Start-up of Anammox and Effect of Fe$^{2+}$ on Nitrogen Removal Rate

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Abstract. Anammox reaction was started by inoculating sewage plant digested sludge, and the change of N element in the reactor was detected. It was found that Anammox needed to go through three parts: hydrolysis period, activity lag period and activity increase period. The effect of different Fe$^{2+}$ concentrations on the denitrification rate was investigated with the well-run Anammox sludge as the inoculation sludge. The results showed that Fe$^{2+}$ had a positive effect on the anaerobic ammonium oxidation reaction within a certain concentration range, while the denitrification effect would be inhibited if the concentration was too high. When the concentration of Fe$^{2+}$ was 0.08mmol/L, Fe$^{2+}$ on Anammox had the best denitrification effect.

1. Introduction

With the rapid development of science and technology and the increasing prosperity of industry and agriculture, the excessive use of chemical fertilizers, pesticides and industrial raw materials has caused serious damage to the environment we rely on. While enjoying the convenience brought by advanced science and technology, people also have to face the harm caused by environmental pollution to human beings. Among them, nitrogen is a kind of environmental pollutant that cannot be ignored. In aquatic ecosystems there are three kinds of common solubility inorganic nitrogen ions, such as ammonium (NH$_4^+$), nitrite (NO$_2^-$), nitrate (NO$_3^-$) can not only through the atmospheric degradation, surface and groundwater runoff and other natural effect can be controlled by industrial wastewater and domestic sewage discharge pollution from human activity into aquatic ecosystems$^1$. In recent years, the concentration of inorganic nitrogen in surface water has been increasing, which has a serious impact on aquatic organisms. Moreover, the ecological degradation of fresh water, estuaries and coastal oceans has appeared. In the 2017 China Environment Bulletin, the proportion of poor IV and the type of water accounted for nearly one-third of the total surface water level. Among the monitoring points of underground water, the proportion of very poor or poor level accounted for 66.6%. Ammonia nitrogen is one of the main pollution indicators, in our environment, nitrogen pollution has been very serious.

Anammox reaction is a process in which NH$_4^+$ and NO$_2^-$ are used as reactants to eventually produce N$_2$ and NO$_3^-$, and it does not require aeration or additional carbon source$^2$. Compared with traditional nitrification and denitrification, Anammox has the advantages of low operating cost, less sludge production and higher denitrification conversion rate, which makes it a more ideal biological denitrification way$^3$. However, Anammox bacteria have a long generation time and are sensitive to the environment$^4$. In this paper, the change of nitrogen element and the influence of Fe$^{2+}$ on the denitrification rate of Anammox reaction were investigated during the start-up process.
2. The experiment

2.1. Start of Anammox

2.1.1 Experimental device
The anaerobic SBR with an effective volume of 3L was used as the reactor, and it run one cycle a day. Each reaction cycle includes five processes: water inlet, reaction, precipitation, water outlet and idling.

2.1.2 Simulated waste water and inoculated sludge
The anaerobic digested sludge of sewage treatment plant was used as inoculation sludge, and the waste water used in the experiment was manually configured. Wastewater was mainly composed of NH₄Cl (on demand), NaNO₂ (on demand), NaHCO₃ 1000 mg/L, KHCO₃ 1000 mg/L, KH₂PO₄ 27 mg/L, CaCl₂·2H₂O 136 mg/L, MgSO₄·7H₂O 200 mg/L, 1 mL/L trace elements⁵.

2.2. Exploration Experiment
Serum flask of 100 mL (or 500 mL) specification was selected for the device, which was sealed with screw cap. A circular non-woven cloth with a diameter of 5 cm is built in to adsorb small particles of Anammox sludge. Operating conditions of all devices: the temperature is 32℃, heated by a water bath oscillator to maintain; The influent pH value was 6.5 ~ 6.6, and 0.1 mol· L⁻¹ hydrochloric acid was used to control the influent pH value to maintain the dissolution of Fe ions. Anammox sludge in good operation in SBR was used as inoculation sludge. The concentration of ammonia nitrogen in simulated wastewater was 100mg/L, and the concentration of NO₂⁻-N was 126mg/L. Other elements were the same as in Experiment 1.

3. Results
The performance of the reactor during the start-up process of Anammox was shown in Figure 2, which can be divided into three parts: the hydrolysis stage, the activity stagnation stage and the activity improvement stage. During 1-17d, the average concentration of ammonia nitrogen in the influent was 50.00±0.11mg/L, the average concentration of nitrite nitrogen in the influent was 63±3.2mg/L, the average concentration of ammonia nitrogen in the effluent was 58.29mg/L and the average concentration of nitrite nitrogen in the effluent was 3.2mg/L. At this point, the effluent concentration...
of ammonia nitrogen was higher than the influent concentration, and the denitrification rate was -16.6%. However, the denitrification condition of nitrite nitrogen was very ideal and kept at 96.1%, which was because the bacteria did not adapt to the environment at this time, cell lysis occurred, and the dissolution of organic nitrogen in the body caused the increase of ammonia nitrogen concentration. At the same time, the dissolved COD and a small amount of exopolymer in bacteria were used as carbon sources, and the heterotrophic denitrification bacteria contained in the sludge took place denitrification. Anammox reaction was weak at this stage and denitrification was dominant. During 18-52d, the effluent ammonia nitrogen concentration began to decrease, with an average removal rate of 31%, and the effluent NO$_2^-$-N concentration increased, because denitrification began to be inhibited with the consumption of organic matter. At this point, the concentration of NH$_4^+$-N and NO$_2^-$-N decreased simultaneously, accompanied by the increase of NO$_3^-$, indicating that the Anammox reaction began to appear, but the ratio of NH$_4^+$-N/NO$_2^-$-N and NO$_3^-$-N/NO$_2^-$-N was lower than the theoretical value of Anammox. This was because there was still a part of denitrification in the reaction that competed with the Anammox reaction, and in this stage, Anammox and denitrification exist simultaneously. During 53-97d, the effluent concentration of ammonia nitrogen continued to decrease, and the denitrification rate increased from 31% to 86.2%. At the same time, the concentration of NO$_3^-$-N increased. The results showed that with the consumption of organic matter, denitrification was weakened and anaerobic ammonium oxidation was gradually prevalent.

During (98-110d), the effluent concentration of ammonia nitrogen in the reactor gradually stabilized, with an average value of 2.3mg/L. The average effluent concentration of NO$_2^-$-N was 1.9mg/L, and the effluent concentration of NO$_3^-$-N was 9.7mg/L. NH$_4^+$-N/NO$_2^-$-N and NO$_3^-$-N/NO$_2^-$-N float around the theoretical value, but NO$_3^-$-N was still low, indicating that Anammox reaction had prevailed but there were still a few denitrifying bacteria.

![Figure 2. Change of N concentration in reactor](image-url)
It can be seen from Figure 3 that increasing Fe$^{2+}$ concentration had a significant effect on Anammox reaction. When the concentration of Fe$^{2+}$ was 0.04, 0.06 and 0.08 mmol/L, the effluent concentration of ammonia nitrogen was lower than that of the control group. When the concentration
of Fe$^{2+}$ was 0.08 mmol/L, the best denitrification rate was 89.4%. When the concentration of Fe$^{2+}$ was 0.12 mmol/L, the effect of the reactor was good in the first 7 days, the ammonia nitrogen in the effluent was lower than that in the control group, and the ammonia nitrogen concentration began to rise from the 8th day, indicating that the high concentration of Fe$^{2+}$ at this time inhibited anammox.

4. Conclusion
Anammox reaction was started by inoculating sewage plant digested sludge, and the change of N element in the reactor was detected. It was found that Anammox needed to go through three parts: hydrolysis period, activity lag period and activity increase period. The effect of different Fe$^{2+}$ concentrations on the denitrification rate was investigated with the well-run Anammox sludge as the inoculation sludge. The results showed that Fe$^{2+}$ had a positive effect on the anaerobic ammonium oxidation reaction within a certain concentration range, while the denitrification effect would be inhibited if the concentration was too high. When the concentration of Fe$^{2+}$ was 0.08 mmol/L, Anammox had the best denitrification effect.

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