Nitrogen removal and microbial communities of a completely autotrophic nitrogen removal over nitrite (CANON) sequencing batch biofilm reactor (SBBR) at different inorganic carbon (IC) concentrations

Caimeng Wang, Lirong Lei, Fangrui Cai and Youming Li

ABSTRACT

In this study, the completely autotrophic nitrogen removal over nitrite (CANON) process was initiated in a sequencing batch biofilm reactor (SBBR). Then the reactor was operated under different IC/N ratios. The total inorganic nitrogen removal efficiency (TINRE) at IC/N ratios of 0.75, 1.0, 1.25, 1.5 and 2.0 were 37.0 ± 11.0%, 58.9 ± 10.2%, 73.9 ± 3.2%, 73.6 ± 1.8% and 72.6 ± 2.0%, respectively. The suitable range of IC/N ratio in this research is 1.25–2.0. The poor nitrogen removal performance at IC/N ratio of 0.75 was due to the lack of growth substrate for AnAOB and low pH simultaneously; at IC/N ratio of 1.0 this was because the substrate concentration was insufficient for fully recovering the AnAOB activities. Microbial analysis indicated that *Nitrosomonas*, *Nitrospira* and *Candidatus* Brocadia were the main ammonium oxidation bacteria (AOB), nitrite oxidation bacteria (NOB) and anammox bacteria (AnAOB), respectively. In addition, at IC ratios of 1.25 or higher, denitrification was promoted with the rise of IC/N ratio, which might be because the change of IC concentrations caused cell lysis of microorganisms and provided organic matter for denitrification.

Key words | CANON, inorganic carbon, microbial communities, nitrogen removal, SBBR

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HIGHLIGHTS

- TINRE at IC/N ratio of 0.75 and 1.0 were 37.0 ± 11.0%, 58.9 ± 10.2%, respectively.
- The suitable range of IC/N ratio is 1.25–2.0 with TINRE being about 73%.
- TINRE at IC/N ratio of 0.75 was due to low pH and lack of growth substance.
- TINRE at IC/N ratios of 1.0 was due to insufficiency of growth substance.
- Slight promotion of denitrification was detected at IC/N ratios of 1.5 and 2.0.

INTRODUCTION

The discovery of anaerobic ammonium oxidation (anammox) bacteria (AnAOB) (Strous *et al.* 1998) brought significant changes to the nitrogen removal technology. Compared with the traditional nitrification/denitrification processes, anammox, which can save energy for aeration and demands no organic carbon resource, has emerged as a promising alternative for ammonium-rich wastewater treatment. The single stage system using anammox for nitrogen removal is known as completely autotrophic nitrogen removal over nitrite (CANON) process, in which ammonium oxidizing bacteria (AOB) serves to oxidize ammonium to nitrite, providing substrate for AnAOB; meanwhile, nitrite oxidation bacteria (NOB), which can compete with AnAOB for nitrite, should be inhibited (Augusto *et al.* 2018).

Among factors affecting the CANON process, such as dissolved oxygen (DO), pH, temperature, organic resources and salinity (Ma *et al.* 2016), the inorganic carbon (IC) plays an essential role since previous research has verified that IC serves as the growth substrate for both AOB and AnAOB (Ma *et al.* 2015). Moreover, in anammox reactors, IC also serves as the bicarbonate alkalinity which can neutralize the acidity produced by AOB during the nitrification, which is essential for the anammox reaction considering that suitable alkalinity concentration could improve the

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treatment performance of anammox reactors (Shanahan & Semmens 2015).

Previous research has demonstrated that AOB activities could be limited at low IC concentrations, leading to nitrification being adversely affected. Guisasola *et al.* (2007) indicated that AOB was limited at total inorganic carbon concentrations lower than 3 mmol C/L. Tora *et al.* (2010) demonstrated that AOB inhibitions by free ammonium and free nitrous acid could be amplified under IC limitation conditions. Besides, AnAOB activities could also be influenced by IC concentration as they could be enhanced with the rise of IC concentration, and then inhibited when IC concentration was too high (Liao *et al.* 2008; Kimura *et al.* 2011).

In addition, research aiming at functional microorganisms regarding nitrogen removal has provided different insights regarding the relationship between IC and nitrogen removal. For instance, Jiang *et al.* (2015) proposed that *Nitrosomonas europaea* scavenges residual inorganic carbon through enhancing carbon transport and initial carbon synthesis under gaseous IC supply alone and limiting IC supply. Wei *et al.* (2006) detected that *Nitrosomonas europaea* could up-regulate its HCO_3^- CO_2 machinery in response to IC limitation.

However, despite that, IC concentrations could affect nitrogen removal has been verified in different studies (Chen *et al.* 2012; Yue *et al.* 2018), very little research focused on the variations of microorganisms related to autotrophic removal at different IC conditions. It is supposed that the abundances of functional bacteria related to autotrophic nitrogen removal may differ greatly at different IC concentrations.

In this study, not only was the nitrogen removal performance of a CANON sequencing batch biofilm reactor (SBBR) at different IC/N ratios evaluated, but also were the microbial communities of biomass analyzed by the the 16 s rDNA gene high-thoughput sequencing technology.

MATERIALS AND METHODS

Reactor, synthetic wastewater and seed sludge

The reactor, synthetic wastewater and seed sludge were all depicted in a previous paper studying the start-up of an autotrophic nitrogen removal reactor (Cai *et al.* 2020). Briefly, the reactor was a 600 mL (effective volume) SBBR (Figure 1) divided into part A (aeration part) and part B (biofilm part, plastic carriers, 10 mm of diameter and 10 mm high, effective specific surface area of 500 m^2/m^3, specific gravity of 960 kg/m^3, porosity of 95%). In the reactor, most biomass was presented in the form of biofilm in part B and a small amount of biomass was presented in form of flocculent sludge in part A. The effective volumes of part A and part B were around 450 mL and 150 mL, respectively. An operation cycle of the reactor (8 h) was segmented into reaction phase (470 min) and filling/withdrawing phase (10 min). Internal recycle flow rate was 120 mL/min during the reaction phase, which was further divided into aerobic phase (235 min) and anaerobic phase (235 min) by switching the aerator. Especially, no settling time was set and the continuous feeding was designed for strengthening the biomass wash-out so that NOB can be removed from the reactor with the flocculent sludge.

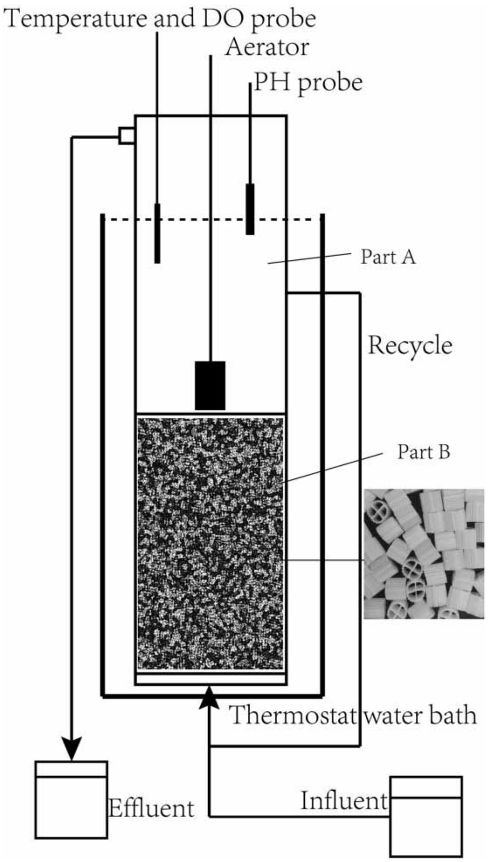

Figure 1 | Scheme of SBBR.

The components of the synthetic wastewater sample are as follow: 0.45–0.49 g/L $(NH_4)_2SO_4$, 0.03 g/L KH_2PO_4, 0.01 g/L $MgSO_4$, 0.03 g/L $CaSO_4{\cdot}2H_2O$, 1.00 g/L $NaHCO_3$, and a 0.40 mL/L trace element solution. The components of trace element solution are as follows: 3.60 g/L

$FeCl_3{\cdot}6H_2O$, 0.36 g/L $MnCl_2{\cdot}4H_2O$, 0.08 g/L $CuSO_4{\cdot}5H_2O$, 0.30 g/L $ZnSO_4{\cdot}7H_2O$, and 0.36 g/L $CoCl_2{\cdot}6H_2O$. No organic matter was added into the synthetic wastewater and ammonium (concentration of NH_4^+-N in influent was around 100 mg/L) was the nitrogen source. $NaHCO_3$ was the sole inorganic carbon resource. The seed sludge contained almost no AnAOB.

Operation strategy

The start-up of CANON process took 100 days. (The IC/N ratio ranged from 1.4 to 1.7). The start-up was further divided into two periods by controlling the hydraulic retention time (HRT) (period 1, 40 days, HRT of 8 h; period 2, 60 days, HRT of 12 h). Then, SBBR was operated for 75 days at different IC concentrations (HRT of 12 h). The stage was further divided into five periods and settings of different periods are shown in Table 1. During the operation of the reactor, DO (about 0.3 mg/L at aerobic phase) and temperature (about 31 °C) remained generally constant (DO and temperature variations are presented in Supplementary Material B).

Analytic methods

The concentrations of NH_4^+-N, NO_2^--N and NO_3^--N were determined according to standard methods (APHA 2005). A portable meter (HQ40d, Hach, Loveland, NJ, USA) with a DO and temperature probe (LDO101, Hach, Loveland, NJ, USA) and a pH probe (PHC101, Hach, Loveland, NJ, USA) was adopted for monitoring DO, temperature and pH.

Calculations

The total inorganic nitrogen concentrations in influent (TIN_{inf}) and effluent (TIN_{eff}) were calculated according to Equations (1) and (2). Ammonium removal efficiency (ARE) and total inorganic nitrogen removal efficiency (TINRE) were calculated according to Equations (3) and (4).

$$TIN_{\mathrm{inf}} = NH_4^+ - N_{\mathrm{inf}} + NO_2^- - N_{\mathrm{inf}} + NO_3^- - N_{\mathrm{inf}} \quad (1)$$

$$TIN_{\mathrm{eff}} = NH_4^+ - N_{\mathrm{eff}} + NO_2^- - N_{\mathrm{eff}} + NO_3^- - N_{\mathrm{eff}} \quad (2)$$

$$ARE = \frac{NH_4^+ - N_{\mathrm{inf}} - NH_4^+ - N_{eff}}{NH_4^+ - N_{\mathrm{inf}}} \times 100\% \quad (3)$$

$$TINRE = \frac{TIN_{\mathrm{inf}} - TIN_{\mathrm{eff}}}{TIN_{\mathrm{inf}}} \times 100\% \quad (4)$$

In the above equations, NH_4^+-N_{inf}, NO_2^--N_{inf}, NO_3^--N_{inf} and TIN_{inf} refer to the concentrations of ammonium, nitrite, nitrate and total inorganic nitrogen in the influent respectively; NH_4^+-N_{eff}, NO_2^--N_{eff}, NO_3^--N_{eff} and TIN_{eff} refer to the concentrations of ammonium, nitrite, nitrate and total inorganic nitrogen in the effluent, respectively.

Microbial analysis

The biomass in part A (samples A, sludge flocs) and part B (samples B, biofilm) of SBBR were sampled at the end of start-up and each period of SBBR operating at different IC/N ratios. Microbial communities of these samples as well as the seed sludge were analyzed by the the 16 s rDNA gene high-thoughput sequencing technology. The analyzing process was also described in our previous research (Cai *et al.* 2020).

RESULTS AND DISCUSSION

Nitrogen removal

Treatment results of SBBR at different IC/N ratios are summarized in Figure 2. During period 2 of the start-up, ARE and TINRE reached 93.9 ± 1.7% and 76.3 ± 2.5%, respectively. (Treatment results during the start-up are shown in Supplementary Material A.)

At IC/N ratio of 0.75, ARE and TINRE were 56.5 ± 9.6% and 37.0 ± 11.0%, respectively, with NO_3^--N_{eff} concentration being 21.1 ± 2.2 mg/L. The results proposed that AnAOB activities were severely inhibited and NOB activities were evidently promoted. In the meantime, according to the research of Strous *et al.* (1998), the ideal molar ratio of nitrite to ammonium for anammox reaction is 1.32, which suggests that around 56.9% of ammonium should be transferred to nitrite in the ideal CANON process. Associating with the ARE, it is proposed that the AOB

Table 1 | Settings of different periods

Item	Period 1	Period 2	Period 3	Period 4	Period 5
Duration (d)	19	14	14	14	14
IC (mg C/L)	75	100	125	150	200
IC/N (L)	0.75	1.0	1.25	1.5	2.0

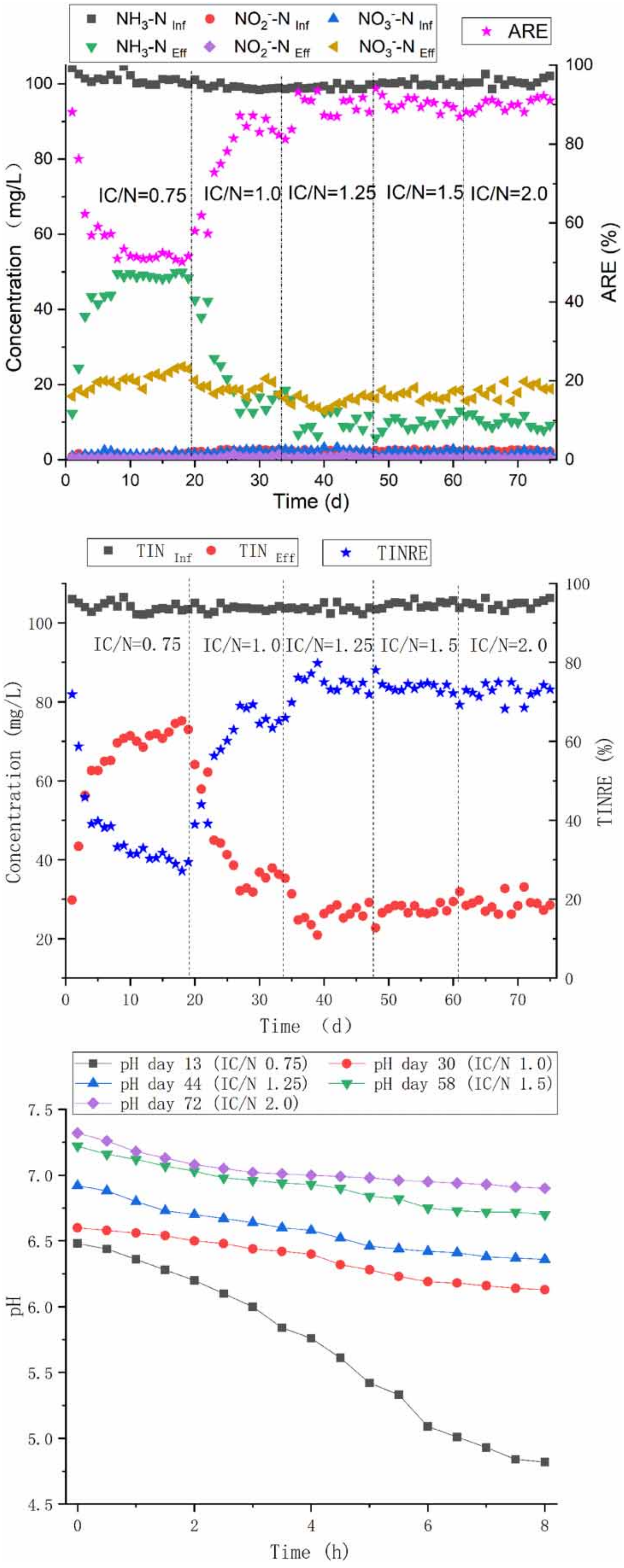

Figure 2 | Treatment results of SBBR at different IC/N ratios (ARE represents the ammonium removal efficiency and TINRE represents the total inorganic nitrogen removal).

activities were barely declined at IC/N ratio of 0.75, which is consistent with the studies of Tora *et al.* (2010) and Guisasola *et al.* (2007) as they both indicated that only when operating at very low IC concentration the real limitation of AOB by IC appeared.

Then, as the IC/N ratio became 1.0, ARE and TINRE were increased to 77.1 ± 10.3% and 58.9 ± 10.2%, respectively. At this ratio, it is proposed that the AnAOB activities were recovered to a certain extent. However, the NO_3^--N_{eff} concentration was still quite high (19.1 ± 1.3 mg/L). Considering that nitrate can be produced by anammox reaction, it is proposed that NOB activities might suffer a decline as well.

Ma *et al.* (2015) indicated that NOB were able to outcompete AnAOB during periods of IC limitation. In this research, it is supposed that AnAOB activities were inhibited due to a dearth of growth substance under IC limitation conditions, which caused nitrite accumulation that availed to the growth of NOB (Li *et al.* 2018). Besides, it has been verified by different scholars that low IC concentrations could cause evidently adverse effect on nitrogen removal. For instance, Zhang *et al.* (2016) speculated that AOB and AnAOB could be significantly suppressed at IC/N ratios lower than 1.0, and Yue *et al.* (2018) detected that ARE and TINRE could be dramatically decreased at IC/N ratios lower than 1.2.

At IC/N ratio of 1.25, ARE and TINRE were 89.0 ± 3.4% and 73.9 ± 3.2%, respectively, which suggested that the CANON process was generally recovered. After that, with the IC/N ratios being increased to 1.5 and 2.0, ARE and TINRE at IC/N ratio of 1.5 were 90.2 ± 1.8% and 73.6 ± 1.8%, respectively, and at IC/N ratio of 2.0 were 90.0 ± 1.4% and 72.6 ± 2.0%, respectively.

Based on these results, it is supposed that the suitable range of IC/N ratio in this study was 1.25–2.0, which was generally consistent with the other studies of Zhang *et al.* (2016) and Yue *et al.* (2018) that speculated stable nitrogen removal of CANON reactors at IC/N ratios at 1.5–2.0 and 1.2–2.5, respectively.

In addition, pH profiles at typical cycles are shown in Figure 2 (pH variations of influent and effluent are shown in Supplementary Material B). The pH value was gradually lowered in a cycle, which could be attributed to the consumption of alkalinity in the CANON process (Tomaszewski *et al.* 2017). At IC/N ratio of 0.75, the pH values ranged 4.7–6.5, which could be ascribed to the lack of bicarbonate alkalinity. A 0.75 IC/N ratio (in gC/gN) translates to 0.88 mol HCO_3^-/mol NH_4^+, which just barely meets the alkalinity requirements of the CANON process according to

Vlaeminck *et al.* (2012). Given that HCO_3^- is the sole carbon source for both AOB and AnAOB and that low pH could inhibit AnAOB activities (Carvajal-Arroyo *et al.* 2014), it is suggested that AnAOB activities were inhibited by the lack of growth substance for AnAOB and low pH simultaneously. However, at IC/N ratio of 1.0, the pH value was generally around 6.5, which has been verified as a suitable value for maintaining AnAOB activities (Jaroszynski *et al.* 2011). With the increases of both IC concentrations and pH values in influent, the inhibition of AnAOB by pH was supposed to have declined since neutral pH would be of benefit to the growth of AnAOB (She *et al.* 2016). Therefore, at IC/N ratio of 1.0, the nitrogen removal inhibition was because the substrate concentration was still insufficient for fully recovering the AnAOB activities. At IC/N ratio of 1.25 or higher, neutral pH was generally maintained, which was consistent with the treatment performance.

Microbial analysis

Microbial richness and diversity

Microbial communities of SBBR sludge samples as well as the seed sludge were analyzed and the relative abundances of samples are shown in Figure 3 at phylum level and genus level, respectively. Samples taken from part A and part B in the reactor at the end of start-up were named as A0 and B0, and at the end of each period of SBBR operating at different IC/N ratios were named as A1, A2, A3, A4, A5 and B1, B2, B3, B4, B5, respectively.

In the high-throughput sequencing analysis, Chao1 and Shannon indices were calculated to reflect the microbial richness and diversity of sludge samples, respectively, and the results are shown in Table 2 (numbers of effective reads and operation taxonomic units (OTUs) are also shown). Estimation of Chao1 index is based on the number of OTUs contained in the sample. Both Chao1 and Shannon indices aim to reflect the alpha diversity of microbial samples.

As is shown in Table 2, the Shannon indices of samples at IC/N ratios of 0.75, 1.0 and 1.25 were evidently lower than Shannon indices of samples A0 and B0, which suggested that the lack of inorganic carbon could lead to the reduction of microbial diversity of biomass. Then, at IC/N ratios of 1.5 and 2.0, both Chao1 indices and Shannon indices were higher than those at IC/N ratios of 1.25 or lower, which suggested the promotion of microbial richness and diversity at high IC concentrations.

Microbial communities

Relative abundances of microbial communities are shown in Figure 3, at phylum and genus level, respectively. At phylum level, the predominant bacteria were *Proteobacteria*, *Bacteroidetes*, *Acidobacteria* and *Chloroflexi* as their proportions amounted to over 80% in each sample. *Proteobacteria*, *Bacteroidetes*, *Acidobacteria* and *Chloroflexi* have all been reported to be related to nitrogen removal (Connan *et al.* 2016). Proportions of *Patescibacteria* (0.8% in B1 and 3.6% in B5) and *Acidobacteria* (4.1% in B1 and

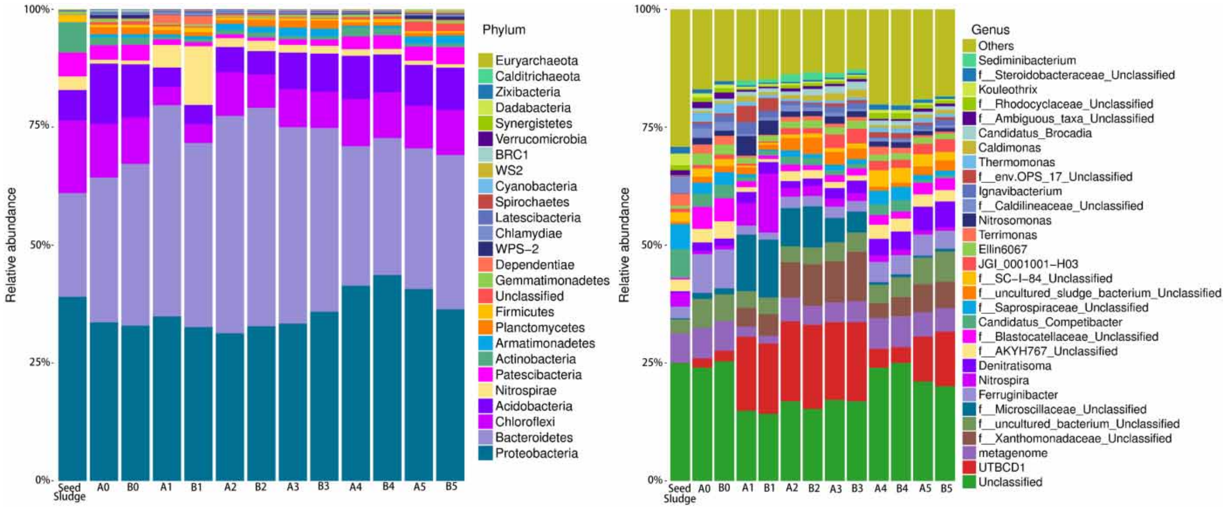

Figure 3 | Relative abundance of microbial communities of sludge samples at phylum and genus levels.

Table 2 | Microbial richness and diversity of sludge samples

Sample	Seed sludge	A0 (start-up)	B0 (start-up)	A1 (IC/N 0.75)	B1 (IC/N 0.75)	A2 (IC/N 1.0)	B2 (IC/N 1.0)	A3 (IC/N 1.25)	B3 (IC/N 1.25)	A4 (IC/N 1.5)	B4 (IC/N 1.5)	A5 (IC/N 2.0)	B5 (IC/N 2.0)
Effective reads	55,071	45,988	50,501	44,623	48,086	49,913	48,705	74,521	49,572	60,859	76,755	63,540	64,504
OTUs	318	318	317	316	317	316	315	315	317	317	318	317	316
Chao1 indices	492	602	592	601	586	610	586	587	586	620	627	616	630
Shannon indices	7.23	7.32	7.20	6.33	6.04	6.48	6.31	6.50	6.39	7.44	7.44	6.91	6.83

9.0% in B5) were found generally to have increased with the rise of IC/N ratio, which suggested that activities of these two phyla could be enhanced by high IC concentrations. Proportions of *Chloroflexi* in samples A1 and B1 were 3.9% and 4.0%, respectively, but in samples A2–A5 and samples B2–B5 ranged 7.9–10.0%. As Sun *et al.* (2019) speculated that *Chloroflexi* interacted with anammox in nitrogen metabolism, the low proportions of *Chloroflexi* in samples A1 and B1 attested that AnAOB activities were severely inhibited at IC/N ratio of 0.75.

At genus level, unclassified bacteria accounted for 15–25% of each sample. UTBCD1, which has been barely reported before, was found abundant in most samples. *Candidatus* Brocadia, a common genera of AnAOB, constituted 1.3% of samples A0 and B0, respectively, but was not detected in sample seed sludge, which proposed the AnAOB enrichment during the initiation of CANON process. In the meantime, *Nitrosomonas* constituted 0.1%, 1.4% and 1.3%, *Nitrospira* constituted 2.9%, 0.9% and 0.9% of samples seed sludge, A0 and B0, respectively, proposing the growth of AOB and suppression of NOB. Furthermore, *Denitratisoma*, a genus of denitrifying bacteria frequently speculated in autotrophic nitrogen removal reactors (Ren *et al.* 2014), accounted for 0.4%, 6.1% and 6.9% of samples seed sludge, A0 and B0, respectively. Variations of these bacteria suggested a successful initiation of CANON process with the enrichment of AOB and AnAOB as well as the inhibition of NOB. In addition, bacteria closely related to nitrogen removal including *Ferruginibacter* and *f__Blastocatellaceae_Unclassified* were detected in samples A0 and B0. *Ferruginibacter* was found playing an important role in denitrification (Liu *et al.* 2018). *Blastocatellaceae* was reported to be related to AnAOB activities in anammox reactors (Pereira *et al.* 2019).

Of samples A1 and B1, *Candidatus* Brocadia held 0.9% and 0.8%, respectively; *Nitrosomonas* held 4.2%, and 3.0%, respectively; *Nitrospira* held 4.8% and 12.5%, respectively. Variations of these bacteria suggested severe inhibition of AnAOB and overgrowth of NOB at IC/N ratio of 0.75. Meanwhile, as the IC/N ratio became 0.75, the relative abundances of *Ferruginibacter* were decreased from over 6.0% to below 2.0%, and that of *f__Blastocatellaceae_Unclassified* were decreased from about 5.0% to around 1.0%, corresponding to the decline of nitrogen removal. In addition, the proportions of *f__Xanthomonadaceae_Unclassified* and *f__Microscillaceae_Unclassified* of samples A1 and B1 were dramatically increased as compared to samples A0 and B0. Both *Xanthomonadaceae* (Zhang *et al.* 2019) and *Microscillaceae* (Khan *et al.* 2019) were related to sludge granulation and organic matter hydrolysis. Therefore, it is supposed that the significant decrease of IC ratio caused the cell lysis of many microorganisms, which provided the organic substance for the proliferation of some heterotrophic bacteria and led to a significant change of bacterial structure. Especially, the proportions of AOB at IC/N ratio of 0.75 were relatively high as compared to the start-up. Associating with the treatment performance, it is proposed that the AOB activities were barely affected by low IC concentrations and the high relative abundances of AOB at samples A1 and B1 might originate from the dramatic decline of relative abundances of AnAOB and other bacteria.

Of samples A2 and B2, *Candidatus* Brocadia constituted 0.8% and 1.4%, respectively; *Nitrosomonas* constituted 1.1% and 1.0%, respectively; *Nitrospira* constituted 1.9% and 2.2%, respectively. Variations of these bacteria were consistent with the treatment performance and attested to the decline of NOB activities as well as the recovery of AnAOB activities at IC/N ratio of 1.0.

In samples A3, B3, A4, B4, A5 and B5, proportions of *Candidatus* Brocadia were 1.6%, 1.7%, 1.0%, 1.0%, 0.7% and 0.8%, respectively; of *Nitrosomonas* were 1.6%, 1.3%, 1.0%, 1.2%, 0.9% and 0.8%, respectively; of *Nitrospira* were 1.7%, 1.6%, 1.4%, 1.2%, 0.9% and 0.7%, respectively; of *Denitratisoma* were 2.3%, 2.7%, 3.5%, 3.8%, 5.0% and 5.5%, respectively. Apparently, as relative abundances of AOB, AnAOB and NOB were gradually decreased simultaneously with the rise of IC/N ratio, variations of *Denitratisoma* experienced stable rises. Associating with the treatment performance, it is proposed that both AOB and AnAOB activities remained generally stable at IC/N ratios over 1.25. Meanwhile, at these IC/N ratios, the proportions of *f__Xanthomonadaceae_Unclassified* were still high (5.6–10.5%), which indicated that the change of IC/N ratio caused the hydrolysis of some bacteria and provided organic substances. Thus, *Denitratisoma* was able to proliferate as a typical genus of heterotrophic denitrifying bacteria. Correspondingly, the relative abundance of *Ferruginibacter* was also increased with the rise of IC/N ratios. Especially, given that denitrifying bacteria are able to compete for nitrite with AnAOB (Jenni *et al.* 2014), the presence of denitrification might cause a slight imbalance between nitrification and anammox reaction and result in the TINRE at at IC/N ratios of 1.25, 1.5 and 2.0 being slightly lower than that of the start-up.

In addition, unlike *f__Xanthomonadaceae_Unclassified*, the proportions of *f__Microscillaceae_Unclassified* as another heterotrophic genus were gradually decreased with the rise of IC/N ratio, which might be related to the pH variations.

Especially, proportions of *Candidatus* Brocadia and *Denitratisoma* in sample A were generally lower than in sample B at the same IC/N ratios, whereas proportions of *Nitrosomonas* and *Nitrospira* in sample A were generally higher than in sample B. These differences are attributed to that biomass in part A and part B was mainly retained in the form of flocculent sludge and biofilm, respectively, and DO concentrations in part A were generally higher than in part B, which enabled AnAOB to be enriched in part B whereas AOB and NOB were inclined to proliferate in part A. In addition, proportions of *Denitratisoma* in samples A were also generally lower than in samples B, indicating that denitrifying bacteria prefer to live in biofilm than in flocculent sludge. However, at IC/N ratio of 0.75, the proportion of *Nitrospira* in part A (4.8%) was much lower than in part B (12.5%), suggesting that considerable NOB proliferation could occur in biofilm under IC limitation condition. Ma *et al.* (2015) also observed that the concentration fractions of *Nitrospira* in the biofilm could increase during IC limitation. Moreover, as the proportions of *Candidatus* Brocadia in samples A0, B0, A1 and B1 were 0.88%, 0.81%, 0.76% and 1.41%, respectively, the recovery of AnAOB in part B was more dramatic than in part A when the IC/N ratio was increased from 0.75 to 1.0. Lotti *et al.* (2015) and Zhang *et al.* (2017) demonstrated that reactor type could impact max specific growth rate of AnAOB. In this research, the biofilm of the SBBR might also enable the AnAOB activities to be rapidly recovered with the rise of IC/N ratio.

CONCLUSION

The TINRE at IC/N ratios of 0.75 and 1.0, 1.25 were 37.0 ± 11.0% and 58.9 ± 10.2%, respectively. The suitable range of IC/N ratio in this research is 1.25–2.0 as TINRE at these ratios were around 73%. The poor nitrogen removal performance at IC/N ratio of 0.75 was due to the lack of growth substrate for AnAOB and low pH; at IC/N ratio of 1.0 was because the substrate concentration was insufficient for fully recovering the AnAOB activities. At IC ratios of 1.25 or higher, the CANON process was recovered. Meanwhile, the denitrification was promoted due to the fact that the change of operational conditions caused cell lysis of microorganisms and provided organic matter for denitrification. *Candidatus* Brocadia, *Nitrosomonas*, and *Nitrospira* were the main AnAOB, AOB and NOB, respectively.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this paper is available online at https://dx.doi.org/10.2166/wst.2020.203.

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