The influence of dissolved oxygen on partial nitritation/anammox performance and microbial community of the 200,000 m³/d activated sludge process at the Changi water reclamation plant (2011 to 2016)

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ABSTRACT

Mainstream partial nitritation/anammox (PN/A), coupled with excess biological phosphorus removal, in a 200,000 m³/d step-feed activated sludge process (Train 2) in the Changi Water Reclamation Plant (WRP), Singapore, has been studied and reported. This paper presents an overview of process performance and the microbial community during the period from 2011 to 2016. The site data showed that, along with the reduction of dissolved oxygen (DO) from 1.7 to 1.0 mg O₂/L in the aeration zones, the concentrations of ammonium and nitrate of the final effluent increased, while nitrite decreased, resulting in an increase of 2.4 mg N/L of total inorganic nitrogen. Autotrophic nitrogen removal was higher than heterotrophic biological nitrogen removal under higher DO concentration conditions, but decreased under low DO operating condition. These macro-scale changes were caused by shifts of the nitrogen-converting microbial community. The ammonia oxidizing bacteria (AOB) population abundance was reduced by 30 times, while the nitrite oxidizing bacteria (NOB) population abundance and specific activity increased significantly with a shift of dominant genus from *Nitrobacter* to *Nitrospira*. The ratio of AOB and NOB specific activities were reduced from 12.8 to 1.6, and the ex situ nitrite accumulation ratio reduced from 76% to 29%. Changes in the microbial community and overall process performance illustrated that, compared to the excellent NOB suppression under high DO conditions, NOB were more active after the DO concentration reduction despite still being partly suppressed. This case study demonstrated, for the first time, the influence of DO reduction on the nitrogen conversion microbial community and PN/A process performance for a suspended growth system. Its relevance to biofilm and hybrid PN/A processes is also discussed.

Key words | ammonium oxidation, Changi WRP, deammonification, mainstream partial nitritation and anammox, NOB suppression, tropical climates

INTRODUCTION

The mainstream partial nitritation/anammox (PN/A) process enables the decoupling of carbon and nitrogen removal and maximizes energy recovery through carbon-concentrating pre-treatment processes that channel more carbon to anaerobic digestion for biogas generation. The result is the provision of a unique opportunity to achieve efficient nitrogen removal and energy-neutral or energy-positive wastewater treatment (Siegrist et al. 2008). Proposals for mainstream PN/A applications have been suggested since the late 1990s (Jetten et al. 1997; Siegrist et al. 2008; Kartal et al. 2010), and since the early 2010s (De Clippeleir et al. 2011; Ma et al. 2011; Winkler et al. 2012) significant research and development has been devoted to this subject, with noticeable progress achieved (Stinson et al. 2013; Cao et al. 2017a).
The Changi water reclamation plant (WRP) is the largest WRP (800,000 m$^3$/d) in Singapore (Daigger et al. 2008) with four process trains. PN/A, coupled with enhanced biological phosphorus removal (EBPR), has been observed and studied in one train (200,000 m$^3$/d) of the five-pass step-feed biological nutrient removal activated sludge process implemented there (Train 2,). (Cao et al. 2013; Cao et al. 2017a). An overall review focusing on the process performance and microbial community for the period from January 2011 to July 2016 has been conducted and is the subject of this manuscript. The focus was on the effects of dissolved oxygen (DO) on changes that occurred during the 5.5 year operational period. The relevance of these results to PN/A biofilm and hybrid system processes is also discussed.

MATERIALS AND METHODS

Details of the configuration of the reactor, along with sampling locations, are presented in Figure 1. Samples were taken from the primary effluent (PE) and return activated sludge (RAS) (50% of the PE), and at the outlet of the anoxic and aerobic zones of the Train 2 activated sludge process. To avoid the interferences of non-ideal mixing and fast initial reaction rate, the influent concentrations in the anoxic zones were calculated from the hydraulic flow data and measured concentrations of primary effluent and RAS (for first anoxic zone only), and the sampling points of the preceding aerobic zone and the primary effluent for the rest of the anoxic zones. Samples were sequenced, taking into account the time sequence as a function of hydraulic flow. The flow and composition data for PE and final effluent are from the plant’s regular sampling programme. For details on the analysis of site sampling programme data and on the conduct of ex situ ammonia oxidizing bacteria (AOB) and nitrite oxidizing bacteria (NOB) batch activity testing, see Cao et al. (2013). For the probes and quantitative polymerase chain reaction (q-PCR) studies of AOB/NOB population abundance and species identifications, see Yang et al. (2016). For fluorescence in situ hybridization procedures and population abundance of phosphorus accumulating organisms (PAO) and glycogen accumulating organisms, see Winkler et al. (2011) and Yang et al. (2016). For the primers, sequences analysis and q-PCR studies of anammox bacteria, see He (2015).

RESULTS

DO control and aeration strategies implemented

Two different DO/air supply strategies were implemented during the 5.5 year period from January 2011 through July 2016. A higher aerobic zone DO concentration was maintained from January 2011 through March 2014. Beginning in March 2014 the aeration air supply was reduced as a result of implementing on-line ammonium-based aeration control. The main purpose of this change was for energy saving, and it could be implemented since the final effluent
NH₄-N concentration was far below 5 mg NH₄-N/L, the control value for NEWater influent. As a consequence, the daily air supply of 660,814 m³/d on average for the period from January 2011 to the end of March 2014 (Phase I) was reduced to 605,130 m³/d on average for the period from April 2014 to the end of July 2016 (Phase II). Correspondingly, the volumetric ratio 3.4 (±0.5) (m³/m³) on average of air supply for aeration to primary effluent hydraulic flow (air/PE) for Phase I was reduced to 3.2 (±0.2) (m³/m³) for Phase II (Figure 2). This corresponded to a reduction in the DO concentration in the aerobic zones (Figure 2). The average DO (measured in the five aerobic zones) was reduced from 1.7 (±0.5) mg O₂/L (high DO) to 1.0 (±0.4) mg O₂/L (low DO). Although the chemical oxygen demand (COD) mass loading rate reduced by 6% (Figure S1, Supplementary information), and total nitrogen (TN) and ammonium (NH₄-N) mass loading rate increased by 9% from Phase I to II (Figure S2, Supplementary information), it could be assumed that there was no substantial change in the total oxygen demands during Phase I and II. Also, no significant variations in other relevant parameters occurred during the whole period, including: temperature, pH and alkalinity of the primary effluent, sludge retention time (SRT, 4.8 ± 0.6 day) (Figure S3, Supplementary information), and the ratio of distribution of feed and RAS in the step-feed activated sludge process. Therefore, DO reduction was identified as the primary parameter causing the changes in process performance and the microbial community. (Figures S1 to S3 are available with the online version of this paper.)

Primary and final effluent quality

Table 1 presents the characteristics of the primary effluent and final effluent, and respective removal efficiencies, from Jan 2011 to July 2016. The COD/N ratio of 8.2 (BOD₅/N of 3.7) is typical for municipal sewage. Given the hydraulic flow rate of 197,000 m³/d, reactor volume of 57,600 m³ and TN of primary and final effluent (Table 1) the nitrogen removal rate (NRR) was 0.12 kg N/m³·d. Total nitrogen and ammonium removal efficiencies of 86.0% and 92.7% were achieved without additional carbon. The TN of the final effluent of 5.7 mg N/L (4.0 mg N/L in 2011) was
about 6–10 mg N/L less than the conventional modified Ludzack–Ettinger process in other WRPs in Singapore (Cao et al. 2014b), demonstrating the performance advantage for the PN/A process.

Figure 3 shows the measured final effluent ammonium, nitrite and nitrate nitrogen concentrations from January 2011 to July 2016. Table 2 shows the average ammonium, nitrite and nitrate concentrations annually and during the two phases. The general tendencies were lower ammonium and nitrate but higher nitrite in Phase I, and vice versa in Phase II. With respect to total inorganic nitrogen (TIN, the sum of ammonium, nitrite, nitrate) the yearly averages were lower in Phase I, and increased in Phase II. The average TIN for Phase II increased by 2.4 mg N/L compared to Phase I, while the maximum annual difference was 3.7 mg N/L comparing 2011 and 2016. The pH and alkalinity of the two phases were almost the same as a result of the compensating effects of the increased final effluent nitrate and ammonium concentrations during Phase II compared to Phase I.

Partial nitritation/anammox

A composite in situ nitrogen profile was developed using the averages of six sets of on-site measurements (29 March 2012; 28 June 2012; 25 November 2013; 29 May 2014; 17 April 2015 and 25 May 2015), as presented in Figure 4. Given hydraulic flow and ammonia concentrations at the

![Figure 3](https://iwa.silverchair.com/wst/article-pdf/78/3/634/482101/wst078030634.pdf)
inlet and outlet of aerobic zones (Figure 4) it was calculated that 70.0% of the influent ammonium, on average, was removed in the aerobic zones, while further removal occurred in the anoxic zones, suggesting that anammox activity was occurring there. Ammonia removal in the anoxic zones was equivalent to an influent flow based value of 5.2 mg NH₄-N/L. Nitrite and nitrate removal was nearly complete in the anoxic zones. It is estimated that 56.5 mg COD/L was consumed in heterotrophic denitrification, based on 4.2 mg NO₂-N/L and 9.2 mg NO₃-N/L denitrified (according to mass balance calculations, not shown here) and stoichiometric coefficients of 2.8 mg COD/mg NO₂-N and 5 mg COD/mg NO₃-N (Henze et al. 2002).

Slightly more nitrite was produced in the aerobic zones than nitrate. The ammonium concentration at the end of the five aerobic zones was: 3.0, 1.5, 1.8, 1.6 and 2.5 mg N/L, with an average of 2.1 mg N/L. These elevated ammonium concentrations helped to maintain the kinetic advantage for AOB growth over NOB growth (Cao et al. 2017a), resulting in the aerobic zone effluent nitrite and nitrate concentrations observed. Note that the nitrogen oxides (NO₂ + NO₃) produced in the aerobic zones were approximately equal to the ammonium oxidized in the aerobic zones during Phase I (Cao et al. 2013), but often were less than the ammonium removed in the aerobic zones during Phase II, apparently due to simultaneous nitrification and denitrification (SND) (data not shown) (Yang et al. 2016).

Nitrite and nitrate

In the aerobic zones nitrite concentrations consistently increased more than the nitrate concentrations (Figure 4) during the whole study period. The nitrite accumulation ratio (NAR) measured at the end of the last (fifth) aerobic zone is represented in Figure 5. The NAR of 79% in Phase I was much larger than the 10% observed in Phase II. The NAR ratio measured in ex situ batch tests during Phases I and II was 76% (Cao et al. 2013) and 29% (data not shown), respectively. The NOB suppression observed during Phase I deteriorated significantly but was still present during Phase II. The higher nitrite concentrations (up to approximately 6 mg N/L) (Cao et al. 2013) in the process during Phase I did not cause deterioration of sludge settling property (Figure S5, Supplementary information, available online) and no inhibition to denitrifying PAO (DPAO) activity (Cao et al. 2017b).

Nitrogen species mass balance and fate

The nitrogen conversions/fate and balance were computed using the plant regular monitoring data for nitrogen, solids and TN/TSS ratios for solids in the PE and final effluent, observed yield (waste sludge) and TN/TSS ratio of waste sludge, and the average on-site nitrification percentage and NAR. From these data, and the stoichiometries of the conventional denitrification (Henze et al. 2002) and anammox
reactions (Lotti et al. 2014), differentiation of ammonium conversions through the autotrophic and conventional denitrification/denitrification pathways was computed, as presented in Cao et al. (2014a). During Phase I, anammox based autotrophic nitrogen removal contributed 37.5% of the total TN, which was higher than the 27.1% for the conventional denitrification contribution (Cao et al. 2014a). This corresponded well with the observed higher ex situ NAR ratio of 79% (Cao et al. 2014a) during Phase I. Autotrophic nitrogen removal contributed 25.4% of the TN, which was less than the 37.2% computed for conventional heterotrophic nitrogen removal in Phase II (calculations not shown). The lower ex situ NAR ratio of 29% in Phase II is representative of the lower NOB repression and larger fraction of heterotrophic denitrification contribution to nitrogen removal during Phase II.

The average aeration energy of 0.13 kWh/m³ in the whole period was the lowest compared to that of the conventional biological nitrogen removal (BNR) process (0.15–0.19 kWh/m³) in Singapore (Cao et al. 2014b). The highest TN removal of 86% versus approximately 60% for conventional BNR (Cao et al. 2014b) indicated the low oxygen demand for nitrogen removal, thus demonstrating the benefit in aeration energy reduction of the PN/A process.

AOB and NOB microbial community

The population abundance and specific activity for AOB, NOB and anaerobic ammonia oxidizing bacteria (AnAOB) are given in Table 3. Nitrosomonas AOB (ß-subdivision) were the dominant AOB during the whole period (Yang et al. 2014). Measured AOB population abundance (16S rRNA) decreased in Phase II by a factor of 30, compared to Phase I. Measured NOB population abundance (16S rRNA) in Phase I was dominated by Nitrobacter NOB. The Nitrobacter NOB population remained similar to Phase I in Phase II, while a substantial increase in Nitrospira NOB occurred, which became the dominant genus (Yang et al. 2014). The measured AOB/NOB population ratio decreased from approximately 1.5 to 0.1% between Phases I and II.

The average AOB specific activity in Phase I (6.7 mg NH₄-N/g VSS·h) (Cao et al. 2013) reduced modestly by 13% in Phase II (5.8 mg NH₄-N/g VSS·h). In contrast, the average specific NOB activity increased almost seven times in Phase II.

Table 3 | Population abundance and activity of AOB, NOB and AnAOB; rRNA copy numbers are based on q-PCR data

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Unit</th>
<th>Phase I</th>
<th>Phase II</th>
</tr>
</thead>
<tbody>
<tr>
<td>AOB</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Population abundance</td>
<td>16S rRNA copies/g VSS</td>
<td>2.3 × 10¹⁰</td>
<td>0.8 × 10⁹</td>
</tr>
<tr>
<td>Specific activity</td>
<td>mg NH₄-N/g VSS·h</td>
<td>6.7</td>
<td>5.8</td>
</tr>
<tr>
<td>Dominant species</td>
<td>Nitrosomonas</td>
<td>Nitrosomonas</td>
<td></td>
</tr>
<tr>
<td>NOB</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Population abundance</td>
<td>16S rRNA copies/g VSS</td>
<td>1.0 × 10¹⁰ Nitrobacter</td>
<td>1.3 × 10¹⁰ Nitrobacter</td>
</tr>
<tr>
<td>Specific activity</td>
<td>mg NO₃-N/g VSS·h</td>
<td>2.0 × 10⁸ Nitrospira</td>
<td>42 × 10⁸ Nitrospira</td>
</tr>
<tr>
<td>Dominant species</td>
<td>Nitrobacter</td>
<td>Nitrospira</td>
<td></td>
</tr>
<tr>
<td>NAR</td>
<td>%</td>
<td>76</td>
<td>29</td>
</tr>
<tr>
<td>AnAOB</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Population abundance</td>
<td>16S rRNA copies/g VSS</td>
<td>5.5 × 10⁸</td>
<td>3.8 × 10⁷</td>
</tr>
<tr>
<td>Population density</td>
<td>%</td>
<td>0.94</td>
<td>0.71</td>
</tr>
<tr>
<td>Dominant species</td>
<td>Ca. Brocadia sp. 40</td>
<td>Ca. Brocadia sp. 40</td>
<td></td>
</tr>
</tbody>
</table>

VSS: volatile suspended solids.
Phase II, compared to Phase I (Cao et al. 2013). The AOB/NOB activity ratio was as high as 12.8 in Phase I, indicating excellent NOB suppression. The ratio in Phase II was 1.6 (see Figure S6, Supplementary information, for example, available online), only one eighth compared to Phase I, indicating a deterioration of NOB community suppression in Phase II, even though the ratio was still higher than 1. These data are consistent with the variations of on-site nitrite and nitrate concentration profiles in Phases I and II, as discussed above.

Anammox bacteria

*Candidatus* Brocadia sp. 40 was the dominant AnAOB species detected over the entire period (He 2015). While often considered to be an extremely slow growing organism, a specific growth rate ($\mu_{\text{max}}$) of 0.33 d$^{-1}$ at 30 °C was recently measured for this organism in a high rate anammox reactor (Lotti et al. 2015). Zhang et al. (2017) also recently reported $\mu_{\text{max}}$ of 0.33 d$^{-1}$ for *Ca*. Brocadia sinica. These values correspond to a doubling time of 2.1 days. Compared to an operating SRT of 5 days at a steady temperature of 30 °C for the Changi WRP step-feed activated sludge process, these recent findings support the observed presence of anammox organisms in the system. The anammox population density (16S rRNA) in Phase II was reduced compared to Phase I. With the growth kinetics of free cell *Ca*. Brocadia sp. 40 (Lotti et al. 2015), the population abundance of 0.71% and 0.94% was sufficient to achieve an NRR of 0.12 kg N/m$^3$d by the autotrophic pathway alone, should the supply of nitrite be adequate.

**DISCUSSION**

**Shift of AOB and NOB microbial community**

The results presented in Table 3 indicate a significant population reduction in AOB, specifically *Nitrosomonas*, during Phase II compared to Phase I. Among others, the reduction of the aerobic fraction of the flocs under low DO in the liquid bulk (Morales et al. 2015) probably played a role. However, despite a 50-fold reduction of the measured AOB population, the specific activity (reactor volume based) was only reduced by 13%, and ammonium oxidation in the aerobic zones was still maintained at a high efficiency during Phase II. The average final effluent ammonium concentration during Phase II increased only by 1.1 mg NH$_4$-N/L compared to Phase I. This suggests that the specific cell activity of AOB during Phase II increased substantially compared to that of Phase I, a similar phenomenon reported in the literature (Coskuner et al. 2005). Further work is needed to resolve the observed difference between the measured AOB population and activity under the respective operating periods (only modest difference in DO concentration). In contrast, the NOB population increased substantially from Phase I to Phase II, corresponding to a significant increase in the K-strategists Nitrospira NOB over the r-strategists Nitrobacter NOB (Nowka et al. 2015). In this instance, the increased measured NOB population correlated well with a significant increase in NOB activity (Table 3). These data also illustrate that, instead of the AOB and NOB population ratio, the ratio of specific activity of these organisms may be an appropriate parameter in study of NOB suppression.

The shift in AOB and NOB populations, and changes in activities from Phase I to Phase II, resulted in reduced NOB suppression during Phase II. This further led to reduced nitrite availability, which would limit PN/A conversion. In addition, a further reduction of nitrite provided to anammox bacteria in anoxic zones occurred due to the SND in the aerobic zones (data not shown) (Yang et al. 2016). A reduction in the anammox population and activity during Phase II was observed, as reported earlier (Zeng et al. 2014). Low DO operation during Phase II was clearly one factor which triggered the shift of the AOB and NOB communities, resulting in deterioration of NOB suppression under the site conditions at the Changi WRP.

**Conflict of interest**

Conflicting reports exist on the effect of low DO (0.87 mg O$_2$/L, Huang et al. 2010; <0.04 mg O$_2$/L, Sliekers et al. 2005; <0.05 mg O$_2$/L, Hu et al. 2015; 0.5 mg O$_2$/L, Zeng et al. 2014) and high DO control (2.5 mg O$_2$/L, Yang et al. 2007; 1.4–1.8 mg O$_2$/L, Cao et al. 2013; 1.5 mg O$_2$/L, Regmi et al. 2014) on NOB suppression in suspended PN and PN/A processes under oxygen limitation conditions. Low DO control, which is based on oxygen saturation constant $K_{O,\text{AOB}} < K_{O,\text{NOB}}$ (Wiesmann 1994), is often suggested. In reality, however, many complicating factors are involved, including variations in selective pressure, e.g. DO, temperature and pH, which create niches favouring different AOB and NOB microorganisms. Studies down to the genus, or even the species, level for AOBs and NOBs (Agrawal et al. 2017), and corresponding intrinsic kinetics (Park & Noguera 2004), may become necessary. Given the complex micro-environments for AOB and NOB competition within flocs, the traditional approach to studies of suspended growth systems, which ignore mass transfer within flocs, appears to be too simplified, as apparent AOB and NOB kinetic
parameters in flocs are related to their distribution patterns and the relative sizes of the microbial colonies (Picireanu et al. 2016) and morphology of the floc particles (Wu et al. 2017). These two topics are, to a large extent, still yet to be fully explored and understood. In this regard, studies on the species, floc morphology, and distribution of AOB and NOB in flocs during Phases I and II would be helpful.

Responses of the process performance

The increase of ammonium in the final effluent in Phase II was probably due to reduced air supply and increased nitrogen loading rate, while the increase of nitrate was a direct response to deterioration of partial nitritation (poor NOB suppression) caused by the shift of the AOB/NOB community in Phase II. Significant reduction of the supply of nitrite to the anammox bacteria resulted in reduced, or even non-detectable, ammonium removal in the anoxic zones during Phase II. As a consequence, the relative contribution of autotrophic nitrogen removal declined and the TN reduction in the DO concentration. Persson et al. (2016), working with a moving bed biofilm reactor process, observed a similar correlation between the relative abundance of AOBs and NOBs and concentrations of nitrogen species in the process. Interestingly, in spite of the reduced NOB suppression in Phase II, and the corresponding increased consumption of COD for denitrification due to reduced PN/A, excellent EBPR was still achieved (Cao et al. 2017b). This suggests the need for studies on the mechanisms and quantitation of competition for nitrite between DPAOs and anammox. Further study of dissimilatory nitrate reduction to ammonium (Guven et al. 2005) and carbon sharing for nitrogen and phosphorus removal by DPAOs in the aeration zones in Phase II is needed to develop a detailed carbon flow picture.

These results suggest that, for a suspended mainstream PN/A process, the reduction of aerobic zone DO concentrations may not favour NOB suppression, resulting in reduced PN/A, even though residual ammonium concentrations, high temperature, short aerobic SRT and transient anoxia conditions still remain. For a single-stage mainstream PN/A biofilm process, a lower DO in the bulk liquid may reduce AOB activity because of reduced DO diffusion into the biofilm and relatively large AOB microcolonies (Picireanu et al. 2016). For a hybrid PN/A process, a lower bulk DO concentration may cause reduced nitrite flux through the biofilm due to heterotrophic denitrification in the bulk liquor, although low DO protects anammox from oxygen (Persson et al. 2014). High DO in the bulk liquid phase can offset the drawback of low DO, but a thick biofilm, which harbours more and active anammox in the deeper internal part, needs to be developed and applied, especially under low temperature (Hao et al. 2002; Gilbert et al. 2015; Cao et al. 2017a).

CONCLUSIONS

A review of the operation and performance of the 200,000 m$^3$/d Train 2 step-feed activated sludge process at the Changi WRP accomplishing mainstream PN/A from 2011 to 2016 illustrated that, after the DO in the aeration zones was reduced from 1.7 to 1.0 mg O$_2$/L on average, the Nitrosomonas AOB population abundance decreased while the NOB population abundance and specific activity increased significantly. Dominance of the NOB genus shifted from Nitrobacter to Nitrospira. The AOB/NOB population ratio was significantly reduced, the ratio of ex situ AOB/NOB specific activity reduced from 12.8 to 1.6, and the in-process nitrite accumulated less in Phase II when the DO concentration was reduced. This also resulted in a reduced anammox bacteria population abundance and activity in Phase II. Nitrogen mass balance analysis indicated a higher contribution of autotrophic nitrogen removal compared to heterotrophic denitrification under high DO concentration conditions, which shifted to increased heterotrophic nitrogen removal under low DO operating conditions. An obvious influence of DO concentration reduction on the nitrogen converting community and process performance for this suspended growth PN/A system was demonstrated.

REFERENCES


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