Treatment performance, nitrous oxide production and microbial community under low-ammonium wastewater in a CANON process

Weixing Mi, Jianqiang Zhao, Xiaoqian Ding, Guanghuan Ge and Rixiang Zhao

ABSTRACT

To investigate the characteristics of anaerobic ammonia oxidation for treating low-ammonium wastewater, a continuous-flow completely autotrophic nitrogen removal over nitrite (CANON) biofilm reactor was studied. At a temperature of 32 ± 1 °C and a pH between 7.5 and 8.2, two operational experiments were performed: the first one fixed the hydraulic retention time (HRT) at 10 h and gradually reduced the influent ammonium concentrations from 210 to 50 mg L^{-1}; the second one fixed the influent ammonium concentration at 30 mg L^{-1} and gradually decreased the HRT from 10 to 3 h. The results revealed that the total nitrogen removal efficiency exceeded 80%, with a corresponding total nitrogen removal rate of 0.26 ± 0.01 kg N m^{-3} d^{-1} at the final low ammonium concentration of 30 mg L^{-1}. Small amounts of nitrous oxide (N_2O) up to 0.015 ± 0.004 kg m^{-3} d^{-1} at the ammonium concentration of 210 mg L^{-1} were produced in the CANON process and decreased with the decrease in the influent ammonium loads. High-throughput pyrosequencing analysis indicated that the dominant functional bacteria '*Candidatus* Kuenenia' under high influent ammonium levels were gradually succeeded by *Armatimonadetes_gp5* under low influent ammonium levels.

Key words | CANON, high-throughput pyrosequencing, low-ammonium wastewater, nitrous oxide

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INTRODUCTION

Anaerobic ammonia oxidation (anammox) plays a key role in the removal of biological nitrogen from ammonium wastewater. To remove ammonium-nitrogen, the completely autotrophic nitrogen removal over nitrite (CANON) process, a typical and popular anammox technique, depends on the harmony and equilibrium of the interactions between aerobic ammonia-oxidizing bacteria (AerAOB) and anammox bacteria (AnAOB) in a single-stage reactor under oxygen-limited conditions (Sliekers *et al.* 2002). First, NH_4^+ is oxidized to NO_2^- by the AerAOB, following which the remaining NH_4^+ and the generated NO_2^- are converted to N_2 by the AnAOB (Liang *et al.* 2014). Compared to traditional nitrification and denitrification processes, the CANON process has numerous advantages, including reduced aeration without additional organic carbon, less sludge production, reduced operational and construction costs, and enhanced sustainability and economic benefits (Zhang *et al.* 2012).

At present, CANON technology has been successfully applied in various industrial wastewater treatments, such as sludge digestion, landfill leachate and other high-ammonium-nitrogen, low-carbon wastewaters (Zhang *et al.* 2013a). Nevertheless, studies on the use of CANON technology to treat low-ammonium wastewater are still in the exploration stage, and several systems have been operated using lower ammonium concentrations (≤100 mg L^{-1}), such as domestic wastewater and secondary effluent sewage. One previous report proved that a CANON reactor initiated with an influent ammonium concentration of 400 mg L^{-1} achieved an excellent performance for treating synthetic wastewater containing 100 mg L^{-1} ammonium (Liu *et al.* 2012a). Another study also confirmed that it was feasible to start up a CANON to treat domestic sewage in a membrane bioreactor (MBR) with a low ammonium concentration (Zhang *et al.* 2013a). Furthermore, a previous study also concluded that the nitrogen removal rate

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achieved a value of 0.26 kg N m^{-3} d^{-1} in a gaslift reactor with anammox biomass when the temperature and influent ammonium concentration were 20 °C and 69 mg L^{-1}, respectively (Hendrickx *et al.* 2012). However, very little information regarding the use of the CANON process to treat different types of low-ammonium wastewater is available. Thus, this study is relevant to the application of the CANON process to various low-ammonium wastewaters.

Nitrous oxide (N_2O), as a powerful greenhouse gas in the atmosphere, is capable of seriously damaging the ozone layer, in addition to having a global warming potential of approximately 310 times that of carbon dioxide (IPCC 2007). At present, the consensus holds that AnAOB do not possess the pathway to produce N_2O. Although no decisive conclusion has been reached, the phenomenon associated with the production of a small amount of N_2O when using AnAOB has been studied in some previous investigations (Lotti *et al.* 2014; Harris *et al.* 2015). Small amounts of N_2O (on average, accounting for an influent total nitrogen (TN) load of 2.7‰) are detected in anammox reactors fed with material with high ammonium concentrations (Kampschreur *et al.* 2009; Okabe *et al.* 2011); therefore, the anammox process is certainly not a major pathway of N_2O production (Okabe *et al.* 2011). One study confirmed that the hydroxylamine oxidation pathways contributed most of the N_2O emissions via the coupling of nitritation and anammox processes in partial nitrification reactors that are supplied with high-ammonium synthetic wastewater (Rathnayake *et al.* 2013). In a single-stage autotrophic nitrogen removal reactor fed with an NH_4^+ concentration of 300 mg L^{-1}, the processes of NH_2OH oxidation and nitrifier denitrification are likely responsible for the majority of the total N_2O production in the aerobic area, and the remaining N_2O produced in the AnAOB-dominated anoxic area is likely generated via heterotrophic denitrification (Muhammad *et al.* 2016). Moreover, heterotrophic denitrification may be the major pathway of N_2O production in the anoxic zone in a sequencing batch reactor fed with an NH_4^+ concentration of 816 ± 13 mg L^{-1} (Wang *et al.* 2014). Nevertheless, information on N_2O production remains relatively rare for the CANON process with respect to the treatment of low-ammonium wastewater; thus, N_2O production shall also be investigated for low influent ammonium conditions.

During the past decades, five genera of anammox bacteria, including 13 species, have been identified. Influent NH_4^+-N is always regarded as an important substrate for the CANON process in the treatment of wastewater because it affects the living environments of AerAOB and AnAOB, thereby giving rise to changes in the community structure (Liang *et al.* 2014). In a CANON biofilter, the microbial characteristics have been found to change with decreasing ammonium concentrations (Liu *et al.* 2012a). Several previous studies have indicated that *Nitrosomonas* (AerAOB) are the dominant bacteria in CANON reactors (Ballinger *et al.* 1998; Zhang *et al.* 2013a). Another study reached the conclusion that '*Candidatus* Kuenenia stuttgartiensis' existed in a CANON reactor fed with a high ammonium concentration (Liang *et al.* 2014). Similarly, '*Candidatus* Kuenenia stuttgartiensis' was also identified as the dominant functional bacteria in the CANON process in association with influent wastewaters with ammonium concentrations of 256–666 mg L^{-1} (Vázquez-Padín *et al.* 2010). Consequently, it is valuable for bacterial engineering work to obtain information on the dominant functional bacteria in a CANON reactor. Nevertheless, for the CANON process, few specific studies have been performed to detect the changes in the dominant functional bacteria with decreasing influent ammonium concentrations. Thus, it is very valuable for us to study the dominant functional bacteria in low-ammonium wastewater.

In this study, the CANON process in a continuous-flow biofilm reactor was conducted to investigate the characteristics of the anammox process in the treatment of low-ammonium wastewater. The treatment performance, nitrous oxide production and the microbial community associated with low-ammonium wastewater were studied. This will provide a valuable reference for future treatment of low-ammonium wastewater.

MATERIALS AND METHODS

Experimental facility

A continuous-flow biofilm reactor was constructed for this experiment. Details of the reactor are shown in Figure 1. It was a rectangular column made of plexiglass (18.3 cm in length, 18.3 cm in width and 37.5 cm in height). The outer wall of this reactor was surrounded by opaque plastic for protection from light exposure. The fillers in the reactor were plastic fibers. The effective working volume of the reactor was 7 L. Because the inlet of the silicone tube was located at the bottom of the reactor, influent was continuously introduced to the bottom of the tank, and discharge was released from the upper outlet. Additionally, a peristaltic pump in the reactor ensured that all substances in the

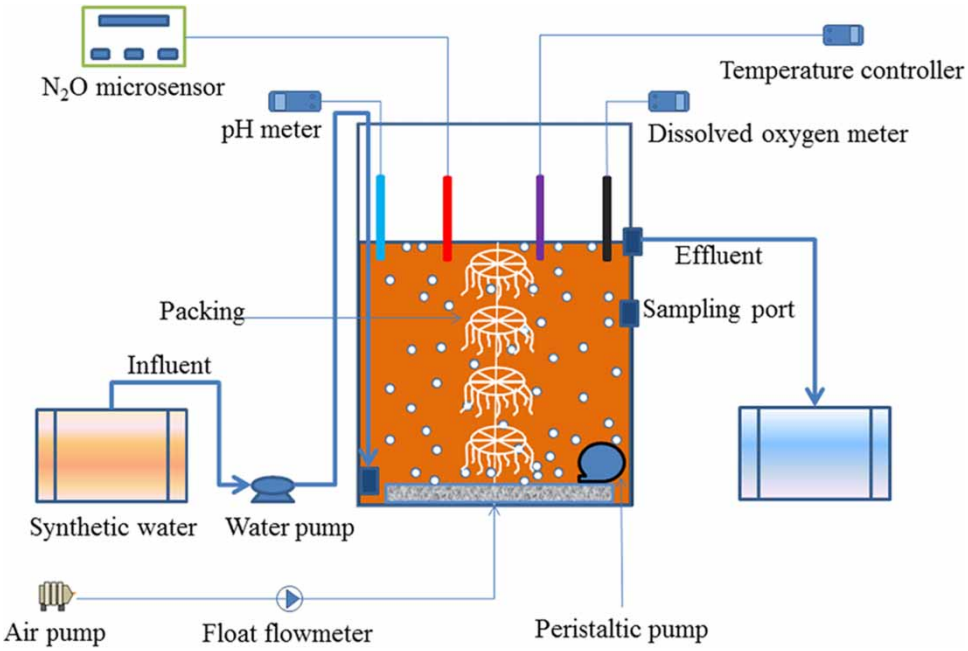

Figure 1 | Experimental facility and process schematic chart.

water were mixed completely while avoiding large disturbances to the biofilm.

Seeding sludge and feeding media

The activated sludge inoculated in this reactor was extracted from the wastewater treatment plant of the Chang'an District in Xi'an, Shaanxi, China. The synthetic wastewater was a mixture of the appropriate amounts of NH_4HCO_3, $NaHCO_3$, anhydrous $CaCl_2$, KH_2PO_4, $MgSO_4{\cdot}7H_2O$ and a trace element solution (Lovley & Phillips 1998) in tap water. Influent NH_4^+-N concentration was gradually decreased from 210 to 136, 100, 70, 50 and 30 mg L^{-1} by reducing the quantity of NH_4HCO_3. The pH was maintained at a relatively stable value by changing the quantity of $NaHCO_3$. The concentrations of other ingredients in the synthetic water were constant: $MgSO_4{\cdot}7H_2O$ 11.07 mg L^{-1}, KH_2PO_4 21.67 mg L^{-1}, anhydrous $CaCl_2$ 11.07 mg L^{-1} and trace element solution 1 mL per 7.5 L. In addition, the tap water contained very little NH_4^+, chemical oxygen demand, NO_2^- and NO_3^-. Additionally, there was no need to drive out the oxygen in the synthetic wastewater.

Experimental background and operation strategy

The original CANON reactor stably treated wastewater with higher influent ammonium-nitrogen concentrations for approximately 5 months and successfully achieved an excellent performance, with a TN removal efficiency and a corresponding total nitrogen removal rate (TNRR) of 80% and 1.02 kg N m^{-3} d^{-1}, respectively. This experiment represents a study based on the original CANON reactor that aims to investigate the performance of the CANON reactor by gradually changing the influent ammonium load.

The temperature was held at 32 ± 1 °C by a thermostatic heater in the water, the pH value varied between 7.5 and 8.2 (mean of 8.0), and the dissolved oxygen (DO) was held at no more than 1.2 mg L^{-1} through adjustments to the aeration rate. Two operational stages were used to change the TN load. The first stage involved a constant hydraulic retention time (HRT) of 10 h and a gradual decrease in the influent ammonium concentration (with values of 210, 136, 100, 70 and 50 mg L^{-1}). The second stage involved a constant influent ammonium concentration of 30 mg L^{-1} and a gradual decrease in the HRT (with values of 7, 5 and 3 h).

Analytical methods

In accordance with the standard methods (Chinese SEPA 2002), concentrations of NH_4^+-N, NO_2^--N and NO_3^--N were measured daily using Nessler's reagent spectrophotometry, the *N*-(1-naphthyl)-ethylenediamine photometric method and UV spectrophotometry, respectively. The DO, temperature and pH were measured with a Hach-HQ30d DO meter, a

TC-058 intelligent temperature measurement device and an S10 portable intelligent pH meter, respectively. TN was calculated based on the sum total of NH_4^+-N, NO_3^--N and NO_2^--N.

The dissolved N_2O was monitored by a N_2O microsensor (Unisense, Denmark). Dissolved N_2O in the CANON reactor would also have been emitted to the air by stirring and air stripping. A previous study has shown that N_2O production can be estimated using a linear equation based on the soluble N_2O (Zhao *et al.* 2016). N_2O production in this continuous-flow reactor was calculated using the following Equation (1):

$$r_g = KC_{N_2O} + \left(\frac{Q}{V}\right)C_{N_2O} \quad (1)$$

where r_g was the total N_2O production rate (mg L^{-1} min^{-1}), and Equation (1) was a combination of two equations: $r_e = KC_{N_2O}$ and $r_c = (Q/V)C_{N_2O}$ in this continuous-flow reactor; r_e was the N_2O emission rate (mg L^{-1} min^{-1}), r_c was the N_2O accumulation rate (mg L^{-1} min^{-1}) C_{N_2O} was the dissolved N_2O concentration (mg L^{-1}), V was the effective volume of the CANON reactor, Q represented the flow rate in this experiment, and K was the N_2O emission coefficient, which was determined through a series of special experiments as shown in Table 1. In a container of the same shape and volume of the reactor, the same volume of tap water was injected, and the same stirring rate and same aeration rate were maintained. Then, N_2O gas with a purity of 99.9% was injected into the reactor. The liquid N_2O concentrations were measured with a N_2O microelectrode, and the release rate of N_2O was obtained. The escape curve at different aeration rates was obtained by changing the aeration rate.

Microbial community analysis

Sludge samples extracted from the CANON reactor were used to identify the microbial community structures when the influent ammonium concentrations were 210 mg L^{-1} (the sludge sample was obtained when the ammonium-nitrogen concentration in influent was decreased from 500 to 210 mg L^{-1} and run for 30 days prior to the present experiment) and 30 mg L^{-1} (the 140th day in this experiment). Microbial communities were identified via the high-throughput 16 S rRNA gene sequencing technique. The DNA of the sludge sample was extracted using the OMEGA DNA kit for soil according to the instructions of the manufacturer. The pyrosequence data were analyzed using the 16 S Ribosomal Database project classifier, and similar sequences were assigned to the same operational taxonomic units (OTUs) based on a 97% similarity.

Table 1 | N_2O emission coefficient (K) under different aeration rates in this experiment

Aeration flow (mL min^{-1})	K (min^{-1})
0	0.006
20	0.006
30	0.009
50	0.012
100	0.015
150	0.018
200	0.025
240	0.036

RESULTS AND DISCUSSION

CANON reactor operation

The influent nitrogen compound content, total nitrogen loading rate (TNLR), and the TNRR are summarized in Figure 2(a). The first stage fixed the HRT at 10 h, and the influent ammonium concentrations were gradually reduced from 210 to 136, 100, 70 and 50 mg L^{-1}; the second stage fixed the influent ammonium concentration at 30 mg L^{-1} and the HRT was gradually reduced from 7 to 5 to 3 h. A clear positive correlation exists between the TNLR and TNRR in Figure 2(a). The effluent nitrogen-compound content, TN concentration, and the removal efficiencies of TN and ammonium-nitrogen are shown in Figure 2(b). The NO_2^--N concentration was low, and the concentrations of ammonium and nitrate fluctuated early on, which led to fluctuations in the removal efficiencies of NH_4^+-N and TN. The CANON reactor took approximately 4 to 7 days to reach a steady state when the TN loads were changed.

The average TNLR and TNRR levels are shown in Table 2. The data in Table 2 were calculated based on the relatively stable and good removal performance stage of the CANON process. Consequently, the optimal TNRR was achieved at 0.26 ± 0.01 kg N m^{-3} d^{-1} when this CANON reactor was fed with an ammonium concentration of 30 mg L^{-1}. In previous studies, TNRR values of 0.26, 0.09 and 0.25 kg N m^{-3} d^{-1} were achieved when the influent ammonium concentrations were 69, 60, and 116.7 mg L^{-1}, respectively (Vázquez-Padín *et al.* 2009; Hendrickx *et al.* 2012; Lotti *et al.* 2014). This study also demonstrated that the CANON process achieved an excellent performance. More precisely, the removal efficiency of the CANON process for each influent ammonium concentration stabilized

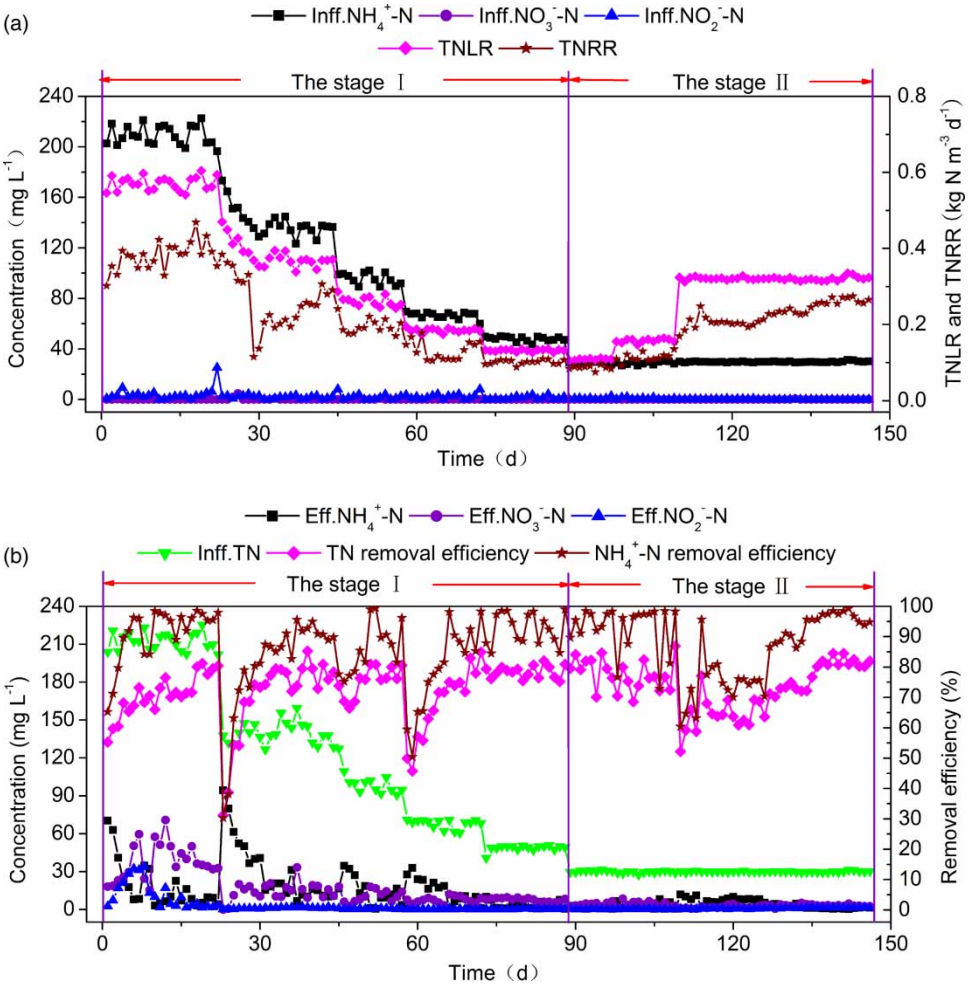

Figure 2 | Effects of the CANON reactor on nitrogen removal: (a) the concentrations of various influent nitrogen compounds and the TNLR and TNRR; (b) the concentrations of various effluent nitrogen compounds and TN, and the TN and NH_4^+-N removal efficiencies.

Table 2 | Summary of the changes in water quality in the CANON process

Stage	Influent NH_4^+ (mg L^{-1})	TNLR (kg N m^{-3} d^{-1})	TNRR (kg N m^{-3} d^{-1})	ΔNH_4^+ (mg L^{-1})	ΔNO_3^- (mg L^{-1})	TN removal efficiency (%)
Stage I	210	0.57 ± 0.02	0.39 ± 0.04	187.97 ± 22.45	33.22 ± 11.27	81.03
	136	0.38 ± 0.03	0.30 ± 0.04	119.87 ± 18.34	16.27 ± 4.94	85.27
	100	0.26 ± 0.01	0.20 ± 0.02	80.94 ± 12.09	10.79 ± 3.16	83.88
	70	0.19 ± 0.01	0.14 ± 0.02	53.16 ± 7.98	8.10 ± 2.62	84.91
	50	0.13 ± 0.01	0.11 ± 0.06	44.50 ± 2.70	6.12 ± 0.92	85.72
Stage II	30	0.11 ± 0.01	0.09 ± 0.01	28.14 ± 1.10	4.16 ± 1.30	84.65
	30	0.16 ± 0.01	0.12 ± 0.01	26.54 ± 2.21	4.33 ± 1.73	86.93
	30	0.32 ± 0.01	0.26 ± 0.01	29.04 ± 0.90	3.08 ± 0.61	84.48

Note: ΔNH_4^+ represents the reduction in the influent ammonium concentration; ΔNO_3^- represents the production of nitrate concentration.

at 90% or more, and the effluent NH_4^+-N was almost zero. The TN removal efficiency also stabilized at approximately 80% under various TN loads, indicating that the CANON process can adapt well to nitrogen load variations and can efficiently remove nitrogen from synthetic wastewater. Several previous studies found maximum TN removal efficiencies of approximately 88.8%, 85%, 89% and 80% (Vázquez-Padín *et al.* 2009; Hendrickx *et al.* 2012; Liu *et al.*

2012a; Zhang *et al.* 2013a, respectively). The putative explanation for this phenomenon can involve a theoretical limit on the maximum TN removal efficiency (Vázquez-Padín *et al.* 2009) in the CANON process and a mass transfer limitation in the biofilm, which requires certain concentrations of ammonium-nitrogen and nitrite-nitrogen.

The production of nitrate (ΔNO_3^-) and the reduction of NH_4^+ (ΔNH_4^+) under each TNLR are presented in Table 2. The molar concentration ratios of ΔNO_3^- to ΔNH_4^+ were 0.16 ± 0.05:1, 0.13 ± 0.06:1, 0.11 ± 0.03:1, 0.14 ± 0.03:1 and 0.13 ± 0.02:1 in stage I and 0.14 ± 0.04:1, 0.15 ± 0.06:1 and 0.11 ± 0.02:1 in stage II. As previously identified, the stoichiometric equation for the CANON process is expressed by the following equation (Third *et al.* 2001):

$$NH_4^+ + 0.85O_2 \rightarrow 0.435N_2 + 0.13NO_3^- + 1.4H^+ + 1.3H_2O \quad (2)$$

According to Equation (2), the theoretical value of ΔNO_3^- to ΔNH_4^+ is 0.13:1. The errors of this test result were +23% and −15% of the theoretical value because of the fluctuation of DO. Moreover, a previous study also reported that the actual ratio of $\Delta NO_3^-/\Delta NH_4^+$ was less than the theoretical ratio obtained in the CANON process as a consequence of the accumulation of nitrite in the CANON reactor (Zhang *et al.* 2013b).

Effect of DO on nitrogen removal in CANON reactor

The DO concentration is an important factor affecting the anammox reaction. To achieve the goal of retaining only ammonia-oxidizing bacteria (AOB) and gradually eliminating nitrite-oxidizing bacteria (NOB) and to finally obtain stable partial nitrification in the CANON reactor, a low DO level is required, as the affinity of AOB for DO exceeds that of NOB (Pathak *et al.* 2007). However, high DO concentrations could improve the efficiency of the partial nitrification process despite increasing the disadvantage of converting NO_2^--N to NO_3^--N, which is harmful to AnAOB activity. Therefore, an optimum DO level should be maintained to balance the interactions between AerAOB and AnAOB. For the different influent TNLRs, different DO levels were used. Based on the measurements of DO concentrations and TN removal efficiencies throughout this experiment, the various DO concentrations and corresponding TN removal efficiencies are shown in Figure 3.

The CANON reactor always had an optimum DO for nitrogen removal in both stage I (constant HRT and decreasing TNLR through decreasing the influent NH_4^+-N concentration) and stage II (constant influent NH_4^+-N concentration and decreasing the HRT to increase the TNLR). The TN removal efficiency was more than 80%, and the NH_4^+ removal efficiency was more than 90%. The optimum DO concentrations corresponding to Figure 3(a)–3(h) were 0.8, 0.75, 0.5, 0.35, 0.28, 0.25, 0.36 and 0.63 mg L^{-1}, respectively. In contrast, a previous study found that the optimum DO concentration for nitrogen removal via an activated sludge process in a CANON reactor supplied with an ammonium-nitrogen concentration of 116.7 mg L^{-1} was 0.5 mg L^{-1} (Vázquez-Padín *et al.* 2009). Another study achieved a TNRR of 0.97 kg m^{-3} d^{-1} by maintaining a DO concentration of 0.15 mg L^{-1} during the treatment of domestic sewage in an MBR-CANON system (Zhang *et al.* 2013a). Additionally, another study indicated that the maximum TNRR values in a CANON biofilter were achieved when the influent ammonium-nitrogen concentrations of 100 and 200 mg L^{-1} had corresponding DO concentrations of 5.06 ± 0.86 and 6.72 ± 0.83 mg L^{-1}, respectively (Liang *et al.* 2014). Differences in experimental parameters (such as the HRT, pH and temperature) and different treatment processes (such as using biofilm or an activated sludge process) between this study and previous studies could account for the large differences in the experimental results. However, the results demonstrate that there is always an optimum DO concentration corresponding to the different influent nitrogen loading rates which results in the maximum TNRR. Moreover, the optimum DO concentration decreases with decreasing TNLR. In addition, because the TNLR was changed by reducing the influent ammonium concentration in stage I and reducing the HRT in stage II, the optimum DO concentration likely decreased with the decrease in the influent ammonium-nitrogen concentration and increased with the reduction in the HRT.

However, the TNRR decreases when an improper aeration rate causes the DO concentration to exceed the optimum value in the CANON reactor. This process is due to the competition between NOB and AnAOB for NO_2^- and to the excessive conversion of NO_2^- to NO_3^-. Eventually, excessive DO concentrations can lead to a decrease in the nitrogen removal efficiency in the CANON process.

N_2O production

Because the N_2O emission coefficient fluctuated under various aeration rates, the amount of N_2O production was calculated using Equation (1) as presented in the 'Analytical methods' section. The dissolved N_2O concentration, N_2O

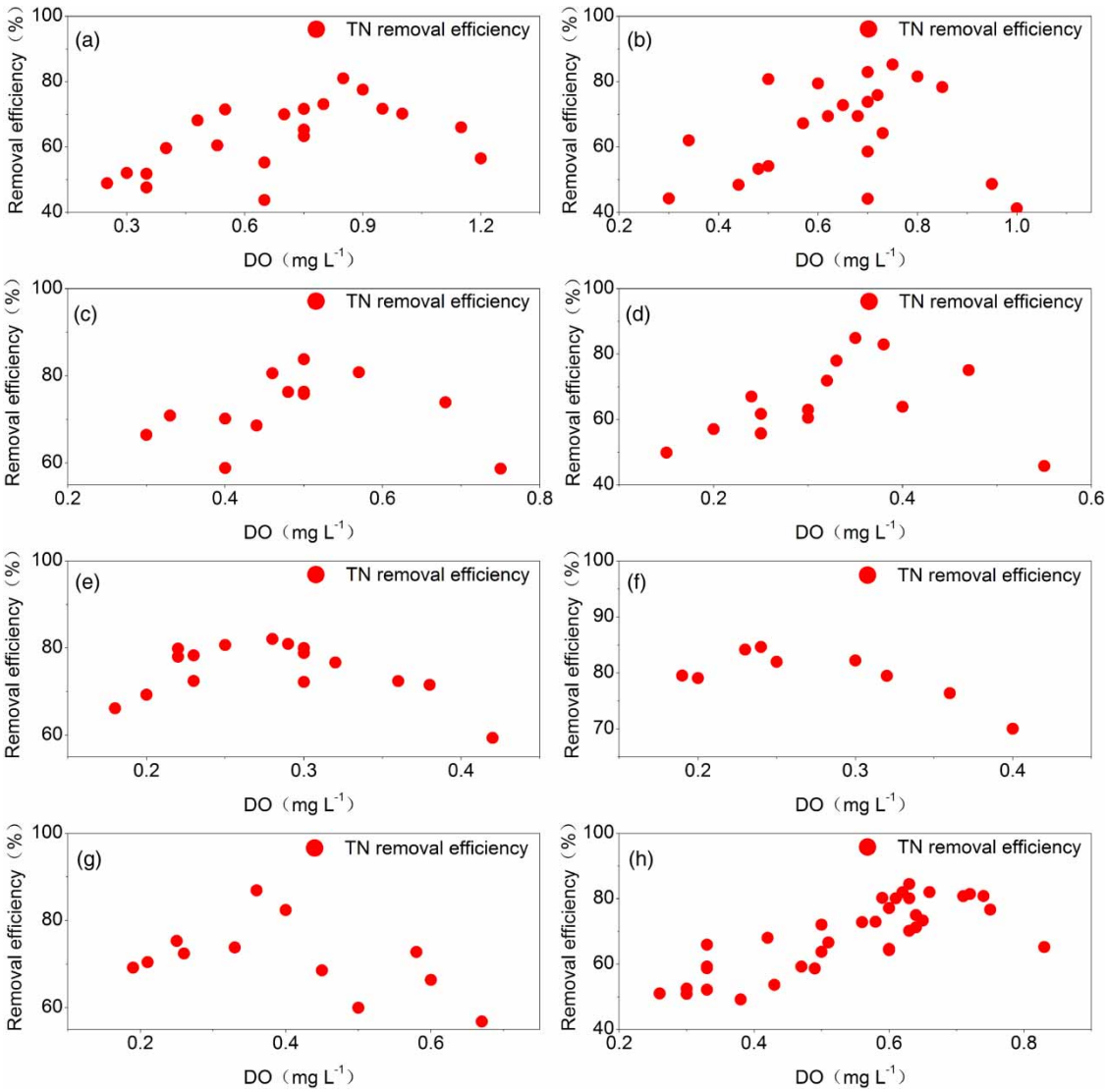

Figure 3 | Effects of DO on TN removal efficiency ((a), (b), (c), (d) and (e) represent the TNLR values of 0.57 ± 0.02, 0.38 ± 0.03, 0.26 ± 0.01, 0.19 ± 0.01 and 0.13 ± 0.01 kg N m^{-3} d^{-1}, respectively, in stage I at an HRT of 10 h; (f), (g) and (h) represent the TNLR values of 0.11 ± 0.01, 0.16 ± 0.01 and 0.32 ± 0.01 kg N m^{-3} d^{-1}, respectively, in stage II at a TN concentration of 30 mg L^{-1}).

emission rate, N_2O production and the correlation between N_2O production and TNLR are depicted in Figure 4.

The correlation between the N_2O production and TNLR is shown in Figure 4(a). Under the conditions of *a*, *b*, *c*, *d* and *e*, the influent TNLR was reduced by decreasing the influent NH_4^+-N concentration, while the proportion of N_2O production accounting for the influent TNLR (2.60 ± 0.52 to 4.49 ± 0.17%) rose. In contrast, the proportion of N_2O production accounting for the influent TNLR (6.00 ± 0.10 to 3.85 ± 0.86%) decreased gradually when the ammonium-nitrogen concentration in influent was fixed at 30 mg L^{-1} under conditions of *f*, *g* and *h*, and the TNLR gradually increased with increasing influent flow. One explanation for these phenomena could be that the change in N_2O production was much smaller than that in the TNLR. One previous study revealed that the proportion of N_2O production accounting for the influent TNLR was 3.0–6.4% in a single-stage nitritation-anammox reactor fed with a TNLR of 0.58–2.7 kg N m^{-3} d^{-1} (De Clippeleir *et al.* 2013). Moreover, according to the calculation, the ratio of N_2O production to the NH_4^+-N removed was 3.10 ± 0.87 to 7.50 ± 0.30% when this reactor was fed with a TNLR of 0.57 ± 0.02 to 0.11 ± 0.01 kg N m^{-3} d^{-1}. In addition, the dissolved N_2O concentration, N_2O emission rate and N_2O

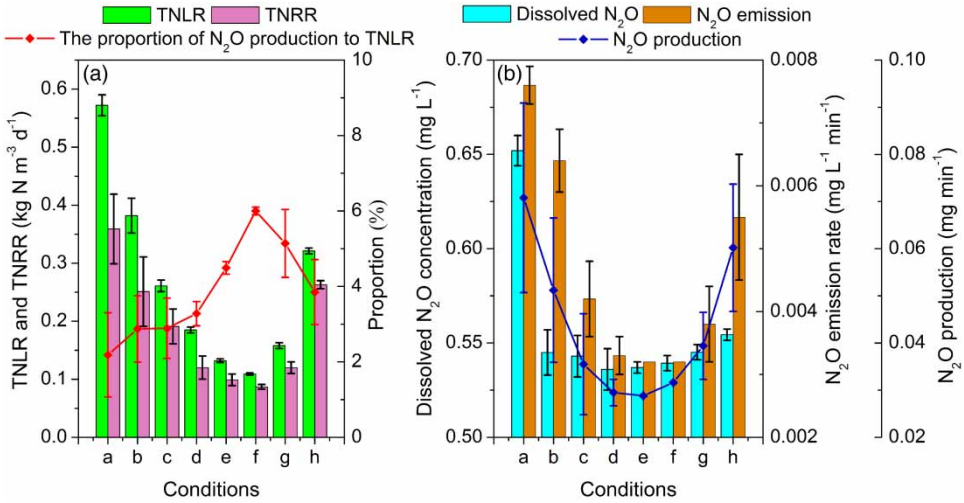

Figure 4 | N_2O production: (a) the correlation between N_2O production and TNLR (*a*, *b*, *c*, *d*, *e*, *f*, *g* and *h* represent different conditions: the TN concentrations of 210 (HRT = 10 h), 136 (HRT = 10 h), 100 (HRT = 10 h), 70 (HRT = 10 h), 50 (HRT = 10 h), 30 (HRT = 7 h), 30 (HRT = 5 h) and 30 mg L^{-1} (HRT = 3 h), respectively); (b) dissolved N_2O concentration, N_2O production and N_2O emission rate.

production appear to have the same tendencies in Figure 4(b). Under the conditions of *a*, *b*, *c*, *d*, *e*, *f*, *g* and *h* in Figure 4(b), when the influent ammonium load was high, the dissolved N_2O concentration and N_2O production were high, and the N_2O emission rate was also high due to the greater aeration rate required. Based on Figure 4(a) and 4(b), the amount of N_2O production was 28.8 ± 1.0 to 70.8 ± 20.0 μg min^{-1} and gradually decreased with the decrease in influent ammonium loads.

Generally, this process would produce a small amount of N_2O, up to 0.015 ± 0.004 kg m^{-3} d^{-1}, at the ammonium concentration of 210 mg L^{-1} in the CANON reactor, which has a minimally negative impact on the environment.

Microbial community structure in the CANON reactor

High-throughput 16S rRNA gene sequencing technology was used to identify the microbial communities at influent ammonium concentrations of 210 and 30 mg L^{-1} to investigate the changes in the microbial community in the CANON reactor. The results provided comprehensive and deeper insight into the microbial community compositions in the CANON reactor and could be very valuable for microbial engineering efforts. Because sequencing analysis suggested complex microbial diversity in the biofilm, the main bacteria at the level of phylum and genus are shown in Figure 5.

As shown in Figure 5(a), the phylum *Planctomycetes* was the most dominant phylum in the biofilm, accounting for 24.37%, when the influent ammonium concentration

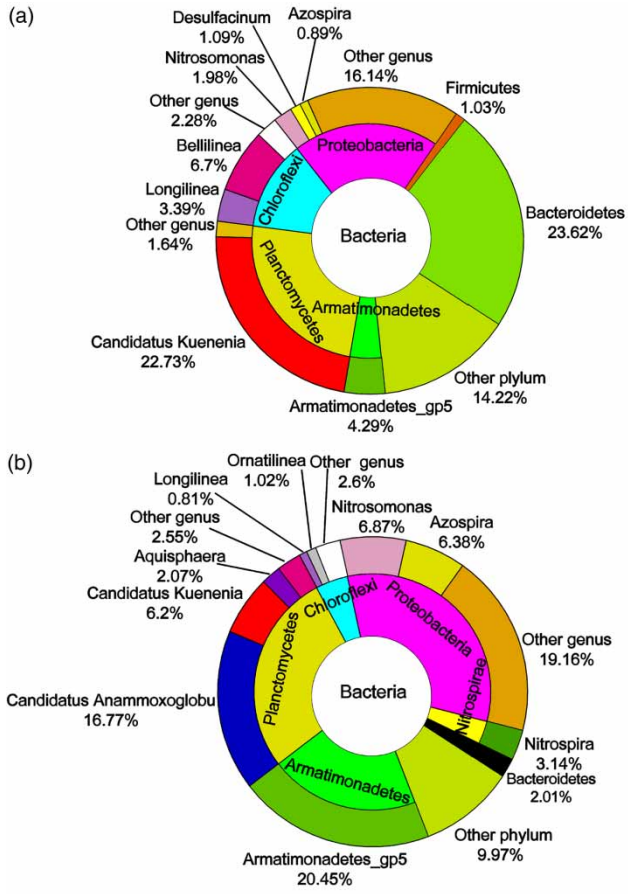

Figure 5 | Pie charts showing the microbial community composition at the level of phylum and genus: (a) the microbial community associated with high influent ammonium concentrations; (b) the microbial community associated with low ammonium concentrations.

was 210 mg L^{-1}. As shown in Figure 5(b), the phylum *Proteobacteria* was the most dominant phylum in this CANON reactor, accounting for 32.41%, when the influent ammonium concentration was 30 mg L^{-1}. One study has revealed that one of the most dominant AerAOB in the CANON process was *Proteobacteria* (Liu *et al.* 2012b); thus, the large proportion of *Proteobacteria* present in this CANON reactor contributes to the partial nitrification process and the nitrite accumulation. The other dominant phyla were *Proteobacteria*, *Armatimonadetes*, *Bacteroidetes*, *Chloroflexi* and *Firmicutes* when the influent ammonium concentration was 210 mg L^{-1}, as shown in Figure 5(a), and *Planctomycetes*, *Armatimonadetes*, *Bacteroidetes*, *Chloroflexi* and *Nitrospirae* when the influent ammonium concentration was 30 mg L^{-1}, as shown in Figure 5(b). The proportions of *Proteobacteria* and *Armatimonadetes* in Figure 5(b) are much larger than those in Figure 5(a), which means *Proteobacteria* and *Armatimonadetes* readily adapted to the low-ammonium and oxygen-limited conditions and consequently promoted the anammox process in the treatment of low-ammonium wastewater in this CANON reactor.

As shown in Figure 5, the genus *Nitrosomonas* was not only the most important AerAOB genus under high NH_4^+-N concentrations, but also the most important AerAOB genus under low NH_4^+-N concentrations in this study. The AerAOB *Nitrosomonas* was previously identified as the dominant functional bacteria in a number of anammox reactors used for nitrogen removal (Ballinger *et al.* 1998; Zhang *et al.* 2013a). The proportion of *Nitrosomonas* (6.87%) in Figure 5(b) is much greater than that in Figure 5(a) (1.98%), which means *Nitrosomonas* played a more important role in low-ammonium environments than in high-ammonium environments.

The AnAOB genus '*Candidatus* Kuenenia' (22.73%) was the most dominant functional bacteria in the high-ammonium environment. A previous study reported that '*Candidatus* Kuenenia' was the main population in an anammox reactor fed with NH_4^+-N concentrations of 256–666 mg L^{-1} (Vázquez-Padín *et al.* 2010). Nevertheless, as the influent NH_4^+-N concentrations changed from high to low, the microbial community gradually altered. Ultimately, the AnAOB genus '*Candidatus* Anammoxoglobus' (16.77%) became the dominant functional bacteria under low ammonium concentrations. Additionally, this study showed that '*Candidatus* Anammoxoglobus' had the ability to adapt to the low ammonium concentration of 30 mg L^{-1} and can outcompete other AnAOB during nitrogen removal.

In addition, the genus *Armatimonadetes_gp5* experienced a large change and increased from 4.29% under the high ammonium concentration to 20.45% under the low ammonium concentration. Furthermore, the genus *Armatimonadetes_gp5* became the most dominant functional bacteria at the ammonium concentration of 30 mg L^{-1} and accounted for the largest proportion. Therefore, these bacteria are inferred to be a new type of AnAOB that promotes the anammox process during the treatment of low ammonium concentrations in the CANON reactor.

Based on Figure 5(a) and 5(b), the NOB *Nitrospira* (3.14%) appeared under the low ammonium concentration but not under the high ammonium concentration, which could be due to the small amount of free ammonia and inhibition mitigation under the low-ammonium conditions (Liu *et al.* 2012a).

CONCLUSIONS

- In the CANON reactor, the influent ammonium concentrations were gradually reduced from 210 to 30 mg L^{-1}. The TN removal efficiency reached 84.48% in the final stages, corresponding to a TNRR of 0.26 ± 0.01 kg N m^{-3} d^{-1}.
- The optimal DO concentrations decreased with decreasing influent ammonium loads and ranged from 0.25 to 0.8 mg L^{-1}, corresponding to TNRR values of 0.09 to 0.47 kg N m^{-3} d^{-1}, respectively.
- The small amount of N_2O produced by the CANON process accounted for 2.60 ± 0.52 to 6.00 ± 0.10% of the influent TNLR when this reactor was fed with a TNLR of 0.57 ± 0.02 to 0.11 ± 0.01 kg N m^{-3} d^{-1}. In addition, the ratio of N_2O production to the NH_4^+-N removed was 3.10 ± 0.87 to 7.50 ± 0.30% when this reactor was fed with a TNLR of 0.57 ± 0.02 to 0.11 ± 0.01 kg N m^{-3} d^{-1}. The production of N_2O reached 0.015 ± 0.004 kg m^{-3} d^{-1} at the ammonium concentration of 210 mg L^{-1} and decreased with decreasing influent ammonium loads.
- The dominant functional bacteria '*Candidatus* Kuenenia' under high influent ammonium conditions was gradually succeeded by *Armatimonadetes_gp5* under low influent ammonium conditions.

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AUTHOR DISCLOSURE STATEMENT

No competing financial interests exist.

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