y., Zhao, Y., Ni, B.J., Liu, Y., 2025. Evaluation of nitrous oxide reduction in solid carbon source-driven counter-diffusional biofilm denitrification system. Water Res. X. 27, 100306.
Liu, Y., He, Y., Lu, Q., Zhu, T., Wang, Y., Tong, Y., Zhao, Y., Ni, B.J., Liu, Y., 2025. Smaller sizes of polyethylene terephthalate microplastics mainly stimulate heterotrophic N_2O production in aerobic granular sludge systems. Water Res. X 27, 100299.
Moussa, M.S., Sumanasekera, D.U., Ibrahim, S.H., Lubberding, H.J., Hooijmans, C.M., Gijzen, H.J., van Loosdrecht, M.C.M., 2006. Long term effects of salt on activity, population structure and floc characteristics in enriched bacterial cultures of nitrifiers. Water Res. 40 (7), 1377–1388.
Ni, B.J., Zeng, R.J., Fang, F., Xie, W.M., Xu, J., Sheng, G.P., Sun, Y.J., Yu, H.Q., 2011. Evaluation on factors influencing the heterotrophic growth on the soluble microbial products of autotrophs. Biotechnol. Bioeng. 108 (4), 804–812.
Shi, S., Lin, Z., Zhou, J., Fan, X., Huang, Y., Zhou, J., 2022. Enhanced thermophilic denitrification performance and potential microbial mechanism in denitrifying granular sludge system. Bioresour. Technol. 344, 126190.
Speirs, L.B.M., Rice, D.T.F., Petrovski, S., Seviour, R.J., 2019. The phylogeny, biodiversity, and ecology of the chloroflexi in activated sludge. Front. Microbiol. 10, 2015.
Tian, B., Hua, Y., 2010. Carotenoid biosynthesis in extremophilic deinococcus–Thermus bacteria. Trends Microbiol 18 (11), 512–520.
Tong, Y., Liao, X., He, Y., Cui, X., Wishart, M., Zhao, F., Liao, Y., Zhao, Y., Lv, X., Xie, J., Liu, Y., Chen, G., Hou, L., 2024. Mitigating greenhouse gas emissions from municipal wastewater treatment in China. Environ. Sci. Ecotechnol. 20, 100341.
Wang, M., He, J., Dong, X., Zhang, J., 2023a. Effect of salinity on performance and microbial community during granulation process in a sequencing batch reactor. Water 15 (22), 3961 (Basel).
Wang, Q., Jiang, G., Ye, L., Pijuan, M., Yuan, Z., 2014. Heterotrophic denitrification plays an important role in N_2O production from nitritation reactors treating anaerobic sludge digestion liquor. Water Res. 62, 202–210.
Wang, X., Zhang, Z., Yang, X., Wang, Y., Li, Y., Zhu, T., Zhao, Y., Ni, B.J., Liu, Y., 2024a. Interaction of poly dimethyl diallyl ammonium chloride with sludge components: anaerobic digestion performance and adaptive changes of anaerobic microbes. Water Res. 266, 122368.
Wang, Y., Chen, F., Guo, H., Sun, P., Zhu, T., Horn, H., Liu, Y., 2024c. Permanganate (PM) pretreatment improves medium-chain fatty acids production from sewage sludge: the role of PM oxidation and in-situ formed manganese dioxide. Water Res. 249, 120869.
Wang, Y., Guo, H., Li, X., Chen, X., Peng, L., Zhu, T., Sun, P., Liu, Y., 2024b. Peracetic acid (PAA)-based pretreatment effectively improves medium-chain fatty acids (MCFAs) production from sewage sludge. Environ. Sci. Ecotechnol. 20, 100355.
Wang, Y., Sun, P., Guo, H., Zheng, K., Zhu, T., Liu, Y., 2022b. Performance and mechanism of sodium percarbonate (SPC) enhancing short-chain fatty acids production from anaerobic waste activated sludge fermentation. J. Environ. Manag. 313, 115025.
Wang, Y., Zheng, K., Guo, H., Tian, L., He, Y., Wang, X., Zhu, T., Sun, P., Liu, Y., 2023b. Potassium permanganate-based advanced oxidation processes for wastewater decontamination and sludge treatment: a review. Chem. Eng. J. 452, 139529.
Wang, Y., Zheng, K., Guo, H., Tong, Y., Zhu, T., Liu, Y., 2022a. Unveiling the mechanisms of how vivianite affects anaerobic digestion of waste activated sludge. Bioresour. Technol. 343, 126045.
Wang, Z., Luo, G., Li, J., Chen, S.Y., Li, Y., Li, W.T., Li, A.M., 2016. Response of performance and ammonia oxidizing bacteria community to high salinity stress in membrane bioreactor with elevated ammonia loading. Bioresour. Technol. 216, 714–721.
Washington, D., Association, C., 1995. Standard methods for the examination of water and wastewater. Am. Phys. Educ. Rev. 24, 481–486.
Xing, W., Li, J., Li, D., Hu, J., Deng, S., Cui, Y., Yao, H., 2018. Stable-isotope probing reveals the activity and function of autotrophic and heterotrophic denitrifiers in nitrate removal from organic-limited wastewater. Environ. Sci. Technol. 52 (14), 7867–7875.
Xu, J., Liu, S., Zhou, J., Li, L., Bi, X., Han, W., Wu, D., 2024. Response of aerobic granular sludge to salinity fluctuations in formation, stability and microbial community structures. Sci. Total Environ. 954, 176471.
Ye, L., Peng, C.Y., Tang, B., Wang, S.Y., Zhao, K.F., Peng, Y.Z., 2009. Determination effect of influent salinity and inhibition time on partial nitrification in a sequencing batch reactor treating saline sewage. Desalination 246 (1), 556–566.
Yuan, Y., Zhou, Z., Jiang, J., Wang, K., Yu, S., Qiang, J., Ming, Q., An, Y., Ye, J., Wu, D., 2021. Partial nitrification performance and microbial community evolution in the membrane bioreactor for saline stream treatment. Bioresour. Technol. 320, 124419.
Zhang, Z., Wang, X., Yang, X., Wang, Y., Li, X., Ding, J., Zhu, T., Sun, P., Ni, B.J., Liu, Y., 2025. Unveiling the synergistic potential of ferrate and pyrophosphate co-pretreatment enhancing anaerobic sludge fermentation: performance and mechanistic insights. Chem. Eng. J. 505, 159612.
Zhang, B., Xu, H., Zhang, H., Chen, Z., Shi, H., Chen, S., Wang, X., 2024a. Dual isotope labelling combined with multi-omics analysis revealing the N_2O source evolution in aerobic biological systems driven by salinity gradient. Sci. Total Environ. 956, 177262.
Zhang, Y., Li, J., Chen, Y., Yang, J., Chen, Z., Wang, X., 2024b. Rapid start-up and stable operation of pilot scale denitrification-partial nitritation/anammox process for treating electroplating tail wastewater. Bioresour. Technol. 409, 131192.
Zhang, Z.J., Chen, S.H., Wang, S.M., Luo, H.Y., 2011. Characterization of extracellular polymeric substances from biofilm in the process of starting-up a partial nitrification process under salt stress. Appl. Microbiol. Biotechnol. 89 (5), 1563–1571.
Zhao, W., Wang, Y., Lin, X., Zhou, D., Pan, M., Yang, J., 2014. Identification of the salinity effect on N_2O production pathway during nitrification: using stepwise inhibition and 15N isotope labeling methods. Chem. Eng. J. 253, 418–426.
Zhao, Y., Ab Hamid, N.H., Reddy, N., Zheng, M., Yuan, Z., Duan, H., Ye, L., 2022. Wastewater primary treatment using forward osmosis introduces inhibition to achieve stable mainstream partial nitrification. Environ. Sci. Technol. 56 (12), 8663–8672.
Zhou, S., Gong, H., Xu, E., Chen, X., Wang, X., Wang, H., Zhu, D., Zhang, Y., Yang, J., Gu, G., Dai, X., 2024. Start-up of a full-scale two-stage partial nitritation/anammox (PN/A) process treating reject water from high solid anaerobic sludge digestion (HSAD). Water Res. X 25, 100259.