water

MDPI

Article

Characteristics of Nitrogen Removal from an Integrated Fixed-Film Activated Sludge (IFAS) System and the Relationship Between Activated Sludge and Biofilm Interactions

Zishuo Tuo [1,2], Long Bai [1,2], Baoping Zhang [1,2], Shuangyi Jing [1,2,*], Chenxi Li [1,2] and Shike Tang [1,2]

[1] School of Energy and Environment, Inner Mongolia University of Science and Technology, Baotou 014010, China; 17736988837@163.com (Z.T.); bailongs@126.com (L.B.); zwcssn@163.com (B.Z.); lcx1808965188@163.com (C.L.); tangsk9186@163.com (S.T.)

[2] Collaborative Innovation Center of Autonomous Region for Ecological Protection and Comprehensive Utilization in the Inner Mongolia Section of the Yellow River Basin, Baotou 014010, China

* Correspondence: 13500622199@163.com

Abstract: In order to investigate the enhancement mechanism of modified three-dimensional elastic filler (MTEF) on the nitrogen removal performance of the integrated fixed-film activated sludge (IFAS) process, and to clarify the interactions between competition and synergy between activated sludge and biofilm in the IFAS system, an IFAS reactor (T2) filled with MTEF was employed for the study, while a sequencing batch reactor activated sludge process (SBR) reactor (T1) was utilized for comparison. IFAS and SBR reactors were operated over an extended period at ambient temperature to assess the enhancement of pollutant removal performance with the addition of the filler to investigate the competitive dynamics between activated sludge and biofilm under varying influent water qualities (C/N, N/P, and organic loading), and to analyze the synergistic relationship between activated sludge and biofilm at the microbial level using high-throughput sequencing technology. The results demonstrate that throughout the entire operational phase, reactor T2 exhibited superior pollutant removal efficiency. Compared to reactor T1, reactor T2 achieved an average increase in the removal rates of COD, ammonia nitrogen, and total nitrogen by 13.07%, 12.26%, and 28.96%, respectively. The findings on the competitive dynamics between activated sludge and biofilm indicate that the nitrification volumetric load of the IFAS system is significantly higher than that of a pure activated sludge system, suggesting that the IFAS system possesses enhanced nitrification capabilities. Furthermore, when dealing with wastewater characterized by low C/N ratios and high phosphorus pollution, or under substantial organic loads, the biofilm holds a competitive edge and the IFAS system exhibits improved stability. High-throughput sequencing data reveal that the microbial community structures in activated sludge and biofilm can influence each other, thereby enabling the IFAS system to effectively enrich denitrification-related functional microbial populations. Additionally, the biofilm has a certain enhancing effect on the expression levels of nitrogen metabolism-related functional genes in the activated sludge phase microorganisms, indicating that, in addition to competitive interactions, there is also a synergistic effect between the biofilm and activated sludge.

Keywords: modified three-dimensional elastic filler (MTEF); integrated fixed-film activated sludge (IFAS); activated sludge–biofilm interactions; nitrogen removal performance; high-throughput sequencing

Citation: Tuo, Z.; Bai, L.; Zhang, B.; Jing, S.; Li, C.; Tang, S. Characteristics of Nitrogen Removal from an Integrated Fixed-Film Activated Sludge (IFAS) System and the Relationship Between Activated Sludge and Biofilm Interactions. *Water* **2024**, *16*, 3040. https://doi.org/10.3390/w16213040

Academic Editor: Daniela Mesquita

Received: 25 September 2024
Revised: 18 October 2024
Accepted: 21 October 2024
Published: 23 October 2024

1. Introduction

Currently, the core process of urban wastewater treatment plants in China predominantly employs the activated sludge method [1]. However, the nitrogen removal efficiency of many wastewater treatment plants in operation is suboptimal [2], with nitrogen pollutant concentrations in the effluent frequently exceeding regulatory standards. Based on this, researchers have sought to address this issue by employing the IFAS process. Yang et al. [3]

employed a Moving-Bed Biofilm Reactor (MBBR) to modify the original Anaerobic-Anoxic-Oxic (A^2O) system of the wastewater plant, finding that ammonia nitrogen and total nitrogen concentrations in the effluent were reduced to 1.28 ± 0.91 and 5.78 ± 1.33 mg/L, respectively; the treatment capacity of the sewage plant was upgraded from 10 × 10^4 m^3/d m^3/d to 15 × 10^4 m^3/d, achieving in situ expansion and enhancement of the sewage plant. Li et al. [4] demonstrated that the nitrification rate of the IFAS system was 2.5 times higher than that of the pure sludge system at temperatures of 4 °C to 6 °C; moreover, the effluent consistently met regulatory standards while treating industrial wastewater after modification of the original A^2O system with the MBBR process. The addition of bio-carriers creates attachment surfaces for microorganisms within the reactor, facilitating biofilm formation, which enhances the biomass of functional flora [5]; this alteration impacts microbial composition, community structure, substrate distribution, and mass transfer mode, thereby optimizing the purification process and improving effluent quality. However, in certain sewage treatment plants, the introduction of suspended carriers into reaction tanks to enhance treatment efficiency can lead to their discharge into sedimentation tanks along with sludge, causing damage to sludge-thickening equipment. This not only incurs economic losses but also disrupts the normal operation of the sewage plants. Consequently, the study of the IFAS process utilizing stationary fillers is of significant importance for achieving in situ upgrades of wastewater treatment plants.

Recent studies have demonstrated that activated sludge and biofilm exhibit different responses to the same water quality factors. Goswami et al. [6] compared the performance of activated sludge and the MBBR process for treating chromium composite tannery wastewater, finding that an increase in influent COD concentration from 300 to 500 mg/L significantly inhibited the COD removal efficiency of the activated sludge system, whereas the MBBR system remained largely unaffected. Song et al. [7] and Jia et al. [8] examined the impact of temperature on the nitrification performance of activated sludge and biofilm, respectively. Their results indicated that both activated sludge and biofilm exhibited inhibited nitrification performance at excessively high influent temperatures, although the degree of inhibition varied. Despite numerous studies investigating the wastewater treatment performance of activated sludge and biofilm, a complex competitive [9] and synergistic [10] relationship exists between activated sludge and biofilm within the IFAS system, wherein the microbial community structures interact with one another. Previous studies primarily focused on the overall pollutant treatment performance of IFAS systems or examined the pollutant removal characteristics of activated sludge and biofilm in isolation, lacking a thorough investigation into the interactions between activated sludge and biofilm.

In response, this paper investigates the IFAS system utilizing modified three-dimensional elastic filler (MTEF) as the research focus, comparing and analyzing the differences in pollutant removal performance between the IFAS system and the SBR system under identical operating conditions while exploring the competition between activated sludge and biofilm in the IFAS system. Simultaneously, the microbial community structure and nitrogen metabolism-related genes in both activated sludge and filler biofilm from the two systems were analyzed using high-throughput sequencing technology. This approach aims to elucidate the enhancement mechanism of MTEF on the denitrification performance of IFAS, analyze the competitive and synergistic interactions between biofilm and activated sludge, and provide a theoretical basis for the application of the IFAS process in practical engineering.

2. Materials and Methods

2.1. Test Setup and Water

Two identical reactors were established to operate concurrently in this experiment, with the T1 reactor lacking a filler and the T2 reactor incorporating a modified filler. The reactor is constructed from organic glass, featuring a cylindrical main body with a diameter of 18 cm and a height of 50 cm, providing an effective volume of 11 L. Two fillers are installed within the reactor, with one end of each filler fixed to a metal frame at the bottom

of the reactor. An aeration disk is positioned at the bottom of the reactor, utilizing an air compressor for aeration, which is controlled by a valve to regulate the dissolved oxygen (DO) levels in the reactor. The inlet water flow is regulated by a peristaltic pump, as illustrated in Figure 1.

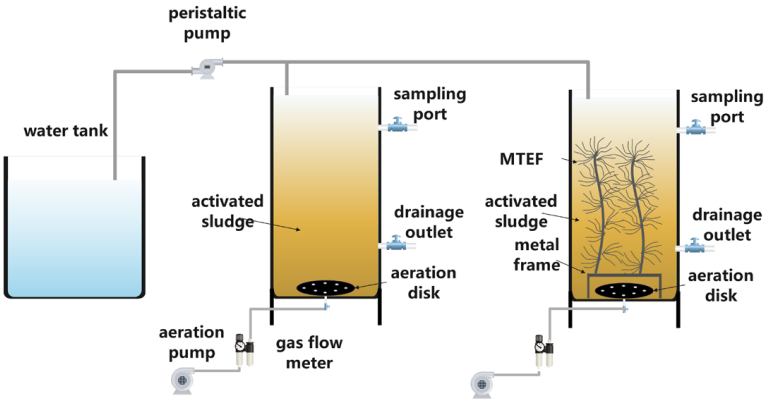

Figure 1. Schematic of the experimental setup.

The homemade modified filler used in T2 consists of polypropylene (PP), iron tetraoxide powder (Fe_3O_4), and polyquaternary ammonium salt-10 (PQAS-10), which are comingled, resembling a black rope brush with a diameter of 15 cm and a length of 40 cm. The appearance of the filler is illustrated in Figure 2. The MTEF used in this study, in a water environment at pH = 7, has a water contact angle of 75.20° and a Zeta potential of −17.53 mV. Compared to the commercial three-dimensional elastic Filler, which has a contact angle of 85.00° and a Zeta potential of −51.11 mV, the MTEF exhibits enhanced hydrophilicity and a reduced negative charge. These properties lead to increased surface biocompatibility, which is more conducive to microbial adhesion and the formation of biofilms.

Figure 2. Modified three-dimensional elastic filler.

The water used in the experiment consisted of manually prepared simulated domestic sewage to which anhydrous sodium acetate (CH_3COONa), ammonium chloride (NH_4Cl), and potassium dihydrogen phosphate (KH_2PO_4) were added to maintain an influent chemical oxygen demand (COD) of 300–400 mg/L, NH_4^+-N levels of 20–30 mg/L, and total phosphorus (TP) levels of 4.0–4.5 mg/L. One liter of water was combined with one milliliter of a micronutrient nutrient solution [11], and sodium bicarbonate ($NaHCO_3$) was added to provide the appropriate level of alkalinity, ensuring that the nitrification reaction in the reactor was not inhibited by pH [12]. The activated sludge was sourced from the aeration tank of a wastewater treatment plant in Baotou City, with a concentration of mixed suspended solids (MLSSs) of approximately 4000 mg/L.

2.2. Operational Programs

The two reactors were operated under identical conditions, differing only in the presence or absence of filler, and both functioned in SBR mode. The experiment consisted of two phases: the first phase involved reactor start-up and long-term operation, during which the reactor was filled with filler material and activated sludge was added to facilitate the domestication of the activated sludge and the initiation of biofilm growth. The hydraulic retention time (HRT) was maintained at 12 h, comprising an aerobic phase of 7.5 h, an anoxic phase of 3.75 h, water in for 0.25 h, water out for 0.25 h, and 0.25 h on standby for the reactor. The mixed liquor suspended solids (MLSSs) were approximately 2000 mg/L, with a pH of 7.5–8 and a dissolved oxygen (DO) concentration in the aerobic stage of 2.5–3.5 mg/L. Influent and effluent water quality were monitored regularly, and the activated sludge and MTEF biofilm in the two reactors underwent 16S rRNA high-throughput sequencing after 120 days of operation. In the second phase, the competition between activated sludge and biofilm in the MTEF-based IFAS system was examined. Two reactors were established: one as a pure sludge SBR system and the other as the MTEF-based IFAS system. The activated sludge for both systems was sourced from the T2 reactor. The cycle time (CT) for the reaction was 4 h, with approximately 2000 mg/L of MLSSs and a DO concentration of 2.5–3 mg/L. The carbon-to-nitrogen ratio (C/N), organic loading, and nitrogen-to-phosphorus ratio (N/P) were utilized as independent variables to investigate the effects of various factors on the competitive relationship between activated sludge and biofilm. The experimental control parameters for each factor are presented in Table 1.

Table 1. Corresponding control parameters for each factor experiment.

Control Variable	COD/mg·L^{-1}	NH_4^+-N/mg·L^{-1}	C/N	N/P
C/N	400/200/100	20	20/10/5	0.2
Organic and nitrogen load	400/800/1600	20/40/80	20	0.2
N/P	400	20	20	5/1/0.2

2.3. Methods of Analysis

2.3.1. General Indicators

In this study, routine water quality indicators were determined following Chinese standard methods [13]. Chemical oxygen demand (COD) was measured using the potassium dichromate method, ammonium nitrogen (NH_4^+-N) was analyzed via Nano reagent spectrophotometry, nitrite nitrogen (NO_2^--N) was assessed using N-(1-naphthalenyl)-ethylenediamine spectrophotometry, nitrate nitrogen (NO_3^--N) was determined by ultraviolet spectrophotometry, and total nitrogen (TN) was measured using UV spectrophotometry with potassium persulfate digestion. Mixed liquor suspended solids (MLSSs) and mixed liquor volatile suspended solids (MLVSSs) were determined using the gravimetric method. Dissolved oxygen (DO) and pH were measured using a Multi8330 portable water quality analyzer.

2.3.2. High-Throughput Sequencing

A section of the filler material was placed in a beaker, to which 50 mL of deionized water was added. Ultrasonic vibration was employed to dislodge the biofilm from the filler. Subsequently, the biofilm and activated sludge samples were stored in dry ice at −80 °C and sent to Shanghai Meiji Biotechnology Company for analysis. Primers 338F (5′-ACTCCTACGGGGAGGCAGCAG-3′) and 806R (5′-GGACTACHVGGGGTWTCTAAT-3′) were selected for PCR amplification of the V3–V4 region of the bacterial 16S rRNA gene. The amplified products served as templates for library construction and sequencing analysis using the Illumina MiSeq platform.

2.4. Calculation of Biofilm Volumetric Loads

Due to the superior mass and oxygen transfer performance of activated sludge, its nitrification volumetric load in both the activated sludge system and the IFAS system is essentially the same when the control conditions are identical [14]. Therefore, the following formula is employed to calculate and characterize this experiment.

$$ARL_V = \frac{\Delta C_{NH_4^+-N}}{CT \times 1000} \times 24 \quad (1)$$

$$ARL_{V(biofilm)} = ARL_{V(composite)} - ARL_{V(sludge)} \quad (2)$$

$$\Phi = \frac{ARL_{V(composite)} - ARL_{V(sludge)}}{ARL_{V(composite)}} \quad (3)$$

ARL_V—Nitrification volumetric load, kgNH_4^+-N/(m^3·d);
$ARL_{V(\text{biofilm})}$—Volumetric load of biofilm nitrification in IFAS system, kgNH_4^+-N/(m^3·d);
$ARL_{V(\text{composite})}$—Overall nitrification volumetric load for IFAS system, kgNH_4^+-N/(m^3·d);
$ARL_{V(\text{sludge})}$—Activated sludge system nitrification volumetric load, kgNH_4^+-N/(m^3·d);
φ—MTEF biofilm nitrification contribution, %;
$\Delta C_{NH_4^+-N}$—NH_4^+-N concentration change before and after system reaction, mg/L;
CT—Reaction cycle time, h.

3. Results and Discussion

3.1. Pollutant Removal Performance

The concentration of pollutants in the effluent stabilized after 72 days of reactor operation; thus, the effluent quality from days 72 to 120 was selected for analysis to investigate the effects of pollutant removal during the long-term operation of each reactor. Figure 3a illustrates that the average concentration of ammonia nitrogen in the influent was 27.01 ± 0.84 mg/L. The average concentrations in the effluent of the T1 and T2 reactors were 4.71 ± 0.20 mg/L and 1.40 ± 0.084 mg/L, respectively, resulting in average removal rates of 82.56 ± 2.40% and 94.82 ± 1.46%. Compared to the initial startup of the reactors (0 days), the ammonium nitrogen removal capacity of reactor T1 decreased, while that of reactor T2 increased. This is due to the long duration of the experiment, which began in the summer with higher environmental temperatures, leading to stronger activity of nitrifying bacteria and better ammonium nitrogen removal. As the experiment progressed into winter, the nitrification performance was affected by lower temperatures, resulting in a decline in the ammonium nitrogen removal capacity of reactor T1. However, a substantial body of research has demonstrated that the impact of low temperatures on biofilm is less pronounced than on activated sludge [4], hence the nitrification performance of reactor T2 was not excessively compromised. Additionally, due to the low activated sludge concentration within the reactor, the small amount of produced activated sludge was insufficient to meet the needs of activated sludge renewal, failing to balance the rate of activated sludge aging. Consequently, the overall activity of the activated sludge gradually decreased, leading to a decline in reactor T1's performance in removing ammonium nitrogen. In contrast, the MTEF in reactor T2, with its larger biomass attachment capacity, increased the biomass of nitrification-related functional microbial communities within the reactor. Furthermore, the shedding of biofilm into the activated sludge during operation mitigated the issue of activated sludge aging, resulting in a slight improvement in the ammonium nitrogen removal performance of reactor T2. Figure 3b depicts the accumulation of nitrite nitrogen in the reactors. As the test water was not supplemented with NO_2^--N, its concentration was not represented in the results. The figure indicates that the concentration of NO_2^--N in the effluent of each reactor was below 0.20 mg/L, with no accumulation of nitrite nitrogen. Figure 3c demonstrates that the concentrations of NO_3^--N in the effluent of the reactors were 7.85 ± 0.24 mg/L and 3.36 ± 0.17 mg/L, respectively. Compared to reactor T1, the T2

reactor with the addition of MTEF showed a decrease of 4.49 mg/L in the concentration of NO_3^--N in the effluent, indicating better denitrification performance in reactor T2. This is attributed to the fact that once the biofilm reaches a certain thickness, its inner layer creates an anaerobic environment that provides conditions conducive to the growth and proliferation of anaerobic denitrifying bacteria. This enables the biofilm to carry out both nitrification and denitrification processes, thereby enhancing the denitrification performance of the reactor. Figure 3d shows that the average concentration of total nitrogen in the influent was 27.97 ± 0.88 mg/L. The average concentrations of total nitrogen (TN) in the effluent from the T1 and T2 reactors were 12.94 ± 0.42 mg/L and 4.84 ± 0.22 mg/L, respectively, yielding average removal rates of 53.75 ± 3.34% and 82.71 ± 5.90%. The installation of MTEF enhanced the nitrification and denitrification performances of the reactor, thereby strengthening the reactor's nitrogen removal capabilities. Figure 3e indicates that the average concentration of COD in the influent was 367.69 ± 5.04 mg/L, and the average concentrations of COD in the effluents of T1 and T2 reactors were 81.59 ± 3.43 mg/L and 33.55 ± 2.05 mg/L, and the average removal rates were 77.76 ± 4.20% and 90.81 ± 1.93%, respectively. The experimental results showed that the IFAS system with MTEF was better than the pure activated sludge system for the removal of all types of pollutants, which was attributed to the fact that the installation of filler increased the number of microorganisms in the reactor [15] and changed the distribution and mass transfer of the substrate in the system.

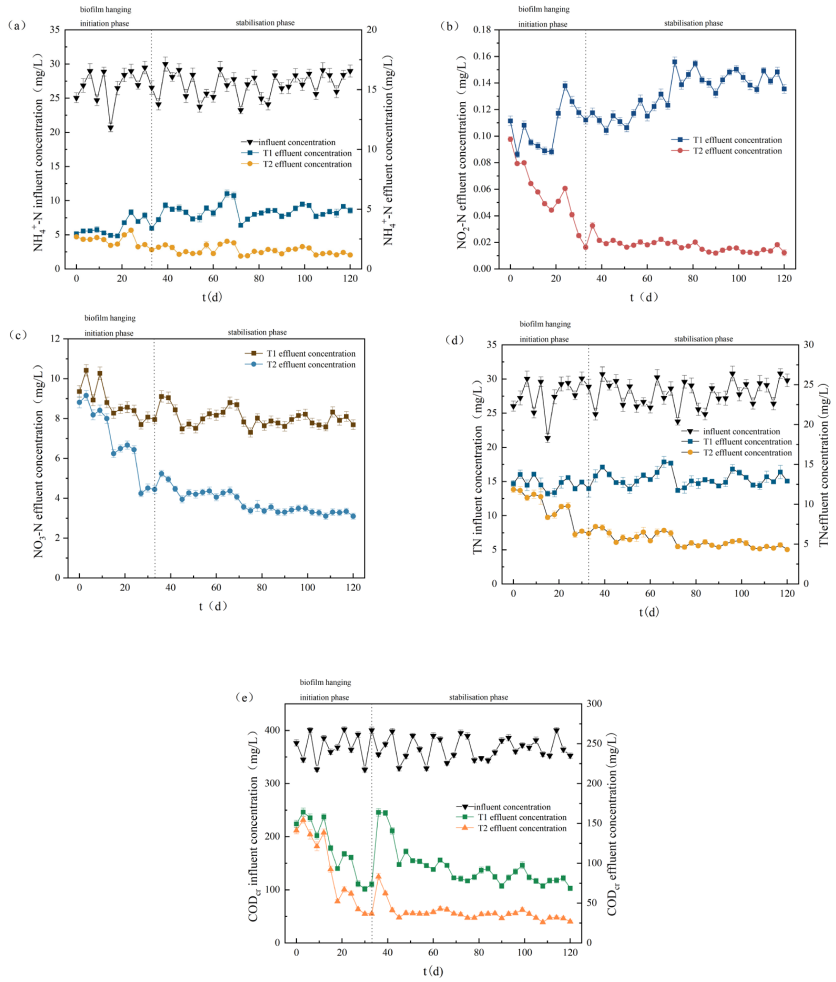

Figure 3. Changes in pollutant concentrations in influent and effluent water. (**a**) Ammonia nitrogen concentration; (**b**) nitrite nitrogen concentration; (**c**) nitrate nitrogen concentration; (**d**) total nitrogen concentration; (**e**) COD concentration.

3.2. Study of the Competition Law between Activated Sludge and Biofilm

3.2.1. Effect of C/N on the Nitrification Performance of the IFAS System

The results illustrating the effect of the carbon-to-nitrogen (C/N) ratio on the nitrification performance of the MTEF-based IFAS system are presented in Figure 4. The nitrification volumetric loads for the activated sludge system at C/N ratios of 20:1, 10:1, and 5:1 were 0.063 ± 0.002, 0.070 ± 0.002, and 0.081 ± 0.003 kgNH_4^+-N/(m^3·d), respectively. The corresponding volumetric loads for biofilm nitrification in the IFAS system were 0.023 ± 0.001, 0.033 ± 0.001, and 0.045 ± 0.001 kgNH_4^+-N/(m^3·d), with contributions of biofilm nitrification at 26.83%, 32.16%, and 35.66%, respectively.

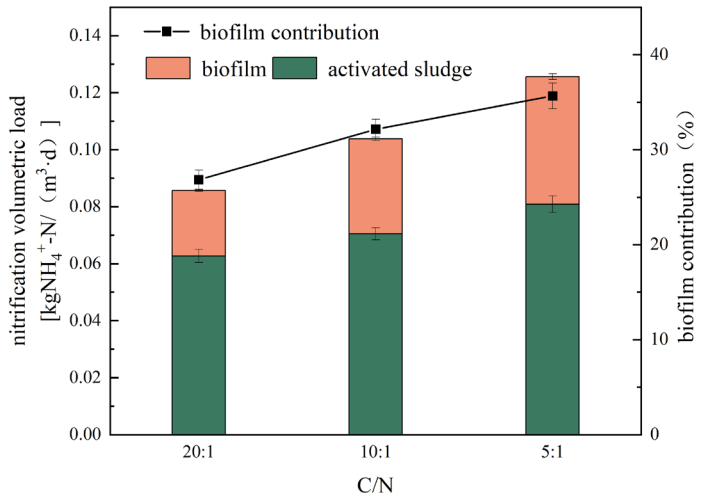

Figure 4. Effect of C/N on the IFAS system.

As the C/N ratio decreased, the nitrification volumetric load of both activated sludge and biofilm exhibited an increasing trend; notably, the biofilm responded more significantly, with its nitrification contribution increasing by 8.83%, thereby positioning it advantageously in the competition between activated sludge and biofilm. It was found that under low carbon-to-nitrogen ratio conditions, nitrogen removal efficiency in municipal wastewater can be enhanced by adjusting the operating parameters and utilizing bio-carriers without the need for additional carbon sources [16]. Therefore, when treating wastewater with a low carbon-to-nitrogen ratio, the IFAS system demonstrates a higher nitrification load.

3.2.2. Effect of Organic Loading on Nitrification Performance of the IFAS System

The results illustrating the effect of organic loading on the nitrification performance of the MTEF-based IFAS system are presented in Figure 5. In this portion of the experiment, the C/N ratio was consistently maintained at 20:1, while the concentrations of influent carbon and nitrogen sources were increased in equal proportions to the influent COD and NH_4^+-N concentrations of 400 mg/L and 20 mg/L, 800 mg/L and 40 mg/L, and 1600 mg/L and 80 mg/L, respectively. The nitrification volumetric loads for the activated sludge system were 0.063 ± 0.002, 0.040 ± 0.002, and 0.021 ± 0.001 kgNH_4^+-N/(m^3·d), respectively. The corresponding nitrification volumetric loads for biofilm in the IFAS system were 0.023 ± 0.001, 0.021 ± 0.001, and 0.017 ± 0.001 kgNH_4^+-N/(m^3·d), with biofilm nitrification contributions of 26.82%, 34.59%, and 45.38%, respectively.

As the organic load gradually increased, the nitrification volumetric loads of both activated sludge and biofilm decreased to varying degrees, with activated sludge being particularly affected, as its nitrification volumetric load decreased by 67.18%, while the biofilm's nitrification volumetric load decreased by 25.59%, resulting in an increased nitrification contribution rate of 18.57%. These results indicate that the biofilm demonstrates greater resistance to shock loading when treating wastewater with high pollutant concentrations [17]. As reported in previous studies, higher abundances of Comammox were

detected in the denitrification system of the attached-growth biofilm process. This can be attributed to the biofilm's ability to enhance the resistance of microorganisms to shock loading, providing a protective effect on these microorganisms; additionally, the oxygen concentration gradient formed within the biofilm is more conducive to the synergistic metabolism of Comammox and various types of nitrifying bacteria [18]. In research utilizing the A/O-MBBR process to treat wastewater from a highway service area, it was found that, compared to the conventional A/O bioreactor, the A/O-MBBR maintains effective total nitrogen (TN) removal efficiency even under higher organic loads. Moreover, it better adapts to changes in water quality when treating wastewater characterized by high ammonia–nitrogen concentrations and low carbon-to-nitrogen ratios, thereby providing more stable treatment performance [19]. In summary, the MTEF-based IFAS system exhibits greater shock load resistance and demonstrates enhanced stability and pollutant removal efficiency when treating wastewater with high pollutant concentrations, with the biofilm playing a crucial role in maintaining the system's normal operation.

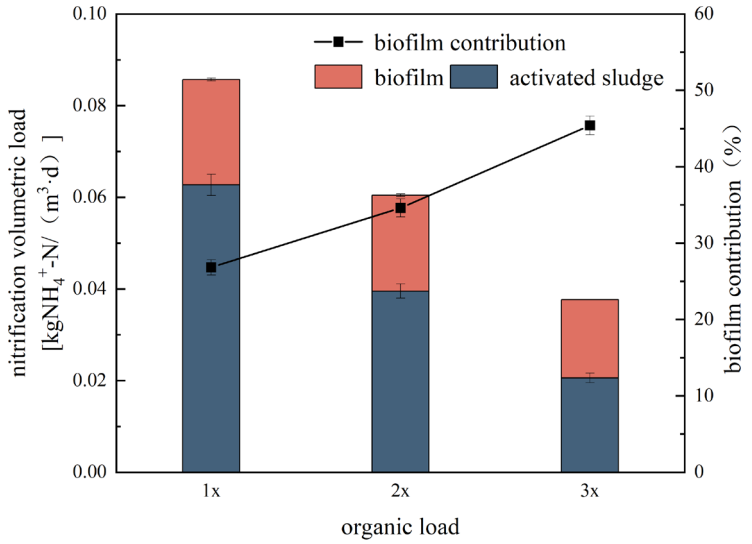

Figure 5. Effect of organic loading on the IFAS system.

3.2.3. Effect of N/P on the Nitrification Performance of the IFAS System

The results illustrating the effect of the nitrogen-to-phosphorus (N/P) ratio on the nitrification performance of the MTEF-based IFAS system are presented in Figure 6. The nitrification volumetric loads of the activated sludge system with N/P ratios of 5:1, 1:1, and 1:5 were 0.063 ± 0.002, 0.045 ± 0.002, and 0.033 ± 0.002 kgNH_4^+-N/(m^3·d), respectively. The corresponding volumetric loads for biofilm nitrification in the IFAS system were 0.023 ± 0.001, 0.021 ± 0.001, and 0.020 ± 0.001 kgNH_4^+-N/(m^3·d), with biofilm nitrification contributions of 26.83%, 31.17%, and 36.83%, respectively.

As the phosphorus concentration in the influent water gradually increased, the nitrification performance of the biofilm was less adversely affected, while the nitrification of activated sludge experienced inhibition; specifically, its nitrification volumetric load decreased by 46.59% whereas that of the biofilm decreased by only 15.10%, resulting in a 10% increase in its nitrification contribution. These results indicate that the biofilm maintains a competitive advantage over activated sludge in the context of increasing phosphorus concentrations. Research indicates that in wastewater treatment utilizing simultaneous nitrification and denitrification in sequencing batch biofilm reactors, nitrification can occur only when aeration time is sufficiently extended, as nitrifying bacteria are less competitive for oxygen compared to polyphosphate-accumulating organisms and heterotrophic bacteria [20]. Consequently, it is presumed that the high phosphorus environment enhances the metabolic activity of phosphate-accumulating organisms [21], which compete with nitrifying bacteria for dissolved oxygen (DO) [22], thereby inhibiting the activity of ni-

trifying bacteria and resulting in decreased nitrification performance within the system. Furthermore, the physical structure of MTEF facilitates the slicing [23] and retention [24] of air bubbles within the water, resulting in a high concentration of DO at the filler's surface. This condition alleviates the competition for DO between polyphosphate-accumulating bacteria and nitrifying bacteria, thereby preserving the nitrification performance of the biofilm and playing a crucial role in maintaining system stability during the treatment of high-phosphorus wastewater.

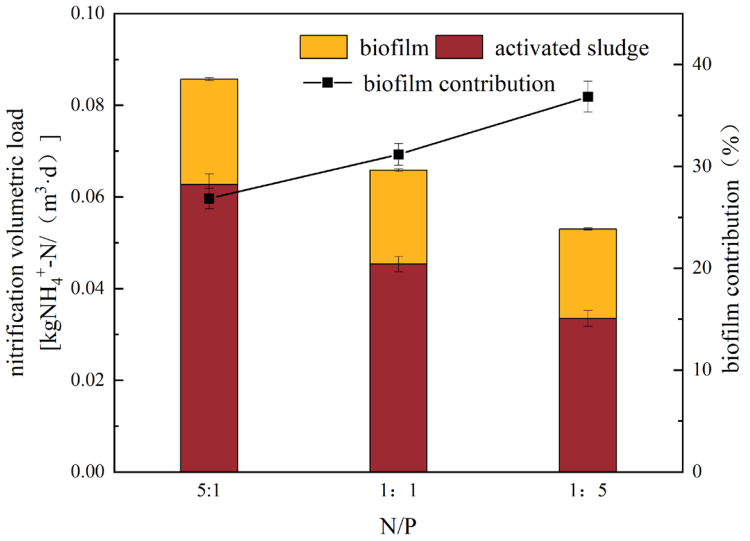

Figure 6. Effect of N/P on the IFAS system.

3.3. Microbial Community Structure

3.3.1. Microbial Community Diversity Analysis

The microbial diversity indices of the biofilm and the activated sludge from both systems are presented in Table 2. The detection results indicate that the microbial species richness and community diversity on the biofilm are greater than those of the activated sludge phase microorganisms in both systems, suggesting that the introduction of MTEF provides an ideal growth environment for microorganisms. Furthermore, due to the partial shedding of the biofilm on the MTEF as the reactor operates, it affects the community diversity of the activated sludge phase microorganisms in reactor T2. Consequently, the species richness and community diversity of the activated sludge phase microorganisms in reactor T2 are greater than those in reactor T1. This result demonstrates that the installation of MTEF not only enhances the biomass within the reactor but also increases the microbial species richness and community diversity and has a regulatory and strengthening effect on the activated sludge within the system.

Table 2. Each factor experiment corresponds to the control parameters.

Sample	Shannon	Simpson	Ace	Chao 1	Coverage
T1 activated sludge	4.45	0.047	1148.18	1116.96	0.99
T2 activated sludge	4.72	0.030	1209.36	1167.94	0.99
T2 biofilm	4.93	0.025	1370.39	1325.56	0.99

3.3.2. Differences in Dominant Genera

Figure 7 presents the dominant genera within the two types of filler biofilms, with relative abundances exceeding 1% in at least one sample. The top five genera with the highest relative abundances in the activated sludge of the T1 reactor were *Paracoccus* (13.68%), *Thauera* (7.09%), OLB12 (5.53%), *Flavobacterium* (5.49%), and *Hydrogenophaga* (3.78%); the five genera with the highest relative abundances in the activated sludge of the

T2 reactor were *Thauera* (12.60%), *OLB12* (8.29%), *Paracoccus* (6.93%), *Azoarcus* (5.20%), and *norank_f__Cryomorphaceae* (5.06%); the five genera with the highest relative abundances in MTEF biofilms were *Thauera* (13.32%), *OLB12* (12.01%), *Azoarcus* (9.05%), *Flavobacterium* (5.17%), and *norank_f__Cryomorphaceae* (3.86%).

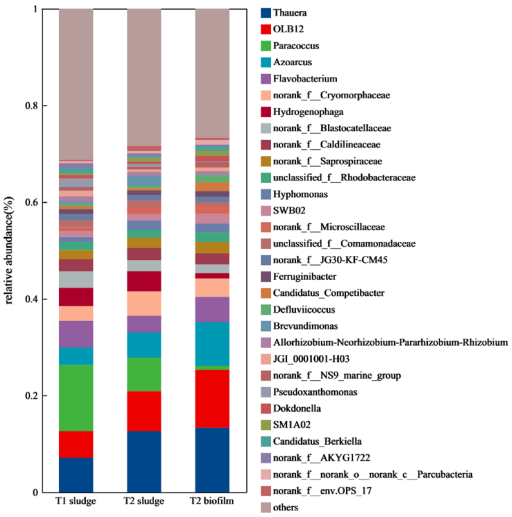

Figure 7. Genus-level microbial community structure.

Thauera was the dominant genus in both MTEF biofilm and T2 reactor sludge, possessing a strong EPS secretion capacity [25], which promotes biofilm formation [26] and participates in denitrification [27]. Its relative abundance in T1 reactor sludge was significantly lower than in T2 reactor sludge, indicating that the microbial community composition of activated sludge and biofilm influences each other. *OLB12* is a class of aerobic nitrifying bacteria [28] that cannot function in anoxic conditions. Its relative abundance in the activated sludge and biofilm of the T2 reactor was greater, indicating that the IFAS system effectively enriches nitrifying bacterial genera, thereby improving the nitrifying performance of the reactor. This is because the MTEF can cut and retain air bubbles, thereby improving the utilization rate of aeration in reactor T2, which is conducive to the growth and proliferation of nitrifying bacteria. This corresponds to the observed lower concentration of ammonia nitrogen in the effluent from the IFAS system, as reported in previous studies. *Azoarcus* is a common anaerobic, parthenogenetic denitrifying [29] bacterium in wastewater treatment systems that can utilize stored carbon sources to denitrify, thus completing nitrogen removal. It ranks among the five dominant genera with the highest abundances in both activated sludge and biofilm of the T2 reactor, while its relative abundance in the sludge of the T1 reactor is lower, further demonstrating the synergistic effect between activated sludge and biofilm. Generally, most of the dominant bacteria in the activated sludge and biofilm of the T2 reactor were involved in nitrogen removal and organic matter degradation. Consequently, the effluent quality of the IFAS system demonstrated a strong ability to remove NH_4^+-N and COD. This also indicates that the installation of filler effectively enriches the functional flora related to denitrification [30], enhancing the system's nitrogen pollutant removal performance.

3.3.3. Characterization of Functional Bacterial Genera

From the previous section, it is evident that the reactor equipped with the MTEF setup exhibits a superior denitrification effect. Additionally, the study of the dominant genera indicated that the MTEF setup effectively enriches the functional bacterial flora. Therefore, this subsection further analyzes the primary functional bacterial genera related to denitrification in the T1 and T2 reactors. These genera are classified into three categories: ammonia-oxidizing bacteria (AOB), nitrite-oxidizing bacteria (NOB), and denitrifying

bacteria (DNB). The relative abundance of each functional bacterial genus is presented in Figure 8.

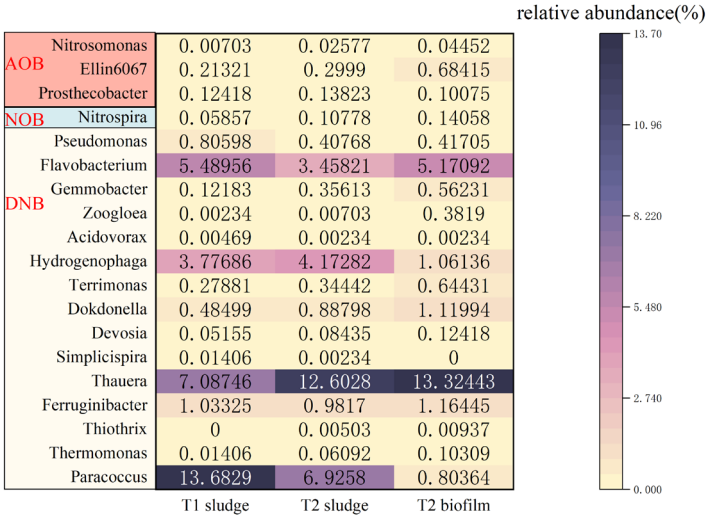

Figure 8. Abundance of functional bacterial genera.

The MTEF setup effectively enriched nitrification-related functional bacterial genera. *Nitrosomonas* is a typical ammonia-oxidizing bacterium (AOB) that can convert ammonia nitrogen in wastewater into nitrite nitrogen, thus completing the ammonia oxidation reaction in wastewater treatment [31]. Its relative abundances in the activated sludge and MTEF biofilm of the T2 reactor were 0.03% and 0.04%, respectively, while its abundance in the activated sludge of the T1 reactor was less than 0.01%. This indicates that the implementation of MTEF favors the enrichment of *Nitrosomonas*, thereby enhancing the reactor's ammonia nitrogen removal capability. Additionally, the synergistic effect between activated sludge and biofilm significantly increased the relative abundance of this genus in the sludge of the T2 reactor, confirming that the ammonia nitrogen removal capacity of the T2 reactor was superior in the previous study. Ellin6067 is another genus of ammonia-oxidizing bacteria, and its relative abundance in the activated sludge and biofilm of the T2 reactor increased compared to that of the T1 reactor. The relative abundances of *Nitrospira*, a genus of nitrite-oxidizing bacteria, in the activated sludge and MTEF biofilms of the T1 and T2 reactors were 0.06%, 0.11%, and 0.14%, respectively. Both the activated sludge and biofilm of the T2 reactor exhibited increased relative abundance compared to the T1 reactor. This indicates that the implementation of MTEF facilitates the enrichment of nitrite-oxidizing bacteria in the reactor, ensuring the smooth progression of the nitrification reaction [32]. This is also consistent with the lower concentration of nitrite nitrogen observed in the effluent of the T2 reactor.

A variety of denitrification-related functional bacterial genera (DNB) were detected in the activated sludge and biofilm of both reactors. In addition to the previously mentioned *Thauera*, *Flavobacterium*, *Paracoccus*, *Pseudomonas*, *Gemmobacter*, *Zoogloea*, and other common DNB genera were also identified. Additionally, several autotrophic denitrifying bacterial genera were identified. *Hydrogenophaga* is a hydrogen-oxidizing autotrophic denitrifying bacterium capable of reducing nitrate nitrogen using hydrogen as an electron donor [33]. *Ferruginibacter*, an iron-autotrophic denitrifying bacterium involved in the conversion between Fe^{2+} and Fe^{3+} and the denitrification process [34], reached a relative abundance of 1.16% in the MTEF biofilm, possibly due to the embedding of ferric iron tetraoxide powder within the MTEF. *Thiothrix*, a genus of sulfur-autotrophic denitrifying bacteria, exhibited a notable relative abundance in both the activated sludge and biofilm of the T2 reactor, whereas its presence was undetected in the sludge of the T1 reactor. This finding further supports the existence of a synergistic effect between activated sludge and biofilm,

indicating that the implementation of MTEF could effectively enrich denitrification-related functional genera, thereby enhancing the denitrification performance of the reactor.

3.4. Nitrogen Metabolism Functional Genes

Nitrogen metabolism encompasses a series of biochemical reactions facilitated by enzymes encoded by specific functional genes. Therefore, to evaluate the enhancement effect of MTEF on the nitrogen removal performance of the IFAS reactor, this study comparatively analyzed the abundance of major nitrogen metabolism-related functional genes in the biofilm and activated sludge. This analysis aimed to investigate the influence of the biofilm on the microorganisms in the activated sludge phase.

Figure 9 illustrates the abundance of major nitrogen metabolism-related functional genes in both biofilm and activated sludge. The functional genes involved in the nitrification process include *amoA*, *amoB*, *amoC*, *hao*, *nxrA*, and *nxrB*. The *amo* gene is recognized as the marker gene for aerobic ammonia oxidation [35], encoding ammonia monooxygenase (AMO), which oxidizes ammonia to hydroxylamine [36]. Hydroxylamine is converted to nitrite by hydroxylamine oxidoreductase (HAO), encoded by the *hao* gene [37], completing the ammonia oxidation process in wastewater treatment [38]. Simultaneously, nitrite is oxidized to nitrate by nitrite oxidoreductase (NXR), encoded by the *nxr* gene [39], thereby completing the nitrification process in wastewater treatment. The abundances of *amo* and *hao* in the activated sludge and biofilm of the T2 reactor were slightly higher than in the T1 reactor. Furthermore, the abundance of *nxr* in both the activated sludge and biofilm of the T2 reactor significantly increased, indicating enhanced nitrite-oxidizing capacity. This timely conversion of nitrite nitrogen, an intermediate product of nitrification, ensures that the nitrification reaction proceeds efficiently. This finding also accounts for the lowest concentrations of ammonia and nitrite nitrogen in the effluent of the T2 reactor.

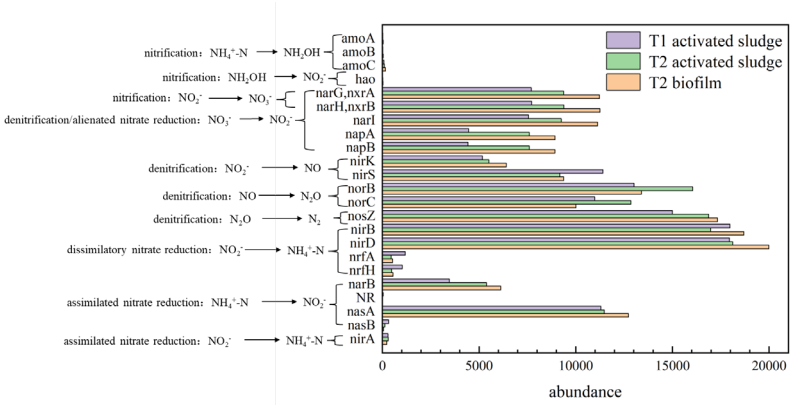

Figure 9. Abundance of functional genes for nitrogen metabolism.

The functional genes involved in the denitrification process include *narG*, *narH*, *narI*, *napA*, *napB*, *nirK*, *nirS*, *norB*, *norC*, and *nosZ*. Among these, the heterotrimeric nitrate reductases (NAR and NAP) encoded by the *nar* and *nap* genes reduce NO_3^- to NO_2^-, completing the first step of the catalytic denitrification process [40], which indicates denitrification performance. Subsequently, nitrite reductase (NIR), encoded by the *nir* gene, reduces NO_2^- to NO, nitric oxide reductase (NOR), encoded by the *nor* gene, converts NO to N_2O, and nitrogen removal from wastewater is completed by nitrous oxide reductase (NOS), encoded by the *nosZ* gene, which converts N_2O to N_2 [41]. In comparison to the T1 reactor sludge, all the aforementioned genes, except *nirS* and *norC*, exhibited greater abundances in both the activated sludge and biofilm of the T2 reactor. This indicates that the synergistic effect between activated sludge and biofilm enhances the expression levels of nitrogen metabolism-related functional genes in the two-phase microorganisms, resulting in improved denitrification performance of the IFAS system. This also explains the lower nitrate nitrogen concentration observed in the effluent from the T2 reactor. Additionally, several

genes related to heterogeneous nitrate reduction (*nirB*, *nirD*, *nrfA*, *nrfH*) and assimilatory nitrate reduction (*narB*, *NR*, *nasA*, *nasB*, *nirA*) were present in both the biofilm and activated sludge. These genes primarily facilitate the conversion of NH_4^+-N and NO_2^--N, with *nirD*, *narB*, and *nasA* being more abundant in the activated sludge and biofilm of the T2 reactor. Overall, the majority of nitrogen metabolism-related genes exhibited the highest abundance in the biofilm, followed by the T2 sludge. This suggests that the biofilm enriches nitrogen metabolism-related genes and increases their abundance in the activated sludge phase of the system, which in turn enhances nitrogen metabolism-related gene expression throughout the reactor.

4. Conclusions

(1) The T2 reactor employing the IFAS process demonstrates superior pollutant removal performance. Compared to the T1 reactor, the average removal rates of COD, ammonia nitrogen, and total nitrogen in the T2 reactor increased by 13.07%, 12.26%, and 28.96%, respectively.

(2) The nitrification volumetric load of the IFAS system was significantly greater than that of the pure sludge system, indicating that the nitrification performance could be effectively enhanced by this combined process. Furthermore, compared to the activated sludge system, the IFAS system exhibits superior stability when treating wastewater with low C/N ratios or high phosphorus levels, as well as when encountering higher organic loads.

(3) The installation of a filler enhances microbial species richness and community diversity within the system. Additionally, activated sludge and biofilm interact within the microbial community structure, allowing the IFAS system to effectively enrich functional flora related to denitrification. The biofilm also enhances the expression of nitrogen metabolism-related functional genes in the activated sludge phase, indicating a synergistic effect between activated sludge and biofilm beyond mere competition.

Author Contributions: Z.T.: Writing—original draft, material preparation, data collection, and analysis; L.B.: Writing—review and editing; B.Z.: Writing—review and editing; S.J.: Investigation, methodology, conceptualization, supervision, Writing—review and editing; C.L.: Review, visualization; S.T.: Project administration and funding updates. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the Natural Science Foundation of Inner Mongolia Autonomous Region (2024MS05046); the Basic Research Operating Costs of Universities Programme (2023QNJS134); and the Inner Mongolia Autonomous Region Postgraduate Research Innovation Programme (S20231166Z).

Data Availability Statement: Some or all data, models, or codes that support the findings of this study are available from the corresponding author upon reasonable request.

Conflicts of Interest: The authors declare no conflicts of interest.

References

1. Que, T.; Wang, Y.; Li, G. Mechanism of carbon nanotubes reducing the efficiency of biological nitrogen and phosphorus removal. *Technol. Water Treat.* **2022**, *48*, 125–130. [CrossRef]
2. Wang, Y.; Du, Z.; Liu, Y.; Wang, H.; Xu, F.; Liu, B.; Zheng, Z. The nitrogen removal and sludge reduction performance of a multi-stage anoxic/oxic (A/O) biofilm reactor. *Water Environ. Res. Res. Publ. Water Environ. Fed.* **2020**, *92*, 94–105. [CrossRef] [PubMed]
3. Yang, P.; Xu, K.; Zhou, J.; Yang, Z.-q.; Han, W.-j.; Wu, D.; Huang, Q.; Zhong, C. Application of MBBR and magnetic coagulation process in upgrading and expansion of a WWTP with A^2/O microporous aeration oxidation ditch. *China Water Wastewater* **2023**, *39*, 67–74. [CrossRef]
4. Li, Z.; Xiao, N.; Lin, M.; Li, D.; Yang, Z. Application of MBBR coupled with MBR process in a stringent discharge standard wastewater treatment plant in northeast China at low temperature. *China Water Wastewater* **2022**, *38*, 77–83. [CrossRef]
5. Ma, Y.; Wang, B.; Li, X.; Wang, S.; Wang, W.; Peng, Y. Enrichment of anammox biomass during mainstream wastewater treatment driven by achievement of partial denitrification through the addition of bio-carriers. *J. Environ. Sci.* **2024**, *137*, 181–194. [CrossRef]

6. Goswami, S.; Mazumder, D. Comparative study between activated sludge process (ASP) and moving bed bioreactor (MBBR) for treating composite chrome tannery wastewater. *Mater. Today: Proc.* **2016**, *3*, 3337–3342. [CrossRef]
7. Song, T.; Sheng, X.; Wang, J.; Liu, R.; Chen, L. Nitrification performance and enhancement method of biological treatment system in wastewater treatment plant under high temperature in summer. *Environ. Sci.* **2019**, *40*, 768–773. [CrossRef]
8. Jia, X.; Sheng, D.; He, S.; Wan, N.; Yu, J. Optimization of pure membrane MBBR process conditions and operation analysis of a large-scale water purification plant. *Water Purif. Technol.* **2024**, *43*, 68–73+94. [CrossRef]
9. Fortunato, L.; Li, M.; Cheng, T.; Rehman, Z.; Heidrich, W.; Leiknes, T. Cake layer characterization in Activated Sludge Membrane Bioreactors: Real-time analysis. *J. Membr. Sci.* **2019**, *578*, 163–171. [CrossRef]
10. Li, W.; Wang, L.; Li, X.; Dai, Y.; Wang, S..; Wang, W.; Yang, J.; Wang, B. Enhanced nitrogen removal from low C/N municipal wastewater through nitrification, partial denitrification and anammox via a step-feed strategy: Synergistic contribution by bacteria in biofilm and floc sludge. *J. Water Process Eng.* **2024**, *66*, 106084. [CrossRef]
11. Chen, X.; Hu, B.; Zhang, R.; Gong, Y.; Cai, H. Effect of superficial gas velocity on the granulation and metabolic characteristics of phosphorus removal sludge. *China Environ. Sci.* **2024**, *44*, 686–698. [CrossRef]
12. Chen, C.; Ai, J.; Chen, L.; Li, Y.; Tang, X.; Li, J. Nitrogen metabolism pathways and functional microorganisms in typical karst wetlands. *Environ. Sci. Pollut. Res. Int.* **2024**, *31*, 22494–22506. [CrossRef]
13. State Environmental Protection Agency. *Analytical Methods for Water and Wastewater Monitoring*, 4th ed.; China Environmental Science Press: Beijing, China, 2002.
14. Zhou, J.; Han, W.; Wu, D.; Sun, X.; He, Q. Factors influencing the competition between activated sludge and biofilm in hybrid MBBR nitrification system. *China Environ. Sci.* **2020**, *40*, 4735–4743. [CrossRef]
15. Zhong, H.; Tang, Y.; Wang, M.; Dong, L. Achieving Simultaneous Nitrification and Denitrification by a Membrane Aerated Biofilm Reactor at Moderate Lumen Pressure. *Separations* **2024**, *11*, 227. [CrossRef]
16. Hou, X.; Li, X.; Zhu, X.; Li, W.; Kao, C.; Peng, Y. Advanced nitrogen removal from municipal wastewater through partial nitrification-denitrification coupled with anammox in step-feed continuous system. *Bioresour. Technol.* **2023**, *391*, 129967. [CrossRef] [PubMed]
17. Qian, L.; Shuang, G.; Qi, Z.; Xu, R.; Li, Z.; Hou, Y.; Sun, Y.; Huang, C. Bio-augmentation of the filler-enhanced denitrifying sulfide removal process in expanded granular sludge bed reactors. *Environ. Res.* **2022**, *212*, 113253. [CrossRef]
18. Xiang, Y.; Shao, Z.; Chai, H.; Ji, F.; He, Q. Functional microorganisms and enzymes related nitrogen cycle in the biofilm performing simultaneous nitrification and denitrification. *Bioresour. Technol.* **2020**, *314*, 123697. [CrossRef]
19. Xing, X.; Yuan, X.; Zhang, Y.; Men, C.; Zhang, Z.; Zheng, X.; Ni, D.; Xi, H.; Zuo, J. Enhanced denitrification of the AO-MBBR system used for expressway service area sewage treatment: A new perspective on decentralized wastewater treatment. *J. Environ. Manag.* **2023**, *345*, 118763. [CrossRef]
20. Xiang, Y. Nitrogen Removal Capacity and Pathways of the Simultaneous Nitrification and Denitrification Process Under Different Oxygen Conditions. Ph.D. Thesis, Chongqing University, Chongqing, China, 2020. [CrossRef]
21. Zhao, Y.; An, P.; Wan, J.; Zhang, X. Enhanced Simultaneous Nitrogen and Phosphorus Removal in a Continuous-Flow Granular Sludge System under Gradient-Controlled Hydraulic Loading. *Water* **2024**, *16*, 1510. [CrossRef]
22. Du, Z.; Lu, B.; Li, D.; Chai, X. Strengthening nitrogen removal of rural wastewater treatment in humus biochemical system under low dissolved oxygen conditions: Sludge and microbial characteristics. *J. Environ. Manag.* **2024**, *366*, 121762. [CrossRef]
23. Han, W.; Zhou, J.; Sheng, D.; Wu, D.; Zhou, H.; Yang, Z.; Yin, J.; Xia, C.; Kan, Y.; He, J. Integration of a pure moving bed biofilm reactor process into a large micro-polluted water treatment plant. *Water Sci. Technol.* **2022**, *86*, 3051–3066. [CrossRef] [PubMed]
24. Forruque, S.A.; Fatema, M.; Adiba, M.; Nuzaba, T. Strategies to improve membrane performance in wastewater treatment. *Chemosphere* **2022**, *306*, 135527. [CrossRef]
25. Zhang, B.; Li, W.; Wu, L.; Shi, W.; Lens, P. Rapid start-up of photo-granule process in a photo-sequencing batch reactor under low aeration conditions: Effect of inoculum AGS size. *Sci. Total Environ.* **2022**, *820*, 153204. [CrossRef] [PubMed]
26. Han, N.N.; Yang, H.J.; Fan, S.N.; Jin, R. Mechanistic insight into microbial interaction and metabolic pattern of anammox consortia on surface-modified biofilm carrier with extracellular polymeric substances. *Bioresour. Technol.* **2024**, *407*, 131092. [CrossRef]
27. Gao, Y.; Shen, J.; Yinzhang, H.; Yang, L. Performance and microbial response to nitrate nitrogen removal from simulated groundwater by electrode biofilm reactor with Ti/CNT/Cu5-Pd5 catalytic cathode. *Water Environ. Res.* **2024**, *96*, e10974. [CrossRef]
28. Hei, S.; Xu, H.; Liu, Y.; Liu, B.; Zhang, S.; Zhu, X.; Lin, W.; Chen, L.; Jiang, H.; Cheng, X.; et al. Redox environment inducing strategy for enhancing biological phosphorus removal in a full-scale municipal wastewater treatment plant. *J. Clean. Prod.* **2022**, *376*, 134237. [CrossRef]
29. Gu, M.; Wang, Y.; Wan, D.; Shi, Y.; He, Q. Electrodialysis ion-exchange membrane bioreactor (EDIMB) to remove nitrate from water: Optimization of operating conditions and kinetics analysis. *Sci. Total Environ.* **2022**, *839*, 156046. [CrossRef]
30. Li, H.; Zhang, Y.; Mi, X.; Wang, D.; Xu, Y. Optimization of nitrogen removal and microbial mechanism of a hydrogen-based membrane biofilm reactor. *Environ. Technol.* **2024**, 11–17. [CrossRef]
31. Song, Z.; Zhang, L.; Yang, J.; Ni, S.; Peng, Y. Achieving high nitrogen and antibiotics removal efficiency by nZVI-C in partial nitritation/anammox system with a single-stage membrane-aerated biofilm reactor. *J. Hazard. Mater.* **2024**, *473*, 134626. [CrossRef]

32. Wang, L.; Zhang, C.; Kang, X.; Liu, Y.; Qiu, Y.; Wanyan, D.; Liu, J.; Cheng, G.; Huang, X. Establishing mainstream partial nitrification in the membrane aerated biofilm reactor by limiting the oxygen concentration in the biofilm. *Water Res.* **2024**, *261*, 121984. [CrossRef]
33. Wang, C.; Liu, S.; Xu, X.; Zhang, C.; Wang, D.; Yang, F. Achieving mainstream nitrogen removal through simultaneous partial nitrification, anammox and denitrification process in an integrated fixed film activated sludge reactor. *Chemosphere* **2018**, *203*, 457–466. [CrossRef] [PubMed]
34. Hao, X.; Zeng, W.; Li, J.; Zhan, M.; Miao, H.; Gong, Q. High-efficient nitrogen removal with low demand of Fe source and mechanism analysis driven by Fe(II)/Fe(III) cycle. *Chem. Eng. J.* **2024**, *481*, 148702. [CrossRef]
35. Li, Y.; Chai, Z.; Song, C.; Chen, J.; Gu, A.; Mu, G.; Ge, R.; Zheng, M. The superiority of hydrophilic polyurethane in comammox-dominant ammonia oxidation during low-strength wastewater treatment. *Sci. Total Environ.* **2024**, *935*, 173017. [CrossRef] [PubMed]
36. Qiang, R.; Wang, M.; Li, Q.; Li, Y.; Li, C.; Zhang, J.; Liu, H. The different responses of AOA and AOB communities to irrigation systems in the semi-arid region of Northeast China. *Front. Microbiol.* **2024**, *15*, 1374618. [CrossRef]
37. Lyu, X.; Guo, Y.; Liu, W.; Tan, H.; Luo, G.; Zhang, H.; Hu, X. Evaluation of long-term operational and production performance of biofloc technology (BFT) system within various particle size. *Aquaculture* **2024**, *578*, 740090. [CrossRef]
38. Chen, G.; Li, J.; Zhang, S.; Gao, X.; Gu, C.; Lv, X.; Peng, Y. Novel Anoxic-Anaerobic-Oxic process successfully enriched anammox bacteria under actual municipal wastewater. *Bioresour. Technol.* **2024**, *412*, 131393. [CrossRef] [PubMed]
39. Xing, W.; Zhang, Z.; Zhang, X.; Liu, J.; Li, J.; Lin, J.; Yao, H. Mainstream partial Anammox for improving nitrogen removal from municipal wastewater after organic recovery via magnetic separation. *Bioresour. Technol.* **2022**, *361*, 127726. [CrossRef]
40. Zhang, Q.; Yang, C.; Qin, S.; Zhang, P.; Hu, Y.; Tian, H.; Liu, B.; Chen, H.; Li, W. The impact of aeration rate on the denitrification performance and microbial community characteristics of the HN-AD bacteria-inoculated MBBR system. *J. Water Process Eng.* **2024**, *66*, 105968. [CrossRef]
41. Fan, Y.X.; Geng, N.W.; Li, X.; Liu, Y.; Cao, S.; Zhang, X.; Wang, Y. Responses of activated sludge to quorum sensing inhibitor and antibiotic compound stress: Interactions among abundant-rare genera, functional genes, and antibiotic resistance genes. *J. Environ. Chem. Eng.* **2024**, *12*, 112199. [CrossRef]