Influence of Temperature and Organics on ANAMMOX and Study on Kinetics Characteristics

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ABSTRACT

The influence of temperature and organics on anaerobic ammonium oxidation (ANAMMOX) bacteria activity was investigated in an up-flow ANAMMOX reactor. The kinetics characteristics of cultured ANAMMOX bacteria were analyzed. The results showed that temperature had a significant effect on ANAMMOX bacteria activity, and it was very important to maintain a high temperature to obtain the high activity of ANAMMOX bacteria. There was substrata competition between the denitrification bacteria and ANAMMOX bacteria when organics in the influent. The half saturation constants for ammonium and nitrite of the ANAMMOX bacteria were 151.97 mg∙L⁻¹ and 42.598 g∙L⁻¹ respectively. The half inhibition constant for ammonium and nitrite of the ANAMMOX bacteria were 555.3 g∙L⁻¹ and 200.6 g∙L⁻¹ respectively.

Keywords: ANAMMOX, Denitrification, Kinetics, Temperature, Organics.

1. INTRODUCTION

Anaerobic Ammonium Oxidation (ANAMMOX) is an efficient, economical and sustainable nitrogen removal technology, which is a hot point in domestic and international research in the field of wastewater treatment at home and abroad in recent years [1-4]. ANAMMOX bacteria grow slowly and are sensitive to environmental conditions, and organics, temperature and other factors have an important impact on ANAMMOX bacteria [5]. In order to optimize the reaction conditions of ANAMMOX process and improve nitrogen removal efficiency of ANAMMOX process. In this study, the influence of temperature and organics on ANAMMOX bacteria activity were investigated in an up-flow ANAMMOX reactor and the kinetics characteristics of experiment Cultured ANAMMOX bacteria were analyzed by sequencing batch experiments. Theoretical and technical guidance for the optimization and performance evaluation of ANAMMOX reactor were provided in this paper.

The reactor is shown in Figure 1, which was made of polymethyl methacrylate with a diameter of 234 mm, 750 mm high, and an effective volume of 30 L. The reactor packing rack for inner ring winding curtain type fiber biomass carrier, effective filling rate was 7.49 g∙L⁻¹. Temperature was controlled by the heater (SX-265, Guangdong Jin Lijia electromechanical Co., Ltd.) automatically. pH was measured through the on-line monitoring installation (SIN-PH160, Hangzhou SinoMeasure Automation technology Co., Ltd.) and linkage control of fluid infusion pump to carry on the addition of acid and alkali solution. The feed solution was introduced to the reactor with a peristaltic pump.

2. MATERIALS AND METHODS

2.1 Experimental installation

The reactor was inoculated sludge from laboratory cultured mixed sludge, which contained some ANAMMOX bacteria. When the temperature was 35 °C, the hydraulic retention time was 20 h and TN load was 0.7 kg∙m⁻³∙day⁻¹, after operating stably in the conditions of stable operation for 60 d, the removal rates of NH₄⁺-N, NO₂⁻-N and TN were 85%, 94% and 80% respectively.
2.3 Experimental wastewater

The composition of synthetic wastewater was as follows: NH$_4^+$-N, NO$_2^-$-N respectively by (NH$_4$)$_2$SO$_4$, NaNO$_2$ distributing according to need, KH$_2$PO$_4$ 54 mg L$^{-1}$, FeSO$_4$·7H$_2$O 9 mg L$^{-1}$, EDTA 5 mg L$^{-1}$, the mineral element solution 2 ml L$^{-1}$, trace element stock 1 ml L$^{-1}$. The mineral element concentration was composed of CaCl$_2$ 0.7 g L$^{-1}$, KCl 0.7 g L$^{-1}$, MgSO$_4$ 0.5 g L$^{-1}$, NaCl 0.5 g L$^{-1}$. The trace element stock was composed of CoCl$_2$·6H$_2$O 0.24 mg L$^{-1}$, CuSO$_4$·5H$_2$O 0.25 mg L$^{-1}$, H$_3$BO$_3$ 0.014 mg L$^{-1}$, MnCl$_2$·4H$_2$O 0.99 mg L$^{-1}$, Na$_2$MoO$_4$·2H$_2$O 0.22 mg L$^{-1}$, NiCl$_2$·6H$_2$O 0.19 mg L$^{-1}$, ZnSO$_4$·7H$_2$O 0.43 mg L$^{-1}$.

2.4 Experimental methods

All water quality indexes were measured in accordance with the Methods for Monitor and Analysis of Water and Wastewater [6].

2.4.1 The influence of different temperatures on ANAMMOX bacteria

In order to explore the influence of different temperatures on ANAMMOX bacteria, the experiment was carried out at 35°C, 30°C and 25°C. The temperature increased firstly and then decreased. Each temperature was stable operated for 5 days. The influence of temperatures on ANAMMOX bacteria were detected by the change of nitrogen content in influent and effluent water. During the experiment, the reactor hydraulic retention time was 20h, influent NH$_4^+$-N and NO$_2^-$-N concentration were unchanged, both 260 g L$^{-1}$.

2.4.2 The influence of organics on ANAMMOX bacteria

In order to explore the influence of organics on ANAMMOX process, a certain concentration of glucose was added in the water (COD was 200 g L$^{-1}$). The influence of organics on the ANAMMOX bacteria was reflected by the change of the water quality of import and export. In the experimental process, the rest of the operating conditions kept the same as the stable operation in addition to the intake of a certain concentration of glucose.

2.4.3 Kinetics experiment

The concentration of NH$_4^+$-N and NO$_2^-$-N in the substrate could affect ANAMMOX bacteria activity, and the kinetics characteristics of NH$_4^+$-N and NO$_2^-$-N of cultured ANAMMOX bacteria were analyzed by sequencing batch experiments. In the 100 ml of a conical flask with a slurry mixture of 25 ml, the culture medium of 50 ml. Concentration of NO$_2^-$-N and NH$_4^+$-N in the culture medium were distributed according to demand, and the remaining nutrients were the same with continuous flow reactor. The conical flask by adding nitrogen to remove oxygen with a rubber plug plugged in a constant temperature table without light training. The concentrations of NH$_4^+$-N and NO$_2^-$-N were measured in 0, 4, 8 and 12 h, respectively. The reaction rate of each concentration was calculated, and the Haldane model was used to fit the reaction rate. Then calculate the relevant parameters.

3. RESULTS AND DISCUSSION

3.1 The influence of temperature on ANAMMOX bacteria

The changes of NH$_4^+$-N and NO$_2^-$-N concentration and removal rate of the effluent at different temperatures are shown in Figure 2. As shown in Fig. 2, the NH$_4^+$-N and NO$_2$-N concentrations in the effluent were significantly changed when the temperature decreased. When the temperature was decreased, the NH$_4^+$-N and NO$_2$-N concentrations were increased. When the temperature was increased, the concentration of NH$_4^+$-N and NO$_2$-N decreased significantly. The results showed that the temperature had a significant effect on the ANAMMOX bacteria. The activity was significantly higher when ANAMMOX bacteria at a high temperature (35°C) than the lower temperature (30°C, 26°C). The optimum temperature of ANAMMOX bacteria recorded in the literature was 30~35°C [7]. The results of this experiment are consistent with the conclusions of the literature.

During the experiment, when the temperature decreased from 35°C to 30°C, it was found that the effluent NH$_4^+$-N concentration first increased and then decreased, while the NO$_2$-N concentration was increased, but the amplitude was significantly less than NH$_4^+$-N. And when the temperature decreased from 30°C to 26°C, the NH$_4^+$-N removal rate increased significantly. This may be the seed sludge that was used in this research mixed the ammonia oxidizing bacteria, the denitrification bacteria and ANAMMOX bacteria. The optimum temperature of these three kinds of bacteria are not the same, ammonia oxidizing bacteria at 25°C to achieve the highest activity [8], the most appropriate temperature of the denitrification bacteria at 30°C [9]. Temperature changes could cause changes in the activity of each bacterium. When the temperature was decreased from 35°C to 30°C, the activity of the denitrification bacteria increased and the activity of ANAMMOX decreased, denitrification bacteria using NO$_2$-N as electron acceptor for denitrification, consuming part of NO$_2$-N, therefore, the NO$_2$-N concentration of the effluent due to the decrease of the activity of ANAMMOX bacteria was not significantly increased as NH$_4^+$-N concentration. When the temperature continued to decline to 26°C, ammonia oxidizing bacteria activity was higher than the other two bacteria, while the water in the reactor still contained a certain amount of dissolved oxygen. Therefore, part of NH$_4^+$-N was converted to NO$_2$-N so that the NH$_4^+$-N removal rate rises.
On the whole, the temperature had a significant effect on the ANAMMOX, maintaining a high temperature had a very important to the high activity of ANAMMOX bacteria.

![Graph](image1)

(a) NH$_4^+$-N and NO$_2^-$-N content of effluent

3.2 The influence of organics on ANAMMOX bacteria

Glucose is the most common organics. Macromolecular organics is first converted to glucose in the further decomposition of digestion in wastewater treatment process. Therefore, glucose was chosen as the research object, to investigate its effect on the nitrogen removal performance of ANAMMOX bacteria.

As shown in Figure 3 (a), when adding glucose into the influent water, the removal rate of NH$_4^+$-N decreased gradually from the second day, the lowest rate fell by 14% compared with the rate before the experiment, The removal rate of NO$_2^-$-N had no obvious change, still maintained at about 96%. Liu Jinling et al. [10] found that add a small amount of glucose in the ANAMMOX sludge could promote the removal of the substrate, but the high concentration of glucose could inhibit the activity of ANAMMOX bacteria. Yang Yang et al. [11] found that there were heterotrophic denitrification bacteria in the ANAMMOX sludge, which would have the substrate competition with ANAMMOX bacteria because of the presence of organics. The removal rate of NH$_4^+$-N and NO$_2^-$-N showed that there was a significant denitrification in the reactor. NO$_2^-$-N was used by the denitrification bacteria to carry out denitrification, so that the NO$_2^-$-N removal rate was not obvious. However, ANAMMOX bacteria could not normally carry on reaction due to the lack of NO$_2^-$-N, so that NH$_4^+$-N removal rate decreased significantly. In addition, the effluent COD and NO$_2^-$-N concentrations were significantly decreased compared to the influent, as shown in Figure 3 (b). It could also prove that the activity of the denitrification bacteria was gradually enhanced, and formed a substrate competition relationship with the ANAMMOX bacteria, so the activity of ANAMMOX bacteria was inhibited. And after sixth days to stop adding glucose, the denitrification bacteria activity decreased due to the lack of organic carbon source, the ability to compete for the substrate decreased, ANAMMOX bacteria repossessed enough NO$_2^-$-N to react, so the removal rate of NH$_4^+$-N gradually recovered, and the inhibitory effect of glucose on ANAMMOX bacteria was gradually relieved.

At higher concentrations (COD 200 g L$^{-1}$), organics would have inhibitory effect on ANAMMOX bacteria activity. The main reason for the inhibition is that the denitrification bacteria used NO$_2^-$-N to carry on the denitrification, formed a substrate competition relationship with the ANAMMOX bacteria.

![Graph](image2)

(b) Removal rate of NH$_4^+$-N and NO$_2^-$-N

**Figure 2.** Changes of NH$_4^+$-N and NO$_2^-$-N content and removal rate of effluent

3.3 Substrate inhibition kinetics characteristics of ANAMMOX bacteria

The kinetics characteristics of ANAMMOX bacteria cultured in laboratory are shown in Figure 4, from which it can be seen that the Haldane model [12], which is consistent with the substrate inhibition kinetics model:

$$ v = \frac{V_{\text{max}} S}{K_s + S + \frac{K_i S}{K_m}} $$

(1)

Among them, $V_{\text{max}}$ is the maximum reaction rate, $S$ is the substrate concentration, $K_m$ is the half saturation constant of ammonia nitrogen, and $K_i$ is the inhibition constant of ammonia nitrogen.

The Haldane model was used to fit the experimental data. The dynamics equation of ANAMMOX bacteria on ammonia (Figure. 4 (a)) and nitrite (Figure. 4 (b)) was obtained.
respectively. The fitting correlation coefficient R² was 0.94621 and 0.97835 respectively. The ammonium of V<sub>max</sub>, K<sub>m</sub>, K<sub>i</sub> was 0.05039 mg mg⁻¹ h⁻¹, 151.97 mg L⁻¹, 555.3 mg L⁻¹ respectively. The nitrite of V<sub>max</sub>, K<sub>m</sub>, K<sub>i</sub> was 0.0803 mg mg⁻¹ h⁻¹, 42.598 mg L⁻¹, 200.6038 mg L⁻¹ respectively.

![Graph](image.png)

**Figure 4.** Dynamics characteristics of ANAMMOX bacteria

### 4. CONCLUSIONS

1. Temperature had a significant effect on ANAMMOX bacteria activity. The decreasing of temperature could inhibit ANAMMOX bacteria activity. When the temperature gradually recovered to 35°C, the ANAMMOX bacteria activity gradually recovered. 35°C is a suitable temperature for ANAMMOX process.

2. When mixed with denitrification bacteria in ANAMMOX sludge, the ANAMMOX bacteria are faced with the substrate and space competition of the denitrification bacteria under the condition of a certain concentration of organics.

3. The half saturation constants K<sub>m</sub> of experiment cultured ANAMMOX bacteria on substrate of ammonia and nitrite were 151.97 mg L⁻¹ and 42.598 mg L⁻¹ respectively, and the inhibition constant K<sub>i</sub> were 555.3 mg L⁻¹ and 200.6038 mg L⁻¹ respectively.

### ACKNOWLEDGMENT

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### REFERENCES


### NOMENCLATURE

- V<sub>max</sub>: maximum reaction rate
- S: substrate concentration