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Performance Analysis of Partial Nitrification Fillers under Different Ammonia Concentration and Dissolved Oxygen Conditions

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Abstract

This study explores the long-term, efficient, and stable performance of partial nitrification (PN) achieved through gel immobilization technology for ammonia-oxidizing bacteria (AOB). Comprehensive research was conducted on the PN characteristics of the packing material under high and low ammonia concentrations and varying apparent dissolved oxygen (DO) conditions. We investigated the microbial community structure within the packing material at different DO levels and identified the optimal DO concentration for biologically active partial nitrification fillers, analyzing the impact of the surface gel layer on oxygen transfer. Results indicate that the biological activity of the packing material can rapidly initiate and exhibit strong shock resistance and adaptability. Once stabilized, the nitrite accumulation rate (NAR) exceeds 95%, with a maximum ammonia oxidation rate of 53.7 mg/(L·h). The high abundance of AOB and the dynamic changes in the functional microbial community are the main factors influencing the stable and efficient partial nitrification performance. The competition among microorganisms and the combined effect of the surface gel effectively inhibit the growth of nitrite-oxidizing bacteria (NOB), ensuring the dominance of AOB. This research provides a reliable theoretical basis for the practical application of partial nitrification processes.

Subject Areas

Biological Engineering, Biotechnology

Keywords

Partial Nitrification, Influencing Parameter, Gel Immobilization, Reactor Configuration, Control Strategy

1. Introduction

In recent years, the application of partial nitrification (PN) processes in urban wastewater treatment has received significant attention. Compared to traditional nitrogen removal methods, the partial nitrification-denitrification technology is a low-energy, high-efficiency nitrogen removal solution [1]. It also demonstrates excellent compatibility with other nitrogen removal techniques such as anaerobic ammonium oxidation (Anammox) and partial denitrification (PDN) [2] [3]. The PN/Anammox (PN/A) process can reduce energy costs by 60%, decrease sludge production by 90%, and lower the demand for electron donors [4]. A key technical challenge for achieving efficient PN is controlling the growth of nitrite-oxidizing bacteria (NOB) to maintain long-term efficiency and stability in the PN process.

Several studies have demonstrated that factors such as adjusting dissolved oxygen (DO), high free ammonia (FA), or free nitrous acid (FNA) can facilitate nitrite accumulation [5]-[7]. However, mainstream municipal wastewater often fails to reach the necessary concentrations of FA or FNA to effectively inhibit nitrite-oxidizing bacteria (NOB), typically relying on sidestreams with high ammonia nitrogen concentrations instead. Therefore, controlling DO has become the most commonly adopted strategy in current PN processes [8]-[10]. The lower oxygen half-saturation coefficient indicates that the oxygen affinity of ammonia-oxidizing bacteria (AOB) is higher than that of nitrite-oxidizing bacteria (NOB) [4]. Numerous experiments have confirmed the feasibility of achieving stable PN through DO control. Akaboci *et al.* [11] demonstrated that in mainstream PN/Anammox processes, stable PN can be rapidly achieved when DO levels drop below 0.5 mg/L. However, the approach of maintaining low DO often compromises the growth of functional bacteria and ammonia oxidation rate (AOR) [12], allowing only for short-term maintenance of partial nitrification [13] [14]. In addition to aeration strategies, NH_4^+-N loading is also considered an important factor influencing the PN process. Studies have shown that both low-strength wastewater (75 mg NH_4^+-N/L) and high-strength wastewater (greater than 250 mg NH_4^+-N/L) can achieve NOB inhibition [15].

Additionally, the long startup times and high process control requirements associated with activated sludge systems significantly limit the scalability of partial nitrification (PN) processes. In contrast to activated sludge, using immobilization technology can effectively maintain high microbial concentrations [16]-[18], enhancing operational efficiency and reactor stability. Microorganisms fixed within the immobilization matrix can be retained indefinitely in the reactor, promoting the growth of autotrophic microorganisms [19]. Consequently, gel-immobilized fillers can ensure a substantial population of AOB is involved in the PN process, thereby guaranteeing high stability and efficiency in PN performance [20]. The polymer matrix within the gel filler provides a stable and suitable growth environment for functional microorganisms, facilitating the enrichment of AOB and other functional microbial communities [21]. This enables the biofiller to effectively respond to variations in water quality. Immobilized fillers also exhibit tolerance to environmental conditions [22]. Actual wastewater often contains chemical oxygen demand (COD), which can promote the proliferation of heterotrophic bacteria, creating competition with AOB. Immobilization provides microorganisms with a relatively stable microenvironment, mitigating the effects of environmental fluctuations [23]. Therefore, gel-immobilized biofillers have significant advantages in achieving stable PN processes for urban wastewater treatment. However, there has yet to be detailed research on the adaptability and stability of PN fillers under fluctuations in water quality and dissolved oxygen, particularly in cases of drastic changes in wastewater characteristics.

In this study, we conducted a comprehensive investigation of the performance of partial nitrification (PN) and microbial community composition in a gel-immobilized bioreactor under fluctuating water quality and dissolved oxygen (DO) conditions by using synthetic wastewater. The objectives of the study are as follows: (1) to evaluate the stability and adaptability of PN fillers under different ammonia nitrogen concentrations; (2) to explore the reactor's performance under both high and low DO conditions and determine the optimal operating conditions; (3) to elucidate the main mechanisms by which DO influences the stable and efficient operation of PN by examining the bioactivity and community composition characteristics of functional microorganisms; and (4) to analyze the advantages and application prospects of PN bio-fillers, providing feasible methods for achieving a stable and efficient PN process.

2. Materials and Methods

2.1. Seed Sludge and Synthetic Wastewater Sources

Ammonia-oxidizing sludge (MLSS = 6950 mg/L) was obtained from a continuously operated fermentation reactor with an effective volume of 2.8 m^3 that had been running for one year. The maximum ammonia oxidation rate (AOR) recorded was 324 mg/(L·h), with a nitrite accumulation rate (NAR) of ≥86%. This sludge served as the seed material for the preparation of hollow ring-shaped immobilized fillers (diameter 10 - 20 mm, thickness 3 - 5 mm) using the PVA-H_3BO_4 cross-linking method [24]. The synthetic wastewater used in the experiments contained NH_4Cl as the nitrogen source and KH_2PO_4 as the phosphorus source, maintaining a ratio of nitrogen to phosphorus is 5:1, with influent NH_4^+-N levels ranging from 40 to 300 mg/L. Sodium carbonate (Na_2CO_3) was added to adjust the pH and serve as a carbon source. **Table 1** lists the components and concentrations of the trace element concentrate. The synthetic wastewater and trace element concentrate were mixed at a volume ratio of 1000:1.

Table 1. Composition of the concentrated trace element mixture.

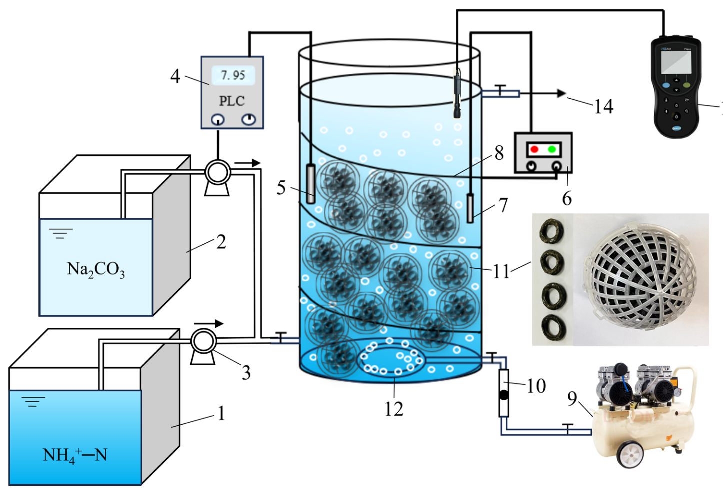
Component	Concentration (mg/L)
EDTA-2Na	5000
H3BO4	0.03
$ZnSO_4·7H_2O$	0.5

Continued

$Na_2MoO_4{\cdot}2H_2O$	0.1
$CoCl_2{\cdot}6H_2O$	0.2
$MnCl_2{\cdot}4H_2O$	0.5
$NiCl_2{\cdot}6H_2O$	0.7
$CuSO_4{\cdot}5H_2O$	0.03
$FeCl_3{\cdot}6H_2O$	0.8
$MgSO_4{\cdot}7H_2O$	3
$CaCl_2$	2
$NaSeO_4$	0.21

2.2. Reactor Setup and Operation

The partial nitrification fillers were encapsulated using polypropylene floating balls (diameter 80 mm) to prevent packing and were loaded into a PMMA PN reactor with an effective volume of 24 L, achieving a packing density of 10%. Synthetic wastewater was pumped from the influent tank into the PN reactor using a metering pump (WS-50-01-L, Beijing, China). A microbubble aeration ring was installed at the bottom of the reactor and connected to an air pump. Apparent dissolved oxygen levels were measured using a portable dissolved oxygen meter (ACH-HQ40d, USA). Heating tape and insulation were wrapped around the reactor, with temperature controlled by a thermostat. An online pH monitoring system maintained the pH within the range of 7.85 to 8.10. Na_2CO_3 was injected into the reactor via a metering pump controlled by a PLC automatic control cabinet to maintain pH balance. The schematic diagram of PN reactor is shown in Figure 1.



1. Inlet tank 2. Alkali tank 3. Metering pump 4. On-line controller 5. pH probe 6. Thermostat 7. Temperature probe 8. Heating band 9. Air compressor 10. Gas flowmeter 11. Immobilized filler 12. Aeration ring 13. DO detector 14. Outlet

Figure 1. Schematic of the PN immobilized filler reactor.

2.3. Analytical Methods and Calculations

Daily monitoring of the influent and effluent of the partial nitrification (PN) reactor was conducted. Water samples were filtered using qualitative filter paper with a specified pore size to eliminate interference. The concentrations of three types of inorganic nitrogen salts were determined using standard methods (AWWA, 2006) [25], and the Pearson correlation coefficient was used to assess the correlation among the data results. The calculation formulas for AOR, NAR, FA, and FNA are as follows (1)-(4):

$$\mathrm{AOR}\left(\mathrm{mg}/(\mathrm{Lgh})\right)=\left(\mathrm{NH_4^+\text{-}N_{inf}}-\mathrm{NH_4^+\text{-}N_{eff}}\right)\Big/\mathrm{HRT} \tag{1}$$

$$\mathrm{NAR}\left(\%\right)=\frac{\left(\mathrm{NO_2^-\text{-}N_{eff}}-\mathrm{NO_2^-\text{-}N_{inf}}\right)}{\left[\left(\mathrm{NO_2^-\text{-}N_{eff}}-\mathrm{NO_2^-\text{-}N_{inf}}\right)+\left(\mathrm{NO_3^-\text{-}N_{eff}}-\mathrm{NO_3^-\text{-}N_{inf}}\right)\right]} \tag{2}$$

$$\mathrm{FA}\left(\mathrm{mg/L}\right)=17\left(\mathrm{NH_4^+\text{-}N}\times 10^{\mathrm{pH}}\right)\Big/14e^{\left(\frac{6334}{273+t}\right)+10^{\mathrm{pH}}} \tag{3}$$

$$\mathrm{FNA}\left(\mathrm{mg/L}\right)=46\left(\mathrm{NO_2^-\text{-}N}\right)\Bigg/14\left(e^{\left(\frac{-2300}{273+\mathrm{T}}\right)\times 10^{\mathrm{pH}}}\right) \tag{4}$$

The subscripts "Inf" and "Eff" refer to the concentrations of $\mathrm{NH_4^+}$-N, $\mathrm{NO_2^-}$-N, $\mathrm{NO_3^-}$-N, and TIN in the influent and effluent (mg/L), respectively.

2.4. Microbial Analysis

2.4.1. DNA Extraction

After the dissolved oxygen testing concluded, immobilized fillers were collected in three phases for metagenomic sequencing. The sample pretreatment method was as follows: all samples were washed three times with PBS, and the immobilized biomass was frozen in liquid nitrogen for grinding. Total community genomic DNA was extracted from the samples using the E.Z.N.ATM Mag-Bind Soil DNA Kit (Omega, M5635-02, USA) according to the manufacturer's instructions. DNA integrity was assessed using agarose gel electrophoresis, and DNA concentration was measured with a Qubit 4.0 fluorometer (Thermo, USA). This process ensures the acquisition of sufficient genomic DNA for subsequent high-throughput sequencing.

2.4.2. Illumina High-Throughput Sequencing

The microbial composition of the samples was analyzed using the high-throughput Illumina MiSeq sequencing platform (Illumina, San Diego, USA). The V3-V4 region of the 16S rRNA gene was amplified and compared against the National Center for Biotechnology Information (NCBI) database. The MEGAN sequencing software was employed to analyze the S sequences of the 16 microbes, with sequence clustering into operational taxonomic units (OTUs) established at a 99% identification threshold. The OTU results were further classified, including alpha diversity and species diversity analyses, which were visualized accordingly. This work was conducted by Shanghai Shenggong Biotechnology Co., Ltd. All raw sequences are stored in the NCBI Sequence Read Archive under the accession number 16S2404690BJ. Wang *et al.* described the processes for quality filtering, read assembly, and functional annotation [26].

3. Results and Discussion

3.1. Start-Up of PN Reactor

3.1.1. PN Performance of the Reactor

The PN reactor was operated in four phases over a span of 25 days, with the operational parameters detailed in **Table 2**. In Phase I (days 1 - 5), the reactor was initiated with influent ammonia levels controlled at 50 ± 5 mg N/L (**Figure 2(a)**). During this period, the PN media increased the ammonia oxidation rate (AOR) from 1.0 mg/(L·h) to 24.9 mg/(L·h), achieving a nitrite accumulation rate (NAR) of ≥ 95%, and the effluent ammonia concentration dropped from 53.4 mg N/L to undetectable levels. The final effluent state indicated that the reactor had achieved rapid startup, with a startup time of 5 days, demonstrating faster startup efficiency compared to traditional activated sludge systems [27] [28]. This rapid initiation was attributed to the favorable PN performance of the seed sludge within the media. On the last day of Phase I, the dissolved oxygen (DO) level was lowered to 5.83 mg O/L, resulting in an AOR significantly greater than in the previous four days, likely due to the lower initial ammonia concentration making higher DO levels unfavorable for the growth of ammonia-oxidizing bacteria (AOB) [9]. After completing Phase I, the influent ammonia load was gradually increased to 100 ± 5 mg N/L to explore the maximum AOR at this concentration. In Phase II (days 6 - 12), the AOR initially dropped to 18.9 mg/(L·h) on the first day but then gradually increased to 38.7 mg/(L·h) by days 11-12, while the effluent ammonia concentration decreased to 9 mg N/L, achieving an oxidation rate of 90%. During this phase, the AOR and DO levels maintained a positive correlation, with the NAR consistently above 95%. This indicates that, when substrate concentrations are sufficient, an appropriate increase in DO can effectively sustain the dominance of AOB populations, further demonstrating that the immobilized bio-media can maintain microbial abundance [16]-[18]. On day 13, the ammonia concentration was further increased to 150 ± 5 mg N/L, resulting in an effluent ammonia level of 31.1 mg N/L, with corresponding AOR and NAR values of 39.5 mg/(L·h) and 99.5%, respectively. On Day 21, by reducing the flow rate of the influent pump, the hydraulic retention time (HRT) of the reactor was increased to 4 hours, resulting in undetectable levels of effluent ammonia nitrogen. This demonstrates that extending the HRT can effectively address the issue of ammonia in the effluent. Additionally, on Day 21, the nitrite accumulation rate (NAR) slightly decreased to 98%. Following this, the effluent ammonia nitrogen concentration remained stable between 30 and 40 mg N/L. Throughout Phase III, the NAR consistently ranged from 95% to 100%. Thus, gradually increasing the ammonia load effectively stimulated an increase in the AOR of the filler. No suppression of the PN filler activity was observed, indicating that the reactor successfully withstood the impacts of high ammonia concentrations, achieving a highly efficient and stable PN process. In Phase IV, the influent NH_4^+-N concentration was raised to 300 ± 10 mg N/L, and the HRT was adjusted to 5 hours. At this point, the AOR increased to 50 mg/(L·h), indicating that the biologically active filler exhibited strong adaptability to fluctuations in ammonia nitrogen concentrations, making it suitable for the variable characteristics of rural wastewater [29] [30]. During this phase, adjustments to the DO levels had minimal impact on the AOR, suggesting that, under sufficient substrate conditions, dissolved oxygen is no longer a major limiting factor affecting AOB activity.

Table 2. Reactor parameters and purposes for the four different stages.

Stage	Time (d)	HRT (h)	DO (mg/L)	Temperature (°C)	pH	Purpose
I	1 - 5	2 - 6	5 - 8	22 - 24	7.85 - 8.1	Start-up period
II	6 - 12	2	5 - 8	19 - 24	7.85 - 8.1	Efficiency improvement period
III	13 - 23	2 - 4	5 - 8	22 - 24	7.85 - 8.1	High NH_4^+ −N examination period
IV	24 - 25	5	5 - 7	24	7.85 - 8.1	NH_4^+ −N change adaptation period

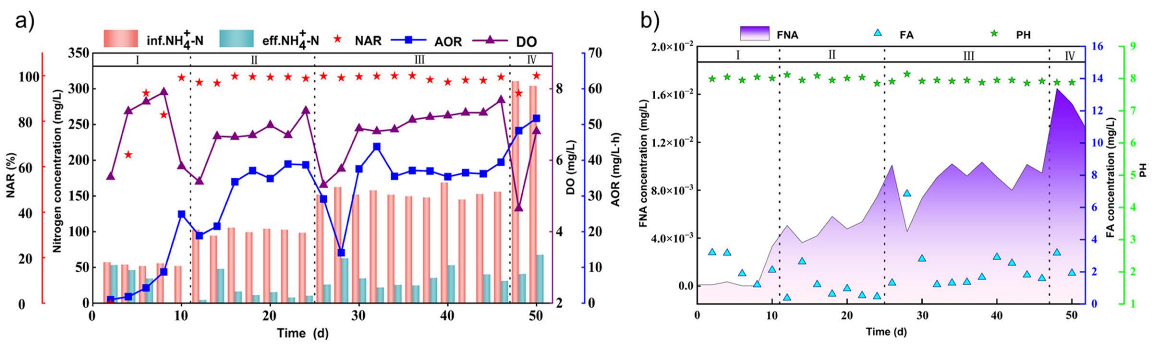


Figure 2. Performance of the PN immobilized filler reactor at various stages: a) NH_4^+ -N concentration, AOR, and NAR; b) FA, FNA, and pH.

On the sixteenth day, Flocculent sludge was observed on the PN filler. To investigate the composition of this flocculent sludge and its impact on the ammonia oxidation rate (AOR), the PN reactor and filler were washed. Sampling and analysis were conducted after 24 hours, the Aerobic Oxidation Rate (AOR) decreased from 43.8 mg/(L·h) to 35.5 mg/(L·h), before gradually recovering to 39.5 mg/(L·h). Throughout this process, the Nitrification Accumulation Rate (NAR) remained consistently above 95%, showing almost no change. This result aligns with the findings of Hu *et al.* [31]. The data indicate that the sludge on the surface of the carriers has minimal impact on the reactor's AOR and recovers quickly. Therefore, during subsequent operations, the reactor can be left unwashed unless excessive external sludge growth severely affects the diffusion of dissolved oxygen.

3.1.2. Free Ammonia and Free Nitrous Acid during Operation

Fulvic acid (FA) and formic acid (FNA) were consistently present throughout all operational phases (Figure 2(b)). The inhibitory threshold of FA on ammonia-oxidizing bacteria (AOB) and nitrite-oxidizing bacteria (NOB) is related to their abundance within the system [32]. Most studies indicate that when FA concentrations exceed 1 mg/L, they can inhibit NOB in the activated sludge of conventional wastewater treatment plants, with the inhibitory effect increasing as FA concentrations rise. Most studies indicate that when FA concentrations exceed 1 mg/L, they can inhibit NOB in the activated sludge of conventional wastewater treatment plants, with the inhibitory effect increasing as FA concentrations rise [33] [34]. Additionally, Vadivelu *et al.* [35] found that AOB activity in activated sludge is unaffected at FA concentrations of 16 mg/L, but further increases in FA concentration do lead to inhibition. In the early stages of the experiment, FA levels were below 1 mg/L, yet the Nitrification Accumulation Rate (NAR) still exceeded 90%, likely due to the abundant AOB within the carriers. In later experimental phases, FA primarily inhibited NOB, playing a positive role in maintaining a stable NAR.

Furthermore, throughout the operation of the reactor, the concentration of formic acid (FNA) varied between 0.0001 and 0.016 mg/L (Figure 2(b)), which is significantly lower than the inhibitory concentration for nitrite-oxidizing bacteria (NOB) (0.02 mg/L) [35]. Therefore, the stable Nitrification Accumulation Rate (NAR) of the carriers cannot be attributed primarily to the effects of FNA. On the contrary, FNA may enhance the potential activity of nitrite-denitrifying bacteria [36]. This finding is corroborated at the biological level by the presence of Pseudomonas in the high-throughput sequencing results.

3.1.3. Effect of Ammonia Concentration with DO on AOR and NAR

Batch experiments were conducted using the active carriers from the start-up phases I, II, and III to simulate the effects of varying ammonium nitrogen concentrations on the AOR and NAR as influenced by dissolved oxygen (DO) levels. The experiments were completed within one day to avoid the impact of AOB proliferation on the results. As shown in Figure 3, during the initial phase with NH_4^+-N at 50 mg N/L, AOR exhibited a positive correlation with apparent dissolved oxygen, reaching a maximum oxidation rate of 17.4 mg/(L·h). The NAR peaked at only 86.4% when DO was at 3 mg O/L, likely due to the presence of a gel layer on the surface of the carriers, which hindered the diffusion of dissolved oxygen into the interior at lower DO levels. Although AOB have a lower oxygen saturation coefficient than NOB [37], they still struggled to obtain sufficient electron acceptors, thus limiting their performance. As DO levels increased, the accumulation rate remained above 90%. Furthermore, the limited hydraulic retention time (HRT) of only 2 hours during this phase resulted in elevated effluent ammonium nitrogen concentrations. At DO = 4 mg O/L, extending the HRT to 5.5 hours significantly reduced the effluent ammonium nitrogen levels; however, AOR did not increase markedly, indicating that the mass transfer of dissolved oxygen had reached its limit. The improvement in NAR could be attributed to AOB's enhanced ability to utilize substrates effectively with the extended HRT, allowing them to gain a competitive edge over NOB.

During the phase with NH_4^+-N at 100 mg N/L, the AOR increased with rising apparent dissolved oxygen (DO), reaching a maximum ammonia oxidation rate of 55.5 mg/(L·h), while the NAR remained above 95%. The hydraulic retention time (HRT) during this phase was 1.25 hours, which resulted in a relatively high effluent NH_4^+-N concentration due to insufficient ammonia oxidation time. At DO = 8 mg O/L, extending the HRT to 2 hours reduced the effluent NH_4^+-N to just 1 mg N/L, demonstrating that increasing the HRT effectively enhances NH_4^+-N removal. In the phase with NH_4^+-N at 150 mg N/L, the AOR no longer increased with apparent DO, peaking at 51.7 mg/(L·h), while the NAR remained above 90%. At DO = 3 mg O/L, the AOR dropped to 11.6 mg/ (L·h) due to the 2-hour HRT and the presence of the gel layer, resulting in an effluent NH_4^+-N of 132 mg N/L; however, as apparent DO increased, the effluent ammonium concentration gradually decreased. At DO = 7 mg O/L, both the AOR and NAR decreased, likely because the reduction in effluent NH_4^+-N compromised the stability of FA inhibition on NOB, and the NOB population may have developed some tolerance to FNA [38]. This led to competition for dissolved oxygen between NOB and AOB, causing a decline in AOR. These findings suggest that in high-ammonium environments, it may be beneficial to slightly reduce the apparent dissolved oxygen levels to maintain effective ammonia removal performance. Overall, the bio-immobilized carriers demonstrated good adaptability across varying ammonia concentrations, indicating that while ammonia concentration can influence AOB performance to some extent [19] [39], it is not the primary limiting factor.

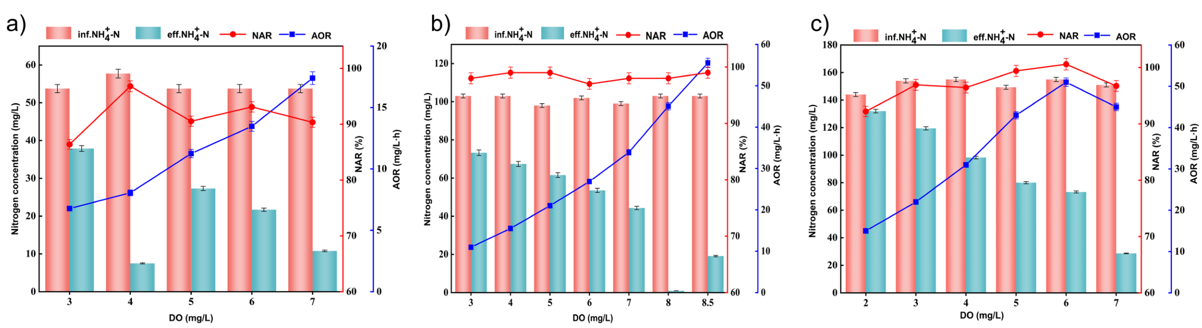


Figure 3. Effect of ammonia concentration on nitrification performance, including: (a) ammonia concentration = 50 ± 5 mg N/L, (b) ammonia concentration = 100 ± 7 mg N/L, (c) ammonia concentration = 150 ± 12 mg N/L.

3.2. Effect of DO on the Performance of Partial Nitrification

3.2.1. AOB Performance Analysis under Different DO

By adjusting the gas-to-liquid ratio, the reactor was maintained at different dissolved oxygen (DO) concentrations to investigate the impact of DO on partial nitrification. **Figure 4** illustrates the performance of a continuous flow bioreactor at NH_4^+-N = 100 mg N/L under varying DO levels. As shown, the ammonia oxidation rate (AOR) is primarily related to the DO concentration in the solution. The 3-hour average AORs at DO levels of 3.0 - 4.0, 5.0 - 6.0, and 7.0 - 8.0 mg O/L were 19.74, 34.26, and 37.1 mg/(L·h), respectively. At DO concentrations of 5.0-6.0 and 7.0 - 8.0

mg/L, the effluent ammonia concentration dropped to undetectable levels, yet the AOR did not reach the maximum levels observed in previous experiments. This may be attributed to substrate limitation, which restricts the progress of ammonia oxidation. Subsequently, by shortening the hydraulic retention time (HRT) to 2 hours, the effluent NH_4^+-N concentration was maintained at 30 - 40 mg N/L to assess the maximum nitrification rate. Correspondingly, the AOR increased to 37.0 and 53.4 mg/(L·h), consistent with the previously determined maximum AOR. During the phase with DO at 3.0 - 4.0 mg O/L, the AOR stabilized around 20 ± 2 mg/(L·h). This was likely due to a complete gel layer on the surface of the immobilized packing, which limited oxygen transfer when the DO gradient between the inside and outside of the packing was low. Additionally, as AOB gradually consumed the dissolved oxygen during the internal mass transfer process, the internal DO levels decreased, becoming insufficient to meet the aerobic requirements of AOB.

In the three DO stages mentioned above, although the Average Oxygen Requirement (AOR) varies slightly, the Nitrifying Activity Rate (NAR) can consistently remain above 90%. This clearly demonstrates that the embedded fillers can sustain the dominance of Ammonia-Oxidizing Bacteria (AOB). At high DO levels, the dominant AOB can utilize more dissolved oxygen, promoting their proliferation and maintaining their competitive status. Conversely, when DO decreases , the oxygen saturation constant for Nitrate-Oxidizing Bacteria (NOB) is significantly higher (1.1 mg O/L) than that of AOB (0.3 mg O/L) [37], making it difficult for NOB to compete with AOB for dissolved oxygen, thus impairing their ability to efficiently oxidize nitrite. Comprehensive studies indicate that DO is a key factor limiting the nitrification performance of AOB [40]-[42]. Hagedorn-Olsen *et al.* found that high concentrations of DO can enhance the activity of nitrifying bacteria in biofilm reactors [43]. However, the optimal dissolved oxygen concentration varies slightly with different concentrations of NH_4^+-N. According to this study, maintaining DO levels between 5 - 7 mg O/L, along with suitable Hydraulic Retention Time (HRT), can achieve efficient and stable removal of ammonia nitrogen from water.

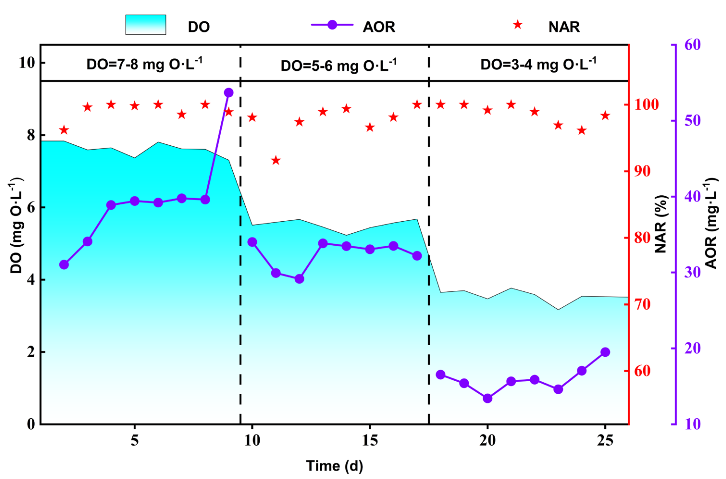


Figure 4. PN performance of immobilized packing under different dissolved oxygen conditions.

3.2.2. Dissolved Oxygen Distribution of Biological Fillers

The gel membrane on the surface of the fillers restricts the mass transfer of dissolved oxygen, causing its concentration to gradually decrease as it passes through the gel layer [44]. Meanwhile, aerobic bacteria embedded in the gel utilize dissolved oxygen, exacerbating this phenomenon. As shown in **Figure 5**, the interior of the filler is filled with mass transfer pathways of varying sizes, allowing the dissolved oxygen within to remain homogeneous. The limitation of oxygen diffusion occurs only within the gel layer; however, due to the complex internal structure of the filler, some areas may still develop hypoxic or anaerobic conditions. Consequently, functional bacteria adapted to different dissolved oxygen concentrations can thrive in the inner layer of the filler. Microbial community analysis provides direct evidence of the various oxygen microenvironments within the filler, detecting AOB, NOB, Denitrifying Bacteria (DNB), and fermentative bacteria. DNB can utilize dissolved cellular products and metabolic byproducts as electron donors for growth and reproduction, which rationalizes nitrogen loss. Given the limited penetration of oxygen, a dissolved oxygen concentration gradient forms between the interior and exterior of the filler. When this gradient is significant, the mass transfer rate of dissolved oxygen increases, allowing AOB to obtain sufficient electron acceptors. Conversely, when the apparent dissolved oxygen is low, the gradient is insufficient to support nitrification reactions in the inner layer of the filler, which explains why the AOR is only 20 ± 2 mg/(L·h) when DO is between 3.0 - 4.0 mg O/L. The surface gel membrane creates a unique microenvironment within the embedded filler, where oxygen levels are strictly regulated. This setup prevents oxygen-sensitive microbial communities from direct exposure to high dissolved oxygen levels, safeguarding them from potential damage due to excess oxygen. This not only helps regulate the internal environment of the filler but also maintains bacterial diversity and abundance, enhancing the resilience of the microbial community.

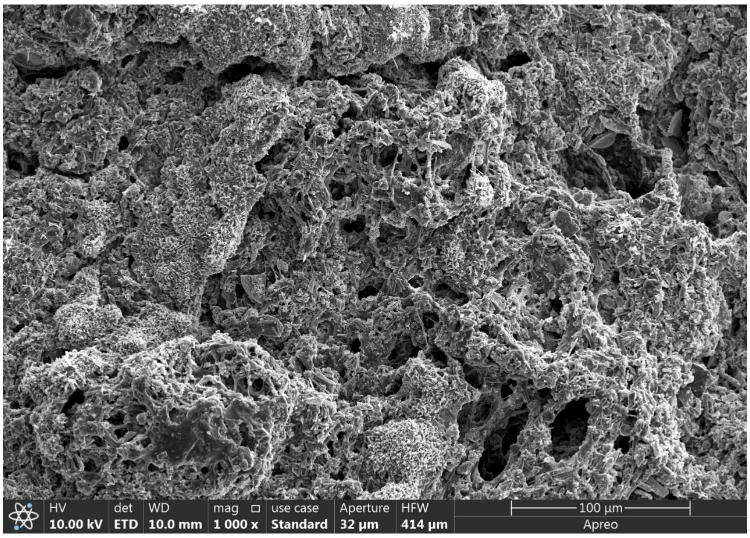


Figure 5. SEM scanning electron microscope for packing.

3.3. Microbial Community Analysis

3.3.1. Microbial Changes under Different Dissolved Oxygen States

The fillers and exogenous sludge are primarily composed of eight phyla (**Figure 6(a)**). Throughout the operation, the relative abundance of the Proteobacteria phylum fluctuated but consistently remained the dominant microbial group, followed by Bacteroidetes and Chloroflexi. The Proteobacteria primarily include Ammonia-Oxidizing Bacteria (AOB), Nitrate-Oxidizing Bacteria (NOB), which utilize soluble microbial products (SMP) and extracellular polymeric substances (EPS) secreted by microorganisms for nourishment [45]. Bacteroidetes play a significant role in the PN process, as they metabolize carbohydrates and degrade various complex organic compounds [46] produced through microbial growth and death. Thus, even when the reactor operates on synthetic wastewater, a diverse array of heterotrophic microorganisms from the Proteobacteria and Bacteroidetes phyla are present. Chloroflexi are commonly found in anaerobic ammonium oxidation systems [47], and Wang *et al.* [48] reported a high abundance of Chloroflexi in such systems. Research by Chen *et al.* [49] found that Chloroflexi are facultative anaerobes, which favor the formation of granular sludge by anaerobic ammonium oxidizing bacteria. As the apparent dissolved oxygen levels increase, ammonia nitrogen is efficiently oxidized to nitrite, providing electron acceptors for Chloroflexi during anaerobic ammonium oxidation, thereby promoting their growth. The enrichment of Chloroflexi serves as biological evidence for the existence of anaerobic environments within the filler. Additionally, during the operation of the reactor, the abundance of Bacteroidetes in the filler increased from 7.97% (DO = 3.0 - 4.0 mg O/L) to 12.73% (DO = 7.0 - 8.0 mg O/L). This increase may be related to the support of the bacterial community within the filler, which aids AOB in functioning efficiently under high dissolved oxygen conditions.

The distribution of bacteria at the genus level more clearly illustrates the differences in microbial community structure during reactor operation (**Figure 6(b)**). **Figure 7** presents the sample dilution curves, with each curve representing a distinct sample. All curves plateau, indicating that the sequencing data is sufficient and representative. The AOB detected in the fillers primarily comprised Nitrospira, with their relative abundance reaching 34.27%, 34.37%, and 36.31% at DO levels of 3.0 - 4.0, 5.0 - 6.0, and 7.0 - 8.0 mg O/L, respectively. This indicates a significant enrichment of AOB from an initial state of 0.57%. The consistent and abundant presence of Nitrospira across different dissolved oxygen stages suggests that the variation in Ammonia Oxidation Rate (AOR) is primarily driven by DO levels, which restrict the functional capacity of AOB. At higher DO levels, the majority of Nitrospira can access sufficient electron acceptors for ammonia oxidation, enhancing the oxidation rate. Flavobacterium, belonging to the Bacteroidetes phylum [50], comprises mostly facultative anaerobic or aerobic bacteria that can thrive under low oxygen or even anaerobic conditions, though they prefer aerobic environments. At DO levels of 3.0 - 4.0 mg O/L, Flavobacterium reached an abundance of 5.28%, which may contribute to the ability of the biofillers to maintain a high AOR even in well-oxygenated conditions.

The immobilized fillers possess the ability to preserve functional bacteria, with their diverse oxygenation environments greatly facilitating microbial diversity. The microbial community also included Denitrifying Bacteria (DNB) and Heterotrophic Bacteria (HB), primarily represented by Rhodobacter and Pseudomonas. This presence may be a key reason for the occurrence of N loss in the reactor. Additionally, other DNB, such as Pseudorhodobacter and Thauera, were found, likely inhabiting the hypoxic zones formed within the fillers. These bacteria can convert NO_3-N to N_2 using organic compounds primarily generated by the metabolic products of other bacteria, thereby enhancing the system's denitrification. These results indicate that the fillers not only effectively retain the PN functional microbial community but can also adjust the bacterial community structure in response to environmental changes.

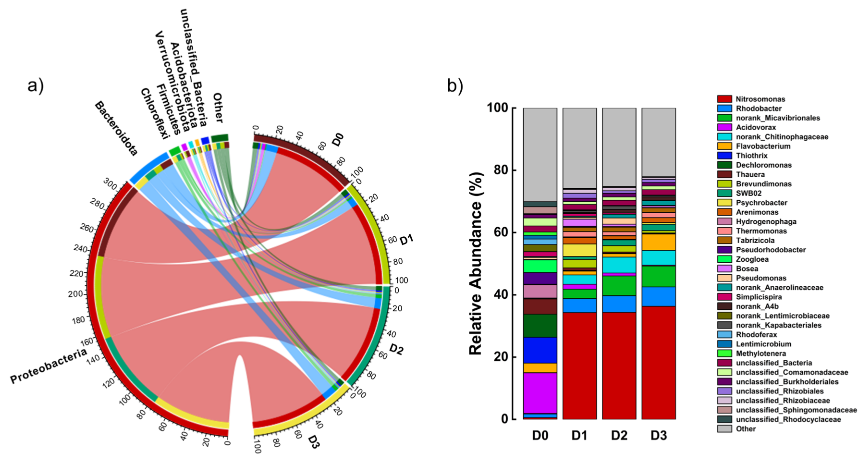


Figure 6. Microbial community structure analysis: (a) community abundance at the phylum level; (b) community abundance at the genus level.

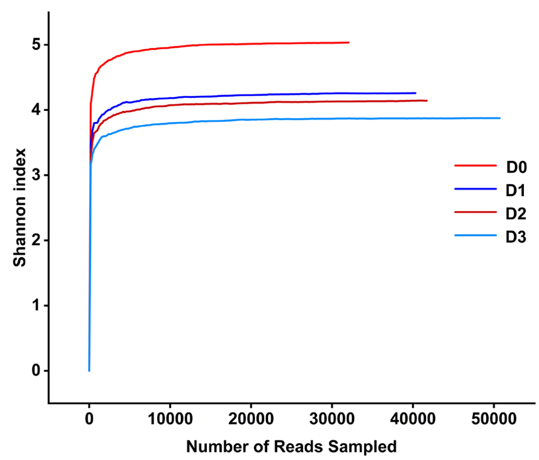


Figure 7. Sample sparsity graph.

3.2.2. Dissolved Oxygen Distribution of Biological Fillers

During the operation of the reactor, AOB achieved rapid enrichment and growth, with AOR exceeding 30 mg/(L·h) within just 8 days. This rapid performance is fundamental to achieving efficient and stable PN. In the PN reactor, a robust source of AOB provides a solid foundation for the quick activation of the fillers. The complex oxygenation environment within the fillers offers ample reproductive space for microbial growth, leading to the formation of a diverse and resilient biological community capable of withstanding fluctuations in ammonia concentration and dissolved oxygen, thereby ensuring high PN efficiency. Additionally, throughout the reactor operation, a small number of unclassified Proteobacteria and Bacteroidetes were detected within the fillers, along with trace amounts of fermentative bacteria in the external sludge [51]. These bacteria participate in the overall nitrogen cycling process in the reactor, contributing to efficient and stable partial nitrification, allowing AOB to maintain their dominant position throughout the operation.

3.4. Advantages and Prospects

Typically, small-scale wastewater treatment plants need to manage low to moderate concentrations of wastewater. In contrast to large-scale treatment facilities, small-scale plants face complex fluctuations in flow rates and water quality, which impose greater demands on the professional skills of operators. This study focuses on a gel-immobilized PN reactor, comprehensively investigating the efficient and stable PN process under varying concentrations (50 - 300 mg N/L) and dissolved oxygen conditions. The study reveals the reactor's performance response to changes in ammonia nitrogen levels. These findings provide reliable practical references for the application of PN processes in wastewater nitrogen removal, as well as for processes that require PN as a critical component. This can be summarized in several key aspects:

Firstly, the filler plays a crucial role in the long-term retention of ammonia-oxidizing bacteria (AOB), providing a stable and independent habitat for their re-enrichment [23]. This environment supports the maintenance of high bacterial abundance and biological activity of AOB, enhancing their resistance to fluctuating ammonia concentrations and excessive aeration. These advantages ensure that AOB remains dominant throughout the partial nitrification (PN) process.

Secondly, the instability in inhibiting nitrite-oxidizing bacteria (NOB) in traditional wastewater treatment processes is often a major factor leading to low nitrogen removal rates within the system. In contrast, the immobilized filler can effectively suppress NOB growth over an extended period without the need for additional inhibition methods such as free ammonia (FA)/free nitrous acid (FNA) side-stream inhibition or physical ultrasound. This significantly facilitates the practical application of the PN process, especially in the treatment of low-concentration ammonia nitrogen wastewater.

Finally, when faced with dramatic fluctuations in ammonia concentration and high aeration rates, the immobilized filler demonstrates excellent adaptability and shock resistance. By automatically adjusting the microbial composition within the filler, it effectively removes key pollutants from the wastewater, thereby reducing the operational complexity of the process and providing significant advantages and operational convenience in engineering applications.

4. Conclusion

Through gel-immobilization technology, stable and efficient PN performance can be achieved under both fluctuating ammonia concentrations and varying dissolved oxygen conditions. The results indicate that the immobilized fillers enable rapid activation within 7 days, with a maximum oxidation-reduction rate reaching 53.7 mg/(L·h) and NAR exceeding 95%. The biofillers provide an optimal space for the retention and enrichment of Ammonia-Oxidizing Bacteria (AOB), while the presence of surface gel and various functional microorganisms restricts the growth of Nitrate-Oxidizing Bacteria (NOB). This ensures that AOB remains dominant, maintaining the advantage and stability of the functional microbial community, which contributes to excellent PN performance. Over time, active sludge gradually forms on the surface of the fillers during the process, and cleaning of the reactor may not be necessary unless excessive growth of external sludge occurs. Furthermore, the abundant AOB and diverse microbial community within the fillers enhance their resilience to shock loads and adaptability to high dissolved oxygen environments.

Conflicts of Interest

The authors declare no conflicts of interest.

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