

Effect of carbon source on nitrogen removal in anaerobic ammonium oxidation (anammox) process

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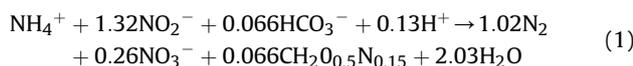
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Anaerobic ammonium oxidation (anammox) has been regarded as an efficient process to treat high-strength wastewater without organic carbon source. To investigate nitrogen removal performance of anammox in presence of organic carbon source can broaden its application on organic wastewater treatment. In this work, effect of carbon source on anammox process was explored. Operating temperature was set at $35 \pm 1^\circ\text{C}$. Influent pH and hydraulic retention time were 7.5 and 6 h, respectively. Effluent $\text{NH}_4^+ - \text{N}$ was affected little with COD no more than 480 mg/L. Independent of carbon source content, nitrite removal rate was around 99%. The variation of $\Delta\text{NO}_2^- - \text{N}/\Delta\text{NH}_4^+ - \text{N}$ lagged behind $\Delta\text{NO}_3^- - \text{N}/\Delta\text{NH}_4^+ - \text{N}$ at high COD content, and pH could be used as an indicator for $\text{NH}_4^+ - \text{N}$ removal. Specific anammox activity dropped from 0.39 to 0.19 $\text{kgNH}_4^+ - \text{N}/(\text{kgVSS} \cdot \text{d})$ at $\text{COD} = 720 \text{ mg/L}$. The remodified logistic model was quite appropriate for describing the nitrogen removal kinetics and predicting the performance of anammox process in presence of carbon source.

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[Key words: Nitrogen removal; Anaerobic ammonium oxidation; Carbon source; Inhibitory effect; Kinetic analysis]

When anaerobic ammonium oxidation (anammox) was first discovered in 1995 (1), as a promising technology, it attracted much attention in the field of wastewater treatment. As shown in Eq. 1 (2), anammox was a lithoautotrophic biological conversion process (3). With anaerobic condition, anaerobic ammonium oxidizing bacteria (AAOB) can use $\text{NO}_2^- - \text{N}$ as electron acceptor to oxidize $\text{NH}_4^+ - \text{N}$ to N_2 (3,4). Compared to traditional technology, it has many advantages such as low sludge production (5) and without requirement for hydrogen donor (6) and aeration. Besides, the nitrogen removal rate of anammox process was high though the inoculated sludge was diverse in different study (6–8), and its maximal value reached 74.3–76.7 $\text{kg}/(\text{m}^3 \cdot \text{d})$ (9), which was much higher than that in nitrification–denitrification process.



However, AAOB was quite sensitive to external environment (10). Organic matter (OM) was one of harmful substances for AAOB. In general, industrial wastewater and living sewage contained lots of OMs, e.g., anaerobic digested fish canning effluent (1000–1300 mg/L) (11) and turtle breeding wastewater (10–700 mg/L) (12). Previous studies found that OM affected

anammox process adversely (4,13). As a result, effect resulting from organic carbon source on anammox process should be investigated.

To date, there were two points about the effect of OM on anammox process. One was that AAOB and heterotrophic denitrification bacteria (HDB) could compete with each other in presence of OM. The activity of AAOB was inhibited at high COD content (14–16). This could be described as the appearance of out-competition (17). Chamchoi et al. (15) observed that anammox would be significantly inhibited with COD higher than 300 mg/L. Ni et al. (13) reported that AAOB decreased and HDB increased with COD higher than 400 mg/L. Tang et al. (18) also found that the performance of autotrophic nitrogen removal would be severely impaired with 800 mg/L COD. Yuan et al. (19) suggested that $\text{NH}_4^+ - \text{N}$ removal efficiency (ARE) was higher than 90% with 100 mg/L COD, and it decreased to 69% when 300 mg/L COD was added. However, these values varied greatly. The other viewpoint was that AAOB was still able to dominate the reactor with OM added. AAOB could coexist with HDB and even degrade OM, which suggested diversity in metabolic pathway (20,21). Sabumon observed that the conversion process of $\text{NH}_4^+ - \text{N}$ to $\text{NO}_3^- - \text{N}$ was accomplished by a mechanism unlike ordinary anammox process (22). Besides, AAOB could use $\text{NO}_2^- - \text{N}$ or/and $\text{NO}_3^- - \text{N}$ as electron acceptor to convert propionate to CO_2 (23). Some other scholars reached similar results (24–26).

In this work, a sequencing batch reactor (SBR) was used to study the effect of organic carbon source on anammox process. Specific anammox activity (SAA) was analyzed throughout the process. In addition, a new kinetic model was constructed to describe nitrogen removal performance within a typical operating cycle.

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MATERIALS AND METHODS

Reactor configuration and operation An SBR made of polymethyl methacrylate was used in this study. The reactor was double-jacketed with an effective volume of 1.5 L (presented in Fig. 1). Its temperature was controlled at $35 \pm 1^\circ\text{C}$ by a water bath with water recirculation through the outer chamber. The reactor was covered with a black cloth to prevent the potential growth of phototrophic microorganisms. The operating mode of the reactor consisted of 0.5 h influent feeding, 6 h anoxic stirring reaction, 0.5 h sludge settling and 0.5 h effluent discharging. The seeding sludge was taken from another lab-scale SBR which had been operated for two years with influent $\text{NH}_4^+ - \text{N}$ and $\text{NO}_2^- - \text{N}$ concentrations of 80 and 105 mg/L, respectively. The concentration of mixed liquor suspended solids was 1.5 g/L after inoculation. In this study, synthetic wastewater was used as the influent, which contained desired concentrations of COD, $\text{NH}_4^+ - \text{N}$, $\text{NO}_2^- - \text{N}$ and trace elements with pH adjusted to 7.5. COD was simulated by starch, with concentrations ranging from 40 to 720 mg/L at different operation phases, and the inhibition threshold of it was defined when $\text{NH}_4^+ - \text{N}$ removal efficiency decreased to 80% (13,27). $\text{NH}_4^+ - \text{N}$ and $\text{NO}_2^- - \text{N}$ concentrations were fixed at around 80 and 105 mg/L, respectively. Trace elements were added according to previous study (13).

Specific anammox activity batch assay Completely closed vials with a working volume of 100 mL were used to perform specific anammox activity (SAA) batch assay. $\text{NH}_4^+ - \text{N}$ and $\text{NO}_2^- - \text{N}$ concentrations were 80 and 105 mg/L, respectively. The influent pH and temperature were 7.5 and 35°C . N_2 was purged into vials to remove O_2 . The concentrations of $\text{NH}_4^+ - \text{N}$ and $\text{NO}_2^- - \text{N}$ were determined at regular intervals from vials, and SAA was calculated by the ratio of maximal substrate consumption rate to sludge concentration (28).

Analytical methods Samples were withdrawn at the beginning and end of each operation cycle and centrifuged at 6000 rpm for 30 min. The supernatants were analyzed for $\text{NH}_4^+ - \text{N}$, $\text{NO}_2^- - \text{N}$, $\text{NO}_3^- - \text{N}$ and COD concentrations according to the standard methods (29). Temperature and pH were measured by WTW portable multi-parameter measuring meter. Sludge concentration was measured after filtering the washed samples through $0.45 \mu\text{m}$ membrane filter and drying at 105°C to constant weight. Each sample analytical result presented in present research was performed in triplicate and the mean value was reported. Quality control/quality assurance procedure was according to Tomar and Gupta (30). Free ammonia (FA) and free nitrous acid (FNA) were calculated according to Eqs. 2 and 3 (31).

$$\text{FA} = \frac{17}{14} * \frac{\text{TAN} * 10^{\text{pH}}}{\left[\exp\left(\frac{6334}{273+\text{pH}}\right) + 10^{\text{pH}}\right]} \quad (2)$$

$$\text{FNA} = \frac{47}{14} * \frac{\text{TNN}}{\left[\exp\left(\frac{-2300}{273+\text{pH}}\right) * 10^{\text{pH}}\right] + 1} \quad (3)$$

where TAN was total $\text{NH}_4^+ - \text{N}$ and TNN was total $\text{NO}_2^- - \text{N}$.

Kinetic analysis The modified logistic model (Eq. 4) was applied to describe the progress of hydrogen production in batch tests (32). Compared to logistic model,

it obtained lag time directly by fitting research data (32). In this work, the modified logistic model was used to simulate $\text{NH}_4^+ - \text{N}$ removal in a typical operating cycle.

$$\text{ARE} = \frac{\text{ARE}_{\text{max}}}{1 + \exp[4\text{R}_{\text{max}}(\lambda - t)/\text{ARE}_{\text{max}} + 2]} \quad (4)$$

where ARE was $\text{NH}_4^+ - \text{N}$ removal efficiency (%); ARE_{max} was the maximum ARE (%); R_{max} was the maximum removal rate (%/h); λ was lag time of $\text{NH}_4^+ - \text{N}$ removal in a typical operating cycle; t was operating time (h).

SAA was calculated by the ratio of maximum substrate consumption rate (MSCR) to sludge concentration (Eq. 5) (33). As presented in Eq. 4, the parameter R_{max} was the maximum removal rate (%/h) of $\text{NH}_4^+ - \text{N}$. $\text{R}_{\text{ammonia}}$ was calculated according to Eq. 6 and SAA was also calculated according to Eq. 7. As a result, the modified logistic model could be remodified as Eq. 8. The remodified logistic model was used to describe the relationship between $\text{NH}_4^+ - \text{N}$ removal and SAA.

$$\text{SAA} = \frac{\text{MSCR}}{\text{S}_c} \quad (5)$$

$$\text{R}_{\text{ammonia}} = \text{R}_{\text{max}}(\text{S}_i - \text{S}_e) \quad (6)$$

$$\text{SAA} = \frac{\text{R}_{\text{max}}\text{S}_i\text{ARE}_{\text{max}}}{\text{S}_c} \quad (7)$$

$$\text{ARE} = \frac{\text{ARE}_{\text{max}}}{1 + \exp\left[4\text{SAA}(\lambda - t)\text{S}_c / \left(\text{S}_i(\text{ARE}_{\text{max}})^2\right) + 2\right]} \quad (8)$$

where SAA was special anammox activity ($\text{kgNH}_4^+ - \text{N}/(\text{kgVSS} \cdot \text{d})$); MSCR was maximum substrate consumption rate ($\text{kgNH}_4^+ - \text{N}/(\text{L} \cdot \text{d})$); $\text{R}_{\text{ammonia}}$ was maximum $\text{NH}_4^+ - \text{N}$ removal rate ($\text{kgNH}_4^+ - \text{N}/(\text{L} \cdot \text{d})$); S_c was sludge concentration (kgVSS/L); S_i was $\text{NH}_4^+ - \text{N}$ concentration in influent (mg/L); S_e was $\text{NH}_4^+ - \text{N}$ concentration in effluent (mg/L).

RESULTS AND DISCUSSION

Nitrogen removal performance of anammox in presence of carbon source

Nitrogen removal performance of anammox in presence of carbon source is presented in Fig. 2. When COD was no more than 200 mg/L, anammox process was affected little by organic carbon source. With 240 mg/L COD, effluent $\text{NO}_3^- - \text{N}$ decreased to 29.5 mg/L, and it decreased further with increasing COD. When COD was more than 480 mg/L, effluent $\text{NH}_4^+ - \text{N}$ increased and peaked at 36 mg/L with 720 mg/L COD. With high carbon source content, denitrification occurred and removed nitrate and nitrite. Our results agreed with previous report that organic matter would negatively affect AAOB and lead to less $\text{NH}_4^+ - \text{N}$ removal (15). However, it affected nitrite removal little and promoted nitrate removal due to the co-existence of HDB.

Independent of carbon source content, nitrite removal rate (NiRE) was around 99%. When COD was no more than 400 mg/L, ARE was affected little. Then it dropped with carbon added further. This was similar with Tang et al. (18) that anammox could be dominant in reactor when COD was less than 400 mg/L. However, Chamchoi et al. (15) reported that ARE decreased with increasing COD and anammox was completely inhibited at $\text{COD} \geq 300 \text{ mg}/\text{L}$. Ni et al. (13) found that the inhibition threshold of COD concentration was 308 mg/L. Both were lower than this work. Nitrogen removal performances resulting from different carbon sources varied. Starch used in this work belonged to macromolecular organic carbon source. It was hard to be hydrolyzed. As a result, AAOB had good tolerance to it and anammox process was affected little by it. In terms of total nitrogen removal efficiency (TNRE), when COD was less than 200 mg/L, it was around 82%. Then it increased with growing COD and peaked at 89% with 560 mg/L COD. However, it dropped again when carbon source was added further.

The stoichiometric ratios of nitrite and nitrate to ammonium (i.e., $\Delta\text{NO}_2^- - \text{N}/\Delta\text{NH}_4^+ - \text{N}$ and $\Delta\text{NO}_3^- - \text{N}/\Delta\text{NH}_4^+ - \text{N}$) could indicate the performance of anammox (34–36). When COD was no more than 200 mg/L, $\Delta\text{NO}_2^- - \text{N}/\Delta\text{NH}_4^+ - \text{N}$ was stabilized at 1.29 while $\Delta\text{NO}_3^- - \text{N}/\Delta\text{NH}_4^+ - \text{N}$ was 0.31. When COD was 200–400 mg/L, $\Delta\text{NO}_2^- - \text{N}/\Delta\text{NH}_4^+ - \text{N}$ was still around 1.29, but

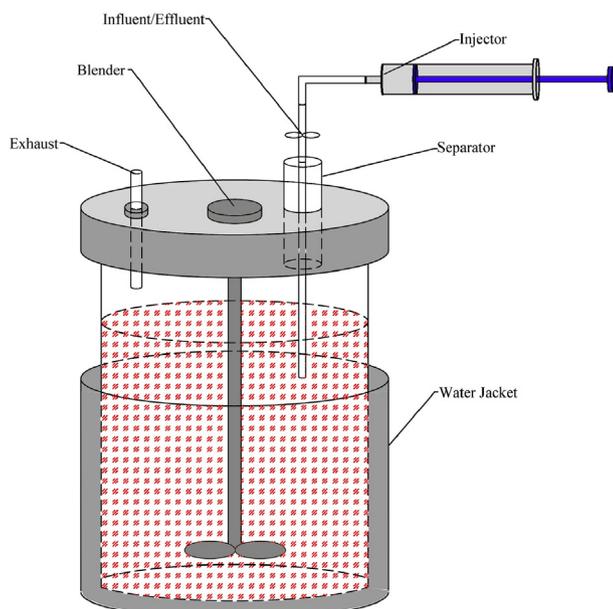


FIG. 1. Schematic diagram of the SBR.

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