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Effect of temperature on anaerobic ammonia oxidation performance of bio-sand filter

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Abstract. A bio-sand filter was used as a reactor for the start-up of anaerobic ammonia oxidation (ANAMMOX), and the effect of temperature change on ANAMMOX performance and its recoverability were investigated. The results showed that the start-up of ANAMMOX could be successfully achieved in 92 days in the bio-sand filter under the temperature of 30°C. When the average influent concentrations of NH\textsubscript{4}+-N and NO\textsubscript{2}--N were 19.7 and 25.5 mg/L respectively, the average removal rates of NH\textsubscript{4}+-N, NO\textsubscript{2}--N and TN were 98.1\%, 98.6\% and 91.5\% respectively. The bio-sand filter could maintain high efficiency and stable performance of ANAMMOX under the temperature of 20~35°C. When the temperature dropped to 15°C for 7 days, total nitrogen (TN) removal rate dropped to 83\%. When the temperature was reduced to 10°C for 11 days, the removal rates of NH\textsubscript{i}+-N, NO\textsubscript{2}--N and TN were dropped to 73.5\%, 72.7\% and 68.5\% respectively. After resuming the temperature to 30°C for 19 days, the ANAMMOX activity could basically resumed to the original. When the temperature increased to 40°C for 9 days, both the NH\textsubscript{i}+-N and NO\textsubscript{2}--N removal rate decreased to 86.5\%. The ANAMMOX activity be gradually recovered after resuming the temperature to 30°C for 5 days. The effect of low temperature on the ANAMMOX performance of bio-sand filter was more significant than that of high temperature, and the time needed for recovery after the shock of low temperature was longer.

1. Introduction
Anaerobic ammonia oxidation (ANAMMOX) is a biological oxidation process in which NH\textsubscript{4}+-N is used as electron donor and NO\textsubscript{2}--N as electron acceptor to convert them into N\textsubscript{2} in anaerobic or anoxic environment [1,2]. In 1998, Strous \textit{et al.} [3] carried out material balance calculation in sequencing batch reactor (SBR) for the nitrogen-containing substances (NH\textsubscript{i}+, NO\textsubscript{2}+, NO\textsubscript{3}+, N\textsubscript{2}, etc.) and carbon-containing substances (HCO\textsubscript{3}−, CO\textsubscript{2}, CH\textsubscript{4}, etc.) in the influent and effluent of ANAMMOX process, and obtained the stoichiometric equation of ANAMMOX reaction as follows:

\[
\text{NH}_4^+ + 1.32\text{NO}_2^- + 0.066\text{HCO}_3^- + 0.13\text{H}^+ \rightarrow 1.02\text{N}_2 + 0.26\text{NO}_3^- + 0.066\text{CH}_4O_{0.15} + 2.03\text{H}_2\text{O}
\] (1)

According to the Equation (1), the proportions of NH\textsubscript{4}+-N consumption (\(\Delta\text{NH}_4^+\)-N), NO\textsubscript{2}--N consumption (\(\Delta\text{NO}_2^\text{--N}\)) and NO\textsubscript{3}--N production (\(\Delta\text{NO}_3^\text{--N}\)) were 1:1.32:0.26. This ratio is regarded as an important basis for judging the occurrence of ANAMMOX. Compared with traditional nitrification and denitrification biological nitrogen removal, ANAMMOX is an economical and convenient denitrification method without providing organic carbon source as electron donor [4]. As a
result, ANAMMOX has become a hot topic in the field of wastewater treatment with high efficiency and low consumption in recent years [5-7].

Anaerobic ammonia-oxidizing bacteria (AAOB) play a key role in ANAMMOX process. According to the maximum activity and biomass of microorganisms, the maximum growth rate of AAOB was 0.0027±0.0005 h⁻¹, and the generation cycle was 11 days. However, AAOB is sensitive to environmental conditions, which leads to poor operation stability of the process, and greatly restricts the application of the technology [8-10]. Temperature is an important parameter affecting the ANAMMOX performance. Only at suitable temperature can AAOB show high activity. When the temperature is too high or too low, the metabolic process of AAOB will be inhibited or even destroyed, leading to the degradation of the ANAMMOX performance. In recent years, the research focusing on the effect of temperature on ANAMMOX has been increasing. While most of the previous researches were focused on activated sludge system, there were few reports on fixed biofilm system.

In this study, a typical fixed biofilm reactor, bio-sand filter, was selected as the research object. Based on the successful start-up of ANAMMOX, the effects of temperature rise and decrease on ANAMMOX performance were investigated, and the recoverability of ANAMMOX activity after temperature shock was also investigated. It is expected to provide scientific references for the efficient and stable operation of ANAMMOX in bio-sand filter and other similar fixed biofilm systems.

2. Materials and methods

2.1. Experimental device

The experimental device is shown in Figure 1. The filter was 70 cm high and the inner diameter was 7 cm. The layer of filter media was 50 cm high, which was composed of natural river sand, shell sand and zeolite sand (0.5~1.0 mm in diameter and 6:3:1 in volume ratio). The gravel layer was located at the top and bottom of the filter media, with a thickness of 2.5 cm and a diameter of 5~18 mm. The wastewater entered from the bottom of the filter, and was discharged from the outlet on the right side above the filter, and the gas produced was discharged and collected from the top outlet. The hydraulic load was set as 1.0 m/d and the temperature was controlled by constant temperature cycle water bath.

![Figure 1. Schematic diagram of experimental device.](image)

2.2. Influent and inoculated sludge

Using simulated wastewater as experimental influent. NH₄⁺-N and NO₂⁻-N were supplied by NH₄Cl and NaNO₂ respectively, and 500 mg/L KHCO₃, 10 mg/L KH₂PO₄, 5.6 mg/L CaCl₂, 300 mg/L MgSO₄·7H₂O, 1 mL/L trace element I and 1 mL/L trace element II were added into the tap water at the same time. The components of trace element I were as follows: EDTA 5000 mg/L, FeSO₄ 5000 mg/L. The components of trace element II were as follows: EDTA 15000 mg/L, EDTA 15000 mg/L,
H₃BO₄ 14 mg/L, MnCl₂·4H₂O 990 mg/L, CuSO₄·5H₂O 250 mg/L, ZnSO₄·7H₂O 430 mg/L, NiCl₂·6H₂O 190 mg/L, Na₂MoO₄·2H₂O 220 mg/L, Na₂SeO₄·10H₂O 210 mg/L. The dissolved oxygen concentration in the influent was controlled below 0.5 mg/L. The inoculated sludge was taken from the mixed sludge of aerobic nitrifying sludge and heterotrophic denitrifying sludge in the SBR system which was running stably in the laboratory. The MLVSS of the two inoculated sludge was about 6100 mg/L and 7000 mg/L, respectively, and the volume ratio of the mixed sludge was 1:2.

2.3. Analysis methods
The concentrations of NH₄⁺-N, NO₂⁻-N and NO₃⁻-N in wastewater were determined by Nessler spectrophotometry, N-(1-naphthyl)-ethylenediamine spectrophotometry and ultraviolet spectrophotometry respectively.

3. Results and discussion

3.1. Nitrogen removal performance during stable ANAMMOX operation
After inoculating the mixed sludge, the bio-sand filter could start the ANAMMOX process successfully after 92 days operation by gradually increasing the influent NO₂⁻-N concentration under the temperature of 30°C. The nitrogen removal efficiency of the stable operation period is shown in Table 1. The results showed that the average removal rates of NH₄⁺-N, NO₂⁻-N and total nitrogen (TN) were 98.1%, 98.6% and 91.5% respectively when the average influent concentrations of NH₄⁺-N and NO₂⁻-N were 19.7 and 25.5 mg/L respectively.

| Index   | Influent (mg L⁻¹) | Effluent (mg L⁻¹) | Removal rate (%) |
|---------|------------------|------------------|-----------------|
| NH₄⁺-N  | 19.7±0.4         | 0.4±0.3          | 98.1±1.6        |
| NO₂⁻-N  | 25.5±0.5         | 0.4±0.2          | 98.6±0.9        |
| TN      | 45.2±0.8         | 3.8±0.3          | 91.5±0.7        |

3.2. Effect of temperature reduction on ANAMMOX
The temperature of the filter was gradually reduced from 30°C to 25°C, 20°C, 15°C and 10°C in turn, and each temperature condition was operated for several days to investigate the effect of temperature reduction on the ANAMMOX performance of bio-sand filter. When the nitrogen removal rate decreased significantly, the temperature was raised back to 30°C to investigate the recovery performance of ANAMMOX after temperature reduction shock.

The effluent NH₄⁺-N, NO₂⁻-N and NO₃⁻-N concentration of bio-sand filter changed little when the temperature dropped from 30°C to 25 and 20°C, and the TN removal rate remained about 90% (Figure 2). It can be seen that the bio-sand filter can maintain stable and efficient ANAMMOX performance in this temperature range. When the temperature dropped to 15°C, the concentration of NH₄⁺-N and NO₂⁻-N in effluent began to increase, and the removal rate of NH₄⁺-N and NO₂⁻-N decreased gradually. As a result, the TN removal rate dropped to 83% after 7 days of operation at 15°C. The concentration of NH₄⁺-N and NO₂⁻-N in effluent increased further when the temperature dropped to 10°C. After 11 days of operation at 10°C, the concentration of nitrogen pollutants in effluent tended to be stable. At this time, the removal rates of NH₄⁺-N, NO₂⁻-N and TN were 73.5%, 72.7% and 68.5% respectively. After resuming 30°C operation, the concentration of NH₄⁺-N and NO₂⁻-N in effluent gradually decreased, and the removal rates of NNH₄⁺-N, NO₂⁻-N and TN returned to the original level after 19 days.
Figure 2. Variations of nitrogen pollutants during temperature reduction.

Figure 2(c) shows the ratios of $\triangle \text{NO}_2^--\text{N}/\triangle \text{NH}_4^+-\text{N}$ were between 1.26 and 1.33, which was close to the theoretical ratio of ANAMMOX (1.32). It indicated that the decrease of temperature has no great influence on the consumption ratio of $\text{NH}_4^+-\text{N}$ and $\text{NO}_2^--\text{N}$. The ratios of $\triangle \text{NO}_3^--\text{N}/\triangle \text{NH}_4^+-\text{N}$ were between 0.12 and 0.19, which was slightly lower than the theoretical ratio of ANAMMOX (0.26). This may be due to the existence of other ways (e.g. assimilation) of consuming $\text{NO}_3^--\text{N}$ in the bio-sand filter, resulting in the decrease of $\text{NO}_3^--\text{N}$ production and the ratio of $\triangle \text{NO}_3^--\text{N}/\triangle \text{NH}_4^+-\text{N}$ [11].
3.3. Effect of temperature increase on ANAMMOX

The reaction temperature was raised from 30°C to 35°C and 40°C, and each temperature condition was operated for several days. When the removal rates of NH$_4^+$-N and NO$_2^-$-N decreased substantially, the temperature was lowered back to 30°C to investigate the recovery performance of ANAMMOX after a higher temperature shock.

![Figure 3. Variations of nitrogen pollutants during temperature increase.](image-url)
The effluent concentrations of NH$_4^+$-N and NO$_2^-$-N were still low when the temperature increased to 35°C, and the TN removal rate remained above 90% (Figure 3). The nitrogen removal efficiency of bio-sand filter at 35°C was similar to that of 30°C. When the temperature increased to 40°C, the concentrations of NH$_4^+$-N and NO$_2^-$-N in effluent increased gradually, and both the removal rate of NH$_4^+$-N and NO$_2^-$-N decreased to 86.5% after 9 days of operation under this temperature condition. After 5 days of recovery operation at 30°C, the nitrogen removal ability of the bio-sand filter gradually returned to its original level. Overall, the effect of low temperature on the performance of ANAMMOX was more significant than that of high temperature, and the reduction of nitrogen removal efficiency caused by low temperature required a longer time to recover. Additionally, the ratios of $\frac{\Delta \text{NO}_2^-\text{N}}{\Delta \text{NH}_4^+\text{N}}$ and $\frac{\Delta \text{NO}_3^-\text{N}}{\Delta \text{NH}_4^+\text{N}}$ were 1.28~1.32 and 0.10~0.18 respectively, which was similar to the case during the temperature reduction.

Several studies on the effect of temperature change on the performance of ANAMMOX have been reported, but the optimum temperature ranges to maintain the activity of ANAMMOX is different. Yang et al. [12] showed that the optimum temperature range for ANAMMOX was 30~35°C, in which the rate of ANAMMOX was as high as 0.171~0.174 mg/(mg·d). When the temperature rose to 40°C, the rate of ANAMMOX decreased to 0.091 mg/(mg·d), and when the temperature dropped to 25°C and 20°C, the rates of ANAMMOX decreased to 0.132 and 0.064 mg/(mg·d) respectively. Dosta et al. [13] investigated the effect of temperature on the stability of ANAMMOX process in SBR system. The results showed that the the stability of ANAMMOX process in SBR system could be maintained when the temperature was reduced from 30°C to 18°C. The accumulation of NO$_2^-$-N would occurred when the temperature was raised to 15°C, and the microbial cracking would occurred when the temperature was raised to 45°C. In this case, the loss of ANAMMOX activity was irreversible.Yao et al. [14] studied the impact of temperature changes on the UASB-biofilm anaerobic ammonia oxidation reactor, and found that the best temperature for ANAMMOX was 31°C. When the temperature reduced from 31°C to 17°C, the average removal rates of NH$_4^+$-N, NO$_2^-$-N and TN decreased from 97.0%, 94.1%, 86.0% to 46.2%, 41.8%, 35.5% respectively. The ANAMMOX activity could be restored in 2 months when the reaction temperature was gradually raised from 17°C to 31°C. Song et al. [15] studied the effect of temperature step descent on the ANAMMOX activity in SBR system. The results showed that the system could maintain high efficiency and stability when the temperature was 20~33°C. The maximum specific ammonia oxidation activity was 91% lower than that of 33°C when the temperature was reduced to 10°C.

The optimum temperature range for ANAMMOX in this study was 20~35°C, indicating that the system had a wide range of adaptability to temperature changes. This was due to the use of filter material to fill the bio-sand filter, the microorganisms on the biofilm had certain buffering and resisting ability when facing the condition of temperature change, thus providing favorable conditions for nitrogen removal from wastewater under low temperature.

4. Conclusions

The bio-sand filter could start the ANAMMOX process successfully under the temperature of 30°C and maintain high efficiency and stable performance of ANAMMOX under the temperature of 20~35°C. When the temperature dropped to 15°C, the activity of ANAMMOX began to be adversely affected. When the temperature continued to drop to 10°C, the activity of ANAMMOX began to be seriously inhibited, and could be gradually restored to its original level after 19 days of operation at 30°C. ANAMMOX would be unfavorably affected when the temperature increased to 40°C, and it could be basically recovered after 5 days of operation at 30°C. The effect of low temperature on the performance of ANAMMOX was more significant than the high temperature, and the time needed for recovery was also longer.

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