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

Liang Qiyu [1], Jia Ruotan [1], Qu Wanxin [1], Zhang Deyuan [2], Ma Lin [3], Zhang Qun [4], Li Xianjin [1], Xie Yuanhua [1], Ma Yongguang [1] and Zhu Tong [1*]

[*1] School of Mechanical Engineering and Automation, Northeastern University, Shenyang 110819, Liaoning, China
[2] Machinery Factory, Northeastern University, Shenyang 110819, Liaoning, China
[3] School of Sciences, Northeastern University, Shenyang 110819, Liaoning, China
[4] School of Metallurgy, Northeastern University, Shenyang 110819, Liaoning, China

Email: tongzhu@mail.neu.edu.cn

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.

2. MATERIALS AND METHODS

2.1 Experimental installation

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.

2.2 Seed sludge

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 NH_4^+-N, NO_2^--N and TN were 85%, 94% and 80% respectively.

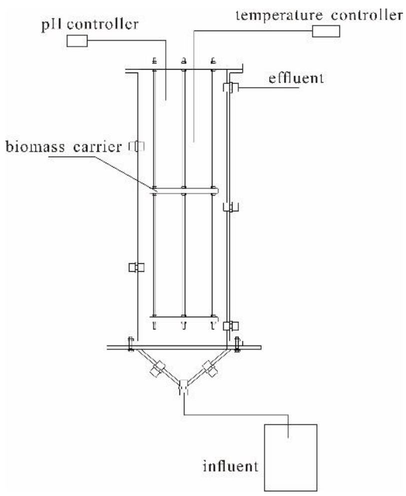


Figure 1. Schematic diagram of experimental installation

2.3 Experimental wastewater

The composition of synthetic wastewater was as follows: NH_4^+-N, NO_2^--N respectively by $(NH_4)_2SO_4$, $NaNO_2$ distributing according to need, KH_2PO_4 54 mg·L^{-1}, $FeSO_4·7H_2O$ 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 0.5g·L^{-1}. The trace element stock was composed of $CoCl_2·6H_2O$ 0.24 mg·L^{-1}, $CuSO_4·5H_2O$ 0.25 mg·L^{-1}, H_3BO_3 0.014 mg·L^{-1}, $MnCl_2·4H_2O$ 0.99 mg·L^{-1}, $Na_2MoO_4·2H_2O$ 0.22 mg·L^{-1}, $NiCl·6H_2O$ 0.19 mg·L^{-1}, $ZnSO_4·7H_2O$ 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.

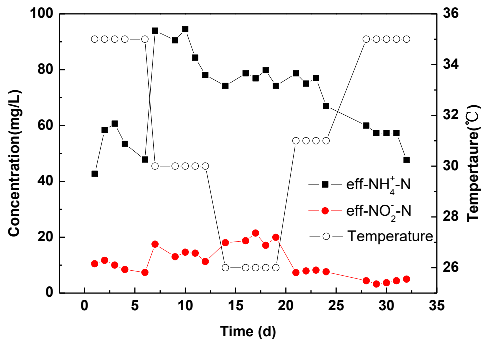
3. RESULTS AND DISCUSSION

3.1 The influence of temperature on ANAMMOX bacteria

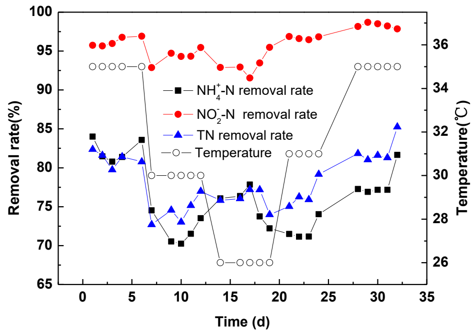
The changes of NH_4^+-N and NO_2^--N concentration and removal rate of the effluent at different temperatures are shown in Figure 2. As shown in Fig. 2, the NH_4^+-N and NO_2^--N concentrations in the effluent were significantly changed when the temperature decreased. When the temperature was decreased, 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℃ 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 ℃ 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.



(a) NH_4^+-N and NO_2^--N content of effluent



(b) Removal rate of NH_4^+-N and NO_2^--N

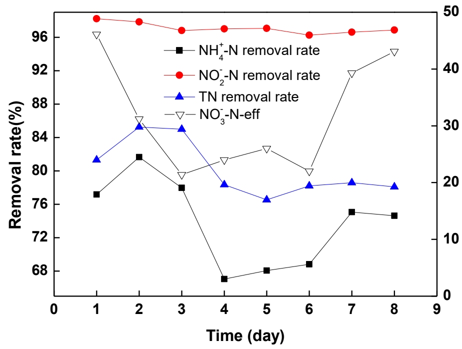
Figure 2. Changes of NH_4^+-N and NO_2^--N content and removal rate of effluent

3.2 The influence of organics on ANAMMOX bacteria

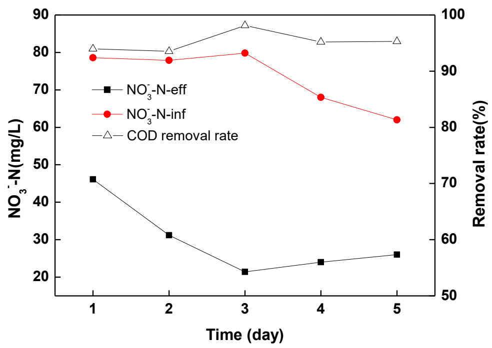
Glucose is the most common organics. Macromolecular organics is first converted to glucose in the further decomposition of digestion in wastewater treatment process. Therefore, glucose was chosen as the research object, to investigate its effect on the nitrogen removal performance of ANAMMOX bacteria.

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 $NH4^+$-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, ANANMMOX 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 ANAMMMOX bacteria was gradually relieved.

At higher concentrations (COD 200 $g \cdot L^{-1}$), 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.



(a) Nitrogen removal rate and variation of nitrate nitrogen concentration in effluent water



(b) Nitrate nitrogen concentration in influent and effluent water and the removal rate of COD

Figure 3. The influence of glucose on ANAMMOX

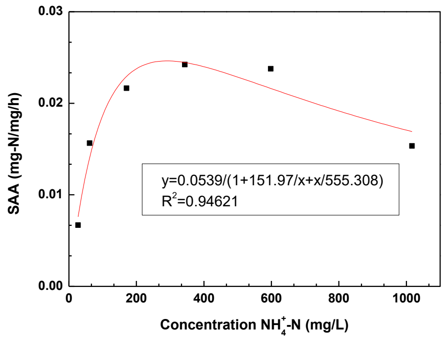
3.3 Substrate inhibition kinetics characteristics of ANAMMOX bacteria

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:

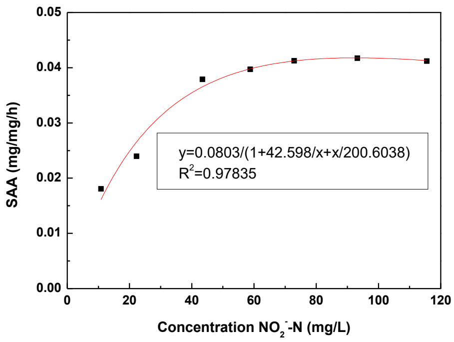
$$v = \frac{v_{max}}{1+\frac{K_m}{S}+\frac{S}{K_i}} \quad (1)$$

Among them, V_{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 R^2 was 0.94621 and 0.97835 respectively. The ammonium of V_{max}, K_m, K_i was 0.05039 mg·mg^{-1}·h^{-1}, 151.97 mg·L^{-1}, 555.3 mg·L^{-1} respectively. The nitrite of V_{max}, K_m, K_i was 0.0803 mg·mg^{-1}·h^{-1}, 42.598 mg·L^{-1}, 200.6038 mg·L^{-1} respectively.



(a) NH_4^+-N



(b) NO_2^--N

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℃, the ANAMMOX bacteria activity gradually recovered. 35 ℃ 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_m 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_i were 555.3 mg·L^{-1} and 200.6038 mg·L^{-1} respectively.

ACKNOWLEDGMENT

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NOMENCLATURE

V_{max}	maximum reaction rate
S	substrate concentration