I COBBIND
I CONGRESSO BRASILEIRO DE BIOTECNOLOGIA INDUSTRIAL
XXIV SINAFERM ** XV SHEB ** XV ENZITEC FLORIANÓPOLIS ** 2024
Creating connections between biotechnology and industrial sustainability
August 25 to 28, 2024
Costão do Santinho Resort, Florianópolis, SC, Brazil
ENVIRONMENTAL BIOTECHNOLOGY

BIOSTIMULATION STRATEGY FOR RAPID START-UP OF ANAMMOX PROCESSES

Heloisa C. Rodrigues[1], Julia E. Serpa[2], Nicholas L. P. dos Santos[2], Alice C. Bolsan[3], Deborah C. de A. Leite[1], Marina C. De Prá[1,*]

[1] *Graduate Program in Biotechnology (PPGBIOTEC), Federal Technological University of Paraná, Dois Vizinhos, Brazil.*
[2] *Undergraduate Bioprocess Engineering and Biotechnology course, Federal Technological University of Paraná, Dois Vizinhos, Brazil.*
[3] *Graduate Program in Urban Environmental Sustainability (PPGSAU), Federal Technological University of Paraná, Curitiba, Brazil.*
** Corresponding author's email address: marinacdepra@gmail.com*

ABSTRACT

Processes involving anaerobic ammonia-oxidation bacteria (anammox) have emerged as highly efficient and cost-effective compared to conventional nitrogen removal technologies. However, the practical application of this process on a large scale faces challenges due to the low growth rate and a scarcity of mature sludge for the start-up. In this study, the anammox bacteria was successfully self-enriched through biostimulation of biomass derived from a municipal wastewater activated sludge system. A strategy was established for faster reactor start- without the need for anammox bacteria inoculation. The anammox process was achieved in 98-100 days with a desirable nitrogen removal efficiency of 81 ± 8%. Additionally, the nitrogen ratio obtained was close to the stoichiometric coefficients of the reaction, with an average ratio of 1.2 for $N\text{-}NO_2^-$: $N\text{-}NH_4^+$ and 0.27 for $N\text{-}NO_3^-$: $N\text{-}NH_4^+$. These results, combined with the formation of red granules in the system, highlight the feasibility and practicality of an economical method for establishing the anammox process without specific inoculation requirements.

Keywords: Anammox. Self-enrichment. Biostimulation.

1 INTRODUCTION

Since the discovery in 1995, processes involving the action of anaerobic ammonium-oxidizing bacteria (anammox) have demonstrated great biotechnological potential for the efficient removal of ammonia nitrogen from wastewater. This process consists of converting NH4+ and NO2- into nitrogen gas and a small portion of NO_3^-, according to Equation 1 [1].

$$NH_4^+ + 1{,}32\ NO_2^- + 0{,}066\ HCO_3^- + 0{,}13\ H^+ \rightarrow 1{,}02\ N_2 + 0{,}26\ NO_3^- + 0{,}066\ CH_2O_{0,5}N_{0,15} + 2{,}03\ H_2O \quad (1)$$

Compared to conventional nitrification and denitrification processes, anammox reduces the oxygen demand by around 60%, external organic carbon by 100%, operational costs for sludge disposal by 80%, and greenhouse gas emissions [2, 3]. However, the large-scale application still represents a technological obstacle since anammox bacteria have a low duplication rate and can take 9 to 11 days to generate new cells [1, 4]. This implies a long time to start up reactors, in addition to a dependency on large quantities of pre-acclimated anammox biomass, which incurs additional costs and presents a global challenge for process consolidation.

In this regard, techniques such as biostimulation have garnered attention. Biostimulation involves the addition of nutrients and adjustments to environmental conditions to modify the system's homeostasis and enhance the activity of target microorganisms. Several studies have focused on the self-enrichment of bacteria with anammox activity in different sludges from sewage and effluent treatment plants, obtaining good removal results and stoichiometric coefficients close to those expected [5–7]. Consequently, this study seeks to investigate the viability of employing a biostimulation strategy with activated sludge biomass to facilitate the rapid start of the anammox process.

2 MATERIAL & METHODS

Biomass was collected from an activated sludge reactor operating at a municipal wastewater treatment plant (Paraná, Brazil). An upflow reactor with a working volume of 1.2 L was inoculated at a concentration of 2 g L^{-1} of volatile suspended solids (VSS). Synthetic wastewater was used as a nutrient source and prepared according to [8], and anoxic conditions were maintained by purging dissolved oxygen (DO) using N_2. The NH_4^+ and NO_2^- were added as NH_4Cl and $NaNO_2$, and from day 0 to 75, the total nitrogen (TN) concentration was 100 mg L^{-1}, rising to 200 mg L^{-1} after this period.

The reactor was operated continuously with hydraulic retention time (HRT) of 6 h, pH maintained at 8,10±0,10, and temperature at 29,0±2°C. Samples were collected daily, and the concentrations of NH_3-N, NO_2^--N, and NO_3^--N were determined according to Standard Methods [9].

3 RESULTS & DISCUSSION

Figure 1 illustrates the nitrogen removal performance and the stoichiometric coefficients obtained during biostimulation.

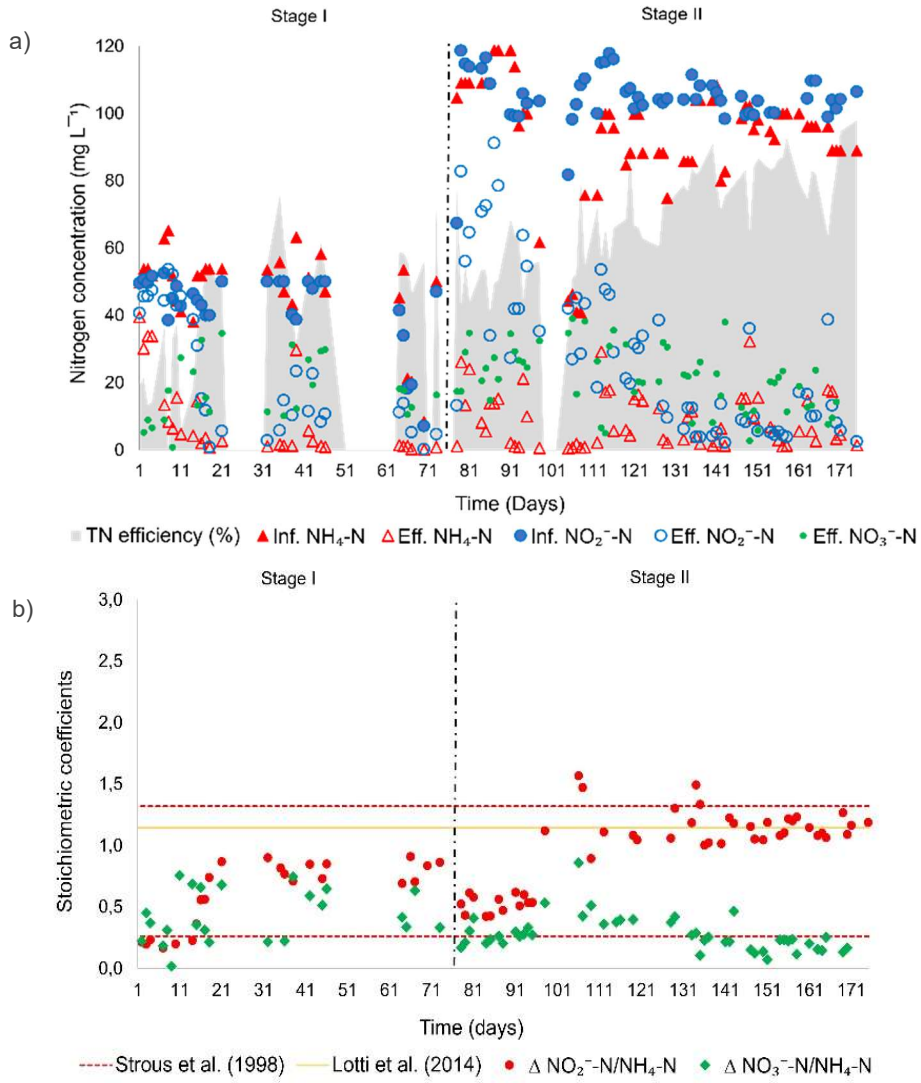

Figure 1 (a) Changes in nitrogen compounds during anammox biostimulation; (b) Variation in stoichiometric ratios.

The biostimulation of anammox can be divided into two stages. Stage 1 (Day 1 -78) consisted of an adaptation period, and it was different from that described by other authors [6, 10], who reported the predominance of denitrification via NO_2^- and an increase in NH_4^+ in the effluent due to cell lysis. In this study, the reactor showed a decrease in effluent NH_4^+ from day 1 and a decrease in effluent NO_2^- after day 10. The NO_3^- output varied throughout this phase but remained lower than the TN concentration, indicating low nitrifying activity and predominance of the anammox process. Although there was a trend towards stoichiometric coefficients from the 20th day, operational challenges such as power outages and hydrodynamic system failures hindered the establishment of the anammox process.

Phase 2 (Days 78-175) is marked by an increase in TN concentration in the influent. Initially, this load increase led to elevating the concentrations of NH_4^+, NO_2^-, and NO_3^- in the effluent, indicating an imbalance among the predominant processes. However, following the load shock, stoichiometric coefficients approached the theoretical after day 98, leading to an enhanced removal efficiency that peaked at 87% on day 120. Once the process was established, the average coefficients settled at 1.2 for $N\text{-}NO_2^-$: $N\text{-}NH_4^+$ and 0.27 for $N\text{-}NO_3^-$: $N\text{-}NH_4^+$, close to that described by [11].

In addition to the results obtained from the physical-chemical analyses, it was possible to observe (Figure 2) a change in the color of the sludge. At the beginning of the biostimulation process, the sludge was dark. Over time, it became lighter with a flocular characteristic and the presence of red granules, indicating bacteria with anammox activity.

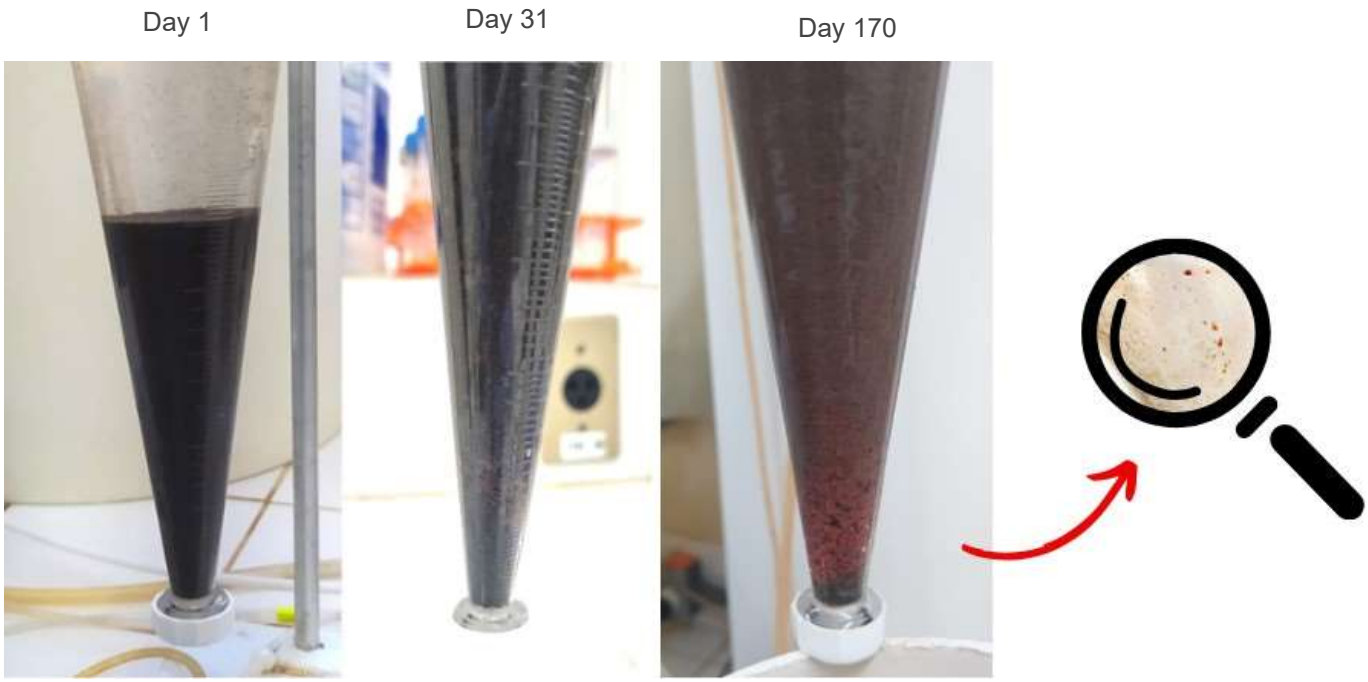

Figure 2 Changes in biomass over time.

Although the start-up period was 98 days, the results obtained are close to those described by some authors who obtained stat up in 95 days [12] or 112 days [13]. Self-enrichment without the need to add mature sludge is an alternative to enable the application of the process, allowing the process to be established in a more viable way.

4 CONCLUSION

The study successfully used the biostimulation strategy to prospect for bacteria with anammox activities in biomass from activated sludge systems treating municipal effluent. The anammox process was established after 98 days with a TN removal efficiency of 81±8%.

REFERENCES

1 STROUS M., HEIJNEN J. J., KUENEN J.G., JETTEN M. S. M. M. 1998. Appl Microbiol Biotechnol 50:589–596. https://doi.org/10.1007/s002530051340
2 CAO Y., VAN LOOSDRECHT M. C. M., DAIGGER G. T. 2017. Appl Microbiol Biotechnol 101:1365–1383. https://doi.org/10.1007/s00253-016-8058-7
3 LACKNER S., THOMA K., GILBERT E. M., et al. 2015. Water Sci Technol 71:553–559. https://doi.org/10.2166/wst.2014.421
4 LI D., DANG Z., ZHANG J. 2022. Chem Eng J 435:134791. https://doi.org/10.1016/j.cej.2022.134791
5 GAO Z., BI X., ZHAO J., ET AL. 2024. Bioresour Technol 397:130458. https://doi.org/10.1016/j.biortech.2024.130458
6 DEORE R., KUMAR R., MIRZA M. W., ALI KHAN A. 2022. Bioresour Technol 347:126719. https://doi.org/10.1016/j.biortech.2022.126719
7 MIRZA M. W., D'SILVA T. C., GANI K. M., et al (2021) Cultivation of anaerobic ammonium oxidizing bacteria (AnAOB) using different sewage sludge inoculums: process performance and microbial community analysis. J Chem Technol Biotechnol 96:454–464. https://doi.org/10.1002/jctb.6560
8 VAN DE GRAAF A. A., DE BRUIJN P., ROBERTSON L. A., et al. 1996.. Microbiology 142:187–189
9 APHA (2023) Standard methods for the examination of water and wastewater. Americam Public Health Association, American Water Works Association, Water Environmental Federation, 24th ed. Washington.
10 SU Y., DU R., WANG J., et al. 2023. P Bioresour Technol 387:129693. https://doi.org/10.1016/j.biortech.2023.129693
11 LOTTI T., KLEEREBEZEM R., LUBELLO C., VAN LOOSDRECHT M. C. M. 2014. Water Res 60:1–14. https://doi.org/10.1016/j.watres.2014.04.017
12 VERMA S., DAVEREY A., LIN J. G. 2021. Int Biodeterior Biodegrad 156:105132. https://doi.org/10.1016/j.ibiod.2020.105132
13 ÇELEN-ERDEM. 2020. Desalin Water Treat 175:43–48. https://doi.org/10.5004/dwt.2020.24898

ACKNOWLEDGEMENTS

The authors would like to thanks The Araucária Foundation to Support the Scientific and Technological Development of the State of Paraná (FA) for funds available, the Laboratory of Food and Environmental Biotechnology from Universidade Tecnológica Federal do Paraná (UTFPR – Dois Vizinhos) for the analytical support, and the *Coordenação de Aperfeiçoamento de Pessoal de Nível Superior* (CAPES) for financial support.