Effect of Fe\textsuperscript{2+} on the performance of Anammox at low temperature

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**Abstract.** The effects of the addition of a certain concentration of Fe\textsuperscript{2+} on the activity of anammox bacteria in the system at neutral low temperature were investigated by detecting the contents of nitrate, conductance values and protein in the system. The results showed that when the temperature was set at 15\degree C and the concentration of Fe\textsuperscript{2+} was 0.16 mmol/L, the system operated in the best condition. After 60h, the denitrification rate increased by 40\%, the electrical conductivity value decreased the fastest, and the protein content increased by 24.5 mg/g compared with the control group. Zero valent iron has been widely concerned as a reducing agent for the treatment of nitrate wastewater, but the treatment efficacy is severely affected by temperature. The addition of anammox bacteria coupled with Fe0 can not only mutually promote the reaction activity to adapt to more non ideal environments but also reduce the secondary pollution, which provides a new idea for the practical application of nitrate wastewater treatment.

**1 Introduction**

The issue of environmental pollution caused by the rapid development of science and technology cannot be ignored. In recent decades, with the development of industrial and agricultural production, there are different degrees of nitrogen pollution in the groundwater in rural and urban areas. The excessive use of agricultural fertilizer, especially the excessive use of nitrogen fertilizer and improper disposal of animal waste, make the nitrate nitrogen content in surface water and groundwater in many parts of the world increasing. The quality and safety of soil and groundwater in the gas inclusion zone have been endangered, and nitrate pollution is becoming more and more serious.

Nitrate wastewater not only causes ecological destruction but also the intermediate generation of nitrite is a great threat to human health. Nitrate in soil is mainly absorbed by crops. Irrigation also makes nitrate seep into and pollute groundwater. Vegetables are very easy to absorb and enrich nitrate. From the survey results, excessive application of nitrogen fertilizer can significantly increase the content of nitrate in soil, which directly leads to excessive accumulation of nitrate in vegetables, especially in leafy vegetables. The well-developed industrial and agriculture in China has inundated chemical fertilizer use, and the contamination of groundwater by nitrate has become an environmental pollution problem.

Nitrate has high solubility and good stability in water, so it is difficult to form coprecipitation or adsorption. Therefore, the traditional simple water treatment technology, such as lime softening and filtration, is difficult to remove nitrate from water. Nitrate wastewater treatment has been widely studied, and although the physical treatment method is quite effective\textsuperscript{1}, it requires frequent medium replacement and is not environmentally friendly. Iron powder is cheap and easy, and has strong reducing power, making it a good wastewater treatment agent. Some authors have achieved the effect of reducing nitrate into nitrite and ammonia through Fe/anammox coupling, but anammox bacteria are mesophilic bacteria, the actual engineering is mostly low temperature water, and it is not suitable for the reaction of anammox bacteria. Some scholars have found that administration of Fe\textsuperscript{2+} can help to improve the activity of anammox bacteria at low temperatures\textsuperscript{2}.

In 2013, Oshik found that Fe\textsuperscript{2+} can reduce nitrate to nitrite and ammonia in the Anammox system. Xing et al. found that the addition of anaerobic ammonium oxidation sludge can enhance the reduction of nitrate by Fe\textsuperscript{0}.

In this paper, we investigated by setting up batch experiments whether the addition of a certain concentration of Fe\textsuperscript{2+} could promote the efficiency of nitrate reduction by Fe\textsuperscript{0}/anammox bacteria and provided experimental data for practical applications in nitrate wastewater treatment.

**2 Experiments and Materials**

**2.1 batch experiment**

An anaerobic serum bottle with a capacity of 110 ml was used as the experimental setup, and the volume of the culture solution was set as 100 ml. The serum bottles were aerated with 99\% pure nitrogen for 30 min before use to
remove dissolved oxygen and were rapidly sealed by rubber stoppers and aluminum caps at the mouth of the bottles, placed in a constant temperature oscillator protected from light, maintained at a rotation rate of 100 rpm, and continuously sampled during the reaction for assay.

2.2 inoculated sludge and experimental water quality

The inoculated sludge used in this paper was anammox sludge, taken from an anaerobe ammonia oxidation reactor operating well for a long period in the laboratory and having a good sludge trait as a brick red colour. Experimental wastewater the artificial configuration of the simulated wastewater with the following main components: NaNO₃, KHCO₃, KH₂PO₄ and trace elements (including CaCl₂ 2H₂O, NaCl, MgSO₄ 7H₂O, KCl) was used. The Fe⁶⁺ used in this experiment were all iron powders with a particle size of 150 μm, activated before use, and their surface oxides were removed by washing with 0.5 mol/lhcl followed by deoxygenated primary water repeatedly until the leaching solution pH was neutral. To avoid air oxidation, activated iron powder after treatment was immediately used.

2.3. analytical methods

During the reaction, the pH value was determined using a pH meter, NH₄⁺-N using nanodrop reagent spectrophotometry, NO₃⁻-N employing n-one-Naphthyl Ethylenediamine photometry, NO₃⁻ UV light photometry was used and EPS was collected by centrifugation³.

3 Results

The coupling of anammox bacteria with Fe⁰ can increase the NO₃⁻ removal rate under neutral conditions. Because the suitable pH for anammox bacteria is 6-8, in addition to the chemical reaction of Fe⁰ with NO₃⁻ in the reaction there are biological reactions in which anammox reduces NO₃⁻. But anammox bacteria are mesophilic bacteria and very sensitive to temperature. At pH=7 and temperature between 20 and 45℃, the best denitrification can reach more than 75% in all cases. Nitrate removal decreased rapidly when the temperature exceeded the suitable range, for example, the lowest denitrification rate was only 20% at 15℃. This is because, too low a temperature both leads to inactivation of anammox bacteria, halting the biological response. But the temperature of common industrial wastewater is at the normal or low temperature, which has a large effect on the treatment effect of anaerobic ammonia oxidation processes⁴. If warming or holding increases energy consumption and is not environmentally friendly. Studies have found that Fe²⁺ is not only an essential trace element but also a growth promoter for anaerobic ammonia oxidizing bacteria. We experimentally explored whether the improvement of the denitrification rate could be achieved by the administration of Fe²⁺ under cryogenic conditions⁵. The experimental temperature was set to 15℃, and 0.08, 0.16, 0.4 mmol/L Fe²⁺ were exceptionally added to the reaction, and other conditions were the same as experiment one. As shown in Fig. 2, nitrate removal was changed compared with the blank group after the addition of iron ions, but the effect was not proportional to the amount of Fe²⁺ added. The best results were obtained when the Fe²⁺ content was 0.16mmol/L, and the denitrification rate increased by 40% compared with the control after 60h. It was slightly less effective at a concentration of 0.08 mg/L, but there was still a 25% improvement. When the concentration of Fe²⁺ was 0.4mmol/L, the denitrification rate was lower than that of the blank group. The results indicated that it was not the higher Fe²⁺ concentration that caused the greater NO₃⁻ removal. Only Fe²⁺ within the suitable range promoted the treatment efficiency in anammox bacteria. Further examination of the effect of different concentrations of Fe²⁺ on the conductivity in the reaction system is shown in Figure 3. The original conductivity in each reactor was (3.10 ± 0.01), At a Fe²⁺ concentration of 0.4 mmol/L, the conductance decrease was the slowest, and after 60h, the highest conductance value was close to 2.3. For example, at the 30th h, the conductivity of R (Fe²⁺ 0.08) was 2.6, while that of R (Fe²⁺ 0.16) was 2.3. It is known that when Fe²⁺ was 0.16 mmol/L at 15℃, the conductivity decreases the fastest and the water conductivity value was the smallest, which proved that anammox bacteria have the best activity and the best denitrification effect at this time.

EPS is a macromolecular compound secreted by cells and encapsulated on the outside of the cell. Its main components are polysaccharides (PS) and proteins (PN). Yanjun Li believed that the total amount of EPS and the content of PS, PN can reflect the state of sludge in the system. As can be seen from table 1, after the administration of 0.16 mmol/LFe²⁺, the total EPS and PN were increased, the polysaccharide content decreased, and the PS / PN ratio decreased, which indicated that Fe²⁺ promoted the activity of the species and the sludge settling property. When Fe²⁺ was 0.08mmol/L, both EPS and PN were elevated but to a lesser extent. When Fe²⁺ was 0.4 mmol / L, the related indexes were lower than those of the blank group, which indicated that too high concentration of Fe²⁺ had an inhibitory effect on anammox bacteria. It is known that if one wants to utilize Fe²⁺ to promote the activity of anammox bacteria, it is essential to control the Fe²⁺ concentration within a suitable range, too high or too low to achieve the desired effect.

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**Figure 1** When PH=7, the influence of temperature on Fe0/Anammox coupling treatment of nitrate wastewater

**Figure 2** The effect of Fe\(^{2+}\) on the combined treatment of nitrate wastewater by Fe\(^{0}\)/Anammox bacteria under low temperature and neutral conditions

**Figure 3** The influence of different concentrations of Fe\(^{2+}\) on system conductivity under low temperature and neutral conditions
Table 1 The influence of different concentrations of Fe$^{2+}$ on PN and PS at 15℃

| c(Fe$^{2+}$)'/ (mmol·L$^{-1}$) | W/(mg·g$^{-1}$) | W(ps)/ w (pn) |
|-----------------------------|-------------|-------------|
|                            | PN         | PS         |
| 0                           | 21.1       | 9.3        | 0.441 |
| 0.08                        | 32.7       | 7.13       | 0.218 |
| 0.16                        | 45.6       | 6.4        | 0.140 |
| 0.4                         | 20.1       | 9.6        | 0.478 |

4 Conclusions

Neutral and low temperature environment, the addition of different concentrations of Fe$^{2+}$ has different effects on the denitrification rate of the Fe$^0$/anammox bacterial system. When the Fe$^{2+}$ loading was 0.16 mmol/L, the denitrification rate was the highest, the conductivity value decreased the fastest, and the protein content was the largest. When the Fe$^{2+}$ addition was 0.4 mmol/L, it inhibited anammox bacteria due to the excessive metal concentration, and all the performances were worse than those of the blank group. It was experimentally proved that the appropriate concentration of Fe$^{2+}$ when dosed at 15℃ contributed to anaerobic sludge activity, and inhibited it vice versa.

Nitrate itself is easily absorbed and excreted by organisms, and does not pose a direct hazard to mammals. However, under hypoxic conditions, such as in the digestive tract, it can be reduced to toxic nitrite. Nitrite can oxidize low ferritin into methemoglobin in the human body, causing it to lose its ability to transport oxygen. In addition, nitrite Salts can also react with secondary amine compounds to produce carcinogenic nitrosamines. Therefore, the treatment of nitrate wastewater is of great significance.

Acknowledgments

1. Study on three-phase synergistic phosphorus recovery mechanism in anaerobic treatment of phosphorus rich wastewater (2019-ZD-0298), Project of Science and Technology Department of Liaoning Province, 2019.
2. Food waste induces surplus sludge to enhance the fermentation performance of L-lactic acid bacteria (1nqn202011), Project of Education Department of Liaoning Province, 2020

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