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Enhanced Enrichment Characteristics and Inhibition Kinetics Characteristics of the Anammox Granular Sludge

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ABSTRACT

The anammox granular sludge was enriched by shortening the hydraulic retention time (HRT) (from 27 h to 6.67 h) in the UASB reactor, which was fed with ammonium chloride and nitrite as the substrates, and the effect of different HRTs on the nitrogen removal performance of anammox granule sludge was studied. After 159 d of operation, the total nitrogen loading rate (NLR_{TN}) reached 1.72 Kg/(m^3·d), the total nitrogen removal rate (NRR_{TN}) reached 1.33 Kg/(m^3·d), and the removal efficiencies of NH_4^+-N ($NRE_{NH+ 4-N}$) and NO_2^--N ($NRE_{NO^-_2-N}$) were over 95%. The ratio of ΔNO_2^--N/ΔNH_4^+-N was 1.31 and ΔNO_3^--N/ΔNH_4^+-N was 0.24, which complied with the chemical reaction stoichiometry of anammox. The colour of anammox granular sludge changed from light red to deep red; the percentage of granular sludge larger than 1.5 mm was the highest, which proportionately accounted for 62.32%; and the surface of the granular sludge was found, via Fourier transform infrared spectroscopy (FTIR), to contain abundant functional groups. The inhibitory effect of substrates (NH_4^+-N and NO_2^--N) on anammox was studied via an inhibition kinetics batch test using anammox granular sludge (Day 159) in the UASB reactor, and the test results were fitted in the Haldane inhibition model with correlation coefficients (R^2) of 0.9912 and 0.9949.

INTRODUCTION

The development of petrochemical, food and pharmaceutical industries, as well as the improvement of people's living standards, has sharply driven up the nitrogen content in urban sewage, industrial sewage, and landfill leachate, which has caused the severe nitrogen pollution problem in China's bodies of water. Anammox is a novel biological nitrogen removal technology. In the anaerobic condition, NO_2^--N is used as an electron acceptor, NH_4^+-N is used as an electron donor, and the reaction product is N_2 (Van de Graaf et al. 1996). The reaction formula is shown as Eq. (1), in which NH_4^+-N:NO_2^-:NO_3^- is 1:1.32:0.26. When compared with the traditional biological nitrogen removal technology, the anammox process has the advantages of low energy consumption, high efficiency, no addition of organic carbon sources, and low operating costs (Mulder et al. 1995).

$$NH^+_4 + 1.32NO_2^- + 0.066HCO_3^- + 0.13H^+ \rightarrow 1.02N_2 + 0.26NO_3^- + 0.066CH_2O_{2.5}N_{0.15} + 2.03H_2O \quad \ldots(1)$$

Currently, more than 200 full-scale anammox reactors have been applied in wastewater treatment plants around the world, and anammox has broad application prospects as a new biological nitrogen removal technology (Kang et al. 2019, Cao et al. 2017). The granulation of anammox bacteria have excellent sedimentation, can retain high biomass, and can improve the impact load resistance and the exchange of substances and information between anammox bacteria. However, anammox growth is slow, the doubling time is 11 d (Strous et al. 1998), and anammox bacteria are extremely sensitive to environmental conditions, including the environmental temperature (30-40°C), pH (7.5-8.0), and loading (Tang et al. 2017). Investigating how anammox granular sludge can be enriched has become a research hotspot (Lin & Wang 2017).

Although ammonia and nitrite were used as substrates for anammox bacteria, more than a certain concentration will inhibit the growth of anammox bacteria. Chen et al. (2011) used EGSB as a reactor and anammox granular sludge with the MLVSS of 31.31 g/L as inoculated sludge. The Haldane model was used to describe the degradation kinetics of inhibition by the test. When the concentration of ammonia reached 707.9 mg/L and nitrite reached 768.1 mg/L, their degradation rates were at maximum with ammonia reaching 381.2 mg/gVSS·d and nitrite reaching 304.7 mg/gVSS·d. Therefore, studying the inhibition kinetics of anammox has significance for guiding microbial growing.

Consequently, this paper will combine the continuous flow and sequencing batch test to study (1) the effects of

HRT on the enrichment characteristics of anammox granular sludge and its nitrogen removal performance; (2) granular sludge morphology and its functional group composition; and (3) the inhibition kinetics of substrate concentration on anammox granular sludge, to provide a theoretical basis and technical support for the practical engineering application of the anammox process.

MATERIALS AND METHODS

Anammox UASB reactor: Fig. 1 shows the UASB reactor, which was composed of Plexiglass with a diameter of 11 cm and a height of 110 cm. The reactor's upper part was equipped with a three-phase separator for the separation of sludge, water and gas. The reactor was enwrapped with soft black material for heat preservation and protection from light, a water bath cycle was arranged outside the main reactor, two heating rods were arranged in the water bath's circulation tank for controlling the temperature inside the reactor, and the reactor had external reflux for controlling its rising velocity.

Test water and inoculated sludge: In this experiment, a continuous culture was carried out, and the anammox granule sludge was further enriched with anammox flocculating sludge and anaerobic granule sludge, both of which were cultured for 160 days under inorganic environmental conditions as inoculated sludge.

Synthetic wastewater was used in the test. The concentration of ammonia nitrogen and nitrite nitrogen was 1:1.32, the concentration of KH_2PO_4 was 27.2 mg/L, the concentration of $CaCl_2{\cdot}2H_2O$ was 180 mg/L, and the concentration of $MgSO_4{\cdot}7H_2O$ was 300 mg/L. The concentrations of trace elements I and II were both 1 mL/L. Trace element I (g/L) consisted of 5 EDTA and 5$FeSO_4$ and trace element II (g/L) consisted of 15 EDTA, 0.43 $ZnSO_4{\cdot}7H_2O$, 0.24 $CoCl_2{\cdot}6H_2O$, 0.99 $MnCl_2$ $4H_2O$, 0.25 $CuSO_4{\cdot}5H_2O$, 0.22 $NaMoO_4{\cdot}2H_2O$, 0.19 $NiCl_2{\cdot}6H_2O$, 0.21 $NaSeO_4{\cdot}10H_2O$ and 0.014 H_3BO_3.

Analysis methods and calculation formulas: Measurements were made every other day, and the water samples were filtered through 0.45 μm filter paper. Test methods were determined via standard methods (APHA 1998), with NH_4^+-N, NO_2^--N, and NO_3^--N determined by a spectrophotometry determination of Nessler's Reagent, N-(1-naphthyl)-ethylenediamine spectrophotometric determination, and ultraviolet spectrophotometry. DO and pH were determined by German Multi3630, and MLSS and MLVSS were determined by the gravimetric method.

Fourier infrared spectroscopy analysis method: The dried anammox granular sludge and KBr were tableted at a dose of 1:100, then analysed by Fourier transform infrared spectroscopy. The collected data were analysed by origin 8.0.

Granular sludge particle size determination method: The particle size distribution of the granular sludge was determined by wet sieving (Laguna et al. 1999) with stainless steel meshes with mesh aperture diameters of 3.0, 2.5, 2.0, 1.5, 1.0 and 0.5 mm. The sludge mixture was taken out of

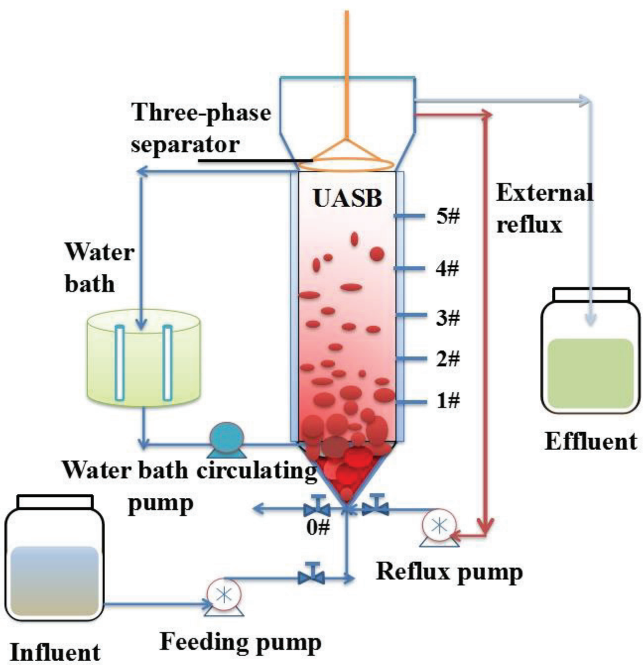


Fig. 1: Schematic diagram of the anammox granular sludge-based UASB reactor.

the reactor, and the steel sieve was positioned vertically from top to bottom according to pore size. A container was placed under the bottom 0.5 mm sieve to hold the fine particles, and the sludge sample was slowly poured into the sieve. After screening, the granular sludge of different particle size ranges was collected into different containers, and the MLSS of each screen that intercepted the granular sludge was measured.

Inhibition kinetic sequencing batch test: The anammox granular sludge (159 d) was taken from the UASB reactor, rinsed three times with clean water to remove the surface matrix, and filtered with filter paper. About 12 g of wet sludge were weighed with an electronic balance, then placed into a 250 mL serum bottle, as shown in Fig. 2. The water bath temperature was controlled to about 33°C, the pH value was controlled at 7.3-7.6, a 10 mL syringe was used for sampling, and the solution was fully mixed with a magnetic stirrer. In the single factor inhibition experiment with NH_4^+-N, other conditions must be controlled. The NH_4^+-N gradients were about 70, 100, 200, 320, 500, and 600 mg/L. In the single factor inhibition experiment with NO_2^--N and other conditions must also be controlled. The NO_2^--N gradients were about 80, 110, 210, 270, 300, and 400 mg/L. Five mL was taken with a 10 mL syringe every 1 h, and the mixture was centrifuged to determine the NH_4^+-N, NO_2^--N and NO_3^--N of the supernatant, while the degradation rate of NH_4^+-N and NO_2^--N were calculated.

RESULTS AND DISCUSSION

Effect of different HRTs on the nitrogen removal performance of anammox granular sludge: The UASB reactor temperature was maintained at 32~34°C, the influent pH value was controlled at 7.3~7.8, and the return flow was kept constant. The test was run under different HRT conditions, and when $NRE_{NH+4\text{-}N}$ and $NRE_{NO_2^-\text{-}N}$ reached 97% or more, HRT conditions were adjusted, along with the concentrations of ammonia and nitrite as given in Table 1.

Removal efficiency of NH_4^+-N and NO_2^--N by HRTs: The removal efficiency of NH_4^+-N and NO_2^--N under different HRT conditions are shown in Fig. 3(a) and (b). In phase I (HRT was 27 h), the anammox granular sludge was adapted to the environment because the inoculated sludge HRT was 27h. After 19 d of operation, $NRE_{NH_4^+\text{-}N}$ was 99.03%, and $NRE_{NO_2^-\text{-}N}$ was 99.18%, while the anammox granular sludge was not inhibited. The removal loading of NH_4^+-N ($NRR_{NH_4^+\text{-}N}$) was 0.17 kg/m^3·d and NO_2^--N ($NRR_{NO_2^-\text{-}N}$) was 0.22 kg/m^3·d. In stage II, the other conditions were essentially unchanged. When HRT was shortened to 20.86 h, the substrate loading suddenly increased. The $NRE_{NH_4^+\text{-}N}$ decreased to 93.42% and

Table 1: Operating parameters of anammox granular sludge reactor at different stages.

Stage	Time (d)	HRT (h)	Velocity (m/h)	Reflux ratio	NH_4^+-N concentration (mg/L)	NO_2^--N concentration (mg/L)	NLR (kg/m^3·d)	NRR (kg/m^3·d)
I	1~19	27.00	4.78	121	187.29	242.48	0.40	0.34
II	20~47	20.86	4.79	90	198.24	253.60	0.54	0.45
III	48~81	15.50	4.80	70	177.96	233.70	0.70	0.55
IV	82~119	8.63	4.86	40	175.56	234.39	1.16	0.95
V	120~159	6.67	4.89	30	173.19	234.17	1.72	1.33

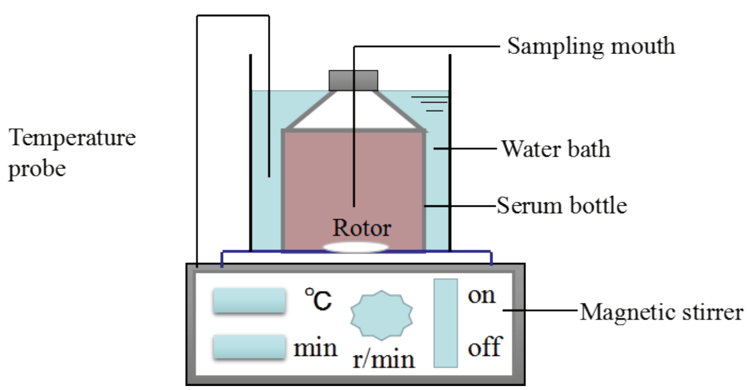


Fig. 2: Schematic diagram of the batching test.

$NRE_{NO_2^--N}$ to 95.31%, while $NRR_{NH_4^+-N}$ rose to 0.21kg/m^3·d and $NRR_{NO_2^--N}$ rose to 0.29kg/m^3·d. After 27 d, $NRE_{NH_4^+-N}$ reached 98.15% and $NRE_{NO_2^--N}$ reached 98.72%.

When HRT was shortened to 15.50 h, in Phase III, the $NRE_{NH_4^+-N}$ began to decrease due to the sudden shortening of HRT and fluctuated around 90%. However, the decrease of NO_2^--N was large, and $NRE_{NO_2^--N}$ decreased from 98.72% in the previous phase to 87.74%, indicating that the inhibition of nitrite nitrogen was more obvious. After 33 d of operation, the activity of anammox bacteria was restored, $NRE_{NH_4^+-N}$ was 97.54% and $NRE_{NO_2^--N}$ was 97.37%, and $NRR_{NH_4^+-N}$ reached 0.27 kg/m^3·d and $NRR_{NO_2^--N}$ reached 0.36 kg/m^3·d. In phase IV (HRT was 8.63 h), the anammox granular sludge activity was better, and at the end of phase IV, $NRE_{NH_4^+-N}$ was 98.27% and $NRE_{NO_2^--N}$ was 98.68%.

In phase V, the HRT was shortened to 6.67 h, $NRE_{NH_4^+-N}$ was significantly reduced to 86.05%, and $NRE_{NO_2^--N}$ was decreased to 79.95%. The reasons for analysis are as follows: (1) The shorter the HRT, the higher the loading of the ammonia nitrogen and nitrite nitrogen, and the anammox activity was inhibited, resulting in a decrease of the treatment effect; (2) The HRT was short and part of the ammonia and the nitrite were discharged outside of the reactor without reaction, increasing the effluent concentration, and a decrease of the removal loading of the ammonia and nitrite. After 39 days, $NRE_{NH_4^+-N}$ was 97.05% and $NRE_{NO_2^--N}$ was 98.21%, and the anammox activity was further improved.

Removal efficiency of TN by HRT: The removal efficiency of TN (NRE_{TN}) under different HRT conditions is shown in Fig. 3(c) and (d). HRT was 27h in phase I. On the first day

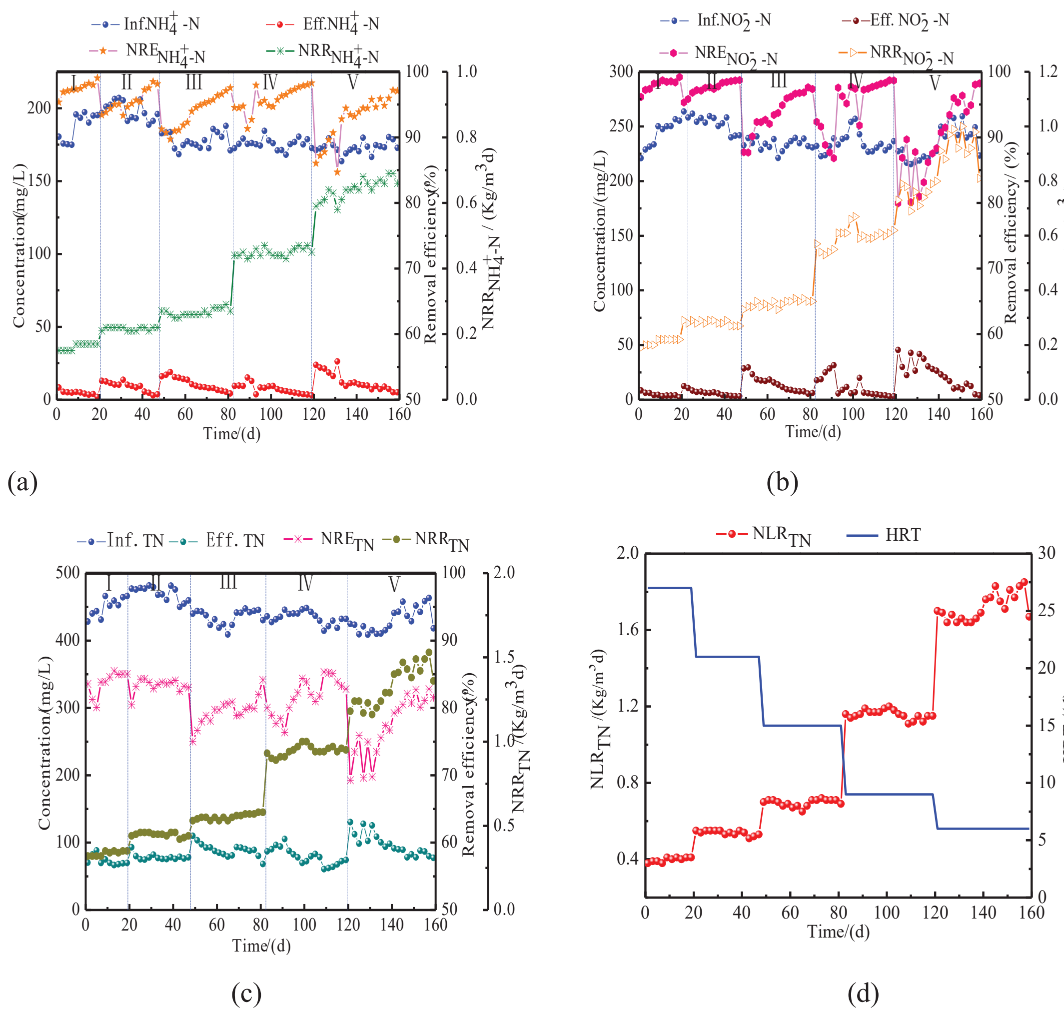


Fig. 3. Under different HRTs (a) NH_4^+-N, (b) NO_2^--N, (c) TN nitrogen removal performance, and (d) the variation of NLR.

of operation, TN concentration was 428.04mg/L, total nitrogen output was 70.33 mg/L, NRR_{TN} was 0.32 kg/m^3·d, and NRE_{TN} was 83.57%. After 19 d of operation, NRE_{TN} reached 85.02%, and NRR_{TN} was increased to 0.35 kg/m^3·d. Other conditions remained unchanged. When HRT was shortened to 20.86 h, on the first day of operation NRE_{TN} decreased to 83.77%, and NRE_{TN} continued to drop to 80.47% on the second day. The anammox activity was slightly inhibited due to the shortening of HRT, however, after 27 d of operation, NRE_{TN} reached 83.01%, NRR_{TN} reached 0.44 kg/m^3·d, and NLR_{TN} reached 0.53 kg/m^3·d. The anammox activity was also improved. Then HRT was shortened to 15.50 h, and NRE_{TN} was reduced to 75.01%. After 33 d, NRE_{TN} was increased to 84.16%, NRR_{TN} reached 0.58 kg/m^3·d, and NLR_{TN} was 0.69 kg/m^3·d. In phase IV, HRT was 8.63 h, anammox activity was high, NRR_{TN} was reduced by about 2%, and NRE_{TN} was maintained at about 80%. When HRT was shortened to 6.67 h, NRE_{TN} was decreased by 69.27%, and activity was severely inhibited, resulting in a significant decrease in the removal efficiency. After 39 d, anammox activity was essentially restored, NRE_{TN} reached 81.53%, NRR_{TN} was 1.33kg/m^3·d and NLR_{TN} was 1.72 kg/m^3·d. This showed that anammox activity was further improved and the ability to withstand the loading of nitrogen was strengthened. Yu et al. (2014) studied the effect of nitrogen performance on different HRTs, with HRTs of 72 h, 48 h, 24 h, 12 h and 6 h, NLR_{TN} was increased from 0.28 kg/m^3·d to 1.28 kg/m^3·d and the anammox activity was further improved with the shorting of HRT.

Changes in stoichiometry under different HRT conditions: Fig. 4 shows the changes of ΔNO_2^--N/NH_4^+-N and ΔNO_3^--N/NH_4^+-N under different HRT conditions. Table 2 shows the average NO_2^--N/NH_4^+-N values and ΔNO_3^--N/NH_4^+-N values in different phases. Formula (1) shows the chemical reaction equation of anammox, the ratio of ΔNO_2^--N/ΔNH_4^+-N is 1.32 and ΔNO_3^--N/ΔNH_4^+-N is 0.26. These ratios can determine the basis of the anammox reaction. Fig. 4 shows that the ratio of ΔNO_2^--N/ΔNH_4^+-N fluctuated around 1.32 and ΔNO_3^--N/ΔNH_4^+-N fluctuated around 0.26, which complied with the chemical reaction stoichiometry of anammox. Kang et al. (2019) concluded that the ratio of ΔNO_2^--N/ΔNH_4^+-N was 1.22 and ΔNO_3^--N/ΔNH_4^+-N was 0.22, and the results of this experiment were similar to Kang et al. (2019).

The Morphology of Anammox Granular Sludge

Apparent characteristics: Fig. 5 shows the apparent characteristics of anammox granular sludge at the initial phase of inoculation and operation for 159 d. The colour of anammox granular sludge was light red, and the average particle size was small at the initial phase of inoculation; when the reactor was operated for 159 d, the colour of anammox granular sludge turned dark red. Tang et al. (2011) concluded that the contents of heme C increased with the increase of NRR_{TN}, which lead to the deepening of colour in a similar result to this experiment. Anammox bacteria exhibit the flocculation effect; under the shearing force of a large rising flow rate, the

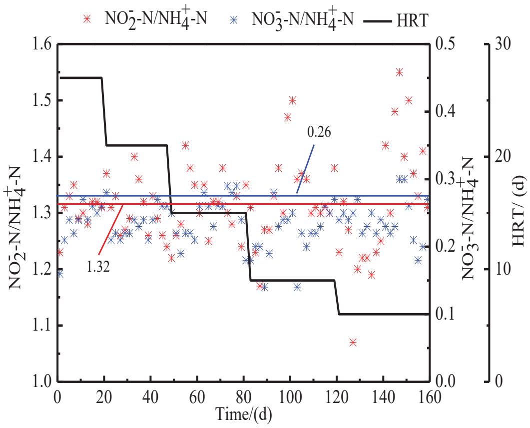


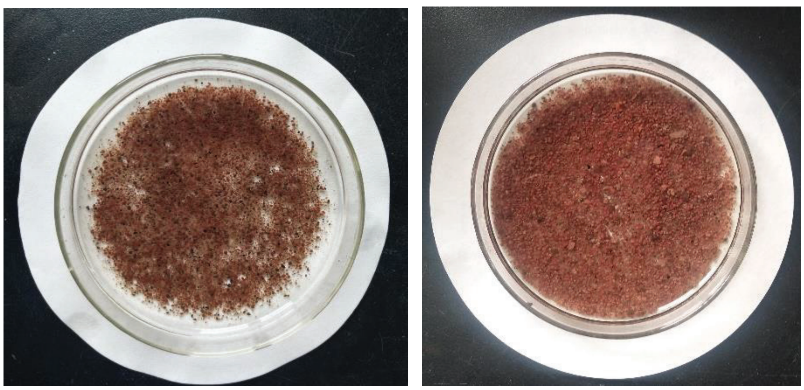
Fig. 4: ΔNO_2^--N/NH_4^+N and ΔNO_3^--N/NH_4^+N under different HRT conditions.

Table 2: Average values of ΔNO_2^--N/NH_4^+N and ΔNO_3^--N/NH_4^+N under different HRT conditions.

HRT/h	27.00	20.86	15.50	8.63	6.67
ΔNO_2^--N/ΔNH_4^+-N	1.30	1.31	1.31	1.33	1.31
ΔNO_3^--N/ΔNH_4^+-N	0.23	0.24	0.24	0.22	0.24

cells gather together and are tired, and anammox granular sludge continues to increase (Lin et al. 2019).

Change in particle size distribution: Fig. 6 shows the particle size distribution of the granular sludge at the bottom of the UASB reactor. The particle size distribution of the granular sludge was characterized by the percentage of the total MLSS of the measured sludge in the range of the particle size gradient. In the initial inoculation, the particle size was 0.50-1.00 mm of granular sludge which accounted for the highest percentage (specific gravity of 27.42%), followed by 1.00-1.50 mm of granular sludge (specific gravity of 25.84%), and the percentage of sludge with a particle size less than 0.50 mm was as high as 13.89%. However, granular sludge with a particle size greater than 3.00 mm has the lowest proportion with only 1.09%. Shortening HRTs increase the rising flow rate, and granular sludge growth was promoted. Cong et al. (2014) showed that the particle diameter was in the range of 0.5-2.0 mm by adjusting the rising flow rate to 9.0 m·h^{-1} in the EGSB reactor, where granular sludge accounted for more than 65%. After 159 d operating, the particle size of the granular sludge in the reactor showed an increasing trend. Granular sludge with a particle size larger than 1.5mm accounted for 62.32%, with the particle size of 1.50-2.00 mm accounting for the highest proportion of the test (27.53%). The specific gravity of 2.00-2.50 mm was 18.47%, the proportion of 2.50-3.00 mm was 11.03%, particle size greater than 3 mm increased by 4.1 percentage points from the initial inoculation, and the specific gravity of granular sludge with the particle size of 1.00-1.5 mm was 14.04%. The specific gravity of granular sludge with particle size less than 0.50 mm was only 8%, which was 5.89 percentage points lower than the initial inoculation. An et al. (2013) obtained the physical properties of anammox granules with different sizes. 1.0~1.5 mm granular sludge



(a) (b)

Fig. 5: The variation of anammox granular sludge in (a) 0 d and (b) 159 d.

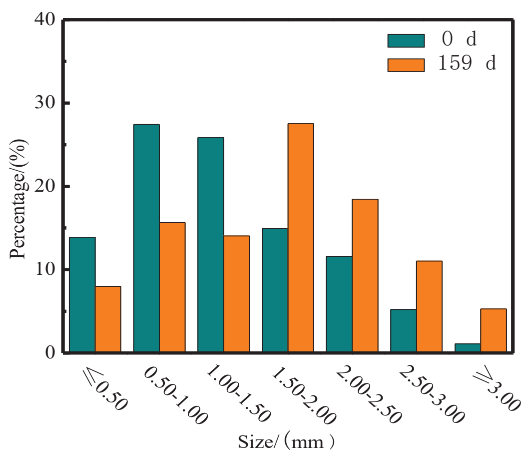


Fig. 6: The changes in the size distribution of anammox granules at different phases.

has the highest activity, and larger granular sludge was more resistant to temperature and nitrogen load shock. However, larger particles also showed larger gas passages and internal voids, reducing the stability of the granular sludge. It can be seen that on the 159 d of the reactor operation, the particle size of the anammox granular sludge gradually changed from small to large. In this experiment, the anammox granular sludge was mainly concentrated in the range of less than 1.00 mm, and the domesticated granular sludge was obtained. The diameter was concentrated in the range of more than 1.50 mm, which was essentially in the particle size range with the highest activity of anammox granular sludge.

FT-IR analysis of granular sludge: Secretions during the cell grew, the shedding of the cell surface, cell lysis and the adsorption of substances from the external environment can carry a large number of functional groups on the surface of the sludge. There are currently many studies on the compositions of EPS and the characteristics of anammox granular sludge (Lotti et al. 2019, Feng et al. 2019, Chen et al. 2016, Fang et al. 2018), but few scholars have reported on the surface functional group properties of anammox granular sludge.

Fig. 7 shows that the characteristics of the functional groups' composition of anammox granular sludge by infrared spectroscopy. In the beginning, the vibration near 3393 cm^{-1} mainly represented O-H stretching vibration and represented the alkane organic matter and C-H in the polysaccharide molecule near 2930 cm^{-1}. O-H stretching vibration in the carboxyl functional group was represented at around 2525 cm^{-1}, C=O stretching vibration in protein amide I was represented at 1600-1700 cm^{-1}, and the peak near 1040 cm^{-1} represented C-O-C stretching of polysaccharide or similar polysaccharide substance vibration. In addition, an obvious absorption peak was also found near the wavelengths of 880 cm^{-1}, 710 cm^{-1}, and 600-900 cm^{-1} which were the fingerprint areas (Yan et al. 2015, Badireddy et al. 2010). Infrared spectroscopy revealed sugar and protein in the surface of anammox granular sludge. As the reaction progressed, a more obvious peak appeared near 3290 cm^{-1}, which was mainly caused by N-H stretching vibration in amides. The peak intensities of 2930 cm^{-1} and 1788 cm^{-1} increased, indicating C-H stretching in the alkane organic matter and polysaccharide molecules. C=O stretching vibration in the vibration and protein amide I were enhanced, and the sugar and protein contents were further enhanced.

Substrates Inhibition of Anammox and Its Kinetic Analysis

Anammox bacteria used ammonia and nitrite as substrates. At low concentrations, anammox bacteria used the substrates to react. However, at high concentrations, the activity of anammox bacteria is inhibited.

Substrates inhibition kinetics can be described using the Haldane model, which was:

$$\nu = \frac{\nu_{\max}}{1 + \frac{Ks}{S} + \frac{S}{K_h}} \qquad \ldots(1)$$

Where ν is the removal rate of substrates, mg/(mg·d); ν_{max} is the maximum conversion rate, mg/(mg·d); S is the substrate concentration, mg/L; k_S is a half-saturation constant, mg/L; and K_h is the inhibition kinetic constant of Haldane, mg/L.

Since the Haldane model was a kinetic simulation of a single substrate reaction, for anammox reactions under dual substrates conditions, it was first necessary to control a substrate concentration to examine the effect of another substrate concentration. Firstly, the NO_2^--N concentration was controlled to 100 mg/L, and the effect of NH_4^+-N concentration on anammox activity was studied. Table 3 shows that in the range of experimental concentration with the increase of NH_4^+-N concentration, the degradation rate increased at first and then decreased.

Fig. 8 shows the kinetic characteristics of ammonium nitrogen inhibition. The correlation kinetic data of the measured ammonium was fitted to the Haldane model using Origin 8.0 software. The correlation coefficient (R^2) of the fitted curve was 0.9912, and the correlation was good. When the NH_4^+-N concentration reached 303.03 mg/L, the anammox

Table 3: Effect of NH_4^+-N concentrations on Anammox bacterial activity.

NO_2^--N concentration (mg/L)	NH_4^+-N concentration (mg/L)	The degradation rate of NH_4^+-N [mg/(mg.d)]	The degradation of NO_2^--N [mg/(mg.d)]
100	70	0.075	0.130
100	100	0.105	0.098
100	200	0.126	0.109
100	320	0.139	0.113
100	500	0.127	0.108
100	600	0.121	0.084

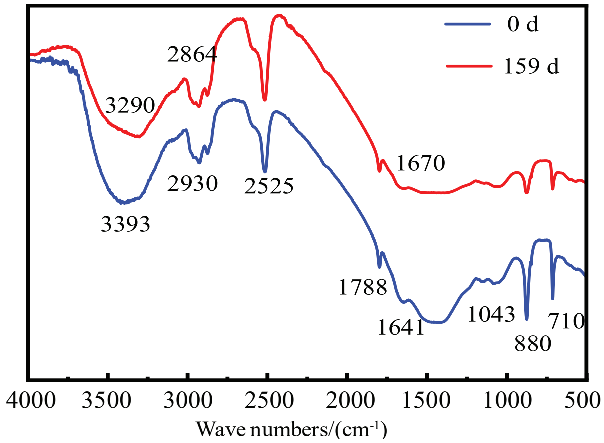


Fig. 7: The FT-IR spectra of granular sludge.

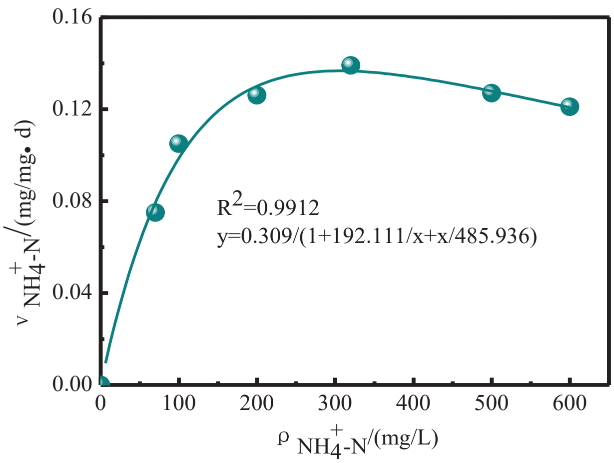


Fig. 8: Inhibition kinetic characteristics of NH_4^+-N concentration on anammox granular sludge.

activity was the highest, and the NH_4^+-N degradation rate was 0.137 mg/(mg.d). When the NH_4^+-N concentration was 600 mg/L, the degradation rate of NH_4^+-N decreased to 0.121 mg/(mg.d), whereas the maximum reaction rate (v_{max}) was 0.309 mg/(mg.d), the half-saturation constant (Ks) was 192.111 mg/L, and the inhibition kinetic constant was 485.936 mg/L.

NH_4^+-N concentration was controlled to be 70 mg/L, and the effect of NO_2^--N concentration on anammox activity was studied. Table 3 shows that in the range of experimental concentration with the increase of NO_2^--N concentration, the degradation rate increased at first and then decreased.

Fig. 9 shows the kinetic characteristics of nitrite inhibition. The correlation inhibition kinetic data of the measured nitrite was fitted to the Haldane model using Origin 8.0 software. The correlation curve (R^2) of the fitted curve was 0.9949 and the result was high. When the concentration of NO_2^--N reached 169.70 mg/L, anammox activity was highest, and the degradation rate of NO_2^--N was 0.115 mg/(mg.d). When the concentration of NO_2^--N reached 400 mg/L, the degradation rate of NO_2^--N decreased to 0.068 mg/(mg.d). The maximum reaction rate (v_{max}) was 0.737 mg/(mg.d), the half-saturation constant (Ks) was 456.739 mg/L, and the inhibition kinetic constant was 61.968 mg/L.

Results showed that when the inhibitor was ammonium, the maximum inhibitory concentration was 303.03 mg/L, the NH_4^+-N degradation rate was 0.137 mg/(mg.d), and the maximum reaction rate was 0.309 mg/(mg·d). When nitrite was an inhibitor, the maximum inhibitory concentration was 169.70 mg/L, the degradation rate of NO_2^--N was 0.115 mg/

Table 4: Effect of NO_2^--N concentrations on Anammox bacteria activity.

NH_4^+-N concentration (mg/L)	NO_2^--N concentration (mg/L)	The degradation rate of NH_4^+-N [mg/(mg.d)]	The degradation of NO_2^--N [mg/(mg.d)]
70	80	0.086	0.092
70	110	0.958	0.104
70	210	0.114	0.111
70	270	0.097	0.108
70	300	0.088	0.101
70	400	0.068	0.083

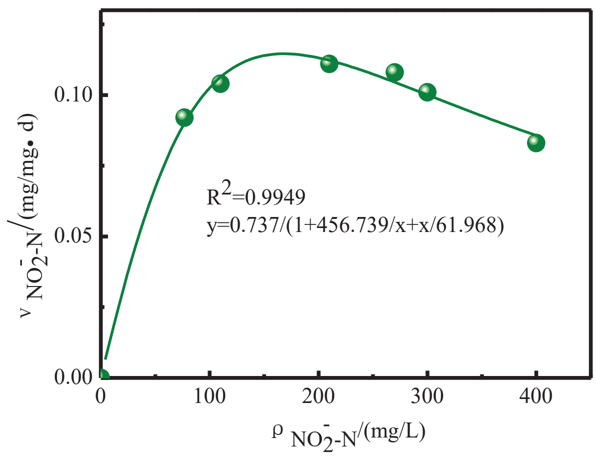


Fig. 9: Inhibition kinetic characteristics of NO_2^--N concentration on anammox granular sludge.

(mg.d), and the maximum reaction rate was 0.737 mg/(mg·d). In contrast, the inhibitory effect of nitrate was more significant in both substrates. Similarly, Li et al. (2016) showed that the removal rate of ammonia and nitrite by inhibition kinetic sequencing batch tests increased at first and then decreased. The ammonia nitrogen concentration was 295.62 mg/L, anammox activity was the highest, and the ammonia nitrogen degradation rate was 0.1540 mg/(mg·d). The nitrite degradation rate was 0.1649 mg/(mg·d) at the concentration of 151.10 mg/L, and anammox activity was the highest.

CONCLUSIONS

(1) Anammox granular sludge that was used UASB as the reactor by shorting hydraulic retention time (HRT shortened from 27 h to 6.67 h) was enriched. After 159 d of operation, the $NRE_{NH+ 4-N}$ and $NRE_{NO_2^- -N}$ reached more than 95%, the NRE_{TN} was 81.53%, and the NRR_{TN} reached 1.33 kg/(m^3.d).

(2) In the initial phase of inoculation, the percentage of granular sludge with the particle size of 0.50-1.00 mm was the highest (specific gravity was 27.42%), and the proportion of granular sludge with particle size greater than 3.00 mm was the lowest, only 1.09%. At 159 d, the average particle size of the granular sludge in the reactor showed an increasing trend, with the highest percentage of 1.50-2.00 mm (specific gravity 27.73%), and the particle size larger than 3 mm accounted for 5.29%, which was 4.1 percentage points higher than the initial inoculation.

(3) Analysing the anammox granular sludge via the Fourier transform infrared spectroscopy revealed that mainly sugar and protein were on the surface of anammox granular sludge. After 159 d of reactor operation, the sugar and protein content further increased.

(4) By inhibition of the kinetic sequencing batch test, the maximum reaction rate was 0.309 mg/(mg·d), the half-saturation constant (*K*s) was 192.111 mg/L, and the inhibition kinetic constant was 485.936mg/L. The nitrite maximum reaction rate was 0.737 mg/(mg·d), the semi-saturation constant (*K*s) was 456.739 mg/L, and the inhibition kinetic constant was 61.968 mg/L. In contrast, the anammox bacteria in the two substrates were more significantly inhibited by nitrate.

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