Chemical Engineering Journal 370 (2019) 110–119

Contents lists available at ScienceDirect

Chemical Engineering Journal

journal homepage: www.elsevier.com/locate/cej

The anammox process at typical feast-famine states: Reactor performance, sludge activity and microbial community

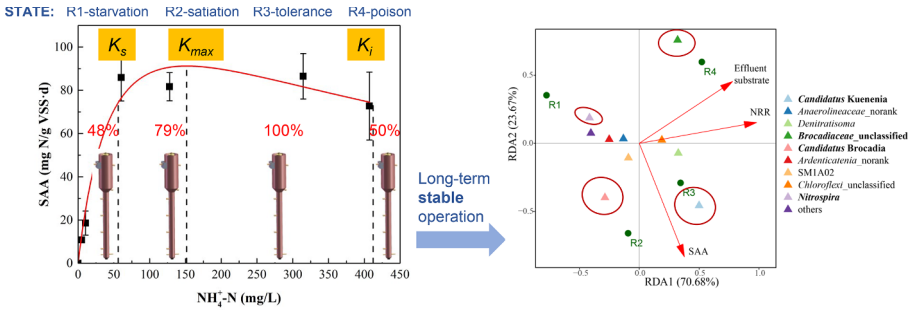
Da Kang[a], Tao Yu[a], Dongdong Xu[a], Zhuo Zeng[a], Aqiang Ding[b], Meng Zhang[c], Shengdao Shan[d], Wudi Zhang[e], Ping Zheng[a,*]

[a] Department of Environmental Engineering, College of Environmental & Resource Sciences, Zhejiang University, China
[b] Department of Environmental Science, College of Resource and Environmental Science, Chongqing University, China
[c] Advanced Environmental Biotechnology Centre, Nanyang Environment & Water Research Institute, Nanyang Technological University, Singapore
[d] School of Environmental and Natural Resources, Zhejiang University of Science & Technology, China
[e] School of Energy & Environmental Science, Yunnan Normal University, China

HIGHLIGHTS

- The anammox process was divided to starvation, satiation, tolerance, poison states.
- Four respective anammox bioreactors were operated stably over a year at each state.
- The relative specific anammox activities of sludge were 48%, 79%, 100%, 50%.
- The functional bacteria evolution could indicate the performance of bioreactors.

GRAPHICAL ABSTRACT



ARTICLE INFO

Keywords:
Anammox process
Feast-famine state
Process performance
Sludge activity
Microbial community

ABSTRACT

Anaerobic ammonium oxidation (Anammox) is a chemolithotrophic bioprocess which has been widely applied in the treatment of different concentrations of ammonium-containing wastewaters. However, there is less attention on the problem that the instantaneous growth rate (or metabolic rate) and equilibrium growth rate were un-synchronous for anammox bacteria due to their long generation time and self-immobilization of the granular sludge which could lead to the inaccurate estimation. In this study, the anammox process was firstly divided to four typical feast-famine (starvation, satiation, tolerance and poison) states based on the combination of both off-site and in-situ anammox reaction kinetics. Then, four respective lab-scale bioreactors were operated at each state for over a year to achieve stable anammox performance. The results showed that the nitrogen removal rates of bioreactors were 0.53, 2.24, 9.30 and 12.96 kg N/(m^3·d); and the specific anammox activities of granular sludge were 188.94 (48%), 313.29 (79%), 397.50 (100%) and 198.60 (50%) mg N/(g VSS·d) which could reflect the reactivity of each feast-famine state. The stable microbial communities of bioreactors were cultured and analyzed, whose species diversity went down with the decrease of Shannon and ACE index. The relative abundance of anammox bacteria increased from 11% to 57% from starvation to poison state. *Candidatus* Brocadia/*Nitrospira*, *Candidatus* Kuenenia and *Brocadiaceae*_unclassified were revealed to be the distinctive functional bacteria, which could serve as the indicator of each state. The setting up of the typical feast-famine states could be regarded as the landmark to help the design, control and optimization of anammox process.

* Corresponding author.
E-mail address: pzheng@zju.edu.cn (P. Zheng).

https://doi.org/10.1016/j.cej.2019.03.111
Received 9 December 2018; Received in revised form 6 March 2019; Accepted 12 March 2019
Available online 13 March 2019
1385-8947/

1. Introduction

Anaerobic ammonium oxidation (Anammox) is a biological process converting ammonium and nitrite to dinitrogen gas via anaerobic ammonium-oxidizing bacteria (AnAOB) [1,2]. Anammox process has now been acknowledged to be a cost-saving process because the need for organic carbon decrease by 100% compared to denitrification and the aeration requirement for partial nitritation/anammox decrease by 60% compared to conventional complete nitrification [3–5]. Nowadays, more than 200 full-scale anammox facilities have been put into use around the world [6]. Anammox process has shown its broad application prospects in the wastewater nutrient removal [7,8].

In the actual wastewater, nitrogen mainly exists in the form of ammonium. The ammonium concentration could vary from 30 to 100 mg/L of municipal wastewaters to 500–1500 mg/L of industrial wastewaters, even higher than 5000 mg/L [9,10]. Based on this, anammox process can find its application both in the mainstream and sidestream nitrogen removal process [11–13]. The nitrogen removal rate and effluent nitrogen concentration of anammox process are the most concerned by environmental engineers. Microorganism are the functional core of wastewater treatment process. According to microbial kinetics, the substrate concentration determines the substrate utilization rate. Due to the substrate self-inhibitory effect of anammox process [14], the relationship of substrate utilization rate and substrate concentration can be expressed by Haldane model as Eq. (1) [15]:

$$q = \frac{q_{max}}{1 + \frac{K_s}{S} + \frac{S}{K_i}} \quad (1)$$

where q-rate of substrate utilization; q_{max}-maximum specific rate of substrate utilization; S-substrate concentration; K_s-the saturation concentration giving half the maximum rate; K_i- the inhibition concentration giving half the maximum rate.

The different substrate concentration level not only determines the removal rate of anammox process, but also can selectively shape the specific microbial community. Since there is no pure culture to date, AnAOB usually exist in the mixed flora of anammox systems [16]. From the view of interspecies relationship, many nitrogen-related bacteria could coexist. Under the low-strength ammonium condition, nitrite oxidizing bacteria (NOB) could easily compete with AnAOB for nitrite in the partial nitritation/anammox process causing the decrease of nitrogen removal efficiency [17,18]. Under the high-strength ammonium condition, AnAOB could also be affected by heterotrophic bacteria metabolizing the biomass decay products or soluble microbial products [19]. From the view of intraspecific relationship, six candidate genera of AnAOB have been reported so far based on phylogenetic analysis: five of them have been recorded in Bergey's Manual of Systematic Bacteriology including *Candidatus* Brocadia, *Candidatus* Anammoxoglobus, *Candidatus* Jettenia, *Candidatus* Kuenenia, *Candidatus* Scalindua [20] and another genus, *Candidatus* Anammoximicrobium, has been found later from the Moscow River silt [21]. The ecology and physiology of all kinds of AnAOB have been critically reviewed, which showed that different anammox bacteria genera exist with substrate affinity constant divergence [22]. It was hypothesized that “Kuenenia” is a k-strategist with strong affinity, while “Brocadia” is a r-strategist with fast growth, which adapt to different substrate conditions [23].

In theory, the microbial community structure essentially determines the function of nitrogen conversion. However, AnAOB are slow-growing microorganism with typical doubling times as long as 15–30 days [24,25]. They are prone to forming the granular sludge to immobilize the bacteria via extracellular polymeric substance (EPS) [26,27]. Therefore, the change of AnAOB community structure and its metabolic activity often lags behind the change of environmental factors, leading to the un-synchronization of instantaneous growth rate (or metabolic rate) and equilibrium growth rate of AnAOB. Only when the bioreactor goes through a long time (at least 3–5 doubling times) at one state, can the instantaneous growth rate reach the equilibrium growth rate. However, the bioreactor was often operated with a short-retained time (< a month) under each working condition to investigate the process performance and microbial community in previous studies. The microbial community structure may not reach its equilibrium state and can cause the misunderstanding. Therefore, it is necessary to keep bioreactors stable under each condition for an enough long time (> 5 doubling times, ~150 days) to establish the structure-activity relationship of anammox process.

In this study, four lab-scale bioreactors were set up to separately simulate different states of anammox process at different ammonium concentrations. The objectives of this research were: (1) to divide and establish the starvation, satiation, tolerance and poison states of anammox process based on the anammox reaction kinetics; (2) to investigate the reactor performance, sludge activity and microbial community at each steady state; (3) to reveal the relationship between process performance and microbial community structure.

2. Materials and methods

2.1. Anammox granular sludge

The anammox granular sludge was withdrawn from a 10 L fermenter operated stably in the lab. The synthetic wastewater was used as the influent, the composition was (g/L): $KHCO_3$ 0.24, $NaHCO_3$ 0.8, $MgSO_4{\cdot}7H_2O$ 0.3, KH_2PO_4 0.0175, $CaCl_2$ 0.0175. Ammonium and nitrite were added in the form of $(NH_4)_2SO_4$ and $NaNO_2$. The influent total nitrogen (TN) was 520 mg/L and nitrogen loading rate (NLR) was 3.7 kg $N/m^3{\cdot}d$. The suspended solids (SS) and volatile suspended solids (VSS) were 28.88 g/L and 26.22 g/L, respectively.

2.2. Anammox reaction kinetics

2.2.1. Off-site batch test of anammox sludge

2.5 g wet sludge of Section 2.1 was washed and re-suspended in 0.1 M phosphate buffering solution for three times (0.22 g/L KH_2PO_4, 1.46 g/L K_2HPO_4). 50 mL soluble substrates (same as Section 2.1) were added to 75 mL serum bottle. Then, the serum bottles were sealed with butyl rubber stoppers and aluminum caps. The headspace and liquid phase were flushed for 6 min for each bottle with 95%Ar + 5%CO_2 to remove the oxygen. pH was set as 8.0. Ammonium was chosen as the inhibitor and the initial concentrations of ammonium were 5 mg N/L, 10 mg N/L, 50 mg N/L, 150 mg N/L, 300 mg N/L, 400 mg N/L, respectively. The ratio of nitrite/ammonium was set as 1.2. The serum bottles were placed in a thermostatic shaker at 150 rpm and 35 °C. Samples were taken every hour, filtered through 0.45 μm film before the determination of substrate concentration. At last, VSS in each serum bottle was measured. The tests were set duplicate. The substrate degradation rate was calculated according to the slope of fitted curve. Haldane model (Eq. (1)) was used to simulate the kinetic curve using software OriginPro 2015.

2.2.2. In-situ continuous test of anammox bioreactor

A lab-scale bioreactor was used to evaluate the kinetic performance of anammox process along the nitrogen loading rates gradient. The detailed description of bioreactors referred to Kang et al. [28]. Briefly, the reactor had an effective volume of 1.0 L with a total volume of 3.7 L. The superficial flow rate of the feeding plus the recirculation could reach up to 4.4 m/h with a height-to-diameter ratio as large as 20 and waterflow recycle ratio of 7.5 to simulate the actual upflow rate of full-scale granular sludge bed reactor (3–7 m/h) (Technical specifications HJ 2023-2012, China). The bioreactor was operated in a continuous-flow mode occupied with 0.67 L seed sludge (see 2.1). The sludge can fulfill the reaction zone exactly when it expands. Waste sludge was withdrawn from the bottom of settling zone periodically to keep the biomass stable. NLR was increased by step increase of substrate

concentration with a fixed hydraulic retention time (HRT) of 1.60 h. The influent and effluent substrates were monitored every two days to calculate nitrogen removal rate (NRR) as Eq. (2):

$$NRR = \frac{Q(c_0 - c)}{1000V} \quad (2)$$

NRR- nitrogen removal rate, kg N/(m^3·d); Q-influent flow, L/d; V-the effective volume, L; c_0-influent ammonium and nitrite concentration, mg N/L; c-effluent TN (the sum of ammonium, nitrite and nitrate) concentration, mg N/L.

2.3. Operation of anammox bioreactor

Four identical lab-scale bioreactors (same as Section 2.2.2, named R1, R2, R3 and R4) inoculated with the same seed sludge of Section 2.1 were operated continuously at steady state. The influent ammonium concentrations were set as 25 mg N/L, 150 mg N/L, 280 mg N/L and 420 mg N/L, respectively. The ratio of nitrite/ammonium was set as 1.2. The NLRs were set as 0.8 kg N/m^3·d, 5 kg N/m^3·d, 10 kg N/m^3·d and 15 kg N/m^3·d, respectively. The initial biomass concentrations were 17.48 g VSS/L. Except that, other conditions were all kept the same. All bioreactors were operated over a year under each condition to achieve the stable anammox performance and microbial community structure.

2.4. Specific activity of anammox sludge

The specific anammox activities (SAAs) of granular sludge of four bioreactors were determined at the end of operation. 2 g wet sludge was chosen and the initial concentrations of ammonium and nitrite were 150 mg N/L and 180 mg N/L, respectively. The procedure referred to Section 2.2.1. The tests were set duplicate. The SAA was represented as the total utilization rate of ammonium and nitrite.

2.5. DNA extraction and Illumina high-throughput sequencing

Anammox sludge at different heights of the bioreactor was withdrawn at the end of operation. Each bioreactor had three samples as the repetitions and 12 samples in total. 0.25 g wet sludge of each sample was used to extract the genomic DNA following the instruction of DNeasy PowerSoil Kit (QIAGEN GmbH, Germany) and stored at −20 °C for further analysis.

Bacterial 16S rRNA gene V3-V4 fragments were amplified using the universal PCR primers 338F (ACTCCTACGGGAGGCAGCAG) and 806R (GGACTACHVGGGTWTCTAAT) [29]. The fragments were purified and sequenced by Illumina Miseq PE300 platform (Majorbio Bio-pharm Technology Co.,Ltd, Shanghai, China). 451,904 high-quality reads were obtained in total after quality control and chimera screening with average sequence length of 440 bp (Table S1). The reads were further used to calculate the operational taxonomic units (OTUs) using a 97% sequence identity threshold. The rarefaction curves of each sequencing sample reached the plateau stage, and the coverage index was larger than 0.99, indicating that the depth and quality of the sequencing met the requirements of subsequent analysis (Fig. S1). All the diversity indices were calculated using the mothur software (version v.1.30.1). The

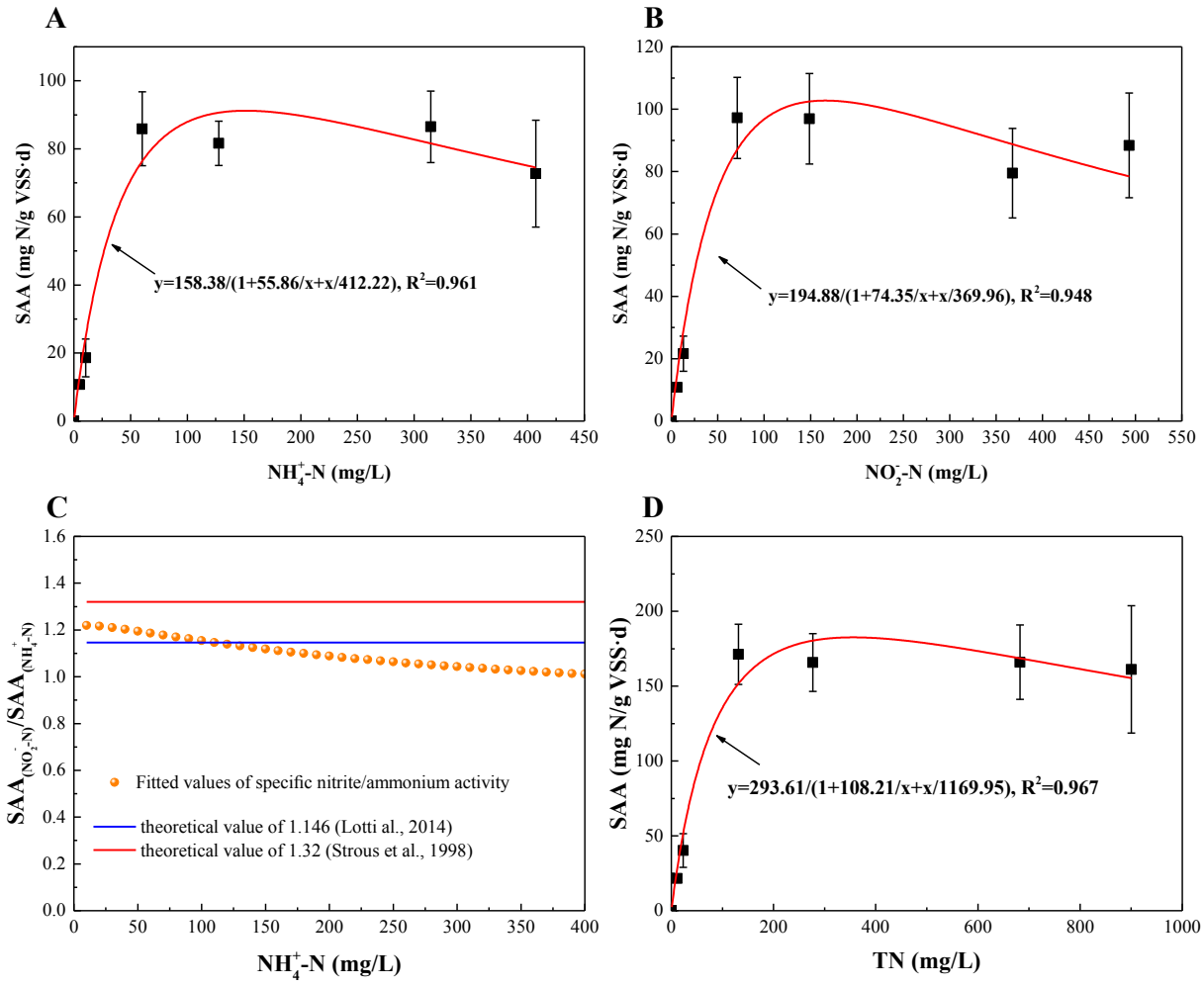


Fig. 1. Performance of anammox reaction kinetics of the batch test.

high-quality reads were then aligned against the bacterial SILVA database. The data were analyzed on the online platform of Majorbio I-Sanger Cloud Platform (www.i-sanger.com). The sequencing data was submitted to NCBI (National Center for Biotechnology Information) with the accession number SRP159318.

2.6. Phylogenetic analysis

The high-quality reads of each sample belong to the family *Brocadiaceae* were screened. All gene sequences were aligned with the known AnAOB 16S rRNA gene sequences downloaded from NCBI by ClustW method using the MEGA software (version 5.0). A phylogenetic tree was constructed using the neighbor-joining method with evolutionary distances computed using the maximum composite likelihood method. All nucleotide sequence data have been deposited in the GenBank database under accession numbers MH918078 to MH918088.

2.7. Other analysis

The ammonium, nitrite, nitrate, TN, SS and VSS concentrations were all determined according to standard methods [30]. Redundancy analysis (RDA) was performed to analyze the linkages between the process performance and microbial community structure using the software R (version 3.5.1).

3. Results

3.1. Anammox reaction kinetics

3.1.1. Off-site reaction kinetics of anammox sludge

Anammox sludge, as the core biocatalyst, determines the bioreactor performance directly. The results showed that the nitrogen reaction kinetics of anammox sludge fitted Haldane model well with $R^2 > 0.9$. For ammonium as the substrate, the half-saturation coefficient (K_s) and half-inhibition coefficient (K_i) was 55.86 mg N/L and 412.22 mg N/L, respectively. K_s and K_i are the value of limiting substrate concentration at which the specific reaction rate is half its maximum value which represents starvation and inhibition level, respectively. The maximum specific ammonium reaction rate (q_{max}) was 158.38 mg N/g VSS·d when the most fitted ammonium concentration (K_{max}) reached 151.75 mg N/L (Fig. 1A). For nitrite as the substrate, the K_s, K_i, K_{max}, q_{max} were 74.35 mg N/L, 369.96 mg N/L, 165.85 mg N/L and 194.88 mg N/g VSS·d (Fig. 1B). For TN, the K_s, K_i, K_{max}, q_{max} were 108.21 mg N/L, 1169.95 mg N/L, 355.81 mg N/L and 293.61 mg N/g VSS·d (Fig. 1D). When the concentration of nitrite/ammonium was 1.2, the reaction ratio of nitrite/ammonium utilization rate was calculated around 1.011–1.220 which was closed to the relevant values reported in previous literatures [24,31] (Fig. 1C). Based on the ammonium reaction kinetic curve, the anammox process can be divided to four typical feast-famine states at different ammonium concentration (mg N/L) level: starvation state [0-K_s (0–55.86), $q/q_{max} < 50\%$)], satiation state [K_s-K_{max} (55.86–151.75), $q/q_{max} > 50\%$], tolerance state [K_{max}-K_i (151.75–412.22), $q/q_{max} > 50\%$] and poison state [> K_i (412.22), $q/q_{max} < 50\%$].

3.1.2. In-situ reaction kinetics of anammox bioreactor

Anammox bioreactor is a functional body which determines the apparent performance of anammox process. NRR is a comprehensive index reflecting the in-situ reaction rate affected by both substrate concentration and operating condition. To verify the ammonium concentration effect on the performance of a practical bioreactor operated in a continuous-flow mode, the in-situ reaction kinetics of anammox bioreactor was evaluated. The results showed that with the step increase of ammonium concentration (nitrite/ammonium = 1.2), NRR could rise linearly from 0.49 ± 0.01 kg N/(m^3·d) to 12.60 ± 0.64 kg N/(m^3·d) fitting the equation: y = 0.014x-0.216, R^2 = 0.994 (Fig. 2B). However, when the nitrogen concentration reached around 1057.67–1184.27 mg N/L, NRR remained stable at 14.35 ± 0.86 kg N/(m^3·d). NRR dropped sharply to 0.23 kg N/(m^3·d) when the nitrogen concentration was slightly elevated to 1234.86 mg N/L. The repeated shock tests confirmed the same trend. Finally, NRR could reach the maximum stable value of 13.16 ± 0.22 kg N/(m^3·d) (Fig. 2A). The NRRs of anammox bioreactors at starvation, satiation, tolerance and poison states were divided as 0–1.50 kg N/(m^3·d), 1.50–4.46 kg N/(m^3·d), 4.46–12.48 kg N/(m^3·d), 12.48–13.16 kg N/(m^3·d).

3.2. Performance of anammox process at different states

3.2.1. Performance of anammox bioreactor

The four respective bioreactors were operated at each feast-famine state and achieved a stable anammox performance for over a year which was shown in Table 1. At the starvation state (R1), the influent ammonium, nitrite and TN concentrations were 22.90 ± 3.41 mg N/L, 23.92 ± 2.50 mg N/L and 51.56 ± 1.52 mg N/L. The biomass concentration was 6.38 ± 3.14 g VSS/L. The effluent ammonium, nitrite, nitrate and TN concentrations were determined as 1.42 ± 1.76 mg N/L, 0.30 ± 0.33 mg N/L, 12.23 ± 3.30 mg N/L and 23.13 ± 0.88 mg N/L. The removal efficiency of ammonium, nitrite and TN was 93.78%, 98.75% and 55.13%. NLR was 0.72 ± 0.11 kg N/

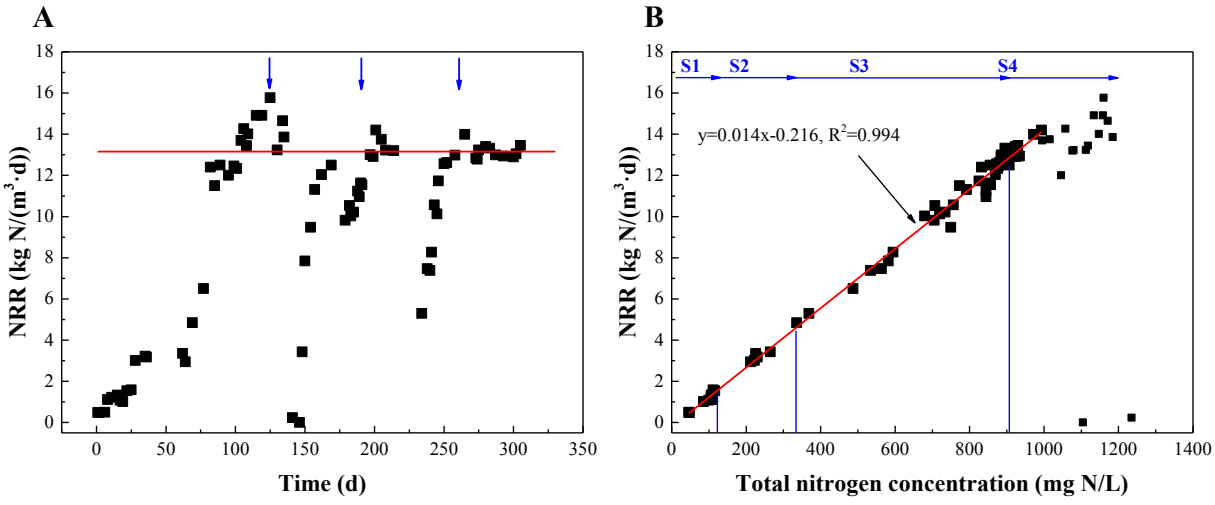


Fig. 2. Performance of anammox reaction kinetics of the continuous-flow bioreactor.

Table 1
The performance of anammox process at different feast-famine states.

	Influent (mg N/L)			Effluent (mg N/L)			Removal efficiency (%)			NLR	NRR	SAA	SAA/SAA_{max}
	Ammonium	Nitrite	TN	Ammonium	Nitrite	TN	Ammonium	Nitrite	TN	kgN/(m^3·d)	kgN/(m^3·d)	mgN/(gVSS·d)	(%)
R1	22.90 ± 3.41	23.92 ± 2.50	51.56 ± 1.52	1.42 ± 1.76	0.30 ± 0.33	23.13 ± 0.88	93.78	98.75	55.13	0.72 ± 0.11	0.53 ± 0.08	188.94 ± 26.58	48
R2	148.99 ± 6.77	170.58 ± 8.74	322.23 ± 7.91	2.12 ± 3.12	2.50 ± 3.69	48.26 ± 2.81	98.58	98.53	85.02	5.05 ± 0.18	2.24 ± 0.10	313.29 ± 53.27	79
R3	296.85 ± 17.10	346.90 ± 18.28	611.35 ± 26.31	1.62 ± 1.80	2.50 ± 3.92	80.76 ± 0.13	99.45	99.28	86.79	9.72 ± 0.45	9.30 ± 0.52	397.50 ± 50.93	100
R4	427.90 ± 46.59	475.86 ± 48.84	941.87 ± 7.80	24.06 ± 12.70	10.18 ± 7.84	121.89 ± 1.34	94.38	97.86	87.06	14.27 ± 2.27	12.96 ± 1.45	198.60 ± 3.12	50

Note: R1, R2, R3, R4 referred to the starvation, satiation, tolerance and poison states of anammox process, respectively.

(m^3·d) with NRR of 0.53 ± 0.08 kg N/(m^3·d) (Fig. 3A).

At the satiation state (R2), the influent ammonium, nitrite and TN concentrations were 148.99 ± 6.77 mg N/L, 170.58 ± 8.74 mg N/L and 322.23 ± 7.91 mg N/L. The biomass concentration was 12.05 ± 5.27 g VSS/L. The effluent ammonium, nitrite, nitrate and TN concentrations were determined as 2.12 ± 3.12 mg N/L, 2.50 ± 3.69 mg N/L, 35.06 ± 3.91 mg N/L and 48.26 ± 2.81 mg N/L. The removal efficiency of ammonium, nitrite and TN was 98.58%, 98.53% and 85.02%. NLR was 5.05 ± 0.18 kg N/(m^3·d) with NRR of 2.24 ± 0.10 kg N/(m^3·d) (Fig. 3B).

At the tolerance state (R3), the influent ammonium, nitrite and TN concentrations were 296.85 ± 17.10 mg N/L, 346.90 ± 18.28 mg N/L and 611.35 ± 26.31 mg N/L. The biomass concentration was 16.80 ± 1.44 g VSS/L. The effluent ammonium, nitrite, nitrate and TN concentrations were determined as 1.62 ± 1.80 mg N/L, 2.50 ± 3.92 mg N/L, 58.22 ± 5.62 mg N/L and 80.76 ± 0.13 mg N/L. The removal efficiency of ammonium, nitrite and TN was 99.45%, 99.28% and 86.79%. NLR was 9.72 ± 0.45 kg N/(m^3·d) with NRR of 9.30 ± 0.52 kg N/(m^3·d) (Fig. 3C).

At the poison state (R4), the influent ammonium, nitrite and TN concentrations were 427.90 ± 46.59 mg N/L, 475.86 ± 48.84 mg N/L and 941.87 ± 7.80 mg N/L. The biomass concentration was 16.60 ± 0.78 g VSS/L. The effluent ammonium, nitrite, nitrate and TN concentrations were determined as 24.06 ± 12.70 mg N/L, 10.18 ± 7.84 mg N/L, 59.49 ± 9.99 mg N/L and 121.89 ± 1.34 mg N/L. The removal efficiency of ammonium, nitrite and TN was 94.38%, 97.86% and 87.06%. NLR was 14.27 ± 2.27 kg N/(m^3·d) with NRR of 12.96 ± 1.45 kg N/(m^3·d) (Fig. 3D).

3.2.2. Activity of anammox sludge

The specific anammox activity (SAA) reflects the reactivity of anammox sludge which was measured by batch tests (Fig. S2). After the long-term stable cultivation, sludge of R1 had the lowest SAA of 188.94 ± 26.58 mg N/(g VSS·d) at the starvation state, while it reached the highest level of 397.50 ± 50.93 mg N/(g VSS·d) at the tolerance state (R3). However, SAA decreased to 198.60 ± 3.12 mg N/(g VSS·d) at the poison state (R4). The relative SAA value from R1 to R4 was 48%, 79%, 100%, 50%, which could be used to indicate the feast-famine states (Table 1).

3.3. Microbial community structure

3.3.1. Microbial diversity

The Alpha diversity of microbial communities includes the species diversity and species richness of microbial community. The species diversity was expressed using the Shannon and Simpson index. The higher the Shannon index, the lower the Simpson index, the higher the community species diversity. The species richness was expressed using the ACE and Chao index. The larger the ACE and Chao index, the greater the community species richness. From R1 to R4 at different states, the Shannon index decreased from 3.72 ± 0.10 to 2.30 ± 0.22, the ACE index decreased from 265.24 ± 3.03 to 149.94 ± 7.05, indicating the decline of microbial community diversity and higher abundance of specific microorganism (Table 2).

3.3.2. Microbial community

At the phylum level, the main microbial phyla were *Planctomycetes*, *Chloroflexi, Proteobacteria*, *Bacteroidetes*, *Nitrospirae* and *Ignavibacteriae* which accounted for more than 80% (Fig. 4A). The relative abundance of *Planctomycetes* phylum which consists of AnAOB increased along the substrate concentration gradient from 21.40% to 57.00%, which became dominant at the poison state. *Chloroflexi* is commonly found in the autotrophic nitrogen removal system and supposed to degrade the dead biomass and promote the cell aggregation, which stayed at 22.53 ± 7.23% [32,33]. The relative abundance of *Nitrospirae* was 6.59% at the starvation state, while almost no *Nitrospirae* were detected

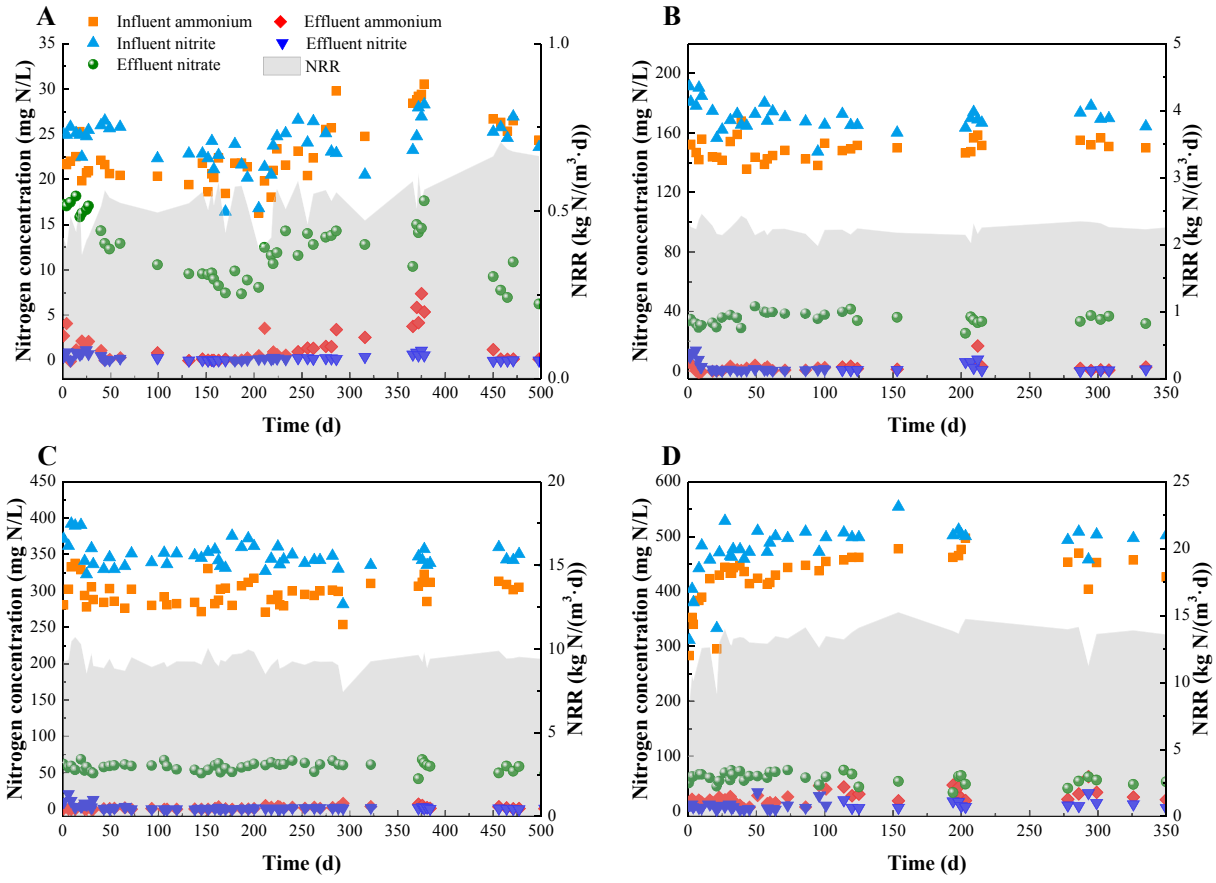


Fig. 3. The stable performance of anammox process at each feast-famine state.

Table 2
The microbial diversity index of anammox process at different feast-famine states.

	Community diversity		Community richness	
	Shannon	Simpson	ACE	Chao
R1	3.72 ± 0.10	0.07 ± 0.01	265.24 ± 3.03	265.76 ± 5.90
R2	2.75 ± 0.22	0.17 ± 0.04	209.52 ± 12.59	211.09 ± 10.03
R3	2.33 ± 0.27	0.25 ± 0.06	182.69 ± 7.86	191.10 ± 8.65
R4	2.30 ± 0.22	0.20 ± 0.04	149.94 ± 7.05	147.93 ± 6.72
Variation[a]	↓	↑	↓	↓

Note: R1, R2, R3, R4 referred to the starvation, satiation, tolerance and poison states of anammox process, respectively.
[a] Variation moved from R1 to R4.

at other states.

At the genus level, the main microbial genus were *Candidatus* Kuenenia, *Anaerolineaceae*_norank, *Denitratisoma*, *Brocadiaceae*_unclassified, *Candidatus* Brocadia, *Ardenticatenia*_norank, SM1A02, *Chloroflexi*_unclassified, *Nitrospira*, which accounted for more than 50% (Fig. 4B). The relative abundance of AnAOB increased from 11% to 57%. *Denitratisoma* was identified as heterotrophic denitrifying bacteria utilizing the bacteria lysates [34,35] and its relative abundance increased with NLR from 1.66% to 11.21%. At the starvation state, *Nitrospira* as one genus of NOB accounted for larger than 6.59% far more than that at other states (< 0.5%). At the poison state, the relative abundance of *Brocadiaceae*_unclassified was 17.19% far more than that at other states (< 2%).

As for AnAOB, five genera were detected in the bioreactor. *Candidatus* Kuenenia was dominated at all states. R1 had the most diversity with 64% *Candidatus* Kuenenia, 20% *Candidatus* Brocadia and 5% others (*Brocadiaceae*_unclassified, *Candidatus* Jettenia, *Candidatus* Anammoxoglobus). In R2, *Candidatus* Kuenenia increased to 81%, while *Candidatus* Brocadia decreased to 17% with others less than 1%. In R3, *Candidatus* Kuenenia became absolutely dominant AnAOB with the relative abundance of 95%, while *Brocadiaceae*_unclassified accounted for 4%. In R4, *Candidatus* Kuenenia was replaced by *Brocadiaceae*_unclassified and decreased to 68%, *Brocadiaceae*_unclassified still increased to 32%. It is noteworthy that the sequence similarity of *Brocadiaceae*_unclassified with other known species (amx8 and amx9) of AnAOB was only 92% by phylogenetic analysis (Fig. 5), which needs a further identification by whole genome analysis.

3.4. Correlation between process performance and microbial community

NRR and SAA were used to represent the bioreactor performance and the sludge activity, respectively. The RDA analysis showed that NRR mainly determined the microbial community distribution and was almost in parallel with RDA1 axis, explaining 70.68% correlations (Fig. 6). *Candidatus* Kuenenia and *Brocadiaceae*_unclassified were suitable for the habitat with a high NRR; while *Candidatus* Brocadia and *Nitrospira* preferred to stay in the habitat with a low NRR. *Candidatus* Kuenenia showed higher reactivity and substrate affinity than *Brocadiaceae*_unclassified. *Brocadiaceae*_unclassified had a low anammox activity, but can tolerate a higher concentration of effluent nitrogen.

4. Discussion

AnAOB could face the different feast-famine situations in the practical anammox systems due to the fluctuation of wastewater quality and quantity. In this study, the typical feast-famine states of anammox process was divided based on the anammox reaction kinetics characteristics. For the batch test, ammonium was chosen as the limiting substrate. There is no uniform kinetic value (K_s, K_i and q_{max}) of

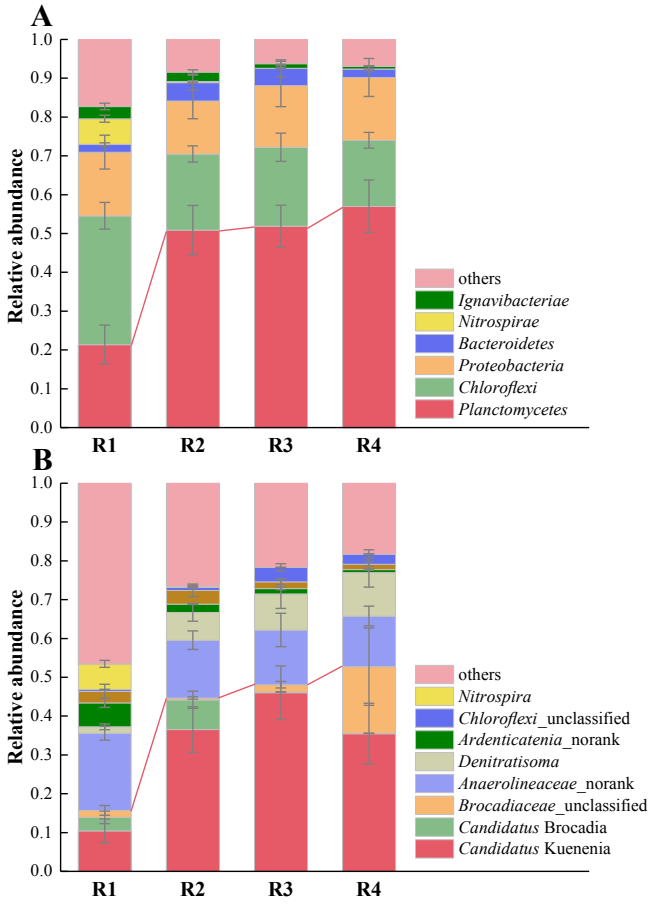


Fig. 4. Microbial community of anammox process at each feast-famine state.

ammonium conversion so far and different results could be obtained as listed in Table 3. The reason might be decided to the difference of functional bacteria diversity and abundance. In essence, free ammonia (FA) was recognized to be the true inhibitor of anammox. The results of kinetic characteristic of FA conversion were consistent with that of ammonium (Fig. S3) and the K_i of FA was consistent with the previous reported value of 40 mg N/L [42]. By the step identification applying continuous-flow test of bioreactor, NRR had an excellent positive relevance with the substrate concentration: y = 0.014x-0.216, R^2 = 0.994 and the maximum value of NRR could reach up to 13.16 ± 0.22 kg N/(m^3·d). Therefore, based on both the off-site and in-situ anammox reaction kinetics, the typical feast-famine states of anammox process could be divided as: starvation state (R1) [NH_4^+: 0–55.86 mg N/L, NRR: 0–1.50 kg N/(m^3·d)]; satiation state (R2) [NH_4^+: 55.86–151.75 mg N/L, NRR: 1.50–4.46 kg N/(m^3·d)]; tolerance state (R3) [NH_4^+: 151.75–412.22 mg N/L, NRR: 4.46–12.48 kg N/(m^3·d)]; poison state (R4) [NH_4^+: > 412.22 mg N/L, NRR: 12.48–13.16 kg N/(m^3·d)].

From the view of energy balance, microorganisms obtain their energy for growth and maintenance from oxidation-reduction reactions. The pathway for bacteria growth involves two kinds of basic reactions, one for energy production and the other for cellular synthesis [15]. In the energy half reactions of anammox metabolism, ammonium acts as the electron donor while nitrite serves as the electron acceptor, producing N_2. In the synthesis half reaction, nitrite is oxidized to nitrate, providing ATP for carbon dioxide fixation [8,43–44]. If the substrate is insufficient lower than the K_s, the energy production pathway is strengthened and more substrates will be converted into N_2 and nitrate to synthesis the energy rather than producing the biomass, causing the reaction ratio change of anammox and the decrease of biomass [28,45]. It has been reported that the theoretical maintenance energy (m) of AnAOB was calculated as ~ 98.14 mg N/g cell·d previously [46] (Text S1). The biomass concentration was positive with NLR (P = 0.804, p < 0.01). For R1 at the starvation state, the biological loading rate

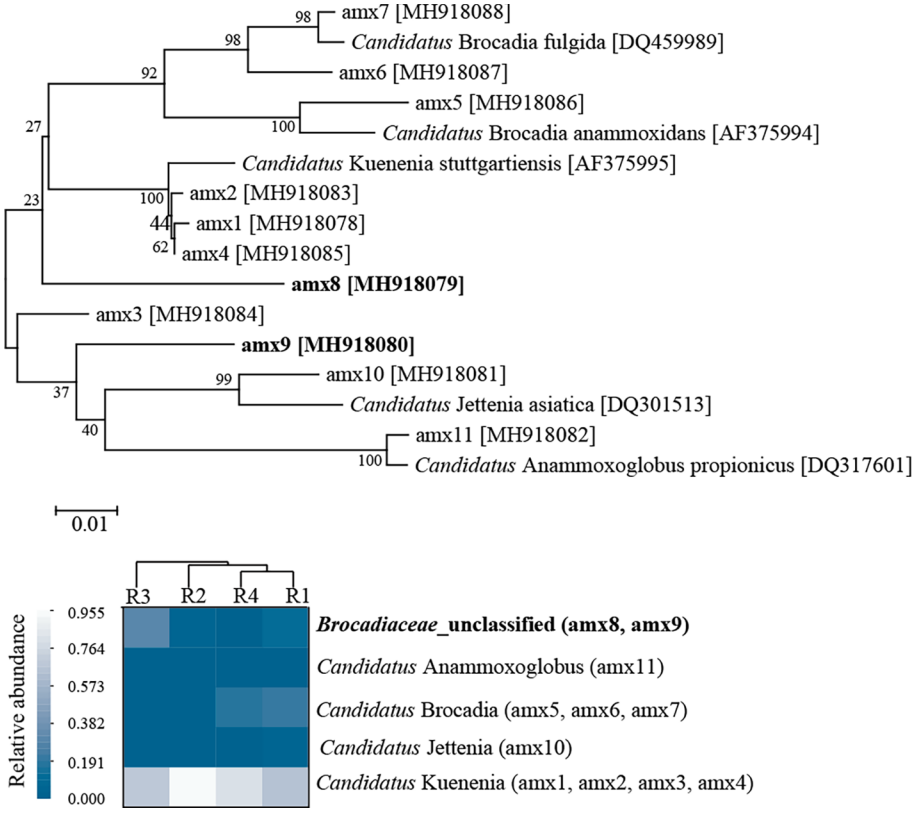


Fig. 5. The phylogenetic analysis and heatmap of AnAOB genus evolution of anammox process at each feast-famine state.

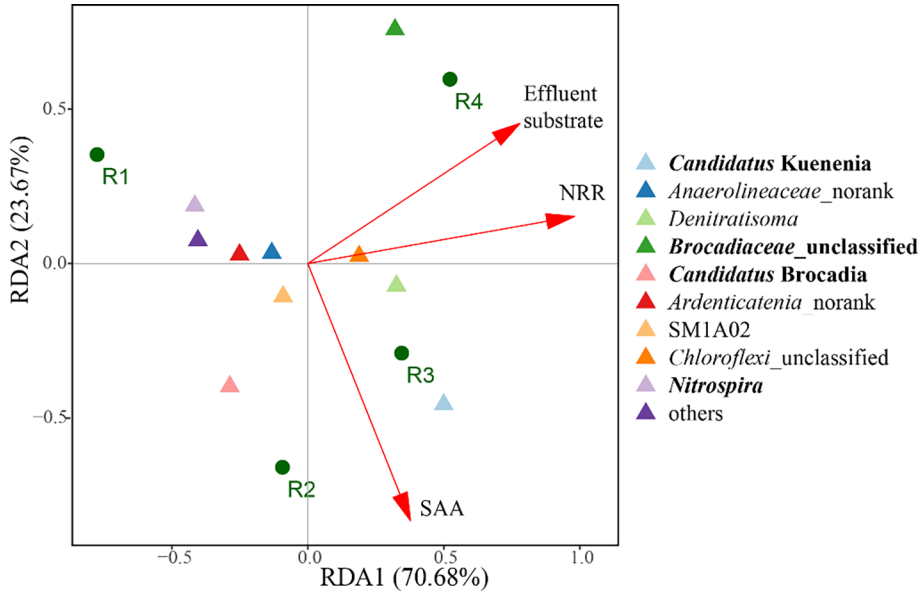


Fig. 6. RDA analysis of process performance and microbial community at genus level.

Table 3
The summary of ammonium kinetic characteristics.

Substrate	Reactor type	Volume (L)	K_s (mg N/L)	K_i (mg N/L)	q_{max} mgN/gVSS·d	Reference
ammonium	fermenter	10.0	55.86	412.22	158.38	This study
ammonium	EGSB	1.2	36.75	887.1	381.2	[36]
ammonium	EGSB	–	11.52	–	298.94	[37]
ammonium	MBR	–	9.54	–	288.58	[37]
ammonium	UASB	1.1	17	11,697	1170	[38]
ammonium	SBR	2.2	25	9016	490	[39]
ammonium	EGSB	1.2	32.85	–	218.4	[40]
ammonium	SBR	89.5	9.5	422	209	[41]
FA[a]	fermenter	10.0	5.69	41.99	158.38	This study
FA	MBBR	3.0	–	40	–	[42]

[a] FA was calculated as N (mg N/L)= $\frac{ammonium\ as\ N\ (mg\ N/L) * 10^{pH}}{e^{6344/(273+T)} + 10^{pH}}$, pH = 8.0, T = 35 °C.

(BLR) was 128.29 mg N/(g VSS·d) which was 1.31 times larger than *m*. The energy obtained was just able to keep balance with their survival and only had the lowest anammox activity of 188.94 mg N/(g VSS·d) and least biomass of only 6.38 ± 3.14 g VSS/L; For R2 and R3 at the satiation and tolerance state, the BLR was 423.38–583.14 mg N/(g VSS·d) which was 4.31–5.94 times larger than *m*. The bacteria could reach the highest anammox activity of 397.50 mg N/(g VSS·d), providing enough energy to sustain the bacteria growth. For R4 at the poison state, the BLR was 906.72 mg N/(g VSS·d) which was almost 10 times larger than *m*. Both the high concentration of ammonium and nitrite could cause the inhibition of anammox activity [47,48]. The SAA dropped to 198.60 mg N/(g VSS·d) and reached the balance between energy production and energy consumption for survival.

At the typical feast-famine states from R1 to R4, the relative value of SAA was 48%, 79%, 100%, 50%, which showed the same trend with off-site reaction kinetics of anammox sludge and could indicate different states of anammox process, respectively. However, the NRR of bioreactor kept increasing from 0.53 to 12.96 kg N/(m^3·d) which seems inconsistent with the trend of SAA. The reason might be ascribed to the high recycle ratio of bioreactor, which caused the dilution of influent wastewater in the reaction zone. Except that, the volume of settling zone was two times larger than the reaction zone, which also caused the dilution of influent wastewater. All above caused the lag effect of substrate inhibition. Although the NRR in R4 was the highest, the sludge activity has already decreased measured by the batch test and the bioreactor became unstable and easily broke down.

The specific culture condition could shape the specific microbial community structure which determined the reactivity of bioreactors. From the starvation to poison state of anammox process, the ability of selective enrichment of functional bacteria was enhanced and the diversity and richness of microorganism decreased. It seemed that *Nitrospira* easily appeared at the starvation state even without extra aeration, which has become one of the bottlenecks of mainstream anammox process application [6]. However, at other states, AnAOB dominated the microbial community with the relative abundance higher than 50%, almost no *Nitrospira* could be detected. The AnAOB species showed an obvious evolution and had the highest diversity at starvation state similar to reference [49,50]. *Candidatus* Brocadia preferred to live at a low NLR (< 5 kg N/(m^3·d)) with a low SAA, while *Candidatus* Kuenenia was more suitable at a high NLR of 10 kg N/(m^3·d) with a high SAA. A new anammox genus-*Brocadiaceae* unclassified was found at the poison state with NLR higher than 15 kg N/(m^3·d), which were supposed to have a higher substrate affinity constant. It has been reported that *Candidatus* Brocadia had low affinities for NH_4^+ and NO_2^- and had a high growth rate. They grow in the substrate-rich environments and are regarded as r-strategists; while *Candidatus* Kuenenia grow in the substrate-poor environments and are regarded as k-strategists [23,51]. But in this study, *Candidatus* Brocadia only existed at starvation state (Fig. 4B). One reason may be that different species of *Candidatus* Brocadia have different affinities varying from 5 to 640 μM,

implying their possible survival at the starvation state [52,53]. Another reason might be that EPS could be the putative energy sources for cell maintenance in starved anammox bacteria [54]. As a result, the binding force between the bacteria in the granular sludge became weak and cells dispersed, which could be easily washed out under the high upflow rate which was more beneficial for r-strategists to stay than k-strategists.

5. Conclusions

1) The anammox process could be divided to four typical feast-famine states based on the anammox reaction kinetics characteristics as: starvation state [NH_4^+: 0–55.86 mg N/L, NRR: 0–1.50 kg N/(m^3·d)]; satiation state [NH_4^+: 55.86–151.75 mg N/L, NRR: 1.50–4.46 kg N/(m^3·d)]; tolerance state [NH_4^+: 151.75–412.22 mg N/L, NRR: 4.46–12.48 kg N/(m^3·d)]; poison state [NH_4^+: > 412.22 mg N/L, NRR: 12.48–13.16 kg N/(m^3·d)].
2) Four lab-scale bioreactors were operated stably for over a year at each feast-famine state. The stable NRRs of bioreactors were 0.53 ± 0.08, 2.24 ± 0.10, 9.30 ± 0.52 and 12.96 ± 1.45 kg N/(m^3·d); the SAAs of granular sludge were 188.94 ± 26.58 (48%), 313.29 ± 53.27 (79%), 397.50 ± 50.93 (100%) and 198.60 ± 3.12 (50%) mg N/(g VSS·d) which could reflect the reactivity of each state.
3) The diversity of microbial community decreased and the relative abundance of AnAOB increased from 11% to 57% from starvation to poison state. *Candidatus* Brocadia/*Nitrospira*, *Candidatus* Kuenenia and *Brocadiaceae*_unclassified were revealed to be the distinctive functional bacteria, which could indicate the different feast-famine states.

Acknowledgments

This research was financially supported by the National Natural Science Foundation of China (51578484 and 51778563) and Research Funds for Central Universities (2017xzzx010-03). Major Scientific and Technological Specialized Project of Zhejiang Province (2015C03013) and Key Research and Development program of Zhejiang Province (2018C03031) were also gratefully thanked.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.cej.2019.03.111.

References

[1] A.A. van de Graaf, A. Mulder, P. Debruijn, M.S.M. Jetten, L.A. Robertson, J.G. Kuenen, Anaerobic oxidation of ammonium is a biologically mediated process, Appl. Environ. Microb. 61 (1995) 1246–1251.
[2] J.G. Kuenen, Anammox bacteria: from discovery to application, Nat. Rev. Microbiol. 6 (2008) 320–326.
[3] M.S. Jetten, M. Wagner, J. Fuerst, M. van Loosdrecht, G. Kuenen, M. Strous, Microbiology and application of the anaerobic ammonium oxidation ('anammox') process, Curr. Opin. Biotechnol. 12 (2001) 283–288.
[4] I. Schmidt, O. Sliekers, M. Schmid, E. Bock, J. Fuerst, J.G. Kuenen, M.S. Jetten, M. Strous, New concepts of microbial treatment processes for the nitrogen removal in wastewater, FEMS Microbiol. Rev. 27 (2003) 481–492.
[5] I. Zekker, A. Kiviräüt, E. Rikmann, A. Mandel, M. Jaagura, T. Tenno, O. Artemchuk, S. dC Rubin, T. Tenno, Enhanced efficiency of nitritating-anammox sequencing batch reactor achieved at low decrease rates of oxidation-reduction potential, Environ. Eng. Sci. (2018).
[6] Y.S. Cao, M.C.M. van Loosdrecht, G.T. Daigger, Mainstream partial nitritation anammox in municipal wastewater treatment: status, bottlenecks, and further studies, Appl. Microbiol. Biotechnol. 101 (2017) 1365–1383.
[7] E. Rikmann, I. Zekker, T. Tenno, A. Saluste, Inoculum-free start-up of biofilm-and sludge-based deammonification systems in pilot scale, Int. J. Environ. Sci. Technol. 15 (2018) 133–148.
[8] P.L. McCarty, What is the best biological process for nitrogen removal: when and why? Environ. Sci. Technol. 52 (2018) 3835–3841.
[9] J. Carrera, J.A. Baeza, T. Vicent, J. Lafuente, Biological nitrogen removal of high-strength ammonium industrial wastewater with two-sludge system, Water Res. 37 (2003) 4211–4221.
[10] S. Lackner, E.M. Gilbert, S.E. Vlaeminck, A. Joss, H. Horn, M.C.M. van Loosdrecht, Full-scale partial nitritation/anammox experiences an application survey, Water Res. 55 (2014) 292–303.
[11] B. Ma, S.Y. Wang, S.B. Cao, Y.Y. Miao, F.X. Jia, R. Du, Y.Z. Peng, Biological nitrogen removal from sewage via anammox: recent advances, Bioresour. Technol. 200 (2016) 981–990.
[12] Y.J. Shi, G. Wells, E. Morgenroth, Microbial activity balance in size fractionated suspended growth biomass from full-scale sidestream combined nitritation-anammox reactors, Bioresour. Technol. 218 (2016) 38–45.
[13] S. Agrawal, D. Seuntjens, P. De Cocker, S. Lackner, S.E. Vlaeminck, Success of mainstream partial nitritation/anammox demands integration of engineering, microbiome and modeling insights, Curr. Opin. Biotechnol. 50 (2018) 214–221.
[14] R.C. Jin, G.F. Yang, J.J. Yu, P. Zheng, The inhibition of the Anammox process: a review, Chem. Eng. J. 197 (2012) 67–79.
[15] B.E. Rittmann, P.L. McCarty, Environmental Biotechnology: Principles and Applications, McGraw-Hill, New York, 2012.
[16] M. Strous, J.A. Fuerst, E.H. Kramer, S. Logemann, G. Muyzer, K.T. van de Pas-Schoonen, R. Webb, J.G. Kuenen, M.S. Jetten, Missing lithotroph identified as new planctomycete, Nature 400 (1999) 446.
[17] E.M. Gilbert, S. Agrawal, S.M. Karst, H. Horn, P.H. Nielsen, S. Lackner, Low temperature partial nitritation/anammox in a moving bed biofilm reactor treating low strength wastewater, Environ. Sci. Technol. 48 (2014) 8784–8792.
[18] G.J. Xu, Y. Zhou, Q. Yang, Z.M.P. Lee, J. Gu, W. Lay, Y.S. Cao, Y. Liu, The challenges of mainstream deammonification process for municipal used water treatment, Appl. Microbiol. Biotechnol. 99 (2015) 2485–2490.
[19] B.J. Ni, M. Ruscalleda, B.F. Smets, Evaluation on the microbial interactions of anaerobic ammonium oxidizers and heterotrophs in Anammox biofilm, Water Res. 46 (2012) 4645–4652.
[20] N.R. Krieg, W. Ludwig, W. Whitman, B.P. Hedlund, B.J. Paster, J.T. Staley, N. Ward, D. Brown, A. Parte, Bergey's Manual of Systematic Bacteriology vol. 4, Springer-Verlag, New York, 2010.
[21] S.V. Khramenkov, M.N. Kozlov, M.V. Kevbrina, A.G. Dorofeev, E.A. Kazakova, V.A. Grachev, B.B. Kuznetsov, D.Y. Polyakov, Y.A. Nikolaev, A novel bacterium carrying out anaerobic ammonium oxidation in a reactor for biological treatment of the filtrate of wastewater fermented sludge, Microbiology+ 82 (2013) 628–636.
[22] M. Oshiki, H. Satoh, S. Okabe, Ecology and physiology of anaerobic ammonium oxidizing bacteria, Environ. Microbiol. 18 (2016) 2784–2796.
[23] W.R.L. van der Star, A.I. Miclea, U.G.J.M. van Dongen, G. Muyzer, C. Picioreanu, M.C.M. van Loosdrecht, The membrane bioreactor: a novel tool to grow anammox bacteria as free cells, Biotechnol. Bioeng. 101 (2008) 286–294.
[24] M. Strous, J. Heijnen, J.G. Kuenen, M. Jetten, The sequencing batch reactor as a powerful tool for the study of slowly growing anaerobic ammonium-oxidizing microorganisms, Appl. Microbiol. Biot. 50 (1998) 589–596.
[25] C. Fux, V. Marchesi, I. Brunner, H. Siegrist, Anaerobic ammonium oxidation of ammonium-rich waste streams in fixed-bed reactors, Water Sci. Technol. 49 (2004) 77–82.
[26] X.L. Hou, S.T. Liu, Z.T. Zhang, Role of extracellular polymeric substance in determining the high aggregation ability of anammox sludge, Water Res. 75 (2015) 51–62.
[27] M. Ali, D.R. Shaw, L. Zhang, M.F. Haroon, Y. Narita, A.-H. Emwas, P.E. Saikaly, S. Okabe, Aggregation ability of three phylogenetically distant anammox bacterial species, Water Res. 143 (2018) 10–18.
[28] D. Kang, D.D. Xu, T. Yu, C.D. Feng, Y.Y. Li, M. Zhang, P. Zheng, Texture of anammox sludge bed: composition feature, visual characterization and formation mechanism, Water Res. 154 (2019) 180–188.
[29] G. Muyzer, E.C. De Waal, A.G. Uitterlinden, Profiling of complex microbial populations by denaturing gradient gel electrophoresis analysis of polymerase chain reaction-amplified genes coding for 16S rRNA, Appl. Environ. Microbiol. 59 (1993) 695–700.
[30] APHA, Standard Methods for the Examination of Water and Wastewater. 23rd ed., Washington, DC: American Public Health Association, 2017.
[31] T. Lotti, R. Kleerebezem, C. Lubello, M. Van Loosdrecht, Physiological and kinetic characterization of a suspended cell anammox culture, Water Res. 60 (2014) 1–14.
[32] Y. Miura, Y. Watanabe, S. Okabe, Significance of Chloroflexi in performance of submerged membrane bioreactors (MBR) treating municipal wastewater, Environ. Sci. Technol. 41 (2007) 7787–7794.
[33] Y.Y. Wang, J. Chen, S. Zhou, X.D. Wang, Y. Chen, X.M. Lin, Y. Yan, X. Ma, M. Wu, H.C. Han, 16S rRNA gene high-throughput sequencing reveals shift in nitrogen conversion related microorganisms in a CANON system in response to salt stress, Chem. Eng. J. 317 (2017) 512–521.
[34] M. Fahrbach, J. Kuever, R. Meinke, P. Kämpfer, J. Hollender, Denitratisoma oestradiolicum gen. nov., sp. nov., a 17β-oestradiol-degrading, denitrifying betaproteobacterium, Int. J. Syst. Evol. Microbiol. 56 (2006) 1547–1552.
[35] S.B. Cao, R. Du, B.K. Li, N.Q. Ren, Y.Z. Peng, High-throughput profiling of microbial community structures in an ANAMMOX-UASB reactor treating high-strength wastewater, Appl. Microbiol. Biotechnol. 100 (2016) 6457–6467.
[36] T.T. Chen, P. Zheng, L.D. Shen, S. Ding, Q. Mahmood, Kinetic characteristics and microbial community of Anammox-EGSB reactor, J. Hazard. Mater. 190 (2011) 28–35.
[37] D. Puyol, J. Carvajal-Arroyo, B. Garcia, R. Sierra-Alvarez, J. Field, Kinetic characterization of Brocadia spp.-dominated anammox cultures, Bioresource, Technol. 139 (2013) 94–100.
[38] C.J. Tang, P. Zheng, S. Ding, H.F. Lu, Enhanced nitrogen removal from ammonium-rich wastewater containing high organic contents by coupling with novel high-rate

ANAMMOX granules addition, Chem. Eng. J. 240 (2014) 454–461.
[39] C.J. Tang, P. Zheng, L.Y. Chai, X.B. Min, Thermodynamic and kinetic investigation of anaerobic bioprocesses on ANAMMOX under high organic conditions, Chem. Eng. J. 230 (2013) 149–157.
[40] T.T. Chen, P. Zheng, C.J. Tang, S. Wang, S. Ding, Performance of ANAMMOX-EGSB reactor, Desalination 278 (2011) 281–287.
[41] Z.M. Zheng, J. Li, J. Ma, J. Du, F. Wang, W. Bian, Y.Z. Zhang, B.H. Zhao, Inhibition factors and Kinetic model for ammonium inhibition on the anammox process of the SNAD biofilm, J. Environ. Sci. 53 (2017) 60–67.
[42] L. Jaroszynski, N. Cicek, R. Sparling, J. Oleszkiewicz, Impact of free ammonia on anammox rates (anoxic ammonium oxidation) in a moving bed biofilm reactor, Chemosphere 88 (2012) 188–195.
[43] B. Kartal, L. van Niftrik, J.T. Keltjens, H.J.O. den Camp, M.S. Jetten, Anammox-growth physiology, cell biology, and metabolism, Adv. Microbiol. Physiol. 60 (2012) 211–262.
[44] B. Kartal, N.M. de Almeida, W.J. Maalcke, H.J. Op den Camp, M.S. Jetten, J.T. Keltjens, How to make a living from anaerobic ammonium oxidation, FEMS Microbiol. Rev. 37 (2013) 428–461.
[45] Y.L. Zhang, H.Y. Ma, R. Chen, Q.G. Niu, Y.Y. Li, Stoichiometric variation and loading capacity of a high-loading anammox attached film expanded bed (AAEEB) reactor, Bioresource. Technol. 253 (2018) 130–140.
[46] D. Kang, Q.J. Lin, D.D. Xu, Q.Y. Hu, Y.Y. Li, A.Q. Ding, M. Zhang, P. Zheng, Color characterization of anammox granular sludge: chromogenic substance, microbial succession and state indication, Sci. Total Environ. 642 (2018) 1320–1327.
[47] A. Dapena-Mora, I. Fernandez, J. Campos, A. Mosquera-Corral, R. Mendez, M. Jetten, Evaluation of activity and inhibition effects on Anammox process by batch tests based on the nitrogen gas production, Enzyme Microbiol. Technol. 40 (2007) 859–865.
[48] T. Lotti, W.R.L. van der Star, R. Kleerebezem, C. Lubello, M.C.M. van Loosdrecht, The effect of nitrite inhibition on the anammox process, Water Res. 46 (2012) 2559–2569.
[49] Z.Z. Zhang, Y.F. Cheng, Y.Y. Liu, Q. Zhang, B.Q. Zhu, R.C. Jin, Deciphering the evolution characteristics of extracellular microbial products from autotrophic and mixotrophic anammox consortia in response to nitrogen loading variations, Environ. Int. 124 (2019) 501–510.
[50] Z.Z. Zhang, Y.F. Cheng, Y.H. Bai, J.J. Xu, Z.J. Shi, Q.Q. Zhang, R.C. Jin, Transient disturbance of engineered ZnO nanoparticles enhances the resistance and resilience of anammox process in wastewater treatment, Sci. Total Environ. 622 (2018) 402–409.
[51] R. Connan, P. Dabert, H. Khalil, G. Bridoux, F. Béline, A. Magri, Batch enrichment of anammox bacteria and study of the underlying microbial community dynamics, Chem. Eng. J. 297 (2016) 217–228.
[52] M. Oshiki, M. Shimokawa, N. Fujii, H. Satoh, S. Okabe, Physiological characteristics of the anaerobic ammonium-oxidizing bacterium '*Candidatus* Brocadia sinica', Microbiology 157 (2011) 1706–1713.
[53] Y. Narita, L. Zhang, Z.I. Kimura, M. Ali, T. Fujii, S. Okabe, Enrichment and physiological characterization of an anaerobic ammonium-oxidizing bacterium '*Candidatus* Brocadia sapporoensis', Syst. Appl. Microbiol. 40 (2017) 448–457.
[54] X. Ma, Y.Y. Wang, S. Zhou, Y. Yan, X.M. Lin, M. Wu, Endogenous metabolism of anaerobic ammonium oxidizing bacteria in response to short-term anaerobic and anoxic starvation stress, Chem. Eng. J. 313 (2017) 1233–1241.