Advances in research of Anammox process in MBR

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Abstract Membrane bioreactor (MBR) is an efficient wastewater treatment technology, which is produced by combining membrane treatment technology and biological treatment technology, and has the characteristics of stable water quality and shock load resistance, and has been widely used in domestic wastewater treatment. Anaerobic ammonia oxidation (ANAMMOX) is a new type of biological denitrification technology with the advantages of low energy consumption, low oxygen demand, carbon source saving and good economic benefits. This paper introduces the principle and characteristics of the MBR nitrification-anaerobic ammonia oxidation reactor process analysis process. Prospects of anaerobic ammonia oxidation technology in MBR are foreseen.

1. Introduction

Anaerobic ammonium oxidation (Anammox) is a reaction in which anaerobic ammonia oxidizing bacteria directly convert NH_4^+-N and NO_2^-N to nitrogen at low oxygen concentrations[1]. Membrane bioreactor (MBR) and anaerobic ammonia oxidation (Anammox) are two popular and efficient wastewater treatment technologies in recent years, which provide practical solutions to the problem of water wastage and shortage. MBR process is widely used with good removal effect and simple process flow[2,3].

Anaerobic ammonia oxidation technology is a new type of nitrogen removal process that has become more popular in recent years, which refers to the process of directly converting ammonia nitrogen to N_2 by using nitrite as an oxidant under insufficient oxygen supply conditions, and is a resource-saving and environment-friendly organic combination of biological nitrogen removal pathway with broad application prospects[4,5]. Combining membrane bioreactor with anaerobic ammonia oxidation process can efficiently retain anaerobic ammonia oxidation bacteria in the reactor, which not only makes up for the shortcomings of slow growth of anaerobic ammonia oxidation bacteria, but also makes the reactor achieve good denitrification performance, using the separation effect of membrane bioreactor to replace the effect of secondary sedimentation tank, so that the anaerobic activated sludge can be returned in time, which cleverly solves the problem of anaerobic activated sludge loss in anaerobic ammonia oxidation process[6]. This paper focuses on the principle and characteristics of the MBR anaerobic ammonia oxidation reactor process analysis process, highlighting the influencing factors of anaerobic ammonia oxidation, and the future development prospects of anaerobic reactors.

2. Membrane bioreactor.

2.1 Membrane Bioreactor principle

Membrane bioreactor (MBR) is a new and efficient wastewater treatment process that integrates high-efficiency separation technology with the traditional activated sludge method. The membrane unit (ultrafiltration membrane or microfiltration membrane) is used to trap the activated sludge and suspended matter and other substances in the biochemical reactor to improve the rate of biodegradation, so that less residual sludge is produced, and the membrane is able to retain a certain amount of organic matter in the wastewater, so that the microbial load of the reactor is always maintained at a high concentration, which results in higher efficiency of nitrogen and phosphorus removal[7]. Ma et al. investigated the feasibility of SNADF(simultaneous partial nitrification, anammox, de nitrification and fermentation process) in a single reactor, where is a carbon source for partial denitrification reactions, facilitating partial denitrification anammox reactions while achieving the goals of sludge reduction and denitrification performance[8]. We usually classify MBR membrane bioreactors into two categories according to the location of the MBR membrane modules, as external and internal. The basic structure is shown in Figure 1:

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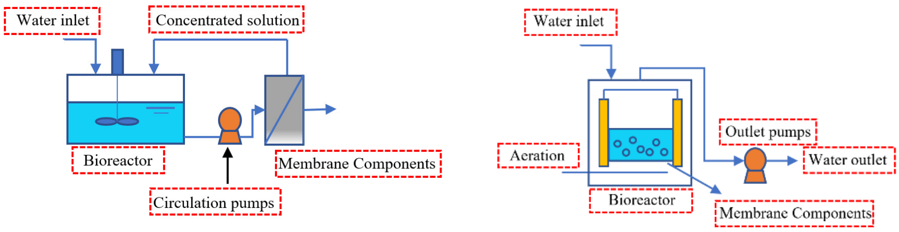

(a) External MBR (b) Internal MBR

Fig. 1 MBR's reaction system (a)

2.2 Advantages of MBR technology

(1) The effluent quality of MBR technology is good. MBR can carry out solid-liquid separation with high efficiency, and the membrane can retain macromolecules and microorganisms, making the suspended matter and turbidity of the effluent close to zero, and BOD, COD and other indicators are better than the general wastewater treatment process, microbial adsorption and degradation of pollutants for high quality and stable water discharge.

(2) Shock load resistance. Due to the high concentration of microorganisms in the reactor, the volume load that can be used in the reactor is larger, which is conducive to the smooth operation of the reactor.

(3) Small footprint. The main reason is that the process has fewer structures, which makes its arrangement more compact and concentrated, and the operation process is simple and convenient for management.

(4) The effect of removing ammonia nitrogen and hard-to-degrade organic matter is better than that of the traditional activated sludge method[9].

In summary, MBR membrane bioreactor is a new choice for wastewater treatment with high efficiency and low pollution in today's increasingly scarce water resources, and membrane contamination will be an important hurdle for MBR promotion and application in the future.

3. Anammox

3.1 Principle of Anaerobic Ammonia Oxidation (Anammox)

Anaerobic ammonia oxidation is a reaction in which microorganisms convert nitrite nitrogen to nitrogen gas under conditions of little or no dissolved oxygen through ammonia as an electron donor and nitrite as an electron acceptor[10]. And Strous et al. [11,12] derived the relationship between reactants and products of anaerobic ammonia oxidation based on the principle of material balance and deduced the chemical equation of the anaerobic ammonia oxidation process:

$$NH_4^+ - N + 1.32NO_2^- + 0.066HCO_3^- + 0.13H^+ \rightarrow$$
$$1.02N_2 + 0.26NO_3^- + 0.066CH_2O_{0.5}N_{0.15} + 2.03H_2O \quad (1)$$

We can see from equation (1) that the core in Anammox is that the ratio between the consumption of NH_4^+-N, the consumption of NO_2^-N ions and the production of NO_3^--N ions is maintained at 1:1.32:0.26. Meanwhile, anaerobic ammonia oxidation can oxidize ammonia nitrogen to nitrite, saving oxygen consumption, and it also has the characteristics of no additional carbon source, shock load resistance, and low operating cost, which has received wide attention[13]. However, the anaerobic ammonia oxidation process also has certain disadvantages, firstly, the growth rate of ammonia oxidizing bacteria (AOB) is slow, with a bacterial multiplication time of 11 days, while the culture conditions of AOB are harsh and the wastewater contains substances that inhibit AOB, leading to some limitations in the promotion of the anaerobic ammonia oxidation process[12].

3.2 Factors affecting anaerobic ammonia oxidation (Anammox)

(1) Temperature.

Most of the studies found that the most suitable temperature for anaerobic ammonia oxidation bacteria(AAOB) growth and reproduction was in the range of 30 to 40 °C, and the researchers basically conducted their studies on the process with Anammox in the environment above 30 °C.[14]. Decrease in anammox activity at temperatures above 45°C[15]. Some studies have also reported that the temperature range between 40-45°C may lead to bacterial cell lysis due to high temperatures, thus reducing anammox activity[16]. At lower temperatures, anammox system ladderane fatty acids concentrations increase, leading to a decrease in membrane permeability and preventing ATP synthesis, thus reducing metabolism[17]. Temperature instability in the anammox process can affect the efficiency of the anammox process.

(2) pH

pH affects Anammox in two main ways: firstly, it disrupts the enzymatic activity of AAOB electrolyte homeostasis, thus affecting bacterial activity; secondly, the balance of NH_4^+-N, free ammonia (FA), NO_2^--N and free nitrite (FNA) concentrations in water varies with pH, thus affecting the Anammox reaction. pH in the anaerobic ammonia oxidation process is generally between 6~8[12]. Too high or low pH will affect the normal progress of the reaction. Due to low pH (less than 7), the concentration of free ammonia decreases and the concentration of un-ionized nitrite increases, and conversely, at high pH, the concentration of free nitrite increases and the concentration of free nitrite decreases[18,19]. Controlling the pH range close to neutral or weakly alkaline is most suitable for AAOB growth and reproduction, and is also conducive to better and more stable operation of the anaerobic ammonia oxidation process.

(3) Substrate concentration.

It was shown that the high substrate concentration would inhibit the effect of anaerobic ammonia oxidation. Increased anammox metabolic activity and energy supply at low nitrogen strength, increased biological production, creation of a more favorable environment, more suitable for bacterial survival, and potentially more active interactions between species with complementary functions[20]. A researcher designed a new composite carrier to provide a relatively high ammonia microenvironment. The start-up of partial nitritation-anammox (PN-A) system for treating low strength wastewater was achieved by adding a composite carrier. The composite carrier promoted the enrichment of AOB and anaerobic ammonium oxidizing bacteria (AnAOB) the composite carrier in the PN-A system enhanced the microbial metabolism[21]. High substrate concentration inhibits anaerobic ammonia oxidation, and substrate is important for anammox biomass growth, but low substrate concentration in the influent water hinders the application of anammox process in treating nitrogen-rich wastewater.

4. Application of membrane bioreactor in anaerobic ammonia oxidation

4.1 partial nitritation-anammox process

The partial nitritation-anammox process is a combination of a nitritation process preceded by an anaerobic ammonia oxidation process to achieve efficient removal of ammonia nitrogen in one step. Compared to conventional nitrification and denitrification processes, the partial nitritation-anammox process has significant advantages such as no consumption of organic carbon, a 60% reduction in aeration and low sludge production rates[22]. A 500 kg N/day two-stage partial nitritation/anaerobic ammonia oxidation process comprising a zeolite-filled PN reactor and a biofilm anaerobic ammonia oxidation reactor was successfully started up within 45 days and operated consistently for over one year for the liquid-ammonia mercerization wastewater treatment achieving an average ammonia removal rate (94.3±2.3%), total nitrogen removal rate (89.4±2.7%) and nitrogen removal rate (1.003±0.386 kg N/m^3 /day)[23].

The partial nitritation-anammox process does shading treatment, and after the wastewater enters the nitrification reactor, NH_4^+-N and oxygen combine to produce NO_2^--N. Meanwhile, the low carbon to nitrogen ratio wastewater is pumped into the reactor, and the NH_4^+-N in the water is nitrosated to NO_2^--N, reaching a ratio of approximately NH_4^+-N: NO_2^--N = 1:1. The nitrification tank is enriched with a large number of AOB bacteria at the same time, and the nitrosated wastewater flows into the After the anaerobic ammonia oxidation tank, the anaerobic ammonia oxidation bacteria use the NO_2^--N in the wastewater as the acceptor for the anaerobic ammonia oxidation reaction and the remaining NH_4^+-N in the wastewater as the electron donor, converting both to N_2. The equation for this test reaction is shown in equation (2)(3):

$$NH_4 + 1.5O_2 \rightarrow NO_2^- + 2H^+ + H_2O \quad (2)$$

$$NH_4^+ + NO_2^- \rightarrow N_2 + 2H_2O \quad (3)$$

Stable operation of the partial nitritation-anammox process requires the synergistic action of ammonia oxidizing bacteria (AOB) and anaerobic ammonia oxidizing bacteria (AnAOB), while inhibiting the proliferation of nitrite oxidizing bacteria (NOB). In a single-stage PNA system, it is difficult to consider the physiological characteristics of both AOB and AnAOB. Improperly regulated operating conditions can easily lead to microbial community imbalance and reactor instability or even collapse[24]. Chen et al. found that a high denitrification efficiency of 72.7 ± 8.4% for 230 days at an aeration rate of 0.28 L/min and a dissolved oxygen (DO) concentration of 0.10-0.20 mg/L was successfully achieved during the operation of the nitrosation-anaerobic ammonia oxidation reactor. The color of anaerobic ammonia oxidation bacteria appeared distinctly red. The specific activity of anaerobic ammonia oxidation and ammonia oxidation increased to 1.02 and 0.93 g-N/g-VSS/d, respectively[25].

In conclusion, this reactor, despite its shortcomings, still has a good prospect to play an important role in the field of wastewater treatment, especially in the field of high ammonia to nitrogen ratio wastewater treatment. Although the partial nitritation-anammox process has advantages in terms of economic feasibility and environmental sustainability, the process control is complex and stringent, and long-term operation is difficult to ensure NO_2^--N accumulation and stable operation of the process[26].

5. Outlook and conclusion

Anammox process in the form of MBR is a cost-effective biological wastewater treatment technology, which suppresses the competition of NOB growth by maintaining low DO and controlling the thickness of biofilm. In practical application, there are still problems such as long AAOB doubling time, insufficient supply of nitrite nitrogen, and fluctuating operation to be solved. To achieve wider engineering application and technology diffusion, we need to continue to To achieve wider engineering application and technology diffusion, the following aspects of research should be continued:

(1) On the basis of microbial community structure and abundance, the mechanism of physiological metabolic response to AAOB needs to be continued to be elucidated, and the growth of anammox bacteria needs to be continuously studied in depth at the microscopic level, especially at low temperatures, and the active species can be screened by constructing metabolic networks of different anammox species.

(2) In the mainstream, the ammonia concentration is low, resulting in a slow growth rate. The slow growth

rate of anammox bacteria led to long start-up time and low nitrogen removal, while high substrate concentration inhibited anaerobic ammonia oxidation and low substrate concentration hindered the application of anammox process in the treatment of nitrogen-rich wastewater.

(3) New carrier materials are needed to solve the problem of membrane contamination caused by EPS and SMP, for example. New carrier materials or composite carriers with both adsorption effect and high affinity for microorganisms should be developed.

Reference

1. Bagchi, S.; Biswas, R.; Nandy, T. Autotrophic Ammonia Removal Processes: Ecology to Technology. *CRITICAL REVIEWS IN ENVIRONMENTAL SCIENCE AND TECHNOLOGY* **2012**, *42*, 1353-1418.
2. Gao, M.; Wang, S.; Ren, Y.; Jin, C.; She, Z.; Zhao, Y.; Yang, S.; Guo, L.; Zhang, J.; Li, Z. Simultaneous removal of perchlorate and nitrate in a combined reactor of sulfur autotrophy and electrochemical hydrogen autotrophy. *Chemical Engineering Journal* **2016**, *284*, 1008-1016.
3. Ren, L.-F.; Liang, S.; Ngo, H.H.; Guo, W.; Ni, S.-Q.; Liu, C.; Zhao, Y.-K.; Hira, D. Enhancement of anammox performance in a novel non-woven fabric membrane bioreactor (nMBR). *RSC Advances* **2015**, *5*, 86875-86884.
4. Zhang, M.; Gu, J.; Wang, S.; Liu, Y. A mainstream anammox fixed-film membrane bioreactor with novel sandwich-structured carriers for fast start-up, effective sludge retention and membrane fouling mitigation. *Bioresource Technology* **2022**, *347*, 126370.
5. Al-Hazmi, H.E.; Lu, X.; Grubba, D.; Majtacz, J.; Badawi, M.; Mąkinia, J. Sustainable nitrogen removal in anammox-mediated systems: Microbial metabolic pathways, operational conditions and mathematical modelling. *Science of The Total Environment* **2023**, *868*, 161633.
6. Trigo, C.; Campos, J.L.; Garrido, J.M.; Méndez, R. Start-up of the Anammox process in a membrane bioreactor. *Journal of Biotechnology* **2006**, *126*, 475-487.
7. Wang, T.; Zhang, H.; Gao, D.; Yang, F.; Zhang, G. Comparison between MBR and SBR on Anammox start-up process from the conventional activated sludge. *Bioresource Technology* **2012**, *122*, 78-82.
8. Ma, G.; Yu, D.; Zhang, J.; Miao, Y.; Zhao, X.; Li, J.; Zhang, Y.; Dong, G.; Zhi, J. A novel simultaneous partial nitrification, anammox, denitrification and fermentation process: Enhancing nitrogen removal and sludge reduction in a single reactor. *Bioresource Technology* **2023**, *369*, 128484.
9. Xiao, K.; Liang, S.; Wang, X.; Chen, C.; Huang, X. Current state and challenges of full-scale membrane bioreactor applications: A critical review. *Bioresource Technology* **2019**, *271*, 473-481.
10. Chen, F.; Qian, Y.; Cheng, H.; Shen, J.; Qin, Y.; Li, Y.Y. Recent developments in anammox-based membrane bioreactors: A review. *Sci Total Environ* **2023**, *857*, 159539.
11. Strous M, H.J.J., Kuenen J G. The sequencing batch reactor as a powerful tool for the study of slowly growing anaerobic ammonium-oxidizing microorganisms. *Applied Microbiology & Biotechnology* **1998**, *50*, 589-596.
12. Strous M, K.J.G., Jetten M S M. Key Physiology of Anaerobic Ammonium Oxidation. *Applied & Environmental Microbiology* **1999**, *65*, 3248-3250.
13. Xu, X.; Xue, Y.; Wang, D.; Wang, G.; Yang, F. The development of a reverse anammox sequencing partial nitrification process for simultaneous nitrogen and COD removal from wastewater. *Bioresource Technology* **2014**, *155*, 427-431.
14. Tang, C.J.; Zheng, P.; Wang, C.H.; Mahmood, Q. Suppression of anaerobic ammonium oxidizers under high organic content in high-rate Anammox UASB reactor. *BIORESOURCE TECHNOLOGY* **2010**, *101*, 1762-1768.
15. Cao, Y.S.; van Loosdrecht, M.C.M.; Daigger, G.T. Mainstream partial nitritation-anammox in municipal wastewater treatment: status, bottlenecks, and further studies. *APPLIED MICROBIOLOGY AND BIOTECHNOLOGY* **2017**, *101*, 1365-1383.
16. Han, P.; Huang, Y.T.; Lin, J.G.; Gu, J.D. A comparison of two 16S rRNA gene-based PCR primer sets in unraveling anammox bacteria from different environmental samples. *APPLIED MICROBIOLOGY AND BIOTECHNOLOGY* **2013**, *97*, 10521-10529.
17. Cho, S.; Kambey, C.; Nguyen, V.K. Performance of Anammox Processes for Wastewater Treatment: A Critical Review on Effects of Operational Conditions and Environmental Stresses. *WATER* **2020**, *12,*1.
18. Jung, J.Y.; Kang, S.H.; Chung, Y.C.; Ahn, D.H. Factors affecting the activity of anammox bacteria during start up in the continuous culture reactor. *WATER SCIENCE AND TECHNOLOGY* **2007**, *55*, 459-468.
19. Mosquera-Corral, A.; Gonzalez, F.; Campos, J.L.; Mendez, R. Partial nitrification in a SHARON reactor in the presence of salts and organic carbon compounds. *PROCESS BIOCHEMISTRY* **2005**, *40*, 3109-3118.
20. Guo, Y.; Zhao, Y.; Zhu, T.; Li, J.; Feng, Y.; Zhao, H.; Liu, S. A metabolomic view of how low nitrogen strength favors anammox biomass yield and nitrogen removal capability. *Water Research* **2018**, *143*, 387-398.
21. Lv, Y.; Pan, J.; Huo, T.; Zhao, Y.; Liu, S. Enhanced microbial metabolism in one stage partial nitritation-anammox system treating low strength wastewater by novel composite carrier. *Water Research* **2019**, *163*, 114872.

22. Yang, W.; He, S.; Han, M.; Wang, B.; Niu, Q.; Xu, Y.; Chen, Y.; Wang, H. Nitrogen removal performance and microbial community structure in the start-up and substrate inhibition stages of an anammox reactor. *J Biosci Bioeng* **2018**, *126*, 88-95.
23. Chen, Z.; Wang, X.; Zhou, S.; Fan, J.; Chen, Y. Large-scale (500 kg N/day) two-stage partial nitritation/anammox (PN/A) process for liquid-ammonia mercerization wastewater treatment: Rapid start-up and long-term operational performance. *J Environ Manage* **2023**, *326*, 116404.
24. Wang, H.; Gong, H.; Dai, X.; Yang, M. Metagenomics reveals the microbial community and functional metabolism variation in the partial nitritation-anammox process: From collapse to recovery. *Journal of Environmental Sciences* **2024**, *135*, 210-221.
25. Chen, H.; Wang, H.; Chen, R.; Chang, S.; Yao, Y.; Jiang, C.; Wu, S.; Wei, Y.; Yu, G.; Yang, M.; et al. Unveiling performance stability and its recovery mechanisms of one-stage partial nitritation-anammox process with airlift enhanced micro-granules. *Bioresour Technol* **2021**, *330*, 124961.
26. Liu, T.; Hu, S.; Guo, J. Enhancing mainstream nitrogen removal by employing nitrate/nitrite-dependent anaerobic methane oxidation processes. *Crit Rev Biotechnol* **2019**, *39*, 732-745.