Proceedings of the 11th World Congress on New Technologies (NewTech'25)
Paris, France - August, 2025
Paper No. ICBB 144
DOI: 10.11159/icbb25.144

Novel Nitrification Process Enabling the Production of High-Concentration Nitrate Solution from Exhaust Ammonia Gas

Mitsuhiko Koyama[1], Arisa Horiuchi[1]
[1]Graduate School of Integrated Science and Technology, Nagasaki University
1-14 Bunkyo-machi, Nagasaki-shi, Nagasaki 852-8521, Japan
koyama.m@nagasaki-u.ac.jp; bb40121503@ms.nagasaki-u.ac.jp

Abstract This study investigated a novel nitrification system designed to recover ammonia gas and convert it into high-concentration nitrate while maintaining microbial activity under high osmotic pressure. In Study 1, activated sludge from a municipal wastewater treatment plant was used to initiate nitrification under increasing nitrate concentrations. Nitrifiers remained active even at nitrate levels exceeding 7,000 mg-N/L, and community analysis revealed a shift from *Nitrospira* to *Nitrobacter*-like and *Nitrosomonas* species. In Study 2, sludge was acclimated to initial nitrate concentrations above 2,000 mg-N/L, promoting the enrichment of nitrifiers tolerant to osmotic stress. In Study 3, the maximum stable ammonia loading rate was determined to be 529 mg-N/L/d at pH 7.0 and 25°C. While the volumetric ammonia removal rate decreased with increasing osmolarity, biomass-specific rates were comparable to the highest benchmark values. These results provide a foundation for optimizing compact systems that recover ammonia gas at low cost and upcycle it into concentrated algal media or nitrate fertilizers.

Keywords**:** nitrification; high-concentration nitrate accumulation; ammonia gas recovery; nitrifying bacterial community; 16S rRNA gene sequencing

1. Introduction

Major sources of ammonia gas emissions are composting and livestock facilities, which account for over 60% of global atmospheric ammonia release [1]. These emissions contribute to the degradation of conditions for living, working, and animal rearing environments, both inside and outside the facilities. Furthermore, ammonia gas serves as a key precursor to PM2.5, significantly contributing to air pollution [2]. Reducing ammonia gas emissions in the agricultural sector is therefore an urgent global challenge. Technologies for recovering and utilizing ammonia gas from composting and livestock facilities are essential, not only for odour control but also for promoting nitrogen resource recycling.

Volatilized ammonia gas, though waste-derived, can serve as a clean nitrogen source for high-value microalgae cultivation [3]. Carotenoids such as astaxanthin, valued at USD 6,000 per kg, are among the key compounds produced [4]. However, the application of ammonia is constrained by the low tolerance of many microalgal species. Nitrate is generally regarded as a less toxic and more favourable nitrogen form. Converting ammonia to nitrate is therefore expected to facilitate the high-density cultivation of a wider range of species, while also reducing odour emissions and enhancing economic sustainability.

We have proposed a novel nitrification system that recovers ammonia gas and transforms it into an extremely high concentration of nitrate while maintaining nitrifying bacterial activity and preventing acidification. This process is based on three key principles: (1) maintaining a neutral pH while co-accumulating high concentrations of nitrate and ammonium by neutralizing nitrate with ammonium and alkaline agents within the reactor; (2) developing a nitrifying bacterial community that remains active under high osmotic pressure; and (3) utilizing a pH control system with electrodialysis to recycle neutralizing agents and automatically adjust pH in response to fluctuations in ammonia gas supply. Highly concentrated nitrate solutions, due to their high portability and long-term stability, have strong potential for widespread use as concentrated nitrogen media for cultivating high-value microalgae and as liquid fertilizers for hydroponics. This innovative approach combines microbial adaptability and precise chemical control to achieve efficient and stable high-nitrate accumulation. By enabling efficient ammonia recovery and nitrogen reuse, this system has the potential to improve nitrogen management in remote and resource-limited settings. With further research and optimization, it may be adaptable to closed environments like polar bases and space stations.

Conventional nitrification technologies have been developed primarily as a pretreatment for denitrification processes, where nitrate is regarded as a target for removal. As such, the accumulation of high concentrations of nitrate within the reactor has not been considered, and typical nitrate concentrations remain around 10–100 mg-N/L. In recent years, studies have explored the use of human urine as a nitrogen source for nitrification to recover nitrate as fertilizer, reporting nitrate concentrations of 2,000–4,000 mg-N/L by continuously supplying alkaline agents [5], [6]. However, these concentrations are limited by the nitrogen content of urine, and the behaviour of nitrifying bacteria under even higher nitrate concentrations remains unclear. In contrast, ammonia gas is highly soluble in water and allows for higher nitrogen input, suggesting the potential for further nitrate accumulation. Nevertheless, no studies to date have experimentally examined this possibility.

The aim of this study was to develop a novel nitrification system capable of stably accumulating high-concentration nitrate while maintaining the activity of nitrifying microorganisms under high osmotic pressure. To achieve this, we examined microbial adaptation, reactor operation strategies, and ammonia loading limits using activated sludge acclimated to nitrate-rich conditions.

2. Materials and methods

2.1. Fundamental investigation on a novel nitrification system (Study 1)

Seed sludge was obtained from the aerobic tank of a municipal wastewater treatment plant in Omura City, Nagasaki Prefecture, Japan. A single 20-L polyethylene (PE) tank was used as the nitrification bioreactor, with an effective volume of 15 L. In this system, ammonia is oxidized to nitrite and nitrate, causing a decrease in pH. To maintain pH within the range of 7.0–7.5, a pH controller (PPH-2108, SATOTECH, Japan) equipped with a pH probe (PE-21, SATOTECH, Japan) was employed. Ammonia water (0.5–1.0 M) was used as both the substrate and an alkaline agent to simulate high concentrations of gaseous ammonia. When nitrite began to accumulate—indicating an imbalance in the nitrification process—the ammonia water supply was temporarily stopped and replaced with a NaOH solution (1–2 mol/L) solely for pH control. The ammonia supply was resumed once the nitrite concentration decreased. Both ammonia water and NaOH were supplied at high concentrations to minimize the liquid volume added to the system. This approach allowed the evaporation rate of water from the reactor and the liquid supply rate to be roughly balanced, enabling the reactor to operate under a near-infinite hydraulic retention time (HRT) condition. The reactor was operated in a temperature-controlled room maintained at 25 ± 2°C. Electrodialysis was not employed in this study, as the focus was on investigating fundamental parameters and analysing transitions and dynamics within the nitrifying bacterial community.

2.2. Enrichment of nitrifier community tolerable to high-concentration of nitrate salt (Study 2)

Activated sludge collected from a municipal wastewater treatment plant in Omura City, Nagasaki Prefecture, Japan, was used as the seed. Three nitrification reactors were prepared, each supplemented with sodium nitrate to establish initial nitrate concentrations of 1,000, 2,000, and 3,000 mg-N/L. The initial ammonia concentration was set at 100 mg-N/L using ammonium chloride as the substrate. Semi-continuous operation was performed, starting with an ammonia loading rate of 10 mg-N/L/d, which was then stepwise increased to 50 and 100 mg-N/L/d. The pH of the reactors was maintained at 7.0 using a pH controller. Samples were periodically collected from each reactor, and batch activity tests were conducted at a nitrate concentration of 4,000 mg-N/L to evaluate nitrification performance. Ammonia-oxidizing activity and nitrite-oxidizing activity were independently monitored in separate batch reactors. The time-course data of ammonia and nitrite concentrations were fitted to a first-order reaction model, and the rate constant k was calculated using the least-squares method.

2.3. Evaluation of tolerable ammonia loading rate (Study 3)

As inoculum, nitrifying sludge that had been acclimated in our laboratory to high-nitrate conditions over approximately six months was used. A single nitrification reactor was operated at 25°C with pH maintained at 7.0. Ammonia water (1–2 mol/L) was continuously supplied as the substrate, starting with an ammonia loading rate of 100 mg-N/L/d. The rate was then gradually increased while monitoring nitrogen species concentrations to evaluate the maximum ammonia loading rate tolerable by the microbial community under stable nitrification conditions. To minimize the liquid volume added to the

system and achieve near-infinite HRT, both ammonia water and NaOH were supplied at high concentrations, similar to Study 1. This setup allowed the evaporation rate and liquid supply rate to remain roughly balanced.

2.4. Microbial community analysis

DNA was extracted from sludge samples for 16S rRNA gene analysis to investigate microbial community composition. DNA extraction was performed using the ISOIL for Beads Beating Kit (NIPPON GENE, Japan). To comprehensively and cost-effectively assess microbial community dynamics across multiple samples, the V3–V4 regions of the 16S rRNA gene were amplified according to the Illumina 16S metagenomic sequencing library preparation protocol, and sequencing was outsourced. Sequence data were analysed using QIIME2 (ver. 2024.10). Noise-filtered reads were assigned to operational taxonomic units (OTUs) using a Naive Bayes classifier trained on the SILVA database (ver. 138, 99% OTUs, full-length).

3. Results and discussion

3.1. Fundamental investigation on a novel nitrification system (Study 1)

Fig. 1 shows the time course of pH and inorganic nitrogen species in the nitrification bioreactor. During the period of ammonia water addition, NO_3^--N concentrations gradually increased and reached their peak between days 60 and 90 of operation. In Reactor #1, the concentration rose to approximately 6,000–7,000 mg-N/L, which corresponds to an osmolality of about 0.9–1.0 Osmol/L, assuming complete neutralization with NaOH. These concentrations exceed those commonly reported in prior nitrification studies. For example, recent research on urine nitrification processes has demonstrated the production of relatively high-concentration nitrate (2,000–4,000 mg-N/L)[5], [6], resulting in estimated osmolality of 0.3–0.6 Osmol/L. Since nitrate accumulation in such systems is limited by the nitrogen content of urine, few studies have explored nitrifier behavior under higher concentrations. This study shows that nitrifiers from low-salinity sludge remained active even under nitrate levels comparable to seawater osmolality.

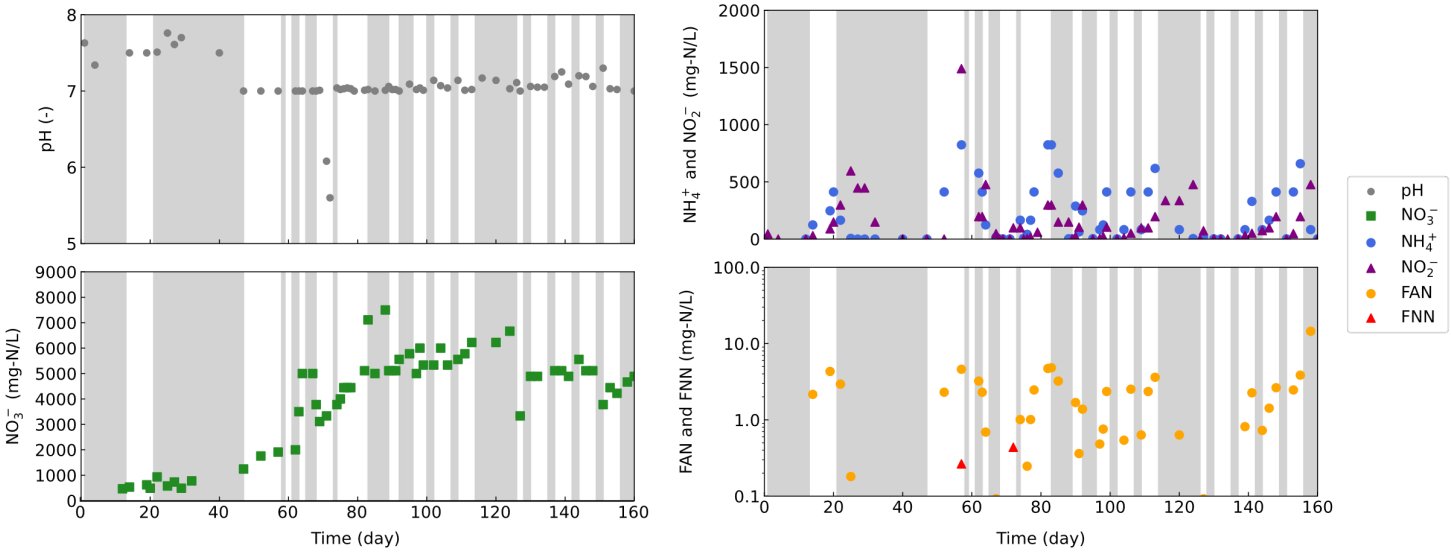


Fig. 1: Time course of pH, NO_3^--N, NO_2^--N, NH_4^+-N, free ammonia nitrogen (FAN), and free nitrite nitrogen (FNN) concentrations in the nitrification bioreactor. The reactor was seeded with activated sludge freshly collected from a municipal wastewater treatment plant. Shaded regions indicate periods during which ammonia supply was interrupted and NaOH was automatically added for pH adjustment.

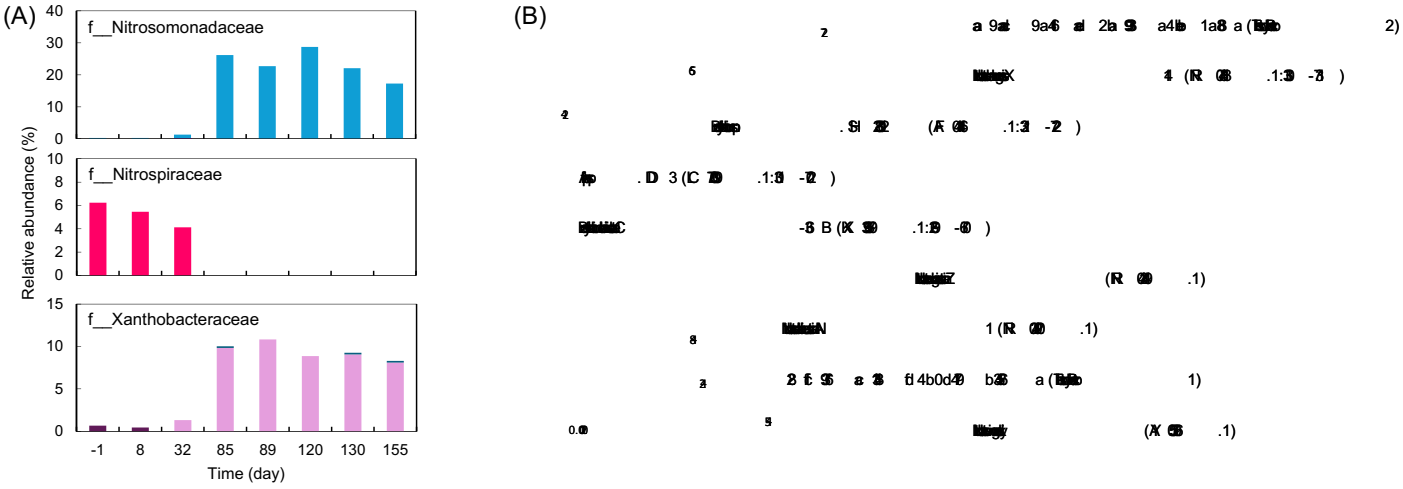
Temporal fluctuations in TAN and TNN were observed during reactor operation (Fig.1), with peak values of 823 mg-N/L and 1,489 mg-N/L, respectively. While ammonium and nitrite ions are selectively transported, their unionized forms—free ammonia nitrogen (FAN) and free nitrite nitrogen (FNN)—can diffuse across cell membranes and exert toxic effects. In

this study, FAN and FNN averaged 4 and 0.02 mg-N/L, with occasional spikes (e.g., 159 mg-N/L of FAN), yet remained mostly below inhibitory thresholds (25–36 mg-N/L for FAN [7], 0.2–2.8 mg-N/L for FNN [8]). Maintaining pH at 7.0–7.5 likely minimized the formation of these species. Notably, ammonia functioned both as a substrate and an alkaline agent, enabling proportional dosing in response to nitrification. However, this dual role led to variable supply rates that were difficult to monitor. For improved stability and clearer evaluation of ammonia loading limits, future studies should employ constant ammonia supply and rely on inorganic alkaline agents such as NaOH for pH control.

An increase in nitrate concentrations was accompanied by shifts in the bacterial community, particularly among nitrification-related families. *Nitrosomonadaceae* showed a marked increase during the mid-to-late stages of operation. This family includes two genera: *Nitrosomonas* (ammonia-oxidizing bacteria, AOB) and *Nitrosospira* (nitrite-oxidizing bacteria, NOB). Genus-level analysis revealed that nearly all *Nitrosomonadaceae* members belonged to *Nitrosomonas* (Fig. 2A). In contrast, *Nitrospira* was not detected after day 32, likely due to inhibition from accumulated nitrate. No known NOB were detected under high nitrate conditions, raising the question of which organisms were responsible for nitrite oxidation. Within *Xanthobacteraceae*, *Nitrobacter* is a known NOB genus. However, this family includes seven other genera and at least 28 additional species [9]. Genus-level classification using a Naive Bayes classifier trained on the SILVA 138 database indicated that most sequences remained unclassified (Fig. 2A), suggesting that higher-resolution analysis is needed to confirm *Nitrobacter* dominance.

To investigate the function of these unclassified *Xanthobacteraceae*, amplicon sequence variants (ASVs) were analysed, and a phylogenetic tree was constructed using ASVs and known *Nitrobacter* 16S rRNA sequences (Fig. 2B). The dominant ASVs were closely related to salt-tolerant *Nitrobacter winogradskyi* [10]. *Nitrobacter* has a higher Km for nitrite than Nitrospira, indicating lower substrate affinity [11]. In summary, this study observed a shift from *Nitrospira* to *Nitrobacter* dominance under high nitrite concentrations, likely driven by Nitrobacter's superior ability to adapt to high-nitrite conditions.

Fig. 2: Nitrifying bacteria community. (A) Relative abundance of bacterial genera within the major families



Nitrosomonadaceae, *Nitrospiraceae* and *Xanthobacteraceae*, (B) phylogenic tree of a *Xanthobacteraceae* feature ID and their closely related bacteria.

3.2. Enrichment of nitrifier community tolerable to high-concentration of nitrate salt (Study 2)

During the early phase of operation, reactors with higher initial nitrate concentrations exhibited transient nitrite accumulation, suggesting that nitrite-oxidizing bacteria (NOB) were not yet adapted to high-nitrate conditions (data not shown). However, after day 20, nitrite accumulation was no longer observed, indicating the onset of NOB adaptation. No accumulation of nitrite was observed during the semi-continuous supply of ammonia, suggesting efficient nitrification and a

gradual increase in nitrate concentrations. In Reactor #1, #2, and #3, nitrate levels reached 3,310, 4,954, and 6,784 mg-N/L, respectively, by day 70. Fig. 3A shows the results of batch nitrification tests using ammonia or nitrite as substrates. The reaction rate constants (k), estimated using a first-order kinetic model, increased during the mid-to-late stages of operation. Ammonia oxidation followed the order Reactor #3 > #2 > #1, whereas nitrite oxidation showed a different trend: Reactor #2 > #1 > #3. These results suggest that acclimating sewage sludge to high-nitrate environments (>2,000 mg-N/L) is effective for establishing a nitrification process capable of high nitrate accumulation. They also indicate that ammonia-oxidizing bacteria (AOB) adapt more readily to high-nitrate conditions than NOB. In the later stages, shifts in the microbial community were observed, with an increased abundance of both AOB and NOB adapted to elevated nitrate concentrations.

Fig. 3B shows the succession of the nitrifying bacteria community. In all reactors, *Nitrospira* was the dominant NOB, and in Reactor #1, which had the lowest nitrate concentration, it was the only nitrifying taxon detected. Although traditionally recognized as a NOB, the *Nitrospira* in this reactor may have been a complete ammonia oxidizer (COMAMMOX), capable of oxidizing both ammonia and nitrite. In contrast, in Reactors #2 and #3, which operated under higher nitrate concentrations, the relative abundance of *Nitrospira* declined. Instead, *Nitrosomonas* (an AOB) and members of *Xanthobacteraceae* closely related to *Nitrobacter* (a NOB), both previously observed in Study 1, became more dominant. These findings suggest that acclimation at initial nitrate concentrations of 2,000 mg-N/L or higher promotes the enrichment of nitrifying populations capable of adapting to high-osmotic-pressure environments.

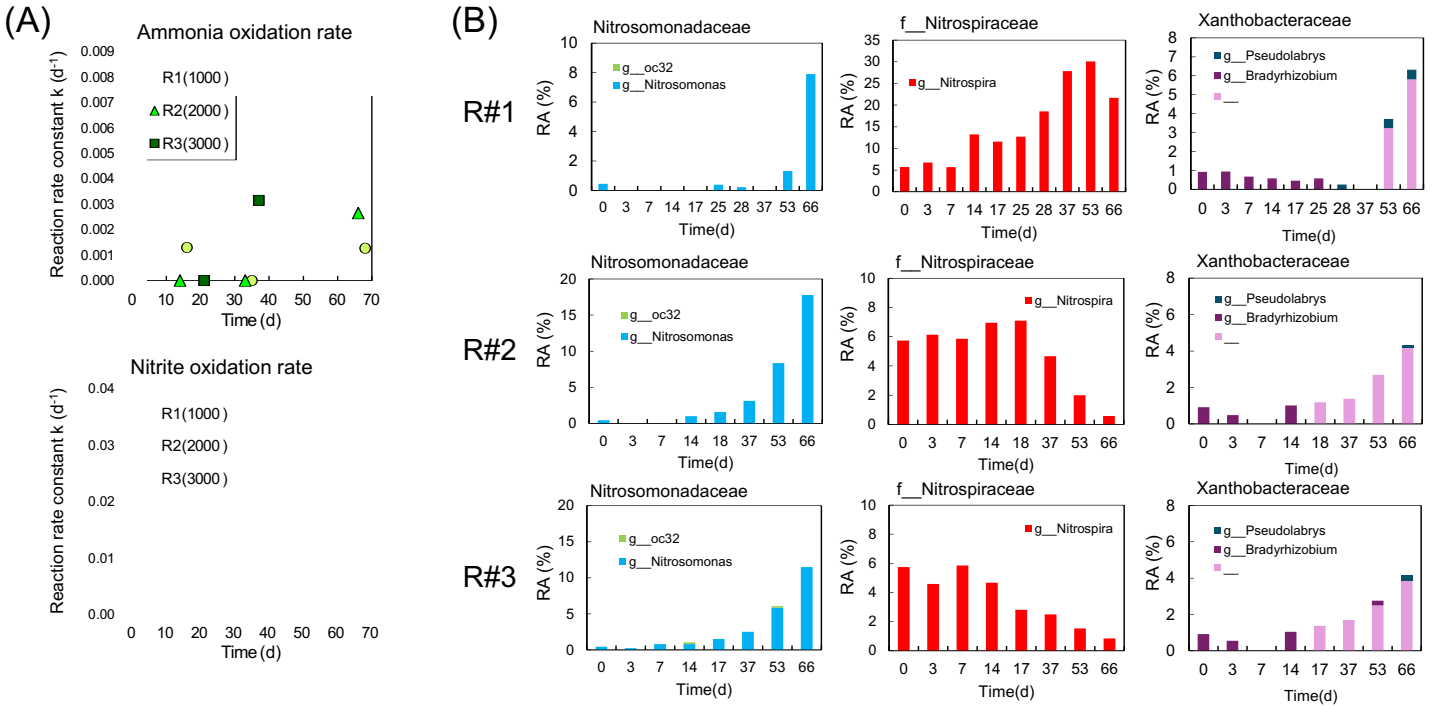
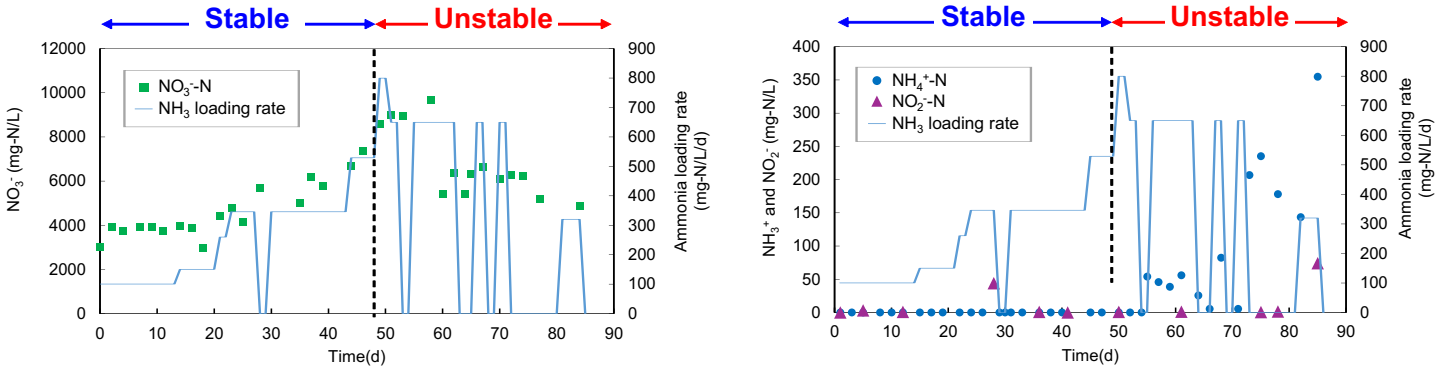


Fig. 3: Nitrification performance and microbial community dynamics under different initial nitrate concentrations. (A) Reaction rate constants (k) for ammonia and nitrite oxidation obtained from batch nitrification tests using a first-order kinetic model. (B) Succession of nitrifying bacterial community composition at the genus level.

3.3. Evaluation of tolerable ammonia loading rate (Study 3)

Fig. 4 shows the time course of nitrate concentrations in a continuous nitrification experiment, where ammonia water was supplied continuously, and the loading rate was gradually increased. Stable nitrification was achieved without the accumulation of nitrite or ammonia up to an ammonia loading rate of 529 mg-N/L/d. However, when the loading rate exceeded this value, both nitrite and ammonia began to accumulate, indicating that the nitrification process had become unstable. These results suggest that the upper limit of the optimal ammonia loading rate under the conditions of pH 7.0 and 25°C is 529 mg-N/L/d. The ammonia removal performance obtained in this study was also compared with those reported in previous studies (data not shown). The volumetric ammonia removal rate tended to decrease with increasing osmolarity, a proxy for osmotic pressure. However, when recalculated on a per biomass basis, the removal rate in this study was comparable to the highest values reported in the literature. These findings suggest that increasing the microbial biomass in the reactor could elevate the volumetric ammonia removal rate to the highest benchmark values observed in previous studies.

Fig. 4: Time course of NO_3^--N, NO_2^--N, and NH_4^+-N concentrations in the nitrification bioreactor during a continuous experiment with stepwise increases in ammonia loading rate.



4. Conclusion

This study demonstrated that a high-concentration nitrification system can accumulate over 7,000 mg-N/L of nitrate while maintaining microbial activity. Nitrifying bacteria derived from activated sludge remained active under osmotic pressures comparable to seawater. Acclimation at nitrate concentrations above 2,000 mg-N/L promoted the enrichment of nitrifiers tolerant to high osmotic stress. The upper limit of the stable ammonia loading rate was identified as 529 mg-N/L/d at pH 7.0 and 25°C. Biomass-specific removal rates were comparable to the highest values reported previously, indicating potential for further improvement by increasing microbial density. This study highlights the potential of a high-nitrate nitrification system to recover ammonia gas at low cost and upcycle it into concentrated algal medium or nitrate fertilizer for use in closed and remote environments.

Acknowledgements

This research was supported by a 2023 research grant from the Amano Institute of Technology, the Nagasaki University Research Fund (2023 Integrated Science and Technology Research Project), and a JSPS KAKENHI Grant-in-Aid for Scientific Research (B) [Grant Number: to be assigned]. We would like to express our sincere gratitude to all those involved. We also thank the Omura City Sewerage Department for providing sewage sludge samples used in this research.

References

[1] R. B. Bist, S. Subedi, L. Chai, and X. Yang, “Ammonia emissions, impacts, and mitigation strategies for poultry production: A critical review,” *J Environ Manage*, vol. 328, p. 116919, Feb. 2023,

[2] S. R. Lee and G. Kim, “Assessing Ammonia (NH_3) Emissions, Precursor Gas (SO2, NOx) Concentrations, and Source Contributions to Atmospheric PM2.5 from a Commercial Manure Composting Facility,” *Applied Sciences 2024, Vol. 14, Page 11467*, vol. 14, no. 23, p. 11467, Dec. 2024,

[3] M. Koyama, N. Nagao, F. Syukri, A. Abd. Rahim, T. Toda, QNM. Tran, K. Nakasaki, "Ammonia recovery and microbial community succession during thermophilic composting of shrimp pond sludge at different sludge properties," *J Clean Prod*, vol. 251, p. 119718, Apr. 2020,

[4] A. Dawidziuk, D. Popiel, M. Luboinska, M. Grzebyk, M. Wisniewski, and G. Koczyk, "Assessing contamination of microalgal astaxanthin producer Haematococcus cultures with high-resolution melting curve analysis," *J Appl Genet*, vol. 58, no. 2, pp. 277–285, 2017,

[5] V. Faust, S. E. Vlaeminck, R. Ganigué, and K. M. Udert, "Influence of pH on Urine Nitrification: Community Shifts of Ammonia-Oxidizing Bacteria and Inhibition of Nitrite-Oxidizing Bacteria," *ACS ES and T Engineering*, vol. 4, no. 2, pp. 342–353, Feb. 2024,

[6] K Janiak, A Jurga, A Wizimirska, S Miodoński, M Muszyński-Huhajło, K Ratkiewicz, B Zięba, "Urine nitrification robustness for application in space: Effect of high salinity and the response to extreme free ammonia concentrations," *J Environ Manage*, vol. 279, p. 111610, Feb. 2021,

[7] F. Zhang, H. Yang, J. Wang, Z. Liu, and Q. Guan, "Effect of free ammonia inhibition on NOB activity in high nitrifying performance of sludge," *RSC Adv*, vol. 8, no. 56, pp. 31987–31995, Sep. 2018,

[8] Y. Zhou, A. Oehmen, M. Lim, V. Vadivelu, and W. J. Ng, "The role of nitrite and free nitrous acid (FNA) in wastewater treatment plants," *Water Res*, vol. 45, no. 15, pp. 4672–4682, Oct. 2011,

[9] A. Oren, "The Family Xanthobacteraceae," *The Prokaryotes: Alphaproteobacteria and Betaproteobacteria*, vol. 9783642301971, pp. 709–726, Aug. 2014,

[10] J. Zou, K. Zhang, S. Wang, M. Li, Z. Wang, S. Wang, Y. Li, Y. Deng, X. Li, D. Wang, Y. Yang, Y. Feng, C. Hu, Z. Wang, "The elevation of salinity above 1% deteriorated nitrification performance and reshaped nitrifier community of an MBR: An often overlooked factor in the treatment of high-strength ammonium wastewater," *Chemosphere*, vol. 335, p. 139072, Sep. 2023,

[11] Z. Su, T. Liu, J. Guo, and M. Zheng, "Nitrite Oxidation in Wastewater Treatment: Microbial Adaptation and Suppression Challenges," *Environ Sci Technol*, vol. 57, no. 34, pp. 12557–12570, Aug. 2023,