Nitrogen removal performance of anaerobic ammonia oxidation (ANAMMOX) in presence of organic matter

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Abstract A sequencing batch reactor (SBR) was used to test the nitrogen removal performance of anaerobic ammonium oxidation (ANAMMOX) in presence of organic matter. Mesophilic operation (30 ± 0.5 °C) was performed with influent pH 7.5. The results showed, independent of organic matter species, ANAMMOX reaction was promoted when COD was lower than 80 mg/L. However, specific ANAMMOX activity decreased with increasing organic matter content. Ammonium removal efficiency decreased to 80% when COD of sodium succinate, sodium potassium tartrate, peptone and lactose were 192.5, 210, 225 and 325 mg/L, respectively. The stoichiometry ratio resulting from different OM differed largely and R_1 could be as an indicator for OM inhibition. When COD concentration was 240 mg/L, the loss of SAA resulting from lactose, peptone, sodium potassium tartrate and sodium

succinate were 28, 36, 50 and 55%, respectively. Sodium succinate had the highest inhibitory effect on SAA. When ANAMMOX process was used to treat wastewater containing OM, the modified Logistic model could be employed to predict the NRE_{max} .

Keywords Nitrogen removal · Organic matter · ANAMMOX · Denitrification · Kinetic model

Introduction

Over the last decades, nitrogen removal from sewage has gained wide concerns owing to its fatal nature to wildlife and causing eutrophication. Autotrophic nitrogen removal process is promising in sewage treatment. There are photoautotrophic system (Regmi et al. 2015), autotrophic denitrification (Liu et al. 2015) and anaerobic ammonium oxidation (ANAMMOX) reaction (Strous et al. 1998). Among these autotrophic reactions, ANAMMOX was regarded as an efficient process for nitrogen removal. In ANAMMOX process, NH_4^+ -N was oxidized to N_2 with NO_2^- -N as electron acceptor, which was devoted to treating NH_4^+ -N rich wastewater. Although many advantages of ANAMMOX made it popular in wastewater treatment (Okamoto et al. 2012; Qiao et al. 2009), it still faced some problems such as inhibitory effect resulting from organic matter (OM).

OM were unfavorable to ANAMMOX process, however, previous studies indicated that anaerobic

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ammonia oxidizing bacteria (AAOB) were still able to dominate the reactor with OM (Dapena-Mora and Campos 2007; Fernández et al. 2009; Kartal et al. 2007). ANAMMOX was successfully applied to sewage treatment with low C/N (Vlaeminck et al. 2010; Yamamoto et al. 2008). However, when C/N was higher than 1.0, AAOB could not compete with heterotrophic denitrifying bacteria (HDB) (Güven et al. 2005). Besides, Xu et al. (2009) stated that AAOB had advantages over HDB when treating sewage with low C/N. It reported that the activity of AAOB was completely inhibited with 3–4 mM methanol (Jensen et al. 2007). However, Isaka et al. (2008) came to the different conclusion that methanol only inhibited 71% of the AAOB activity when the concentration was 5 mM. In addition, Güven et al. (2005) revealed that low methanol concentrations led to complete, immediate and irreversible inhibition on ANAMMOX reaction. For acetate, Dapena-Mora and Campos (2007) reported 50 mM acetate resulted in 70% inhibition on ANAMMOX. However, Güven et al. (2005) found that acetate was potentially substrate for AAOB. Phenol, chloramphenicol, penicillin, antibiotics and propionate had been studied, too (Fernández et al. 2009; Güven et al. 2005; Toh et al. 2002; van de Graaf et al. 1996). However, effects of various OM on ANAMMOX were diverse, and inhibitory threshold values differed largely even for the same OM.

In this work, a sequencing batch reactor (SBR) was used to study the nitrogen removal performance of ANAMMOX in presence of OM. Specific ANAMMOX activity (SAA) was analyzed throughout the process. In addition, kinetic and thermodynamic analyses were performed to study inhibitory characteristics.

Materials and methods

Experimental set-up

Five parallel SBRs made of polymethyl methacrylate were used in this work. The reactors were double-jacketed with an effective volume of 1.5 L. Operating temperature was kept at 35 °C. All the reactors were covered with aluminum caps to prevent light. Hydraulic retention time (HRT) was 8 h. During the experiment, reactors were stirred at 100 rpm. N₂ (99.99%) was purged in influent to remove O₂. The influent was

pumped into reactors through peristaltic pump and influent pH was around 7.5 by adding 0.1 mol/L hydrochloric acid or sodium hydroxide.

Inoculated sludge and synthetic wastewater

Each reactor was inoculated with 1 L ANAMMOX sludge which was obtained from two lab-scale reactors (with an effective volume of 7 L) operated steadily over one year at 35 ± 1 °C. The inoculating sludge was mixed and added to each reactor. The sludge was brightly red with good settling property (presented in Fig. S1) and the concentration of it was 1500 mg/L. The diameter of the granular sludge was 0.5–5.0 mm, with a mean value of 1.72 mm. Influent NH₄⁺-N and NO₂⁻-N were 80 and 105 mg/L, respectively. The removal efficiencies of both substrates were higher than 98%. Composition of synthetic wastewater was (g/L): KH₂PO₄ 0.028; CaCl₂ 0.14; KHCO₃ 2.0; MgSO₄·7H₂O 0.2; NH₄Cl 0.336; NaNO₂ 0.573. Trace element solutions I and II (1.2 ml/L) were also added according to previous report (van de Graaf et al. 1995).

Experimental procedures

Lactose, peptone, sodium potassium tartrate and sodium succinate were added into the synthetic wastewater as influent. The control one started with inoculation of ANAMMOX sludge without any OM addition. The effect resulting from OM on ANAMMOX was studied simultaneously. The influent COD gradually increased from 40 mg/L to the concentration which caused ANAMMOX inhibition. And the inhibition threshold of COD was defined when NH₄⁺-N removal efficiency decreased to 80% (Molin-uevo et al. 2009; Ni et al. 2012). OM concentration was based on COD and six cycles were operated in each OM concentration.

Kinetic evaluation

The modified Logistic model and modified Boltzmann model (Yang and Jin 2013) were employed to quantify the nitrogen removal characteristics of ANAMMOX performance in a cycle.

$$H = \frac{H_{\max}}{1 + \exp[4 \times R_m \times (\lambda - t)/H_{\max} + 2]} \quad (1)$$

$$H = H_{\max} + \frac{H_{\min} - H_{\max}}{1 + \exp[(t - t_c)/t_d]} \quad (2)$$

where R_m represents maximum nitrogen removal rate (%/h); λ represents lag time (h); t represents operating time (h); t_c represents center value (h); t_d represents time constant (h). When Eqs. 1 and 2 were used to demonstrate the progress of substrate degradation in batch tests, H (NRE, nitrogen removal efficiency) and $H_{\max}$ ($NRE_{\max}$, maximum nitrogen removal efficiency) describe the cumulative degraded substrate value and maximum degraded substrate value, respectively (Wang and Wan 2009).

Analytical methods

The influent and effluent samples were collected each day and then analyzed immediately. NH_4^+ -N, NO_2^- -N and NO_3^- -N concentrations were measured with the Nessler's reagent spectrophotometry method, ultraviolet spectrophotometric screening method and N-(1-naphthyl)-ethylenediamine spectrophotometric method, respectively (APHA 1998). Sludge concentration, COD and TN were all analyzed according to the standard methods (APHA 1998). Temperature and pH were measured by the WTW 340i/SET pH/Oxi portable multi-parameter measuring meter. Each analytical result reported in this work was analyzed in triplicate. The activity inhibition was described as Eq. 3.

$$C_I = (SAA - SAA_c)/SAA_c \quad (3)$$

$$\Delta_r G'_m = \Delta_r G_m^\theta + RT \cdot \ln Q_a \quad (4)$$

where C_I represents inhibition degree; SAA represents specific ANAMMOX activity, SAA_c represents SAA in control group; $\Delta_r G_m^\theta$ represents standard Gibbs free energy; R represents ideal gas constant and its value is 8.314 J/kg/K; T represents Kelvin.

Completely closed vials were used to perform SAA batch assays. The influent NH_4^+ -N and NO_2^- -N concentrations were 80 and 105 mg/L, respectively. The sludge concentration was 1500 mg/L. The influent pH value was 7.5 and the temperature was maintained at 35 °C. N_2 was purged into vials to remove O_2 . NH_4^+ -N and NO_2^- -N were determined at regular intervals (0.5 h). Eventually, SAA was calculated by the ratio of maximum substrate consumption rate to sludge concentration (Qiao et al. 2014).

Results and discussion

Nitrogen removal performance of ANAMMOX process

Nitrogen removal performances of ANAMMOX processes with different OMs were presented in Fig. 1. When lactose was added, effluent NH_4^+ -N and NO_2^- -N varied little at $COD \leq 160$ mg/L. With 360 mg/L COD, effluent NH_4^+ -N increased to 20 ± 2 mg/L while ARE and ammonia removal rate (ARR) were $75 \pm 3\%$ and 0.175 ± 0.01 kg/(m³ day), respectively. In addition, effluent NO_3^- -N was lower than that in influent. The maximum NRE and nitrogen removal rate (NRR) reached $94.5 \pm 1\%$ and 0.535 ± 0.01 kg/(m³ day), respectively, with 280 mg/L COD. When peptone was added, effluent NH_4^+ -N was 18 ± 2 mg/L with 240 mg/L COD. ARE and ARR were $77.5 \pm 2.5\%$ and 0.188 ± 0.01 kg/(m³ day), respectively. NRE and NRR peaked at $87 \pm 1\%$ and 0.50 ± 0.005 kg/(m³ day) with 160 mg/L COD and then decreased to $81.5 \pm 1.5\%$ and 0.485 ± 0.01 kg/(m³ day), respectively. When sodium potassium tartrate was added, effluent NH_4^+ -N and NO_2^- -N varied little with $COD \leq 120$ mg/L. When COD was 240 mg/L, effluent NH_4^+ -N increased to 21 ± 2 mg/L and ARE and ARR were $71.5 \pm 2.5\%$ and 0.177 ± 0.012 kg/(m³ day), respectively. The maximum NRE and nitrogen removal rate (NRR) reached $87 \pm 1\%$ and 0.485 ± 0.005 kg/(m³ day), and then decreased to $83 \pm 1\%$ and 0.455 ± 0.02 kg/(m³ day). When sodium succinate was added, effluent NH_4^+ -N increased when COD was more than 120 mg/L. NRE decreased from 83 ± 1 to $77.5 \pm 2\%$. All were lower than the control group, which indicated the inhibition effect throughout the whole process.

Low OM content did not affect ANAMMOX greatly. However, high OM concentration would apparently inhibit the ANAMMOX process. The inhibition threshold values of lactose, peptone, sodium potassium tartrate and sodium succinate were 325, 240, 210 and 192.5 mg/L. The inhibitory effect resulting from different OMs varied. AAOB had more affinity to micro-molecular OMs than macro-molecular ones. With OM added, nitrogen was removed through competition between ANAMMOX and denitrification (Anjali and Sabumon 2014; Ma et al. 2016). At low OM concentration, AAOB could coexist with HDB. However, at high OM concentration,

Fig. 1 Nitrogen removal performance of ANAMMOX process in presence of OM: **a** peptone; **b** sodium potassium tartrate; **c** sodium succinate; **d** lactose; **e** removal efficiency of $\text{NH}_4^+\text{-N}$ and TN; **f** removal rate of $\text{NH}_4^+\text{-N}$ and TN

increasing HDB competed for NO_2^- -N with AAOB. Our results agreed with Chamchoi et al. (2008) that HDB could rapidly grow with high COD concentration (300 mg/L) and adversely affect AAOB. Comparison of inhibition in presence of various OMs was presented in Table 1. Independent of OM species, in general, it was the same that ANAMMOX reaction

was inhibited when high OM concentration was added.

When OM was removed from the reactor in the last period of research, ANAMMOX activities in all SBRs recovered in 8 days. NH_4^+ -N and NO_2^- -N were completely removed. The value of ARR and NRR were back to 0.23 ± 0.008 and 0.47 ± 0.012 kg/

Table 1 Comparison of inhibition in presence of various OM

OMs	Operation mode	Concentration	Effect	References
Non-fat dry milk	Continuous, UASB	308 mg/L	−20%	Ni et al. (2012)
Pig manure effluent (after UASB-post-digestion)	Semi-continuous, UASB	Higher than 237 mg/L ¹	−100%	Molinuevo et al. (2009)
Pig manure effluent (after UASB-post-digestion)	Semi-continuous, UASB	Higher than 290 mg/L ^a	−100%	Molinuevo et al. (2009)
Glucose	Continuous, UASB	100 mg/L	Lower than −10% ^a	Yuan et al. (2015)
Glucose	Continuous, UASB	300 mg/L	−31%	Yuan et al. (2015)
Glucose	Batch, Vials	1 mM	5%	Oshiki et al. (2011)
Methanol	Continuous ³	0.5 mM	−100%	Guyen et al. (2005)
Methanol	Batch, Vials	1 mM	−86%	Oshiki et al. (2011)
Methanol	Batch	1.7 mM	−30%	Isaka et al. (2008)
Methanol	Batch	3.3 mM	−49%	Isaka et al. (2008)
Methanol	Batch	5 mM	−71%	Isaka et al. (2008)
Ethanol	Batch, Vials	1 mM	−91%	Oshiki et al. (2011)
Ethanol	Batch ³	1.1 mM	−3%	Isaka et al. (2008)
Ethanol	Batch ³	3.3 mM	−10%	Isaka et al. (2008)
Ethanol	Continuous ³	2 mM	−30%	Guyen et al. (2005)
Acetate	Batch ³	10 mM	No significant effect	Dapena-Mora and Campos (2007)
Acetate	Batch, Vials	1 mM	−2%	Oshiki et al. (2011)
Acetate	Batch, Vials	10 mM	No significant effect	Dapena-Mora and Campos (2007)
Acetate	Batch, Vials	25 mM	−22%	Dapena-Mora and Campos (2007)
Acetate	Batch, Vials	50 mM	−70%	Dapena-Mora and Campos (2007)
Sucrose	Continuous, UASB	700 mg/L	−45%	Tang et al. (2010)
Propionate	Batch, Vials	1 mM	−1%	Oshiki et al. (2011)
Formate	Batch, Vials	1 mM	−36%	Oshiki et al. (2011)
Tetracycline hydrochloride	Continuous	10 mg/L	−60%	Fernández et al. (2009)
Chloramphenicol	Batch	200 mg/L	−68%	van de Graaf et al. (1995)
Lactose	Batch, SBR	360 mg/L	−26%	This study
Peptone	Batch, SBR	240 mg/L	−25.5%	This study
Sodium potassium tartrate	Batch, SBR	240 mg/L	−28%	This study
Sodium succinate	Batch, SBR	240 mg/L	−38%	This study

^a When the COD was higher than that concentration, the results were all suitable

(m^3 day). These results revealed that OM inhibition on ANAMMOX was reversible. Besides, HDB did not exist in reactor due to absence of carbon source, and the competition for NO_2^- -N between AAOB and HDB disappeared. AAOB dominated the reactor again easily. The ability of AAOB to resist OM shock was good and ANAMMOX process could also be used to treat wastewater containing low OM content.

Stoichiometry

The stoichiometry can be used as evidence for ANAMMOX inhibition (Sun et al. 2011). The variations of R_1 (the ratio of consumed NO_2^- -N to consumed NH_4^+ -N) and R_2 (the ratio of produced NO_3^- -N to consumed NH_4^+ -N) with different OM were presented in Fig. 2. The R_1 and R_2 of control group were 1.29 and 0.315, respectively. When OM was added, the R_1 varied little with $\text{COD} \leq 80$ mg/L. Independent of OM species, R_1 increased with

growing COD. When COD was no more than 40 mg/L, R_2 varied little. Then it increased with increasing COD. Our results agreed with previous report that both stoichiometric ratios deviated from theoretical values with increasing COD (Li et al. 2016). It demonstrated that OM addition affected ANAMMOX process and heterotrophic denitrification occurred with high COD content. When OM was removed from the reactor, R_1 and R_2 became 1.30 ± 0.03 and 0.315 ± 0.02 , respectively. ANAMMOX activity recovered quickly.

The stoichiometry ratio resulting from different OM differed largely. When COD was 240 mg/L, the R_1 of sodium succinate addition was the most of all. It was in line with previous result that the inhibitory effect of sodium succinate was the most of all. R_1 could be as an indicator for OM inhibition. In addition, the change of R_1 was slower than R_2 with increasing COD. There were two possibilities leading to the phenomenon. Firstly, the metabolic pathways of

Fig. 2 Stoichiometric ratio in presence of OM

AAOB might be transformed (Dalsgaard and Thamdrup 2002; Dosta et al. 2008). Secondly, other electron acceptor except NO_2^- -N might exist when OM was added.

Previous study indicated that, as the result of fast growth rate and cell yield of HDB, AAOB could lose their activity or even vanish from the reactor when OM was added (Kuenen 2008). Tomar et al. (2015) reported that HDB had higher affinity to NO_2^- -N than AAOB. HDB could outcompete AAOB when OM content was high. Both resulted in the increase of R_1 and decrease of R_2 . Comparison of stoichiometric ratios with various OM was presented in Table 2. Independent of OM species, in general, it was the same that R_1 increased and R_2 decreased when OM was added.

Activity of AAOB

Batch test was conducted to determine SAA in presence of OM. The results were presented Fig. 3. When peptone was added, SAA increased with COD

no more than 100 mg/L. Then it decreased to $0.22 \text{ kgNH}_4^+\text{-N/kgVSS day}$ with 240 mg/L COD. Similar results were found when lactose or sodium potassium tartrate was added. SAA increased with COD no more than 40 mg/L, and then decreased with increasing COD. However, when sodium succinate

Fig. 3 Variations of SAA with COD in presence of OM

Table 2 Comparison of stoichiometric ratio in presence of OM

OM	Control group		Concentration (mg/L)	Experimental result		Reference
	R_1	R_2		R_1	R_2	
Propionate	1.30	–	296	1.60	–	Guyen et al. (2005)
Non-fat dry milk	1.01	0.18	100	1.04	0.12	Ni et al. (2012)
Non-fat dry milk	1.01	0.18	200	1.10	–	Ni et al. (2012)
Non-fat dry milk	1.01	0.18	400	1.39	0.06	(Ni et al. 2012)
Glucose	1.29 ± 0.09	0.26 ± 0.03	100	1.33 ± 0.10	0.24 ± 0.03	Tang et al. (2013)
Glucose	1.29 ± 0.09	0.26 ± 0.03	400	1.39 ± 0.12	0.05 ± 0.02	(Tang et al. 2013)
Glucose	1.29 ± 0.09	0.26 ± 0.03	800	4–5	–	Tang et al. (2013)
UASB-post-digested effluent	1.32	–	95	1.49	–	Molinuevo et al. (2009)
UASB-post-digested effluent	1.32	–	237	3.03	–	Molinuevo et al. (2009)
Lactose	1.29	0.315	80	1.293	0.25	This study
Lactose	1.29	0.315	240	1.40	0.02	This study
Lactose	1.29	0.315	360	1.70	–0.11	This study
Peptone	1.29	0.315	80	1.295	0.27	This study
Peptone	1.29	0.315	240	1.69	0.03	This study
Sodium potassium tartrate	1.29	0.315	80	1.298	0.26	This study
Sodium potassium tartrate	1.29	0.315	240	1.72	0.07	This study
Sodium succinate	1.29	0.315	80	1.288	0.31	This study
Sodium succinate	1.29	0.315	240	2.05	0.12	This study

was added, SAA decreased with increasing COD throughout the whole process. Different OM had different effects on ANAMMOX process. Activity of AAOB could be enhanced when some OM were added (peptone, lactose and sodium potassium tartrate). However, independent of OM species, SAA decreased sharply when COD was high. This also agreed with previous report that SAA sharply decreased from 0.27 kgNH₄⁺-N/kgVSS day without OM to 0.07 kgNH₄⁺-N/kgVSS day with 700 mg/L COD (Tang et al. 2013).

Comparison of SAA with different OM both in previous studies and this work was presented in Table 3. Inhibitory effects resulting from different OM differed largely. In this work, SAA changed slowest when lactose was added. It indicated that lactose had little effect on ANAMMOX process. When COD concentration was 240 mg/L, the loss of SAA resulting from lactose, peptone, sodium potassium tartrate and sodium succinate were 28, 36, 50 and 55%, respectively. Sodium succinate had the highest inhibitory effect on SAA. As a result, both the OM species and concentration affected the activity of AAOB.

Kinetic and thermodynamic analyses

The removal performance of TN was described by modified Logistic model and modified Boltzmann model. The results were presented in Tables 4 and 5. R² were all higher than 0.95, which indicated that both models could simulate the process well in a typical operation. Compared to modified Boltzmann model, modified Logistic model described nitrogen removal performance better. Independent of OM species and concentration, NRE_{max} values simulated from the model were quite similar with ones achieved from experiment. Besides, unlike the modified Boltzmann model, the modified Logistic model was a three-parameter model. It was simpler and easier to simulate experimental process and the solution of three-parameter model was more stable (Zwietering et al. 1990). When ANAMMOX process was used to treat wastewater containing OM, the modified Logistic model could be employed to predict the NRE_{max} very well.

When OM was added, heterotrophic denitrification reaction occurred in the reactor. The equations of ANAMMOX (van de Graaf et al. 1995), nitrite-based

Table 3 Comparison of SAA in presence of OM

OM	Influent nitrogen concentration (mg/L)		COD concentration (mg/L)	SAA (kg NH ₄ ⁺ -N/kg VSS d)	References
	NH ₄ ⁺ -N	NO ₂ ⁻ -N			
Glucose	250–350	320–430	0	0.27	Tang et al. (2013)
	350	430	200	0.24	
	350	430	400	0.19	
	350	430	800	0.07	
Sucrose	240	240	0	0.317	Tang et al. (2010)
	240	240	Recover from 700	0.122	
Chloramphenicol	150	150	0	0.25	Fernández et al. (2009)
	150	150	20	0.05	
Tetracycline hydrochloride	150	150	0	0.26	Fernández et al. (2009)
	150	150	10	0.10	
Control group	80	105	0	0.36	This study
Lactose	80	105	360	0.21	
Peptone	80	105	240	0.23	
Sodium potassium tartrate	80	105	240	0.18	
Sodium succinate	80	105	240	0.16	

Table 4 Simulated values through the modified Logistic model

Lactose	0	40	80	120	160	200	240	280	320	360
NRE _{max} (%)	82.64	83.71	85.74	88.57	91.84	93.05	92.99	93.30	91.42	89.28
R _{max} (%/h)	25.02	26.76	26.24	25.00	27.22	27.91	27.48	26.65	26.74	26.36
λ (h)	1.00	1.05	0.97	0.80	0.87	0.86	0.90	0.91	0.86	0.74
R ²	0.9947	0.9926	0.9948	0.9968	0.9947	0.9955	0.9967	0.9954	0.9953	0.9937
Peptone	0	40	80	120	160	200	240			
NRE _{max} (%)	82.64	82.53	84.73	86.26	87.29	83.23	83.18			
R _{max} (%/h)	25.02	27.22	32.52	35.30	33.94	28.77	22.50			
λ (h)	1.00	0.77	0.83	0.91	1.10	0.65	0.89			
R ²	0.9947	0.9922	0.9954	0.9965	0.9978	0.9950	0.9854			
Sodium potassium tartrate	0	40	80	120	160	200	240			
NRE _{max} (%)	82.64	82.98	84.42	86.77	84.10	82.12	79.27			
R _{max} (%/h)	25.02	26.86	26.99	29.52	33.46	26.95	24.75			
λ (h)	1.00	0.76	0.87	0.90	1.00	0.60	0.65			
R ²	0.9947	0.9952	0.9935	0.9968	0.9952	0.9951	0.9928			
Sodium succinate										
NRE _{max} (%)	82.64	81.87	82.77	82.93	81.59	80.21	75.91			
R _{max} (%/h)	25.02	24.46	25.03	22.64	25.54	26.67	23.79			
λ (h)	1.00	0.87	0.78	0.56	0.64	0.65	0.62			
R ²	0.9947	0.9942	0.9952	0.9876	0.9897	0.9943	0.9941			

heterotrophic denitrification and nitrate-based heterotrophic denitrification (in presence of lactose) were as follows:

ANAMMOX

Denitrification

$\Delta_r G_m^\theta$ was described as the Gibbs free energy in standard state (temperature was 298.15 K and atmospheric pressure was 101.325 kPa). Among them, NO_2^- -N was the common substrate for both ANAMMOX and nitrite-based heterotrophic denitrification. Likewise, the addition of OM might have provoked the competition between nitrite-based heterotrophic denitrification and nitrate-based heterotrophic denitrification. Thus, the competition for substrate might have existed among these three bio-reactions.

As thermodynamic analysis showed above, for nitrite competition, $\Delta_r G_m^\theta$ of ANAMMOX reaction ($-357.79 \text{ kJ/mol NO}_2^-$) is higher than that of nitrite-based heterotrophic denitrification ($-406.03 \text{ kJ/mol NO}_2^-$). However, it is undeniable that AAOB is more likely to receive NO_2^- -N at the beginning of dosing lactose where the amount of HDB was still low (Tang et al. 2013) which can be revealed by the promotion of low COD concentration on ANAMMOX reaction. However, based on the thermodynamic analysis of $\Delta_r G_m^\theta$, at high COD of lactose, the nitrite-based heterotrophic denitrification occurs more easily than ANAMMOX reaction which is in line with the results revealed above.

Table 5 Simulated values through the modified Boltzmann model

Lactose	0	40	80	120	160	200	240	280	320	360
$NRE_{\max}$ (%)	83.68	84.81	86.90	89.69	93.22	94.21	94.05	94.84	92.72	90.34
$NRE_{\min}$ (%)	−8.83	−9.67	−9.73	−8.86	−12.04	−10.74	−8.78	−11.80	−10.96	−11.15
t_c	2.43	2.39	2.37	2.36	2.28	2.29	2.40	2.39	2.32	2.17
t_d	0.99	0.95	0.99	1.05	1.04	1.00	0.99	1.08	1.04	1.02
R^2	0.9978	0.9966	0.9983	0.9990	0.9986	0.9986	0.9990	0.9991	0.9985	0.9966
Peptone	0	40	80	120	160	200	240			
$NRE_{\max}$ (%)	83.68	83.41	85.27	86.76	87.71	84.11	85.95			
$NRE_{\min}$ (%)	−8.83	−11.43	−7.82	−6.64	−4.86	−11.26	−18.81			
t_c	2.43	2.02	1.98	2.01	2.29	1.85	2.27			
t_d	0.99	0.93	0.76	0.70	0.72	0.89	1.28			
R^2	0.9978	0.9961	0.9979	0.9985	0.9991	0.9985	0.9941			
Sodium potassium tartrate	0	40	80	120	160	200	240			
$NRE_{\max}$ (%)	83.68	83.90	85.39	87.22	84.55	82.55	80.51			
$NRE_{\min}$ (%)	−8.83	−10.40	−10.54	−5.29	−5.72	−6.28	−14.20			
t_c	2.43	2.06	2.19	2.25	2.14	1.97	1.90			
t_d	0.99	0.94	0.95	0.82	0.71	0.86	1.02			
R^2	0.9978	0.9987	0.9973	0.9978	0.9969	0.9961	0.9981			
Sodium succinate	0	40	80	120	160	200	240			
$NRE_{\max}$ (%)	83.68	83.00	83.94	84.89	83.09	81.13	77.16			
$NRE_{\min}$ (%)	−8.83	−10.82	−11.65	−21.78	−17.66	−11.89	−13.92			
t_c	2.43	2.27	2.15	1.82	1.81	1.87	1.86			
t_d	0.99	1.02	1.02	1.25	1.06	0.93	1.03			
R^2	0.9978	0.9979	0.9988	0.9950	0.9965	0.9984	0.9991			

Similarly, thermodynamic analyses suggested that the $\Delta_r G_m^\theta$ of nitrate-based heterotrophic denitrification (-5212.9 kJ/mol $C_{12}H_{22}O_{11}$) is higher than nitrite-based heterotrophic denitrification (-6496.48 kJ/mol $C_{12}H_{22}O_{11}$). Organic matter was initially consumed by nitrite oxidizing bacteria. Then it was consumed by nitrate oxidizing bacteria. This could explain that adding extra NO_2^- -N benefited ANAMMOX reaction in presence of OM.

Conclusions

SBRs were used to test nitrogen removal performance of ANAMMOX in presence of different OMs. Independent of OM species, ANAMMOX reaction was promoted when COD was lower than 80 mg/L. However, SAA decreased with increasing organic matter content. The values of ARE decreased to 80% when COD of sodium succinate, sodium potassium

tartrate, peptone and lactose were 192.5, 210, 225 and 325 mg/L, respectively. The stoichiometry ratio resulting from different OM differed largely and R_1 could be as an indicator for OM inhibition. When COD concentration was 240 mg/L, the loss of SAA resulting from lactose, peptone, sodium potassium tartrate and sodium succinate were 28, 36, 50 and 55%, respectively. Sodium succinate had the highest inhibitory effect on SAA. The competition for NO_2^- -N and living space between AAOB and HDB could weaken ANAMMOX process. In wastewater engineering, adding extra NO_2^- -N benefited ANAMMOX reaction in presence of OM. When ANAMMOX process was used to treat wastewater containing OM, the modified Logistic model could be employed to predict the $NRE_{\max}$ very well.

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