Study on Start-up Performance of Anammox Process

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Abstract. Anammox, as one of the most revolutionary new denitrification technologies so far, has broad application prospects. In order to explore the start-up process of the anaerobic ammonia oxidation process, the experiment was conducted to domesticate anaerobic sludge in an up-flow anaerobic sludge bed reactor. The results showed that the reactor reached a stable operation stage from the 95th day to the 112th day. The removal rate of NH$_4^+$-N and the removal rate of NO$_2^-$-N were stabilized at 94.12% and 97.70%, respectively. According to the removal of NH$_4^+$-N and NO$_2^-$-N in the reactor, the start-up process can be divided into 4 stages, and there is denitrification and synergistic anaerobic ammonia oxidation in the reactor.

1. Instruction
The anaerobic ammonia oxidation (ANAMMOX) process relies on its short process flow, low energy consumption and cost-effective denitrification advantages, and has gradually realized engineering operation after decades of research and development. But it is undeniable that the process is not yet mature in actual engineering. The AnAOB generation has a long reproductive cycle (11-20 days), and its extremely environmentally sensitive characteristics mean that the process operation conditions are relatively harsh [1]. Therefore, how to quickly start and operate the anaerobic ammonia oxidation process has always been a hot topic in the field of wastewater treatment.

In this paper, the Up-flow Anaerobic Sludge Bed (UASB) is used to study the ANAMMOX reaction, and artificial simulated wastewater is used as the feedwater of the UASB reactor to explore the start of the reactor. It is intended to provide strong data support for the application promotion of ANAMMOX.

2. Materials and Methods
2.1. Test Device
The device diagram of the UASB reactor is shown in Figure 1. The reactor is composed of double-layer cylindrical organic glass. The overall height of the reactor is 60cm. It is divided into two parts: the upper and lower precipitation zone and the reaction zone. The total volume is 9L and the effective volume is 7L. The upper sedimentation zone has a diameter of 15 cm and a height of 40 cm. The precipitation zone is equipped with a three-phase separator, and the gas outlet is connected with a gas scrubber; The lower reaction zone has a diameter of 8cm and a height of 60cm; and the bottom end is a hemispherical mud bucket with a diameter of 8cm to settle the sludge; 5 sampling ports are arranged along the height of the reactor column with an interval of 10cm for water discharge and sampling and testing.
2.2. Test Water
The artificial sewage is adopted to simulate the domestic sewage. No carbon source is added in the experiment, while ensuring that the ratio of NH₄⁺-N to NO₂⁻-N in the influent water is 1:1.32. The water quality is shown in Table 1.

| Water quality index | concentration (mg/L) |
|---------------------|----------------------|
| NH₄Cl               | 46-56                |
| NaNO₂              | 60-74                |
| NaHCO₃             | 50                   |
| MgSO₄·7H₂O          | 300                  |
| CaCl₂·2H₂O         | 136                  |
| KH₂PO₄            | 30                   |

2.3. Test Method
The experiment used the anaerobic nitrification sludge of Shenyang Zaohua Wastewater Treatment Plant as the inoculation sludge. The sludge was a suspended floc with a loose structure and dark brown. The mud was allowed to stand for 24 hours before inoculation to removed the suspended solids and various impurities in the upper layer. Afterwards, the supernatant should be discarded and the inoculated sludge should be further cleaned with a filter. Finally, the remaining sludge was washed with NaHCO₃ buffer to remove the residual matrix in the sludge. Observing the sludge at this time, a large number of filamentous bacteria and transparent sticky substances were found. It is shown in Figure 2. The reactor was inoculated with 2L of the above sludge. Afterwards, the NH₄⁺-N concentration in the influent of the reactor was controlled to 50mg•L⁻¹, the NO₂⁻-N concentration was 66mg•L⁻¹, and the pH value was controlled within the range of 7.5 to 8.0. The water temperature in the reactor was controlled at 30°C, and the peristaltic pump was adjusted to make the HRT initially set to 48h, and gradually increased to 12h as the reaction time progressed. Samples are taken regularly every 3 days to detect various nitrogen indicators in the incoming and outgoing water to investigate the denitrification performance of the reactor.
3. Results and discussion
Observing Figures 3 and 4, it is not difficult to find that the start-up process of the UASB reactor can be divided into four stages: the cell lysis phase, the lag phase, the activity-enhanced phase and the steady running phase.

(1) In the initial start-up period (1~16d), the average concentration of influent NH$_4^+$-N was 51.98mg•L$^{-1}$, and the average concentration of effluent NH$_4^+$-N was 57.31 mg•L$^{-1}$. In the cell lysis phase, the average removal rate of NH$_4^+$-N from the reactor was -10.04%. The effluent NH$_4^+$-N concentration was higher than the influent NH$_4^+$-N concentration, and the effluent water quality was very bad. The reason was that the environment of the inoculated sludge had changed from a rich substrate condition to a poor substrate condition. The growth and reproduction of a large number of heterotrophic bacteria were severely restricted, and then the endogenous respiration period would cause death and autolysis [2]. The degradation of organic nitrogen in the body caused the concentration of NH$_4^+$-N in the effluent to be higher than the concentration of NH$_4^+$-N in the influent. However, observing Figure 4, it could be found that the NO$_2^-$-N removal rate in the reactor was very considerable, with an average removal rate of 97.06%; the NO$_3^-$-N concentration in the effluent was very low, with an average concentration of 1.01 mg•L$^{-1}$. It was because the inoculated sludge contained some heterotrophic denitrifying bacteria. Although the artificial simulation configuration did not contain organic matter in the inlet water, the heterotrophic denitrifying bacteria could use the COD produced by the hydrolysis and autolysis of the bacteria and the external polymer contained in a small amount of anaerobic granular sludge as a carbon source for denitrification [3]. Therefore,
NH$_4^+$-N and NO$_2^-$-N could not be removed at the same time in the hydrolysis stage of the bacteria. The anaerobic ammonia oxidation was negligible, and denitrification was dominant at this time.

(2) In the early stage of start-up (17~49d), a small amount of fine sludge was found in the outlet bucket, which showed that bacteria, not suitable for the inorganic environment, had been washed out of the reactor with the outlet water. Observing the sludge in the reactor, it was found that the sedimentation level seemed to stop falling, and the autolysis of bacteria gradually disappeared. In the lag phase, the NH$_4^+$-N removal effect was gradually strengthened, the effluent NH$_4^+$-N concentration was reduced from 50.51 mg•L$^{-1}$ to 27.68 mg•L$^{-1}$, and the average removal rate of NH$_4^+$-N was 28.35%. In comparison, the removal rate of NO$_2^-$-N dropped slightly, with an average removal rate of 92.62%, and the concentration of NO$_3^-$-N in the effluent also increased compared to the previous cycle [4]. At the same time, autotrophic bacteria were gradually enriched, so that NH$_4^+$-N and NO$_2^-$-N began to be removed simultaneously, and anaerobic ammonia oxidation began to appear. It was observed that in this phase, the ratios of $\frac{\Delta \text{NO}_2^-}{\Delta \text{NH}_4^+}$ and $\frac{\Delta \text{NO}_3^-}{\Delta \text{NH}_4^+}$ still deviated from the theoretical values of 1.32 and 0.26, indicating that there might be short-cut denitrification in the reactor to strengthen NO$_2^-$-N removal effect [5].

(3) In the middle of the start-up phase (50~94d), it was found that the removal rate of NH$_4^+$-N increased greatly, from 40.89% to 89.12%, and the simultaneous removal ratio of NH$_4^+$-N and NO$_2^-$-N gradually increased; At the same time, it could be seen that the effluent NO$_3^-$-N had increased. In the activity-enhanced phase, the ratio of $\frac{\Delta \text{NO}_2^-}{\Delta \text{NH}_4^+}$ gradually approached the theoretical value of 1.32. The experimental data of the appeal showed that due to the lack of organic substrate in the reactor, the activity of heterotrophic bacteria was inhibited and the denitrification effect was gradually weakened; due to the suitable environment, the richness of AnAOB increased rapidly and maintained a high activity, and anaerobic ammonia oxidation took the lead.

(4) In the late start-up phase (95~112d), the reactor reaches a steady running phase. The final NH$_4^+$-N removal rate and NO$_2^-$-N removal rate stabilized at 94.12% and 97.70%, respectively. The ratio of $\frac{\Delta \text{NO}_2^-}{\Delta \text{NH}_4^+}$ fluctuated around the theoretical value of 1.32, while the ratio of $\frac{\Delta \text{NO}_3^-}{\Delta \text{NH}_4^+}$ still deviated from the theoretical value of 0.26. At this stage, the average value of $\frac{\Delta \text{NO}_2^-}{\Delta \text{NO}_3^-}$ was 1.35:0.04:1, which indicated that some denitrifying bacteria still existed in the reactor [6]. Observing the sedimentation zone in the reactor, it was found that a small
amount of red spherical sludge floated on the top of the three-phase separator with fine particles. In general, the ANAMMOX reactor was successfully started.

4. Conclusion
The anaerobic nitrification sludge of Shenyang Zaohua Wastewater Treatment Plant was used as inoculation sludge. By controlling the temperature to 30°C and the pH value of 7.5 to 8.0, adjusting the peristaltic pump so that the HRT was initially set to 48h and gradually increased to 12h as the reaction time progressed, the ANAMMOX reactor was successfully started after 112 days. The inoculated sludge was transformed from the initial dark-brown flocculent sludge to red-brown fine particle sludge, which confirmed that the AnAOB enrichment in the reactor was successfully achieved.

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