Nitrogen Removal Performance of an IFAS System under Low C/N Municipal Wastewater Conditions

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Abstract—To overcome the limited total nitrogen removal and dependence on external carbon sources in conventional treatment of low C/N municipal wastewater, a continuous-flow integrated fixed-film activated sludge (IFAS) reactor was established to investigate its nitrogen and carbon removal performance and underlying mechanisms under different organic loading conditions. The reactor was operated for 203 days with progressively increased influent COD, combined with dissolved oxygen regulation, intermittent aeration, and hydrazine-assisted inhibition. The results showed that nitrogen removal gradually improved after start-up. Under higher organic loading, the reactor achieved an average total nitrogen removal efficiency of about 80%, with a maximum of 89%, and showed good recovery after a 45-day starvation period. Activity tests suggested the coexistence of anammox, partial nitritation-anammox, and partial denitrification-anammox pathways. Overall, the stratified microenvironment in the IFAS system promoted synergistic interactions among functional microorganisms, indicating its potential for efficient and stable nitrogen removal from low-C/N municipal wastewater.

1. Introduction

Nitrogen pollution can cause eutrophication and threaten human health and ecological systems[1]. Conventional biological nitrogen removal (BNR) processes generally perform well for municipal wastewater with a C/N ratio above 5. However, increasingly stringent discharge standards and the growing proportion of low-C/N municipal wastewater (<5) have exposed the limitations of conventional BNR, particularly in total nitrogen removal and its dependence on external carbon sources, which increase both operating costs and carbon emissions in wastewater treatment plants (WWTPs).

Partial nitritation-anammox (PNA) is an energy-efficient autotrophic nitrogen removal process in which ammonium-oxidizing bacteria (AOB) convert NH_4^+ to NO_2^-, and anaerobic ammonium-oxidizing bacteria (AnAOB) subsequently use NO_2^- and residual NH_4^+ for nitrogen removal. Compared with conventional processes, PNA can reduce aeration energy demand by about 60%[2, 3] and does not require external carbon addition, making it a promising strategy for low-carbon wastewater treatment. However, its application under mainstream low-ammonium and low-C/N conditions remains challenging, mainly because effective suppression of nitrite-oxidizing bacteria (NOB) under low free ammonia (FA) conditions[4] and stable NO_2^- supply for AnAOB are difficult to achieve[5].

For the PNA nitrogen removal system, it is critical to inhibit NOB activity while improving AnAOB performance during the treatment of low-ammonia-nitrogen wastewater. Hydrazine (N_2H_4) is a commonly used chemical reagent and also occurs as a metabolic intermediate of AnAOB. Exogenous supplementation of N_2H_4 has been proven to efficiently suppress NOB without notably affecting AOB. According to the study by Xiang[6, 7], hydrazine inhibits nitrite oxidase activity and activates the nitrite reduction pathway in AnAOB. Meanwhile, the optimal N_2H_4 dosing conditions were determined, and the strongest NOB inhibition was obtained at a dosage of 5 mg/L.

In China, most WWTPs still rely on activated sludge systems for nitrogen removal, although their practical performance is often insufficient to consistently meet discharge standards. To address this, integrated fixed-film activated sludge (IFAS) systems, which combine suspended sludge and biofilm processes, have been increasingly explored[8]. IFAS offers low energy consumption, reduced excess sludge production, strong resistance to shock loading, and considerable potential for simultaneous nitrogen and carbon removal.. In addition, aerobic heterotrophs and nitrifiers tend to accumulate in suspended sludge, whereas AnAOB and some anoxic heterotrophs are preferentially retained within the biofilm[9]. Therefore, IFAS provides a feasible platform for establishing stratified microenvironments and enhancing nitrogen removal under low-C/N conditions.

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2. Materials and Methods

2.1 Experimental Setup

In this study, a continuous-flow integrated fixed-film activated sludge (IFAS) reactor was used. The reactor body was made of transparent acrylic, with a total effective working volume of 5.5 L. Three WPT carriers were installed in the reactor to establish a hybrid suspended sludge–biofilm system. Each carrier measured 6 × 26 cm and contained approximately 50 biofilm filaments. A schematic diagram of the IFAS reactor is presented in Figure 1.

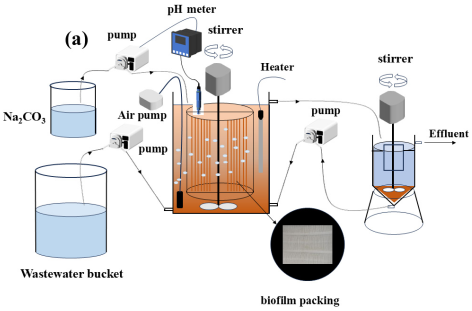

Figure. 1 IFAS reactor configuration

2.2 Inoculum Sludge and Influent Composition

Both the suspended sludge and biofilm were taken from a laboratory-scale PNA reactor that had been operated continuously. The influent to this reactor contained 60–70 mg/L ammonia nitrogen and 30–50 mg/L COD. After inoculation, the mixed liquor suspended solids (MLSS) concentration of the suspended sludge was 2700 mg/L, whereas that of the biofilm was 1900 mg/L.

The influent used in this study was simulated municipal wastewater prepared manually. NH_4^+-N and COD were supplied by $(NH_4)_2SO_4$ and glucose, respectively. Hydrazine (N_2H_4) was supplied as hydrazine sulfate.

2.3 Chemical Analysis

Water samples collected during the experiment were filtered through 0.45 μm membrane filters prior to analysis. NH_4^+-N was determined using the Nessler reagent spectrophotometric method, NO_2^--N was measured by the N-(1-naphthyl)-ethylenediamine spectrophotometric method, and NO_3^--N was analyzed using the dilute hydrochloric acid spectrophotometric method. In this study, total inorganic nitrogen (TIN) was defined as the sum of NH_4^+-N, NO_2^--N, and NO_3^--N. COD was determined by the potassium dichromate method. Dissolved oxygen (DO) was measured using a portable dissolved oxygen meter (JPBL-610L), and pH was measured using a pH meter (Sartorius PB-20).

2.4 Sludge Activity Tests

At the end of Phase IV (day 203), in-situ batch tests in triplicate were performed at 30 °C (pH = 7.5 ± 0.4) to assess sludge activities regarding anammox, PNA, and PDA pathways. All tests were terminated once the NH_4^+-N concentration dropped below 5 mg N/L, with water samples collected every 10 min.

For the anammox test, aeration and influent pumps were shut down, and both NH_4^+-N and NO_2^--N were initialized at 25 mg N/L. For the PNA test, aeration remained on (DO=4.5–5.3mg/L) with an initial NH_4^+-N of 25 mg N/L. For the PDA test, all pumps were turned off; the initial NH_4^+-N and NO_3^--N were set at 25 mg N/L, accompanied by an initial COD of 200 mg/L.

2.5 16S rRNA gene sequencing

Suspended sludge and biofilm samples were collected on days 0, 76, 107 and 203 for microbial community analysis. Illumina MiSeq sequencing platform was used for 16S rRNA gene sequencing to characterize the microbial community succession of the system during different operational stages.

3. Results and Discussion

3.1 Performance of the IFAS Reactor

The reactor was operated for 203 days in total. By progressively increasing the influent COD concentration and adjusting the operating parameters accordingly, the experimental period was divided into four phases. The nitrogen and COD removal performance of the IFAS reactor during each phase is presented in Figure 2.

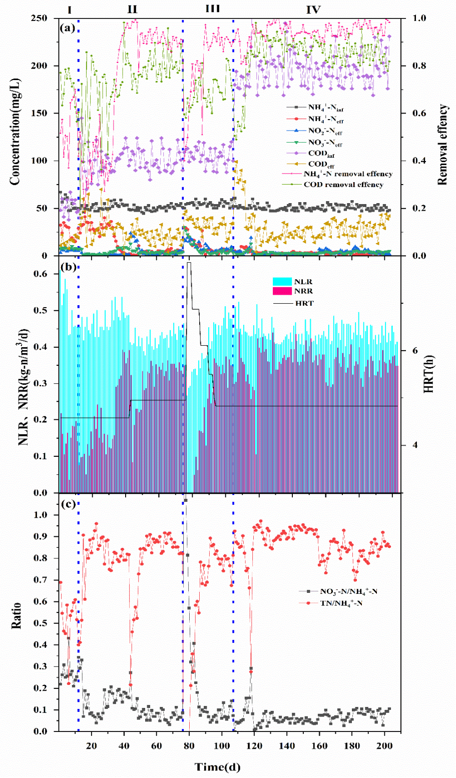

Figure. 2 Performance of the IFAS Reactor: Performance of the IFAS Reactor: (a) long-term changes in nitrogen and COD concentrations and removal efficiencies; (b) variations in NRR, NLR, and HRT; (c) variations in stoichiometric ratios.

During Phase I (0–12 d), the reactor showed relatively poor nitrogen removal, with high effluent NH_4^+-N and TN concentrations, because the microbial community was still acclimating to the new influent conditions. As operation continued, the activities of AOB and AnAOB gradually recovered, leading to improved NH_4^+-N oxidation and TN removal. By day 12, both removal efficiencies increased markedly, indicating that the reactor had reached a relatively stable state.

In Phase II (13–76 d), increasing influent COD caused NO_3^--N accumulation, suggesting enhanced NOB activity and suppression of the PNA pathway. To control NOB, 5 mg/L N_2H_4 was continuously added and intermittent aeration was applied. The reactor was initially operated under a 70/20 min (aeration/non-aeration) mode at a DO of approximately 0.8 mg/L. As higher COD promoted heterotrophic oxygen consumption and weakened nitrogen removal, the DO was increased to 2.8 mg/L. By day 37, effluent TN decreased to 10.9 mg/L, with a TN removal efficiency of 78%. After the aeration mode was adjusted to 70/30, the system regained stable performance, with effluent NO_2^--N and TN decreasing to 4.2 mg/L and 10.8 mg/L, respectively. Under stable conditions, the ΔNO_3^--N/ΔNH_4^+-N ratio was approximately 0.08, lower than the theoretical PNA value, whereas the ΔTN/ΔNH_4^+-N ratio approached the theoretical value of 0.88, indicating that autotrophic nitrogen removal remained dominant.

In Phase III (77–107 d), the reactor was re-fed after a 45-d starvation period to evaluate its recovery potential. With influent NH_4^+-N and COD concentrations of about 55 mg/L and 100 mg/L, respectively, and an HRT of 4.8 h, the reactor rapidly recovered its nitrogen removal performance. The TN removal efficiency remained around 70%, while ammonium removal efficiency and the nitrogen removal rate (NRR) gradually stabilized, demonstrating the strong resilience and recovery capacity of the IFAS system.

In Phase IV (108–203 d), the influent COD was increased to approximately 200 mg/L while NH_4^+-N remained nearly constant, increasing the C/N ratio to 4. Although the sudden COD increase initially reduced TN removal, the reactor gradually adapted and achieved stable performance, with an average TN removal efficiency of about 80% and a maximum of 89%. After hydrazine addition was terminated on day 150 and the aeration mode was adjusted from 70/30 to 90/40, the reactor maintained efficient nitrogen removal, with effluent NO_3^--N remaining around 4.5 mg/L and the ΔNO_3^--N/ΔNH_4^+-N ratio stabilizing near 0.10. The additional TN removal beyond that expected from the PNA pathway was likely associated with the PDA pathway, together with the stratified microenvironment formed within the IFAS system under higher organic loading.

3.2 Microbial Activity Tests

At the end of Phase IV, activity tests targeting anammox, partial nitritation–anammox, and partial denitrification–anammox were conducted to clarify the potential nitrogen removal pathways and microbial interactions in the IFAS system during stable operation (Figure 3).

In the anammox activity test, NH_4^+-N decreased from 26.1 mg/L to 4.1 mg/L within 70 min, while total nitrogen decreased from 53.3 mg/L to 15.4 mg/L. The specific nitrogen removal rate and specific ammonium removal rate were 10.64 mg N/g VSS/h and 6.18 mg N/g VSS/h, respectively, indicating the presence of anaerobic zones within the biofilm and a considerable potential for anammox activity.

In the partial nitritation–anammox activity test, NH_4^+-N decreased from 26.4 mg/L to 4.4 mg/L within 70 min, and total nitrogen decreased from 26.4 mg/L to 12.2 mg/L. The corresponding specific nitrogen removal rate and specific ammonium removal rate were 4.49 mg N/g VSS/h and 6.18 mg N/g VSS/h, respectively. These results indicate that aerobic ammonium oxidation and anaerobic nitrogen removal could occur simultaneously in the IFAS system, likely due to the stratified microenvironments formed within the biofilm and the coexistence of suspended sludge and biofilm.

In the partial denitrification–anammox activity test, NH_4^+-N decreased from 26.6 mg/L to 4.1 mg/L within 60 min, while total nitrogen decreased from 51.1 mg/L to 8.6 mg/L. The specific nitrogen removal rate and specific ammonium removal rate reached 13.99 mg N/g VSS/h and 7.33 mg N/g VSS/h, respectively, suggesting a strong nitrate reduction potential in the presence of organic carbon and a possible synergistic interaction between heterotrophic bacteria in suspended sludge and anaerobic microorganisms in the biofilm.

Overall, these activity tests suggest that the IFAS system likely developed a coupled nitrogen removal pathway involving partial nitritation, partial denitrification, and anammox.

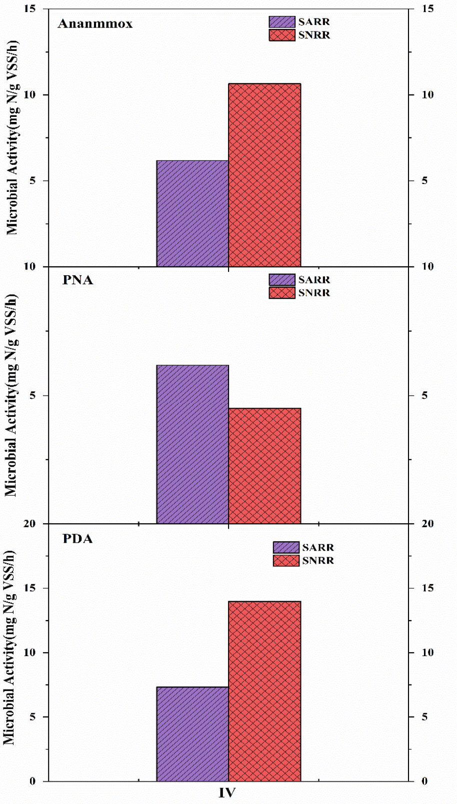

Figure. 3 Activity Measurement during the IFAS Operation Process

3.3 Microbial community analysis

During the entire operational period, the microbial community underwent obvious succession responding to varying C/N ratios and hydrazine regulation(Figure 4). In the initial adaptation stage (Phase I), the system possessed basic anammox, ammonia oxidation and heterotrophic denitrification capacities, with *Candidatus_Kuenenia* as the dominant AnAOB, and high abundances of Nitrosomonas and *Denitratisoma*. When the C/N ratio increased to 2 in Phase II, combined with hydrazine and intermittent aeration, AnAOB abundance declined due to environmental stress; nevertheless, AOB abundance slightly increased while NOB (*Nitrospira*) was consistently suppressed. After starvation recovery (Phase III), the anammox community shifted from *Candidatus_Kuenenia* to *Candidatus_Jettenia* and *Candidatus_Brocadia*, and organic carbon activated heterotrophic denitrifiers. In Phase IV with a C/N ratio of 4, functional microbial differentiation was achieved. The further enrichment of *Candidatus_Jettenia* and *Candidatus_Brocadia* ensured effective AnAOB retention, and the remarkably increased *Nitrosomonas* guaranteed stable ammonia oxidation capacity. Heterotrophic bacteria including *Hydrogenophaga* were enriched to consume dissolved oxygen and convert nitrate into nitrite, which facilitated the coupling of multiple nitrogen removal pathways. Additionally, the cooperation between suspended sludge and biofilm stabilized anaerobic functional microorganisms.

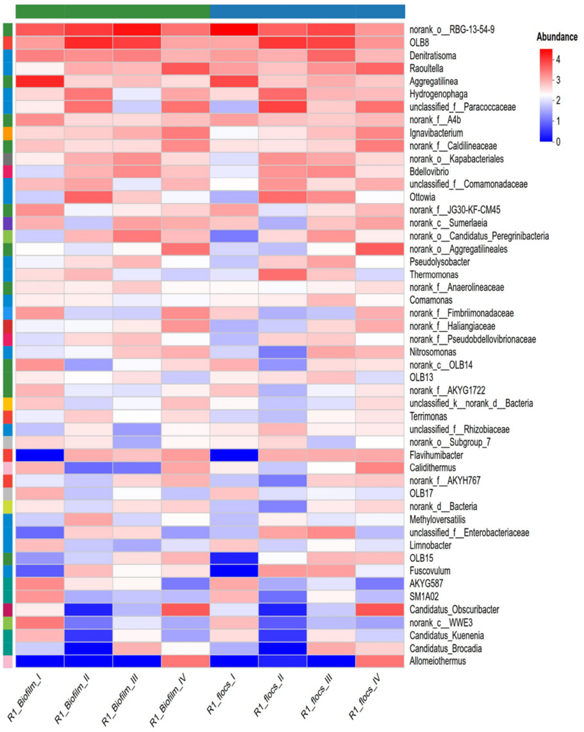

Figure. 4 Microbial community structure heatmap

4. Results and discussion

This study systematically investigated the nitrogen removal performance and microbial response of an IFAS system treating low C/N municipal wastewater. Reasonable regulation of organic substrates, dissolved oxygen, and aeration modes effectively strengthened the nitrogen removal capacity of the reactor. The system exhibited outstanding operational stability under elevated organic loading and presented excellent resilience after long-term starvation stress. Biological activity tests demonstrated that multiple nitrogen removal pathways, including anammox, partial nitritation–anammox, and partial denitrification–anammox, jointly contributed to nitrogen transformation. Microbial community structure underwent distinct succession in response to operational manipulation. The selective enrichment of dominant functional microorganisms, such as *Candidatus_Jettenia*, *Candidatus_Brocadia*, and *Nitrosomonas*, ensured persistent ammonia oxidation and anammox activity. Beneficial heterotrophic microorganisms synergistically optimized the microenvironmental conditions for nitrogen metabolism. Furthermore, the stratified biofilm structure and the cooperation between suspended sludge and attached biomass stabilized anaerobic functional bacteria. Collectively, the IFAS system constructed a synergistic nitrogen removal microbial consortium, showing considerable application potential for deep nitrogen removal from low C/N municipal wastewater.

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