Assessing the potential of anammox bacteria as an alternative bioenergy resource

T. Sari[1], D. Akgul[2], B. Mertoglu[1]

[1]Department of Bioengineering, Marmara University, Istanbul, Goztepe, 34722, Turkey
[2]Department of Environmental Engineering, Marmara University, Istanbul, Goztepe, 34722, Turkey
Keywords: hydrazine, anammox, bioenergy, biotechnology
Presenting author email: tgbasarii@gmail.com

Hydrazine (N_2H_4) is a highly energetic molecule because of its molecule chemistry; therefore, it has been extensively used as rocket fuel and propellants in the aerospace industry. Hydrazine and its derivatives have also widespread usage in different industries including pharmaceuticals, textile, chemical, and environment (Patil and Rattan, 2014). The global hydrazine market value in 2020 was reported at $510.95 million, and global hydrazine demand is continuously increasing (Prakhar *et al*, 2022). Presently, the commercial production of hydrazine has been performed by only chemical processes, which have several disadvantages such as needing high amounts of energy input and producing inorganic salts as by-products (Oshiki *et al*, 2020). It has also been found that, hydrazine is hazardous to both the normal ecosystem and living organisms including humans, water and soil microorganisms. Despite all, hydrazine has remained its place in worldwide applications as it is often difficult to find its alternatives (Eimoori *et al*, 2020).

Anaerobic ammonium oxidation (anammox) bacteria utilize this extremely toxic substance as an intermediate. In their metabolisms, hydrazine formation is catalysed by a unique enzyme, hydrazine synthase (HZS), such that HZS has been used as a phylogenetic marker to detect anammox bacteria in nature (Peeters and van Niftrik, 2019). Thus, anammox has gained the utmost attention by virtue of its distinctive cell structure and metabolism in nature.

In recent decades, anammox technology has surpassed the traditional biological removal process. To be brief, anammox is a short-cut process to produce dinitrogen gas by ammonium oxidation with nitrite under anoxic conditions and utilize inorganic carbon as a carbon source. Thereof, it eliminates external carbon requirements and facilitates up to 60% energy saving in wastewater treatment plants (WWTPs). The slow growth rate of anammox bacteria also saves the sludge treatment cost. Moreover, it provides less greenhouse gas emissions. (Lin *et al*, 2016). Based on its all superiorities, the anammox process has become a cost-effective, eco-friendly, and sustainable technology and has been successfully integrated into many WWTPs to treat ammonium-rich wastewaters (Adams *et al*, 2022).

From a different point of view, the main question is: Could anammox bacteria be used as microbial cell factories for hydrazine bioproduction? Because if the answer is yes, the adverse effects of hydrazine and its chemical production on the environment might be minimized, and biosynthesized hydrazine might be also used in different industries together with current environmental applications. Although addressing all statements above requires much research, the current study aims to reveal the starting point of the research question by assessing the potential of anammox bacteria as an alternative bioenergy resource. In this context, the limits of anammox bacteria to intermediate hydrazine have been investigated in a long-term manner with respect to nitrogen treatment capacity and changes in microbial structure.

The anammox seed sludge was taken from an up-flow anammox bioreactor which has been operated in our laboratory, and used to inoculate a lab-scale bioreactor with a 2 L working volume. The bioreactor has been operated in a sequencing batch reactor mode with fill, reaction, settling, and effluent withdrawal times of 25 min, 46.58 h, 30 min, and 25 min, respectively. Following the start-up period of the anammox bioreactor, hydrazine has been started to supplement the system. The hydrazine application strategy has been determined to be 20 mg/L for 1 d, 100 mg/L for 5 d, and 150 mg/L for the rest of the case study considering our previous studies. During the whole operation period, influent and effluent concentrations of ammonium nitrogen, nitrite nitrogen, and hydrazine have been analysed. It should be noted that total nitrogen removal efficiency (TNRE) represents the sum of ammonia and nitrite nitrogen removal, and the results are given average ± standard deviation. For the first 120 days of the operation period, the TNRE of the bioreactor has been obtained to be 79.72 ± 10.62%. Although TNRE of the anammox population has shown a slowly decreasing profile over time, >50% TNRE has been observed (Figure 1).

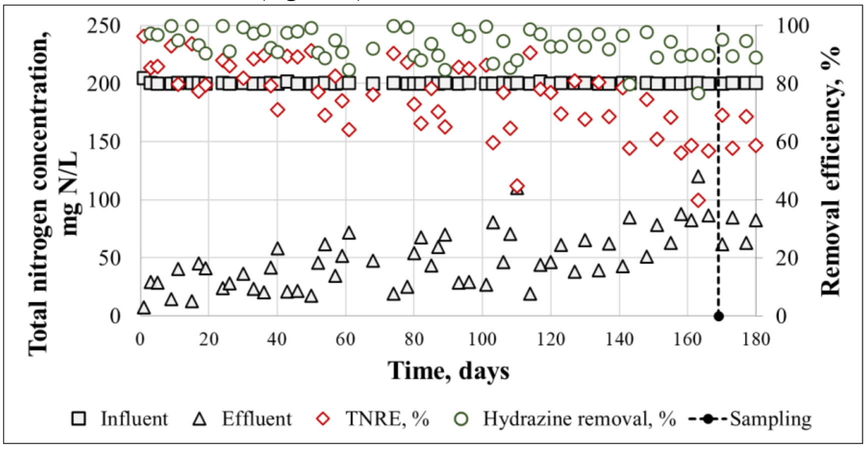

Figure 1. Nitrogen consumption profile of the anammox bioreactor

Up to now, 20.83 mg/L of hydrazine has been reported as the maximum produced concentration in the current literature (Oshiki *et al*, 2020). However, in the current study, a total of 8.3 g/L hydrazine has been added to the system so far. Anammox bacteria appear to might be able to withstand a lot higher hydrazine intermediate, as pointed out in this study. Therefore, with the appropriate experimental plan, bioaccumulation of hydrazine within the anammox metabolism may be boosted. Moreover, enriched anammox culture has consumed 93.17 ± 5.16% of supplied hydrazine contrary to the decrement in TNRE (Figure 1). The probable reason for it is that the mortality rate of anammox bacteria might have been reduced by hydrazine, which is associated the energy metabolism (Ganesan and Vadivelu, 2020). The study has continued to observe a complete loss of anammox activity.

During the 180 days operation period, the microbial composition of the bioreactor at the class level is represented in Table 1. Both the classes of Brocadiae and Phycisphaerae are in the phylum Planctomycetes (Kallscheuer *et al*, 2020; Peeters and van Niftrik, 2019). The relative abundance of Brocadiae representing the anammox bacteria in the enriched culture has been promoted by continuous hydrazine supplementation. Interestingly, Phycisphaerae quantity has been boosted by 6.83%, which is much more than that of Brocadiae. The SM1A02 genus has taken a large portion of class Phycisphaerae. Former studies have revealed that this species might have the anammox ability and represent a potential uncultured anammox strain (Yang *et al*, 2020). As for classes of Anaerolineae and Ignavibacteria and Proteobacteria, they seem to be vulnerable to hydrazine.

Table 1. Microbial structure during the operation period

Class name	**Initial sample (%)**	**Sampling (%)**
Anaerolineae	27.73	25.33
Ignavibacteria	20.25	21.21
Brocadiae	10.82	12.95
Phycisphaerae	9.9	16.73
Gammaproteobacteria	9.11	7.18
Alphaproteobacteria	8.78	7.37
Others (<5%)	13.42	9.22

Acknowledgement

This work was financially supported by the Scientific and Technological Research Council of Turkey [TUBITAK- Project no.: 122Y095]; and Marmara University Scientific Research Committee [Project no.: FDK-2022-10756]. Tugba Sari is also supported by the Turkish Council of Higher Education (CoHE) 100/2000 PhD Scholarship Program.

References

Adams, M., Xie, J., Kabore, A.w.J., Chang, Y., Xie, J., Guo, M. and Chen, C. 2022. Research advances in anammox granular sludge: A review. Critical Reviews in Environmental Science and Technology 52(5), 631-674.

Eimoori, R., Zolala, J., Pourmohiabadi, H., Noroozian, E. and Mansouri, H. 2020. Contribution of Azolla filiculoides to hydrazine elimination from water. Wetlands Ecology and Management 28(3), 439-447.

Ganesan, S. and Vadivelu, V.M. 2020. Effect of storage conditions on maintaining anammox cell viability during starvation and recovery. Bioresource Technology 296.

Kallscheuer, N., Wiegand, S., Kohn, T., Boedeker, C., Jeske, O., Rast, P., Müller, R.-W., Brümmer, F., Heuer, A., Jetten, M.S.M., Rohde, M., Jogler, M. and Jogler, C. 2020. Cultivation-Independent Analysis of the Bacterial Community Associated With the Calcareous Sponge Clathrina clathrus and Isolation of Poriferisphaera corsica Gen. Nov., Sp. Nov., Belonging to the Barely Studied Class Phycisphaerae in the Phylum Planctomycetes. Frontiers in Microbiology 11.

Lin, J.-G., Daverey, A., Dutta, K., Guo, W. and Ngo, H.H. (2016) Green Technologies for Sustainable Water Management, pp. 419-453.

Oshiki, M., Yamada, K., Kato, I., Okoshi, K., Imai, T., Yamaguchi, T. and Araki, N. 2020. Biosynthesis of hydrazine from ammonium and hydroxylamine using an anaerobic ammonium oxidizing bacterium. Biotechnology Letters 42(6), 979-985.

Patil, K.C. and Rattan, T.M. (2014) Inorganic hydrazine derivatives: synthesis, properties and applications, Wiley, New York.

Peeters, S.H. and van Niftrik, L. 2019. Trending topics and open questions in anaerobic ammonium oxidation. Current Opinion in Chemical Biology 49, 45-52.

Prakhar, J., Marpu, V. and Yerukola, P. 2022 Hydrazine Market by Application (Pharmaceuticals, Water Treatment, Blowing Agents, Agrochemicals, Others): Global Opportunity Analysis and Industry Forecast, 2020-2030.

Yang, R., Mao, W., Wang, X., Zhang, Z., Wu, J. and Chen, S. 2020. Response and Adaptation of Microbial Community in a CANON Reactor Exposed to an Extreme Alkaline Shock. Archaea 2020, 8888615.