Frontiers in Marine Science

TYPE Original Research
PUBLISHED 22 May 2024
DOI 10.3389/fmars.2024.1408774

OPEN ACCESS

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RECEIVED 28 March 2024
ACCEPTED 19 April 2024
PUBLISHED 22 May 2024

CITATION
Ma X, Yin R, Yang C, Cai C, Zhao J, Lv C, Wang X, Chen S, Ren J and Zhang B (2024) Application of a biofilm-enhanced A_2O system in the treatment of wastewater from mariculture.
Front. Mar. Sci. 11:1408774.
doi: 10.3389/fmars.2024.1408774

Application of a biofilm-enhanced A_2O system in the treatment of wastewater from mariculture

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Development of environment-friendly and efficient aquaculture effluent treatment system is crucial for sustainable intensification of aquaculture, in the face of the rapidly increasing environmental pressure in the mariculture industry. In this study, mariculture wastewater was treated by the anoxic-anaerobic-oxic biochemical treatment system (A_2O system) with traditional activated sludge replaced by nitrifying bacteria, denitrification bacteria and phosphorus accumulating bacteria absorbed on PBS carrier biofilms suitable for saline/brackish water. The results showed that biofilm-enhanced A_2O system can effectively remove pollutants from aquaculture wastewater. The removal efficiencies of COD_{Mn}, NH_4^+-N, TN and TP in A_2O system were approximately 86.3%-90.8%, 97.7%-99.5%, 94.6%-95.2% and 97.0%-98.1%. The results further showed that COD_{Mn}, NH_4^+-N, and TN were mainly removed in anaerobic tank and anoxic tank, while TP was mainly removed in the anoxic tank and oxic tank. The biofilm-enhanced A_2O system by adding nitrifying bacteria and phosphorus accumulating bacteria biofilms using PBS as carriers instead of conventional activated sludge could be applied to the treatment of circulating aquaculture wastewater. This study provides a feasible scheme for enhancing the efficiency of A_2O system in the treatment of aquaculture tail water, and provides a reference for the immobilization of microorganisms.

KEYWORDS

biofilm-enhanced A2O system, mariculture wastewater, total nitrogen, total phosphorus, PBS

Introduction

With the rapid development of population and economy, seafood such as fish and shrimp from aquaculture plays a very important role in China's food supply (Yaseen et al., 2022; Fong et al., 2023). Statistically, China is the only country in the world where aquaculture production exceeds fishery production, accounting for more than 30% of the world's total aquatic products. However, the rapid development of aquaculture industry produced a large amount of aquaculture wastewater containing organic matter and ammonia-nitrogen. The direct discharge of marine aquaculture wastewater caused serious environmental pollution, such as eutrophication (Zhang et al., 2023b). Furthermore, it has been confirmed that marine aquaculture wastewater was an oligotrophic wastewater, with low concentrations of ammonia-nitrogen (NH_4^+-N) and organic matter, but also with high salinity (about 30). At present, the environmental pollution caused by the direct discharge of aquaculture wastewater has attracted more and more attention. Many treatment methods of aquaculture wastewater have been explored and reported. For instance, Ma et al. (2021) realized effective treatment of aquaculture wastewater by the constructed wetland system (CWs) and Zhou et al. (2022) found that macroalga could adsorb nutrients in aquaculture water. Although the CWs has a good treatment effect on aquaculture wastewater, it has the disadvantages of large area and high investment cost (Ren et al., 2021). Aerobic denitrifying bacteria isolated from aquaculture farms, such as *Vibrio* spp. AD2. These bacteria can effectively remove ammonia and nitrate from aquaculture wastewater under aerobic conditions. However, aerobic denitrifying bacteria are heterotrophic microorganisms, and their growth and nitrogen removal performance depend on high C/N ratio. The growth and energy metabolism of aerobic denitrifying bacteria require sufficient supply of carbon sources to meet their growth and energy metabolism needs (ref). Aquaculture wastewater was an oligotrophic wastewater, which further limited the application of aerobic denitrification in aquaculture wastewater treatment (Tang et al., 2020). These characteristics of marine aquaculture wastewater are not conducive to the growth and nitrogen removal performance of traditional nitrifying bacteria, such as *Nitrosomonas*, *Nitrosospira*, and *Nitrospira* (Li et al., 2020; Nunzia et al., 2021). Therefore, it is difficult for marine aquaculture wastewater to be effectively treated by traditional biological methods.

However, faced with the rapidly increasing environmental pressure of the seawater aquaculture industry, there is an urgent need for efficient and low-cost wastewater treatment technologies. The A_2O process has been used for urban sewage treatment. By adding an anoxic tank to the AO process, the organic matter, nitrogen and phosphorus in the water can be removed while denitrification. The process has a simple structure and the total hydraulic retention time is shorter than other similar processes. Currently, research on the A_2O process has shifted from improving wastewater treatment efficiency to treating special wastewater, such as high-strength, highly recalcitrant wastewater from the production of polyphenylene sulfide (PPS) resins and their composite chemicals (Guo et al., 2023), brewery wastewater, meat processing wastewater, pharmaceutical wastewater, etc (Sun et al., 2015; Gallardo-Altamirano et al., 2019). Alternatively, research can improve the treatment efficiency of polluted wastewater by modifying the A_2O process or combining it with other processes. For example, Congcong Zhang (Zhang C. et al., 2023) combined the side flow sludge fermenter with the A_2O system. Yongqing Gao et al. (2011) combined two-step alkaline sludge fermentation with A_2O system. Likun Huang et al. (2022) studied the A_2O -MBR -BAF -O_3 combined process. The A_2O-MBR combined process has also been studied by many scholars (Na et al., 2017; Abyar et al., 2018; Li et al., 2019). Chunhong Na (Na et al., 2017) studied the combination of anaerobic-anoxic-oxic (A_2O) and deep oxidation processes. IFAS (Gallardo-Altamirano et al., 2021) studied an integrated fixed membrane activated sludge system. A full-scale biofilm system using fluidized-carriers integrated with anaerobic-anoxic–aerobic process was used for municipal wastewater treatment (Xiao et al., 2016).The sludge age (residence time of biological solids) is the control objective of wastewater nitrification management. In order for the nitrifying bacterial community to survive in a continuous flow system, the SRT of the system must be greater than the specific growth rate of autotrophic nitrifying bacteria. Short sludge age can lead to the loss of nitrifying bacteria or a decrease in nitrification rate. In actual denitrification projects, the sludge age generally selected should be greater than the actual SRT. Research has shown that for activated sludge denitrification, the sludge age is generally not less than 15 days. However, excessive sludge age was not conducive to phosphorus removal (Wang et al., 2019). The A_2O system was relatively mature in the treatment of freshwater wastewater. But there are few reports on its use in saline/semisaline water treatment, which might have a significant relationship with activated sludge (Xiao et al., 2020; Ma et al., 2023). Additionally, the traditional A_2O system was not suitable for the treatment of the nutrient-poor seawater aquaculture wastewater (Rajesh Banu et al., 2009). The traditional A_2O system mainly removes nitrogen and phosphorus by microorganisms in the activated sludge. The existence of activated sludge in the system is limited, and when the activated sludge exists in the high-salt and poor-nutritional water body, the microorganisms in the sludge will also limit the efficiency of nitrogen and phosphorus removal due to the lack of carbon source. Therefore, strengthening the A_2O system by taking some measures may be an alternative approach. A suitable treatment system for saline/brackish water aquaculture wastewater was conducted by combined the A_2O system with nitrifying bacteria, denitrification bacteria and phosphorus accumulating bacteria biofilm systems suitable for saline/brackish water, i.e. Biofilm-enhanced A_2O system. Biofilm-enhanced A_2O system has the advantage of providing organic carbon source independently and has strong salt tolerance, which can solve the shortcomings of traditional A_2O system in the treatment of mariculture tail water, small floor area, high treatment efficiency, free operation time, and low investment cost. Biofilm-enhanced A_2O system has extensive potential in the treatment of aquaculture wastewater.

At present, the main limitation of applying A_2O system to treat the marine aquaculture wastewater with the characteristics of oligotrophic was that the organic carbon source required by denitrifying bacteria is insufficient, which limits their normal growth and energy metabolism (Wu et al., 2023). It is believed that denitrifying microorganisms are heterotrophic microorganisms, which not only require sufficient carbon sources for their growth, but also require carbon sources as electron donors to achieve complete nitrogen removal in denitrification (Zhang et al., 2023a). The effluent from PBS carrier contained mainly protein-like and soluble microbial product-like substances, which can be used as the growth carrier of microorganisms and the source of organic carbon (Díaz et al., 2014). The proportion of methyl and hydroxyl functional groups in PBS materials decreased, while other functional groups did not change significantly (Zhang C. et al., 2023). Starch and ethylene, the main monomer components of PBS, could be used as carbon sources by denitrification microorganisms. Scanning electron microscope observation showed that after the attachment and growth of denitrification biofilm, holes would appear on the surface of PBS particles, expanding the surface area of biofilm biological attachment and growth, which was conducive to the formation of dense denitrification biofilm, Protection against denitrifying bacteria (Wang et al., 2012). The application of PBS particles as a denitrification carbon source and biofilm carrier in a packed bed reactor for A_2O system has certain feasibility (Zeng et al., 2010; Tang et al., 2021). Therefore, it is of great significance to use PBS as biofilm carrier to strengthen the A_2O system to remove the marine aquaculture wastewater with the characteristics of oligotrophic wastewater.

In this study, the A_2O system was strengthened by biofilms of microorganisms adsorbed on PBS carriers to evaluate the treatment effect of A_2O system on aquaculture wastewater, including chemical oxygen demand (COD_{Mn}), NH_4^+-N, total nitrogen (TN), total phosphorus (TP), etc. This study provides a practical method for aquaculture wastewater treatment.

Experimental materials and methods

Experimental setup and operation

An A_2O urban domestic sewage treatment (A_2O UDST) system with five units was set up in the laboratory, including inlet sedimentation tank, anaerobic tank, anoxic tank, oxic tank, and outlet sedimentation tank (Figure 1). The working volume of the inlet tank, anaerobic tank, anoxic tank, oxic tank, and outlet sedimentation tank were 1000 L, 20 L, 25 L, 53 L, and 10 L, respectively. The bottom of outlet sedimentation tank was provided with a return pipe to return the sludge to the anaerobic tank and the return ratio was about 300%. A circular distribution bucket with a working volume of 1000 L was used to hold simulated aquaculture wastewater. A peristaltic pump connected to a distribution bucket and an inlet settling tank was used to pump aquaculture waste into the A_2O system. Besides, an aeration pump was installed at the bottom of the oxic tank for continuous aeration to maintain a high dissolved oxygen concentration.

The entire operation of the A_2O system consisted of two phases: initial startup phase (0–15 days) and biofilm strengthening phase (16 –180 days). In the initial startup phase, 200 g of the mature PBS biofilm was further added to anaerobic tank, anoxic tank, oxic tank every 5 days (i.e., 5 days of sludge age), respectively. After 15 days of stable operation, the A_2O system entered the second phase, i.e. the biofilm strengthening stage. The peristaltic pump continuously pumps aquaculture waste into the A_2O system, and controls the HRT time to 8 h. The effluent flow rate was 10 L/h. A_2O system was equipped with agitator, and the shaking speed was controlled at 1000 rpm/min. Water samples were collected every 7 days from each reaction tank of the A_2O system for the determination of COD_{Mn}. The collected samples were filtered through a 0.45 μm filter membrane to determine the concentration of NH_4^+-N, NO_2^-N, NO_3^-N, and TP.

Biofilm culture

To obtain mature biofilms, the enhanced microorganisms were placed in the same container with PBS and cultured for approximately 14 days and biofilms were formed on PBS. Anaerobic biofilm and oxic biofilm were cultured in oxic and anaerobic environments respectively. Hypoxia: phosphorus-accumulating bacteria, nitrifying bacteria, and PBS were added to a sealed container. Oxic: phosphorus-accumulating bacteria, nitrifying bacteria, and PBS were added to the open container. In this experiment, a microorganism isolated from the sea area of Haizhou Bay by laboratory separation and purification and send to 16s rRNA sequence, at last confirmed that the microorganism is named *Halomonas* sp.; nitrifying bacteria (rich in nitrifying bacteria and denitrification bacteria) were purchased from Beihai Yeshengwang Biotechnology Co., Ltd., liquid (Biozym); PBS (polybutylene succinate) (HO-(CO-$(CH_2)_2$-CO-O-$(CH_2)_4$-O) n-H) was a white or light yellow crystalline cylinder with a diameter of approximately 2 mm and a height of approximately 4 mm.

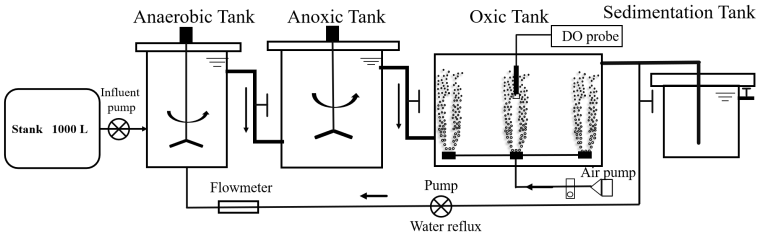

FIGURE 1
Diagram of the biofilm-enhanced A_2O system.

Analytical method

The aquaculture wastewater was taken from aquaculture farms and originated from Haizhou Bay. The characteristics of aquaculture wastewater were as follows: the concentrations of COD, NH_4^+-N, TN/NOx and TP were 1.2 mg/L, 0.89 mg/L, 0.98 and 0.1 mg/L. The concentration of COD_{Mn}, NH_4^+-N, NO_2^-N, NO_3^-N, and TP were measured using the method specified in the seawater monitoring standard GB/T 17378.4-1998.

Results

Water quality characteristics change

The characteristics of water quality change in A_2O system were measured. As shown in Figure 2A, the influent COD_{Mn} concentration of the A_2O process were 1.13 ± 0.04 mg/L, the effluent COD_{Mn} concentration of the anaerobic tank was 0.65 ± 0.02 mg/L, the effluent COD_{Mn} concentration of the anaerobic tank was 0.21 ± 0.03 mg/L, and the effluent COD_{Mn} concentration of the oxic tank was 0.13 ± 0.02 mg/L. In the first three months, the total COD_{Mn} removal efficiencies of A_2O system were 86.3-87.7%. In the last three months, the total COD_{Mn} removal efficiencies of A_2O system were rose to 90.7-90.8%. The removal of NH_4^+-N in the A_2O system was shown in Figure 2B. The concentrations of influent water in the A_2O system were 0.75-0.89 mg/L, the effluent from the anaerobic tank was 0.18-0.21 mg/L, the effluent from the anaerobic tank was 0.05-0.13 mg/L, and the effluent from the oxic tank was 0.004-0.02 mg/L. The total removal efficiencies were approximately 97-99.5%. The removal of TN/NOx in the A_2O system were shown in Figure 2C. The influent concentrations of TN in the A_2O system were 0.82-0.98 mg/L. the NO_3^-N concentrations in the anaerobic tank outlet were 0.64-0.7 mg/L, the NO_3^-N concentrations in the anaerobic tank outlet were 0.02-0.03 mg/L, the NO_3^-N concentrations in the oxic tank outlet were 0.03-0.04 mg/L, the NO_2^-N concentrations in the oxic tank outlet were 0.001-0.01 mg/L, the TN concentrations in the oxic tank outlet were 0.04-0.05 mg/L, and the TN removal efficiencies were 94.7-95.2%. The removal of TP in the A_2O system was shown in Figure 2D. The influent concentrations of TP in the system were 0.07-0.1 mg/L, the concentrations of TP in the effluent of the anaerobic tank were 0.06-0.09 mg/L, the concentrations of TP in the effluent of the anaerobic tank were 0.003-0.04 mg/L, and the concentrations of TP in the effluent of the oxic tank were 0.001-0.002 mg/L. The TP removal efficiencies were 97-98%.

The removal efficiencies of COD_{Mn}, NH_4^+-N, TN/NO_x, and TP in the A_2O system

The removal efficiencies of COD_{Mn}, NH_4^+-N, TN/NO_x, and TP in the A_2O system were shown in Figure 3. As shown in Figure 3A, the

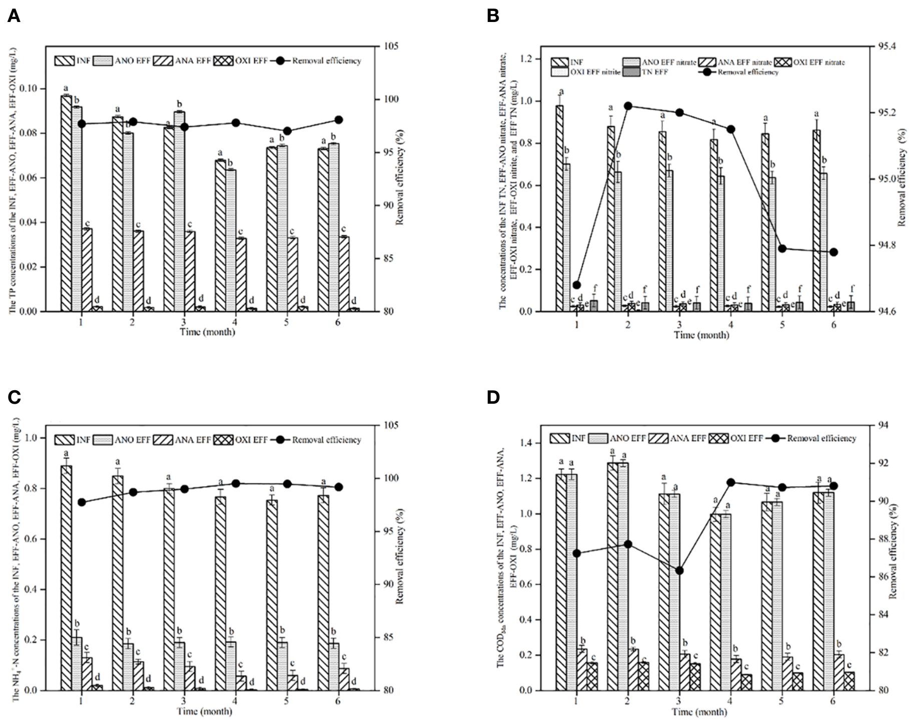

FIGURE 2
Concentrations and total removal efficiency of COD_{Mn}, NH_4^+-N, TN/NO_x, and TP in each unit of the A_2O system, including: anaerobic tank, ANA; anoxic tank, ANO; oxic tank, OXI; influent, INF; effluent, EFF. **(A)** Concentrations and total removal efficiency of COD_{Mn} in each unit of the A_2O system; **(B)** Concentrations and total removal efficiency of NH_4^+-N in each unit of the A_2O system; **(C)** Concentrations and total removal efficiency of TN/NO_x in each unit of the A_2O system; **(D)** Concentrations and total removal efficiency of TP in each unit of the A_2O system. The same letter indicates no significant difference(P>0.05), while different letters indicate significant difference(P<0.05).

removal efficiency of COD_{Mn} in the anaerobic tank and the anoxic tank remained stable at 39.2-46.9% and 65.7-70.6%, respectively. The removal efficiency of COD_{Mn} in oxic tank gradually decreased in the first three months and the removal efficiencies were about 26.2-33.6%. After that, the removal efficiency of COD_{Mn} increased significantly to 49.4% in the fourth month. The removal efficiencies of NH_4^+-N were showed in Figure 3B. The removal efficiencies of NH_4^+-N in anaerobic tank gradually increased from 38.1% to 70.3% in the first four months and then gradually decreased to approximately 53.5% in the sixth month. The removal efficiencies of NH_4^+-N in anoxic tank remained stable, approximately 76.4%. The removal efficiencies of NH_4^+-N in oxic tank maintained high level, approximately 91.1%. The removal efficiencies of TN were showed in Figure 3C. The removal efficiencies of TN in anaerobic tank, and anoxic tank remained stable, approximately 62.6% and 21.1%. The removal efficiencies of TP were showed in Figure 3D. The removal efficiencies of TP in anaerobic tank and oxic tank were 55.7% and 94.7%, respectively.

Water quality removal analysis of anoxic, anaerobic and oxic tanks

The removal distribution of different indicators (COD_{Mn}, NH_4^+-N, TN and TP) in anaerobic tank, anoxic tank, and oxic tank were shown in Figure 4. The A_2O system can remove a total of 88.9% of COD_{Mn}, where anaerobic tank, anoxic tank, and oxic tank can remove approximately 39%, 42.7%, and 7.2%, respectively. The A_2O system can remove a total of 98.9% of NH_4^+-N, where anaerobic tank, anoxic tank, and oxic tank can remove approximately 12.6%, 76.1%, and 10.2%, respectively. The A_2O system can remove a total of 99.8% of TN, where anaerobic tank, anoxic tank, and oxic tank can remove approximately 24.1%, 73%, and 2.7%, respectively. The A_2O system can remove a total of 99.8% of TP, where anaerobic tank, anoxic tank, and oxic tank can remove approximately 3.5%, 55.3%, and 41%, respectively.

Discussion

In this study, the results showed that the A_2O system by adding nitrifying bacteria and phosphorus accumulating bacteria biofilms using PBS as carriers instead of conventional activated sludge could effectively treat aquaculture wastewater. Compared to the application of the A_2O system in freshwater wastewater treatment (Ravishankar et al., 2019; Chen et al., 2022; Choi et al., 2022) or the combination of A_2O with MBR (Hao et al., 2022), BAF (Xi et al., 2022), SBR (Dai et al., 2022), the application in saline/semisaline water could achieve relatively ideal results.

As shown in Figure 2, biofilm-enhanced A_2O system can effectively remove COD_{Mn}. In the first three months, the COD_{Mn} removal efficiency of the A_2O system was about 87.2%. After three months of PBS biofilm enhancement of A_2O system, the removal efficiency of COD_{Mn} was further improved to approximately 91%. The results in Figure 3A further also showed that the removal

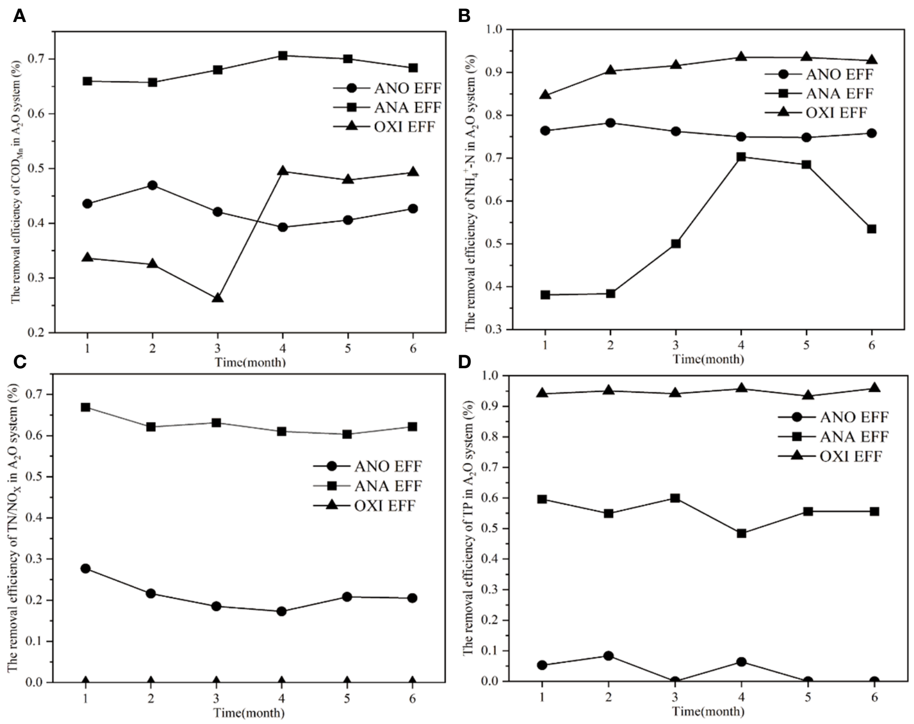

FIGURE 3
The removal efficiencies of COD_{Mn}, NH_4^+-N, TN/NO_x, and TP in each unit of the A_2O system, including: anaerobic tank, ANA; anoxic tank, ANO; oxic tank, OXI. **(A)** The removal efficiency of COD_{Mn} in each unit of the A_2O system; **(B)** The removal efficiency of NH_4^+-N in each unit of the A_2O system; **(C)** The removal efficiency of TN/NO_x in each unit of the A_2O system; **(D)** The removal efficiency of TP in each unit of the A_2O system.

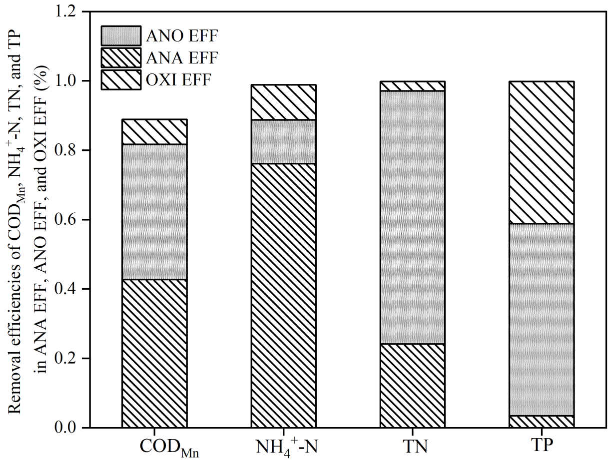

FIGURE 4
The removal distribution of different indicators (COD_{Mn}, NH_4^+-N, TN and TP) in anaerobic tank (ANA EFF), anoxic tank (ANO EFF), and oxic tank (ANO EFF).

efficiency of COD_{Mn} in the oxic tank gradually decreased in the first three months and gradually increased thereafter. This may be due to the gradual growth of heterotrophic microorganism after 3 months of A_2O system operation, which further improved the utilization of organic matters (Choi et al., 2022). Nevertheless, the results of Figure 4 further showed that COD_{Mn} was mainly removed in anaerobic tank and anoxic tank, accounting for about 92.7% of the total COD_{Mn} removal. Simultaneously, the removal efficiency of COD_{Mn} in the anaerobic tank and the anoxic tank remained stable (Figure 3A). It has been proved that heterotrophic nitrifying bacteria was more sensitive to oxygen, and heterotrophic nitrifying bacteria was mainly distributed in anaerobic and anoxic environment in A_2O systems (Ravishankar et al., 2019). Heterotrophic nitrifying bacteria require organic matter as an energy source for growth and metabolism (Chen et al., 2022). Moreover, it has been confirmed that many autotrophic nitrifying bacteria were mainly distributed in the oxic tank of the A_2O system, such as *Nitrosomonas*, *Nitrosospira*, *Nitrospira*, and *Nitrotoga* (Hao et al., 2022). These bacteria mainly rely on an autotrophic lifestyle for growth and metabolism resulting in a low need for organic matter (Xi et al., 2022). Therefore, the removal of COD_{Mn} in the A_2O system may be mainly due to the growth of heterotrophic nitrifying bacteria. The results in Figure 2, the removal efficiencies of NH_4^+-N and TP remained stable, and the removal efficiencies were 98.9% and 97.7%, respectively. This indicated that biofilm-enhanced A_2O system had a good performance in removing NH_4^+-N and TP from aquaculture wastewater. Autotrophic nitrifying bacteria can use oxygen as electron acceptor to oxidize ammonia to nitrite or nitrate under oxic conditions by autotrophic nitrification (NH_4^+-N→NH_2OH→NO_2^-N→NO_3^-N) (Dai et al., 2022). Therefore, the nitrification of autotrophic nitrifying bacteria may be one of the reasons for the increased removal efficiency of NH_4^+-N. As shown in Figure 3B, the results showed that the removal efficiency of NH_4^+-N in the anaerobic tank of the A_2O system decrease from 70.3% to 53.5%, which may be due to the release of intracellular nitrogen after the ganglionic formation of some dead bacterial cells, resulting in an increase in NH_4^+-N concentration (Yue et al., 2023). Nevertheless, some studies also believed that this may also be due to the reduction of nitrate and nitrite to ammonia caused by the reduction of ammonia nitrogen removal rate (Xiang et al., 2023). The results in Figure 4 showed that nearly 76.1% of NH_4^+-N was removed under anoxic conditions, which was similar to Peng et al. (2020). This was mainly due to the existence of a large number of facultative anaerobic and oxic microorganisms in the anoxic environment, which can effectively use ammonia nitrogen (Marazuela et al., 2023).

As shown in Figure 2, the results showed that COD_{Mn} and NH_4^+-N decreased significantly in the anaerobic tank, while the reduction of nitrate nitrogen and TP were not significant and even slightly increased. The reason was that the aquaculture wastewater in the tank was raw sewage after sedimentation and returned phosphorus-containing sludge. Dissolved organic matter and NH_4^+-N were absorbed by microorganisms for their own cell synthesis and respiratory metabolism (Zhang et al., 2023a), but the change in NO_3^-N content was not significant (Figure 2C). The characteristics of phosphorus released by phosphorus-accumulating bacteria in the anaerobic tank made the concentration of TP in wastewater not significantly reduced (Figure 3D). The results in Figure 4 also confirmed that approximately 96.3% of TP was removed in anoxic tank and oxic tank. The results in Figure 2 also showed that the concentration of NO_3^-N and phosphorus decreased significantly. However, as the nitrification process increased the concentration of NO_3^-N, TP also decreased at a faster rate with excessive uptake by phosphorus-accumulating bacteria. In the anoxic tank, PBS could not only be used as a carrier of microorganisms to help the growth of nitrifying bacteria/denitrification bacteria/phosphorus accumulating bacteria but could also properly supplement the carbon source required for microbial growth due to its own degradation (Zhang et al., 2019). The denitrification bacteria used the organic matter in the sewage and part of the products degraded by PBS as the carbon source to bring a large amount of NO_2^-N and NO_3^-N into the reflux mixture and reduced them to N_2 or released N_XO to the air.

Nitrogen and phosphorus removal mechanisms in three different phases of the reactor were shown in Figure 5. In anaerobic period, the sludge returned from the oxic zone entered the anaerobic zone. NH_4^+-N in the oxic zone was converted by AOB and NOB to nitrite and nitrate through nitrification. NO_2^-N and NO_3^-N were further removed to NO_X/N_2 heterotrophic nitrification bacteria (HNB). Simultaneously, it has been reported that HNB can use carbon source as electron donor and energy source to achieve denitrification (Abyar et al., 2018; Li et al., 2020). Therefore, nitrogen and COD are simultaneously removed by organic matter during the anaerobic phase of the reactor. Besides, phosphorus accumulating bacteria release phosphorus from the cell into the activated sludge (Ravishankar et al., 2019). Phosphorus and nitrogen released into the sludge entered the anoxic zone and were further removed by phosphorous-accumulating bacteria (PAOs) and Glycanogen organisms (GAOs). Some studies have confirmed that PAOS and GAOS can store carbon sources in the form of PHB

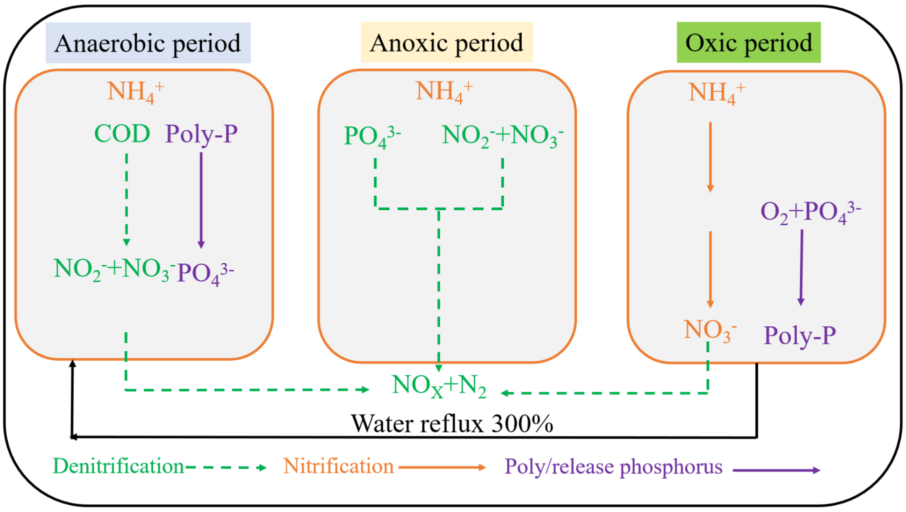

FIGURE 5
The removal pathway of COD_{Mn}, NH_4^+-N, TN/NO_x, and TP in the A_2O system.

in microbial cells, and achieve nitrogen and phosphorus removal through endogenous denitrification (ED).

In this study, by adding nitrifying bacteria and phosphorus accumulating bacteria biofilms using PBS as carriers instead of conventional activated sludge, the A_2O system could effectively solve the inherent contradiction between nitrogen (long HRT) and phosphorus (short HRT) on HRT and improve treatment efficiency. In contrast to the methods mentioned above, biofilm-enhanced A_2O system had the advantages of good treatment effect, low cost and easy practical application. Therefore, the biofilm-enhanced A_2O system has wide application potential in aquaculture wastewater treatment.

Conclusion

The nitrifying bacteria, denitrifying and phosphorus accumulating bacteria biofilms were used to enhance the A_2O system to evaluate the removal efficiency for the aquaculture wastewater. The results showed that Biofilm-enhanced A_2O system can effectively remove pollutants from aquaculture wastewater. The removal efficiencies of COD_{Mn}, NH_4^+-N, TN and TP in A_2O system were approximately 86.3%-90.8%, 97.7%-99.5%, 94.6%-95.2% and 97.0%-98.1%. The results further showed that COD_{Mn}, NH_4^+-N, and TN were mainly removed in anaerobic tank and anoxic tank, while TP was mainly removed in the anoxic tank and oxic tank. This study provides a practical method for aquaculture wastewater treatment.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material. Further inquiries can be directed to the corresponding author.

Author contributions

XM: Writing – review & editing, Data curation, Project administration. RuY: Formal analysis, Writing – review & editing. CY: Formal analysis, Writing – review & editing. CC: Writing – original draft, Writing – review & editing. JZ: Resources, Writing – review & editing. CL: Writing – review & editing. XW: Writing – review & editing. SC: Writing – review & editing. ReY: Writing – review & editing. BZ: Writing – original draft.

Funding

The author(s) declare financial support was received for the research, authorship, and/or publication of this article. This research was supported by the Project Funded by the Priority Academic Program Development of Jiangsu Higher Education Institutions (PAPD), Study on Removing Heavy Metal Pollution in Porphyra Cultivation Areas (Z2009050); Ecological Effects of Characteristic Pollutants in Typical Seafood in Haizhou Bay (2013HS011); the Natural Science Foundation of Jiangsu Province (No. BK20220682); the Project funded by China Postdoctoral Science Foundation (2022M721398); the Project funded by Postdoctoral Science Foundation of Lianyungang (LYG20230005); Postgraduate Research & Practice Innovation Program of Jiangsu Province; the Doctoral Program of Entrepreneurship and Innovation in Jiangsu Province (JSSCBS20221618).

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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