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# Correlation of anaerobic ammonium oxidation and denitrification

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**Abstract:** The feasibility of the nitrous organic wastewater treated was studied in seven anaerobic sequencing batch reactors(ASBRs) ($0^{\#}$—$6^{\#}$) which had been run under stable anaerobic ammonium oxidation (Anammox). By means of monitoring and data analysis of COD, $NH_4^+$-N, $NO_2^-$-N, $NO_3^-$-N and pH, and of microbial test, the results revealed that the optimal Anammox performance was achieved from $2^{\#}$ reactor in which COD/ $NH_4^+$-N was 1.65, Anammox bacteria and denitrification bacteria could coexist, and Anammox reaction and denitrification reaction could occur simultaneously in the reactors. The ratio of $NH_4^+$-N consumed : $NO_2^-$-N consumed : $NO_3^-$-N produced was 1:1.38:0.19 in $0^{\#}$ reactor which was not added glucose in the wastewater. When different ratio of COD and $NH_4^+$-N was fed for the reactors, the ratio of $NO_2^-$-N consumed: $NH_4^+$-N consumed was in the range of 1.51—2.29 and the ratio of $NO_3^-$-N produced: $NH_4^+$-N consumed in the range of 0—0.05.

**Keywords:** anaerobic ammonium oxidation (Anammox); anaerobic sequencing batch reactor (ASBR); biological denitrification; denitrification

## Introduction

In recent years, anaerobic ammonium oxidation (Anammox) process has been put forward as a promising alternative to treat ammonium rich wastewaters. In this process, Anammox bacteria combine ammonium and nitrite to produce nitrogen gas under anoxic condition(van de Graff *et al.*, 1996). Compared with the conventional nitrogen removal system, less oxygen is required, the addition of organic matter is not necessary, and the low amount of surplus sludge is produced, which would lead to a reduction in the operational costs (Jetten *et al.*, 2001). However, Anammox bacteria grow slowly which doubling time is about 11 d. In order to bring the process into practical use, a reactor with high biomass retention such as sequencing batch reactor (SBR) (Strous *et al.*, 1998; Dapena-Mora *et al.*, 2004a, b) is required.

Anammox is a biological conversion process. Based on several basic studies, the stoichiometry of Anammox reaction was represented by the following reaction(Strous *et al.*, 1998).

$$NH_4^+ + 1.32NO_2^- + 0.066HCO_3^- + 0.13H^+ \longrightarrow 1.02N_2 + 0.26NO_3^- + 0.066CH_2O_{0.5}N_{0.15} + 2.03H_2O \quad (1)$$

Denitrification bacteria can convert nitrite or nitrate to nitrogen gas while treating carbonaceous wastewater under anoxic condition, according to reaction 2 or reaction 3(the glucose as organic matter).

$$C_6H_{12}O_6 + 8NO_2^- \longrightarrow 4N_2 + 6CO_2 + 2H_2O + 8OH^- \quad (2)$$

$$5C_6H_{12}O_6 + 24NO_3^- \longrightarrow 12N_2 + 30CO_2 + 18H_2O + 24OH^- \quad (3)$$

Anammox reaction was influenced less by the degree of partial denitrification, and the Anammox bacteria did not compete with denitrification bacteria (Ahn *et al.*, 2004).

Considering denitrification reaction of nitrite or nitrate is possible with the organic matter as an electron donor under anoxic conditions, if denitrification bacteria and Anammox bacteria can coexist and Anammox and denitrification reaction could occur in Anammox reactor, the organic matter and ammonium nitrogen can be removed at the same time, which will promote the treatment of the nitrous organic wastewater.

Based on these considerations, using anaerobic sequencing batch reactor (ASBR) as Anammox reactor, the objectives of this study are: (1) to assess the feasibility of the nitrous organic wastewater treated; and (2) to demonstrate the correlation of denitrification and Anammox in ASBR.

## 1 Materials and methods

### 1.1 Reactor and seed sludge

The ASBRs ($0^{\#}$—$6^{\#}$) were made of glass with working volume of 6.8 L (gas room 0.8 L, Fig.1), run at mesophilic(35℃), and mixed by mechanical stirrers (70—80 r/min). The pH of the influent was ranged between 7.5 and 8.0 without any deliberate control. The reactors were operated intermittently. Each cycle comprised four phases, namely influent (0.42 h), stirring, sedimentation (1.5 h) and effluent (0.08 h). Every phase time was controlled by means of the time relay. The influent volume and the effluent volume were 3 L. The samples were taken simultaneously from the uppermost sampling port. The samples were analyzed after 1.5 h sedimentation. The data in figures were the average value of three analysis results under the stable operation condition. The reactors were flushed with nitrogen gas to maintain anaerobic

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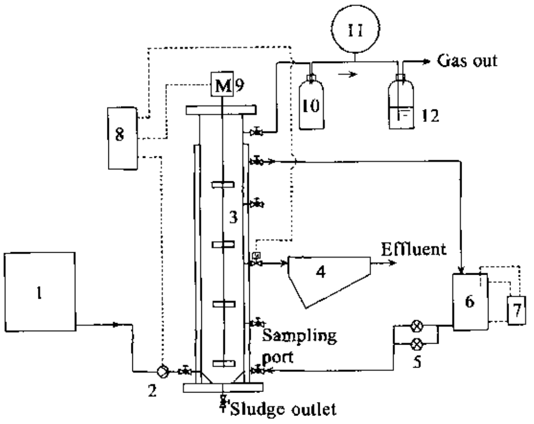

Fig.1 ASBR system and its diagram
1. influent tank; 2. gauging pump; 3. SBR reactor; 4. settling tank; 5. pipeline pump; 6. calefaction water tank; 7. thermostat; 8. time control system; 9. stirrer; 10. buffer; 11. gas bag; 12. waterseal

conditions and covered with black clothes to avoid the inhibition of light on Anammox activity.

The ASBRs had been run under stable Anammox, which had been indicated by ammonium nitrogen removal, nitrite nitrogen removal, ratio of tri-nitrogen and microbial test (Liang, 2005).

The sludge collected from a previously developed Anammox ASBR was inoculated for the ASBRs. The VSS/TSS (%) of the seed sludge was 39.28%. It was close to 39.13% (Zheng *et al.*, 2004). The initial biomass concentration inside the reactors was 1.15 gVSS/L.

### 1.2 Feeding medium

The ASBRs were fed with synthetic medium described in Table 1 (Luo, 2003). Water quality indices and ratios of carbon(COD) and nitrogen of the influent for the reactors are described in Table 2 and Table 3, respectively. The COD was produced only by glucose(Table 2 and 3).

**Table 1 Composition of synthetic wastewater for the reactors**

| Component | Concentration, g/L | Component | Concentration, ml/L |
|---|---|---|---|
| $KH_2PO_4$ | 0.027 | $NH_4Cl$ | Variation |
| $MgSO_4 \cdot 7H_2O$ | 0.300 | $NaNO_2$ | Variation |
| $KHCO_3$ | 0.500 | Fe-EDTA$^1$ | 1.25 |
| $CaCl_2$ | 0.136 | Trace elements solution$^2$ | 1.00 |

Notes: 1. Fe-EDTA solution contained(g/L): EDTA 5.000, $FeSO_4 \cdot 7H_2O$ 5.000; 2. described by van de Graaf *et al.*(1996)

**Table 2 Water quality indices in the influent for the reactors**

| Index | $0^{\#}$ | $1^{\#}$ | $2^{\#}$ | $3^{\#}$ | $4^{\#}$ | $5^{\#}$ | $6^{\#}$ |
|---|---|---|---|---|---|---|---|
| COD, mg/L | 0 | 93.11 | 214.15 | 325.22 | 436.95 | 702.97 | 917.13 |
| $NH_4^+$-N, mg/L | 123.73 | 127.70 | 129.56 | 127.17 | 130.88 | 131.94 | 135.12 |
| $NO_2^-$-N, mg/L | 180.24 | 193.35 | 132.79 | 131.85 | 129.35 | 121.86 | 135.28 |
| pH | 7.86 | 7.85 | 7.85 | 7.83 | 7.82 | 7.83 | 7.83 |

**Table 3 Ratio of COD and nitrogen in the influent for the reactors**

| Index | $1^{\#}$ | $2^{\#}$ | $3^{\#}$ | $4^{\#}$ | $5^{\#}$ | $6^{\#}$ |
|---|---|---|---|---|---|---|
| COD/$NH_4^+$-N | 0.73 | 1.65 | 2.56 | 3.34 | 5.33 | 6.79 |
| COD/$NO_2^-$-N | 0.48 | 1.61 | 2.47 | 3.38 | 5.77 | 6.78 |

### 1.3 Denitrification bacteria culture

Culture medium composition of denitrification bacteria is described in Table 4.

**Table 4 Culture medium composition of denitrification bacteria**

| Component | Concentration, g/L |
|---|---|
| Glucose | 10.0 |
| $KNO_3$ | 1.0 |
| $CaCl_2 \cdot 2H_2O$ | 0.5 |
| $K_2HPO_4$ | 0.5 |

### 1.4 Analysis

The concentration of $NH_4^+$-N, $NO_2^-$-N, $NO_3^-$-N, and COD were determined using the standard method issued by State Environmental Protection Administration of China. The pH was determined using glass electrodes connected to Ecoscan pH 5/6 pH-meter. TSS was determined by drying the sample at 105℃ for at least 24 h. After burned at 600℃ for 2 h, the ash was measured. The difference between TSS and ash was termed VSS. Most possible number (MPN) method was used to take count of denitrification bacteria.

## 2 Results and discussion

### 2.1 Denitrification bacteria test

Denitrification bacteria concentration was $2.5 \times 10^6$ entries/ml in seed sludge for the reactors by MPN method. The result verified that denitrification bacteria existed in $0^{\#}$—$6^{\#}$ reactors sludge. With the organic matter as an electron donor under 35℃ and anoxic conditions, denitrification reaction could occur in the reactors.

### 2.2 Nitrite nitrogen removal

During the overall period, nitrite nitrogen removal in $0^{\#}$—$6^{\#}$ reactors are shown in Fig.2. The experiment results indicated that nitrite nitrogen removals were more than 68% for the first 16 h in $0^{\#}$—$6^{\#}$ reactors. On hour 26, nitrite nitrogen removals in $0^{\#}$—$2^{\#}$ reactors were more than 79% , nitrite nitrogen removal in $3^{\#}$ reactor was 99%, and nitrite nitrogen removals in $4^{\#}$—$6^{\#}$ reactors were 100%.

Nitrite nitrogen was consumed not only by Anammox reaction, but also by denitrification reaction. According to reaction 2, the more COD in the influent was for the reactor, the more nitrite nitrogen removal happened in the reactor.

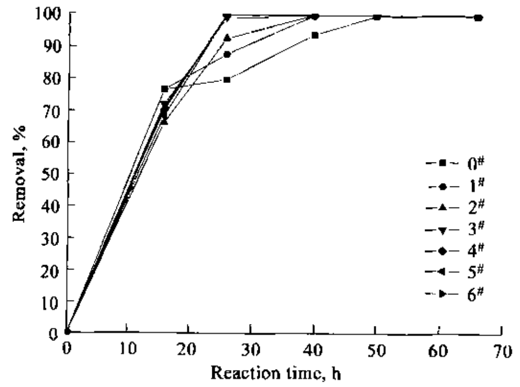

Fig.2 Variation of nitrite nitrogen removal in the reaction period

### 2.3 Ammonium nitrogen removal

As shown in Fig.3, ammonium nitrogen removals were more than 49% for the first 26 h in $0^{\#}$—$6^{\#}$ reactors. From 26 h onwards, the trendlines rose for $0^{\#}$—$1^{\#}$ reactors and dropped for $2^{\#}$—$6^{\#}$ reactors.

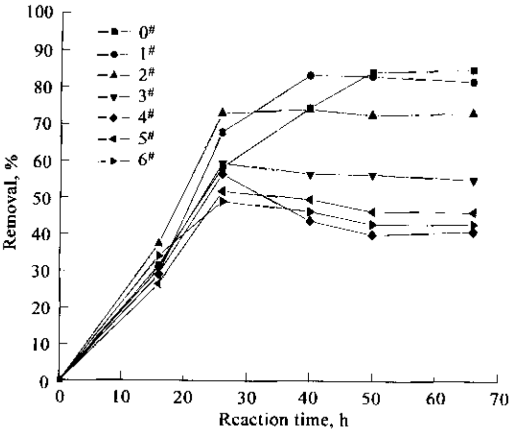

Fig.3 Variation of ammonium nitrogen removal in the reaction period

Because $0^{\#}$—$6^{\#}$ reactors had run at stable Anammox, ammonium nitrogen was removed according to reaction 1. For the first 26 h, Anammox reaction could be maintained because of the presence of ammonium nitrogen and nitrite nitrogen in the reactors. From 26 h onwards, Anammox reaction was inhibited because of few or complete depletion of nitrite nitrogen in $2^{\#}$—$6^{\#}$ reactors, which resulted in very little removal of ammonium nitrogen. At the last period, the curve of ammonium nitrogen removal dropped in $2^{\#}$—$6^{\#}$ reactors, which might be resulted from bacteria disaggregation. And the reason needs further investigation.

### 2.4 COD removal

The removal of COD was one of the characteristics of denitrification process. The change of COD was the indication of denitrification reaction. COD removal in $1^{\#}$—$6^{\#}$ reactors are shown in Fig.4. COD concentration was reduced rapidly in $1^{\#}$—$6^{\#}$ reactors for the first 40 h. On hour 40, COD removal was more than 80% in $1^{\#}$—$4^{\#}$ reactors and more than 65% in $5^{\#}$ and $6^{\#}$ reactors.

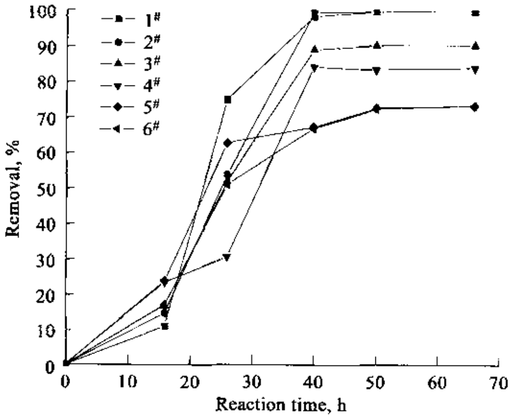

Fig.4 Variation of COD removal in the reaction period

Because the same sludge was inoculated in $1^{\#}$—$6^{\#}$reactors, the quantity of denitrification bacteria was the same at the beginning. The lower COD in the influent was for the reactor, the higher COD removal happened in the reactor. At the last period, nitrite nitrogen became the limiting factor of denitrification reaction, which resulted in very little removal of COD in the reactors.

Due to the inoculation sludge collected from a previously developed Anammox ASBR, Anammox bacteria existed in $0^{\#}$—$6^{\#}$ reactors. According to the data of COD removal and the result of denitrification bacteria test, it could be confirmed that denitrification bacteria existed in $1^{\#}$—$6^{\#}$ reactors. This means that denitrification bacteria and Amammox bacteria could coexist in Anammox reactors.

### 2.5 Ratio of carbon and nitrogen

Denitrification bacteria could compete with Anammox bacteria for nitrite nitrogen in virtue of denitritation reaction, which would affected the Anammox process of the reactors.

As shown in Fig.3, ammonium nitrogen removals of $2^{\#}$ reactor increased quickly and was of the optimal Anammox performance for the first 26 h in $0^{\#}$—$6^{\#}$ reactors. Thereafter, Anammox reaction was inhibited because nitrite nitrogen became the limiting substrate in the reactors, which resulted in very little removal of ammonium nitrogen. Thus, in $0^{\#}$—$6^{\#}$ reactors, the optimal Anammox performance was achieved firstly from $2^{\#}$reactor in which COD/NH$_4^+$-N was 1.65.

### 2.6 Variation of pH value

Because Anammox reaction could consume a little acidity(reaction 1), denitrification reaction could produce alkalinity(reaction 2 and 3), and acidification of glucose could produce acidity, the predominated reaction could be indicated by means of the change of pH value.

As shown in Fig.5, in $0^{\#}$ reactor without COD in the influent, the curve of pH value went up firstly, and then came down. But in 1#—6# reactors, the pH value was reduced for the first 16 h because of acidification of glucose at a temperature of 35℃. The more COD was in the influent for the reactor, the faster pH value dropped in the reactor. Thereafter, due to the denitrification reaction and the consumption of glucose, pH value was ascended. At the last period, the curve of pH value went down because both Anammox reaction and denitrification reaction were inhibited, and with the possible contribution of bacteria disaggregation.

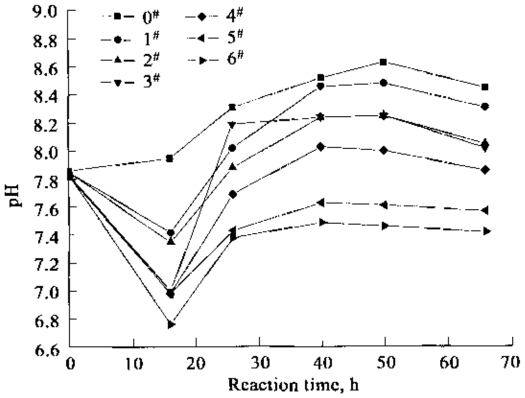

Fig.5 Variation of pH value in the reaction period

## 2.7 Ratios of $NO_2^-$-N consumed:$NH_4^+$-N consumed and $NO_3^-$-N produced:$NH_4^+$-N consumed

Ammonium nitrogen and nitrite nitrogen can be removed in proportion according to Anammox reaction (reaction 1), and some nitrate can be formed from nitrite nitrogen. The ratio of $NH_4^+$-N consumed: $NO_2^-$-N consumed: $NO_3^-$-N produced is 1:1.32:0.26. At the same time, denitrification bacteria can convert nitrite or nitrate to nitrogen gas while treating carbonaceous wastewater under anoxic condition (reaction 2 or reaction 3). If Anammox reaction and denitrification reaction could occur simultaneously in 1#—6# reactors, the ratio of $NO_2^-$-N consumed: $NH_4^+$-N consumed would be more than 1.32 and the ratio of $NO_3^-$-N produced: $NH_4^+$-N consumed would be less than 0.26.

The ratios of $NO_2^-$-N consumed: $NH_4^+$-N consumed and $NO_3^-$-N produced: $NH_4^+$-N consumed during the whole experimental period are shown in Fig.6.

The results revealed the ratio of $NH_4^+$-N consumed:$NO_2^-$-N consumed:$NO_3^-$-N produced was 1: 1.38:0.19 in 0# reactor which was not added glucose in the influent. In 1#—6# reactors which were added glucose in the influent, the ratios of $NO_2^-$-N consumed: $NH_4^+$-N consumed and $NO_3^-$-N produced:$NH_4^+$-N consumed were in the range of 1.51—2.29 and 0—0.05, respectively.

Based on the above results, Anammox reaction and denitrification reaction could occur simultaneously was confirmed, which explained Anammox bacteria and denitrification bacteria could coexist in the reactions once more.

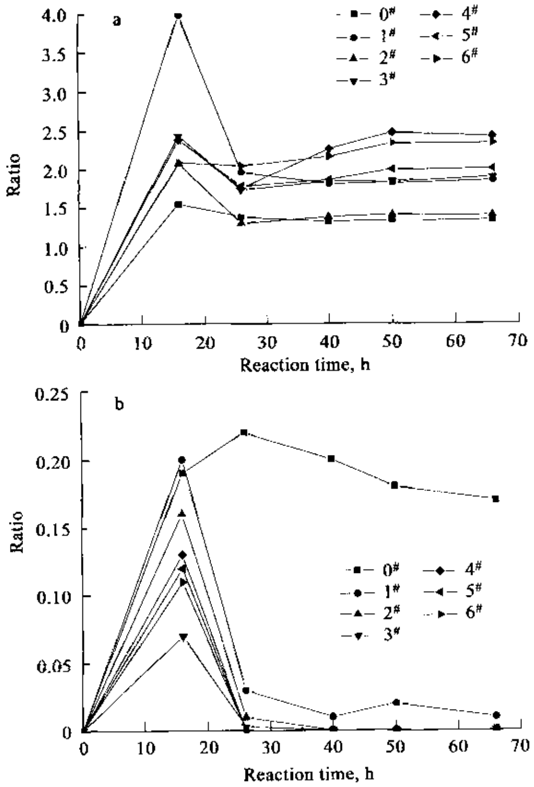

Fig.6 Ratio of nitrite nitrogen and ammonium nitrogen
a. $NO_2^-$-N consumed : $NH_4^+$-N consumed; b. $NO_3^-$-N produced : $NH_4^+$-N consumed

## 3 Conclusions

The treatment of nitrous organic wastewater with a certain ratio of carbon and $NH_4^+$-N by Anammox process was feasible. The Anammox performance of reactor related to the COD/$NH_4^+$-N. The optimal Anammox performance was achieved from the reactor in which COD/$NH_4^+$-N was 1.65.

Anammox bacteria and denitrification bacteria could coexist, and Anammox reaction and denitrification reaction could occur simultaneously in the same Anammox reactor.

When glucose was not added in the influent, the ratio of $NH_4^+$-N consumed: $NO_2^-$-N consumed: $NO_3^-$-N produced was 1:1.38:0.19. When different ratio of COD and $NH_4^+$-N was fed for the reactors, the ratio of $NO_2^-$-N consumed: $NH_4^+$-N consumed was in the range of 1.51—2.29 and the ratio of $NO_3^-$-N produced: $NH_4^+$-N consumed in the range of 0—0.05.

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## Correction Note

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## Decomposition kinetics of dimethyl methylphospate(chemical agent simulant) by supercritical water oxidation

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The affiliation and acknowledgement part will be corrected as follows.

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