Determine the operational boundary of a pilot-scale single-stage partial nitritation/anammox system with granular sludge
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ABSTRACT
The partial nitritation/anammox (PN/A) process has been applied to ammonium-rich wastewater treatment, but the operational boundary has not been well determined for long-term stability. This pilot-scale study was targeted at a single-stage PN/A process using a sequencing batch reactor (SBR) (volume: 53 m$^3$) and granulated activated sludge. The maximum nitrogen removal rate reached 0.83 kg N/(m$^3$/d). Microbial analysis suggested that ammonium oxidizing bacteria were mainly present in small sludge flocs while anammox bacteria were prone to grow in large sludge granules. The PN/A performance was enhanced when dissolved oxygen (DO) was increased from 0.25 to 0.76 mg/L, and deteriorated at DO higher than 1.15 mg/L. The PN/A was inhibited at free ammonia (FA) over 77.0 mg/L. High DO or FA concentrations inhibited anammox activity and further induced high and inhibitory nitrite concentrations. Therefore, appropriate DO and FA concentrations should be controlled to achieve single-stage PN/A in SBRs.

INTRODUCTION
The partial nitritation/anammox (PN/A) process has become one of the most innovative biological wastewater treatment processes in recent years. It is generally applied to treat ammonium-rich wastewater (>300 mg N/L) such as sludge digestion liquid, landfill leachate and other industrial wastewaters (Daverey et al. 2013; Lackner et al. 2014; Shalini & Joseph 2013). Among the around 100 full-scale PN/A implementations worldwide by 2014, the sequencing batch reactor (SBR) is the most commonly used type (Lackner et al. 2014). Compared to the continuous stirred tank reactor and plug-flow reactor, SBR has two advantages for PN/A: good biomass retention due to prolonged settling time and flexible operation based on pH, oxidation–reduction potential, and ammonium (NH$_4^+$-N) control (Wett 2007; Joss et al. 2011). In addition, the formation of granules could further enhance biomass retention and consequently improve nitrogen conversion rate (Abma et al. 2007).

Despite the success of the granular PN/A SBRs (Abma et al. 2007, 2010), the operational boundary has not been well elucidated for high nitrogen removal rate and stable operation, especially how to balance the occurrence of partial nitritation and anammox. Dissolved oxygen (DO) is considered as one of the key operational parameters in the single-stage PN/A process (Zubrowska-Sudol et al. 2011). Low DO concentration limits the activity of ammonium-oxidizing bacteria (AOB) and reduces nitrogen removal rate (Zubrowska-Sudol et al. 2011), while high DO concentration inhibits anammox activity (Strous et al. 1997) and promotes the growth of nitrite-oxidizing bacteria (NOB) (Peng & Zhu 2006). The optimal DO range of single-stage PN/A reactors varied with the sludge morphology. In the floc-based reactors, the optimal DO concentration is 0.15–0.3 mg/L (Ni et al. 2014). In the biofilm-based reactors, the operational
DO is up to 1.5 mg/L (Lackner et al. 2014). So far, DO optimization has been rarely reported in granule-based PN/A SBR systems, especially at pilot-scale and full-scale (Winkler et al. 2011).

In addition, the free ammonia (FA) concentration, an inhibitor to most bacteria (Martinelle et al. 1996), is commonly high in the wastewaters suitable for PN/A systems. The quantification of the FA inhibitory threshold is important for the operational boundary. The effects of FA concentration on individual species, such as AOB and NOB, have been extensively investigated. The FA inhibitory threshold of AOB (10–150 mg/L) is much higher than that of NOB (0.1–1 mg/L) (Anthonisen et al. 1919). As to anammox bacteria, a decrease in anammox activity of 50% has been reported at FA concentrations of 20–40 mg/L (Fernández et al. 2012; Lackner et al. 2014). All these evaluations were conducted in two-stage anammox systems. Until now, the effect of FA on nitrogen removal performance in a single-stage PN/A system has not been determined.

By targeting these existing challenges, the objective of this study was to elucidate the operational boundary of a pilot-scale single PN/A SBR system (volume: 53 m³) with granular sludge treating ammonium-rich wastewater. The tasks of the study were: (i) to investigate nitrogen removal performance, granulation, and microbial distribution in the pilot-scale PN/A SBR system; and (ii) to evaluate the effects of FA and DO concentration on nitrogen removal performance and determine the operational boundary.

MATERIAL AND METHODS

Set-up and operation of the pilot-scale PN/A SBR

A pilot-scale SBR system was constructed at the Gaobeidian wastewater treatment plant (WWTP) (Beijing, China). The SBR had a working volume of 53 m³ with a diameter of 5.5 m and a height of 5.5 m (Figure 1(a) and 1(b)). The reactor was aerated through a fine-bubble aerator and equipped with online sensors to monitor DO, pH, NH₄⁺-N, NO₂⁻-N, and NO₃⁻-N. Temperature was maintained at 30–35 °C with a thermostatic heater.

Each cycle of the SBR consisted of feeding (1 h), aeration (8.5–86.7 h), settling (1 h), and decanting (1 h). DO concentration during aeration period was controlled at 0.2–0.4 mg/L. Aeration time was adjusted based on NH₄⁺-N online sensors to keep the effluent ammonium concentration between 10 and 25 mg/L. The volume exchange ratio of the reactor was set at several values (1/8, 1/4, 1/2 and 3/4) in the operation period to evaluate nitrogen removal performance at different initial ammonium concentrations (ammonium concentrations in the reactor at the start of aeration).

Wastewater and seeding sludge

Synthetic ammonium-rich wastewater was prepared by adding NH₄HCO₃ into municipal sewage collected at the effluent of the primary sedimentation tank in Gaobeidian WWTP. The main water characteristics of the feeding during SBR operation were: total chemical oxygen demand (TCOD) = 204 ± 43 mg/L, NH₄⁺-N = 1615.0 ± 68.5 mg/L, NO₂⁻-N = 0.0 ± 0.1 mg/L, NO₃⁻-N = 0.2 ± 0.3 mg/L, TP = 5.9 ± 1.4 mg/L, SS = 130.5 ± 32.6 mg/L.

The SBR was inoculated with 30 m³ activated sludge taken from the aeration tanks at the Gaobeidian WWTP and 0.5 m³ anammox granular sludge taken from an upflow anaerobic sludge bed (UASB)-anammox reactor (volume: 20 m³) treating ammonium-rich wastewater. The average diameter of the seeding activated sludge and anammox granules were 59 μm and 507 μm, respectively. The anammox bacteria abundance in the anammox granules were 2.49 × 10⁸ copies/mg VSS (volatile suspended solids). After inoculation, the biomass concentration in the SBR reached 3.6 g VSS/L.
Operational boundary batch tests

Two critical operational parameters, FA and DO concentrations, were examined in the SBR system when nitrogen removal performance stabilized, to determine the operational boundary for the used PN/A process. In both FA and DO batch tests, temperature was maintained at 30.0 ± 0.5 °C and pH was controlled at 7.8–8.0. The SBR procedure during batch tests was identical with that of normal operation. The exchange ratio of the reactor was 25%. Aeration was terminated when ammonium concentrations in the reactor reached 10–25 mg/L, resulting in various aeration times (6.5–46.5 h).

For the FA tests (day 117–121), influent ammonium concentrations were 2,315, 3,364, and 4,372 mg/L, resulting in initial FA concentrations of 40.4, 77.0 and 94.1 mg/L, respectively (Hansen et al. 1998). DO concentrations were controlled at 0.3–0.4 mg/L in all FA levels.

For the DO tests (day 128–131), the SBR was allowed to recover from the inhibition during FA batch tests by decreasing influent ammonium concentration to around 1,200 mg/L and DO concentrations to 0.2–0.3 mg/L. Then four DO levels (0.25 ± 0.05, 0.45 ± 0.07, 0.76 ± 0.05, and 1.15 ± 0.08 mg/L) were examined in the aeration stage of the SBR system. Influent ammonium concentration was maintained at around 1,200 mg/L and the FA concentrations in the reactor were below 16.4 mg/L in the DO batch tests.

Assimilation and heterotrophic denitrification were considered negligible in the batch tests. Therefore, all total nitrogen (TN) removal was processed by anammox in the reactor. In situ activities of AOB and anammox bacteria (in the form of nitrite conversion rate) were calculated by Equations (2) and (3) according to the anammox stoichiometry (Equation (1)) (Strous et al. 1998).

\[
\begin{align*}
\text{NH}_4^+ + 1.32\text{NO}_2^- & + 0.066\text{HCO}_3^- + 0.13\text{H}^+ = 1.02\text{N}_2 \\
& + 0.26\text{NO}_3^- + 0.066\text{CH}_2\text{O}_{0.5}\text{N}_{0.15} + 2.03\text{H}_2\text{O} \\
\end{align*}
\]

\[
\text{Anammox rate} = \frac{1.32 \times \text{TN removal rate}}{1 + 1.32 - 0.26} = \frac{1.32 \times \text{TN removal rate}}{2.06}
\]

\[
\text{Nitritation rate} = \frac{\text{ammonium removal rate} - \text{TN removal rate}}{2.06}
\]

Analysis methods

The influent and effluent samples of the PN/A SBR system were taken every cycle. TCOD, \(\text{NH}_4^+\)-N, \(\text{NO}_2^-\)-N, \(\text{NO}_3^-\)-N, TN, mixed liquor suspended solids, and mixed liquor volatile suspended solids were measured according to Standard Methods (APHA et al. 2005). DO, pH and temperature were measured using in situ online sensors (WTW, Germany). Sludge morphology was observed using a microscope (Olympus BX51, Tokyo, Japan). The size distribution of sludge aggregates was analyzed using a laser particle size analyzer (Malvern Mastersizer 2000, Malvern, UK) (detection range 0–2,000 μm) on day 2 (initial stage), day 78 (middle stage), and day 116 (final stage).

The mixed liquor was taken from the SBR on day 116 (final stage) and separated into flocs and granules using a 0.20 mm sieve. These three samples, namely mixed liquor, flocs, and granules, were later preserved at −20 °C before molecular biology analysis. The abundances of AOB, Nitrobacter, Nitrospira, anammox bacteria and total bacteria were determined by quantitative PCR (polymerase chain reaction). DNA extraction and quantitative PCR procedure were conducted as previously reported (Zeng et al. 2014). Each sample was measured three times and the average value was calculated as the final result.

RESULTS AND DISCUSSION

Correlation of nitrogen removal with granulation and microbial stratification in the PN/A SBR system

TN removal rate of the SBR system improved from 0.12 to 0.44 kg N/(m³·d) within 1 month after inoculation (Figure 2).
indicating a fast PN/A start-up could be achieved by inoculating a certain amount of anammox sludge. Moreover, a good ammonium and TN removal was maintained in the system despite the variation of initial ammonium concentrations (200–800 mg/L) (Figure 2), with the average ammonium and TN removal efficiency of 99 and 95%. TN removal rate increased with initial ammonium concentrations, with the maximum rate of 0.83 kg N/(m^3·d), which was comparable to the values in other actual PN/A applications (Lackner et al. 2014).

Granulation progress in the PN/A SBR system was examined by measuring the sludge size distribution on three typical dates during the operational period (initial stage: day 2; middle stage: day 78; and final stage: day 116) (Figure 3). On day 2, the average diameter of sludge was 86 μm, and sludge size distribution profile had two peaks mainly representing the seeding activated sludge and anammox granules with the proportion of 95 and 7%. After 76 days operation, the sludge size distribution profile had only one peak and fitted well with Gaussian normal distribution with a mean diameter of 190 μm. On day 116, the granules steadily increased to the mean diameter of 209 μm, with the diameter larger than 200 μm being 51% of total biomass, which was eight times as on day 2. Meanwhile, biomass concentration increased from 3.6 g VSS/L on day 2 to 4.8 g VSS/L on day 116, indicating that new granules were formed in the PN/A SBR system over 116 days operation.

There were two major sludge morphologies in the PN/A SBR system: tawny flocs and red granules with a clear boundary. The quantitative PCR showed the different distribution of anammox bacteria and AOB in granules and flocs (Figure 4). Anammox bacteria were more abundant in granules (11.1% of total bacteria) than in flocs (0.2% of total bacteria). In contrast, the relative abundance of AOB in flocs was 29.6%, much higher than in particles (6.0%). These results confirmed that AOB were prone to grow in smaller aggregates while anammox bacteria preferred to reside in larger aggregates (Vlaeminck et al. 2010; Zhang et al. 2015). This might be attributed to the spatial distribution of DO in flocs and granules. Due to the oxygen penetration limitation, granules had a higher anoxic fraction, suitable for anammox bacteria growth, than flocs. In contrast, since microorganisms in flocs can more easily obtain oxygen than those in granules, AOB were more prone to grow in flocs. Both flocs and particles contained a small amount of NOB with an abundance of 0.8 and 0.7% of total bacteria (Figure 4), indicating that NOB were effectively inhibited in the PN/A system due to low DO, high FA concentration, and high temperature (Peng & Zhu 2006).

**Effects of FA concentration on nitrogen removal performance**

The effect of FA concentrations on nitrogen removal rate and efficiency as well as the effluent N-compound concentrations of the PN/A SBR system was studied (Table 1). A good nitrogen removal performance was achieved at FA concentration of 40.4 mg/L, with the TN removal rate and efficiency being 0.59 kg N/(m^3·d) and 91%, respectively, and negligible nitrite concentration in the effluent (Table 1). Increasing FA concentration to 77.0 mg/L did not weaken the reactor performance. However, at FA concentration of 94.1 mg/L, a nitrite accumulation of 272.5 mg/L occurred in the effluent, TN removal rate decreased to 0.39 kg N/(m^3·d), and TN
removal efficiency declined to 63%, indicating a clear inhibitory effect of FA on the PN/A process.

The activities of AOB and anammox bacteria were calculated at different FA concentrations (Table 1). Increasing FA concentration from 40.4 to 94.1 mg/L did not suppress the AOB activity but promoted the nitritation rate from 0.33 to 0.39 kg NO₂⁻/(m³·d), which were probably attributed to higher substrate availability. High nitrite production also improved the anammox rate at FA levels below 77.0 mg/L. Nevertheless, when FA concentration was further raised to 94.1 mg/L, anammox rate dropped to 0.26 kg NO₂⁻/(m³·d), about 72% of at the FA of 77.0 mg/L. Therefore, the inhibition threshold of FA on anammox bacteria in this study was 77.0 mg/L, which was higher than previous reports (Waki et al., 2010; Fernández et al., 2015) in which 50% of anammox activity was suppressed at 38 mg/L FA, and a complete loss of anammox activity occurred at 13–90 mg/L FA. Notably, all these FA inhibition studies were conducted in two-stage anammox systems (either flocculent, biofilm or granular sludge) where anammox bacteria were directly exposed to FA in the bulk liquid. In contrast, anammox bacteria were mainly present in the inner layer of granules shielded by an AOB outer layer in a single-stage granular system (Winkler et al. 2011), which probably enhanced the resistance to FA inhibition.

Variations of nitrogen compounds in the reactor during FA batch tests were investigated. Interestingly, there was a significant break-point in the TN profile at the aeration time of 33rd hour when initial FA concentration was 94.1 mg/L (Figure 5). TN removal rate before the break-point was 0.50 kg N/(m³·d), lower than that with FA concentrations of 40.4 and 77.0 mg/L, indicating the weakened anammox activity at high FA levels. TN removal rate after the break-point dropped to 0.12 kg N/(m³·d) even though the FA concentration was below 27.3 mg/L, indicating that anammox activity was repressed by other aspects (e.g. high concentration of nitrite: 152.0 mg/L) (Puyol et al. 2013).

Based on the FA tests in the PN/A SBR system, the FA inhibition at high levels should be avoided when treating wastewaters with high ammonium concentration. Feasible strategies could be the dilution of the influent, the reduction

<table>
<thead>
<tr>
<th>Parameter</th>
<th>40.4 mg/L</th>
<th>77.0 mg/L</th>
<th>94.1 mg/L</th>
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<td>Reactor performance</td>
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<tr>
<td>Initial NH₄⁺-N concentration (mg/L)</td>
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<td>Effluent TN concentration (mg/L)</td>
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<td>352.5</td>
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<td>1.5</td>
<td>272.5</td>
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<td>87.4</td>
<td>53.2</td>
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<td>Aeration duration (h)</td>
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<td>28.0</td>
<td>46.5</td>
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<tr>
<td>TN removal rate (kg N/(m³·d))</td>
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<td>0.64</td>
<td>0.39</td>
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<tr>
<td>TN removal efficiency (%)</td>
<td>91.0</td>
<td>88.0</td>
<td>63.0</td>
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<tr>
<td>Activity calculation</td>
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<tr>
<td>Nitritation rate (kg NO₂⁻N/(m³·d))</td>
<td>0.33</td>
<td>0.35</td>
<td>0.39</td>
</tr>
<tr>
<td>Anammox rate (kg NO₂⁻N/(m³·d))</td>
<td>0.33</td>
<td>0.36</td>
<td>0.26</td>
</tr>
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*Nitritation and anammox rates were calculated based on data in the first 5 h of the cycle.

![Figure 5](https://iwaponline.com/wst/article-pdf/73/9/2085/183898/wst073092085.pdf)
of the exchange ratio in SBRs, or the recycle of effluent in continuous reactors. Until now, the FA inhibition to the PN/A process was rather limited (Jin et al. 2012), and more studies should be required.

**Effects of DO concentration on nitrogen removal performance**

The effect of DO on the nitrogen removal performance in the PN/A system was investigated at four DO levels (0.25 ± 0.05, 0.45 ± 0.07, 0.76 ± 0.05, and 1.15 ± 0.08 mg/L). The TN removal rate increased nearly four times to 1.02 kg N/(m³·d) when DO was increased from 0.25 to 0.76 mg/L (Figure 6(a)), while TN removal efficiency slightly declined from 90 to 88% and nitrite began to accumulate. At the DO concentration of 1.15 mg/L, the TN removal rate sharply dropped to 0.06 kg N/(m³·d), TN removal efficiency decreased dramatically to 6%, and the effluent nitrite accumulated up to 307.0 mg/L.

Nitritation rate and anammox rate at different DO concentrations were calculated (Figure 6(b)). Anammox rate was positively correlated with DO concentration and matched well with nitritation rate at DO levels below 0.76 mg/L, indicating that anammox activity was limited by nitrite production at low DO levels and nitritation was the rate-limiting step. When DO was elevated to 1.15 mg/L, nitritation rate increased to 0.89 kg NO₂-N/(m³·d) while anammox rate decreased to 0.04 kg NO₂-N/(m³·d), indicating a strong inhibition of DO on anammox bacteria. This mismatch of AOB and anammox activity resulted in a high nitrite accumulation and a sharp decrease of TN removal efficiency.

![Figure 6](https://iwaponline.com/wst/article-pdf/73/9/2085/183898/wst073092085.pdf)
The optimum concentration and operational boundary of DO (DO < 0.76 mg/L) in this study were lower than the reported DO (DO > 1.5 mg/L) of granular and biofilm reactors (Winkler et al. 2011; Zubrowska-Sudol et al. 2011). In a single-stage biofilm and granular system, the outer AOB layer could protect anammox bacteria from oxygen (Winkler et al. 2011), inducing a higher optimum DO than the floc-based system. Compared to the exclusively granular or biofilm systems, floc sludge presented in this study might have a significant impact on microbial distribution (Hubaux et al. 2015; Zhang et al. 2015). AOB were prone to grow in flocs while anammox bacteria preferred to reside in granules, and shrank the outer AOB layer of granules (Hubaux et al. 2015). This would lower the tolerance of granules to DO in the bulk liquid (Hao et al. 2002). The lower optimum DO concentration system in this study than the exclusively granular system indicated the reduction of aeration energy, but the single-stage PN/A system could be less resistant to DO fluctuation and requires a more precise control of aeration for stable operation.

CONCLUSIONS

The operational boundary of FA and DO concentrations were studied in a pilot-scale PN/A SBR system with granular sludge. The 116-day operational tests showed that sludge granulation promoted the TN removal rate to 0.83 kg N/(m²·d). Microbial analysis suggested that AOB were mainly present in small sludge flocs while anammox bacteria were prone to grow in large sludge granules. The inhibitory threshold of the FA concentration on the PN/A process was 77.0 mg/L, which was higher than previous reports on anammox reactor without nitritation. Increasing DO appropriately could improve nitrogen removal performance. Maximum TN removal rate was achieved when nitrite was slightly accumulated. When DO concentration was elevated to 1.15 mg/L, the PN/A process was severely deteriorated, mainly caused by the loss of anammox activity and high and inhibitory nitrite accumulation.

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REFERENCES


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