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The Study on Nitrogen Removal Enhancement from Secondary Biochemical Effluent by Sulfur Autotrophic Denitrification Composite Filler

Yi Rong[1] & Hua Fang[1]

[1] School of Environmental Science and Engineering, Nanjing University of Information Science and Technology, Nanjing, China

Correspondence: Yi Rong, School of Environmental Science and Engineering, Nanjing University of Information Science and Technology, Nanjing, China.

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Abstract

Experiments were conducted using simulated secondary effluent from wastewater treatment plants. A composite filler was prepared by mixing sulfur and pyrite (FeS_2) as electron donors for microorganisms to evaluate the nitrate removal efficiency under different filler compositions. Three iron-carbon-based composite sulfur autotrophic denitrification fillers were synthesized with varying pyrite-to-sulfur mass ratios (2:1, 1:1, and 1:2, labeled as FS1, FS2, and FS3, respectively). A synergistic autotrophic-heterotrophic denitrification biofilter system was established to compare the operational conditions and nitrogen removal performance among mixed autotrophic-heterotrophic, heterotrophic, and autotrophic denitrification processes. The results showed that the FS2 system (FeS_2:S = 1:1) achieved the shortest start-up time (7 days), 33% and 50% faster than FS1 (FeS_2:S = 2:1) and FS3 (FeS_2:S = 1:2), respectively. During the start-up phase, FS2 exhibited the highest NO_3^--N removal rate (1.82 mg/(L·h)), significantly outperforming FS1 (1.25 mg/(L·h)) and FS3 (0.98 mg/(L·h)). The optimal HRT for pyrite-based systems was 24 h, with FS2 achieving the highest NO_3^--N removal efficiency (94.2%), followed by FS3 (84.2%) and the pure sulfur system (80%). When HRT was reduced to 12 h, FS2 maintained a removal efficiency of 81.3%, while FS1 and FS3 declined to 78.5% and 65.2%, respectively. Within pH 6.5–8. 0, FS2 demonstrated stable NO_3^--N removal (95%–98%) with minimal effluent pH fluctuation (±0.3 from initial pH 7.0), whereas FS1 and FS3 exhibited larger pH variations (±1.2 and ±1.8, respectively), indicating inferior buffering capacity. The FS2 filler (FeS_2:S = 1:1) exhibited superior denitrification efficiency, rapid system start-up, and robust pH stability, making it a promising candidate for enhancing nitrogen removal in secondary effluent treatment.

Keywords: sulfur-iron-based filler, synergistic denitrification, microbial community structure

1. Introduction

1.1 Research Background and Significance

In the context of rapid industrialization and urbanization, nitrogen pollution control in water bodies has become a critical issue in China's ecological environment governance [1]. Despite secondary biological treatment, effluent from existing wastewater treatment facilities still exhibits significant nitrogen pollution loads (TN: 15-40 mg/L) and shows prominent carbon-to-nitrogen imbalance (C/N <3.0), with nitrate nitrogen accounting for over 60% of the water quality characteristics [2,3], making it difficult to meet the new demands for improving water environmental quality. In response to this situation, some local ecological departments have implemented strict discharge standards, with emission limits in key watersheds approaching the Class IV water quality requirements of the Surface Water Environmental Quality Standards [4-6]. Under these circumstances, constructing an efficient deep nitrogen removal system has become a technological necessity in the field of water treatment.

The current nitrogen-containing wastewater treatment technology system encompasses traditional biological treatment, physicochemical separation, and novel biological denitrification processes [7]. Among these, the new biological denitrification technology based on sulfur autotrophic denitrification and composite nutrient-type denitrification has become a research hotspot due to its ability to overcome carbon source limitations [8]. While traditional physicochemical methods can achieve nitrogen separation, they are constrained by high treatment costs and significant secondary pollution risks, limiting their practical application in engineering projects. In contrast, biological denitrification technology achieves nitrogen conversion through microbial metabolism, demonstrating

significant advantages in sustainability [9]. Heterotrophic denitrification removes nitrate nitrogen by converting it into nitrite through microbial-mediated nitrogen transformation. The core mechanism involves heterotrophic bacteria using organic carbon sources as electron donors to gradually reduce nitrate nitrogen to gaseous nitrogen for release from the system [10]. This process has strict requirements for the carbon-to-nitrogen ratio; however, effluent from wastewater treatment systems often lacks sufficient carbon sources (COD/ρ(TN) <3.5) [11], necessitating the addition of external carbon sources to maintain denitrification efficiency during operation, which not only increases treatment costs but also poses risks of water quality fluctuations. By comparison, autotrophic denitrification systems, through the metabolic activities of hydrogen autotrophs or sulfur/iron-oxidizing bacteria, can use reduced inorganic substances to replace organic matter for electron transfer, showcasing unique process advantages. However, autotrophic denitrification commonly faces issues such as unstable pH, sulfate accumulation, and iron ion leaching, requiring subsequent treatment [12].

For the synergistic application of heterotrophic and autotrophic denitrification technologies, the field of wastewater treatment has developed composite nutrient-based nitrogen removal systems. Current research primarily focuses on optimizing process parameters, while systematic analysis of synergistic metabolic mechanisms remains insufficient. This study uses simulated secondary effluent from a wastewater treatment plant for experiments. Sulfur and pyrite are mixed to form a substrate, serving as an electron donor source for microorganisms. The denitrifying bacterial populations previously cultivated in the laboratory were tested in batch experiments to evaluate the effectiveness of different substrates in removing nitrate from water.

1.2 Materials and Methods

1.2.1 Experimental Equipment and Packing

Figure 1 shows the experimental setup. The main reaction unit of the experimental setup is a sequential batch biofilter column, with structural parameters including a column height of 500 mm, an inner diameter of 64 mm, and an effective volume of 1.6 L. The system's packing layer uses porous ceramic granules with a particle size of 3-5 mm, forming a filter bed that is 350mm high and has a volume of 1.1 L. The device is equipped with a fluid control system, which achieves hydraulic circulation through a dual-channel peristaltic pump: the influent unit diffuses substrate through a bottom distributor to form a closed hydraulic circulation path, ensuring normal microbial activity and growth within the reactor. The reactor creates an ideal space for microorganisms to survive and reproduce under anaerobic conditions. Throughout the experiment, constant flow delivery is maintained to ensure the stable metabolic activity of the microorganisms.

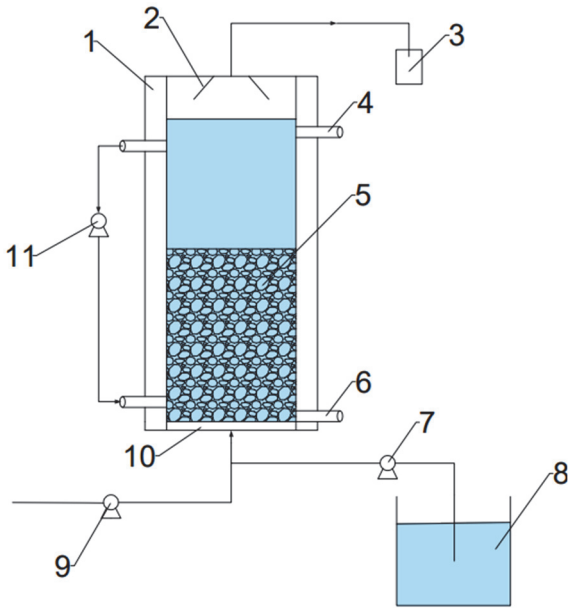

Figure 1. Autotrophic denitrification reactor (1 insulation layer; 2 three-phase separator; 3 gas collection device; 4 outlet; 5 packing layer; 6 sludge outlet; 7 water pump; 8 water inlet bucket; 9 backflush pump; 10 water distribution device; 11 circulation pump)

Sulfur and pyrite are ground and sieved through a 100-mesh standard sieve; cement is selected as the binder; sulfur powder and pyrite powder are weighed in certain mass ratios (1:2, 1:1, 2:1), with 10% of the total weight of cement added to each portion. After mixing evenly, add water at 10-15% of the solid weight and mix again until uniform. Add this mixture to a pill-making machine for granulation, then place it in a vacuum drying chamber and dry at 200-220°°C for two hours. Once cooled to room temperature, remove the product. Prepare sulfur: composite fillers with sulfur-to-pyrite mass ratios of 2:1, 1:1, and 1:2 (marked as FS1, FS2, FS3).

1.2.2 Experimental Water Intake and Sludge Acclimation

In this experiment, the effluent from the laboratory A/O process was used as the influent of the denitrification reaction system.

The concentration of each component and the characteristics of water quality parameters are shown in Table 1-1, and the inlet water of denitrification experimental device is shown in Table 1-2.

Table 1-1. Water quality indexes of influent to SBR reactor

metric	water temperature	pH	PO43--P (mg/ L)	COD (mg/ L)	NH4+-N (mg/ L)	COD/TN
potency	18-27	7.5-7.9	0.8-1.5	150-200	35-40	2.78-5
mean	22.5	7.7	1.15	175	37	3.89

Table 1-2. Water quality indexes of autotrophic denitrification reactor

metric	water tempe rature	pH	COD (mg/ L)	NH4+-N(mg / L)	NO3--N (mg/ L)	PO43--P (mg/ L)
potency	25-30	7.5-7.9	35-65	2-5	18-25	0.4-0.8

The microbial strains used in this study originate from the activated sludge of the anoxic zone in a municipal wastewater treatment plant that employs the A²/O process. A 200mL sample of sludge was mixed with nine times its volume of nutrient solution and inoculated into a 1L standard anaerobic reactor, with elemental sulfur added as the electron donor. The cultivation process was carried out in a constant temperature shaker, maintaining a temperature of 30°C and a shaking speed of 150 rpm to optimize solid-liquid contact. The enrichment culture lasted for two weeks, during which the reaction system was tightly wrapped with three layers of light-blocking material to eliminate any adverse effects of light on chemolithoautotrophic microorganisms.

1.2.3 Test Indicators and Methods

Nitrate nitrogen, sulfate, sulfate ion chromatography, Ph acid meter, nitrite nitrogen-N-(1-naphthyl) ethylenediamine photometry

2. Results

2.1 Start the Membrane Hanging

The experiment set up five denitrification reactor units. Three of these were filled with composite media prepared from sulfur and pyrite, while the other two used pyrite and sulfur alone as electron donors to make the media. All reactors operated in batch mode, completing one water intake and discharge cycle every 12 hours. During the start-up phase, laboratory-cultured sulfur autotrophic denitrifying bacteria were employed. These microorganisms were initially cultured in constant temperature flasks and gradually adapted to the pollutant environment over 14 days before being transferred to the reactors. The influent maintained a nitrate concentration between 20-23 mg/L, and the microbial growth status was assessed by real-time monitoring of denitrification efficiency. The specific criteria for assessment are: when three consecutive tests show no significant fluctuation in nitrate removal rate, it confirms that the microorganisms have stably survived on the surface of the media. The changes in NO3-N effluent from the five reactors during the biofilm formation start-up phase are shown in Figures 2-1 and 3-2:

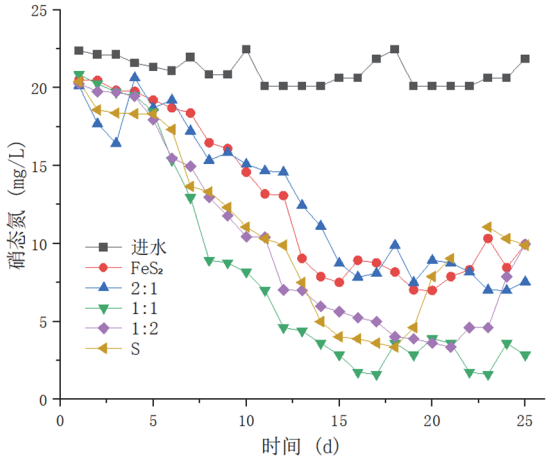

Figure 2-1. Denitrification effect of different packing filter membrane start-up

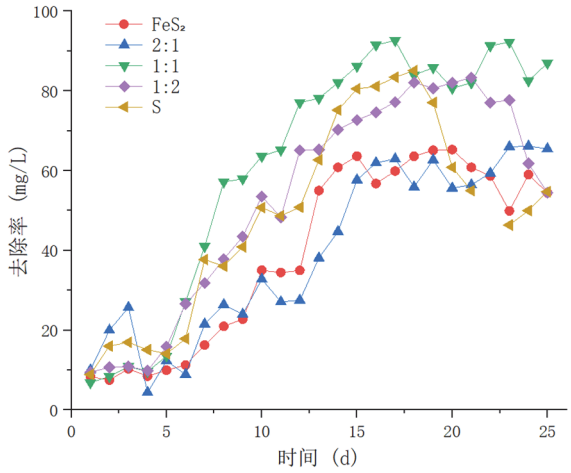

Figure 2-2. Denitrification removal rate of different packing materials

As shown in the figure, during the biofilm operation period, all five packing systems exhibited differentiated nitrogen removal characteristics. The effluent concentration of the pyrite elemental system (FeS_2) was the highest, showing a progressive removal feature. From day 1 to day 10, the effluent concentration decreased from 20.52mg/L to 14.59mg/L (removal rate 34.8% → 53.4%), consistent with the dual-electron transfer pathway of Fe^{2+}/SO_4^{2-} oxidation and release in FeS_2 (Equation 3-1). After day 15, a plateau period appeared, indicating that the formation of a surface passivation layer limited the electron release rate.

$$FeS_2 + 15NO_3^- + 14H^+ \rightarrow Fe^{3+} + 2SO_4^{2-} + 15NO_2^- + 7H_2O \quad (2\text{-}1)$$

FeS_2/S^0 = 2:1 ratio system had a removal rate of only 7.3% in the first 8 days, but began to degrade more rapidly from day 9, with the final concentration dropping to 1.60 mg/L, indicating the sustained-release characteristics dominated by pyrite; FeS_2/S^0 = 1:1 ratio system exhibited optimal synergistic effects, with the removal rate dropping to 15.36 mg/L on day 5 and reaching 1.75 mg/L on day 15, showing a 42% increase in the overall removal rate constant compared to the elemental sulfur system; the elemental sulfur (S^0) system maintained a 18.33-13.33 mg/L during the early stage (1-10 days), then suddenly dropped to 3.62 mg/L in the later stage (11-18 days), exhibiting typical characteristics of the lag phase in autotrophic denitrification.

The material system formed by the 1:1 composite of pyrite and elemental sulfur constructs an electron transfer network through Fe-S synergy, thereby facilitating chemical reduction reactions. After an 18-day treatment period, the nitrate nitrogen concentration in the effluent decreased to 1.75 mg/L, with a denitrification efficiency of 92.3%, fully demonstrating the significant advantages of the composite filler in enhancing denitrification performance.

2.2 Analysis of Operation Effects Under Different HRTs

This experimental study selects five typical filter bed systems and sets three HRT gradients of 8 h, 16 h, and 24h for comparative research. By synchronously monitoring the pollutant removal efficiency of each filter under different HRT conditions, the quantitative relationship between hydraulic retention time and denitrification efficiency is systematically analyzed. This comparative study can effectively reveal the metabolic regulation mechanism of denitrifying functional bacteria under HRT, providing theoretical support for process parameter optimization. The denitrification effects of the filters under different HRT conditions are shown in Figures 2-3 to 2-7.

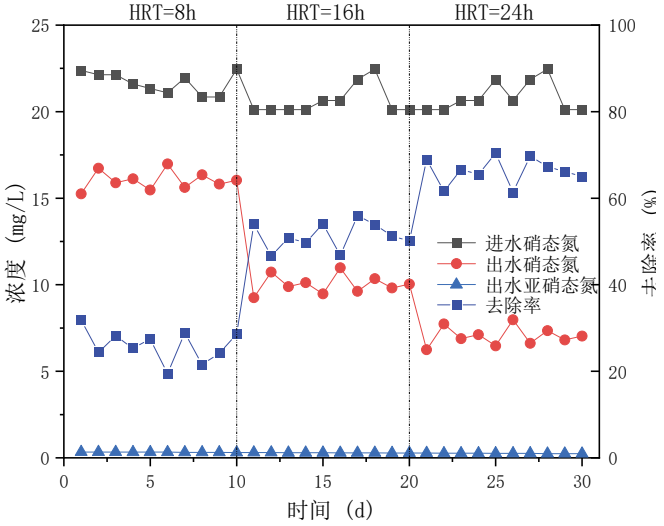

Figure 2-3. Effect of HRT on nitrogen removal efficiency of hematite elemental system

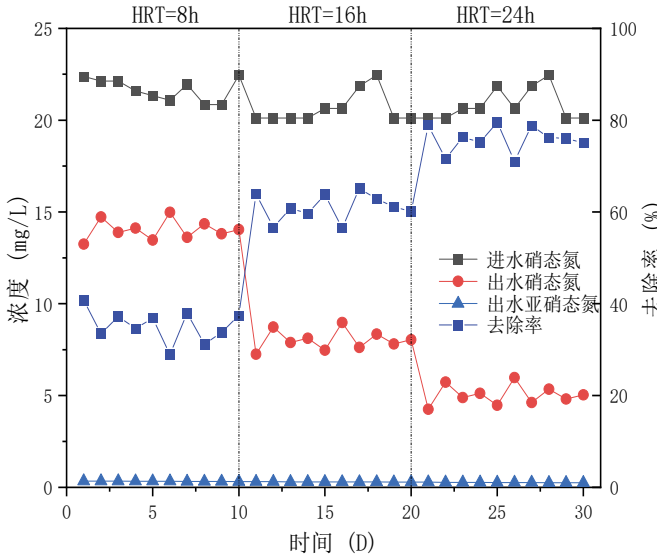

Figure 2-4. Influence of HRT on nitrogen removal efficiency of FeS_2:S=2:1 system

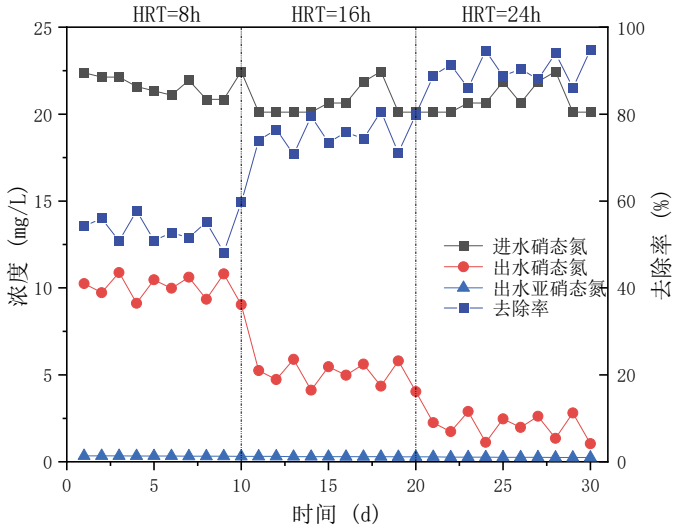

Figure 2-5. Influence of HRT on nitrogen removal efficiency of FeS_2:S=1:1 system

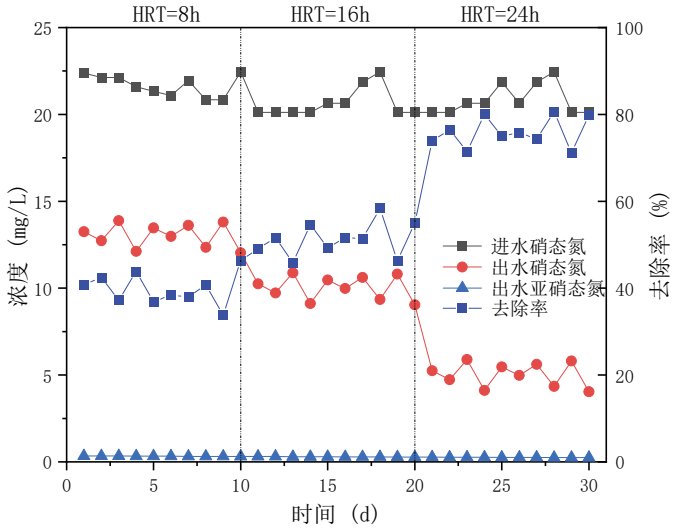

Figure 2-6. Influence of HRT on nitrogen removal efficiency of FeS_2:S=1:2 system

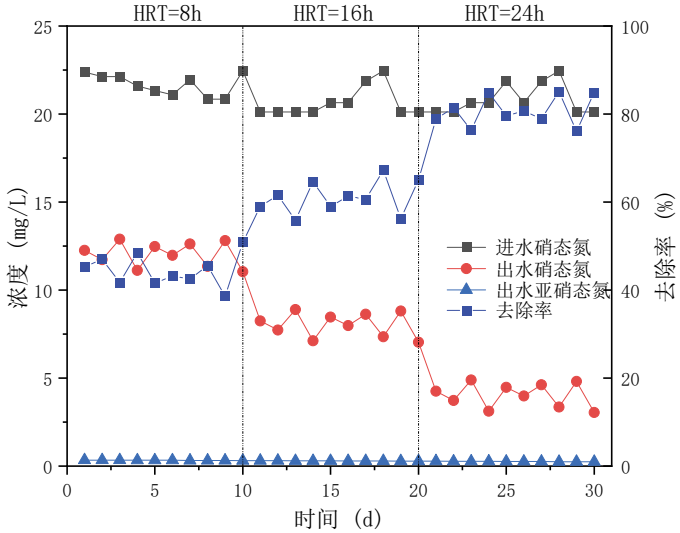

Figure 2-7. Effect of HRT on nitrogen removal efficiency of sulfur

From Figures 2-3 to 2-7, it can be seen that the NO_3^--N removal rate in all denitrification systems significantly increases with the extension of HRT. The influent NO_3^--N concentration in all systems ranges from 20 to 25 mg/L. When HRT is 8 h, the effluent NO_3^--N concentration drops to 10 to 15 mg/L, with a removal rate between 40% and 60%, and the effluent NO_2^--N concentration remains consistently low. As HRT increases to 16 h, the influent NO_3^--N concentration slightly decreases (possibly due to enhanced system stability), and the effluent NO_3^--N concentration further decreases, with an increased removal rate. However, NO_2^--N does not accumulate significantly, indicating high conversion efficiency of denitrification intermediate products. When HRT extends to 24 h, the nitrogen removal efficiency peaks for all systems, with the effluent NO_3^--N concentration further decreasing, and the removal rate increasing to over 80% (for the FeS_2:S=2:1 system) or around 80% (for other systems). The NO_2^--N concentration remains low, suggesting sufficient reaction time and complete denitrification.

Pyrite elemental system The removal rate is highest when HRT = 24 h. Based on the patterns of other systems, the removal rate may be similar to that of FeS_2:S = 1:1 or 1:2 systems (approximately 80%). For shorter HRTs (8 h), the removal rate is only 48%-65%, indicating that microorganisms require more time to complete the full denitrification process of NO_3^--N; when HRT = 24 h, the removal rate in the FeS_2:S = 1:1 system exceeds 90%, outperforming other ratio systems, possibly due to the synergistic effect between pyrite and elemental sulfur: a higher proportion of FeS_2 can provide more electron donors, enhancing denitrifying bacterial activity, while elemental sulfur supplements the sulfur source, optimizing the microbial metabolic environment; for both FeS_2:S = 1:1 and 1:2 systems, the removal rate at HRT=24h is about 80%, but the system with a higher proportion of elemental sulfur (1:2) may have a faster oxidation rate of sulfur, leading to temporary excess electron supply, resulting in slightly lower NO_3^--N utilization efficiency by denitrifying bacteria compared to the 2:1 system; the nitrogen removal trend in the elemental sulfur system is consistent with that of the pyrite system, but the removal rate at HRT=24h is around 80%, slightly lower than the FeS_2:S = 2:1 system, suggesting that a single sulfur source may limit the diversity of electron donors, affecting the metabolic efficiency of denitrifying bacteria.

2.3 Analysis of Operation Effect Under Different pH

The specific denitrification effect is shown in Figure 2-8 to 3-12:

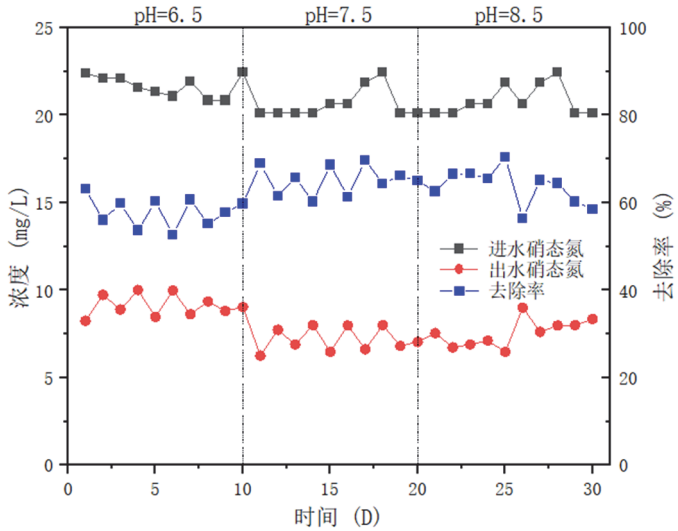

Figure 2-8. Effect of pH on nitrogen removal efficiency of pyrite system

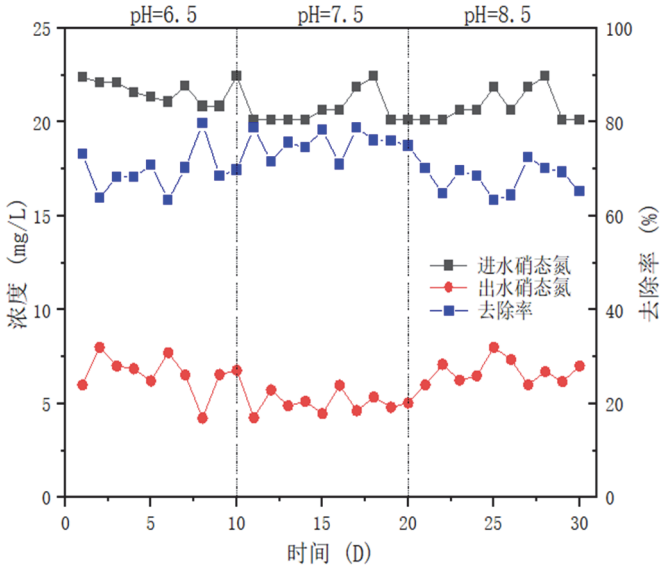

Figure 2-9. pH Effect of FeS_2:S=2:1 system on nitrogen removal efficiency

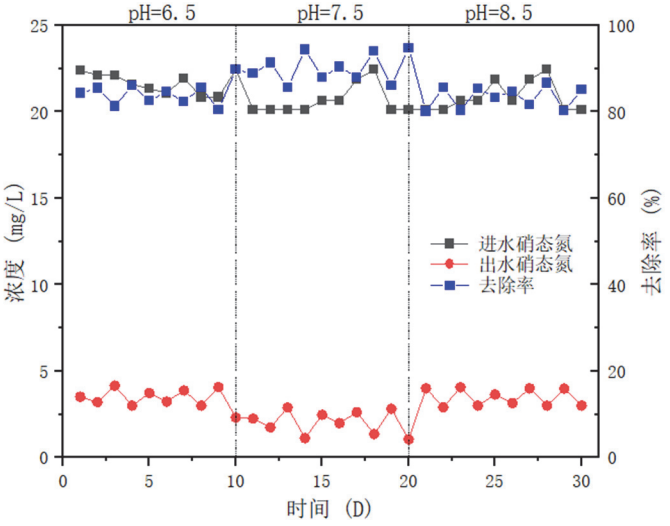

Figure 2-10. Effect of pH on FeS_2:S=1:1 system denitrification efficiency

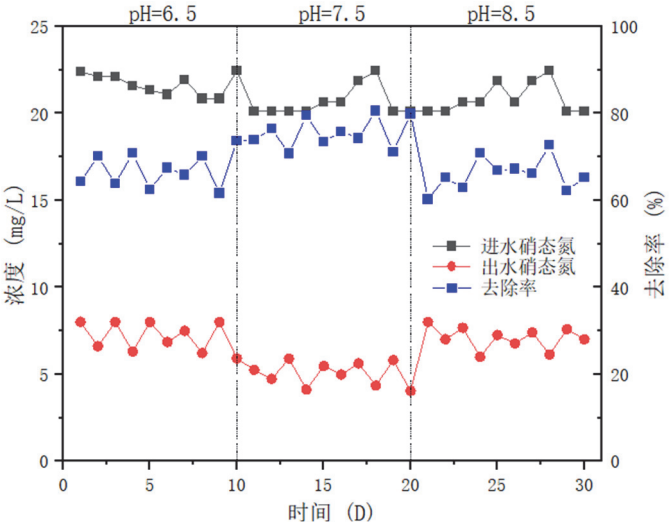

Figure 2-11. Effect of pH on FeS_2:S=1:2 system nitrogen removal efficiency

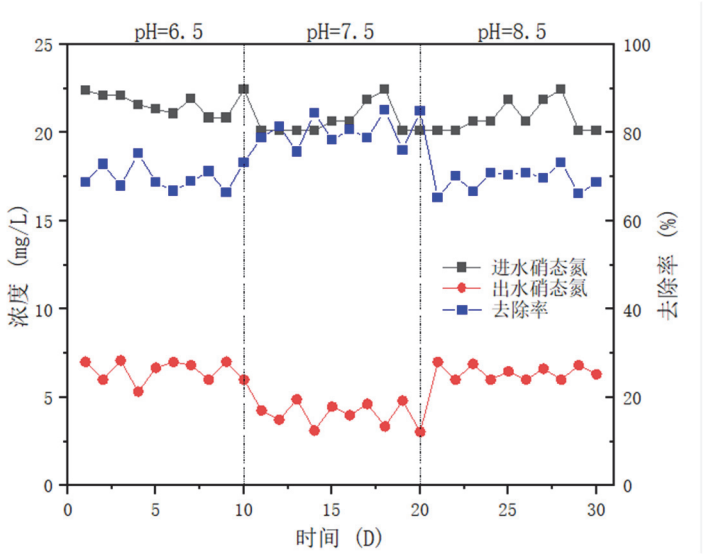

Figure 2-12. Effect of pH on nitrogen removal efficiency of elemental sulfur

Figure 2-8 shows that the denitrification efficiency of the pyrite elemental system is significantly regulated by pH. At pH = 7.5, the system exhibits optimal performance, with nitrate nitrogen concentration in the effluent stabilized at 4-6 mg/L and removal rates fluctuating between 70%-90%. However, at pH=6.5 and pH = 8.5, the effluent concentrations rise to 5-7 mg/L and 5-7 mg/L, respectively, while removal rates drop to 60%-80%. This trend aligns with the typical characteristics of sulfur autotrophic denitrification: a neutral environment (pH = 7.5) promotes the activity of sulfur-oxidizing bacteria, whereas excessively low or high pH levels may inhibit enzyme activity or alter the chemical properties of the pyrite surface, leading to decreased denitrification efficiency.

Figure 2-9 shows that the impact of pH on the denitrification efficiency of the FeS_2:S=2:1 system is evident when the ratio of FeS_2 to sulfide is 2:1 (Figure 2-9). The denitrification efficiency is optimal at pH=7.5, with nitrate nitrogen concentration in the effluent (5-7 mg/L) and removal rate (70%-90%) being better than under other pH conditions. Notably, compared to the elemental pyrite system, the introduction of FeS_2 slightly improves the denitrification efficiency at pH=6.5 and 8.5.

Figure 2-10 shows that the FeS_2:S=1:1 system is more sensitive to pH. When pH=7.5, the denitrification efficiency of this system is significantly better than other ratios, with the lowest nitrate nitrogen concentration in the effluent (3-5 mg/L) and removal rates as high as 80%-97%. At some time points, complete denitrification was even achieved. This phenomenon suggests that the balance ratio between FeS_2 and sulfides (1:1) may optimize the synergistic effect of sulfur autotrophic denitrification and iron chemical reduction.

Figure 2-11 shows that in the system with FeS_2:S = 1:2, the denitrification efficiency remains optimal at pH=7.5 (outlet concentration 4-6 mg/L, removal rate 70%-90%), but it slightly decreases compared to the FeS_2:S = 1:1 system. Additionally, when pH = 8.5, the changes in outlet concentration and removal rate are similar to those of the pyrite elemental system, indicating that a high proportion of sulfides may reduce the system's tolerance to alkaline conditions.

Figure 2-12 shows that the denitrification efficiency of the elemental sulfur system is generally lower than that of the FeS_2-containing system. When pH = 7.5, the nitrate nitrogen concentration in the effluent is 6-8 mg/L, with a removal rate of 60%-80%; however, when pH=6.5 and 8.5, the removal rate further decreases to 50%-70%. This result highlights the critical role of FeS_2 in the denitrification process: the presence of iron not only may directly participate in denitrification through chemical reduction but also enhances system stability by modulating the metabolic environment of sulfur-oxidizing bacteria (such as neutralizing acidic byproducts). In contrast, the pure sulfur system lacks the buffering capacity mediated by iron, making its denitrification efficiency more sensitive to pH fluctuations.

According to the analysis of the above results, the optimal pH values for each system show consistency: all systems achieve peak denitrification efficiency at pH = 7.5, which aligns with the optimal growth pH (in neutral conditions) of sulfur autotrophic denitrifying bacteria. The ratio of FeS_2 to sulfide significantly affects denitrification efficiency. When the ratio is 1:1 (Figure 2-10), the system exhibits optimal performance, indicating that there is an optimal

ratio for the synergistic effect between iron and sulfur; deviations from this ratio may lead to electron donor imbalance or accumulation of reaction byproducts. The FeS_2 system enhances sulfur oxidation through the release of Fe^{2+}, while sulfides maintain the stability of the electron donor, and the presence of iron may neutralize H^+ produced by sulfur autotrophic denitrification (e.g., $Fe^{2+} + 2H^+ \rightarrow Fe^{3+} + H_2$), thereby enhancing the system's buffering capacity in acidic environments. Pure sulfur systems lack the chemical reduction and buffering effects mediated by iron, resulting in significantly lower denitrification efficiency and adaptability to pH fluctuations compared to systems containing FeS_2. Therefore, when the ratio of FeS_2 to sulfide is 1:1 and the pH is 7.5, the system can achieve efficient denitrification (removal rate> 80%, effluent concentration <5 mg/L) through the synergistic effect of iron and sulfur.

2.4 The Concentration of Ammonia Nitrogen in Effluent Changes with Time

The removal effect of ammonia nitrogen by five autotrophic denitrification packing systems with respect to time is shown in Figure 2-9.

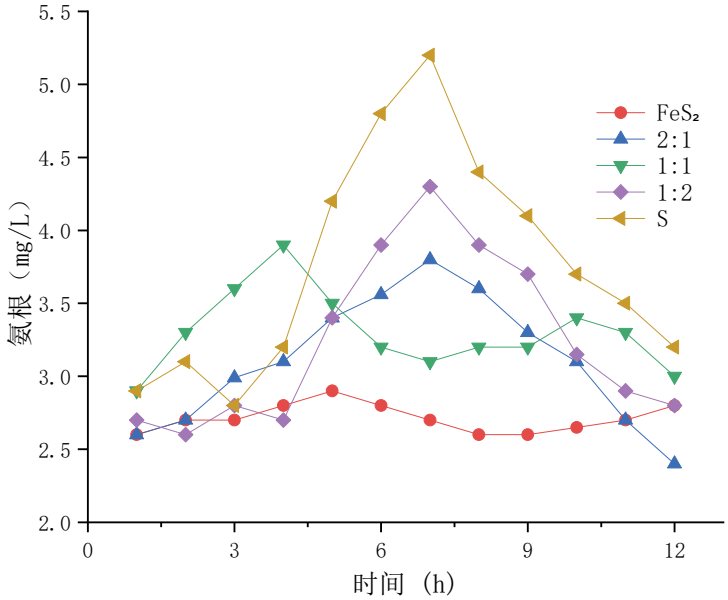

Figure 2-13. Changes of ammonia nitrogen concentration in different packing materials

As shown in Figure 2-13, compared to the influent ammonia nitrogen baseline value (2.5 mg/L), each modified filler system (FeS_2, FS1, FS2, FS3, S) exhibits distinct ammonia nitrogen enrichment characteristics. The elemental sulfur system shows a peak in ammonia nitrogen concentration (5.61 mg/L) during the initial reaction phase (7 h), which is closely related to the activation of the denitrification nitrate reduction to ammonium (DNRA) metabolic pathway [57,58]. After 12 h, the ammonia nitrogen concentration gradually decreases and stabilizes at 3.51 mg/L. Analyzing the dynamic changes in ammonia nitrogen reveals that the electron supply properties of sulfur-based fillers significantly influence the nitrogen transformation pathway selection: the ammonia nitrogen accumulation in the composite pyrite system (FS1-FS3) is 18.7-32.4% lower than that in the pure sulfur system, which is closely related to the buffering of pH fluctuations and maintaining a better redox potential. The pyrite component effectively regulates electron transfer efficiency through the Fe^{2+}/Fe^{3+} cycle, inhibiting the competitive consumption of reduction equivalents by the DNRA pathway. The effluent ammonia nitrogen concentration in the FeS_2 experimental group is slightly higher than the influent, maintained within the range of 3.0-3.5 mg/L. This may be due to the fact that using FeS_2 alone might not effectively support autotrophic denitrification functions, or it could lead to slight ammonia nitrogen accumulation due to limited iron ion dissolution or surface adsorption.

The ammonia nitrogen concentration in the effluent of Group FS1 fluctuated dramatically between 2.0 and 4.5 mg/L, peaking at 4.5 mg/L at the 3rd hour, then gradually decreasing to 2.0 mg/L after the 10th hour. This may be due to insufficient electron donors when sulfur content is low (S ratio 33%), leading to limited microbial metabolic activity initially, and later inhibition of denitrification reactions due to the accumulation of sulfur oxides (SO_4^{2-}). The ammonia nitrogen concentration in the effluent of Group FS2 remained the most stable throughout, ranging from 2.5 to 3.0 mg/L, with a minimum value of 2.5 mg/L at the 8th hour, close to the influent level without significant peaks. Its peak was much lower than that of the sulfur group, possibly because the optimized balance

ratio of FeS_2 to sulfur (S ratio 50%) in the composite filler improved electron transfer efficiency, resulting in stable ammonia removal performance. This process consumes some NH4+, preventing a rapid increase in NH4+ concentration. The ammonia nitrogen concentration in the effluent of Group FS2 fluctuated between 2.5 and 4.0 mg/L, increasing initially and peaking at 4.0 mg/L at the 5th hour, but improving later and dropping to 2.5 mg/L at the 12th hour. This may be due to the high sulfur ratio causing an initial rapid sulfur oxidation rate (creating an acidic environment or sulfate inhibition), followed by a recovery in denitrification efficiency as microorganisms adapt. In summary, Group FS2 (FeS_2:S = 1:1) demonstrated the most stable ammonia removal capability, with effluent concentrations approaching those of the influent, making it suitable for long-term denitrification systems.

2.5 SO42-Generation and NO3-Reduction Analysis

The results of NO3--N variation along the process in the five autotrophic denitrification packing systems under optimal operating conditions are shown in Figure 2-14.

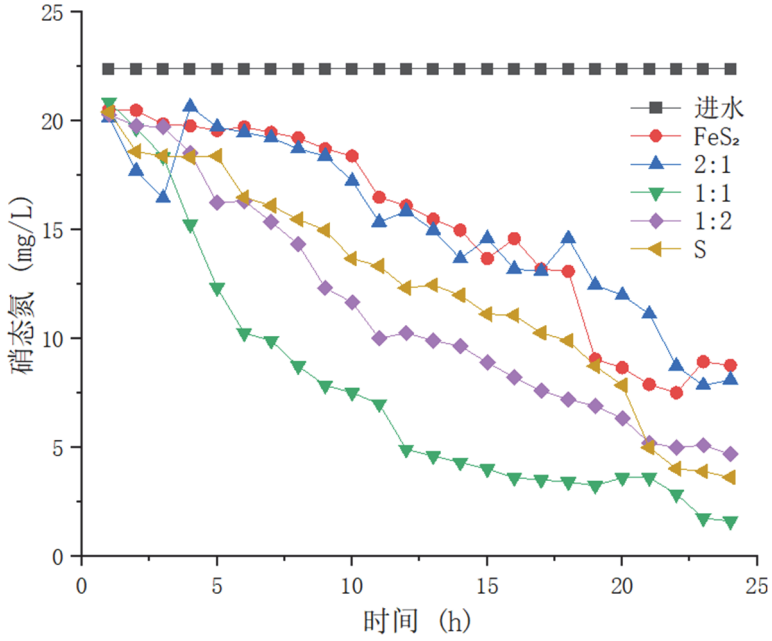

Figure 2-14. NO3-N along the course

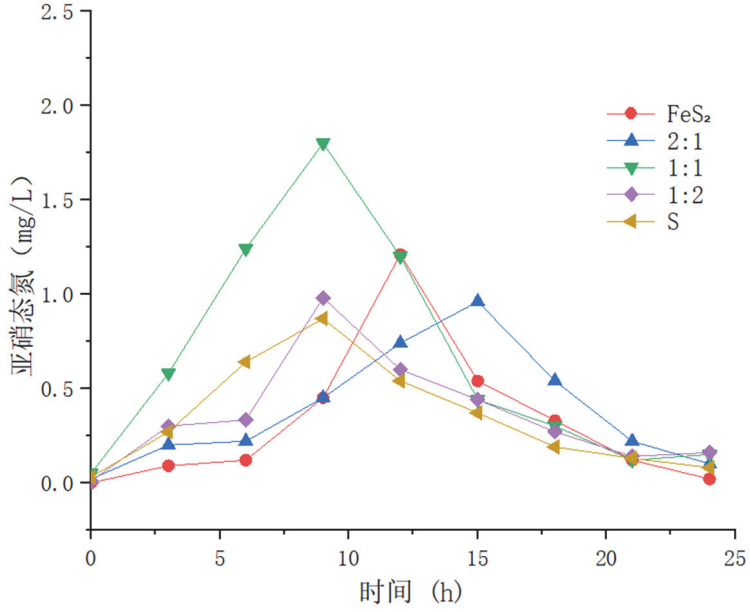

Figure 2-15. Variation of NO2-N along the course

As shown in Figure 2-14, for the FeS_2 system, the nitrate concentration decreases slowly during the initial stage (0-5 hours), but increases denitrification efficiency in the later stage (15-25 hours), ultimately reducing the nitrate concentration to around 4 mg/L. For the FS1 system, the nitrate concentration decreases rapidly at the beginning (0-5 hours) and then levels off. The final nitrate concentration drops to about 5 mg/L, demonstrating good removal efficiency; for the FS2 system, the trend of nitrate concentration decrease is relatively stable, showing consistent removal effects throughout the experiment. The final nitrate concentration reaches approximately 7 mg/L, slightly lower than FS1 but better than FS3; for the FS3 system, the nitrate concentration decreases slowly, especially in the later stage (15-25 hours), with a significant reduction in rate. The final nitrate concentration drops to around 10 mg/L, indicating relatively poor removal efficiency; for the elemental sulfur system, denitrification efficiency remains consistently high, ultimately reducing the nitrate concentration to about 3.6 mg/L.

As shown in Figure 2-15, during the optimal process operation of the five autotrophic denitrification media systems, the NO_2^--N concentration showed a trend of first increasing and then decreasing along the flow path. The FeS_2 system reached its peak NO_2^--N concentration at 10 hours, at 1.5 mg/L; the FS1 system and elemental sulfur system both peaked at 10 hours, with concentrations of 2.0 mg/L and 1.5 mg/L, respectively; the FS2 system slightly exceeded the FeS_2, reaching 1.8 mg/L at 10 hours; the FS3 system had the latest peak, with the highest concentration at 2.5 mg/L at 15 hours. Ultimately, the NO_2^--N concentrations of all media systems were reduced to lower levels: the effluent concentration from the FeS_2 system was 0.1 mg/L, while those from FS1, FS2, and FS3 were 0.125 mg/L, 0.13 mg/L, and 0.2 mg/L, respectively; the effluent concentration from elemental sulfur (S) was 0.15 mg/L.

By analyzing the concentration changes of nitrate nitrogen and nitrite nitrogen along the process, it was found that under optimal hydraulic retention time conditions, no significant accumulation of nitrite nitrogen was observed in the effluent of each system. However, during the initial stage of reactor operation, all experimental groups showed a noticeable increase in nitrite nitrogen concentration. This may be due to the sufficient substrate supply at the beginning of the reaction, which created favorable metabolic conditions for microbial communities, promoting efficient conversion of nitrate nitrogen to nitrite nitrogen; secondly, as an intermediate product in the nitrogen transformation chain, the conversion process of this intermediate to gaseous nitrogen exhibits a time delay effect; furthermore, the dissolved oxygen carried by the influent inhibits the activity of nitrite-reducing bacteria, preventing timely completion of subsequent conversion of nitrite nitrogen. When the system operates into the mid-to-late stages, with the gradient of dissolved oxygen and nitrate nitrogen concentration tending to be depleted, the metabolic inhibition of nitrite-reducing bacteria is relieved, facilitating the final conversion of nitrite nitrogen to gaseous nitrogen.

$$a^2+b^2=c^2 \tag{1}$$

$$x = \frac{-b \pm \sqrt{b^2 - 4ac}}{2a}$$

3. Discussion

1) The FS2 system performs optimally during the biofilm initiation phase, completing biological maturation in just 7 days (a reduction of 33% and 50% compared to FS1 and FS3, respectively). By day 18, the nitrate nitrogen (NO_3^--N) concentration in the effluent drops to 1.75 mg/L, with a removal rate as high as 92.3%, representing increases of 12.3% and 38.9% over the pure sulfur system (80%) and the pure FeS_2 system (53.4%), respectively. This may be due to the direct participation of Fe^{2+} in denitrification through chemical reduction ($Fe^{2+} + NO_3^- \rightarrow Fe^{3+} + NO_2^-$), while sulfur is provided by sulfur-oxidizing bacteria (such as denitrifying sulfur bacteria), forming a cascade electron transfer chain.

2) For pyrite and its composite systems, the optimal HRT is 24 hours, at which point the removal rate of NO_3^--N can exceed 80%, significantly reducing the effluent NO_3^--N concentration without any accumulation of NO_2^--N. The FS2 (FeS_2:S = 1:1) has the highest removal rate (94.2%), followed by FS3 (84.2%) and the pure sulfur system (80%). Under short HRTs (6 h), the electron donor release rate is insufficient, resulting in removal rates of only 40-60% for FS1 and FS3; however, FS2 maintains a high removal rate of 81.3% even at HRT=12 h due to the synergistic effect of pyrite and sulfur, demonstrating stronger adaptability. This may be because the dissolution rate of pyrite is relatively slow (FeS_2 easily forms a passive layer on its surface), requiring a longer time to release electrons; while the biological oxidation of sulfur depends on the metabolic activity of sulfur-oxidizing bacteria, balancing HRT and electron donor release rate is necessary under their synergistic effect.

3) In the pH=6.5-8.0 range, the NO_3^--N removal rate of FS2 stabilized at 95%-98%, with the effluent pH fluctuating by only 0.3 (initial pH = 7.0), while FS1 and FS3 showed fluctuations of 1.2 and 1.8, respectively. This may be due to the alkaline dissolution of FeS_2 neutralizing H^+ produced by sulfur autotrophic denitrification, preventing the accumulation of acidic byproducts (such as SO_4^{2-} and H^+ from S^0 oxidation) and maintaining the optimal metabolic environment for microorganisms. In contrast, FS3 experienced acid accumulation due to an excessively high sulfur content (effluent pH dropping to 6.2), which inhibited the activity of sulfur bacteria; FS1, on the other hand, saw excessive dissolution of Fe^{2+} (concentration reaching 2.1 mg/L) by FeS_2, leading to hydroxyl radicals that inhibited microbial metabolism.

4) Under optimal HRT conditions, no significant residual nitrite nitrogen was detected in the effluent of any packing system, but all systems exhibited characteristics of nitrite accumulation during the initial reaction phase. Taking the FS2 system as an example, its ammonia nitrogen concentration remained stable within the range of 2.5-3.0 mg/L, roughly equal to the influent concentration. The nitrite nitrogen concentration peaked at 1.8 mg/L at the 10th hour and then steadily decreased, eventually stabilizing at 0.13 mg/L. The intrinsic mechanism of this dynamic change can be summarized into three aspects: First, in the initial reaction stage, the high concentration of nutrients in the influent provided a suitable metabolic environment for microbial communities, promoting efficient conversion of nitrate nitrogen to nitrite nitrogen; Second, as an intermediate product of the nitrification-denitrification chain reaction, there is a noticeable kinetic hysteresis effect in the conversion of nitrite nitrogen to gaseous nitrogen.

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