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 m³/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/m³-d, producing an average effluent NO₂⁻–N/NH₄⁺–N ratio of 1.04. The average nitrogen removal rate of the system was 3.91 kgN/m³-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 an average NLR of 1.86 kgN/m³-d (1.64 kgN/m³-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/m³-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

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/m³-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
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. 2003). 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/m³·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/m³·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/m³·d in this configuration, the NLR for the entire process is approximately 0.36 kgN/m³·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 m³/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 m³/d was installed and operated at the Busan G STP, Korea. The Busan G STP treats 450,000 m³/d of sewage using an anaerobic/anoxic/oxic (A²/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 (NH₄⁻–N), and the influent total chemical oxygen demand (TCOD) to NH₄⁻–N ratio averaged 0.56. This carbon/nitrogen (C/N) ratio is low compared to the range of 5–6 normally required for typical nitrification and heterotrophic denitrification processes (Christenson 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 m³ (top

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Characteristics of the reject water</th>
</tr>
</thead>
<tbody>
<tr>
<td>Item</td>
<td>Operation period average (mg/L)</td>
</tr>
<tr>
<td>TN</td>
<td>1,059 ± 246a</td>
</tr>
<tr>
<td>NH₄⁻–N</td>
<td>983 ± 233</td>
</tr>
<tr>
<td>Alkalinity (CaCO₃)</td>
<td>3,808 ± 735</td>
</tr>
<tr>
<td>TCOD</td>
<td>485 ± 140</td>
</tr>
<tr>
<td>SS</td>
<td>215 ± 88</td>
</tr>
</tbody>
</table>

*aAverage ± standard deviation.*
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 m³ (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 m²/m³ (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.

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

**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.
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

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

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

$^a$Average ± standard deviation.
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/m³-d from the initial NLR of 0.6 kgN/m³-d (Figure 3(c)). Fluctuating reject water TN concentrations led to changes in the effluent NH₄⁺–N and NO₂⁻/NO₃⁻–N concentrations, but the NO₂⁻/NH₄⁺–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/m³-d would require a MLSS concentration of 3,800 mg/L to achieve an effluent NO₂⁻/NH₄⁺–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 NO₂⁻/NH₄⁺–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/m³-d (3.0–3.27 kgN/m³-d) for days 350–457 with stable operation and an effluent average NO₂⁻/NH₄⁺–N ratio of 1.04.
The average SVI remained at 32 mL/g for days 350–457 (Figure 5(d)). During days 405–410, the granule size distribution in the PN–AGR was analyzed three times. Granules with sizes of 0.2–0.5 mm, 0.5–3.0 mm, and greater than 3.0 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/m³·d for a biofilm reactor (Okabe et al. 2011) and 1.2 kgN/m³·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/m³·d to 1.8 kgN/m³·d during this period. During the last 109 days (days 139–247), the A–MBBRs were operated at an NLR of 1.85 kgN/m³·d with a flow rate of 53 m³/d, producing a volumetric NRR of 1.62 kgN/m³·d and a stable NRE of 87.2% (Table 3, Figure 5).

**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 m³/d to 80 m³/d. As a result, the HRT was reduced from 9 h to 6 h, and the NLR increased from 2.0 kgN/m³·d to 4.5 kgN/m³·d. At this point the entire flow (81 m³/d) from the PN–AGR was introduced to the A–MBBR in service. An NLR of 4.5 kgN/m³·d was maintained over the following 90 days (days 368–457), resulting in a NRR of 3.91 kgN/m³·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/m³·d to 0.9 kgN/m³·d within 3 weeks. Dapena-Mora et al. (2007) found that for an SBR reactor, the NLR could be increased from 0.3 kgN/m³·d to 1.0 kgN/m³·d within 4 weeks. Van Dongen et al. (2001) reported that, for a full-scale installation in Rotterdam, the design load of 7 kgN/m³·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 NO₂–N was removed for every mg of NH₄⁺–N removed, and 0.088 mg of NO₃–N was produced for every mg of NH₄⁺–N removed. This compares to the known stoichiometry of the Anammox reaction where 1 mg of NH₄⁺–N is oxidized with 1.32 mg of NO₃–N as the electron acceptor, producing 1.02 mg of N₂–N and 0.26 mg of NO₂–N (Jetten et al. 2001). The NO₂–N/NH₄⁺–N ratio for...
### Table 3 | A-MBBR loading and removal performance

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

*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.
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. 2013; 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 Anammox. 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. (2022) 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).

### Table 4 | Comparison of SAA for PN/A process

<table>
<thead>
<tr>
<th>Type of reactor</th>
<th>SAA (kgN/kgVSS-d)</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Two-stage AMX®</td>
<td>0.29 ± 0.05$^a$</td>
<td>This study</td>
</tr>
<tr>
<td>Two-stage PN/A (SBR)</td>
<td>0.27</td>
<td>Dosta et al. (2015)</td>
</tr>
<tr>
<td>Only Anammox reactor</td>
<td>0.5 ± 0.03</td>
<td>Dapena-Mora et al. (2007)</td>
</tr>
<tr>
<td>Single-stage PN/A (MBBR)</td>
<td>0.12</td>
<td>Andrea (2010)</td>
</tr>
<tr>
<td>Single-stage PN/A (SBR)</td>
<td>Low DO (under 0.1 mg/L) 0.3</td>
<td>Chen et al. (2020)</td>
</tr>
<tr>
<td></td>
<td>Optimum DO (0.2 mg/L)  0.2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>High DO (over 2 mg/L)  0.05</td>
<td></td>
</tr>
</tbody>
</table>

$^a$Average ± standard deviation.

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
average HRT of 17 h (16–19 h) with a NRR of 1.62 kgN/m³-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/m³-d and an average HRT of 19 h, and the NRR was 1.43 kgN/m³-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/m³-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/m³-d, which was 1.6 times

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

<table>
<thead>
<tr>
<th>Process</th>
<th>Process configuration</th>
<th>Vol.NLR (kgN/m³-d)</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Two-stage PN/A</td>
<td>Two-stage AMX® Granule(PN) + MBBR(A)</td>
<td>1.86 (1.8–2.0)a</td>
<td>This study</td>
</tr>
<tr>
<td></td>
<td>TERRAMOX® Attached(PN) + Granule(A)</td>
<td>0.4–1.0</td>
<td>Bowden et al. (2015)</td>
</tr>
<tr>
<td></td>
<td>SHARON–ANAMMADX® Suspended(PN) + Granule(A)</td>
<td>0.6</td>
<td>Bowden et al. (2015)</td>
</tr>
<tr>
<td>Single-stage PN/A</td>
<td>ANAMMADX® Granule (CSTR)</td>
<td>1.0–2.33</td>
<td>Lackner et al. (2014)</td>
</tr>
<tr>
<td></td>
<td>DEMON® Granule (SBR)</td>
<td>0.3–0.6</td>
<td>Bowden et al. (2015)</td>
</tr>
<tr>
<td></td>
<td>SBR Suspended (SBR)</td>
<td>0.18–0.4</td>
<td>Bowden et al. (2015)</td>
</tr>
<tr>
<td></td>
<td>DeAmmon® MBBR (CSTR)</td>
<td>0.6 (design)</td>
<td>Bowden et al. (2015)</td>
</tr>
<tr>
<td></td>
<td>ANITA™ Mox MBBR (CSTR)</td>
<td>1.0–1.2</td>
<td>Bowden et al. (2015)</td>
</tr>
</tbody>
</table>

aAverage (minimum – maximum).
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 Anammox 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)). NH₄⁺–N and NO₂–N concentrations were 671 and 390 mg/L, respectively, and the NO₂–N/NH₄⁺–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 NO₂–N/NH₄⁺–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.

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**Figure 8** | Recovery for high influent TSS at PN–AGR. (a) Influent TSS and MLSS. (b) Nitrogen concentration of PN–AGR effluent and NO₂–N/NH₄⁺–N.
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/m³-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/m³-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/m³-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.

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DATA AVAILABILITY STATEMENT

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

REFERENCES


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