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

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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 COD = 720 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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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 μm 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).

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

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

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.

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

where ARE was $\text{NH}_4^+ - \text{N}$ removal efficiency (%); $\text{ARE}_{\max}$ 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 $\text{NH}_4^+ - \text{N}$. R_{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.

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

$$R_{\text{ammonia}} = R_{\max}(S_i - S_e) \quad (6)$$

$$\text{SAA} = \frac{R_{\max} S_i \text{ARE}_{\max}}{S_c} \quad (7)$$

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

where SAA was special anammox activity ($\text{kgNH}_4^+ - \text{N}/(\text{kgVSS} \cdot \text{d})$); MSCR was maximum substrate consumption rate ($\text{kgNH}_4^+ - \text{N}/(\text{L} \cdot \text{d})$); R_{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 $\text{COD} \geq 300 \text{ mg/L}$. 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.

FIG. 2. Nitrogen removal performance of anammox process in presence of carbon source: (A) influent and effluent concentrations of nitrogen species; (B) removal efficiencies of nitrogen species; (C) stoichiometric ratios; (D) removal rates of nitrogen species.

$\Delta\text{NO}_3^- - \text{N}/\Delta\text{NH}_4^+ - \text{N}$ decreased. With COD more than 400 mg/L, $\Delta\text{NO}_2^- - \text{N}/\Delta\text{NH}_4^+ - \text{N}$ increased. Besides, the variation of $\Delta\text{NO}_2^- - \text{N}/\Delta\text{NH}_4^+ - \text{N}$ lagged behind $\Delta\text{NO}_3^- - \text{N}/\Delta\text{NH}_4^+ - \text{N}$. Previous report indicated that, in presence of organic matter, AAOB not only showed anammox metabolic characteristics, but also displayed denitrifying metabolic mechanism (21). Nitrogen removal through anammox might be versatile in presence of carbon source.

When COD was no more than 480 mg/L, ammonia removal rate (ARR) was stabilized around 0.31 kg/(m³·d). Then, it dropped gradually with increasing COD. When COD was 720 mg/L, it was 0.19 kg/(m³·d). Independent of COD content, nitrite removal rate (NiRR) was around 0.41 kg/(m³·d). Total nitrogen removal rate (TNRR) was around 0.62 kg/(m³·d) at COD ≤ 200 mg/L. Then it increased with growing COD and peaked at 0.68 kg/(m³·d) with 560 mg/L COD. An early study reported that simultaneous partial nitrification, anammox and denitrification process was affected more by nitrogen loading rate (NLR) than by organic loading rate (OLR) (37). However, our results were similar to simultaneous anammox and denitrification process that nitrogen removal was affected greatly by OLR (38).

Feature of pH and FA pH variations of anammox process with different carbon source concentrations are presented in Fig. 3. In general, independent of COD content, effluent pH was higher than that in influent. When COD was no more than 120 mg/L, ΔpH was around 0.68. Then it grew with increasing COD. Based on Eq. 1, each mole $\text{NH}_4^+ - \text{N}$ removal consumed 0.13 mol H⁺. With increasing carbon source content, denitrification occurred in reactor. Heterotrophic denitrification reaction also consumed H⁺. As a result, effluent pH was higher than influent pH and ΔpH increased with growing carbon source. However, ΔpH decreased again when COD was more than 400 mg/L. With 720 mg/L COD, ΔpH was around 0.4. When carbon source content was high, anaerobic fermentation occurred in the reactor (Eqs. 9 and 10). COD was removed and H⁺ was produced. As a result, anammox, denitrification and anaerobic fermentation coexisted in reactor when carbon source content was high.

The relationship between pH and $\text{NH}_4^+ - \text{N}$ removal within each operating cycle was also explored and the data are presented in Fig. 4. In order to study pH- $\text{NH}_4^+ - \text{N}$ correlation, linear fitting of pH- $\text{NH}_4^+ - \text{N}$ was performed with each carbon source concentration. Independent of carbon source content, there was good correlation between $\text{NH}_4^+ - \text{N}$ concentration and pH value (date not

FIG. 3. Variation of pH with carbon source concentration.

FIG. 4. The relationship between pH and $\text{NH}_4^+ - \text{N}$.

shown). Besides, both the slope and intercept of fitting equation were quite similar to each other. As a result, a new formula ($y = 8.18 - 0.0086x$) was deduced. $\text{NH}_4^+ - \text{N}$ concentration could be calculated through determining pH value, and pH could be as an indicator for $\text{NH}_4^+ - \text{N}$ removal.

The pH increase could enhance FA concentration and high FA concentration could inhibit anammox reaction (39,40). FA variation within each operating cycle is presented in Fig. 5. At COD ≤ 400 mg/L, FA concentration decreased with reaction time and there was little FA in effluent. However, effluent FA concentration increased with carbon source added further. When COD was 720 mg/L, effluent FA concentration was 4.1 mg/L. Previous study indicated that anammox process could be operated well when FA was less than 20–25 mg/L (40). Similar result was reported by Waki et al. (39) that 13–90 mg/L FA was toxic to AAOB. However, Jung et al. (41) and Jaroszynski et al. (42) found that anammox process would be inhibited when FA concentration was only 1.4 mg/L. When anammox process was operated in presence of carbon source, double effects resulting from organic matter and FA might inhibit AAOB.

Activity of AAOB Batch test was conducted to determine SAA in presence of carbon source. The results are presented in Fig. 6. When COD was less than 200 mg/L, SAA was stabilized at about 0.35 kg $\text{NH}_4^+ - \text{N}$ /(kgVSS·d). Then it increased with increasing carbon source content and peaked at 0.39 kg $\text{NH}_4^+ - \text{N}$ /(kgVSS·d) with 400 mg/L COD. When carbon source was added further, it decreased greatly. It was only 0.19 kg $\text{NH}_4^+ - \text{N}$ /(kgVSS·d) with 720 mg/L COD, which led to a loss around 50%. High carbon source concentration inhibited the activity of AAOB.

Regardless of carbon source concentration, Chamchoi et al. (15) indicated that activity of AAOB dropped gradually with increasing COD and organic matter inhibited anammox process. Tang et al. (18) reported that SAA sharply decreased from 0.27 kg $\text{NH}_4^+ - \text{N}$ /kgVSS·d (COD = 0 mg/L) to 0.07 kg $\text{NH}_4^+ - \text{N}$ /kgVSS·d (COD = 700 mg/L). However, our results agreed with Du et al. (43) that activity of AAOB increased when a small amount of organic matter was added. Carbon source content was a key parameter and proper COD concentration even benefited anammox process. Xu et al. (44) found that anammox was not suitable for wastewater with a COD/ $\text{NH}_4^+ - \text{N}$ ratio >1, because the autotrophic AAOB were no longer able to outperform the heterotrophic denitrifying bacteria. In this work, the activity of AAOB was strengthened at COD/ $\text{NH}_4^+ - \text{N}$ ratio = 5 and nitrogen removal performance was good in presence of 400 mg/L carbon source. With COD/ $\text{NH}_4^+ - \text{N}$ ratio >5, the activity of AAOB was affected.

FIG. 5. FA concentrations in a typical operating cycle at different carbon source concentrations.

And the loss of activity was more than 50% at $\text{COD}/\text{NH}_4^+ - \text{N}$ ratio = 9.

This index gives us the reasonable range of COD level in influent, and it is very useful to maintain the stability of sewage treatment plant which applying the anammox technology. According to the results obtained, we should take caution when the influent $\text{COD}/\text{NH}_4^+ - \text{N} > 5$. In extreme cases that $\text{COD}/\text{NH}_4^+ - \text{N}$ ratio much higher than 5, the dilution of influent is necessary.

Kinetic analysis The relationship between $\text{NH}_4^+ - \text{N}$ removal and SAA in an operating cycle was described by remodified logistic model (Eq. 8). The results are presented in Fig. 7. Independent of carbon source content, all plots of the remodified logistic model showed a high correlation (R^2 more than 0.98). 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.

From the plots, the values of $\text{ARE}_{\max}$, SAA and lag time of $\text{NH}_4^+ - \text{N}$ removal (λ) at different CODs could be acquired. The λ

values with different carbon concentrations were 0.69–1.93 h, which were in good agreement with experimental data. In general, there was a lag phase before $\text{NH}_4^+ - \text{N}$ was removed. The value of λ mainly depended on the external surroundings and it increased with growing COD. High carbon source content not only led to low activity of AAOB, but also lengthened reaction time.

Various other substrate inhibition models such as, Haldane, Bhatia et al., Parkin and Speece models could be applied for predicting inhibition kinetics in anammox SBR, and they were all well predicted the inhibition of anammox process (45,46). However, different to these models, the remodified Logistic model used in present research could simulate the nitrogen removal performance in presence of carbon source in a typical operation cycle. Meanwhile, the SAA and the λ could be predicated. In presence of carbon source, we not only found the effect on SAA intuitively, but also revealed the influence on λ which decreased with the anammox activity.

The validity of remodified logistic model was also analyzed by comparing experimental data and model-predicted values. The results are presented in Fig. 8. When carbon source content was more than 560 mg/L, the experimental SAA values were higher than predicted ones. However, the high value of correlation coefficient ($R^2 = 0.9337$) suggested good linear relationship between the experimental data and the predicted value of SAA. The t-value was 0.97 which was lower than that of the t_{crit} (2.201). According to Tomar and Gupta (30), this result demonstrated that the model was unbiased and it could be suitably applied for determining the SAA in SBR. With regard to ARE, in dependent of carbon source concentration, the predicted values were in quite good agreement with experimental ones. The linear fitting relationship between experimental ARE values and predicted ones was very good ($R^2 = 0.9805$). The t-value was 0.10 which was also much lower than that of the t_{crit} . This result also demonstrated that the model was unbiased and it could be suitably applied for determining the ARE in SBR. In summary, the remodified logistic model was validated further to assess its applicability for describing and predicting nitrogen removal performance of anammox in presence of carbon source.

FIG. 6. Variation of SAA with carbon source concentration.

FIG. 7. Kinetic characteristics at different carbon source concentrations.

FIG. 8. Comparison of predicted value and experimental data: (A) SAA; (B) ARE.

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