A high-rate and stable nitrogen removal from reject water in a full-scale two-stage AMX® system

Minki Jung, Taeseok Oh, Daehwan Rhu, Jon Liberzon, S. Joh Kang, Glen T. Daigger and Sungpyo Kim

ABSTRACT

This paper reports long-term performance of a two-stage AMX® system with a capacity of 70 m^3/d treating actual reject water. An air-lift granulation reactor performed partial nitritation (PN–AGR) at an average nitrogen loading rate (NLR) of 3.1 kgN/m^3-d, producing an average effluent NO_2^-–N/NH_4^+–N ratio of 1.04. The average nitrogen removal rate of the system was 3.91 kgN/m^3-d following an anaerobic ammonium oxidation (Anammox) stage moving bed biofilm reactor (A–MBBR). Although the total nitrogen concentrations in the reject water fluctuated seasonally, overall nitrogen removal efficiency (NRE) of the two-stage AMX® system was very stable at over 87%. The two-stage AMX® system, consisting of a PN–AGR followed by an A–MBBR, operated at a stable NLR of 1.86 kgN/m^3-d (1.64 kgN/m^3-d including the intermediate tank), which is 1.8 times higher (1.6 times including the intermediate tank) than other commercialized single-stage partial nitritation/Anammox (PN/A) processes (which operate at a NLR of about 1 kgN/m^3-d). The PN–AGR was affected by high influent total suspended solids (TSS) loads, but was able to recover within a short period of 4 days, which confirmed that the two-stage PN/A process is resilient to TSS load fluctuations.

Key words | air-lift granulation reactor, anaerobic ammonium oxidation, high nitrogen loading rate, reject water, side-stream treatment, two-stage AMX® system

Minki Jung
Taeseok Oh
BKT Co. Ltd.,
25 Yuseong-daero 1184 beon-gil,
Yuseong-gu,
Daejeon 34109,
Korea

Minki Jung
Sungpyo Kim (corresponding author)
Department of Environmental System Engineering,
Korea University,
Sejong 30019,
Korea
E-mail: *ub1905ub@korea.ac.kr*

Daehwan Rhu
Jon Liberzon
Tomorrow Water (BKT),
1225 N. Patt St.,
Anaheim,
CA 92801,
USA

S. Joh Kang
Water & Energy Advisors. Llc.,
3418 Mills Ct,
Ann Arbor,
MI 48104,
USA

Glen T. Daigger
Department of Civil and Environmental Engineering,
University of Michigan,
Ann Arbor,
MI 48109,
USA

HIGHLIGHTS

- The two-stage AMX® system has been successfully operated for 2 years.
- Increased granule-based MLSS in the PN–AGR was able to achieve high NLR.
- Anammox activity was maximized with independent PN and A bioreactor configurations.
- The system had been operated at an average NLR of 1.86 kgN/m^3-d and reached a stable NRE of 86.9%.
- High-solids influent load was affected the system but was able to recover within 4 days.

INTRODUCTION

Biological systems incorporating autotrophic nitrification and heterotrophic denitrification have become the primary technology for removing nitrogen from wastewater due largely to the reliability, robustness, ease of operation, and favorable economics of these technologies when compared to available alternatives (Gao *et al.* 2015). Recent discovery of anaerobic ammonium-oxidizing bacteria (AOB), which use ammonia as an electron donor and nitrite as an electron acceptor to produce nitrogen gas and some nitrate, has created significant interest as an alternative approach to biological nitrogen removal. Anaerobic ammonium

doi: 10.2166/wst.2021.002

oxidation (Anammox) reactions must be coupled with partial nitritation (PN), in which a portion of influent ammonia is converted to nitrite, to accomplish nitrogen removal (Jetten *et al.* 2001). The resulting PN/Anammox (PN/A) process offers the potential to reduce process oxygen requirements for biological nitrogen removal by more than 60%, to eliminate the need for external carbon, and thereby to significantly reduce operating costs (Van Dongen *et al.* 2001). The PN/A process has been developed and commercialized, and about 114 plants have been installed worldwide (Muhammad & Okabe 2015).

The PN/A process can be deployed in either a two-stage PN/A configuration, where a PN reactor is followed by an Anammox reactor, or in a single-stage configuration, where PN/A occur in a single bioreactor. Both approaches are used in PN/A processes commercialized to date, such as moving bed biofilm reactor (MBBR) systems (Rosenwinkel & Cornelius 2005), granular sludge processes (Abma *et al.* 2010), and sequencing batch reactor (SBR) configurations (Wett 2007; Joss *et al.* 2009). Important considerations for selecting a PN/A configuration include the nitrogen loading rate (NLR), the nitrogen removal rate (NRR), and the stability of the system. The SBR-based DEMON® system is the most widely implemented single-stage PN/A processes, and has been operated at an NLR in the range of 0.04–0.65 kgN/m^3-d. Single-reactor MBBR-based ANITAMox and granule-based ANAMMOX® systems have been operated at NLRs in the range of 1.0–1.1 kgN/m^3-d (Lackner *et al.* 2014). Two-stage PN/A processes are known for their quick start-up and rapid recovery from system upsets (Jaroszynski & Oleszkiewicz 2011). The SHARON-ANAMMOX® system is one such two-stage PN/A process. Although the NLR for the Anammox stage ranges up to 7 kgN/m^3-d in this configuration, the NLR for the entire process is approximately 0.36 kgN/m^3-d because of the long hydraulic retention time (HRT) required during the PN stage (Lackner *et al.* 2014).

The recently developed two-stage AMX® process is proposed to offer the advantages of two-stage processes, such as quick start-up, rapid recovery from upsets, etc., while avoiding the disadvantage of a long HRT in PN stage. This performance is achieved using an air-lift granulation reactor (AGR), which reduces the HRT by granulating AOB in the PN stage, followed by an MBBR to achieve stable Anammox performance (Jung *et al.* 2019). While successfully demonstrated at the pilot scale, commercial-scale data are needed to further evaluate the system's performance. This paper thus presents long-term performance results from a two-stage AMX® system at commercial scale (70 m^3/d) treating actual reject water from a sewage treatment plant (STP). The objective of this installation was to demonstrate stable nitrogen removal while operating at a NLR 1.5 times higher than the design factor for other commercial systems.

MATERIALS AND METHODS

Characteristics of reject water treated

A two-stage AMX® system with a capacity of 70 m^3/d was installed and operated at the Busan G STP, Korea. The Busan G STP treats 450,000 m^3/d of sewage using an anaerobic/anoxic/oxic (A^2/O) process, and produces reject water with high ammonium concentrations from dewatering of anaerobic digestate.

The characteristics of the reject water are shown in Table 1. The total nitrogen (TN) concentration averaged 1,059 mg/L over the period of study, 837 mg/L in the summer, and 1,235 mg/L in the winter. Ninety-three percent of TN was ammonium-nitrogen (NH_4^+–N), and the influent total chemical oxygen demand (TCOD) to NH_4^+–N ratio averaged 0.56. This carbon/nitrogen (C/N) ratio is low compared to the range of 3–6 normally required for typical nitrification and heterotrophic denitrification processes (Christensson *et al.* 1994). The molar ratio of alkalinity to ammonia was 1.1, which is sufficient for PN but insufficient for complete oxidation of ammonia to nitrite, which normally requires a C/N ratio of 2.

Two-stage AMX® system

The two-stage AMX® system (illustrated in Figure 1) was composed of a PN stage AGR (PN–AGR), followed by an Anammox stage MBBR (A–MBBR). System loading was scaled up based on optimal operating conditions determined from a previous study (Jung *et al.* 2019). Three PN–AGRs were installed, each with an effective volume of 9 m^3 (top

Table 1 | Characteristics of the reject water

Item	Operation period average (mg/L) (n = 305)	Summer (19.04–19.10) (mg/L) (n = 135)	Winter (18.12–19.03, 19.11–20.03) (mg/L) (n = 170)
TN	1,059 ± 246[a]	837 ± 111	1,235 ± 169
NH_4^+–N	983 ± 233	776 ± 107	1,148 ± 164
Alkalinity ($CaCO_3$)	3,808 ± 735	3,122 ± 489	4,384 ± 233
TCOD	485 ± 140	423 ± 139	526 ± 126
SS	215 ± 88	177 ± 60	243 ± 95

[a]Average ± standard deviation.

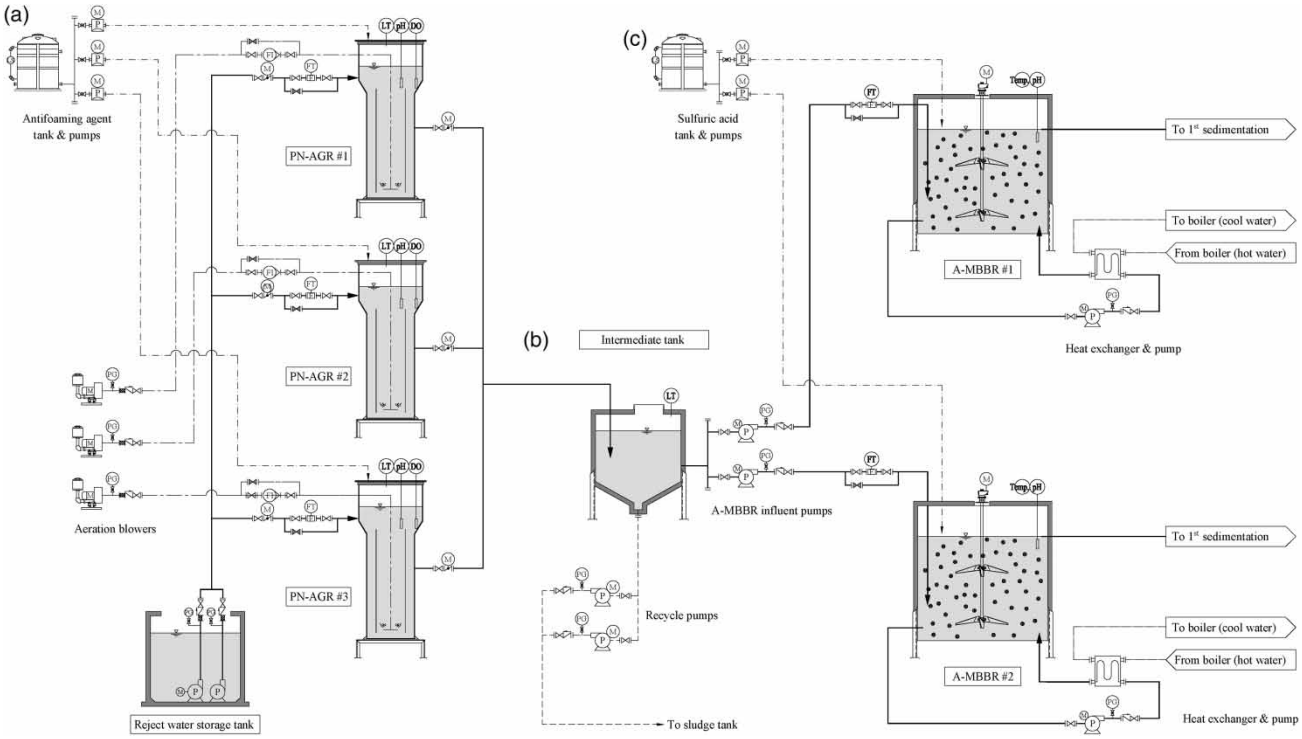

Figure 1 | Schematic of the two-stage AMX® plant. (a) PN–AGR. (b) Intermediate tank. (c) A–MBBR.

diameter 2.1 m, body diameter 1.5 m, height 5.3 m, and water depth 4.6 m). The PN–AGRs were operated as SBRs using fill to discharge cycle times totaling between 3.5 h and 5 h, depending on the influent nitrogen loading (from 63 to 88 kgN/d, respectively). The operation modes of the PN–AGR were feeding (12 min), aeration (168–248 min, adjusted for N loading), settling (20–30 min) and discharge (10 min). Effluent was discharged from the middle of each PN–AGR with the discharge volume based on sludge settleability. The typical volume exchanged in each cycle was 45–50%.

Two A–MBBRs were installed, each with an effective volume of 18 m^3 (width 2.5 m, length 2.5 m, height 3.5 m, and water depth 2.9 m). Each A–MBBR was filled to 50% of total volume with plastic bio-carrier media having a specific surface area of 860 m^2/m^3 (Figure 2). Feed was pumped from the intermediate tank to the A–MBBR at a constant flow rate. The A–MBBRs were insulated and heat was added via a heat exchanger to maintain a water temperature of 32 °C. In addition to equalization of flow from the upstream PN–AGR, the intermediate tank also allowed for settling and removal of suspended solids (SS) in order to minimize the impact of influent SS on the A–MBBR. Settled solids collected in the intermediate tank were periodically pumped to the Busan G STP solids handling facility.

An antifoaming agent (CAS No. 63148-62-9, 1–30%) was dosed at a concentration of 1% to the PN–AGR at a rate of 1 min/h during the aeration to prevent foam discharge from the reactor. The A–MBBR's pH was controlled by a pH meter (HPS-P2A, HANCHANG, Korea), which triggered an acid dosing pump each time the pH reached 7.8. The pump dosed 9% sulfuric acid for 20 s at each trigger. A SCADA system provided for automatic operation of the two-stage AMX® plant.

Start-up and operation

Activated sludge from the secondary aeration basin at the Busan G STP was used to seed the PN–AGRs. The initial mixed liquid suspended solids (MLSS) concentration was 7,600 mg/L. The PN–AGRs were operated at an HRT of 2 days for the first week to stabilize the seeded sludge. The settling time was shortened step by step during days 8–50 to select for fast-settling granules. Bio-carriers from a full-scale Anammox reactor at the Daejeon D STP in Korea were added to the A–MBBR to initiate operation. Acclimated and virgin bio-carriers were added at fill ratios of 8% and 42%, respectively (total fill fraction was 50%). Flow to the A–MBBR's was increased stepwise to determine the maximum NLR.

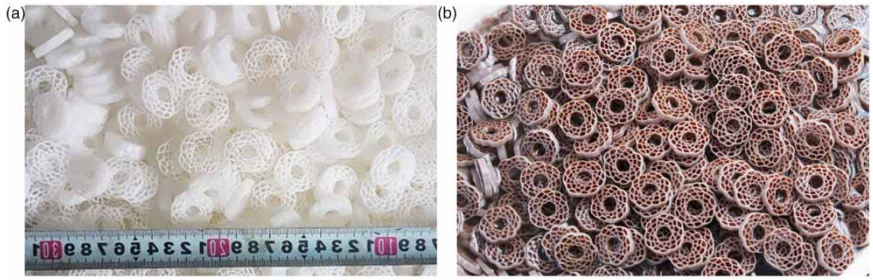

Figure 2 | Bio-carriers for AOB. (a) Before attachment of bacteria. (b) Bio-carriers at the conclusion of the study.

In this study, operation can be divided into Phase I (days 1–247), operated with three PN–AGRs and two A–MBBRs, and Phase II (days 248–457), operated with three PN–AGRs and one A–MBBR (Table 2). Phase I was focused on maximizing operational stability. Nitrogen removal efficiency (NRE) and operational stability were evaluated during days 139–247, after stable operation had been achieved. Phase II focused on achieving the maximum NLR by shortening the HRT of the system. During days 248–367, the PN–AGR effluent flow to one A–MBBR was increased gradually (2–3% per day) from 50% to 100% while maintaining a stable NRE. NRE was evaluated at the highest NLR on days 368–457 and compared with the values of other commercialized systems.

Analytical methods

Total suspended solids (TSS), volatile suspended solids (VSS), chemical oxygen demand (COD), TN, ammonium, nitrite, and nitrate as nitrogen (NH_4^+–N, NO_2^-–N, NO_3^-–N) were all measured according to standard methods (APHA 2005). PN–AGR granule size analysis was performed using standard sieves (KS A5101-1-3). Granules were sampled from the bottom of the PN–AGR, and sieving was performed in order, beginning with the largest pore size. Sieves with pore sizes of 0.5 mm (sieve no. #40), 0.3 mm (sieve no. #50), 0.2 mm (sieve no. #80), and 0.1 mm (sieve no. #100) were used. Sieved granules were weighed and used to determine the mass distribution ratio.

Specific Anammox activity (SAA) was determined by sampling 10–15 bio-carriers at random. The attached growth biomass was detached from each bio-carrier by first shaking the carrier in a 200-mL plastic bottle containing phosphate buffer at pH 7.2, and subsequently collected by scraping with a stainless steel knife. Scraped bio-carriers were finally cleaned with distilled water. Batch SAA analysis was then performed on each biomass vial at a working volume of 100 mL according to the procedure of Jin *et al.* (2011). The temperature was maintained at 35 °C. The test was performed using equivalent NH_4^+–N and NO_2^-–N concentrations of 100 mg/L. Bae *et al.* (2016) reported high Anammox activity at these concentrations, and these concentrations were selected because they are acceptable concentrations of each compound that do not affect Anammox activity.

RESULTS AND DISCUSSION

System performance of the full-scale two-stage AMX® process

Long-term performance of PN–AGR

The full-scale two-stage AMX® system began operation in December 2018 and operated for 457 days. The system was operated by changing the flow rate of PN–AGR to increase the nitrogen load step by step, as shown in

Table 2 | Operational conditions for the two-stage AMX® system

	Operation period (day)	Flowrate (m^3/d)	Influent TN conc. (mg/L)	N load (kgN/d)	Note
Phase I	1–138 ($n = 70$)	44.4 ± 15.5[a]	1,199 ± 181	51.2 ± 12.9	PN–AGR 3 each A–MBBR 2 each
	139–247 ($n = 64$)	84.4 ± 9.4	831 ± 115	69.0 ± 3.0	
Phase II	248–367 ($n = 92$)	89.2 ± 7.5	878 ± 129	77.4 ± 5.3	PN–AGR 3 each A–MBBR 1 each
	368–457 ($n = 79$)	63.0 ± 2.5	1,331 ± 55	83.7 ± 1.7	

[a]Average ± standard deviation.

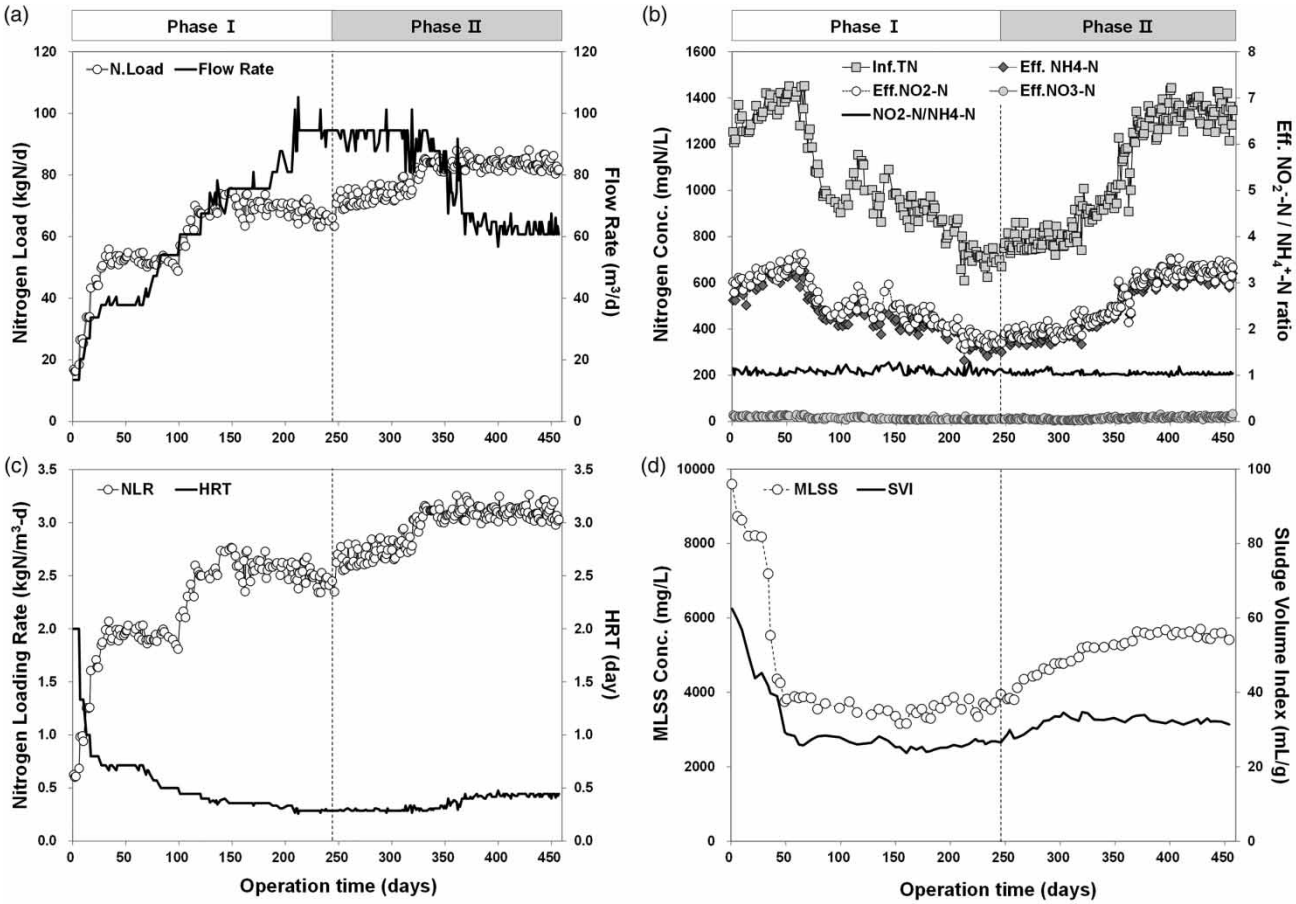

Figure 3 | Performance of PN–AGR. (a) Input nitrogen load and flow rate. (b) Nitrogen concentration in the influent and the effluent, effluent nitrite/ammonium ratio. (c) NLR and HRT. (d) MLSS and SVI.

Figure 3(a). Seasonal variations in the reject water TN concentration (Table 1) also contributed to NLR variations.

Phase I. Phase I was operated with three PN–AGRs during days 1–247. Only 50 days were required for the PN–AGR to achieve an NLR of 2.0 kgN/m^3-d from the initial NLR of 0.6 kgN/m^3-d (Figure 3(c)). Fluctuating reject water TN concentrations led to changes in the effluent NH_4^+–N and NO_2^-–N concentrations, but the NO_2^-–N/NH_4^+–N ratio was maintained at 1–1.1 by changing the aeration time (Figure 3(b)). Quick start-up was achieved because of the short settling time used. The settling time was reduced from 1 h to 20 min over the first 50 days of operation so that granules with a high settling velocity were retained in the PN–AGR. A sludge volume index (SVI) of 30 mL/g or less was quickly achieved and maintained throughout the remainder of Phase I, as shown in Figure 3(d). The NLR increased rapidly through the first half of Phase I as the short settling time for the granules allowed an increased in the number of cycles per day and it could treat more reject water (Figure 3(c)). Jung *et al.* (2019) had previously reported that operation of the PN–AGR at an NLR of 2.2–2.4 kgN/m^3-d would require a MLSS concentration of 3,800 mg/L to achieve an effluent NO_2^-–N/NH_4^+–N ratio of 1–1.2 (as required for the downstream Anammox reaction). During days 50–247, the average MLSS was 3,600 mg/L and the average NO_2^-–N/NH_4^+–N ratio was 1.1.

Phase II. Phase II was operated with three PN–AGRs during days 248–457. The PN–AGR was operated during days 248–349 by increasing the settling time to 30 min to increase the MLSS. The MLSS increased gradually to approximately 5,600 mg/L, allowing the NLR to increase to an average of 3.1 kgN/m^3-d (3.0–3.27 kgN/m^3-d) for days 350–457 with stable operation and an effluent average NO_2^-–N/NH_4^+–N ratio of 1.04.

The average SVI remained at 32 mL/g for days 350–457 (Figure 3(d)). During days 405–410, the granule size distribution in the PN–AGR was analyzed three times. Granules with sizes of 0.2–0.3 mm, 0.3–0.5 mm, and over 0.5 mm represented 23%, 47%, and 13% of the total MLSS (5,600 mg/L), respectively (Supplement 1). Kreuk & Bruin (2004) reported that increased MLSS and improved settleability could be achieved using aerobic granular sludge, which can shorten the HRT required for nitrification. During Phase II, 83% of MLSS consisted of granules with a size of 0.2 mm or greater, promoting a low SVI. Accordingly, the increased MLSS based on granules improved the reaction rate and was able to achieve high NLR. Based on these results, the high NLR of PN–AGR achieved in this study was 1.8 and 2.6 times higher than the previously reported NLRs of 1.76 kgN/m^3-d for a biofilm reactor (Okabe *et al.* 2011) and 1.2 kgN/m^3-d for a suspended growth reactor (Soliman & Eldyasti 2016).

Long-term performance of A–MBBR

The A–MBBR was operated continuously from day 7–457. The initial seven-day period allowed the PN–AGR to begin producing effluent which could be fed to the A–MBBR. Figure 4 presents the A–MBBR influent flow rate and nitrogen loading during this entire operating period.

Phase I. Phase I was operated with two A–MBBRs during days 7–247. The A–MBBR nitrogen load during days 7–138 was increased from 25 kgN/d to 66 kgN/d by increasing the influent flow rate. A steady nitrogen load of 67 kgN/d was subsequently maintained during days 139–247, corresponding to an average HRT of 10.4 h (8.2–12.8 h). The A–MBBRs were operated for the first week at an HRT of 43 h to establish a stable process. The HRT was then gradually decreased to 12 h. The NLR increased from an initial value of 0.5 kgN/m^3-d to 1.8 kgN/m^3-d during this period. During the last 109 days (days 139–247), the A–MBBRs were operated at an NLR of 1.85 kgN/m^3-d with a flow rate of 33 m^3/d, producing a volumetric NRR of 1.62 kgN/m^3-d and a stable NRE of 87.2% (Table 3, Figure 5).

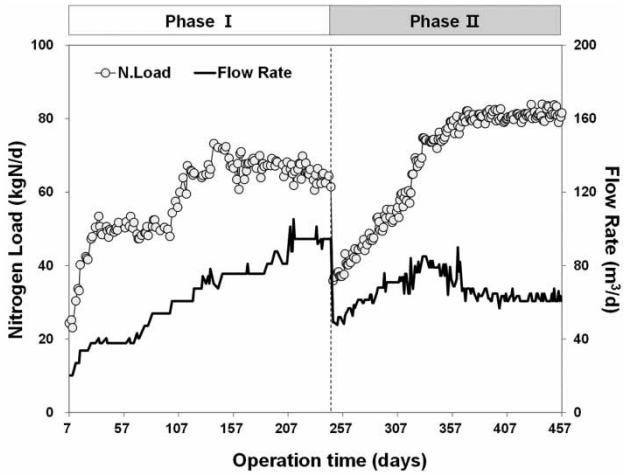

Figure 4 | Input nitrogen load and flow rate of A–MBBRs (two A–MBBR bioreactors were operated in Phase I, and one A–MBBR bioreactor was operated in Phase II).

Phase II. One A–MBBR bioreactor was taken out of service to begin Phase II. Influent flow to the remaining unit was increased slowly (2–3% per day) to maintain an NRE of 85% or more. The nitrogen load to this single A–MBBR was increased during days 248–367 from 34 kgN/d to 80 kgN/d by increasing the flow rate of PN–AGR effluent from 36 m^3/d to 80 m^3/d. As a result, the HRT was reduced from 9 h to 6 h, and the NLR increased from 2.0 kgN/m^3-d to 4.5 kgN/m^3-d. At this point the entire flow (81 m^3/d) from the PN–AGR was introduced to the A–MBBR in service. An NLR of 4.5 kgN/m^3-d was maintained over the following 90 days (days 368–457), resulting in a NRR of 3.91 kgN/m^3-d, a NRE of 86.8% (Table 3, Figure 5), and an average HRT of 6.9 h.

Similar to other studies, it was observed that the NLR could be increased rapidly when a sufficient Anammox population was present. Isaka *et al.* (2006) found that for an attached growth reactor, the NLR could be increased from 0.1 kgN/m^3-d to 0.9 kgN/m^3-d within 3 weeks. Dapena-Mora *et al.* (2007) found that for an SBR reactor, the NLR could be increased from 0.3 kgN/m^3-d to 1.0 kgN/m^3-d within 4 weeks. Van Dongen *et al.* (2001) reported that, for a full-scale installation in Rotterdam, the design load of 7 kgN/m^3-d was achieved in around 3 weeks after a sufficient Anammox population was developed. Thus, it appears that the high NLR was achieved rapidly in Phase II because sufficient Anammox population was cultivated during Phase I.

Overall. As shown in Figure 6, throughout Phases I and II, 1.28 mg of NO_2^-–N was removed for every mg of NH_4^+–N removed, and 0.088 mg of NO_3^-–N was produced for every mg of NH_4^+–N removed. This compares to the known stoichiometry of the Anammox reaction where 1 mg of NH_4^+–N is oxidized with 1.32 mg of NO_2^-–N as the electron acceptor, producing 1.02 mg of N_2–N and 0.26 mg of NO_3^-–N (Jetten *et al.* 2001). The NO_2^-–N/NH_4^+–N ratio for

Table 3 | A–MBBR loading and removal performance

	Operation period (day)	Flow rate (m^3/d)	HRT (hr)	NLR (kgN/m^3-d)	NRR (kgN/m^3-d)	NRE (%)
Phase I	7–138 ($n = 66$)	25.4 ± 5.0[a]	20.6 ± 7.0	1.41 ± 0.28	1.19 ± 0.24	84.2 ± 2.4
	139–247 ($n = 64$)	33.4 ± 1.4	10.4 ± 1.1	1.85 ± 0.08	1.62 ± 0.08	87.2 ± 1.7
Phase II	248–367 ($n = 92$)	59.2 ± 14.2	6.4 ± 1.0	3.29 ± 0.79	2.80 ± 0.68	85.1 ± 2.3
	368–457 ($n = 79$)	81.0 ± 1.2	6.9 ± 0.3	4.50 ± 0.07	3.91 ± 0.09	86.8 ± 1.6

[a]Average ± standard deviation.

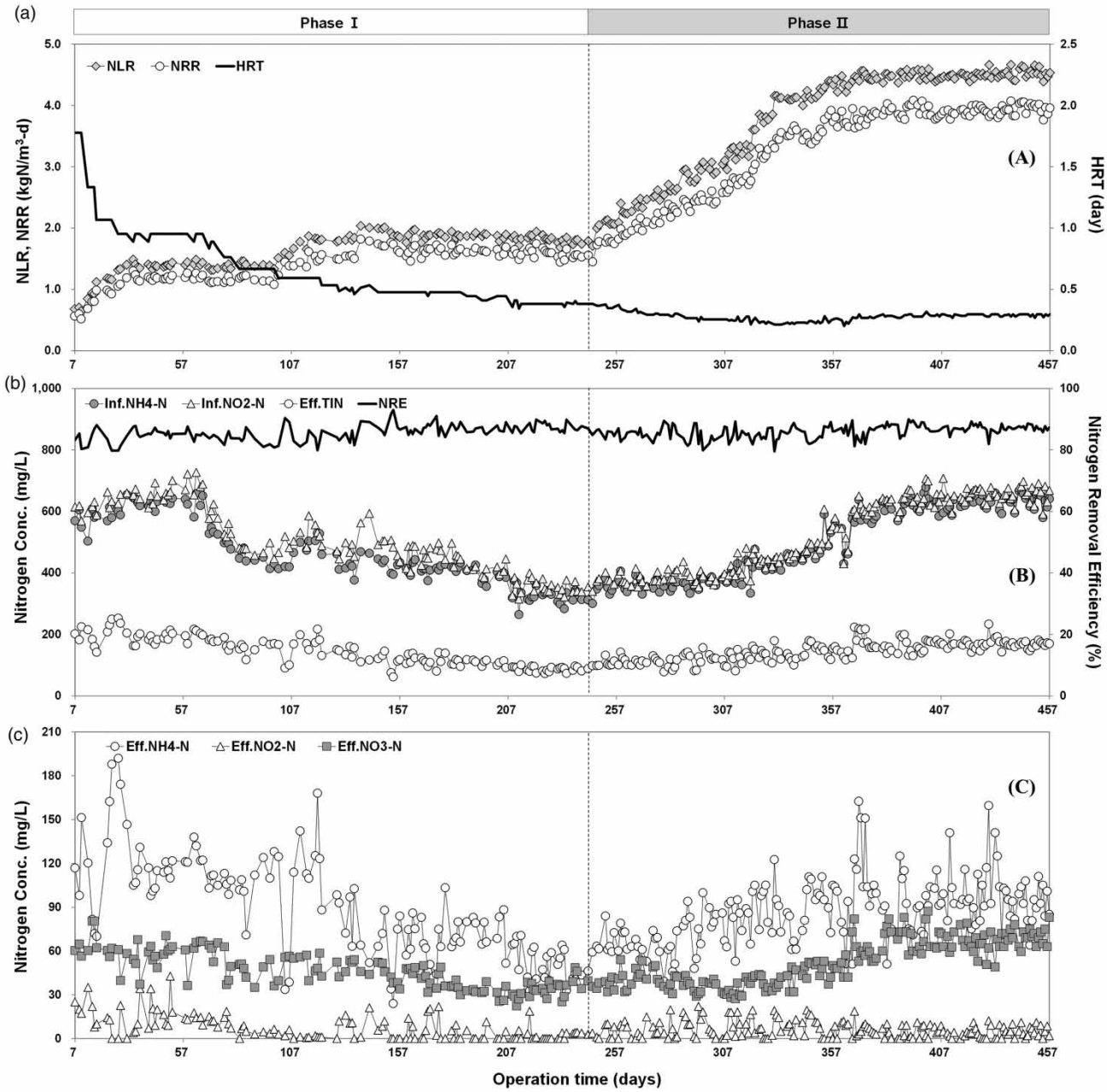

Figure 5 | Nitrogen removal performance of A–MBBR. (a) NLR, NRR, and HRT for one A–MBBR. (b) Concentration of NH_4^+–N and NO_2^-–N in the influent, and total inorganic nitrogen in the effluent, NRE. (c) Concentration of nitrogen compounds (NH_4^+–N, NO_2^-–N, NO_3^-–N) in the effluent.

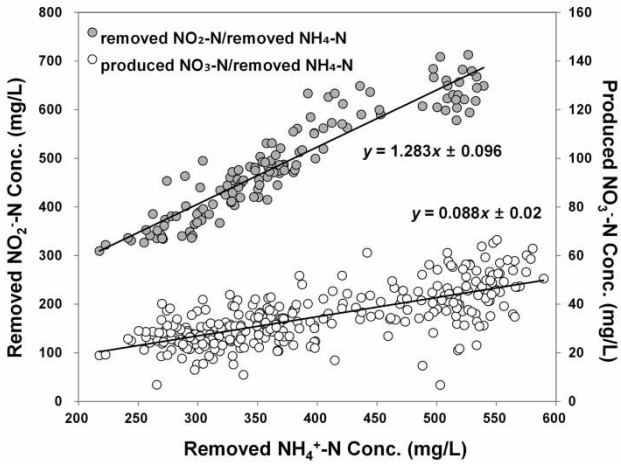

Figure 6 | The correlation of NO_2^-–N removed, NO_3^-–N produced, and NH_4^+–N removed in the A–MBBRs.

the A–MBBR process was lower than this theoretical value, but was consistent with values of 1.26–1.29 reported in other studies (Tang *et al.* 2009; Ni *et al.* 2011; Yu *et al.* 2014). The NO_3^-–N/ NH_4^+–N ratio was significantly less than the theoretical value, which suggests that heterotrophic denitrification may have contributed to NO_3^-–N removal (Langone *et al.* 2014; Ibrahim *et al.* 2016).

Results of SAA assays for the A–MBBR bio-carrier biomass are compared to published results from previous studies in Table 4. This study found an SAA of 0.29 kgN/kgVSS-d, consistent with results obtained from other separate-stage Anammox bioreactors, and higher than those of single-stage PN/A studies. Single-stage PN/A processes may be able to achieve similar SAA to this study's A–MBBR, but they would need to maintain a reactor dissolved oxygen (DO) concentration of 0.2–0.5 mg/L (or lower) to prevent inhibition of Annamox. Chen *et al.* (2020) reported that the activity of AOB (specific ammonia oxidizing bacteria activity, SAOA) was 0.7–1.0 kgN/kgVSS-d at a DO of 2 mg/L, but SAA was as low as 0.05 kgN/kgVSS-d. On the other hand, they also reported that the SAA increased to 0.3 kgN/kgVSS-d when the DO was maintained as low as 0.1 mg/L but the SAOA was reduced to below 0.2 kgN/kgVSS-d. Jin *et al.* (2012) reported that DO should be strictly controlled in the Anammox reactors to avoid DO inhibition. An advantage of two-stage systems such as AMX® is the separation of partial nitritation from Anammox, so that the DO in the Anammox stage can be kept at a minimum (below 0.05 mg/L).

NLR and NRR of combined PN–AGR and A–MBBR process

Figure 7 presents the nitrogen removal performance of the two-stage AMX® system. The NLR, NRR, and HRT are calculated based on the total working volume of the system either without the intermediate tank (WO/I.T) (Figure 7(a)) or including the intermediate tank (W/I.T). During Phase I (days 7–247), when three PN–AGRs and two A–MBBRs were operated, the total HRT excluding the HRT of the intermediate tank was reduced from 75 h to 21 h, and NLR increased from 0.4 kgN/m^3-d to 1.1 kgN/m^3-d. For the last 109 days (days 139–247) of Phase I, the HRT was adjusted in response to the influent nitrogen concentration so that the NLR was maintained at an average of 1.1 kgN/m^3-d, giving an HRT of 18 h (14–22 h). The resulting NRR was 0.96 kgN/m^3-d, and the NRE was stable with an average of 87.3%. Including the volume of the intermediate tank, the NLR averaged 1.0 kgN/m^3-d, the HRT averaged 20 h, and the system NRR was 0.87 kgN/m^3-d. During Phase II (days 248–457), when three PN–AGRs and one A–MBBR were operated, the total HRT (excluding the intermediate tank) was reduced from 18 h to 14 h. The NLR was increased from 1.1 kgN/m^3-d to 1.86 kgN/m^3-d on days 248–367, and for about 90 days (days 248–457) of Phase II, the system was operated at an average NLR of 1.86 kgN/m^3-d and an

Table 4 | Comparison of SAA for PN/A process

Type of reactor		SAA (kgN/kgVSS-d)	References
Two-stage AMX®		0.29 ± 0.05[a]	This study
Two-stage PN/A (SBR)		0.27	Dosta *et al.* (2015)
Only Anammox reactor		0.3 ± 0.03	Dapena-Mora *et al.* (2007)
Single-stage PN/A (MBBR)		0.12	Andrea (2010)
Single-stage PN/A (SBR)	Low DO (under 0.1 mg/L)	0.3	Chen *et al.* (2020)
	Optimum DO (0.2 mg/L)	0.2	
	High DO (over 2 mg/L)	0.05	

[a]Average ± standard deviation.

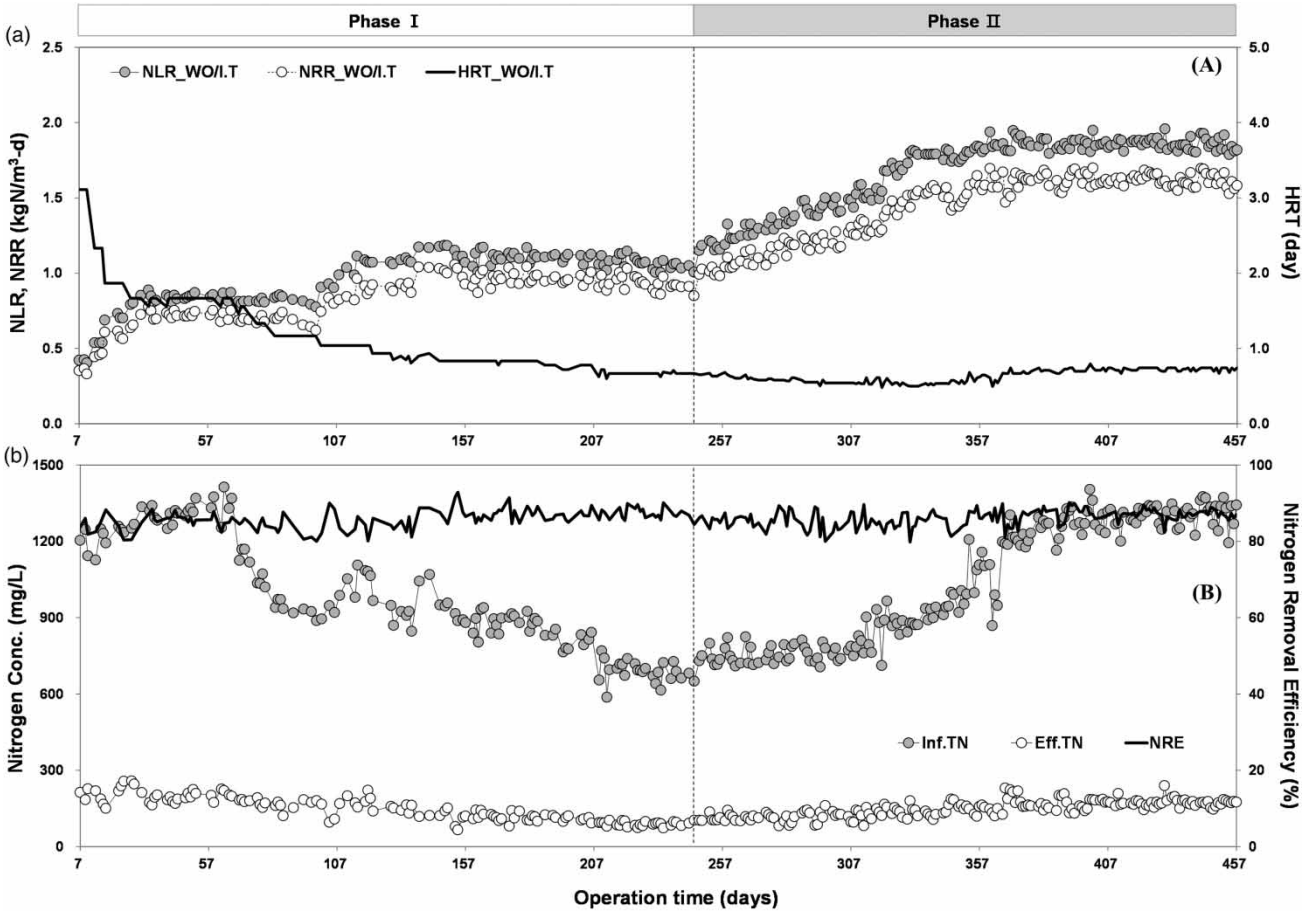

Figure 7 | Performance of two-stage AMX® System. (a) NLR, NRR, and HRT excluding the HRT of the intermediate tank. (b) TN concentration of influent and effluent, and TN removal efficiency.

average HRT of 17 h (16–19 h) with a NRR of 1.62 kgN/m^3-d, and a stable NRE of 86.9%. Including the volume of the intermediate tank, the system was operated during this latter period at an average NLR of 1.64 kgN/m^3-d and an average HRT of 19 h, and the NRR was 1.43 kgN/m^3-d.

Table 5 compares the NLR of the two-stage AMX® system achieved in this study with the NLRs of other commercialized systems. The two-stage AMX® system was operated stably at an NLR of 1.86 kgN/m^3-d (excluding the HRT of the intermediate tank), which is 1.8 times higher than the NLR achieved by other commercialized single-stage PN/A processes. Including the HRT of the intermediate tank in this study, the two-stage AMX® system achieved an NLR of 1.64 kgN/m^3-d, which was 1.6 times

Table 5 | Comparison of operation NLR of full-scale PN/A process

Process		Process configuration	Vol.NLR (kgN/m^3-d)	References
Two-stage PN/A	Two-stage AMX®	Granule(PN) + MBBR(A)	1.86 (1.8–2.0)[a]	This study
	TERRAMOX®	Attached(PN) + Granule(A)	0.4–1.0	Bowden *et al.* (2015)
	SHARON–ANAMMOX®	Suspended(PN) + Granule(A)	0.6	Bowden *et al.* (2015)
Single-stage PN/A	ANAMMOX®	Granule (CSTR)	1.0–2.33	Lackner *et al.* (2014)
	DEMON®	Granule (SBR)	0.3–0.6	Bowden *et al.* (2015)
	SBR	Suspended (SBR)	0.18–0.4	Lackner *et al.* (2014)
	DeAmmon®	MBBR (CSTR)	0.6 (design)	Bowden *et al.* (2015)
	ANITA™Mox	MBBR (CSTR)	1.0–1.2	Lackner *et al.* (2014)

[a]Average (minimum – maximum).

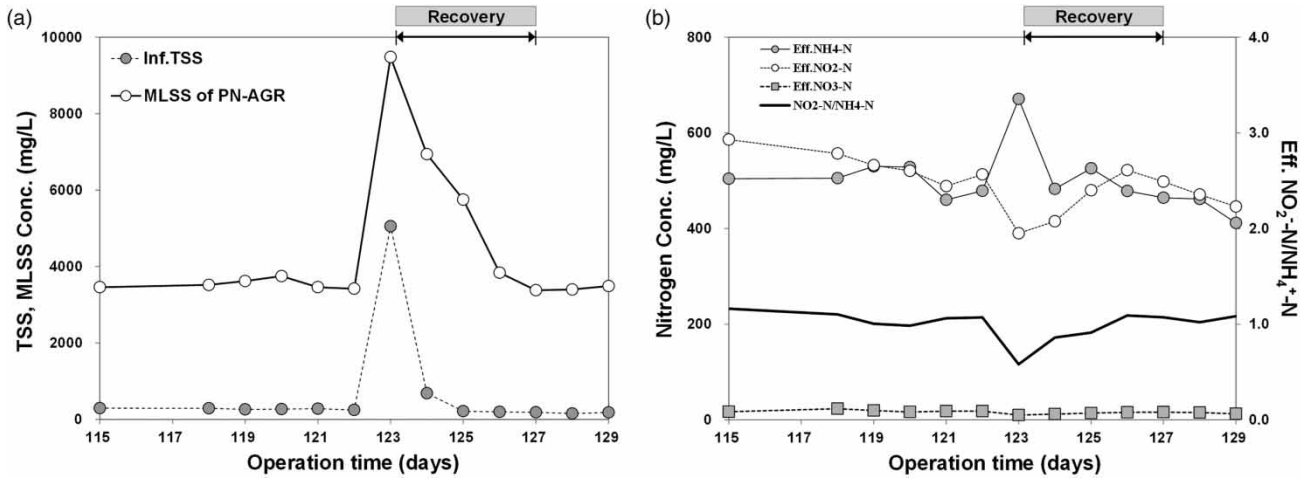

Figure 8 | Recovery for high influent TSS at PN–AGR. (a) Influent TSS and MLSS. (b) Nitrogen concentration of PN–AGR effluent and NO_2^-–N/NH_4^+–N.

higher than the NLR of other commercialized single-stage PN/A processes. Single-stage PN/A processes combine the PN and Anammox reactions in one reactor, typically with a DO concentration of 0.2–0.5 mg/L, in order to maintain both AOB and Anammox activity (Chen *et al.* 2020). High DO and high nitrite concentrations can act as inhibitors to the Anammox bacteria, causing reduced NRR and system upset (Jin *et al.* 2012; Suneethi *et al.* 2014). Single-stage PN/A processes are operated at low DO conditions for this reason, but this adversely impacts the PN reaction rate due to low DO, which subsequently limits the NLR (Jaroszynski & Oleszkiewicz 2011). Two-stage PN/A processes, on the other hand, provide independent PN and Anammox bioreactors, which allows for increased DO and shorter HRTs in the PN stage, without impacting the DO in the subsequent Annamox stage.

Operating issues: high influent TSS

The full-scale two-stage AMX® system was operated without pre-treatment. The maximum TSS concentration in the reject water was 240 mg/L throughout the course of this study. Influent reject water was pumped directly into the PN–AGR from the reject water storage tank. However, an incident did occur when influent TSS increased to 5,050 mg/L suddenly on day 123 due to a dewatering facility upset. As a result, high-TSS influent was fed directly into the PN–AGR. Figure 8 presents the change in the MLSS concentration and system performance in response to this high-solids influent. During this event, the MLSS of the PN–AGR surged to 9,480 mg/L, roughly three times the average value in this study (Figure 8(a)). NH_4^+–N and NO_2^-–N concentrations were 671 and 390 mg/L, respectively, and the NO_2^-–N/NH_4^+–N ratio dropped to 0.58 (Figure 8(b)). Influent TSS concentrations dropped quickly back to normal after the dewatering issue was corrected, but a recovery protocol was necessary to regain full performance. During recovery, the PN–AGR was operated with a shortened settling time of 10–15 min (down from 20 min) in order to decrease the MLSS. Granular sludge was maintained in the PN–AGR, and suspended sludge was washed out. The effluent of the PN–AGR was directed to the sludge storage tank using a recycling pump installed in the intermediate tank, and feeding of the A–MBBR was paused in order to protect the Anammox bacteria. PN–AGR performance recovered within 3 days, with the MLSS concentration returning to 3,400 mg/L, and the NO_2^-–N/NH_4^+–N ratio returning to 1.1. Quick recovery of the PN–AGR was achieved through selective washout of TSS and retention of rapid-settling AOB granules.

High influent TSS can cause severe operational problems for PN/A systems (Lackner *et al.* 2014). A DEMON® system which experienced elevated influent TSS loads showed an increase in nitrate production and required extra sludge withdrawal, which reduced the active biomass in the reactor. Jaroszynski & Oleszkiewicz (2011) reported that single-stage PN/A processes operate with a relatively high SRT of between 20 and 30 days, resulting in a high potential for inert solids accumulation and a reduction in biomass activity by up to 40%. The two-stage AMX® system in this study experienced a high-solids influent load but was able to recover within a short period of about 4 days. These results demonstrate the ability of the two-stage AMX® system to handle dewatering facility upsets without pre-treatment.

CONCLUSIONS

The PN–AGR was effective in achieving a high NLR during the treatment of reject water because of the high concentration of granular sludge established and maintained at steady state. The average NRR achieved in the A–MBBR was 3.91 kgN/m^3-d. The NRE remained over 87% throughout the study period, despite fluctuating TN concentrations in the influent reject water. The two-stage AMX® system provided stable operation at an NLR of 1.86 kgN/m^3-d (excluding the HRT of the intermediate tank), which is 1.8 times higher than the design NLR of most commercialized single-stage PN/A processes. Including the HRT of the intermediate tank, the two-stage AMX® system operated stably at an NLR of 1.64 kgN/m^3-d. PN–AGR performance was adversely affected by high-solids influent following a dewatering facility upset, but was able to recover within about 4 days. Influent feed to the A–MBBR was paused during this period to protect the Anammox bacteria. The AMX® system demonstrated stable performance at a short HRT along with a quick start-up and fast recovery after system upset, thanks to characteristics inherent to the two-stage PN/A technology. The process is able to handle shock loading events such as high influent TSS.

ACKNOWLEDGEMENT

This subject is supported by Korea Ministry of Environment as 'Global Top Project' (Project No. 2019002210001).

DATA AVAILABILITY STATEMENT

All relevant data are included in the paper or its Supplementary Information.

REFERENCES

Abma, W. R., Driessen, W., Haarhuis, R. & van Loosdrecht, M. C. M. 2010 Upgrading of sewage treatment plant by sustainable and cost-effective separate treatment of industrial wastewater. *Water Science and Technology* **61** (7), 1715–1722.

Andrea, B. 2010 *Study on One Stage Partial Nitritation Anammox Process in Moving Bed Biofilm Reactors: A Sustainable Nitrogen Removal*. Master Thesis, KTH Royal Institute of Technology in Sweden.

APHA/AWWA/WEF 2005 *Standard Methods for the Examination of Water and Wastewater*, 21st edn. American Public Health Association/American Water Works Association/Water Environment Federation, Washington, DC, USA.

Bae, H. K., Paul, T., Kim, D. I. & Jung, J. Y. 2016 Specific ANAMMOX activity (SAA) in a sequencing batch reactor: optimization test with statistical comparison. *Environmental Earth Sciences* **75** (22), 1452.

Bowden, G., Tsuchihashi, R. & Stensel, H. D. 2015 *Technologies for Sidestream Nitrogen Removal,* WERF Research Report Series. IWA Publishing, London, United Kingdom.

Chen, G., Zhang, Y., Wang, X., Chen, F., Lin, L., Ruan, Q., Wang, Y., Wang, F. & Cao, W. 2020 Optimizing of operation strategies of the single-stage partial nitrification-anammox process. *Journal of Cleaner Production* **256** (20), 120667.

Christensson, M., Lie, E. & Welander, T. 1994 A comparison between ethanol and methanol as carbon sources for denitrification. *Water Science and Technology* **30**, 83–90.

Dapena-Mora, A., Fernandez, I., Campos, J. L., Mosquera-Corral, A., Mendez, R. & Jetten, M. S. M. 2007 Evaluation of activity and inhibition effects on Anammox process by batch tests based on the nitrogen gas production. *Enzyme and Microbial Technology* **40** (4), 859–865.

De Kreuk, M. K. & De Bruin, L. M. M. 2004 *Aerobic Granule Reactor Technology*. Stowa-Foundation for Applied Water Research, Amersfoort, Netherlands.

Dosta, J., Vila, J., Sancho, I., Basset, N., Grifoll, M. & Mata-Alvarez, J. 2015 Two-step partial nitritation/Anammox process in granulation reactor: start-up operation and microbial characterization. *Journal of Environmental Management* **164**, 196–205.

Gao, J. L., Oloibiri, V., Chys, M., De Wandel, S., Decostere, B., Audenaert, W., He, Y. L. & Van Hulle, S. W. H. 2015 Integration of autotrophic nitrogen removal, ozonation and activated carbon filtration for treatment of landfill leachate. *Chemical Engineering Journal* **275**, 281–287.

Ibrahim, M., Yusof, M. Z., Yusoff, M. & Hassan, M. A. 2016 Enrichment of anaerobic ammonium oxidation (anammox) bacteria for short start-up of the anammox process: a review. *Desalination and Water Treatment* **57** (30), 1–21.

Isaka, K., Date, Y., Sumimo, T., Yoshie, S. & Tsuneda, S. 2006 Growth characteristic of anaerobic ammonium-oxidizing bacteria in an anaerobic biological filtrated reactor. *Applied Microbiology and Biotechnology* **70** (1), 47–52.

Jaroszynski, L. W. & Oleszkiewicz, J. A. 2011 Autotrophic ammonium removal from reject water: partial nitrification and Anammox in one-reactor versus two-reactor systems. *Environmental Technology* **32** (3), 289–294.

Jetten, M. S. M., Wagner, M., Fuerst, J., van Loosdrecht, M., Kuenen, G. & Strous, M. 2001 Microbiology and application of the anaerobic ammonium oxidation ('anammox') process. *Current Opinion in Biotechnology* **12** (3), 283–288.

Jin, R. C., Ma, C., Mahmood, Q., Yang, G. F. & Zheng, P. 2011 Anammox in a UASB reactor treating saline wastewater. *Process Safety and Environmental Protection* **89** (5), 342–348.

Jin, R. C., Yang, C. G., Yu, J. J. & Zheng, P. 2012 The inhibition of the Anammox process: a review. *Chemical Engineering Journal* **197** (15), 67–79.

Joss, A., Salzgeber, D., Eugster, J., Konig, R., Rottermann, K., Burger, S., Fabijan, P., Leumann, S., Mohn, J. & Siegrist, H. 2009 Full-scale nitrogen removal from digester liquid with partial nitritation and anammox in one SBR. *Environmental Science and Technology* **43** (14), 5301–5306.

Jung, M. K., Oh, T. S., Jung, K. B., Kim, J. M. & Kim, S. P. 2019 Study on the optimization of partial nitritation using air-lift granulation reactor for two-stage partial nitritation/ Anammox process. *Membrane Water Treatment* **10** (4), 265–275.

Lackner, S., Gilbert, E. M., Vlaeminck, S. E., Joss, A., Horn, H. & van Loosdrecht, M. C. 2014 Full-scale partial nitritation/ anammox experiences–an application survey. *Water Research* **55**, 292–303.

Langone, M., Yan, J., Haaijer, S. C. M., Op den Camp, H. J. M., Jetten, M. S. M. & Andreottola, G. 2014 Coexistence of nitrifying, anammox and denitrifying bacteria in a sequencing batch reactor. *Frontiers in Microbiology* **5**, 1–12.

Muhammad, A. & Okabe, S. 2015 Anammox-based technologies for nitrogen removal: advance in process start-up and remaining issues. *Chemosphere* **141**, 144–153.

Ni, S. Q., Gao, B. Y., Wang, C. C., Lin, J. G. & Sung, S. 2011 Fast start-up, performance and microbial community in a pilot scale anammox reactor seeded with exotic mature granules. *Bioresource Technology* **102** (3), 2448–2454.

Okabe, S., Oshiki, M., Takahashi, Y. & Satoh, H. 2011 Development of long-term stable partial nitrification and subsequent anammox process. *Bioresource Technology* **102** (13), 6801–6807.

Rosenwinkel, K.-H. & Cornelius, A. 2005 Deammonification in the moving-bed process for the treatment of wastewater with high ammonia content. *Chemical Engineering and Technology* **28**, 49–52.

Soliman, M. & Eldyasti, A. 2016 Development of partial nitrification as a first step of nitrite shunt process in a Sequential Batch Reactor (SBR) using Ammonium Oxidizing Bacteria (AOB) controlled by mixing regime. *Bioresource Technology* **211**, 85–95.

Suneethi, S., Sri Shalini, S. & Joseph, K. 2014 State of the art strategies for successful ANAMMOX startup and development: a review. *Journal of Waste Resources* **4** (4), 1000168.

Tang, C., Zheng, P., Chen, J., Chen, X., Zhou, S. & Ding, G. 2009 Start-up and process control of a pilot-scale Anammox bioreactor at ambient temperature. *Chinese Journal of Biotechnology* **25** (3), 406–412.

Van Dongen, U., Jetten, M. S. M. & van Loosdrecht, M. C. M. 2001 The SHARON®-Anammox® process for treatment of ammonium rich wastewater. *Water Science and Technology* **44** (1), 153–160.

Wett, B. 2007 Development and implementation of a robust deammonification process. *Water Science and Technology* **56** (7), 81–88.

Yu, Y., Tao, Y. & Gao, D. 2014 Effects of HRT and nitrite/ ammonia ratio on anammox discovered in a sequencing batch biofilm reactor. *Royal Society of Chemistry* **4**, 54798–54804.

First received 4 August 2020; accepted in revised form 20 December 2020. Available online 2 January 2021