Nitrogen removal via a single-stage PN–Anammox process in a novel combined biofilm reactor

Yue-mei Han, Feng-xia Liu, Xiao-fei Xu, Zhuo Yan and Zhi-jun Liu

ABSTRACT

This study developed a partial nitrification (PN) and anaerobic ammonia oxidation (Anammox) process for treating high-ammonia wastewater using an innovative biofilm system in which ammonia oxidizing bacteria grew on fluidized Kaldnes (K1) carriers and Anammox bacteria grew on fixed acryl resin carriers. The airlift loop biofilm reactor (ALBR) was stably operated for more than 4 months under the following conditions: 35 ± 2 °C, pH 7.5–8.0 and dissolved oxygen (DO) of 0.5–3.5 mg/L. The results showed that the total nitrogen removal efficiency reached a maximum of 75% and the total nitrogen removal loading rate was above 0.4 kg/(d·m^3). DO was the most efficient control parameter in the mixed biofilm system, and values below 1.5 mg/L were observed in the riser zone for the PN reaction, while values below 0.8 mg/L were observed in the downer zone for the Anammox reaction. Scanning electron microscopy and Fluorescence In Situ Hybridization images showed that most of the nitrifying bacteria were distributed on the K1 carriers and most of the Anammox bacteria were distributed within the acryl resin carriers. Therefore, the results indicate that the proposed combined biofilm system is easy to operate and efficient for the treatment of high-ammonia wastewater.

Key words | ALBR, Anammox, cooperative work, nitrogen removal, PN

Yue-mei Han
Feng-xia Liu
Xiao-fei Xu
Zhuo Yan
Zhi-jun Liu (corresponding author)
R&D Institute of Fluid and Powder Engineering,
Dalian University of Technology,
Dalian 116024
China
E-mail: *liuzj@dlut.edu.cn*

Yue-mei Han
College of Mechanical Engineering,
Dalian University,
Dalian 116622
China

INTRODUCTION

The anaerobic ammonium oxidation (Anammox) nitrogen removal process has been recognized as a cost-effective strategy that exhibits low energy consumption and does not require inputs of organic carbon. In recent years, this process has attracted considerable attention from the scientific community (Sobotka *et al.* 2016), and more than 100 Anammox plants have been built worldwide (Yeshi *et al.* 2016). However, the growth rate of Anammox bacteria is very slow, and the doubling time is longer than 1 month (Bertino *et al.* 2011). Therefore, an optimal environment must be developed for Anammox bacteria, such as by reactor/carrier modification, to improve the Anammox growth rate and facilitate practical operations.

Reactor modifications have been implemented to obtain a faster growth rate and improve mass transfer (Liu *et al.* 2008; Gao *et al.* 2012; Wang *et al.* 2016). Although Anammox bacteria have been observed in fluidized bed reactors (Mulder *et al.* 1995), sponges and nonwoven carriers are the optimal carriers and show improved nitrogen removal loadings for Anammox cultures (Fujii *et al.* 2002; Qiao *et al.* 2012). A combination of partial nitrification (PN) and Anammox can be performed in separate reactors (Van Dongen *et al.* 2001) or within a single reactor (Furukawa *et al.* 2008). Compared with the two-stage PN–Anammox process, single-stage processes, such as completely autotrophic nitrogen removal over nitrite (CANON) (Vázquez-Padín *et al.* 2010), oxygen limited autotrophic nitrification denification (OLAND), deammonification, and single-stage nitrogen removal using Anammox and partial nitritation (SNAP), require smaller spaces and can prevent accumulated nitrite inhibition (Bertino *et al.* 2011; Cho *et al.* 2011; Hoang *et al.* 2014).

The airlift loop biofilm reactor (ALBR) is a kind of multiphase reactor based on the conventional bubble tower and has been applied in chemical engineering, bioengineering and waste water treatments. Compared with other bioreactors the ALBR has obvious advantages such as simple structure, continuous operation, homogeneous mixture and less power consumption (Fu *et al.* 2010; Xu *et al.* 2011; Behin 2012). Furthermore, the homogeneous shear stress inside is suitable for organism growth and promotes ALBR's wide application in biotechnology (Wang *et al.* 2003).

doi: 10.2166/wst.2017.572

The objective of this study was to develop a single-stage ALBR in which a simultaneous PN–Anammox process is easily performed and maintained by controlling the dissolved oxygen (DO) concentrations with the goal of enriching ammonia oxidizing bacteria (AOB) and Anammox bacteria in the different zones, i.e. AOB in the high-DO zones and Anammox bacteria in the low-DO zones.

MATERIALS AND METHODS

Reactor and operation design

An ALBR with fluidized K1-type carriers and fixed acryl resin carriers was designed for a single-stage PN–Anammox process (Figure 1). The double-tube structure effectively separated the two functional zones with different DO conditions. The riser zone was aerated and oxygen limited, whereas the downer zone between the two tubes was anaerobic. The geometrical construction parameters have key influence on mass transfer and flow pattern in ALBR. Zhu *et al*. (Zhu *et al.* 2012) think that the ratio of height to diameter can affect the flow situation of liquid and gas distinctly and the low ratio of height to diameter may lead to vortex and backmixing easily, while high ratio may lead to appearance of bubbles in the downer zone, suggesting that the proper ratio of height to diameter may more than 3. Ratio of draft tube diameter to reactor has a large effect on liquid recirculation velocity. Considering the liquid circulation velocity and gas holdup, the diameter ratio of draft tube to reactor should be in the range of 0.6 to 0.8, and 0. 7 is the optimal. The height of bottom clearance can affect local flow structure in bottom zone, and its height should be equal to the width of the downer. The detail geometrical construction is shown in Figure 1(b) and Table 1. The total working volume of the ALBR was approximately 2.5 L. The hydraulic retention time (HRT) of the ALBR was approximately 17–55 hours and the temperature was controlled at 35 ± 2 °C. The reactor was composed of Plexiglas tubes with a wall thickness of 5 mm; detailed structural parameters are shown in Figure 1(b).

The influent was synthetic wastewater in this study and the process was divided into two phases according to the nitrite (NO_2^-–N) concentration: 50–80 mg/L nitrite in phase I (days 1–51) and 0 mg/L nitrite in the influent starting on day 52.

Seed sludge and carriers

The nitrifying seed sludge was collected from a local wastewater treatment plant in Dalian. The anaerobic sludge was acquired from a laboratory-scale Anammox reactor. The nitrifying carriers were a type of biological particle known as K1 (YUDU Co. Ltd, China) (Figure 1(a) in appendix, available with the online version of this paper). These particles provide a high specific surface area of 500 m^2/m^3

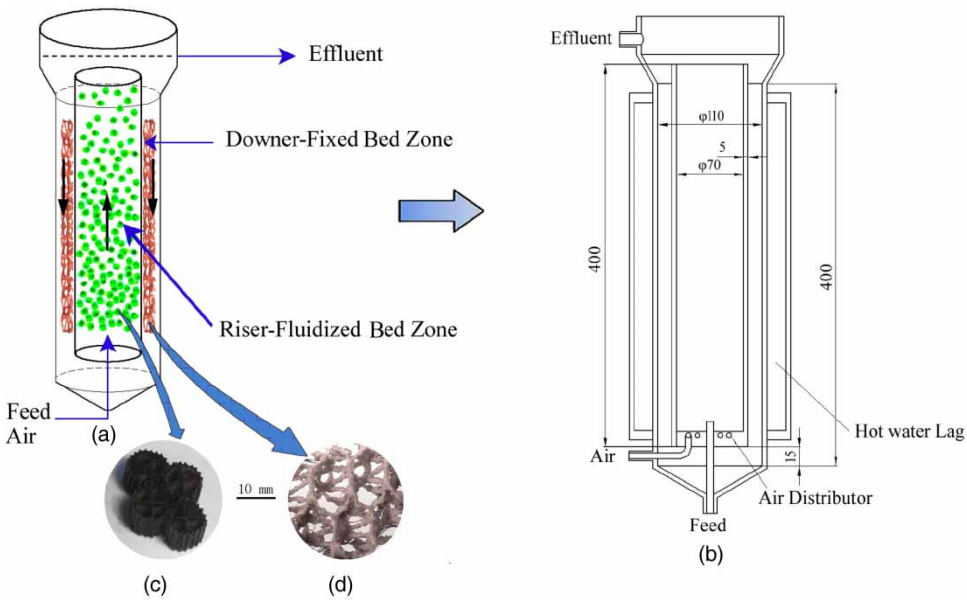

Figure 1 | Schematic and structure of the ALBR: (a) schematic of the hybrid reactor, (b) dimensions and details of the reactor, (c) K1 carrier and (d) acryl resin fibre carrier.

Table 1 | Geometrical construction parameters ratio

Ratio	D_E/D_T	H_T/D_T	A_D/A_R	$H_B/(D_T-D_E/2)$
Proper value	0.7	≤5	0.96	1
Value in study	0.64	4	0.86	1

A_D, cross-sectional area of the downer (m^2); A_R, cross-sectional area of the riser (m^2); D_E, diameter of the draft-tube (m); D_T, diameter of the reactor (m); H_T, height of the reactor (m); H_B, height of the bottom clearance (m).

and a suitable density that is nearly identical to the density of water (Bertino *et al.* 2011). The K1 carriers were placed in a sequencing batch reactor (SBR) for cultivation and biofilm culturing for 40 days. The Anammox carrier was a hydrophilic net-type acryl resin fibre material (BX, NET Co. Ltd, Japan) (Figure 1(b) in appendix) that was pre-filmed in the Anammox reactor for more than 100 days.

Composition of synthetic waste water

The composition of the synthetic wastewater is shown in Table 2 (Gao *et al.* 2012). $NaHCO_3$ was used as the source of inorganic carbon. The pH of the synthetic wastewater was between 7.5 and 8.0. A detailed list of the nitrogen compounds in the influent and the operation strategy is provided in Table 3.

Analytical methods

The concentrations of NH_4^+–N, NO_2^-–N and NO_3^-–N were analyzed by a spectrophotometric method (Cho *et al.* 2011) using an ion chromatograph (UV-1800, Shimadzu, Japan). The DO concentration in the reactor was measured using a portable DO meter (JB-2, Iinesa, China), and the pH was measured using a pH meter (METTLER TOLEDO FE20, USA).

SEM imaging

Scanning electron microscopy (SEM) was used to observe the biofilm on day 120. The samples were first washed three times with distilled water and then frozen at −50 °C for 1 hour. The frozen samples were vacuum dried (pressure less than 30 Pa) for 3 hours in a freeze dryer and then analyzed using a FEI QUANTA 450 SEM (FEI USA, Inc.).

FISH analysis

To provide a more detailed analysis of the AOB and Anammox communities, fluorescence in situ hybridization (FISH) was carried out on the biofilm sample on day 120. SituInconstruct FISH probes NSO190, Nsm156, Nsv443 labelled by red fluorochrome Cy3 (Xu *et al.* 2012) was used to detect AOB bacteria in the samples, and SituInconstruct FISH probe Amx820 (5′-CAAAACCCCTCTAC TTAGT GCCC-3′) labelled with red fluorochrome Texas red-12-dUTP (Invitrogen, Japan) was chosen for Anammox detection. SituInconstruct FISH probes EUB338 labelled with green fluorescein isothiocyanate (FITC)-12-dUTP (Invitrogen, Tokyo) was to mark other bacteria (Mobarry *et al.* 1996). Some K1 from the riser zone and some pieces of acryl resin carrier from the downer zone were picked out to be detected at the end of the operation. Hybridizations of the samples were performed in 20 mM Tris-HCl buffer, pH 7.2 containing 0.9 M NaCl, 0.01% sodium dodecyl sulphate (SDS), 30% formamide, and the labelled probes as described by Amann *et al.* (Amann *et al.* 1996) at 46 °C, and then followed by washing with 20 mM Tris–HCl buffer, pH 7.2 containing 0.112 M NaCl and 0.01% SDS. The pre-treated samples were put under confocal laser scanning microscopy (CLSM) and images were obtained.

RESULTS AND DISCUSSION

Nitrogen removal performance

During the initial period (days 1–51), the influent NO_2^-–N concentration ranged from 30–80 mg/L to ensure high Anammox bacteria activity (Table 2). The nitrogen removal performance is shown in Figure 2. The results showed that the total nitrogen (TN) removal efficiency ranged from 30% to 70% and the ammonium and nitrate oxidation efficiencies were 66.1% and 19.5%, respectively, during this period. In the first 7 days, obvious Anammox reactions were not detected. On day 5, 26.3 mg/L of NO_2^--N and 12.2 mg/L of NO_3^-–N were detected in the effluent, indicating that nitrification was successfully achieved in the reactor. On day 6, 30 mg/L of NO_3^--N was detected in the

Table 2 | Composition of the synthetic wastewater

Component	$(NH_4)_2SO_4$	$NaNO_2$	$NaHCO_3$	$FeSO_4{\cdot}7H_2O$	EDTA	$CaCl_2$	KH_2PO_4
Concentration (mg/L)	50–200	0–80	70–180	18	10	136	27

Table 3 | Concentration of nitrogen compounds in the influent and the operation strategy

T (day)	NH_4^+-N (mg/L)	NO_2^--N (mg/L)	HRT (h)
0–6	50	0	55
7–15	50	30	55
16–30	100	30	55
31–40	200	50	55
41–51	200	80	55
52–68	100	0	33
69–92	100	0	17
93–108	150	0	17
109–120	200	0	17

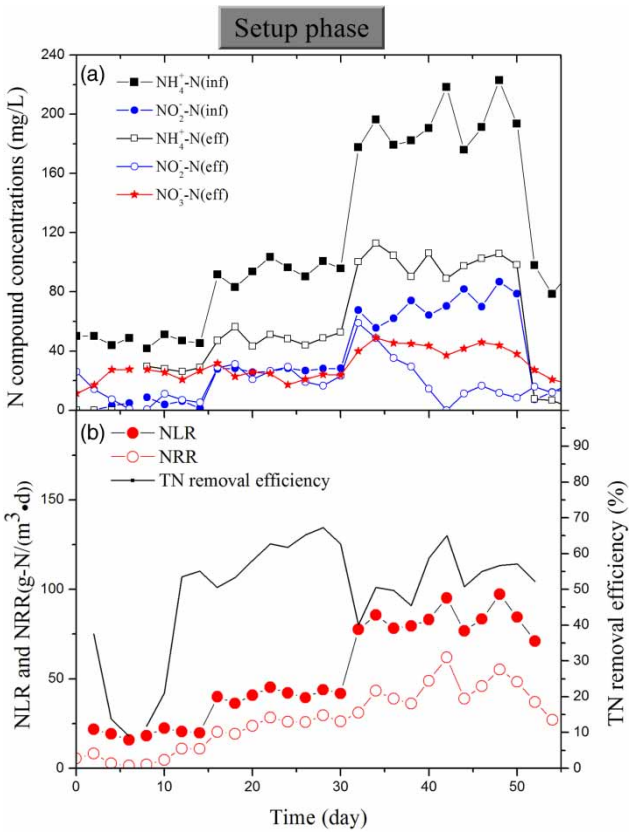

Figure 2 | Nitrogen removal performance in the setup phase.

effluent (Figure 2(a)), which was attributed to the growth of nitrite oxidizing bacteria (NOB) because Anammox bacteria were inhibited under the high aeration conditions. The Anammox bacteria were therefore inhibited by high DO and substrate competition from the AOB and NOB in the absence of nitrite. Over the next 7 days, 30 mg/L of NO_2^--N was added to enhance the Anammox activity. Accordingly, the TN removal efficiency sharply increased, which indicated that the activity of the Anammox bacteria successfully recovered. Approximately 50% of the ammonium and 60% of the nitrite were removed, whereas the NO_3^--N concentration in the effluent decreased slightly (average of 21.2 mg-N/L). This result indicated that the NOB remained activated in the ALBR. In addition, the total inorganic nitrogen removal efficiency was 55%.

Over days 16–30, the concentration of the influent NH_4^+–N increased and the NO_2^--N concentration remained unchanged (Table 3). The TN removal efficiency was 56%, whereas more than 50% of the NH_4^+-N and 10% of the NO_2^--N were removed. The effluent NO_3^--N concentration was only 22 mg/L, which indicated that less nitrogen was oxidized to nitrate and the NOB activity was weakened during this time period. Concurrently, Anammox carriers showed an increase in red colour and the surface of the Anammox carriers was covered in bubbles.

Over the next 3 weeks (days 31–51), the nitrogen volumetric loading rate (NLR) increased to 0.3 g-N/ (m^3·d) and the effluent nitrogen concentrations gradually decreased as shown in Figure 2. Sixty per cent of NH_4^+-N and 80% of NO_2^--N were removed and only 20 mg/L of NO_3^--N was produced. The amount of NO_3^--N produced only accounted for 10% of the total influent nitrogen, which demonstrates that Anammox bacteria became the dominant source of nitrogen removal in the ALBR.

From day 52 to 120, the single-stage PN–Anammox reaction was successfully achieved with NH_4^+-N (100–200 mg/L) as the sole nitrogen source, and the N compound concentrations and nitrogen removal performance is shown in Figure 3. Nitrogen loading rate largely increased and the HRT decreased (17 hours) (Figure 4). Over days 33–55, the HRT was shortened from 55 hours to 33 hours (Figure 4) and the influent NLR increased from 70 g-N/(m^3·d) to 101 g-N/(m^3·d). The nitrogen removal loading rate (NRR) increased from 37.34 g-N/(m^3·d) to 52.85 g-N/(m^3·d) at a TN removal efficiency of 58%, which demonstrates that high AOB and Anammox bacteria activity was achieved in the ALBR. On day 69, although the influent NH_4^+-N was decreased to 100 mg/L, the NLR reached values as high as 194 g-N/(m^3·d) because of the HRT being reduced to 17 hours. On day 82, the NRR first decreased (85 g-N/(m^3·d)) and then increased to a maximum of 145 g-N/(m^3·d). This finding indicates that the Anammox and AOB in the ALBR reached high levels of ammonia nitrogen removal and a stable phase of combined completely autotrophic denitrification.

Over days 80–120, the average NLR increased to 403 g-N/(m^3·d) and the HRT was 17 hours. Figure 4 shows that only 1.3 mg/L of NH_4^+-N, 7.37 mg/L of NO_2^--N and

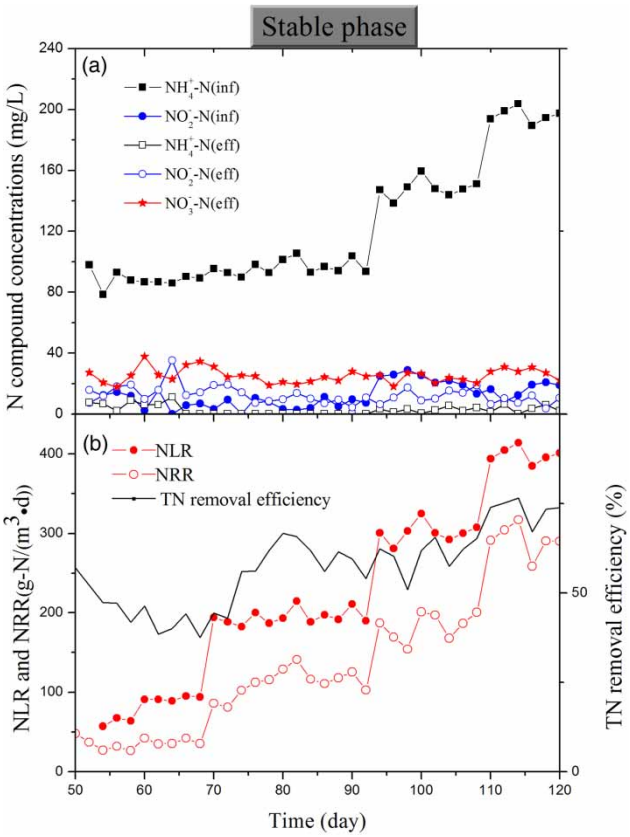

Figure 3 | Nitrogen removal performance in the stable phase.

27.89 mg/L of NO_3^--N were detected in the effluent on day 114. In addition, the amount of NO_3^--N produced (27.9 mg/L) accounted for 14% of the NH_4^+-N that was consumed (203.7 mg/L), which was slightly higher than the standard proportion of the Anammox reaction (Bertino *et al.* 2011). These results demonstrated that the Anammox reaction produced all the observed nitrate and the NOB were inhibited in the ALBR.

On day 120, the K1 carriers with the attached AOB sludge and the acryl resin fibre carriers with the attached Anammox sludge were removed from the reactor, dried for 2 hours at 105 °C in an electrothermal blowing dry box, and subsequently weighed. The total weight of the AOB sludge on 60 K1 carriers was 16.4 g, whereas the total weight of the Anammox sludge was 7.1 g. The average biomass NRR was 2.16 g-N/(g-mixed liquor volatile suspended solids (MLVSS) (d·m^3)) in this process, which was slightly higher than that in the other processes (Lieu *et al.* 2006; Yongtao *et al.* 2015).

NLR and TN removal efficiency

The profiles of the NLR, NRR and TN removal efficiency throughout the setup phase and stable phase are shown in Figures 2(b) and 3(b). Over days 1–25, the average removal efficiency was only 30%, which was a result of the powerful aeration. After modifying the aeration system, the TN removal efficiency increased to more than 60% on day 42. In the following 2 weeks, the NLR reached 102.3 g/(m^3·d) and the NRR reached 61.3 g-N/(m^3·d) (Figure 2(b)) on day 51. From day 52 onward, the NLR increased as the HRT reduced. When the NLR reached 403.2 g/(m^3·d) on day

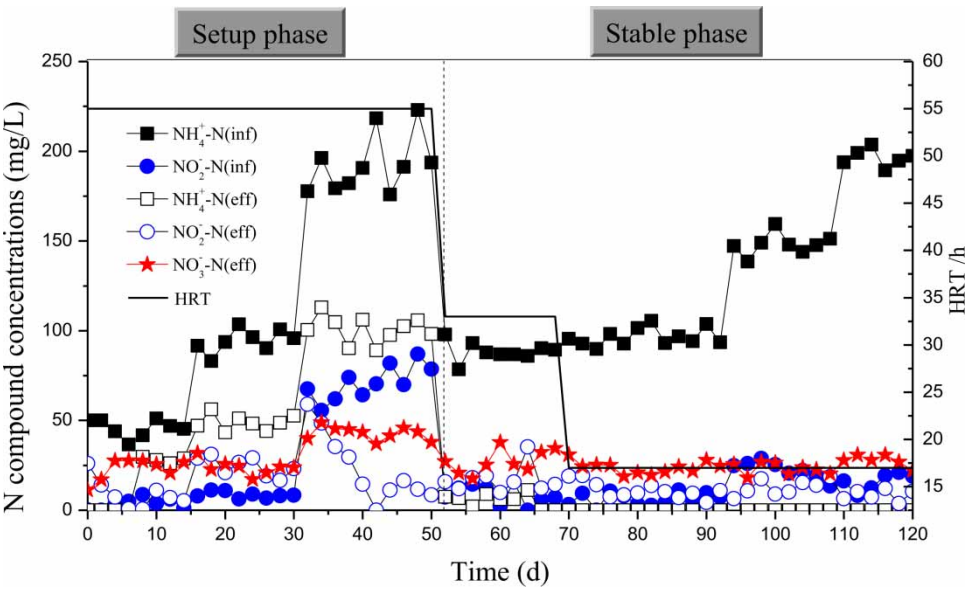

Figure 4 | Concentration of influent and effluent nitrogen compounds and the HRT in the ALBR.

Table 4 | Comparison of the performances of single-stage systems for nitrogen removal

System	Reactor	NLR kg-N/(m³·d)	Nitrogen removal		Reference
			%	kg-N/(m³·d)	
CANON	Moving bed bioreactor	0.79	44.3	0.35	Bertino *et al.* (2011)
SNAP	Up-flow bioreactor	1.35	16.3	0.22	Cho *et al.* (2011)
	Up-flow bioreactor	2.0	17.5	0.35	
CANON	Gas-lift	3.7	42	1.44	Ka *et al.* (2003)
	SBR	0.63	82	0.52	Yongtao *et al.* (2015)
	MBR	0.82	84	0.69	Zhang *et al.* (2014)
SNAP	Up-flow bioreactor	0.6	76.3	0.45	Lieu *et al.* (2006)
OLAND	RBC (synthetic)	2.04	88	1.82	Pynaert *et al.* (2004)
	RBC (raw)	0.91	46.5	0.42	
Combined	ALBR	0.41	75	0.308	This study

120, more than 300 g/(m³·d) of ammonia nitrogen was removed through the single-stage Anammox process and the TN removal efficiency was above 75% (Figure 3(b)).

The results from all other single-stage Anammox processes, such as CANON, OLAND and SNAP, were compared with the results of this study (Table 4). Although Bertino *et al.* (Bertino *et al.* 2011) achieved success with the CANON process by placing K1 carriers in a moving bed bioreactor; the overall nitrogen removal efficiency was only 44.3%. Cho *et al.* (Cho *et al.* 2011) successfully established the SNAP process using two up-flow bioreactors (one with PN and the other with Anammox); however, a lower nitrogen removal efficiency (below 20%) was achieved in both reactors. Ka *et al.* (Ka *et al.* 2003) used a gas-lift reactor in the CANON process and although a 42% nitrogen removal efficiency was obtained the process was not easily attainable at the laboratory or production scale because of the costly requirement of a maximum gas flow of 0.2 L/min for both 95% Ar/5% CO_2 and compressed air. Yongtao *et al.* (Yongtao *et al.* 2015) used an SBR with a working volume 4.6 L as a CANON reactor and 82% nitrogen removal efficiency was obtained; however, the MLVSS was more than 2.8 g/L and the average biomass NRR was 0.20 g-N/(g-MLVSS(d·m³)), which was much less than the average biomass NRR 2.16 g-N/(g-MLVSS(d·m³)) in this study. Some large-scale and complicated bioreactors such as membrane bioreactors (MBR) (Zhang *et al.* 2014) and rotating biological contactors (RBC) (Pynaert *et al.* 2004) have been applied in CANON or OLAND processes in recent years. They all achieved a fairly high NRR and TN removal efficiency of more than 80%; however, the expensive equipment and operational costs are prohibitive at a larger scale.

Table 3 shows that the nitrogen removal efficiency rate was higher than that of most of the other processes, which indicated that the special design of the ALBR was suitable for single-stage autotrophic Anammox processes. The fluidized AOB biofilm attached on the K1 carriers could quickly oxidize ammonium to nitrite because of the high rate of mass transfer, and the Anammox biofilm could maintain a high activity under low DO conditions. However, additional studies on the modification of oxygen zones and carrier packing ratios should be performed to further enhance the nitrogen removal loading and efficiency.

Effect of DO on the PN and Anammox reactions

The aeration rate and mode should be strictly controlled throughout the entire operation because the Anammox reaction is highly anaerobic and AOB have a higher affinity for oxygen than NOB (Corbalárobles *et al.* 2016). Adjustments to the aeration were performed in the first week, and the relationship between the aeration rate and the effluent nitrogen concentration is shown in Figure 5. Under a low aeration rate of 0.01 vvm, approximately 60% of the influent NH_4^+-N (35 mg/L) and a small amount of NO_2^--N (below 6 mg/L) were detected in the effluent because of the insufficient supply of DO to the AOB. When the aeration rate increased to 0.05 vvm, a high NO_3^--N concentration (approximately 35 mg/L) and a low NO_2^--N concentration (below 8 mg/L) were detected in the effluent, indicating that nitrification occurred. However, the Anammox activity sharply decreased because of inhibition under high DO and competition with the NOB (Rongsayamanont *et al.* 2014). The specific growth rate of the NOB was higher than that

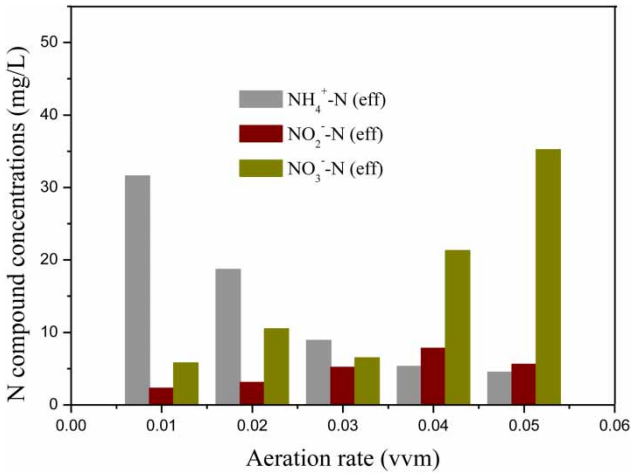

Figure 5 | Effect of the aeration rate on the effluent nitrogen concentration (NH_4^+-N feeding concentration of 50 mg/L).

of the Anammox bacteria under high DO levels, e.g. >3.9 mg/L, and thus most of the NH_4^+-N was oxidized to NO_3^--N. Based on the consumption of NH_4^+-N (41 mg/L) and the concentration of NO_3^--N in the effluent (6.5 mg/L; Figure 5) at the aeration rate of 0.03 vvm, a combined nitrogen removal process was successfully achieved.

Intermittent aeration was adopted starting on day 15 after unstable aeration within the reactor in the first two weeks. The entire aeration period was 4 hours, which included 0.5 hours on and 3.5 hours off (Figure 6). The DO in the inner tube gradually increased to the maximum value (3.5 mg/L) at the end of aeration and then decreased to a stable value (1.5 mg/L). The DO variation in the gap was similar to that in the riser zone, although the maximum value (2.2 mg/L) and minimum value (0.8 mg/L) were lower than those in the inner tube. The DO in the gap continued decreasing below 0.5 mg/L, even after aeration had ceased. The DO distribution was therefore suitable for the co-occurrence of AOB and Anammox in the ALBR.

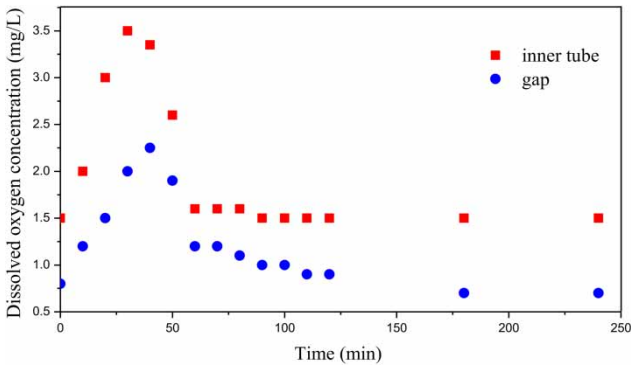

Figure 6 | Concentration of DO in the inner tube and the gap of the ALBR during an aeration period.

The average DO concentration in the riser zone and downer zone was studied throughout the entire operation (Figure 6). The average DO concentration was 1.3–2.0 mg/L in the riser zone and 0.7–1.0 mg/L in the downer zone from day 15 at the start of intermittent aeration. The DO meter probe could not be placed deep enough into the gap because of the reactor structure and only the DO concentration near the gas–liquid separation could be tested. The DO concentration in the middle and bottom of the anaerobic zone would therefore be lower than the observed value in Figure 6 and was always kept below 0.7 mg/L for Anammox (Yin *et al.* 2016). The DO concentration gradient in both the riser zone and the downer zone should be carefully investigated in future studies.

SEM images

The SEM results are shown in Figure 7 and the images indicate that the thickness of the AOB biofilm is between 0.2 mm and 0.7 mm and the main bacteria were coccus and short bacillus (Figure 7(a)–7(c)). The biofilm on the acryl resin fibre carrier in Figure 7(d), 7(e) and 7(f) shows that the compact biofilm aggregated throughout the carrier. Figure 7(f) clearly shows many cauliflower-type aggregates in the carrier, which demonstrates that the Anammox bacteria flourished in the acryl resin.

FISH analysis of the biofilm

FISH and CLSM images of combined process biomass are shown in Figure 8. Figure 8(a) shows that Anammox bacteria (the red) is dominant in the biofilm attached on acryl resin carrier and are wrapped by some AOB bacteria (the blue) and other bacteria (the green), which indicates the Anammox reaction was dominating in the downer zone. A large area of blue can be seen in image Figure 8(b), which indicates AOB stained by fluorochrome Cy3 and was wrapped by a little unknown bacteria (the red). No obvious Anammox (the green) was detected in this image maybe because the strict anaerobism led to their inactivation in riser zone.

CONCLUSION

A novel internal ALBR was designed to achieve nitrogen removal from high-ammonia wastewater via a single-stage

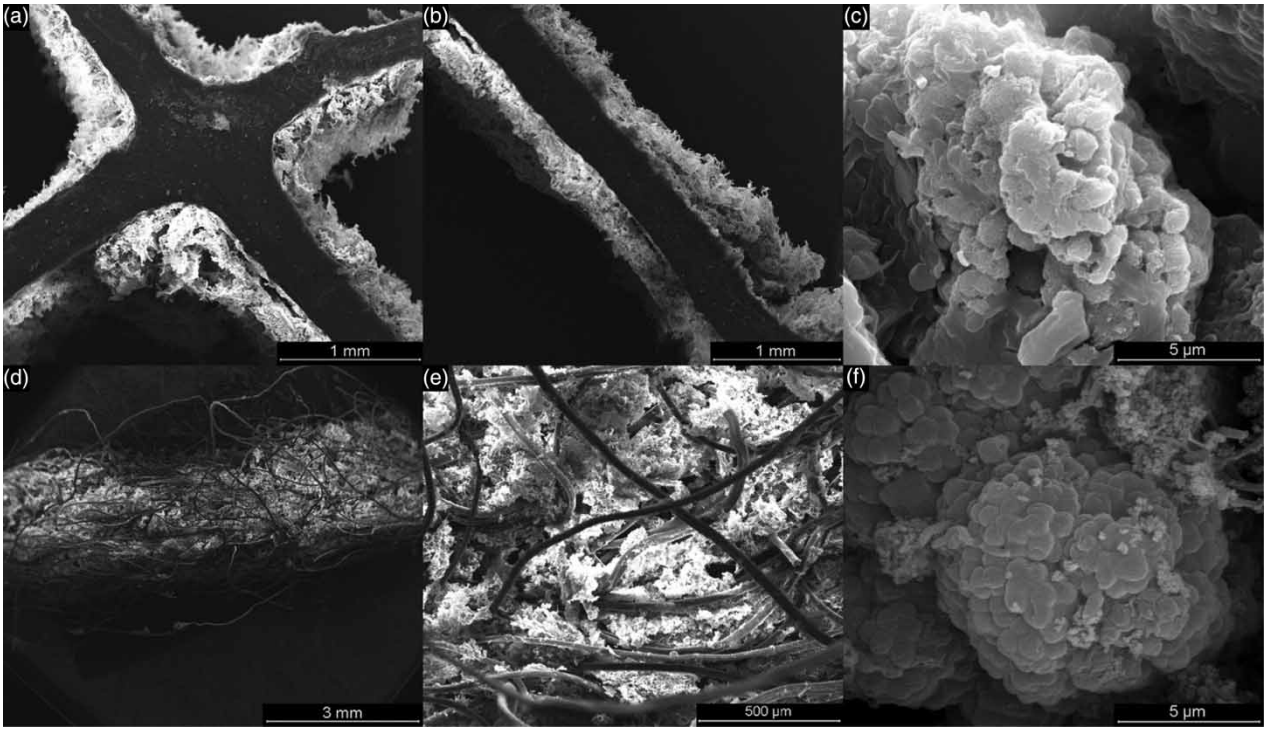

Figure 7 | SEM images: (a) biofilm inside the K1 carrier at 100× magnification; (b) biofilm inside the K1 carrier at 100× magnification; (c) biofilm inside the K1 carrier at 20,000× magnification; (d) biofilm on the acryl resin carrier at 100× magnification; (e) biofilm on the acryl resin carrier at 200× magnification; and (f) biofilm on the acryl resin carrier at 20,000× magnification.

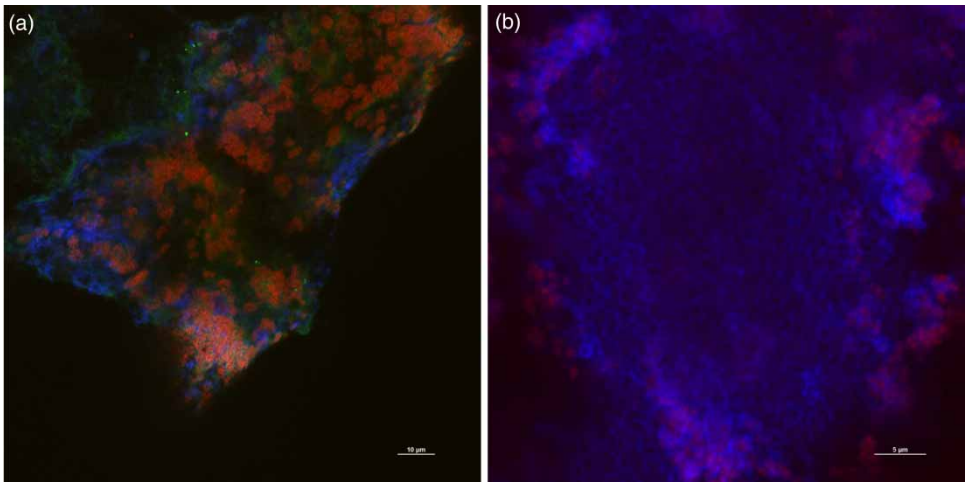

Figure 8 | FISH and CLSM images of combined process biomass taken on day 120 from (a) acryl resin carrier in the downer zone and (b) K1 in the riser zone.

PN–Anammox process. The enriched AOB on the fluidized K1 carriers and Anammox in the fixed acryl resin carrier generated considerable improvements in the NRR, which reached a maximum of 402 g/(m^3·d). The TN removal efficiency also reached above 75%. Moreover, PN was facilitated via intermittent aeration, which did not inhibit the Anammox reaction. It is shown in FISH images that Anammox bacteria dominated in the downer zone while AOB dominated in the riser zone. Ammonium nitrogen removal is therefore feasible with a single-stage PN–Anammox process, and a pilot-scale study is expected in the future.

ACKNOWLEDGEMENTS

The financial support from the Natural Science Foundation of China (Project No. 10972049) and the Natural Science Foundation of Liaoning, China (Project No. 20170540042) is greatly acknowledged.

REFERENCES

Amann, R., Snaidr, J., Wagner, M., Ludwig, W. & Schleifer, K. H. 1996 In situ visualization of high genetic diversity in a natural microbial community. *Journal of Bacteriology* **178** (12), 3496–3500.

Behin, J. 2012 Deinking in bubble column and airlift reactors: influence of wastewater of Merox unit as pulping liquor. *Chemical Engineering Research & Design* **90** (8), 1045–1051.

Bertino, A., Plaza, E., Genon, G. & Onofrio, M. 2011 *Study on One-Stage Partial Nitritation- Anammox Process in Moving Bed Biofilm Reactors: A Sustainable Nitrogen Removal.* POLITECNICO DI TORINO Turin, Italy.

Cho, S., Fujii, N., Lee, T. & Okabe, S. 2011 Development of a simultaneous partial nitrification and anaerobic ammonia oxidation process in a single reactor. *Bioresource Technology* **102** (2), 652–659.

Corbalárobles, L., Picioreanu, C., van Loosdrecht, M. C. & Pérez, J. 2016 Analysing the effects of the aeration pattern and residual ammonium concentration in a partial nitritation-anammox process. *Environmental Technology* **37** (6), 694–702.

Fu, C. C., Fan, L. S. & Wu, W. T. 2010 Flow regime transitions in an internal-loop airlift reactor. *Chemical Engineering & Technology* **30** (8), 1077–1082.

Fujii, T., Sugino, H., Rouse, J. D. & Furukawa, K. 2002 Characterization of the microbial community in an anaerobic ammonium-oxidizing biofilm cultured on a nonwoven biomass carrier. *Journal of Bioscience & Bioengineering* **94** (5), 412–418.

Furukawa, K., Hohman, H., Kashihara, Y. & Pham, K. L. 2008 *Studies on Nitrogen Removal Performances for Single Stage Nitrogen Removal Using Anammox and Partial Nitritation (SNAP) Process*. AnnualReportofFY2007, The Core University Program between Japan Society for the Promotionof Science (JSPS) and Vietnamese Academy of Science and Technology (VAST) P.395-P.402.

Gao, Y., Liu, Z., Liu, F. & Furukawa, K. 2012 Mechanical shear contributes to granule formation resulting in quick start-up and stability of a hybrid anammox reactor. *Biodegradation* **23** (3), 363–372.

Hoang, V., Delatolla, R., Abujamel, T., Mottawea, W., Gadbois, A., Laflamme, E. & Stintzi, A. 2014 Nitrifying moving bed biofilm reactor (MBBR) biofilm and biomass response to long term exposure to 1 °C. *Water Research* **49** (49C), 215–224.

Ka, S. A. T., Abma, W., Kuenen, J. G. & Msm, J. 2003 CANON and Anammox in a gas-lift reactor. *FEMS Microbiology Letters* **218** (2), 339–344.

Lieu, P. K., Hatozaki, R., Homan, H. & Furukawa, K. 2006 Single-stage nitrogen removal using anammox and partial nitritation (SNAP) for treatment of synthetic landfill leachate. *Japanese Journal of Water Treatment Biology* **41** (2), 1627–1628.

Liu, S., Gong, Z., Yang, F., Zhang, H., Shi, L. & Furukawa, K. 2008 Combined process of urea nitrogen removal in anaerobic Anammox co-culture reactor. *Bioresource Technology* **99** (6), 1722–1728.

Mobarry, B. K., Wagner, M., Urbain, V., Rittmann, B. E. & Stahl, D. A. 1996 Phylogenetic probes for analyzing abundance and spatial organization of nitrifying bacteria. *Appl. & Environ. Microbiol.* **62** (6), 2156–2162.

Mulder, A., Graaf, A. A. V. D., Robertson, L. A. & Kuenen, J. G. 1995 Anaerobic ammonium oxidation discovered in a denitrifying fluidized bed reactor. *FEMS Microbiology Ecology* **16** (3), 177–184.

Pynaert, K., Smets, B. F., Beheydt, D. & Verstraete, W. 2004 Start-up of autotrophic nitrogen removal reactors via sequential biocatalyst addition. *Environmental Science & Technology* **38** (4), 1228–1235.

Qiao, S., Fujii, T., Bhatti, Z. & Furukawa, K. 2012 Rapid startup and high rate nitrogen removal from anaerobic sludge digester liquor using a SNAP process. *Biodegradation* **23** (1), 157–164.

Rongsayamanont, C., Limpiyakorn, T. & Khan, E. 2014 Effects of inoculum type and bulk dissolved oxygen concentration on achieving partial nitrification by entrapped-cell-based reactors. *Bioresource Technology* **164** (164), 254–263.

Sobotka, D., Czerwionka, K. & Makinia, J. 2016 Influence of temperature on the activity of anammox granular biomass. *Water Science & Technology A Journal of the International Association on Water Pollution Research* **73** (10), 2518–2525.

Van Dongen, U., Jetten, M. S. M. & van Loosdrecht, M. C. M. 2001 The SHARON®-Anammox® process for treatment of ammonium rich wastewater. *Wat. Sci. Tech.* **44**, 153–160.

Vázquez-Padín, J., Mosquera-Corral, A., Campos, J. L., Méndez, R. & Revsbech, N. P. 2010 Microbial community distribution and activity dynamics of granular biomass in a CANON reactor. *Water Research* **44** (15), 4359.

Wang, T., Wang, J., Ren, F. & Jin, Y. 2003 Application of Doppler ultrasound velocimetry in multiphase flow. *Chemical Engineering Journal* **92** (1–3), 111–122.

Wang, Z., Kim, M., Nakhla, G. & Zhu, J. 2016 Anaerobic fluidized bed digestion of primary and thickened waste activated sludges. *Chemical Engineering Journal* **284**, 620–629.

Xu, Y., Luo, L. & Yuan, J. 2011 CFD simulations to portray the bubble distribution and the hydrodynamics in an annulus sparged air-lift bioreactor. *Canadian Journal of Chemical Engineering* **89** (2), 360–368.

Xu, G., Xu, X., Yang, F., Liu, S. & Gao, Y. 2012 Partial nitrification adjusted by hydroxylamine in aerobic granules under high DO and ambient temperature and subsequent Anammox for low C/N wastewater treatment. *Chemical Engineering Journal* **213** (12), 338–345.

Yeshi, C., Hong, K. B., van Loosdrecht, M. C., Daigger, G. T., Yi, P. H., Wah, Y. L., Chye, C. S. & Ghani, Y. A. 2016

Mainstream partial nitritation and anammox in a 200,000 m^3/day activated sludge process in Singapore: scale-down by using laboratory fed-batch reactor. *Water Science & Technology A Journal of the International Association on Water Pollution Research* **74** (1), 48–57.

Yin, Z., Santos, C. E. D. D., Vilaplana, J. G., Sobotka, D., Czerwionka, K., Xie, L. & Makinia, J. 2016 Importance of the combined effects of dissolved oxygen and pH on optimization of nitrogen removal in anammox-enriched granular sludge. *Process Biochemistry* **51** (9), 1274–1282.

Yongtao, L., Jia, Y., Kai, J., Jie, Z., Rui, M. & Lei, W. 2015 Strengthening cultivation of nitritation granular sludge by adding Ca^{2+}. *Chinese Journal of Environmental Engineering* **9** (9), 4112–4116.

Zhang, X., Li, D., Liang, Y., Zeng, H., He, Y., Zhang, Y. & Zhang, J. 2014 Performance and microbial community of completely autotrophic nitrogen removal over nitrite (CANON) process in two membrane bioreactors (MBR) fed with different substrate levels. *Bioresource Technology* **152** (1), 185–191.

Zhu, J., Zhang, T. & Wei, C. 2012 Numerical optimization on hydrodynamic characteristics of internal-loop fluidized bed based upon structure parameter response relationships. *Acta Scientiae Circumstantiae* **32** (11), 2732–2740.

First received 12 April 2017; accepted in revised form 30 October 2017. Available online 8 December 2017