Desalination and Water Treatment
www.deswater.com
doi: 10.1080/19443994.2015.1084594

57 (2016) 17291–17299
August

Taylor & Francis
Taylor & Francis Group

Novel anammox reactor start-up method using immobilized particles as biocatalyst and its kinetic characteristics

Guanghui Chen[a], Jun Li[a,*], Yonglei Wang[a,b], Hailiang Deng[a], Yanzhuo Zhang[a], Jinping Zeng[a]

[a]*Key Laboratory of Beijing for Water Quality Science and Water Environment Recovery Engineering, Beijing University of Technology, Beijing 100124, China, Tel. +8615652356918; emails: ghui0066@163.com (G. Chen), lijunbjut@163.com (J. Li), wyl1016@sina.com (Y. Wang), 1210793384@qq.com (H. Deng), 83995983@qq.com (Y. Zhang), yufeng569@163.com (J. Zeng)*
[b]*College of Environmental and Municipal Engineering, Shandong Jianzhu University, Jinan 250101, China*

Received 6 January 2015; Accepted 14 August 2015

ABSTRACT

Anaerobic ammonium oxidation (anammox) immobilized particles were added to a reactor as biocatalyst to start an up-flow anammox reactor. The kinetic characteristics of the immobilized particles were studied using batch tests. The results demonstrated that using immobilized particles as the biocatalyst succeeded in starting the anammox reactor in 49 d. The removal rates of NH_4^+-N and NO_2^--N were 80.7 and 83.1%, respectively. The total nitrogen removal load was 0.505 kg m^{-3} d^{-1}. The results of the kinetic characteristics study demonstrated that the half-saturation constants of the immobilized particles for ammonium and nitrite were 1.57 and 1.505 mmol L^{-1}, respectively. These constants were lower than those of flocculent anammox sludge (FAS), which indicated that the immobilized particles utilize the substrate better in adverse environments. The half-suppression constants of the immobilized particles for ammonia and nitrite were 724.2 and 66.65 mmol L^{-1}, respectively, and higher than those of FAS. Thus, the immobilized particles could survive longer in high-concentration substrates. As a biocatalyst in a reactor, the anammox immobilized particles exhibited robust kinetic characteristics and effectiveness, which are important to the development of new biocatalyst techniques for anammox.

Keywords: Anammox; Immobilized particles; Immobilization; Start-up; Inorganic carbon; Kinetic characteristics study

1. Introduction

Anaerobic ammonium oxidation (anammox) is a process in which a type of *Planctomycetes* bacteria uses nitrite as the electron acceptor and oxidizes ammonium salt to produce nitrogen and a small amount of nitrate under anaerobic or anoxic conditions (Eq. (1)) [1]. Compared with traditional biological nitrogen removal processes, the anammox technique is more efficient and less expensive. The anammox technique has a total nitrogen removal rate (NRR) of up to 9.50 kg m^{-3} d^{-1} and can save approximately 60% in the aeration rate and 100% of the organic carbon source [2,3]. Therefore, in recent years, the anammox technique has attracted attention from researchers [4–7]. However, in fed-batch cultures, anammox bacteria grow slowly and with a long generation period,

*Corresponding author.

which typically results in a long anammox start-up period [8–11].

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

Adding anammox bacteria to a reactor has been demonstrated to effectively decrease the start-up time of an anammox reactor (i.e., the biocatalysis technique) [12,13]. Typically, high-rate anammox granules (HAG) are selected as the biocatalyst. However, a long time is required to culture HAG using flocculent anammox sludge (FAS). López [14] and Dapena Mora [15] used a sequencing batch reactor (SBR) to culture anammox particle sludge. The culture time was relatively long (>200 d), and the total nitrogen removal load was less than 2.0 kg m^{-3} d^{-1}. Tang et al. [16] used an expanded granular sludge bed (EGSB) reactor. After steady operation for 230 d, they obtained an anammox particle sludge with a mean particle size of 2.51 ± 0.91 mm. Cong et al. [17] used an EGSB reactor to inoculate aerobic and anaerobic bacteria. They cultured these bacteria for 80 d and obtained a total nitrogen removal load of 4.758 kg m^{-3} d^{-1}. They also obtained a particle-like anammox sludge. However, the mean particle size was only 0.556 mm. These studies demonstrate that anammox sludge particles can be obtained by culturing for a period of time. However, it is impractical to apply anammox sludge particles in practice because of the long culture time. Therefore, the primary goal of the biocatalysis technique is to obtain high-rate anammox bacteria for use as a biocatalyst.

The embedding immobilization technique is a newly developed microbe immobilization technique in biological engineering. Free cells or enzymes are immobilized in a certain area by embedding materials to restrict their activities. Thus, they can be used repeatedly [18,19]. In this study, the embedding immobilization technique was combined with the anammox biocatalysis technique. Common FAS was used to prepare the immobilized particles. Waterborne polyurethane (WPU) was used as the immobilization material, which was previously demonstrated as safe and non-toxic to anammox bacteria. The WPU-immobilized particle exhibits good mechanical strength and good mass transfer performance [20]. Subsequently, the immobilized particles were used to replace HAG as the biocatalyst to be added to an anammox reactor. To provide theoretical support for this novel anammox biocatalysis technique, the reactor's start-up process and the kinetic characteristics of the anammox immobilized particles were investigated.

2. Materials and methods

2.1. Experimental set-up

In this experiment, an up-flow anaerobic sludge bed (UASB) reactor was used. The reactor was constructed of polymethyl methacrylate, and the effective volume of the reactor was 17 L. As shown in Fig. 1, the inner reactor was filled with plastic balls with large holes (with a diameter of 8 cm). Annular braided fiber materials were wrapped inside the balls. Water could flow and expand along the gaps between the plastic balls. Fiberfill was used to reduce sludge loss. The outside of the reactor had a water-bath layer, which was used to maintain the reactor inner temperature at 32°C.

2.2. Inoculation sludge

The inoculation sludge used to start the reactor was collected from the active sludge in the aeration tank of a sewage disposal station in Beijing. The mixed liquor suspended solids (MLSS) of the inoculation were 3,480 mg L^{-1}, the mixed liquor volatile suspended solids (MLVSS) were 2,850 mg L^{-1}, and MLVSS/MLSS were 83.9%, which collectively indicate excellent nitrification.

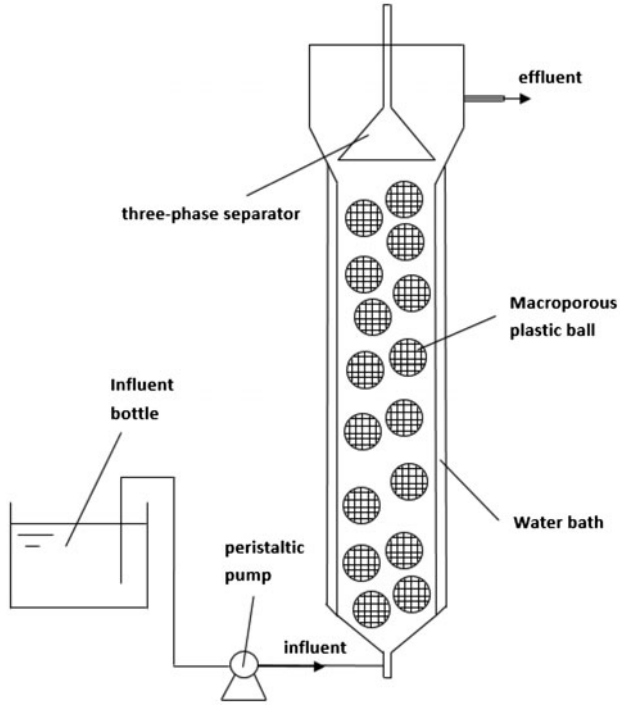

Fig. 1. UASB reactor.

2.3. Preparation of the immobilized particles

The embedding sludge used in this study was FAS. The sludge was obtained from the middle of a UASB reactor that was stably operated for more than two years in our laboratory [21]. The sludge was concentrated to 30 g L^{-1} by centrifugation and mixed with WPU and active carbon power in a beaker. To start polymerization, an initiator (i.e., potassium persulfate) and a promoter (i.e., *N,N,N´,N´*-tetramethylethylenediamine) were added to the beaker. After approximately 30 min, the mixed solution formed a gel, and the gel was cut into 3 mm cubes. The gel carrier contained 8% (w/v) WPU, 0.3% (w/v) initiator, 0.2% (w/v) promoter, 1% activated carbon powder, and 4% (w/v) sludge.

2.4. Water quality

The primary components of the water that was prepared for use in this study are listed in Table 1. The NH_4^+-N and NO_2^--N were provided by NH_4Cl and $NaNO_2$, respectively, with the required concentrations. The trace elements I and II concentrations refer to notations from the literature [22].

2.5. Reaction kinetics measurements

First, 20 mL of anammox immobilized particles (which contained approximately 0.4 g of anammox sludge) and 180 mL of simulated wastewater were mixed in a 300-mL serum bottle. The NH_4^+-N and NO_2^--N concentrations in the simulated wastewater corresponded to the required concentrations. The other components corresponded to the values listed in Table 1 for the anammox period. The mixed solution was ventilated using high-purity N_2 for 20 min to replace the dissolved oxygen (DO). The serum bottle, which was closed with a butyl rubber stopper and wrapped with black reflective paper, was placed in a constant-temperature incubator shaker at 32℃ at a speed of 80 r min^{-1}. Samples were taken every 6 h to calculate the NH_4^+-N and NO_2^--N removal rates. All tests were repeated three times.

The anammox kinetics are limited by the NH_4^+-N and NO_2^--N concentrations. Assuming that the anammox bacteria cell quantities are constant during the experiments and that the reactor contents are well-mixed, the Haldane model [23] can be used to describe the kinetics of the anammox reaction as follows:

$$v = \frac{V_{\max}}{1 + \frac{K_m}{S} + \frac{S}{K_i}} \quad (2)$$

where V is the reaction rate, S is the substrate concentration, $V_{\max}$ is the maximum reaction rate, K_m is the half-saturation constant, and K_i is the half-suppression constant.

All of the batch and continuous-flow experiments were performed under laboratory conditions from March to May.

2.6. Analytical methods

NH_4^+-N, NO_2^--N, NO_3^--N, TN, MLSS, and MLVSS were determined in accordance with standard methods [24]. Temperature and pH values were determined using a WTW/Multi 3,420 multiparameter device. For scanning electron microscopy (SEM), a Hitachi S-4300 was used.

3. Experimental results

3.1. Nitration start-up

Many approaches have been proposed to start an anammox reactor. However, all approaches currently in use involve a microbe activation and expansion

Table 1
Compositions of artificial wastewater

Nitrification culture	Mass concentration (mg L^{-1})	Anammox culture	Mass concentration (mg L^{-1})
$NaHCO_3$	53	$NaNO_2$	134–745
NH_4Cl	712	NH_4Cl	110–705
NaCl	51	$CaCl_2$	118
KCl	22	$KHCO_3$	726
$CaCl_2$	17	KH_2PO_4	31
$MgSO_4{\cdot}7H_2O$	92	$MgSO_4{\cdot}7H_2O$	286
$NaH_2PO_4{\cdot}12H_2O$	1,180	Trace elementI,II	–

Notes: The content of Trace element I and II are 1 mL L^{-1}.

process. Because anaerobic anammox bacteria grow slowly, it is desirable to reduce bacteria loss such that the anammox bacteria can more rapidly become the dominant bacteria in the reactor. In this study, a nitration reactor was used to start the anammox and to inoculate the anammox sludge. Aerobic biofilms were formed on plastic balls with large holes, which enabled the anammox sludge and the biofilms of the nitration to work together to rapidly start the anammox reactor.

During the nitration reaction start-up process, microbes with a substrate affinity can be concentrated by applying a low load (known as the low-load method) based on the biological *K*-strategy. Alternatively, the fast-growing microbes can be concentrated by applying a high load (known as the high-load method) based on the *R*-strategy. In this study, the *R*-strategy (i.e., the high-load method) was adopted. A water solution of NH_4Cl was added to maintain $\rho(NH_4^+\text{-N})$ of approximately 200 mg L^{-1}. The other components are listed in Table 1. In the reactor, an aeration tip was used to maintain a DO level above 7 mg L^{-1}. The hydraulic retention time (HRT) was 6 h. On the 14th day, the NH_4^+-N removal rate was 90.3% in the reactor, the MLSS in the reactor were 4,358 mg L^{-1} and the MLVSS/MLSS were 86.2%. Sludge attached to the braided fillings inside the plastic balls with large holes. Biological membranes were formed, which indicated that the nitration reaction was successfully started. Then, the reactor switched from aerobic to anaerobic operation.

3.2. Start-up of the anammox reactor

After the reactor changed to anaerobic operation, the low-load method was adopted. The HRT was 8 h, and the water was controlled to maintain a $\rho(NH_4^+\text{-N})$ of approximately 30–50 mg L^{-1}. Then, increasing the load, the NH_4^+-N concentration (60–200 mg L^{-1}) of the incoming solution was gradually increased. The results are shown in Fig. 2. The data in Fig. 2(a) show the variation of the NH_4^+-N removal rate over time. During the first three days, the rate was similar to that of the start-up of other anammox reactors, and the outgoing NH_4^+-N concentration was slightly higher than the incoming NH_4^+-N concentration [25]. Then, because of the anaerobic ammonia oxidation, the NH_4^+-N in the outgoing solution decreased. However, the removal effect was not obvious, and the NH_4^+-N removal rate was below 1%. On the 11th day, the NH_4^+-N removal load was only 0.0046 kg m^{-3} d^{-1}. On the 12th day, 500 mL of anammox immobilized particles was added to the reactor, and the anammox

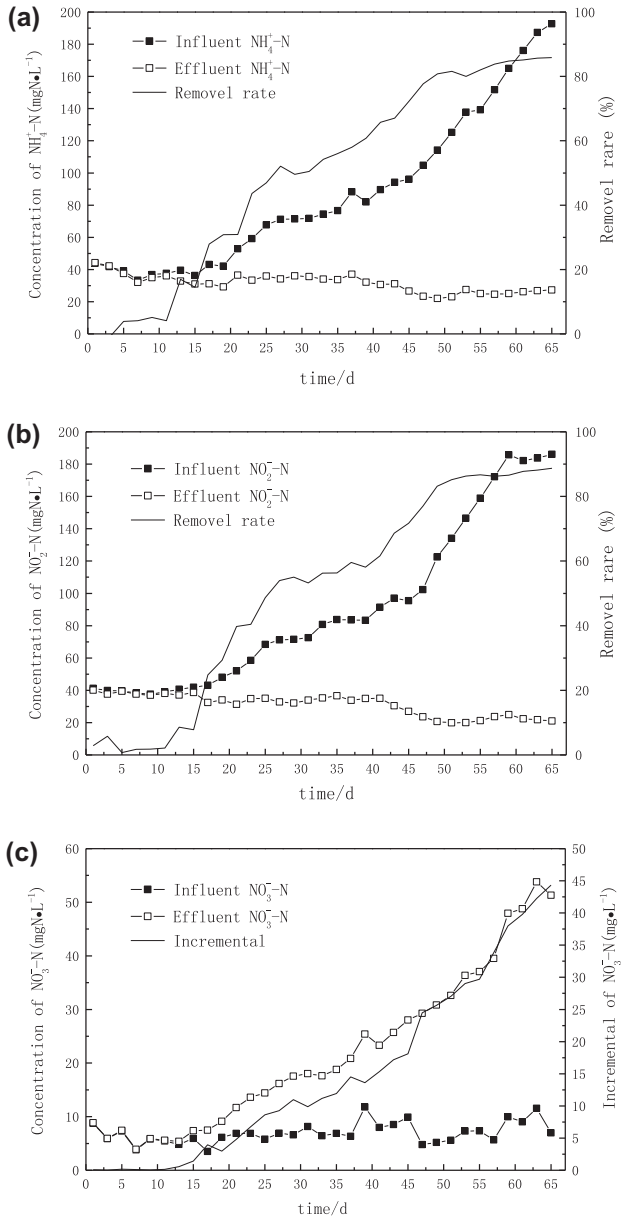

Fig. 2. Time courses of nitrogen concentrations.

function immediately appeared in the reactor. Correspondingly, the NH_4^+-N removal rate gradually increased. From the 20th day to the 50th day, the NH_4^+-N removal rate increased from 0.0489 to 0.3064 kg m^{-3} d^{-1}, which indicated that the quantity of biological matter involved in the reaction increased in the reactor. Thus, the anammox capability increased. On the 65th day, the NH_4^+-N removal rate reached 85.5% with a corresponding removal load of 0.4961 kg m^{-3} d^{-1}, which indicated a strong anammox capability.

Generally, the variation of NO_2^--N resembles that of NH_4^+-N. As shown in Fig. 2(b), the NO_2^--N removal rate increased from 2.1% on the 11th day to 88.7% on the 65th day because of the addition of the immobilized particles. The removal load also increased from 0.0025 kg m^{-3} d^{-1} to 0.4951 kg m^{-3} d^{-1}. The substrates for the anammox reaction were NH_4^+-N and NO_2^--N, and the substrate concentration determines the reaction rate. Therefore, to start the anammox reactor faster, the concentrations of NH_4^+-N and NO_2^--N in the incoming solution were increased. However, a high NO_2^--N concentration can also suppress the anammox process. In a study on anammox bacteria in an SBR reactor, Strous et al. [26] found that if $\rho(NO_2^-\text{-N})$ is higher than 100 mg L^{-1} the anammox reaction is completely suppressed. Jetten et al. [27] found that when $\rho(NO_2^-\text{-N})$ is higher than 280 mg L^{-1}, the anammox reaction is suppressed. However, when $\rho(NO_2^-\text{-N})$ is higher than 140 mg L^{-1}, the anammox reaction is affected. The data in Fig. 2(b) indicate that as the nitrite concentration in the incoming solution was increased, the NO_2^--N removal load in the reactor also gradually increased. From the 53th to 65th day, $\rho(NO_2^-\text{-N})$ in the incoming solution was increased from 146 mg L^{-1} to 186 mg L^{-1}. The anammox bacteria in the reactor tolerate high influent NO_2^--N over the long term. However, the NH_4^+-N and NO_2^--N removal loads in the reactor did not decrease. Therefore, in this study, the high influent NO_2^--N did not suppress the anammox reaction.

Fig. 2(c) shows the variation of NO_3^--N in the reactor. Although the anammox bacteria converted NO_2^--N to NO_3^--N, they also assimilated CO_2 with the obtained reducing force. The increment of the NO_3^--N quantity reflects the increment of the anammox bacteria quantity. The data in the figure indicate that as the NH_4^+-N and NO_2^--N removal loads increased the increment of NO_3^--N also gradually increased and reached 44.32 mg L^{-1} on the 65th day. This outcome indicates that the activity of the anammox bacteria also gradually increased. The data also indicated that on the 49th day, the NH_4^+-N and NO_2^--N removal rates exceeded 80% and the steadily increasing total nitrogen removal load was 0.505 kg m^{-3} d^{-1}. Based on calculations, the NH_4^+-N: NO_2^--N: NO_3^--N ratio involved in the reaction was 1:1.11:0.28 on the 49th day, which is close to the theoretical value of the anammox reaction, i.e., 1:1.32:0.26. This outcome indicates that a steady anammox reaction was proceeding in the reactor. Therefore, the experiment demonstrated that after 49 d, the anammox reactor was successfully started.

3.3. Anammox reaction kinetics

To study the kinetic characteristics of the immobilized particles and the FAS behavior after embedding, the kinetics of the immobilized particles were measured in batch tests. The corresponding correlations among the test results, the embedding particle anammox rates and the substrate concentrations are shown in Table 2. Non-linear fitting was performed on the experimental data listed in Table 2 using the Haldane model in Origin 8.0 to derive the kinetic functions for NH_4^+-N (Fig. 3(a)) and NO_2^--N (Fig. 3(b)). The fitting constants R^2 are 0.949 and 0.983, which indicate good correlations. The data in Fig. 3(a) indicate that the obtained maximum nitration reaction rate of the immobilized particles was 1.99 mg (mg d)$^{-1}$, the ammonium half-saturation constant was 1.57 mmol L^{-1}, and the ammonium half-suppression constant was 724.2 mmol L^{-1}. The data in Fig. 3(b) indicate that the maximum nitrite reaction rate was 2.55 mg (mg d)$^{-1}$, the nitrite half-saturation constant was 1.505 mmol L^{-1}, and the nitrite half-suppression constant was 66.62 mmol L^{-1}.

In Table 3, the kinetic characteristics of the immobilized particles are compared with those of other types of anammox sludge. Zheng et al. [28] and Zu et al. [29] studied the kinetics of anammox mixed cultured bacteria in a biological nitrogen removal bed-batch reactor and in an EGSB reactor, respectively, and obtained markedly different kinetic characteristics parameters. The difference might be caused by the complicated composition of the mixed bacteria in the incubator sludge that could result in a large number of denitrifying bacteria and anaerobic methane bacteria, which would affect kinetic characteristics tests. Tang et al. [30] studied HAG and FAS in a UASB reactor and tested the kinetic characteristics. In Tang's study on embedding particle kinetics, the kinetic characteristics of the immobilized particles were similar to those of HAG because the maximum reactor rates and half-suppression constants were higher (and the half-saturation constants are lower) that those of other types of anammox sludge, which indicated that immobilized particles exhibit better anammox kinetic characteristics.

4. Discussion

Anammox bacteria and aerobic ammonium oxidation bacteria (AOB) have many similar characteristics. Van de Graaf et al. [31] found that certain aerobic AOB are facultative bacteria, which can convert ammonia nitrogen to N_2 to obtain energy via an anaerobic nitration reaction under anaerobic

Table 2
Effect of substrate concentration on Anammox

NH_4^+-N/(mmol L^{-1})	Removal rate of NH_4^+-N/mg (mg d)$^{-1}$	NO_2^--N/(mmol L^{-1})	Removal rate of NO_2^--N/mg (mg d)$^{-1}$
1	0.632	0.05	0.233
2.5	1.326	2.1	1.422
4.8	1.576	4.3	1.858
16.4	1.766	7.2	1.901
22.3	1.824	7.9	1.976
39.6	1.730	10	1.943
52.3	1.813	15.7	1.924
61.1	1.789	20	1.883
80.3	1.802	22.1	1.826
100	1.754	25	1.765

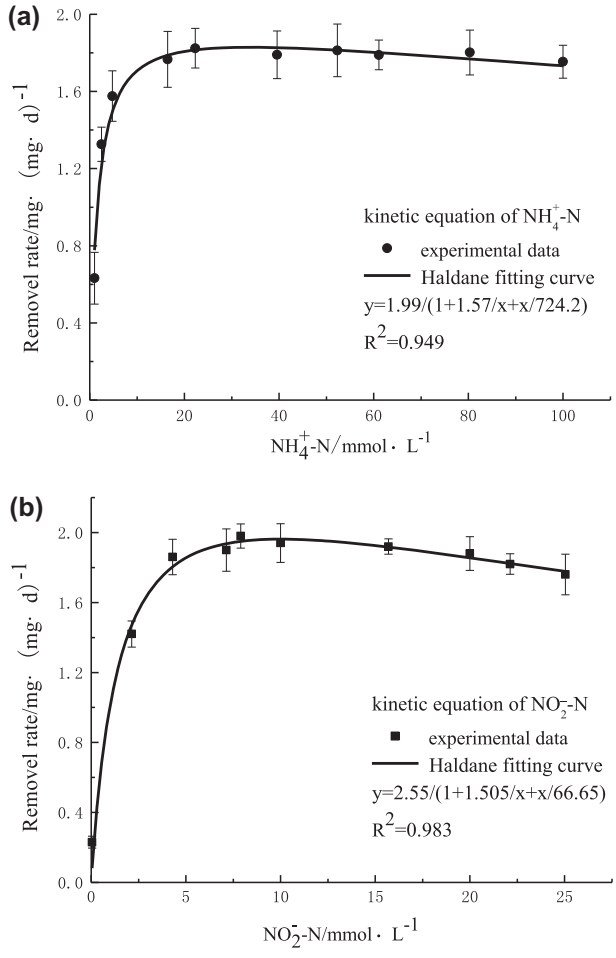

Fig. 3. Anammox kinetics of immobilized granules.

conditions. The anammox bacteria concentration in nitration sludge is approximately 1.7×10^6 copies/mg dry sludge [32]. Therefore, it should be possible to use nitrifying sludge as the inoculation sludge for anammox bacteria. However, in this study, the inoculated sludge did not exhibit anammox characteristics after a certain period of incubation. As shown in Fig. 2, the NH_4^+-N and NO_2^--N were removed from the reactor after adding 500 mL of anammox immobilized particles (approximately 10 g of anammox sludge) on the 12th day. Then, the anammox capability gradually increased, and the total NRR reached 0.86 kg m^{-3} d^{-1} on the 65th day. Relative to the quantity of sludge in the reactor, the added quantity of embedding anaerobic sludge was small (the added sludge contributed approximately 7%), which in theory should not result in such a strong effect. Tang et al. [33] found a similar phenomenon in the anammox reaction started by nitration sludge under meso-scale conditions. Tang did not observe any NH_4^+-N removal after 200 d in the reactor. Tang added HAG on the 214th day, which successfully started the anammox reactor. This phenomenon indicates that although a certain quantity of anammox bacteria was accumulated in the reactor, a certain factor (or factors) limited the anammox. Several studies demonstrated that anammox bacteria exhibit anammox activities only when the cell density reaches 10^{10} per mL [34]. Therefore, although anammox bacteria were cultured, their activities were limited by the cell densities [35].

The immobilized particles and the plastic balls with large holes were removed from the reactor on the 70th day (Fig. 4). Fig. 4(a) and (b) show some of the particle-shaped red sludge found on the embedding particle surfaces and on the braided fillings near the immobilized particles. The red sludge was observed using SEM. Fig. 4(c) shows the SEM image with ×18,000 magnification. The image illustrates that the bacteria are spherical with volcano-shaped hollows on the sides, which is typical of anammox bacteria [36]. These images demonstrate that adding anammox immobilized particles increased the local anammox bacteria density in the reactor. The

Table 3
Comparison of kinetic parameters of different Anammox sludge

Sludge	V_{max} NH_4^+-N/mg (mg d)$^{-1}$	V_{max} NO_2^--N/mg (mg d)$^{-1}$	K_m/(mmol L^{-1})		K_i/(mmol L^{-1})		Refs.
			NH_4^+-N	NO_2^--N	NH_4^+-N	NO_2^--N	
Mixed culture	0.018–0.039	0.203	1.80–4.21	0.468	38.1–98.4	16.656	[28]
Mixed culture	0.082	0.104	6.22	80.21	1.09	11.39	[29]
HAG	1.92	2.63	1.21	1.39	834.2	52.5	[30]
FAS	0.71	1.11	1.78	1.51	643.9	12.80	[30]
Immobilized granules	1.99	2.55	1.57	1.505	724.2	66.65	This study

Fig. 4. Photographs of immobilized granules (a) ribbon-type filler, (b) SEM images of particle sludge in reactor, and (c) ×18 k (70th day).

increased density facilitates anammox particle sludge formation and promotes the metabolism of other types of free-form anammox bacteria, thus starting the anammox reactor in a relatively short period of time. Anammox immobilized particles work as an efficient biocatalyst.

In Fig. 3, the kinetic characteristics of different batches of anammox sludge are compared. The data indicate that the kinetic properties of the sludge are enhanced by embedding FAS to form immobilized particles. The maximum ammonium reaction rate of the immobilized particles is approximately 2.8 times higher than that of FAS, and the maximum nitrite reaction rate is approximately 2.3 times that of FAS. The increased reaction rate promotes the conversion of substrates, relaxes the self-suppression of substrates, and increases anammox sludge production. In the experiment, the ammonium and nitrite half-saturation constants (K_m) of the immobilized particles are lower than those of FAS, which indicates that the immobilized particles have more substrate affinity and can therefore utilize the substrate more readily in competitive environments, which aids the anammox process. It is expected that embedding materials would inhibit mass transfer such that the corresponding half-saturation constant would be greater than that of FAS. However, this study demonstrates that the half-saturation constant with immobilized particles is lower than that of FAS, probably because the embedded anammox sludge contains a higher concentration of the biological quantity. Thus, the anammox bacteria content is greater. The nitrite half-suppression constant (K_i) of the embedding particle is approximately 5.2 times that of FAS and is also higher than that of HAG, which is 66.65 mmol L^{-1} (933.1 mg L^{-1}). This outcome indicates that the embedded anammox sludge can also survive higher nitrite salt concentrations. By differentiating Eq. (2) and setting the first-order derivative to zero, the substrate concentration required to reach the maximum reaction rate is as follows:

$$S_{max} = \sqrt{K_m K_i} \tag{3}$$

Using Eq. (3), $\rho(NO_2^-\text{-N})$ is 140 mg L^{-1} when the immobilized particles reach the maximum nitrite reaction rate. On the 53rd day, when $\rho(NO_2^-\text{-N})$ of the incoming solution is 146 mg L^{-1}, the anammox reaction in the reactor was not suppressed. In the subsequent test, the removal load gradually increased as the incoming solution substrate concentration

increased. Therefore, the addition of immobilized particles enhanced the system's tolerance for nitrites and helped the anammox reactor operate in a highly effective, steady mode.

5. Conclusions

In this study, anammox immobilized particles were used to replace HAG as biocatalyst to rapidly start an anammox reactor. The kinetic characteristics of the immobilized particles were studied, which provided theoretical support for the developing anammox biocatalysis technique. The most important conclusions are listed in the following:

(1) Nitrate membranes and nitrate sludge were used to start the anammox reactor. Anammox immobilized particles were added as biocatalyst. The anammox reactor started on the 49th day and subsequently maintained steady operation. The removal rates for NH_4^+-N and NO_2^--N in the system were 80.7 and 83.1%, respectively. The corresponding removal loads were 0.276 and 0.306 kg m^{-3} d^{-1}.
(2) The facultative bacteria in the nitration sludge formed anammox particle sludge on the surface of the immobilized particles and in their surroundings. This sludge increased local anammox bacteria concentrations in the reactor. It also contributed to the metabolism of other types of free-form anammox bacteria. Therefore, anammox immobilized particles were demonstrated to be efficient as a biocatalyst and also decreased the reactor start-up time.
(3) The anammox immobilized particles exhibited kinetic characteristics similar to those of HAG. The ammonium and nitrite half-saturation constants (k_m) of the immobilized particles were 1.57 mmol L^{-1} (21.98 mg L^{-1}) and 1.505 mmol L^{-1} (21.05 mg L^{-1}), respectively, which were lower than the corresponding FAS values and therefore helped the immobilized particles utilize substrates more easily under adverse conditions. The ammonium and nitrite half-suppression constants of the immobilized particles (K_i) were 724.2 mmol L^{-1} (10,138.8 mg L^{-1}) and 66.65 mmol L^{-1} (933.1 mg L^{-1}), respectively, which were substantially higher than the corresponding FAS values. Therefore, the immobilized particles could better survive in high-concentration substrates and better relax the self-suppression effect.

Acknowledgment

This work was financially supported by National Critical Project for Science and Technology on Water Pollution Prevention and Control of China (2014ZX07201-011).

References

[1] A.A. van de Graaf, P. de Bruijn, L.A. Robertson, M.S. Jetten, J.G. Kuenen, Metabolic pathway of anaerobic ammonium oxidation on the basis of 15N studies in a fluidized bed reactor, Microbiology 143 (1997) 2415–2421.
[2] M.S. Jetten, M. Wagner, J. Fuerst, M. van Loosdrecht, G. Kuenen, M. Strous, Microbiology and application of the anaerobic ammonium oxidation ('anammox') process, Curr. Opin. Biotechnol. 12 (2001) 283–288.
[3] I. Zekker, K. Kroon, E. Rikmann, T. Tenno, M. Tomingas, P. Vabamäe, S.E. Vlaeminck, T. Tenno, Accelerating effect of hydroxylamine and hydrazine on nitrogen removal rate in moving bed biofilm reactor, Biodegradation 23 (2012) 739–749.
[4] I. Zekker, E. Rikmann, T. Tenno, V. Lemmiksoo, A. Menert, L. Loorits, P. Vabamäe, M. Tomingas, T. Tenno, Anammox enrichment from reject water on blank biofilm carriers and carriers containing nitrifying biomass: operation of two moving bed biofilm reactors (MBBR), [MBBR] Biodegradation 23 (2012) 547–560.
[5] I. Zekker, E. Rikmann, T. Tenno, A. Saluste, M. Tomingas, A. Menert, L. Loorits, V. Lemmiksoo, T. Tenno, Achieving nitritation and anammox enrichment in a single moving-bed biofilm reactor treating reject water, Environ. Technol. 33 (2012) 703–710.
[6] L. Vafajoo, M. Pazoki, Model-based evaluations of operating parameters on CANON process in a membrane-aerated biofilm reactor, Desalin. Water Treat. 51 (2013) 4228–4234.
[7] I. Zekker, E. Rikmann, T. Tenno, A. Menert, V. Lemmiksoo, A. Saluste, T. Tenno, M. Tomingas, Modification of nitrifying biofilm into nitritating one by combination of increased free ammonia concentrations, lowered HRT and dissolved oxygen concentration, J. Environ. Sci. 23 (2011) 1113–1121.
[8] C.J. Tang, P. Zheng, T.T. Chen, J.Q. Zhang, Q. Mahmood, S. Ding, X.G. Chen, J.W. Chen, D.T. Wu, Enhanced nitrogen removal from pharmaceutical wastewater using SBA-ANAMMOX process, Water Res. 45 (2011) 201–210.
[9] E.M. Gilbert, S. Agrawal, S.M. Karst, H. Horn, P.H. Nielsen, S. Lackner, Low Temperature Partial Nitritation/Anammox in a Moving Bed Biofilm Reactor treating low strength Wastewater, Environ. Sci. Technol. 48 (2014) 8784–8792.
[10] X. Zhang, D. Li, Y. Liang, H. Zeng, Y. He, D. Fan, J. Zhang, Start-up, influence factors, and the microbial characteristics of partial nitrification in membrane bioreactor, Desalin. Water Treat. 54 (2015) 581–589.
[11] I. Zekker, E. Rikmann, T. Tenno, P. Vabamäe, K. Kroon, L. Loorits, A. Saluste, T. Tenno, Effect of concentration on anammox nitrogen removal rate in a moving bed biofilm reactor, Environ. Technol. 33 (2012) 2263–2271.

[12] W.R. van der Star, W.R. Abma, D. Blommers, J.W. Mulder, T. Tokutomi, M. Strous, C. Picioreanu, M. van Loosdrecht, Startup of reactors for anoxic ammonium oxidation: Experiences from the first full-scale anammox reactor in Rotterdam, Water Res. 41 (2007) 4149–4163.
[13] B. Wett, Solved upscaling problems for implementing deammonification of rejection water, Water Sci. Technol. 53 (2006) 121–128.
[14] H. López, S. Puig, R. Ganigu2, M. Ruscalleda, M.D. Balaguer, J. Colprim, Start-up and enrichment of a granular anammox SBR to treat high nitrogen load wastewaters, J. Chem. Technol. Biotechnol. 83 (2008) 233–241.
[15] A. Dapena Mora, J. Campos, A. Mosquera Corral, M. Jetten, R. Méndez, Stability of the ANAMMOX process in a gas-lift reactor and a SBR, J. Biotechnol. 110 (2004) 159–170.
[16] C.J. Tang, P. Zheng, C.H. Wang, J.Q. Zhang, J.W. Chen, S. Ding, Granulation and characteristics of extracellular polymers of Anammox sludge in high-load EGSB reactor, CIESC J. 61 (2010) 732–739.
[17] Y. Cong, X.L. Huang, X.L. Wang, D.W. Gao, Faster formation of Anammox granular sludge, CIESC J. 65 (2014) 664–671.
[18] Y. Chen, T. Zhou, S. Yao, J.L. Wu, Ethanol production by repeated batch and continuous fermentations by Saccharomyces cerevisiae immobilized in a fibrous bed bioreactor, J. Microbiol. Biotechnol. 23 (2013) 511–517.
[19] C.H. Wang, J.C.W. Liu, K.K. Ng, C.F. Lin, P.K.A. Hong, P.Y. Yang, Immobilized bioprocess for organic carbon and nitrogen removal, Desalin. Water Treat. 37 (2012) 296–301.
[20] G.H. Chen, J. Li, S. Tabassum, Z.J. Zhang, Anaerobic ammonium oxidation (ANAMMOX) sludge immobilized by waterborne polyurethane and its nitrogen removal performance-a lab scale study, Rsc Adv. 5 (2015) 25372–25381.
[21] Z.B. Li, C.J. Liu, B.H. Zhao, J.X. Ma, X.Y. Wang, J. Li, Activity and inhibition characteristics of Anammox and heterotrophic denitrifier bacteria in a multi-substrate system, Chin. Environ. Sci. 4 (2013) 648–654.
[22] M. Strous, E. Van Gerven, P. Zheng, J.G. Kuenen, M.S. Jetten, Ammonium removal from concentrated waste streams with the anaerobic ammonium oxidation (Anammox) process in different reactor configurations, Water Res. 31 (1997) 1955–1962.
[23] C.S. Gee, M.T. Suidan, J.T. Pfeffer, Modeling of nitrification under substrate-inhibiting conditions, J. Environ. Eng. 116 (1990) 18–31.
[24] D.E. Andrew, H.F.M. Ann, Standard Methods for the Examination of Water and Wastewater, twenty-first ed., APHA, 2005, pp. 4–133.
[25] D. Patureau, N. Bernet, T. Bouchez, P. Dabert, J. Delgenes, R. Moletta, Biological nitrogen removal in a single aerobic reactor by association of a nitrifying ecosystem to an aerobic denitrifier, Microvirgula aerodenitrificans, J. Mol. Catal. B: Enzym. 5 (1998) 435–439.
[26] M. Strous, J.G. Kuenen, M.S. Jetten, Key physiology of anaerobic ammonium oxidation, Appl. Environ. Microbiol. 65 (1999) 3248–3250.
[27] M.S. Jetten, M. Strous, K.T. van de Pas-Schoonen, J. Schalk, U.G. van Dongen, A.A. van de Graaf, S. Logemann, G. Muyzer, M. van Loosdrecht, J.G. Kuenen, The anaerobic oxidation of ammonium, FEMS Microbiol. Rev. 22 (1998) 421–437.
[28] P. Zheng, B.L. Hu, Kinetics of Anaerobic Ammonia Oxidation, Chin. J. Biotechnol. 17 (2001) 193–198.
[29] B. Zu, D.J. Zhang, Q. Yan, Effect of trace NO_2 and kinetic characteristics for anaerobic ammonium oxidation of granular sludge, Environ. Sci. 29 (2008) 683–687.
[30] C.J. Tang, L. Xiong, Y.Y. Wang, P. Zheng, Kinetic Characteristics of High-rate ANAMMOX Granules, Environ. Sci. 34 (2013) 3544–3551.
[31] A.A. van de Graaf, P. de Bruijn, L.A. Robertson, M.S. Jetten, J.G. Kuenen, Autotrophic growth of anaerobic ammonium-oxidizing micro-organisms in a fluidized bed reactor, Microbiology 142 (1996) 2187–2196.
[32] I. Tsushima, Y. Ogasawara, T. Kindaichi, H. Satoh, S. Okabe, Development of high-rate anaerobic ammonium-oxidizing (anammox) biofilm reactors, Water Res. 41 (2007) 1623–1634.
[33] C.J. Tang, P. Zheng, Q. Mahmood, J.W. Chen, Start-up and inhibition analysis of the Anammox process seeded with anaerobic granular sludge, J. Ind. Microbiol. Biotechnol. 36 (2009) 1093–1100.
[34] P. Zheng, X. Xu, B. Hu, Novel Biological Nitrogen Removal: Theory and Technology, Science Press, Beijing, 2004.
[35] E. Bock, I. Schmidt, R. Stüven, D. Zart, Nitrogen loss caused by denitrifying Nitrosomonas cells using ammonium or hydrogen as electron donors and nitrite as electron acceptor, Arch. Microbiol. 163 (1995) 16–20.
[36] G.L. Zhu, Y.Y. Hu, Q.R. Wang, Nitrogen removal performance of anaerobic ammonia oxidation co-culture immobilized in different gel carriers, Water Sci Technol. 59 (2009) 2379–2386.