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Performance and sludge characteristics of anammox process at moderate and low temperatures

Jin Li*,†, Dan Wang, Deshuang Yu*, and Peiyu Zhang***

*School of Environmental Science and Engineering, Qingdao University, Qingdao 266071, China
**National Marine Environmental Forecasting Center, State Oceanic Administration, Beijing 100081, China

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Abstract−A sequencing batch reactor (SBR) was used to investigate the performance and sludge characteristics of anammox process at moderate and low temperatures. The initial pH was 7.5 and hydraulic retention time (HRT) was 3 h. When temperature was 25-35 °C, nitrogen removal rate (NRR) fluctuated from 1.67 to 1.82 $kg/m^3 \cdot d$. However, when temperature dropped to 15 °C, NRR suddenly decreased by 0.48 $kg/m^3 \cdot d$. Larger activation energy was acquired at lower temperature, and it was difficult to achieve efficient nitrogen removal under low temperature. When temperature declined to 10 °C, ΔNO_2^--N/ΔNH_4^+-N and ΔNO_3^--N/ΔNH_4^+-N reached 1.02 and 0.27, respectively. Inhibition resulting from low temperature on anammox activity was recoverable, and the modified Boltzmann model was appropriate to analyze recovery feature of anammox process. Low temperature not only led to poor nitrogen removal, but also affected sludge size and feature.

Keywords: Anammox, Nitrogen Removal Performance, Temperature, Activation Energy, Sludge Characteristics

INTRODUCTION

High-strength nitrogen in water can lead to many environmental issues, including eutrophication and toxicity to aquatic animals [1]. As a result, nitrogen removal from wastewater is quite important because it threatens the aquatic ecosystem [2]. Compared to traditional nitrification-denitrification process, anaerobic ammonium oxidation (anammox) technology has many advantages, such as low sludge production and high nitrogen removal efficiency [3-5]. Anammox bacteria belong to autotrophic microbes, and they oxidize NH_4^+ to N_2 with NO_2^- being reduced [6]. During the anammox process, aeration is not required and organic carbon source is not added; therefore, it is cost-effective and environment-friendly. As a promising technology, the anammox process has been employed to remove nitrogen from different kinds of wastewaters such as swine wastewater [7], turtle breeding wastewater [8], landfill leachate [9,10] and high-salinity wastewater [11]. It has also been used to treat municipal wastewater [12]. It is quite suitable for treating wastewater whose ammonia content is high and organic carbon is low [13].

Anammox bacteria can grow in a broad temperature range, between −1 and 43 °C [14]. Previous study indicated that the optimal temperature for them was around 35 °C [15]. Many studies concerning nitrogen removal using anammox have been conducted at relatively high temperatures (30-37 °C) [16,17]. However, it is still difficult to achieve efficient nitrogen removal through anammox at low temperature. Although nitrogen removal performance with temperature below 25 °C has been studied [18], to our knowledge, little improvement has been made when operating temperature is low. Since some wastewaters have low temperatures, it is necessary to study performance and sludge characteristics of anammox process with low operating temperature.

The sequencing batch reactor (SBR) has many advantages, such as operating simplicity and flexibility as well as low cost. As a result, it is quite suitable to enrich slow-growing bacteria [19]. In this work, an SBR was used and the study objectives were (1) to explore the influence resulting from temperature shock on anammox process (10 to 35 °C for long-term effect and 5 to 35 °C for short-term effect), (2) to study the operating stability of anammox process when temperature is low, and (3) to investigate the anammox granular sludge characteristics at moderate and low temperature.

†To whom correspondence should be addressed.
E-mail: ljin0532@126.com

MATERIALS AND METHODS

1. Wastewater Characteristics

According to van de Graaf et al. [20], synthetic autotrophic medium was used in this work. Ammonia and nitrites were added in the form of NH_4Cl and $NaNO_2$. Besides, in order to achieve optimal growth of anammox bacteria, trace element solutions I and II (1 mL/L) were also added. The trace element solution I contained (g/L): $FeSO_4$ $7H_2O$ 5; EDTA 5. The trace element solution II contained (g/L): EDTA 15; H_3BO_3 0.011; $MnCl_2 \cdot 4H_2O$ 0.99; $CuSO_4 \cdot 5H_2O$ 0.25; $ZnSO_4 \cdot 7H_2O$ 0.43; $NiCl_2 \cdot 6H_2O$ 0.19; $Na_2MoO_4 \cdot 2H_2O$ 0.22; $CoCl_2 \cdot 6H_2O$ 0.24; $NaSeO_4 \cdot 10H_2O$ 0.21.

2. Experimental Setup and Operational Strategy

The SBR was fabricated with Plexiglas, and the effective volume was 7 L (presented in Fig. 1). It was covered completely with an aluminum cap to avoid the growth of photosynthetic microorganisms. The initial pH was maintained around 7.5. Moreover, before this work, the reactor had been operated stably for about ten months

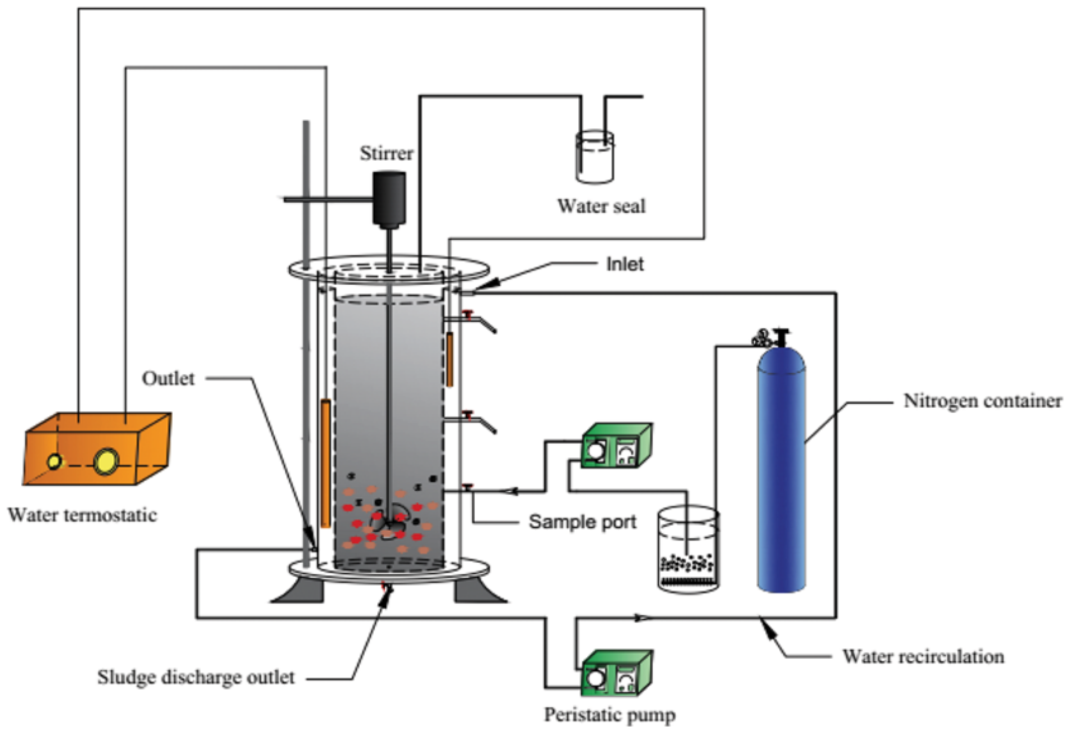

Fig. 1. Schematic diagram of the SBR.

Table 1. Operational condition during entire experimental process

Phase	Operating temperature (°C)	Operating time (d)	Nitrogen rate (kg/m^3·d)		Stoichiometric ratio	
			NLR	NRR	ΔNO_2^--N/ΔNH_4^+-N	ΔNO_3^--N/ΔNH_4^+-N
P_1	35-25	1-46	2.42	1.40-2.05	1.27±0.05	0.23±0.03
P_2	25-18	47-126	2.42	1.23-1.75	1.23±0.08	0.24±0.05
P_3	10-18	127-196	2.42	0.29-1.24	1.02±0.19	0.27±0.08
P_4	18	197-262	1.67-2.42	0.24-1.32	1.22±0.21	0.26±0.15

and a steady state was achieved with nitrogen removal rate (NRR) of 2.06 kg/(m^3·d) at 35 °C. In addition, the maximal specific anammox activity (SAA) in the reactor was 5.53 mg/(g·h).

The influent nitrogen concentration was approximately 302 mg/L for both NO_2^--N and NH_4^+-N (NO_2^--N/NH_4^+-N was 1.32). Additionally, the working hydraulic retention time (HRT) was controlled at 3 h during the process. The operating mode of the reactor consisted of 0.5 h influent feeding, 3 h anoxic stirring reaction, 0.5 h sludge settling and 0.5 h effluent discharging. Considering that a sudden temperature change could lead to destabilization of a biological system, the anammox process was operated by gradually decreasing temperature. The whole operating process was divided into four phases (P_1-P_4) according to temperature variation presented in Table 1.

3. Batch Test

SAA was determined by batch test based on the method reported by Jin et al. [21]. A serum bottle was used in batch test to analyze the short-term effect of temperature on anammox activity. The inoculating sludge was obtained from the test SBR, and the mixed liquor suspended solids (MLSS) was about 2.5 g/L. Both influent NH_4^+-N and NO_2^--N contents were 100 mg/L. Initial pH was controlled around 7.5 by adding HCl or NaOH (0.5 mol/L). The short-term effect of temperature on anammox is presented in Table 2.

Table 2. The short-term effect resulting from temperature on anammox process

Temperature (°C)	SAA (mg/g·h)	Activation energy (kJ/mol)
35	3.82	45.92
30	3.12	45.92
25	2.09	45.92
20	1.17	95.34
15	0.63	95.34
10	0.29	95.34
5	0.14	95.34

4. Analytical Methods

NH_4^+-N, NO_2^--N and NO_3^--N were analyzed based on standard methods [22]. Dissolved oxygen (DO) content, pH and temperature were tested by oxygen, pH and temperature probes (WTW 340i, Germany). Particle size distribution of granular sludge was studied according to previous report [23]. Scanning electron microscopy (SEM) was utilized to analyze granular characteristics [24].

5. Kinetic Analysis

Arrhenius equation:

$$\ln r_T = \frac{-E_a}{RT} + \ln A \quad (1)$$

where A is Arrhenius constant; R is gas constant; T is temperature; E_a is activation energy.

Modified Boltzmann growth model [25]:

$$NRR = NRR_{max} + \frac{NRR_{min} - NRR_{max}}{1 + e^{t - t_c / t_d}} \quad (2)$$

where NRR is nitrogen removal rate; NRR_{max} is maximum NRR; NRR_{min} is minimum NRR; t is operating time; t_c is center value; t_d is time constant.

Modified Gompertz model [26]:

$$NRR = NRR_{max} \cdot \exp\left[-\exp\left(\frac{R_{max} \cdot e}{NRR_{max}} \cdot (\lambda - t) + 1\right)\right] \quad (3)$$

where R_{max} is maximum growth rate; λ is lag time; t is accumulation time.

RESULTS AND DISCUSSION

1. Short-term Effect of Temperature on Anammox

The short-term effect of temperature on anammox activity can be described by the Arrhenius equation. The Arrhenius plot presented in Fig. 2(a) indicates a break at 25 °C and two different activation energy values, 95.34 and 45.92 kJ/mol, respectively, corresponding to temperature ranges of 5-25 and 25-35 °C. In terms of short-term effect, it is clear that the anammox reaction could be operated much more steadily between 25 and 35 °C. However, it was difficult to achieve efficient nitrogen removal when the temperature was low due to higher activation energy.

2. Long-term Effect of Temperature on Anammox

The nitrogen removal performance of anammox resulting from long-term temperature effect is presented in Fig. 3. When temperature was 25-35 °C, nitrogen removal was affected little. A steady performance was achieved. NRR fluctuated from 1.67 to 1.82 kg/m^3·d. When the temperature was around 23 °C, effluent NH_4^+-N and NO_2^--N fluctuated from 15.12 to 38.45 mg/L and 42.23 to 57.53 mg/L, respectively. On day 67, temperature was 20 °C, the NRR dropped to 1.23 kg/m^3·d. However, it could increase to 1.60 kg/m^3·d after 9 days' operation. When operating temperature was 18 °C, the average NRR still stayed at 1.55 kg/m^3·d. The lower effluent substrate concentration (NO_2^--N was below 55 mg/L) indi-

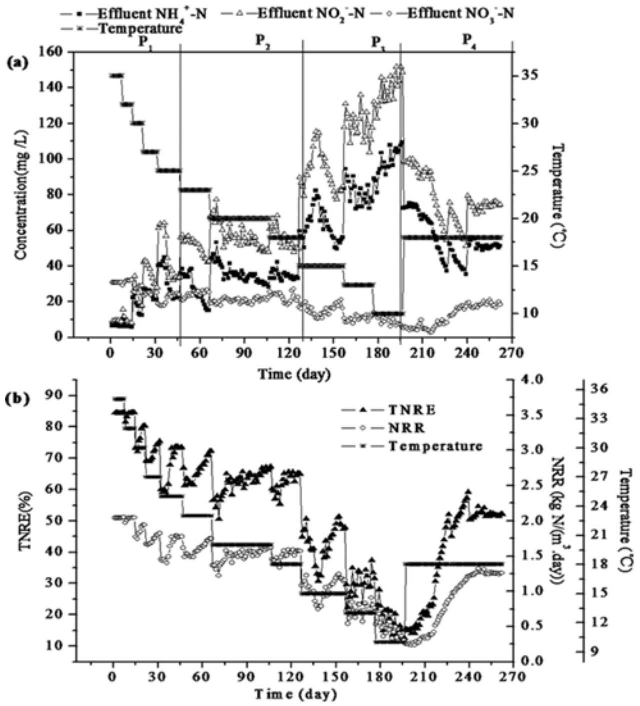

Fig. 3. Nitrogen removal performance of anammox during the whole process. (a) Effluent concentrations of NH_4^+-N, NO_2^--N and NO_3^--N at different temperatures. (b) Nitrogen removal rate (NRR) and total nitrogen removal efficiency (TNRE) at different temperatures.

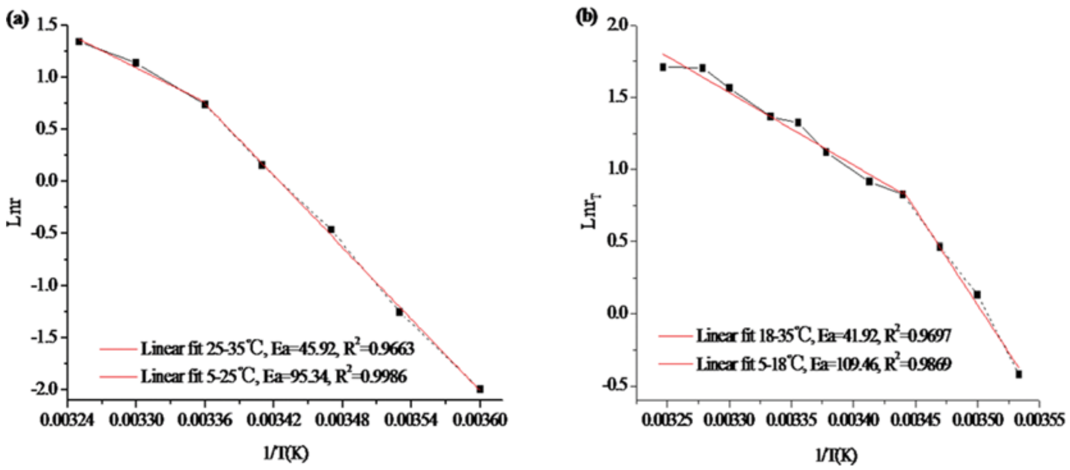

Fig. 2. Relationship between anammox activation and temperature in batch test (a) and continuous test (b).

cated a high potential at low temperature. This could be attributed to the accumulation and adaptation of anammox bacteria to low temperature environment.

However, when operating temperature dropped to 15 °C, nitrogen removal was affected greatly. NRR dropped suddenly by 0.48 kg/m^3·d on day 127. Simultaneously, high NO_2^--N accumulation (115.39 mg/L) was observed, suggesting significant inhibition resulting from low temperature. Previous report indicated that high nitrite levels (>100 mg/L) would cause the loss of anammox activity [16,27]. When temperature was 13 °C, effluent NO_2^--N increased to about 120.09 mg/L. The mean NRR was 0.73 kg/m^3·d. It suggested that the inhibition resulting from low temperature was strengthened by nitrite accumulation. When temperature dropped to 10 °C, nitrogen removal performance deteriorated further. The TNRE was only 19.87%, and the NRR dropped to 0.28 kg/m^3·d. This suggested that the anammox process was severely affected. The recover process of anammox was also investigated. The temperature was kept at 18 °C. NRR increased from 0.24 to 1.32 kg/m^3·d after several days' operation. Besides, TNRE increased from 14.13% to 59.17%. This suggested that anammox performance could have been revived even if anammox bacteria had been restrained with temperature of 10 °C.

The anammox recovery performance and kinetic feature were described by the modified Boltzmann model and modified Gompertz model. The regression of experimental data through two models was calculated by Eq. (4) and (5).

$$NRR=1.28-1.05/[1+\exp(t-27.3)/5.9](R^2=0.9973) \quad (4)$$

$$NRR=1.44\exp[-\exp(0.032e(2.3-t)+1)](R^2=0.9653) \quad (5)$$

Fig. 4 indicates that higher value of R^2 (0.9973) was acquired by the modified Boltzmann model. This suggests that the modified Boltzmann model was appropriate to describe recovery feature of anammox process. The t_c achieved through the modified Boltzmann model was 27.3 d, indicating that there should be a long time to recover anammox performance. This was also quite similar to experimental data (28 d). Additionally, the NRR_{max} predicted by the modified Boltzmann model was 1.28 kg/m^3·d, which was inferior to actual nitrogen removal capacity (1.32 kg/m^3·d). This could be attributed to co-existence of anammox bacteria and denitrifying bacteria. Based on the modified Gompertz model, the λ value produced by model was only 2.4 d, lower than actual time (10 d). Moreover, the predicted NRR_{max} was higher than experimental value. As a result, it was recommended to use the modified Boltzmann model rather than the modified Gompertz model.

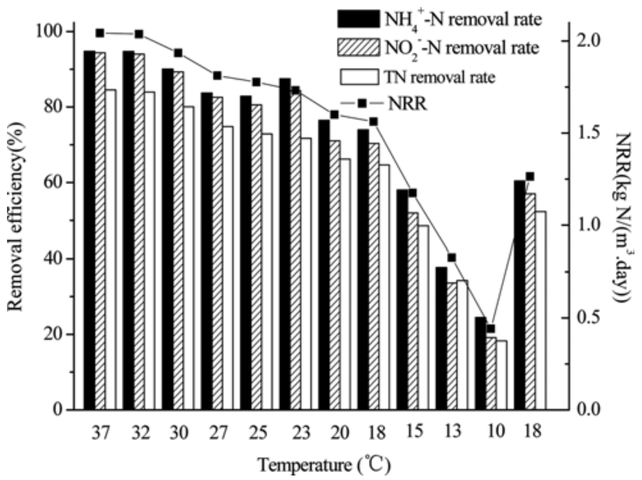

Fig. 5. Comparison of nitrogen removal efficiency and NRR at different temperatures.

3. Nitrogen Removal Performance of Anammox at Different Temperatures

Temperature-impacted growth and metabolic rate of bacteria through affecting activity of enzyme [28]. In addition, low temperature affected the efficiency of material transformation. The comparison of nitrogen removal efficiency and NRR at different temperatures is presented in Fig. 5. The temperature shock affected anammox process greatly. When the reactor was operated during P_1 phase, NRR decreased from 2.04 to 1.78 kg/m^3·d. However, NH_4^+-N removal efficiency, NO_2^--N removal efficiency and TNRE

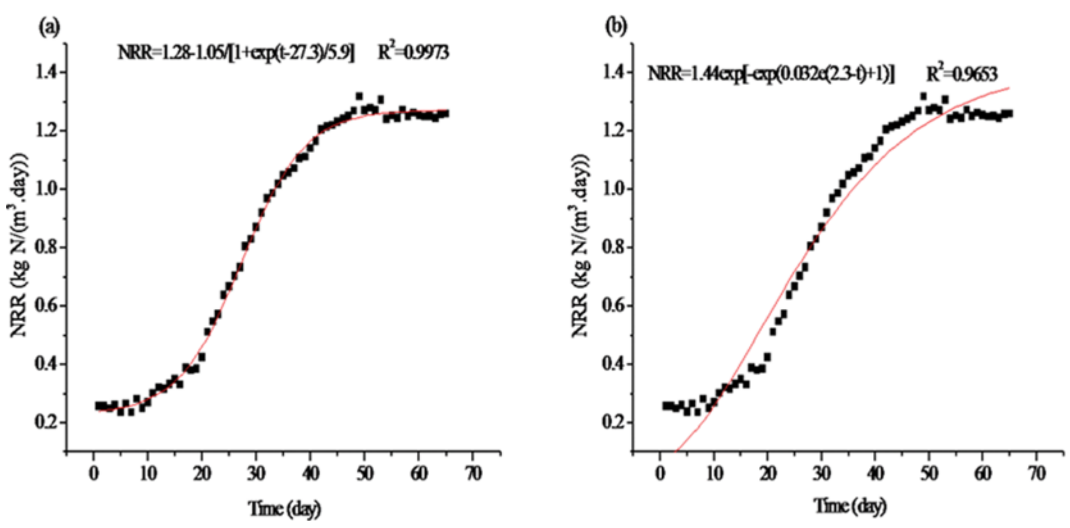

Fig. 4. Regression curve of the modified Boltzmann model (a) and the modified Gompertz model (b).

Table 3. NRR variation with decreasing temperature

Temperature (°C)	Operating time (d)	Amplitude[a] (%)	Amplitude[b] (%)
35-25	46	13.05	13.05
25-18	80	12.03	23.50
18-10	70	71.81	78.43

[a]Amplitude variations with the slope of this phase
[b]Amplitude variations of anammox performance compared with the maximum value
Amplitude=$(NRR_{T1}-NRR_{T2})/NRR_{T1}$

remained at relatively high level (82.89%, 80.61% and 72.88%). Taking operating cost into account, the anammox process was recommended to operate at 25 °C.

When operating temperature gradually decreased from 25 to 18 °C, NRR stayed at 1.51 kg/m^3·d, and TNRE stabilized at 62.49%. Considering the accumulation of nitrite probably resulted in instability of the reactor at low temperature [15], much more attention was paid to effluent nitrite concentration. The NO_2^--N content was less than 55 mg/L after 80 d. Simultaneously, the response curve of NRR was flat, with variation amplitude of 12.03% (presented in Table 3). When temperature decreased from 18 to 10 °C, NRR and temperature indicated a linear relationship with a regression equation of NRR=0.1209T–0.7642 (R^2=0.9973), suggesting that variation of temperature significantly affected anammox performance below 18 °C.

4. Activation Energy

According to the batch test, a larger activation energy value was acquired at lower temperature, while smaller value was achieved at higher temperature, which was in line with previous studies [18,29]. High activation energy might be linked to poor adaptive capacity for rapid change of temperature. It was demonstrated that anammox activation energy was more sensitive at low temperature than at high temperature. However, the tolerance of anammox bacteria could be progressively improved owing to adaptability for low temperature. A comparison of activation energies for anammox bacteria is presented in Table 4. Strous et al. [16] reported that the activation energy was 70 kJ/mol. Rysgaard et al. [30] and Kawagoshi et al. [31] observed 51 kJ/mol and 54 kJ/mol for anammox from marine sediments. Park et al. [32] indicated that the activation energy was 108 kJ/mol at 10-25 °C. However, our results are not in accordance with them. This might result from different inocula. Besides, both short-term and long-term effects indicated that activation energy varied with temperature. This was also in agreement with Yang et al. [33], Osaka et al. [34], Isaka et al. [18] and Kim et al. [35], suggesting that variation in temperature probably led to a shift of dominant anammox bacteria. Still, compared with short-term effect, anammox bacteria could be progressively adapted to low temperature environment with long-term operation. This was in agreement with Isaka et al. [18]. Meanwhile, anammox bacteria had ability to grow from −2 to 85 °C [21], indicating the probability that anammox technology could be applied at low temperature through long-term acclimatization.

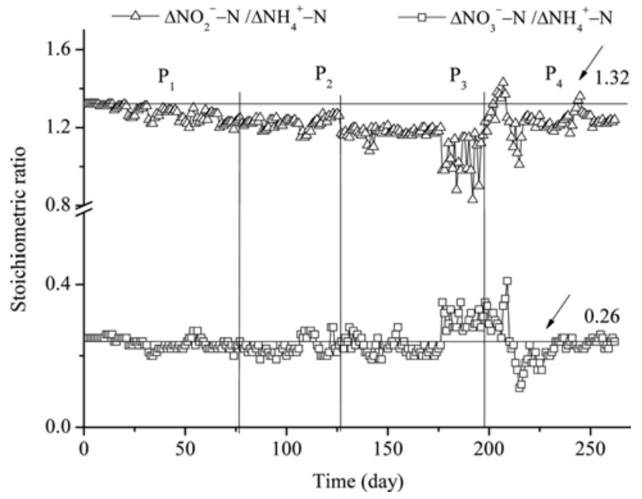

Fig. 6. The stoichiometric ratio during different phases.

5. Stoichiometry

Stoichiometric ratio could be used as an indicator to anammox performance [36]. The theoretical stoichiometric ratio of ΔNO_2^--N/ΔNH_4^+-N and ΔNO_3^--N/ΔNH_4^+-N were 1.32 and 0.26, respectively [6]. As presented in Fig. 6, the stoichiometric ratio fluctu-

Table 4. Comparison of activation energy for Anammox bacteria

Activation energy (kJ/mol)	Seeding sludge	Condition	Temperature (°C)	Reference
51	Marine sediment	Long-term	−2-30	[30]
54	Marine sediment	Long-term	5-30	[31]
70	Anammox sludge	Short-term	20-43	[6]
93	Anammox sludge	Short-term	22-28	[18]
94	Anammox sludge	Long-term	6-22	[18]
33	Anammox sludge	Short-term	28-37	[18]
108	Anammox sludge	Short-term	10-25	[32]
63	Biofilm sludge	Short-term	10-45	[15]
46	Anammox sludge	Short-term	25-35	This work
95	Anammox sludge	Short-term	5-25	This work
42	Anammox sludge	Long-term	18-35	This work
109	Anammox sludge	Long-term	10-18	This work

Table 5. Comparison of different Anammox processes below 25 °C

Reactor	MLSS (g/L)	Temperature (°C)	Feeding media	NRR ($kg/m^3 \cdot d$)	Reference
ABF		20-22	Synthetic wastewater	8.1	[36]
UFCR	22	23	Synthetic wastewater	17.5	[37]
UFCR	35	23-26	Synthetic wastewater	4.5	[37]
UASB		16	Municipal wastewater	2.28	[23]
SBR		18	Municipal wastewater	0.29	[15]
SBR		25	Black water	0.45	[38]
SBR	7.96	18	Synthetic wastewater	1.60	This work

ated with temperature. When temperatures was 35-25 °C, ΔNO_2^--N/ΔNH_4^+-N and ΔNO_3^--N/ΔNH_4^+-N were around 1.27 and 0.23, respectively. This was close to theoretical values, suggesting good anammox performance. With temperature decreased further, a disturbance occurred on ΔNO_2^--N/ΔNH_4^+-N and ΔNO_3^--N/ΔNH_4^+-N. When temperature decreased to 10 °C, ΔNO_2^--N/ΔNH_4^+-N and ΔNO_3^--N/ΔNH_4^+-N reached 1.02 and 0.27, respectively. The possible reason was associated with the excess ammonium production resulting from cell decay. This also agreed with Dosta et al. [15].

6. Comparison of Different Reactors at Moderate and Low Temperature

Comparison of different anammox reactors below 25 °C is presented in Table 5. Isaka et al. [37] used an anaerobic biological filter (ABF) with an NRR of 8.1 $kg/m^3 \cdot d$ at 20-22 °C. Yang et al. [38] re- ported that NRR reached 17.5 $kg/m^3 \cdot d$ at 23 °C in an up-flow column reactor (UFCR). The relatively high NRR obtained by them might be attributed to high anammox sludge concentration. Tang et al. [24] reported high SAA and biomass contents were thought to be key factor for good anammox performance. However, NRRs reported by Dosta et al. [15] and Wang et al. [39] were only 0.29 and 0.45 $kg/m^3 \cdot d$, respectively. This was much lower than the NRR acquired in this work. The reason was that municipal waste and black water are much more complex and unsuitable to anammox treatment.

7. Sludge Characteristics

The SAA variation with temperature is presented in Fig. 7. When

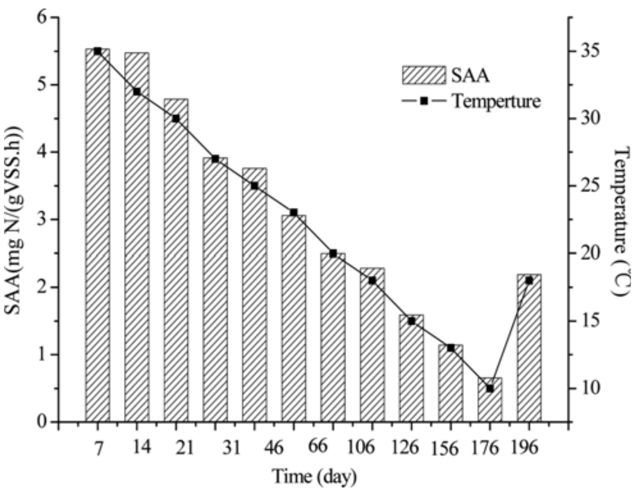

Fig. 7. The SAA variation with temperature.

temperature decreased from 35 to 10 °C, SAA decreased from 5.53 to 0.65 mg/g·h. Besides, the VSS decreased from 7.60 g/L (on day 126) to 4.93 g/L (on day 196), which shows that there was a significant loss of SAA and biomass in the reactor. The simultaneous reduction of SAA and biomass resulted in the acute decline in anammox performance. Moreover, the mean value of effluent SS was 7.23 mg/L. However, when temperature was 10 °C, effluent SS increased to 41.44 mg/L. After long-term recovery period, the anammox activity was maintained at 2.19 mg/g·h. This was higher than the value achieved during strong inhibition period. In addition, VSS increased by 25.56% and effluent SS decreased to 15.43 mg/L, when temperature was 18 °C. These results indicated that the suppression resulting from low temperature on anammox activity was recoverable. SAA and VSS were associated with anammox performance, which was in accordance with previous report [40,41].

Morphological feature of sludge is presented in Fig. 8. Sludge was sampled on day 115 (P_2 phase) and day 190 (P_3 phase). The morphological characteristics of sludge were not identical. From the image of sludge, the sludge consisted of spherical and elliptical bacteria cells with smooth surface on day 115. In terms of the sludge on day 190, abnormal bacilli and filamentous bacteria were also found. Besides, the outer color of granule changed from brick-red to red-brown, which suggested the reduction of heme C content. Heme C content was associated with cell structure damage and anammox activity decrease resulting from low temperature [42]. Still, the size of sludge sampled on day 190 was smaller than that on day 115. After low temperature exposure, granules appeared to be more irregular with a smaller average granule diameter [43]. Sludge characteristics changed with low-temperature operation [44]. Low temperature not only led to poor nitrogen removal, but also affected sludge size and feature.

CONCLUSIONS

An SBR was used to study nitrogen removal performance at moderate and low temperature. Temperature shock greatly affected the anammox process. Taking operating cost into account, the anammox process was recommended to operate at 25 °C. It was difficult to achieve efficient nitrogen removal when temperature was low due to higher activation energy. When temperature decreased to 10 °C, ΔNO_2^--N/ΔNH_4^+-N and ΔNO_3^--N/ΔNH_4^+-N reached 1.02 and 0.27, respectively. Inhibition resulting from low temperature on anammox activity was recoverable, and the modified Boltzmann model was appropriate to describe recovery characteristics

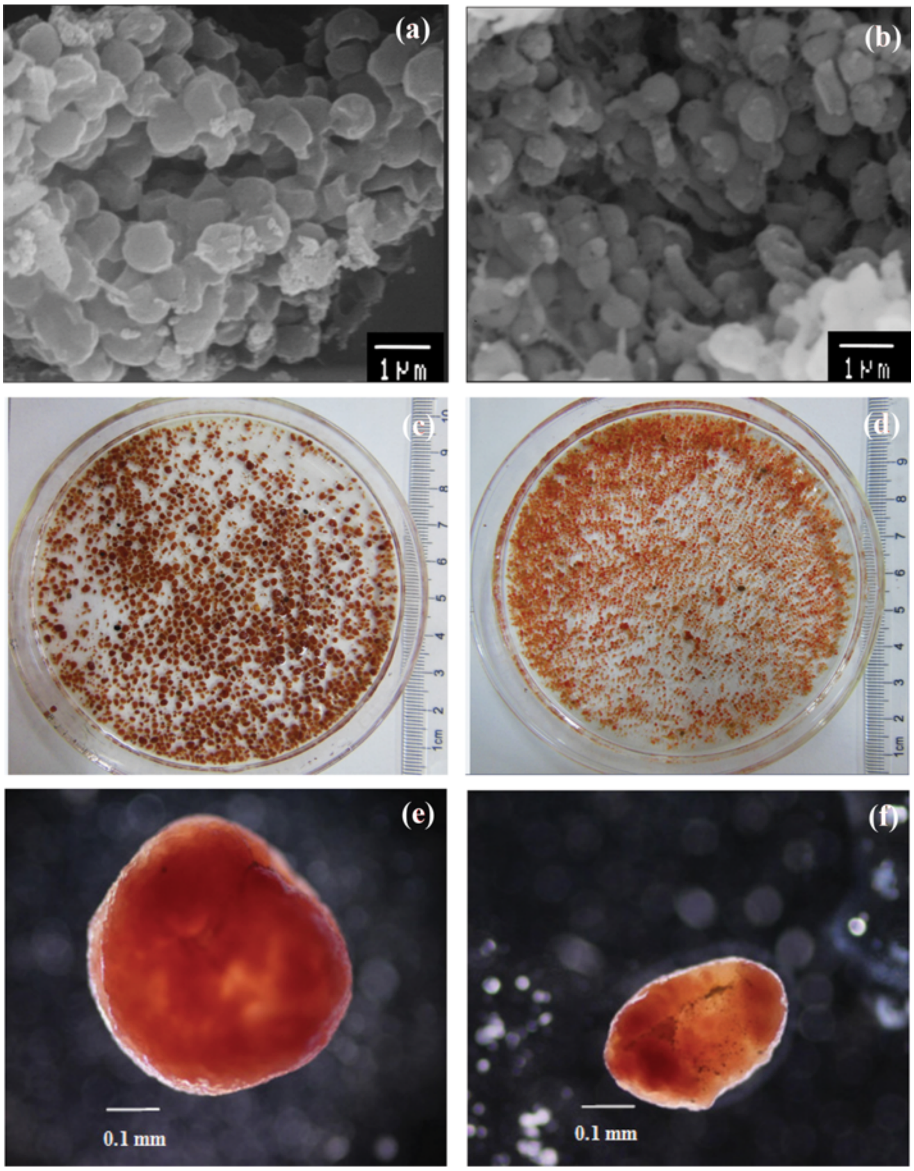

Fig. 8. Morphological feature of sludge sampled on day 115 (a), (c) and (e) and day 190 (b), (d) and (f).

of anammox process. Low temperature not only led to poor nitrogen removal, but also affected sludge size and feature.

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