Desalination and Water Treatment
www.deswater.com
doi: 10.5004/dwt.2019.24466

163 (2019) 317–324
September

Kinetics of temperature effects on autotrophic nitrogen removal from digested swine wastewater

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Received 44 December 2018; Accepted 28 May 2019

ABSTRACT

The temperature is the crucial factor influencing the performance of autotrophic nitrogen removal (ANR) process based on partial nitritation (PN) and anammox. The quantitative effects of temperature on ANR process are not clear. Here, four laboratory-scale bioreactors were initiated to investigate it. The performance of the bioreactors operating at 30°C, 25°C, 20°C and 15°C showed that the total nitrogen removal efficiency of the reactors at 30°C, 25°C, 20°C and 15°C were 77.1%, 65.4%, 50.9% and 34.6%. Anammox bacteria had a stronger temperature dependence in 15°C–25°C range. 25°C is the demarcation point of anammox bacteria temperature dependence. High-throughput pyrosequencing analysis showed that aerobic ammonia-oxidizing bacteria (AOB) – *Nitrosomonas* – and anammox bacteria – Candidatus *Brocadia* – were the main functional microorganisms. The temperature activity coefficient (θ) of AOB and anammox bacteria were 1.101 and 1.185 at 15°C–20°C, 1.073 and 1.084 at 20°C–25°C, 1.026 and 1.052 at 25°C–30°C, respectively. These θ values of multi-interval can provide more information and valuable parameters for future researches and engineering design.

Keywords: Partial nitritation; Anammox; Temperature activity coefficient; Digested swine wastewater; Temperature

1. Introduction

With the rapid expansion of industrial-scale swine farms, large amounts of manure and wash wastewater have been discharged, which have increased the environmental burden and pollution risk [1]. Anaerobic digestion technology has been used widely in the treatment of swine wastewater for removing organic matter and recovering energy [2]. However, anaerobic digestion cannot remove nitrogen efficiently and the high concentrations of ammonium nitrogen (NH_4^+–N) in the digested swine wastewater require advanced treatment [3]. Nitrogen removal is an enormous challenge in the post-treatment of digested swine wastewater [4,5]. Due to the low carbon-to-nitrogen (C/N) ratio and the high NH_4^+–N concentration in digested swine wastewater, the performance of the traditional nitrogen removal using nitrification and denitrification processes is very poor. The removal efficiency was 50%–80% and 20%–60% for NH_4^+–N and total nitrogen, respectively [6,7].

The autotrophic nitrogen removal (ANR) process based on the combination of partial nitritation (PN) and anammox (anaerobic ammonium oxidation) process, is a new, low-energy and cost-effective microbial process that is a promising option for the treatment of digested swine wastewater with a low C/N ratio [8–10]. In recent years, there are large number of researches on ANR, which have the high nitrogen

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removal efficiency of 61% at temperature of 32°C, 55% at temperature of 35°C in treating digested swine wastewater [11], and our previous research obtained a total nitrogen removal efficiency of 73% [12] at the temperature of 30°C. However, these previous works on the treatment of digested swine wastewater by ANR were studied at a moderate reaction temperature (>30°C). It is impossible to maintain 30°C for swine wastewater and its digester for practical application [13]. How about is the performance of ANR process in treating digested swine wastewater at ambient temperature? There exist a significant number of knowledge gaps.

The temperature has a huge influence on anammox bacteria [14,15]. Numerous studies showed that a decrease of temperature would severely suppress the activity of anammox bacteria and cause the deteriorating of the performance of anammox proceses [16,17]. For example, Laureni et al. [18] reported that lowering the temperature from 29°C to 12.5°C resulted in a marked decrease of anammox activity from 0.465 to 0.046 g-N L^{-1} d^{-1}. In addition, anammox bacteria did not show any activity when temperature decreased to 10°C [10]. These studies demonstrated that anammox bacteria have strong temperature dependence. Moreover, for ANR process, besides the anammox bacteria, ammonia oxidizing bacteria (AOB) also plays a key role in nitrogen removal from wastewater [19,20]. However, to our knowledge, no previous report has evaluated the effect of temperature on the performance of ANR systems containing AOB and anammox bacteria.

Therefore, in this study we used four laboratory-scale gas-lift granular sludge reactors, operated at a different temperature (15°C, 20°C, 25°C and 30°C, seperately), to investigate the quantitative influence of temperature on the performance of an ANR system treating digested swine wastewater, and to explore feasibility of ANR process treating digested swine wastewater at ambient temperature.

2. Materials and methods

2.1. Reactors description

Four 10-L reactors were used in the experiments with effective volume of 5 L. Each reactor was maintained at a different incubation temperature (30°C, 25°C, 20°C and 15°C) using a circulating water bath and were designated, respectively, ANR30, ANR25, ANR20 and ANR15. The experimental reactor was described as Wang et al. [12]. All reactors operated in a continuous feeding mode and influent was introduced from the bottom of the reactors using peristaltic pumps (model WT600-2J; Baoding Longer Precision Pump Co. Ltd., China). Ambient air was continuously pumped into the bottom of the reactors using an air pump (ACO-005, Zhejiang Sensen Instrument Factory, China). Driven by airflow, sludge and wastewater flow up in the riser part and down in the down comer zone. Effluent and produced gas were discharged from the top of the reactor. A simple three-phase separator was placed on the top of each reactor to separate gas and liquid. The airflow was controlled using a glass rotameter (Zhejiang Yuyao Instrument Factory, China) according to the concentration of nitrogen of effluent.

2.2. Wastewater and seeding sludge

Digested swine wastewater to be used as influent wastewater in this study was obtained from biogas plant treating swine wastewater in Jianyang, Sichuan, China. The concentration of NH_4^+–N of digested swine wastewater was 500–730 mg L^{-1}, nitrite and nitrate were less than 1 mg L^{-1}. At the start of an experiment, digested swine wastewater was diluted to 50% as influent by adding tap water for starting experiments quickly, and then adjusted to 100% for stabilizing the performance of reactors. The influent flow rate and corresponding hydraulic retention time of the reactors were set at 40.0 L d^{-1} and 3.0 h, respectively. The total nitrogen loading rate was about 3 kg N m^{-3} d^{-1} for four reactors in stable stages. Each reactor was seeded with 1 L of PN-anammox sludge and mixed-liquor volatile suspended solids (MLVSS) was 9.28 g L^{-1}, which was collected from an operating ANR reactor [12].

2.3. Analytical methods

Influent and effluent samples were collected every day and analyzed immediately or stored at 4°C. NH_4^+–N, nitrite nitrogen (NO_2^-–N) and nitrate nitrogen (NO_3^-–N) were determined using an auto-analyzer (AA3, Bran + Luebbe, Norderstedt, Germany). pH was determined using a PHS-3C pH meter (Shanghai Leici Equipment Factory, China) and DO was measured with a dissolved oxygen meter (HQ30d, HACH Co., Ames, IA, USA). Mixed-liquor suspended solids and MLVSS were monitored using standard methods [21].

2.4. Batch activity tests

Sludge samples were taken from each reactor at the end of an experiment to determine the aerobic ammonium conversion activity of AOB, the anaerobic nitrite reducing activity of anammox bacteria, the aerobic nitrite oxidizing activity of nitrite oxidizing bacteria (NOB) and the anaerobic nitrate reducing activity of denitrifying bacteria (DB). Each batch test was conducted at the same temperature used in the corresponding reactor from which the sludge was collected (e.g., the sludge from ANR30 was tested at 30°C and the sludge from ANR25 was tested at 25°C). Each batch test was performed in triplicate. The detailed method for the batch activity tests was described by Wang et al. [12].

2.5. High-throughput pyrosequencing

Samples of biomass were taken from the inoculum and from the ANR15, ANR20, ANR25 and ANR30 reactors at 200 d, and named “Seed”, “T15”, “T20”, “T25” and “T30”, respectively. The total DNA was extracted using the cetyltrimethylammonium bromide method. The amplification of the 16S rRNA gene was performed using the 341F-806R primer for bacteria and the Parch519F-Arch915R primer for archaea. The PCR thermocycling steps were described by Wang et al. [7]. The PCR products for sequencing were carried out using an Illumina Hiseq 2500 platform (Illumina, Inc., San Diego, CA, USA); the detailed methodology and subsequent bioinformatics analysis were as described by

Chen et al. [22]. The raw sequences were deposited in the US National Center for Biotechnology Information Sequence Read Archive database (accession number SRP129053).

2.6. Calculations

According to the batch experiments, the activities of DB were zero, indicating the removal of nitrogen by denitrifying bacteria is negligible. So, the AOB, anammox bacteria and NOB play the key roles in the nitrogen metabolism, the stoichiometry of their reactions in stable stage are listed in Eqs. (1)–(3) [9]. So, the ammonium conversion rate by AOB (AOB-ACR), nitrite conversion rate by anammox bacteria (anammox-NCR) or by NOB (NOB-NCR) and the total nitrogen removal rate (TNRR) of the reactors can be calculated through the concentrations of ammonium, nitrite, nitrate and total nitrogen of influent and effluent.

$$\text{AOB: } NH_3 + 1.5O_2 = NO_2^- + H_2O + H^+ \quad (1)$$

Anammox bacteria:

$$NH_3 + 1.32NO_2^- + 0.066HCO_3^- + 0.13H^+ = 1.02N_2 + 0.26NO_3^- + 0.066CH_2O_{0.5}N_{0.15} + 2.03H_2O \quad (2)$$

$$\text{NOB: } NO_2^- + 0.5O_2 = NO_3^- \quad (3)$$

The quantitative influence of temperature on ANR system was calculated by the Arrhenius equation (Eq. (4)) [23].

$$R(T2) = R(T1)\theta^{(T2-T1)} \quad (4)$$

In this equation, θ is the temperature activity coefficient, T is the temperature (K) and R is the reaction rate. In this study, the reaction rates of reactor performance and bath tests were used to calculate θ.

3. Results and discussion

3.1. Performance of ANR reactors

The influent and effluent characteristics and the performance of four ANR reactors during the 200 d operation are shown in Table S1 and Fig. 1. Because the NO_2^-–N and NO_3^-–N concentrations of the digested swine wastewater (influent) were less than 1 mg L^{-1}, the influent NO_2^-–N and NO_3^-–N concentrations were not shown.

The performance of ANR30 was shown in Fig. 1a. Based on the parameters of the start up period (0–60 d), in phase II (61–200 d), the influent was adjusted to 100% digested swine wastewater with total nitrogen (TN) concentration of 378 ± 47 mg L^{-1}, and the DO concentration was controlled in the range of 0.8–1.2 mg L^{-1}. The average removal efficiencies of NH_4^+–N and TN in the stable stage were 87.5% and 77.1%, respectively. In this period, the NH_4^+–N and TN in the effluent were 48 ± 19 and 87 ± 22 mg L^{-1}, respectively (Table S1). This excellent performance was consistent with previous research [12].

The performance of ANR25 was shown in Fig. 1b. Based on the parameters of the start up period (0–70 d), during phase II (71–200 d), the DO gradually increased to 0.8–1.0 mg L^{-1}, and the influent was adjusted to 100% digested swine wastewater with TN concentration of 373 ± 47 mg L^{-1}. In the stable stage, the NH_4^+–N removal efficiencies of 77.2% was achieved and TN removal efficiencies of the reactor also increased to 65.4% and corresponding concentration of NH_4^+–N and TN in the effluent were 85 ± 14 and 129 ± 20 mg L^{-1}, respectively (Table S1).

The performance of ANR20 was shown in Fig. 1c. Based on the parameters of the start up period (phase I 0–60 d), during phase II (61–200 d), the DO concentration was increased to 0.6–0.9 mg L^{-1}, and the influent was adjusted to 100% digested swine wastewater with TN concentration of 367 ± 31 mg L^{-1}. During this period, the average NH_4^+–N removal efficiency was 68.7% and TN removal efficiency was 50.9%. In this stage, average NH_4^+–N and TN concentrations in the effluent were 115 ± 25 and 180 ± 32 mg L^{-1}, respectively, the nitrite of effluent increased to 53 ± 31 mg L^{-1} (Table S1).

The performance of ANR15 was shown in Fig. 1d. Since low temperature may inhibit the activity of AOB, in the first 7 d, the DO concentration was maintained in excess of 3.0 mg L^{-1} to enhance the rapid activity of AOB at the low temperature. Even so, much NH_4^+–N remained in the effluent (195 ± 10 mg L^{-1}), which resulted in a low NH_4^+–N removal efficiency (38.3%). Meanwhile, the high DO concentration inhibited anammox bacteria, which resulted in a low TN removal efficiency (24.4%). Hence, from day 8 of operation (phase II), the DO concentration decreased to 0.4–0.6 mg L^{-1}. Nevertheless, the decreased DO concentration did not decrease the NH_4^+–N removal efficiency (48.4%), which may be the reason for enhanced AOB activity. During 61–200 d, the influent was adjusted to 100% digested swine wastewater with TN concentration of 382 ± 32 mg L^{-1}, and the DO was maintained at 0.6–0.9 mg L^{-1}. As a result, NH_4^+–N removal efficiency increased to 58.1% and TN removal efficiency increased to 34.6%. In this stage, the average effluent NH_4^+–N and TN were 160 ± 24 and 250 ± 39 mg L^{-1}, the effluent NO_2^-–N concentration was high up to 76 ± 15 mg L^{-1} (Table S1).

The decrease of temperature from 30°C to 15°C decreased the performance of the ANR process treating digested swine wastewater. Furthermore, we found that the residual NO_2^-–N concentration in ANR15 (76 ± 15 mg L^{-1}) was significantly higher than ANR30 (18 ± 9 mg L^{-1}) (Table S1). Dosta et al. [17] also found that nitrite began to accumulate in anammox process when the temperature decreased to 15°C. Therefore, we speculated that the temperature sensibilities of main functional microbes, AOB and anammox bacteria, were not synchronous in ANR process with the decrease of temperature.

3.2. Effect of temperature on the activities of the functional microbes

The specific activities of the sludge in all four reactors were determined in batch tests to investigate the changing rate of the activities of different functional microbes with the decrease of temperature (Fig. 2). The results showed that the activities of AOB and anammox bacteria in all reactors were significantly higher than NOB and DB. In all reactors, the anaerobic nitrate reduction activity of DB is close to zero.

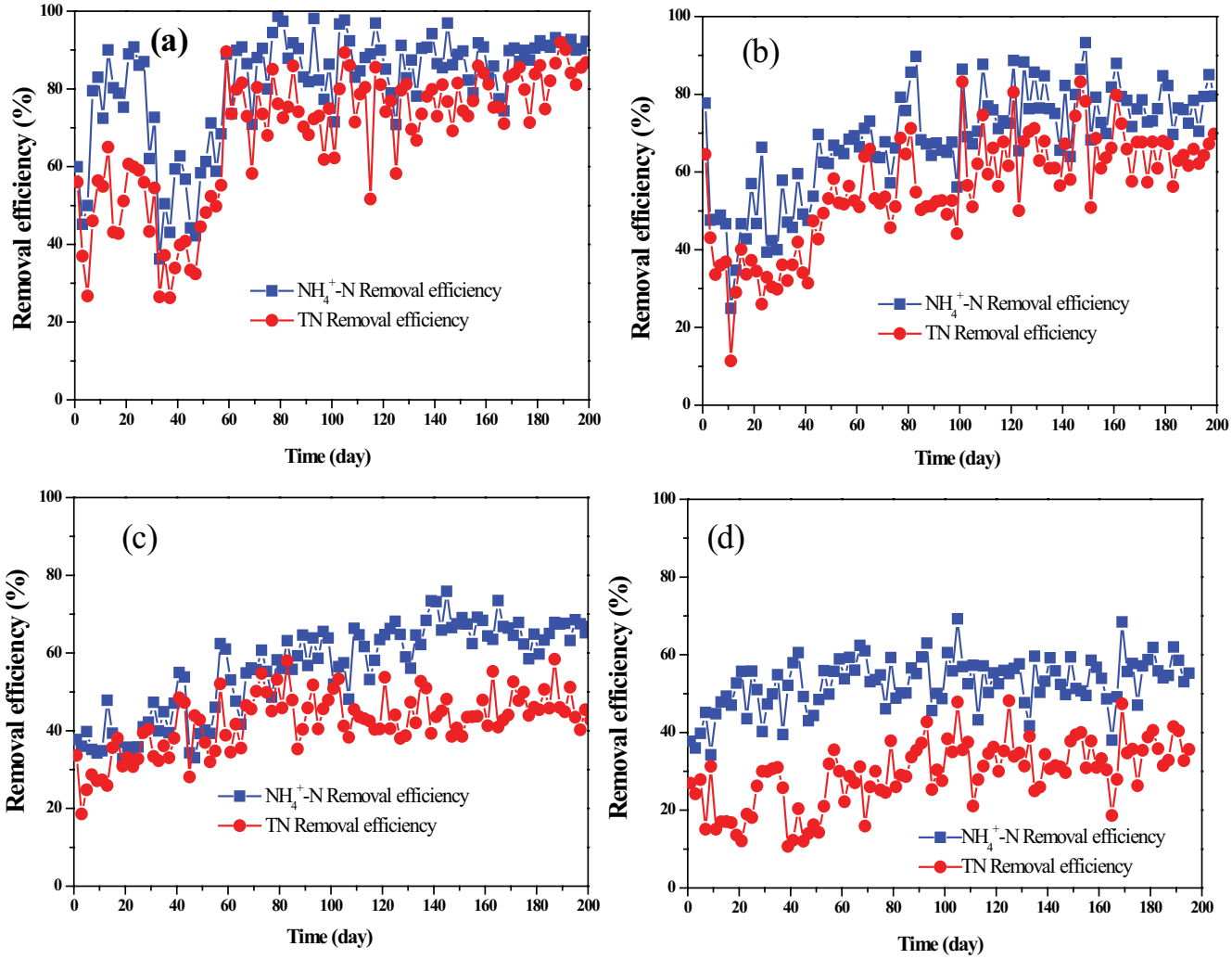

Fig. 1. Performance of autotrophic nitrogen removal processes treating digested effluent at different temperatures. (a–d) are ammonia and total nitrogen removal efficiency of ANR30, ANR25, ANR20 and ANR15.

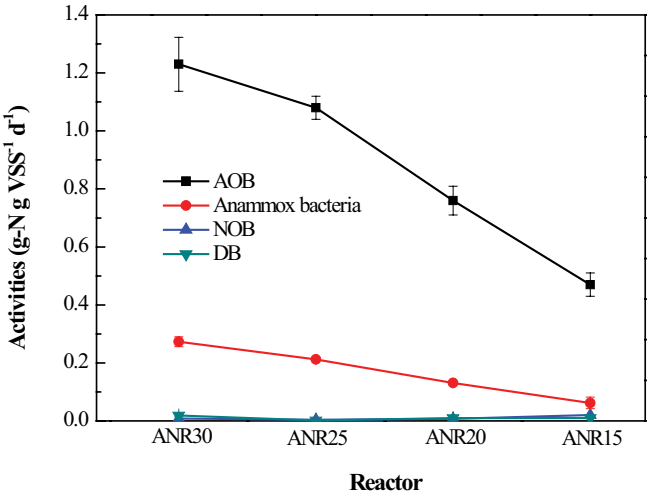

Fig. 2. Activities of the functional microbes in the reactor ANR30, ANR25, ANR20 and ANR15 at 30°C, 25°C, 20°C and 15°C, respectively.

This indicates that nitrogen removal was accomplished through the PN-anammox process, and the activities of NOB and DB were successfully suppressed.

The batch tests indicated that the activities of AOB and anammox bacteria were positively correlated with temperature; that is, maximum biomass-specific activity decreased when temperature decreased. The ammonium oxidizing activity of AOB in the ANR30 reactor at 30°C was 1.23 ± 0.09 g-N g VSS^{-1} d^{-1}, which was the highest ammonium oxidizing activity of sludge from all reactors, and was similar to the values reported previously for ANR processes [12,24]. At 25°C in ANR25, the activity of AOB was 1.08 ± 0.05 g-N g VSS^{-1} d^{-1} and decreased by ~12% compared with ANR30, which is high enough to support a high-rate ANR process [25]. Compared with ANR30, the maximal AOB activity (0.76 ± 0.04 g-N g VSS^{-1} d^{-1}) of ANR20 at 20°C decreased by ~38%, and that in ANR15 (0.47 ± 0.01 g-N g VSS^{-1} d^{-1}) decreased by ~62%. Nevertheless, these activities were significantly higher than observed in previous research (i.e., 0.27 ± 0.01 g-N g VSS^{-1} d^{-1} at 21°C and ~0.26 g-N g VSS^{-1} d^{-1} at 13°C) [24], and resulted in good effluent for both ANR20 and ANR15.

Anammox is the key process in ANR, and the activity of anammox bacteria determined the nitrogen removal efficiency of the reactors in this study. Lower temperature led to severe anammox activity suppression (Fig. 2). Compared with ANR30 (0.27 ± 0.01 g-N g VSS^{-1} d^{-1}), the anammox activity of ANR25 sludge at 25°C (0.21 ± 0.01 g-N g VSS^{-1} d^{-1}), ANR20 sludge at 20°C (0.14 ± 0.01 g-N g VSS^{-1} d^{-1}) and ANR15 sludge at 15°C (0.06 ± 0.01 g-N g VSS^{-1} d^{-1}) decreased by 22%, 52% and 77%, respectively. Hence, the reduction in activity of anammox bacteria was much greater than the reduction of AOB activity, which resulted in heavier nitrite accumulation at lower temperatures. And the results of test batches were consistent with volumetric removal capacity of ANR reactors.

3.3. Quantitative effect of temperature on nitrogen removal

The volumetric removal capacity of each ANR reactor, including the ammonium conversion rate by AOB (AOB-ACR), nitrite conversion rates by anammox bacteria (anammox-NCR) and nitrite conversion rates by NOB (NOB-NCR) on long-term ANR operation were calculated (Fig. 3). NOB-NCRs were approximately zero at every temperature, and the AOB-ACR and anammox-NCR gradually decreased as temperature decreased. However, at 30°C and 25°C, the AOB-ACRs (1.47 ± 0.16 and 1.36 ± 0.14 kg N m^{-3} d^{-1}, respectively) were almost equal to the anammox-NCR (1.52 ± 0.13 and 1.31 ± 0.14 kg N m^{-3} d^{-1}, respectively). The ratio of AOB-ACR and anammox-NCR in ANR30 and ANR25 were 1.03 and 1.04, respectively. Thus, in ANR30 and ANR25, the NO_2^-–N produced by AOB could be timely consumed by anammox bacteria and the NO_2^-–N concentration in effluent was low (18 ± 9 mg L^{-1} and 27 ± 10 mg L^{-1}, respectively). However, at 20°C and 15°C, the anammox-NCR sharply decreased to 1.06 ± 0.07 and 0.61 ± 0.08 kg N m^{-3} d^{-1}, respectively, which was significantly lower than AOB-ACR (1.27 ± 0.16 and 1.10 ± 0.15 kg N m^{-3} d^{-1}, respectively) and the ratio of AOB-ACR and anammox-NCR in ANR20 and ANR15 were high up to 1.20 and 1.80 (Fig. 3), which resulted in the accumulation of NO_2^-–N in effluent (56 ± 10 and 76 ± 15 mg L^{-1}, respectively). These data indicate that when the temperature decreased to 20°C, anammox bacteria were more sensitive to temperature dropping than AOB.

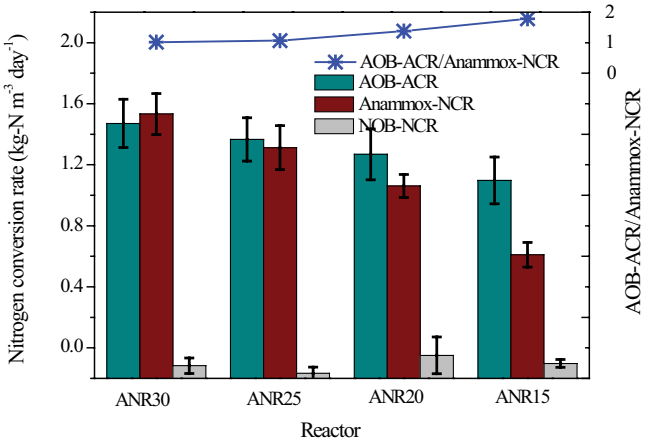

Fig. 3. Performance of different functional microorganisms at different temperatures in reactors (AOB-ACR, the ammonium conversion rate of AOB; Anammox-NCR, nitrite conversion rate of anammox bacteria; NOB-NCR, nitrite conversion rate of NOB).

The quantitative influence of temperature on the nitrogen conversion rate can be described by temperature activity coefficients (θ), the higher value of θ indicated the more significant effect of temperature on the nitrogen conversion efficiency. The results of θ of both long-term reactor operation based on the volumetric removal capacity and bath tests based on the activities of microbes were shown in Tables 1 and 2.

As far as the influence of temperature on the volumetric removal rate (Table 1), as the temperature rises, θ becomes smaller. The θ values of AOB-ACR, anammox-NCR and TNRR in the ranges of 15°C–20°C were clearly higher than those of 20°C–25°C. The results indicated that ammonium conversion by AOB and the nitrite conversion by anammox bacteria were much more sensitive to variation in temperature from 15°C to 25°C than to variation from 25°C to 30°C. The θ of anammox-NCR were significantly higher than those of AOB-ACR, especially in the ranges of 15°C to 25°C, which indicated that the nitrite conversion by anammox bacteria was much more sensitive to the variation of temperature than that of ammonium conversion by AOB. However, the θ values of anammox-NCR were similar to those of TNRR, which implied that the nitrite conversion by anammox bacteria is the rate-limiting step in ANR from digested swine wastewater.

With the increase of temperature, the changed trend of the temperature activity coefficients (θ) of microbe activities was similar to those of the volumetric removal rate (Table 2). However, the temperature activity coefficients (θ) of microbe activities were obviously higher than those of the volumetric removal rate, differences could be explained that latter was the result of combined impact of microorganisms quantity

Table 1
Temperature activity coefficients (θ) of the volumetric removal rate on long-term reactor operation in different temperature ranges

Range of Temperature (°C)	AOB-ACR	Anammox-NCR	TNRR
	θ	θ	θ
15–20	1.029	1.117	1.072
20–25	1.016	1.044	1.055
25–30	1.015	1.031	1.036

Table 2
Temperature activity coefficients (θ) of the activities of microbes on batch tests in different temperature ranges

Range of Temperature (°C)	AOB	Anammox
	θ	θ
15–20	1.101	1.167
20–25	1.073	1.083
25–30	1.026	1.052

and microbial activities. Compared with previous studies (Table 3), although the θ values are different, the variation trends are similar. The research of Sobotka et al. [26] also reported that in the lower temperature range (11°C–15°C), the temperature coefficient of anammox bacteria was higher than higher temperature range (15°C–30°C) (1.65 vs. 1.07). However, the θ values of previous studies were only for a wide range of temperatures, which was not so accurate as a narrow temperature range, and they were just tested in anammox process or nitrification. There were no temperature activity coefficients (θ) for ANR containing AOB and anammox bacteria in single system. In this study, the θ values are for multi-interval (15°C–20°C, 20°C–25°C, 25°C–30°C), and various functional microorganisms (AOB, anammox) and their combined effects. These θ values can provide more information and valuable parameters for future researches and engineering design.

3.4. Effect of temperature on microbial community structure

The microbial community in the reactors was studied using high throughput sequencing of the 16S rRNA gene. The enough effective sequences of bacteria and archaea in Seed, T30, T25, T20 and T15 were obtained after removing low-quality sequences (Table S2). The phylogenetic diversity (PD), Shannon indices and OTU numbers of the bacteria and archaea indicated that the bacterial diversities were significantly greater than the archaeal diversities and showed that bacteria were the predominant microbes in the four reactors. After 200 d operation, the microbial consortia of the ANR30, ANR25, ANR20 and ANR15 reactors were all larger than in the seed sludge. The result of high-throughput sequencing analysis of each reactor indicated that the microbial community diversity followed the increasing order of ANR30 (T30) < ANR25 (T25) < ANR20 (T20) < ANR15 (T15). It suggested that the enrichment of functional microorganisms decreased with the decrease of temperature.

Table 4 shows the relative abundances of the functional microorganisms, including AOB, NOB, anammox bacteria and DB. The relative abundances of AOB obviously increased from 0.11% (seed) to 3.00% in ANR30, 2.85% in ANR25, 6.20% in ANR20 and 1.68% in ANR15 after 200 d operation. Analysis of the community structures using high-throughput pyrosequencing (Fig. S1) indicated that *Nitrosomonas* was the predominant genus of AOB. AOB survived in the low-temperature (15°C) reactor, but the relative abundances in ANR15 were lower than in the other reactors, indicating that growth was inhibited at low temperature. Candidatus *Brocadia* was the major anammox genus, accounting for 0.10% in ANR25 and ANR30, which were significantly greater than in ANR20 and ANR15 (0.01%; Fig. S1). These observations indicated that the growth of anammox bacteria was inhibited at 20°C and 15°C. Although NOB belonging to order *Nitrospira* was detected in all reactors, their relative abundance was severely limited by the reactor operation, and these results were consistent with reactor performance and the microbial activities analysis. On the contrary, heterotrophic denitrifying bacteria in ANR15 were the dominant functional microbes (accounting for 4.22% of the microorganisms), and their abundance was obviously larger than in ANR20 (1.62%), ANR25 (1.31%) and ANR30 (1.37%). These results indicated that heterotrophic denitrifying bacteria could have higher tolerance to low temperature. However, heterotrophic denitrifying bacteria have many metabolic pathways and grow at a much higher rate in the presence of organic matter [31]. Hence, although the abundance of DB was high, the batch tests showed that its specific activity of nitrate reducing was low and they did not use nitrite or nitrate as electron acceptors.

Table 3
Comparison of θ of different temperature range for anammox and nitrification process

Process	Temperature range	θ	Reference
Anammox	11–15/15–30	1.650/1.070	[26]
Anammox	17–23/23–32	1.145/1.064	[27]
Nitrification	5–20/10–20	1.033/1.013	[28]
Nitrification	5.7–16.2	1.16	[29]
Nitrification	7.2–28.3	1.099	[30]

Table 4
Relative abundances of AOB, anammox bacteria, NOB and DB in different sludges

Sludge	AOB	Anammox bacteria	NOB	DB
Seed	0.11%	0.03%	2.28%	1.23%
ANR30	3.00%	0.10%	0.09%	1.37%
ANR25	2.85%	0.10%	0.05%	1.31%
ANR20	6.20%	0.01%	0.06%	1.62%
ANR15	1.68%	0.01%	0.10%	4.22%

The analysis of functional microbial communities showed that the relative abundance of anammox bacteria in ANR30 and ANR25 were almost same. However, when the temperature decreased from 25°C to 20°C, the relative abundance of anammox bacteria in ANR20 and ANR15 decreased one order of magnitude, whereas the relative abundance of AOB decreased just 44% from that in ANR30 to that in ANR15. Hence, consistent with the volumetric removal capacity in reactor performance and microbial activity in batch tests, the results strongly suggest that low temperatures affect anammox bacteria even more than AOB, that is, the temperature dependence of anammox bacteria is stronger than AOB at 15°C and 20°C. However, the abundances of anammox bacteria at 25°C and 30°C do not have any significant difference.

Previous studies showed that when temperature decreased from 30°C to 10°C, the growth rate of anammox bacteria decreased 4–300 times from 0.33 to 0.087–0.0011 d^{-1} [16,18,32–35], which was significantly larger than the decrease of AOB growth rate, almost two times (from 1.37–2.05 d^{-1} to 0.8 d^{-1}) [32,36,37]. In addition, the growth rates of AOB were 4–6 times higher than anammox bacteria at 30°C, but 20–28 times higher at 20°C [18,32,33,35,38,39]. Hence, as these studies showed, anammox bacteria were more sensitive to the decreases of temperature than other microorganisms, and this difference resulted in a sharper decrease in cell numbers and activity of anammox bacteria than of AOB. The consequence was nitrite accumulation and a low

total nitrogen removal performance of the ANR process at low temperature.

4. Conclusions

In this study, we initiated four ANR reactors to treat the digested swine wastewater at 30°C, 25°C, 20°C and 15°C, and the total nitrogen removal efficiency were 77.1%, 65.4%, 50.9% and 34.6%. With the decrease of temperature, the reductions of growth rate, activity and abundance of anammox bacteria were significantly larger than those of AOB. The temperature activity coefficient of AOB and anammox bacteria were 1.101 and 1.185 at 15°C–20°C, 1.073 and 1.084 at 20°C–25°C, 1.026 and 1.052 at 25°C–30°C, respectively. The temperature dependence of anammox bacteria is stronger than AOB. This difference led to a sharper decrease in cell numbers and activity of anammox bacteria. Furthermore it resulted in nitrite accumulation and lower total nitrogen removal rate at the temperature less than 25°C. High-throughput pyrosequencing analysis showed that *Nitrosomonas* and Candidatus *Brocadia* undertake the main task of removing nitrogen.

Acknowledgments

This work was supported by the Natural Science Foundation of China (31500105) and the China Agriculture Research System (CARS-35);

Declaration of interest statement

No potential conflict of interest was reported by the authors.

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Supplementary Information

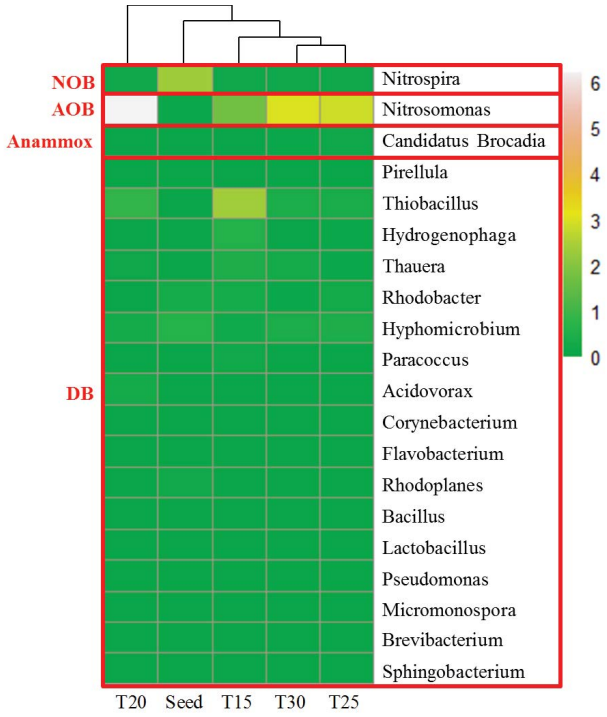

Fig. S1. Heat map of the relative abundances of functional genera in the seed sludge, ANR30 sludge (T30), ANR25 sludge (T25), ANR20 sludge (T20) and ANR15 sludge (T10). The relative abundances of the functional genera in each sludge are labeled on the heat map. The indicator on the right denotes the relationship between the relative abundance of each genus and the color range.

Table S1
Operational parameters and influent and effluent characteristics of four reactors

Reactor	ANR30		ANR25		ANR20		ANR15		
Phase	Stage I	Stage II	Stage I	Stage II	Stage I	Stage II	Stage I	Stage II	Stage III
Time (day)	0–60	61–200	0–70	71–200	0–60	61–200	0–7	8–60	61–200
TN_{inf} (mg L^{-1})	217 ± 31	378 ± 47	197 ± 36	373 ± 47	181 ± 46	367 ± 31	316 ± 25	233 ± 32	382 ± 32
TN_{eff} (mg L^{-1})	111 ± 29	87 ± 22	88 ± 12	129 ± 20	134 ± 24	180 ± 32	239 ± 11	185 ± 37	250 ± 39
NH_4^+–N_{eff} (mg L^{-1})	70 ± 15	48 ± 19	63 ± 7	85 ± 14	96 ± 40	115 ± 25	195 ± 10	120 ± 45	160 ± 24
NO_2^-–N_{eff} (mg L^{-1})	15 ± 7	18 ± 9	14 ± 8	27 ± 10	21 ± 9	53 ± 10	28 ± 16	44 ± 13	76 ± 15
NO_3^-–N_{eff} (mg L^{-1})	26 ± 12	21 ± 7	10 ± 5	17 ± 4	17 ± 5	13 ± 7	16 ± 10	20 ± 9	15 ± 8

Table S2
Summary of sequencing data for sludge samples from seed sludge, ANR30 sludge (T30), ANR25 sludge (T25), ANR20 sludge (T20) and ANR15 sludge (T15)

Sludge	Bacteria					Archaea				
	Seed	T30	T25	T20	T15	Seed	T30	T25	T20	T15
Taxon tags	29,254	19,608	32,050	28,506	28,168	222	736	1,010	844	1,144
OTUs	1,210	1,376	1,518	1,458	1,595	41	37	45	37	38
PD	50.12	56.80	59.82	59.90	62.42	1.8	2.0	1.9	2.0	2.2
Shannon	7.596	7.777	8.202	8.169	8.576	3.9	4.0	4.2	3.8	4.2