Study on the Removal of Nitrogen Content in Wastewater by Autotrophic Denitrification

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Abstract Sulfur autotrophic denitrification technology is a low-carbon and environmentally friendly wastewater treatment technology. The effects of factors such as pH, temperature, S/N and salinity on the efficiency of sulfur autotrophic denitrification reactions were discussed, and the community characteristics of microorganisms were summarized. This article also introduced the future research and development directions of this process.

Key words Autotrophic denitrification; Sulfur; Influencing factors; Microbial community

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In recent years, petrochemical industry has generated a large amount of industrial wastewater, which generally contains a large amount of ammonia nitrogen and organic matter[1–3]. Sulfur autotrophic denitrification process offers a brand-new path for removing nitrogen pollution. This process does not require an external carbon source and can be regarded as an eco-friendly technology with characteristics of low carbon, low cost, and small sludge production. A large number of scholars have studied sulfur autotrophic denitrification technology in recent years. Due to the differences in process operation parameters, the denitrification efficiency of this process usually varies greatly, and the industrial application or promotion of this technology is slow.

1 Types of sulfur autotrophic denitrification

1.1 Autotrophic denitrification of elemental sulfur Elemental sulfur, as a by-product in the petroleum and petrochemical production process, is inexpensive, non-toxic, harmless and easy to transport. Therefore, it is mostly used as an electron donor in autotrophic denitrification processes. Studies have shown that the removal of 1 mg of NO_3^--N will be accompanied by the generation of 7.54 mg of sulfate and the consumption of 4.57 mg of alkalinity, that is, the autotrophic denitrification process of elemental sulfur generated a large amount of sulfate, and its consumed alkalinity was high[4–5]. Zhang *et al.*[6] successfully treated nitrate wastewater with a mass concentration of 30 and 300 – 500 mg/L using an SLAD reactor. They also confirmed that limestone can not only effectively control the pH in the reactor but also provide inorganic carbon for the denitrification process. When the concentration of influent NO_3^--N was 30 mg/L, the removal rate of nitrogen by the autotrophic denitrification process of elemental sulfur can reach over 85%. Xu *et al.*[7] studied the feasibility of using sulfur-eggshell as a mixed filler to replace limestone. The results showed that eggshell is a better alkaline filler for the autotrophic denitrification of elemental sulfur, with more obvious advantages over limestone. The removal rate of nitrates in groundwater could reach 97%, and the production of sulfate also decreased significantly.

1.2 Autotrophic denitrification of sulfides Unlike the autotrophic denitrification of elemental sulfur, the autotrophic denitrification of sulfides has higher efficiency and lower sulfate and sludge production. During the autotrophic denitrification of sulfides, alkalinity is generated, which can save the addition of alkaline substances.

Fajardo *et al.* conducted an experiment on the autotrophic denitrification of sulfides using a sequencing batch reactor. It was found that the applicable pH for the reaction was 7.5 – 8.0, and when the concentration of nitrate nitrogen was less than 450 mg/L, the removal rate could reach 67%[8]. Cardoso *et al.*[9] found that during the treatment of nitrate wastewater by the autotrophic denitrification of sulfides, S^{2-} would be completely oxidized to SO_4^{2-} only when sulfide concentration was configured at a stochemical ratio or lower S/N. Wang Shan *et al.*[10] studied the promoting effect of sulfides on denitrification by using Na_2S as the electron donor. The results showed that when sulfide concentration was less than 64 mg/L, the denitrification effect was obvious, and the removal rate of nitrates could reach over 98%.

1.3 Autotrophic denitrification of thiosulfate Capua *et al.*[11] conducted experiments to compare the advantages and disadvantages of elemental sulfur and thiosulfate as electron donors. The results indicated that as thiosulfate was used as an electron donor, the denitrification rate could reach 52.5 mg NO_3^--N/(L · d), which was ten times the reaction rate when elemental sulfur was as an electron donor. Park *et al.*[12] respectively used FeS, $Na_2S_2O_3$, FeS_2 and S^0 as electron donors to treat nitrate wastewater. After the experiments were conducted for 48 h, the removal rate of nitrates is as follows: S^0 (38.8%) < FeS_2 (58.1%) < FeS (64.1%) <

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$Na_2S_2O_3$ (96.5%). Zhou *et al.* [13] experimentally studied the effects of elemental sulfur, Na_2S and $Na_2S_2O_3$ as electron donors on the removal of nitrate nitrogen. The results revealed that when NO_3^--N concentration was 13 mg/L and HRT was 4 h, the removal rate of nitrate nitrogen is shown as follows: Na_2S (46.7%) < S^0(49.8%) < $Na_2S_2O_3$(92.1%).

1.4 Autotrophic denitrification of pyrite Pyrite is an iron-sulfur mineral existing in the Earth's crust, and its main component is FeS_2. Due to its stable properties and low price, pyrite is often used as an electron donor to drive autotrophic denitrification. Pu *et al.* [14] utilized pyrite to treat nitrate-contaminated groundwater by autotrophic denitrification, and found that the removal rate of nitrates could exceed 99%, with a significant removal effect. Jacobsen *et al.* [15] demonstrated that 50% of NO_3^--N in groundwater containing pyrite was removed by the autotrophic denitrification of pyrite. Tong *et al.* [16] compared the effects of nitrogen removal by the autotrophic denitrification of pyrite and elemental sulfur through experiments. The results showed that when the influent nitrate concentration was 100 mg/L and the hydraulic retention time (HRT) was 2.9 h, the removal rate of nitrate nitrogen by the autotrophic denitrification of pyrite and elemental sulfur was 39.7% and 99.9%, respectively. However, the alkalinity consumption and generation of sulfur by-product during the autotrophic denitrification of pyrite were significantly lower.

2 Influencing factors

2.1 pH Autotrophic denitrifying bacteria require an appropriate pH value to maintain their activity. Both excessively high and low pH values will have adverse effects on the denitrification process. For instance, Yuan Yuling[17] studied the characteristics of autotrophic denitrification using natural pyrite and sulfur as sulfur sources. The results showed that the optimal pH range for the autotrophic denitrification of elemental sulfur and pyrite was 7 – 8 and 7.5 – 9.0, respectively. Fajardo *et al.* [18] found that the optimal pH value for the autotrophic denitrification of sulfides ranged from 7.5 to 8.0. Cui *et al.* [19] found that the optimal pH range for the autotrophic denitrification of thiosulfate was 6.5 – 8.0.

2.2 Temperature Temperature is also an important factor affecting bacteria strains with autotrophic denitrification function. A large number of bacteria strains with denitrification function are mesophilic bacteria[20], and they are suitable for a temperature range of 25 – 35 ℃. Capua *et al.* [21] found that when water temperature gradually rose from 6 to 30 ℃, the denitrification rate of *Thiobacillus* increased exponentially. When the temperature rose to 16 ℃, the denitrification rate increased by three times. The most suitable temperature for the growth of *Thiobacillus* was around 30 ℃.

In addition, changes in temperature can also affect the accumulation of NO_2^--N. Yuan Ying *et al.* [22] found that when the temperature rose from 10 to above 20 ℃, the concentration of NO_2^--N in the effluent of the sulfur autotrophic denitrification system decreased from 1 – 3 to 0.2 – 0.8 mg/L. Zhang Chenxiao *et al.* [23] pointed out that when the temperature increased from 20 to 40 ℃, the content of NO_2^--N in the effluent first declined and then increased, up to the maximum of about 6.0 mg/L at 40 ℃.

2.3 S/N S/N ratio is an important factor influencing the sulfur autotrophic denitrification process. Wang *et al.* [24] found that when the minimum S/N ratio was 1.6 during the autotrophic denitrification process of sulfides, the removal rate of nitrates by autotrophic denitrification could be up to over 90%. When the concentration of sulfides was too high, all nitrates were converted into nitrogen gas and discharged. Due to incomplete oxidation of some sulfides in the wastewater, elemental sulfur might exist in the effluent. However, as the concentration of sulfides was low, the denitrification process was not thorough, and the removal efficiency of nitrogen decreased. Li Jun *et al.* [25] investigated the influence of the influent S/N ratio (0.4, 0.7, 1.0, and 1.3) on the accumulation of nitrite nitrogen. The results showed that when S/N ratio was 1.0, the accumulation of nitrite nitrogen was the maximum, and the rate could reach 92.1%. Liu *et al.* successfully achieved the process coupling of partial autotrophic denitrification of sulfur and Anammox reaction using a UASB reactor. When S/N ratio was 1.3, the accumulation rate of S^0 and NO_2^--N reached peaks of 90% and 70%, respectively[26].

2.4 DO Excessively high DO will inhibit the activity of denitrifying enzymes in the bacterial strain, thereby affecting the nitrogen removal effect of sulfur autotrophic denitrification. Chen *et al.* [27] found that micro-oxygen conditions not only enhance the activity of autotrophic denitrifying bacteria but also inhibit the activity of sulfur-reducing bacteria, thereby significantly improving the removal efficiency of sulfur and nitrogen. Du Fengwei conducted deep nitrogen removal from wastewater using a sulfur autotrophic denitrification filter, and found that during the sulfur autotrophic denitrification process, DO could act as an electron acceptor to compete with NO_3^--N, resulting in a decrease in the removal efficiency of nitrogen[28]. Wang Aijie *et al.* pointed out that in an anaerobic environment, with the addition of a carbon source, an increase in sulfide concentration would significantly inhibit the activity of heterotrophic denitrifying bacteria, leading to the accumulation of a large amount of nitrite[29 – 30].

2.5 Salt The increase in salt content in wastewater can cause disorders in the osmotic pressure of functional microbial cells. In severe cases, it may lead to the plasmolysis of microorganisms and their loss of activity, as well as a significant reduction in the removal efficiency of nitrogen by autotrophic denitrification. Campos *et al.* [31] found that the nitrification process in the biological denitrification process was greatly affected by the salt content in the wastewater. When NaCl concentration was higher than 3%, the nitrification process would be completely destroyed and cannot proceed[31]. Chen *et al.* [32] concluded that the salt tolerance limit of nitrifying bacteria in the activated sludge system was 6.5 g/L. Zhou Peng *et al.* [33] found that when NaCl concentration was 20 g/L, the function of denitrifying bacteria decreased to 75%, while that of heterotrophic bacteria dropped to 21%. Liu Chunshuang *et al.* [34] studied the effect of denitrification desulfurization process on the simultaneous removal of carbon, nitrogen and sulfur in wastewater under high NaCl conditions. The results revealed that as NaCl concentration was 100 g/L, the removal rate of NO_3^--N dropped to 40%, and autotrophic denitrifying bacteria mainly composed of *Thiobacillus* and *Azoarcus* were gradually formed.

3 Microbial community

The normal metabolism of microorganisms with sulfur autotrophic denitrification function is the key to maintaining the high efficiency of autotrophic denitrification. In recent years, it has been found that more than ten types of denitrifying desulfurization bacterial communities have been discovered[35-36], including *Thiobacillus denitrificans*, *Sulfurimonas denitrificans*, *Thiosphaera pantotropha*, *Pseudomonas*, *Ochrobactrum*, *Rhodococcus*, and *Azoarcus*. Among them, *Azoarcus* and *Pseudomonas* belong to heterotrophic sulfur-oxidizing denitrifying bacteria, and *T. denitrificans* belongs to a typical autotrophic sulfur-oxidizing denitrifying bacterium.

Zhao Jinnan *et al.* [37] conducted a rapid start-up experiment on the coupling of sulfur autotrophic denitrification with anammoxidation denitrification system, and analyzed the changes in functional microbial communities during the experimental process. The results indicated that the sulfur autotrophic denitrification and anammoxidation processes could be successfully coupled, and the overall species diversity and richness of the system increased; the dominant functional microorganisms were *Candidatus Brocadia* and *Thiobacillus*.

4 Conclusions and prospects

Sulfur autotrophic denitrification technology is a low-carbon and environmentally friendly denitrification technology. However, this technology still has somewhat shortcomings in engineering application and promotion. It is suggested that the next research and development direction of this technology start from the following aspects.

(1) The processing load of the reactor needs to be further increased. The engineering application of this technology needs to meet the requirements of high water quality fluctuation shock. It is suggested that in the next step, in-depth research should be conducted in combination with the selection of biological fillers, the design of reactors, and the operation of the process, so as to find a rapid start-up method for this process and achieve the long-term stable operation of the system.

(2) The coupled application with other processes should be studied. It is recommended that autotrophic denitrification be simultaneously coupled with other processes for wastewater treatment. For instance, heterotrophic denitrification and autotrophic denitrification are coupled to simultaneously remove carbon, nitrogen and sulfur from wastewater. The coupling of autotrophic denitrification and anammooxidation further enhances the removal efficiency of nitrogen from wastewater.

(3) The stability of functional strains should be studied. It is suggested that the functional strains of sulfur autotrophic denitrification for nitrogen removal should be studied in terms of formulation methods, and the removal efficiency of nitrogen by the process is improved through the optimization of functional strains.

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system of dimethyl succinate (DMSu) and propylene carbonate (PC). The results show that the structure of the prepared PPSG copolyester was correct. Moreover, with the addition of the third monomer EG, the glass transition temperature of the copolyester reduced from −15.6 to −21.4 ℃, and the melting temperature declined from 94.5 to 72.8 ℃. With the increase of the amount of added EG, the biodegradability of PPSG gradually was improved, and the degradation weight loss rate of PPSG-30% within 24 d reached 75.8%. Therefore, the comprehensive performance of copolyester PPSG prepared with EG as the third monomer was superior to the original polyester PPS.

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