Performance and kinetics of ANAMMOX granular sludge with pH shock in a sequencing batch reactor

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Abstract As an efficient and cost-effective nitrogen removal process, anaerobic ammonium oxidation (ANAMMOX) could be well operated at suitable pH condition. However, pH shock occurred in different kinds of wastewater and affected ANAMMOX process greatly. The present research aimed at studying the performance and kinetics of ANAMMOX granular sludge with pH shock. When influent pH was below 7.5, effluent $\text{NH}_4^+\text{-N}$ and $\text{NO}_2^-\text{-N}$ increased with decreasing pH. At pH 6.0, effluent $\text{NO}_2^-\text{-N}$ approached 100 mg/L, and the ratios of $\Delta\text{NO}_2^-\text{-N} : \Delta\text{NH}_4^+\text{-N}$ and $\Delta\text{NO}_3^-\text{-N} : \Delta\text{NH}_4^+\text{-N}$ approached 2.2 and 1.3, respectively. Both greatly deviated from

theoretical values. When influent pH was above 7.5, effluent $\text{NH}_4^+\text{-N}$ and $\text{NO}_2^-\text{-N}$ increased with increasing pH. At pH 9.0, ammonium removal rate (ARR) and nitrite removal rate (NRR) decreased to 0.011 ± 0.004 and $0.035 \pm 0.004 \text{ kg}/(\text{m}^3 \cdot \text{d})$, respectively. Besides, $\Delta\text{NO}_2^-\text{-N} : \Delta\text{NH}_4^+\text{-N}$ deviated from theoretical value. Longer recovery time from pH 9.0 than from pH 6.0 indicated that alkaline surroundings inhibited anaerobic ammonium oxidizing bacteria (AAOB) greater. The sludge settling velocity was 2.15 cm/s at pH 7.5. However, it decreased to 2.02 cm/s when pH was 9.0. Acidic pH had little effect on sludge size, but disintegration of ANAMMOX granule was achieved with pH of 9.0. The Bell-shaped (A) model and the Ratkowsky model were more applicable to simulate the effect resulting from pH shock on ANAMMOX activity ($R^2 > 0.95$), and both could describe ANAMMOX activity well with pH shock. They indicated that $q_{\max}$ was 0.37 kg $\Delta\text{NH}_4^+\text{-N}/(\text{kgMLSS} \cdot \text{d})$ at the optimum pH value (7.47) in present study. The minimum pH during which ANAMMOX occurred was 5.68 while the maximum pH for ANAMMOX reaction was 9.26. Based on nitrogen removal performance with different pH, strongly acidic ($\text{pH} \leq 6.5$) or alkaline ($\text{pH} \geq 8.5$) inhibited ANAMMOX process. Besides, ANAMMOX appeared to be more susceptible to alkaline wastewater. Compared to extremely acidic condition (low pH), extremely alkaline condition (high pH) affected ANAMMOX granules much more.

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Introduction

As a feasible and promising biological wastewater treatment technology, anaerobic ammonium oxidation (ANAMMOX) has attracted more and more attention. During the process, ammonium was oxidized to nitrate with nitrite as electron acceptor according to Eq. 1 (Strous et al. 1998). Many advantages of ANAMMOX made it popular in wastewater treatment (Okamoto et al. 2012; Qiao et al. 2009). However, ANAMMOX process was greatly affected by many factors such as pH (Daverey et al. 2015), organic matter (Li et al. 2015) and free ammonia (Aktan et al. 2012). With regard to pH, Strous et al. (1999) reported that physiological pH range of ANAMMOX bacteria was 6.7–8.3 while Egli et al. (2001) indicated the value was 6.5–9. Although the activity of ANAMMOX bacteria could still be found with extreme alkaline or acidic surroundings (Ahn et al. 2004; Yin et al. 2016), ANAMMOX process was greatly affected by pH shock.

Numerous studies were focused on the effect of pH on wastewater treatment_ENREF_35_ENREF_14_ENREF_37. On the one hand, both the growth and activity of microbes were directly affected by pH (Fux et al. 2004; Strous et al. 1997; Tang et al. 2009; Yin et al. 2016)._ENREF_45_ENREF_37_ENREF_14 On the other hand, pH affected free ammonia (FA) and free nitrous acid (FNA) contents, which influenced biochemical reaction process indirectly (Fernandez et al. 2012; Puyol et al. 2014a, b; Tang et al. 2009; Waki et al. 2007)._ENREF_37_ENREF_42_ENREF_12_ENREF_37_ENREF_42 With regard to direct effect resulting from pH, Strous et al. (1997) reported that specific ANAMMOX activity (SAA) at pH 9 was only 20% of that at pH 8. Fux et al. (2004) found that ANAMMOX activity was completely inhibited at pH 9.3 in a fixed-bed reactor. Besides, Yin et al. (2016) showed that very low nitrogen removal efficiencies

(<7%) were achieved with extreme initial pH values (i.e. 6.0 or 10.5). However, the results varied with different reactor configurations or wastewater characteristics.

As pH-dependent parameters, FA and FNA could indirectly affect ANAMMOX process. With high pH, FA was regarded as an inhibitor. Tang et al. (2009) observed the loss of ANAMMOX stability due to 70 mg/L FA resulting from increasing pH. Waki et al. (2007) suggested that the deterioration of ANAMMOX system occurred at 13–90 mg/L FA. Fernandez et al. (2012) indicated that a 50% decrease of SAA was obtained at 38 mg/L FA while 80% decrease was got at 100 mg/L FA. FNA was also thought to be an important contributor to ANAMMOX inhibition when pH was lower than 7.1 (Puyol et al. 2014a). Jung et al. (2007) reported that 0.8–1.2 µg/L FNA significantly inhibited the ANAMMOX process. Fernandez et al. (2012) found that 6.6 µg/L FNA had no significant inhibition on anaerobic ammonium oxidizing bacteria (AAOB) in batch tests, and a decrease over 50% was obtained when FNA was 11 µg/L.

There were numerous high-strength ammonium wastewater with various pH values (Chung and Bae 2002; Chung et al. 2007). As a result, it is necessary to study nitrogen removal performance of ANAMMOX with pH shocks. The present work aimed at studying pH shock on process stability and recovery process after inhibition. Besides, sludge property and kinetics were analyzed.

Materials and methods

Experimental set-up and operational strategy

Two anaerobic sequencing batch reactors (SBRs) were operated in parallel in this work and each reactor was 1.5 L. Two reactors were covered completely with aluminum caps to avoid the growth of photosynthetic microorganisms. N₂ (99.99%) was purged into the influent in order to maintain anaerobic conditions. Besides, each reactor was bubbled with N₂ gas to remove dissolved oxygen before sludge was inoculated into it. Hydraulic retention time (HRT) was 6 h. During the experiment, SBRs were stirred at 100 rpm. In order to study the effect resulting from FNA and FA on ANAMMOX process, NH₄⁺–N decreased to 40 mg/

L at pH 6 and NO_2^- -N decreased to 53 mg/L at pH 9.0 (Anthonisen et al. 1976). NH_4^+ -N/ NO_2^- -N was kept at 1.32 (according to Strous et al. 1998) except P5 and P11. The detailed operational strategy was presented in Table 1.

Inoculated sludge and synthetic wastewater

ANAMMOX granular sludge was inoculated from two lab-scale reactors (with an effective volume of 7 L). Both reactors were operated steadily over one year at 35 ± 1 °C with influent pH of 7.5. The inoculating sludge was mixed and added into two SBRs. The sludge concentration was 1.55 ± 0.05 g/L in both SBRs and the sludge retention time (SRT) was about 30 days. The granular sludge was brightly red with good settling property (shown in Fig. S1). Synthetic wastewater was used in this study (g/L): KH_2PO_4 0.028; CaCl_2 0.14; KHCO_3 2.0; $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ 0.2; NH_4Cl 0.336; NaNO_2 0.573. Trace element solutions I and II (1.2 ml per litre medium) were also added as previous reported (Van de Graaf et al. 1996).

Specific ANAMMOX activity (SAA) batch assays

To test the effects of pH shocks on ANAMMOX activity, batch assays were carried out under different pH values at 35 °C. Completely closed vials with a total volume of 100 mL of liquid volume were used to perform ANAMMOX batch assays. The NH_4^+ -N/ NO_2^- -N concentrations were 80 and 105 mg/L, respectively. At the initial of test, sludge concentration

was 1.55 g/L. N_2 was purged into reactors to remove O_2 . The determination was performed at regular intervals (0.5 h) and SAA was calculated according to the report from Qiao et al. (2014).

Analytical methods

The supernatants were analyzed for NH_4^+ -N, NO_2^- -N and NO_3^- -N concentrations according to standard methods (APHA 1998). Temperature and pH were measured by WTW 340i/SET pH/Oxi portable multi-parameter meter. Microscopic images were taken with light microscope (Olympus, CX31) and photos were taken with digital camera (Canon, IXUS 500 HS). Sludge concentration was measured after filtering the washed samples through 0.45 μm membrane filter and drying at 105 °C to constant weight. Each analytical result reported in present research was performed in triplicate and the mean value was reported. The size of granular sludge was performed according to method described by Tang et al. (2011). The measure of settling velocity was based on the methods of Mu et al. (2008). The FA and FNA concentrations were acquired according to previous report from Anthonisen et al. (1976).

Kinetic analysis

The kinetic analysis at different influent pH (6.0–9.0) was carried out in batch tests. The influent pH in different tests were adjusted by adding HCl (1 mol/L) and NaOH (2 mol/L).

Table 1 Operational strategy

Reactor	Phase	Cycle	NH_4^+ -N ^a (mg/L)	NO_2^- -N ^a (mg/L)	pH ^b
SBR1	P1	0–15	80	106	7.5
	P2	15–25	80	106	7.0
	P3	25–35	80	106	6.5
	P4	35–45	80	106	6.0
	P5	45–55	80	53	6.0
	P6 ^c	55–75	80	106	7.5
SBR2	P7	0–15	80	106	7.5
	P8	15–25	80	106	8.0
	P9	25–35	80	106	8.5
	P10	35–45	80	106	9.0
	P11	45–55	40	106	9.0
	P12 ^c	55–116	80	106	7.5

^a Influent concentration

^b Influent pH

^c Shaded region correspond to recovery period

The bell-shaped (A) model

This model was applied to describe the inhibition on biological nitrification resulting from pH (Park et al. 2007). In present study, it was applied to analyze pH on ANAMMOX activity. The model was constructed by a cosine bell-shape (A) function based on an assumption that the specific substrate removal rate was zero when pH reached an upper limit ($\text{pH} = \text{pH}_{\text{opt}} + \omega$) or a lower limit ($\text{pH} = \text{pH}_{\text{opt}} - \omega$).

$$q = \frac{q_{\text{max}}}{2} \times \left\{ 1 + \cos \left[\frac{\pi}{\omega} \times (\text{pH} - \text{pH}_{\text{opt}}) \right] \right\} \quad (2)$$

where q and q_{max} were the specific substrate ($\text{NH}_4^+ - \text{N}$ in this study) removal rate at certain pH and the optimum pH_{opt} , respectively; The ' ω ' was defined as pH range in which q was larger than half of q_{max} ; ' 2ω ' was pH range in which ANAMMOX could take place.

The Michaelis model

This model was applied to predict the effect resulting from pH shock on enzymatic reactions (Segel 1975). Besides, Yin et al. (2016) used this model to study the effect of pH shock on ANAMMOX reaction. The equation was presented in Eq. 3.

$$q = \frac{q_{\text{max}}}{1 + 10^{(\text{pK}_{\text{s1}} - \text{pH})} + 10^{(\text{pH} - \text{pK}_{\text{s2}})}} \quad (3)$$

where pK_{s1} was logarithm of the lowest pH at which q was equal to $1/2 q_{\text{max}}$; pK_{s2} was logarithm of the highest pH at which q was equal to $1/2 q_{\text{max}}$.

The Ratkowsky model

The Ratkowsky model (Eq. 4) was a good choice to demonstrate the effect of pH on hydrogen production (Wang and Wan 2009). But it was the first time applied to analyze the effect of pH shock on ANAMMOX activity.

$$q = [A \times (\text{pH} - \text{pH}_{\text{min}})]^2 \times \{1 - \exp[B \times (\text{pH} - \text{pH}_{\text{max}})]\}^2 \quad (4)$$

where A and B were constants; pH_{min} and pH_{max} were upper and lower limits of ANAMMOX reaction.

The Haldane model

Usually, the Haldane (Eq. 5) model was widely used to demonstrate substrate inhibition on ANAMMOX process. However, it could also describe the effect resulting from H^+ concentration on specific hydrogen production rate (Fabiano and Perego 2002; Wang and Wan 2009). In this study, the Haldane model was used to describe the effect of pH shock on ANAMMOX activity.

$$q = \frac{q_{\text{max}} \times S_{\text{H}}}{S_{\text{H}} + K_{\text{a}} + S_{\text{H}}^2/K_{\text{b}}} \quad (5)$$

where K_{a} and K_{b} were two regression coefficients; S_{H} was the concentration of H^+ .

The Bell-shaped (B) model

The function of this model was showed in Eq. 6 (Henze et al. 1995). Van Hulle et al. (2007) used the Bell-shaped (B) model to analyze SHARON kinetics, and they found that the data obtained in experiment showed a better fit to this model.

$$q = q_{\text{max}} \times \frac{K_{\text{pH}}}{K_{\text{pH}} - 1 + 10^{|\text{pH}_{\text{opt}} - \text{pH}|}} \quad (6)$$

where K_{pH} was pH constant; pH_{opt} was the optimum pH value of biological process.

Results and discussion

Effect of pH shock on nitrogen removal performance

Nitrogen removal performance at $\text{pH} < 7.5$

The effect of pH shock (< 7.5) on ANAMMOX granular sludge was presented in Fig. 1. In general, when influent pH decreased, effluent $\text{NH}_4^+ - \text{N}$ and $\text{NO}_2^- - \text{N}$ increased. $\text{NO}_2^- - \text{N}$ was accumulated in reactor. At pH 6.0, effluent $\text{NO}_2^- - \text{N}$ approached 100 mg/L, which was regarded as the inhibition $\text{NO}_2^- - \text{N}$ concentration reported by Strous et al. (1999). With pH of 7.5–6.5, the stoichiometric ratios were similar to the reported value ($\Delta \text{NH}_4^+ - \text{N} : \Delta \text{NO}_2^- - \text{N} : \Delta \text{NO}_3^- - \text{N} = 1 : 1.32 : 0.26$) by Strous et al. (1998). When influent pH was 6.0, the ratios of $\Delta \text{NO}_2^- - \text{N} : \Delta \text{NH}_4^+ -$

Fig. 1 Overall performance of ANAMMOX process at $\text{pH} \leq 7.5$: **a** concentrations of three nitrogen species in influent and effluent, **b** stoichiometric ratios, **c** loading and removal rates of $\text{NH}_4^+\text{-N}$ and $\text{NO}_2^-\text{-N}$, **d** pH, FA and FNA in influent and effluent

N and $\Delta\text{NO}_3^- - \text{N} : \Delta\text{NH}_4^+ - \text{N}$ approached 2.2 and 1.3, respectively, which deviated from theoretical values greatly.

During the period P2–P4, ammonium loading rate (ALR) and nitrite loading rate (NLR) were at 0.34 ± 0.02 and 0.41 ± 0.02 $\text{kg}/(\text{m}^3 \cdot \text{d})$, respectively. With influent pH decreased to 6.0, ammonium removal rate (ARR) and nitrite removal rate (NRR) decreased to 0.015 ± 0.005 and 0.035 ± 0.006 $\text{kg}/(\text{m}^3 \cdot \text{d})$, respectively. The results implied that the ANAMMOX process was seriously impeded at pH 6.0. This agreed with van der Star et al. (2007) that a sharp decrease of ANAMMOX activity was observed at pH lower than 7.2. FA concentration was stabilized at low level (lower than 3.5 mg/L) in this phase. However, FNA concentration was higher than the toxic threshold at pH 6.0. When effluent FNA concentration decreased in period P4, little enhancement of ARR and NRR were acquired. The decrease of FNA concentration could not improve nitrogen removal performance at strongly acidic condition.

H^+ was consumed when AAOB utilized $\text{NO}_2^- - \text{N}$ as electronic acceptor to oxidize $\text{NH}_4^+ - \text{N}$, and it was considered as the main reason for the variation of pH in ANAMMOX process. The shadow zone in the Fig. 1d implied that the effluent pH was much higher than the influent though ANAMMOX process was almost completely inhibited. In addition, the lower alkalinity in influent resulted in decrease of buffering capacity and the degassing of CO_2 from liquid into gas phase (He et al. 2015). During the recovery period P6, ANAMMOX performance could be restored after five cycles' operation.

Nitrogen removal performance at pH > 7.5

The effect of pH shock (>7.5) on ANAMMOX granular sludge was shown in Fig. 2. In general, when influent pH increased, effluent $\text{NH}_4^+ - \text{N}$ and $\text{NO}_2^- - \text{N}$ increased. In addition, effluent $\text{NO}_3^- - \text{N}$ decreased. The stoichiometric ratios approach to theoretical value when pH was 7.5–8.5. However, $\Delta\text{NO}_2^- - \text{N} : \Delta\text{NH}_4^+ - \text{N}$ deviated from theoretical value at pH 9. The lysis of AAOB occurred at pH 9.0 (showed below), which released organic matter. The organic matter was consumed by HDB to remove $\text{NO}_2^- - \text{N}$ and $\text{NO}_3^- - \text{N}$. During the period P8–P10, ALR and NLR maintained at 0.34 ± 0.02 and 0.41 ± 0.02 $\text{kg}/$

$(\text{m}^3 \cdot \text{d})$, respectively. When influent pH was 9.0, ARR and NRR decreased to 0.011 ± 0.004 and 0.035 ± 0.004 $\text{kg}/(\text{m}^3 \cdot \text{d})$, respectively. This indicated that nitrogen removal performance was poor at extremely alkaline condition. This also agreed Jaroszynski et al. (2011) that high pH caused instability of ANAMMOX process.

The FNA concentration was stabilized at low level (lower than 0.0065 mg/L), which was lower than inhibitory threshold. However, FA concentration sharply increased with growing pH. At P10, effluent FA concentration was higher than 30 mg/L, which approached the threshold of 35–40 mg/L. When influent FA concentration decreased to half of that in P10, both ARR and NRR increased little. The decrease of FA could not improve nitrogen removal performance under strong alkaline condition. Puyol et al. (2014a) confirmed that high pH was a much more important inhibition factor than FA, which explained the result in present study.

In addition, the shadow zone in Fig. 2d indicated the effluent pH value was higher than that in influent. In alkaline surroundings, FA generation was promoted. Besides, ANAMMOX process was nearly completely inhibited at pH 9.0. As a result, the consumption of OH^- was higher than the production of it, which led to the decrease of pH value. In addition, the duration time of the recovery phase after strongly alkaline pH shock was much longer than acidic pH shock. 50 cycles' operation was needed to reactivate ANAMMOX process. Longer recovery time from pH 9.0 than from pH 6.0 indicated that alkaline surroundings inhibited AAOB greater.

Mechanism of pH shock on ANAMMOX

Based on the nitrogen removal performance above, both strong acidic ($\text{pH} \leq 6.5$) and alkaline conditions ($\text{pH} \geq 8.5$) inhibited ANAMMOX process. Besides, ANAMMOX appeared to be more susceptible to alkaline wastewater. The mechanism of pH shock on ANAMMOX reaction was as follows: first, extreme pHs destroyed the proton motive force. They could inhibit the associated energy generation and transform metabolic processes, including energy generation by adenosine triphosphatases (ATP) and active transport of proteins (Fig. 3) (Carvajal-Arroyo et al. 2014; Lu et al. 2013). Moreover, the denaturation of original bond cleavage, the permeability of cell membrane and

Fig. 2 Overall performance of ANAMMOX process at $\text{pH} \geq 7.5$: **a** concentrations of three nitrogen species in influent and effluent, **b** stoichiometric ratios, **c** loading and removal rates of $\text{NH}_4^+\text{-N}$ and $\text{NO}_2^-\text{-N}$, **d** pH, FA and FNA in influent and effluent

Fig. 3 Reaction mechanism occurred in AAOB with acidic and alkaline condition. (1) diffusion of FA and FNA; (2) carrier protein; (3) NH_4^+ and NO_2^- not diffuse into cell membrane; (4) passive transport; (5) ANAMMOX process and cell synthesis; (6) transformation between NH_3 and NH_4^+ ; (7) transformation between HNO_2 and NO_2^-

destruction of other biological structures occurred in reactor, which led to the inactivation of enzymes (Yin et al. 2016). Still, the change of pH value would also bring about the change in the net surface charge of AAOB and thus hampered chemical forces responsible for adhesion and aggregation of microorganisms (Sandberg and Ahring 1992). Furthermore, the inhibition of extreme pH was also ascribed to the pH-dependent substrates such as FA and FNA. FA and FNA were hydrophobic and they could diffuse into cytoplasm through cell membrane (Kadam and Boone 1996). pH could affect the equilibrium of $\text{FA}/\text{NH}_4^+ - \text{N}$ and $\text{FNA}/\text{NO}_2^- - \text{N}$ (Kadam and Boone 1996; Mosquera-Corral et al. 2005). When the extracellular and intracellular pH values were different, the concentrations of FA and FNA in both spaces would also differ. Then the concentration gradient would drive the diffusion of FA and FNA across the cell membrane (Jin et al. 2012; Kadam and Boone 1996). That would result in variation of intracellular pH and create a proton imbalance (Almeida et al. 1995; Sprott and Patel 1986; Wittmann et al. 1995). This made FA and FNA (in cell) transformed into $\text{NH}_4^+ - \text{N}$ and $\text{NO}_2^- - \text{N}$ constantly, which led to the further increase (alkaline condition) or decrease (acidic condition) of pH in cell (showed in Fig. 3). Last, extreme pH shock could hamper the utilizability of trace elements (Gadd and Griffiths 1977), which led to the inactivation of AAOB.

Sludge characteristics

The particle size distributions (PSD) of ANAMMOX sludge with pH shocks were presented in Fig. 4. The extreme pH could decrease the size of granular sludge (Xing et al. 2015; Li et al. 2015). The diameter of ANAMMOX sludge was 0–5 mm with the mean diameter of 1.69 mm at pH 7.5. The percentage of sludge with diameter larger than 2 mm was 0.444 at pH 6.0 while it was 0.434 at pH 7.5. Besides, at pH 6.0, the mean diameter of sludge was 1.65 mm. Acidic pH had little effect on sludge size. However, disintegration of ANAMMOX granule was observed when pH shifted to 9.0. Besides, the frequency of sludge with diameter larger than 2 mm decreased to 0.254. The mean diameter of granular sludge was 1.28 mm, which was smaller than that at pH 7.5. Extremely alkaline pH could lead to breakage of AAOB, which resulted in decrease of granular diameter. The results found in present work was also agreed with Gao et al. (2010) that the flocs in anaerobic digestion sludge suspension shifted to smaller size at pH 9.1. Sandberg and Ahring (1992) also detected that extremely alkaline pH could lead to disintegration of microbial granule, and the percentage of smaller granule rose as pH increased. The breakage of AAOB in reactor was attributed to the rupture of polysaccharide fibril, which was the support of different cells (Gao et al. 2010; Parker et al. 1972).

Fig. 4 The PSD of AAOB with pH shock (**a** pH 6.0; **b** pH 7.5; **c** pH 9.0; **d** comparison of granular sludge size simulated by Gaussian model)

The small particles formed with alkaline pH had poor sedimentary property, and the fragment of AAOB was easy to wash out which resulted to decrease of MLSS in reactor. The sludge concentration and settling velocity at different pH (i.e. pH 6.0, 7.5 and 9.0) were presented in Fig. 5. In general, the sludge concentration and settling velocity were just similar at pH 6 and 7.5. However, sludge concentration at pH 9.0 decreased to 1.39 g/L. It was lower than that at pH 7.5. This agreed with Sandberg and Ahning (1992) that the concentrations of TS and VS in UASB reactor decreased when pH increased.

A higher settling velocity implied a better sludge retention ability, which could stabilize and enhance the performance of ANAMMOX. In this work, the

Fig. 5 Sludge concentration and settling velocity at different pH

settling velocity was 2.15 cm/s at pH 7.5. Tang et al. (2011) obtained a settling velocity of 73–88 m/h (2.03–2.44 cm/s) in two ANAMMOX reactors, which was similar to present work. However, the settling velocity decreased to 2.02 cm/s when pH was 9.0. Decreasing size of granular sludge resulted in the decrease of settling velocity. Our results also agreed with Su et al. (2013). In general, the granular sludge became fragile at pH 9.0 and a slight pH increase would lead to further damage on ANAMMOX system. This indicated that compared to extremely acidic condition (low pH), the extremely alkaline condition (high pH) affected ANAMMOX granules much more.

Kinetic analysis

pH variations in a typical operation cycle of SBR1 and SBR2 were presented in Figs. S2 and S3. Even though ARR varied with influent pH, there was no linear correlation between them. Besides, ARR was also not correlated with ΔpH . In alkaline surroundings, FA generation was promoted. ANAMMOX process was nearly completely inhibited at pH 9.0. Kinetic analysis could predict the value which experiment did not easily acquired. Besides, it could provide an effective tool for process control. In present work, five kinetic models were used to simulate the ANAMMOX activity with pH shock.

The Bell-shaped (A) model

Figure 6a presented the simulation of pH shock on ANAMMOX activity using the Bell-shaped (A) model. The correlation coefficient (R^2) of the plot was 0.9563. Bell-shaped (A) model could well simulate ANAMMOX activity with different pH values. According to Eq. 2, specific NH_4^+-N removal rate ($q_{\max}$) and optimum pH value were 0.37 kg $\text{NH}_4^+-\text{N}/(\text{kg MLSS}\cdot\text{d})$ and 7.47, respectively. In addition, the predicted ' ω ' value was 1.90, which indicated that ANAMMOX reaction could occur with pH of 5.57–9.37.

The Michaelis model

Figure 6b depicted the graph plotted between specific NH_4^+-N removal rate ($q_{\max}$) and pH. The values of $q_{\max}$, pK_{s1} and pK_{s2} were acquired according to Eq. 3.

The $q_{\max}$ was 0.53 kg $\text{NH}_4^+-\text{N}/(\text{kgMLSS d})$. According to the predicated values of pK_{s1} and pK_{s2} , ANAMMOX reaction could occur at pH of 6.80–8.12. This was not in agreement with experimental data. Moreover, the R^2 of the plot was only 0.8878, which suggested that the Michaelis model was not appropriate to predict ANAMMOX activity with pH shock.

The Ratkowsky model

The Ratkowsky model was applied to predict ANAMMOX activity at various pH values as shown in Fig. 6c. The values of $\text{pH}_{\min}$ and $\text{pH}_{\max}$ were 5.68 and 9.26, respectively, and R^2 was 0.9608. The simulated values were consistent with experimental data. It suggested applicability of the Ratkowsky model to predict ANAMMOX activity at different pH values.

The Haldane model

Figure 6d described the relationship between specific NH_4^+-N removal rate ($q_{\max}$) and concentration of H^+ . As a result, the Haldane model failed to predict ANAMMOX activity ($R^2 = 0.7895$). The simulated $q_{\max}$ was as high as 0.55 kg $\text{NH}_4^+-\text{N}/(\text{kgMLSS d})$, which greatly deviated from experimental data.

The Bell-shaped (B) model

van Hulle et al. (2007) reported that experimental data could well fit the equation of the Bell-shaped (B) model. In this work, the simulated $q_{\max}$ and pH_{opt} were 0.38 and 7.45, respectively, which were in line with experimental data. However, low R^2 (0.8672) indicated poor regression result. As a result, further study was needed to explore if the Bell-shaped (B) model could describe the pH shock on ANAMMOX process.

Evaluation and validation of kinetic models

The applicable models were evaluated through judging the linear relationship between experimental data and predicted values. In present work, five kinetic models were used to evaluate the pH on ANAMMOX performance. In order to test the validity of five

Fig. 6 The regression curve of five kinetic models

models, experimental data were compared with predicted values calculated from models (shown in Fig. 7). Kinetic parameters determined in this work were summarized in Table 2.

Through analyzing these models, the Bell-shaped (A) model and the Ratkowsky model were more applicable to simulate the effect resulting from pH shock on ANAMMOX activity ($R^2 > 0.95$). Not only R^2 of regression line plotted by the Ratkowsky model was higher than that by the Bell-shaped (A) model in Fig. 6, but also the former one was a three-parameter model which was simpler and easier to use than the four-parameter model (the latter one) (Zwietering

et al. 1990). Both models could simulate the ANAMMOX activity well with pH shock. Moreover, both models could predict the pH range during which ANAMMOX reaction could occur. This was difficult to measure and was important to control nitrogen removal performance. With regard to other three models, though R^2 of the plot curve using the Michaelis model was high, the pH range for ANAMMOX reaction was 6.80–8.12. This deviated from experimental values. In terms of the Haldane model, its application was limited by two factors. Firstly, the independent variable was H⁺ concentration rather than pH value. H⁺ concentration was more

Fig. 7 Comparison of experimental data and predicted value

Table 2 Summary of kinetic parameter determined in ANAMMOX process

Model	Parameter					R^2
	$Q_{\max}$	$H_{\min}$	$H_{\max}$	H_{opt}	ω	
Bell-shaped (A) model	0.37	—	—	7.47	1.90	0.9563
Michaelis model	0.53	6.80	8.12	—	—	0.8878
Ratkowsky model	—	5.68	9.26	—	—	0.9608
Haldane model	0.55	—	—	—	—	0.7895
Bell-shaped (B) model	0.38	—	—	7.45	—	0.8672

difficult to measure than pH value. Secondly, the model only predicted the $q_{\max}$, and the value was not fitted to experimental data precisely. When it came to

the Bell-shaped (B) model, though the simulated $q_{\max}$ and pH_{opt} were in agreement with experimental data, the predicted values were not fitted very well to

experimental data (showed in Fig. 7e). As a result, though the Bell-shaped (B) model was not applicable in this work, it still deserved further study due to well simulation for $q_{\max}$ and pH_{opt} .

The Bell-shaped (A) model and the Ratkowsky model indicated that $q_{\max}$ was 0.37 kg $\text{NH}_4^+\text{-N}/(\text{kgMLSS d})$ at the optimum pH value (7.47) in present study. The minimum pH during which ANAMMOX occurred was 5.68 while the maximum pH for ANAMMOX reaction was 9.26. Egli et al. (2001) observed that ANAMMOX activity took place only at pH value within 6.5–9. Strous et al. (1999) found that the physiological pH range of ANAMMOX was 6.7–8.3 in SBR. Both were not consistent with this work. Comprehensive effects resulting from different reactor configurations, operational conditions, as well as wastewater types devoted to the discrepancies.

Conclusions

Based on nitrogen removal performance with different pH, strongly acidic ($\text{pH} \leq 6.5$) or alkaline ($\text{pH} \geq 8.5$) inhibited ANAMMOX process. When influent pH was below 7.5, effluent $\text{NH}_4^+\text{-N}$ and $\text{NO}_2^-\text{-N}$ increased with decreasing pH. At pH 6.0, effluent $\text{NO}_2^-\text{-N}$ approached 100 mg/L, and the ratios of $\Delta\text{NO}_2^-\text{-N}:\Delta\text{NH}_4^+\text{-N}$ and $\Delta\text{NO}_3^-\text{-N}:\Delta\text{NH}_4^+\text{-N}$ approached 2.2 and 1.3, respectively. Both greatly deviated from theoretical values. When influent pH was above 7.5, effluent $\text{NH}_4^+\text{-N}$ and $\text{NO}_2^-\text{-N}$ increased with increasing pH. At pH 9.0, ARR and NRR decreased 0.011 ± 0.004 and $0.035 \pm 0.004 \text{ kg}/(\text{m}^3 \cdot \text{d})$, respectively. Besides, $\Delta\text{NO}_2^-\text{-N}:\Delta\text{NH}_4^+\text{-N}$ deviated from theoretical value. Longer recovery time from pH 9.0 than from pH 6.0 indicated that alkaline surroundings inhibited AAOB greater. The sludge settling velocity was 2.15 cm/s at pH 7.5. However, it decreased to 2.02 cm/s when pH was 9.0. Acidic pH had little effect on sludge size, but disintegration of ANAMMOX granule was achieved with pH of 9.0. The Bell-shaped (A) model and the Ratkowsky model were more applicable to simulate the effect resulting from pH shock on ANAMMOX activity ($R^2 > 0.95$), and both could describe ANAMMOX activity well with pH shock. They indicated that $q_{\max}$ was 0.37 kg $\text{NH}_4^+\text{-N}/(\text{kgMLSS d})$ at the optimum pH value (7.47) in present study. The minimum pH during which

ANAMMOX occurred was 5.68 while the maximum pH for ANAMMOX reaction was 9.26.

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