The occurrence of enhanced biological phosphorus removal in a 200,000 m³/day partial nitration and Anammox activated sludge process at the Changi water reclamation plant, Singapore

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ABSTRACT

Mainstream partial nitritation and Anammox (PN/A) has been observed and studied in the step-feed activated sludge process at the Changi water reclamation plant (WRP), which is the largest WRP (800,000 m³/d) in Singapore. This paper presents the study results for enhanced biological phosphorus removal (EBPR) co-existing with PN/A in the activated sludge process. Both the in-situ EBPR efficiency and ex-situ activities of phosphorus release and uptake were high. The phosphorus accumulating organisms were dominant, with little presence of glycogen accumulating organisms in the activated sludge. Chemical oxygen demand (COD) mass balance illustrated that the carbon usage for EBPR was the same as that for heterotrophic denitrification, owing to autotrophic PN/A conversions. This much lower carbon demand for nitrogen removal, compared to conventional biological nitrogen removal, made effective EBPR possible. This paper demonstrated for the first time the effective EBPR co-existence with PN/A in the mainstream in a large full-scale activated sludge process, and the feasibility to accommodate EBPR into the mainstream PN/A process. It also shows EBPR can work under warm climates.

Key words | biological nutrient removal, Changi WRP, COD, deammonification, enhanced biological phosphorus removal, mainstream partial nitritation and Anammox, tropical

INTRODUCTION

Changi water reclamation plant (WRP) is the largest WRP (800,000 m³/d) in Singapore (Daigger et al. 2008). There are four step-feed activated sludge (SFAS) process trains in the plant, each with five step feed points and each train treating 200,000 m³/day wastewater. Of the four trains, three are operated for chemical oxygen demand (COD) and biological nutrient removal (BNR), and one for COD removal only. For the BNR process, the total sludge retention time (SRT) is five days; two and a half days each for the anoxic and aerobic zones. The sewage temperature is 28 to 32 °C all year around. The yearly average removal efficiency of total nitrogen (TN) has historically been 86%. Despite the conventional BNR step-feed design, it was observed that partial nitritation occurred in the aerobic zones and Anammox in the anoxic zones of the BNR activated sludge process in Changi WRP (Cao et al. 2013, 2016). Quantitative polymerase chain reaction (qPCR) tests (using modified primers from the primer pair of Amx694F2/Amx960R) found the potential Anammox bacteria concentration of (16S rRNA gene) 3.6 ± 3.2 × 10⁶ copies/mL, and the abundance in 16S rRNA gene was 0.83 ± 0.18% (He 2015). The main species identified was a close relative of Candidatus Brocardia sp. 40, with 98% sequence similarity (Cao et al. 2014a;
Lee et al. 2014; He 2015). It is the same organism with high growth rate cultured at Delft University of Technology (TU Delft) (Lotti et al. 2015). Enhanced biological phosphorus removal (EBPR) co-existing with partial nitritation and Anammox (PN/A) in Changi WRP was reported (Cao et al. 2015, 2016), but no quantitative results and detailed analyses were provided. The popular opinion is that EBPR is not possible or difficult in warm climates (Erdal et al. 2005; Panswad et al. 2005; Rabinowitz et al. 2004; Gu et al. 2005; Lopez-Vazquez et al. 2008; Cao 2011) due to strong competition between glycogen accumulation organism (GAO) and phosphorus accumulation organism (PAO) for volatile fatty acid (VFA) at tropical temperature (25 °C – 30 °C) (Lopez-Vazquez et al. 2008, 2009a). Short SRT (Whang & Park 2006), influent with constant and appropriate carbon (acetate or propionate) supply (Ong et al. 2013, 2014) as well as a higher pH (7.0 – 8.0) (Filipe et al. 2001) or segregation of different sizes of granular sludge (Winkler et al. 2011) were proposed for PAO competitive advantages, but few full-scale cases were reported to verify the ideas, until the reporting of several full-scale EBPR processes under warm climates in recent years (Gineset et al. 2014; Jimenez et al. 2014; Sayi-Ucar et al. 2015).

This paper presents the results of a study focusing on EBPR in the mainstream PN/A process in Changi WRP. The scope included: process (in-situ) performance and kinetics, ex-situ specific activities measurement and mechanism study, microbial community analysis, and the effects of EBPR on the fate of COD, etc. The main objectives were: (i) to understand the EBPR kinetics and mechanisms, (ii) to understand the potential interactions of EBPR and PN/A through analysis of carbon balance and fate, and (iii) to explore the feasibility to accommodate EBPR in mainstream PN/A, especially EBPR in warm climates.

**MATERIALS AND METHODS**

**Site sampling program**

Six sets of site sampling program data (29 March 2012; 28 June 2012; 25 November 2013; 29 May 2014; April 2015 and 27 May 2015) were collected to study process performance and kinetics. Samples were taken from the primary effluent (PE), return activated sludge (RAS, 50% of the influent) and at the outlet of anoxic and inlet of aerobic zones of the Train 2 activated sludge process to take into account the delay as a function of hydraulic flow. The details of reactor configuration and sampling locations can be found in Figure 1. The analytic parameters included NH4-N, NO2-N, NO3-N, sCOD, PO4-P, pH, alkalinity (ALK), and mixed liquor suspended solids (MLSS), etc. Dewatering supernatant was returned to Train 4 (for COD removal only). However, the thickening supernatant (both primary and waste activated sludge) was continuously directed to the RAS pipe where it then entered the head of the first anoxic zone. A special sampling program was conducted for the analysis of PO4-P and VFA of thickening supernatant and RAS. The process reaction kinetics at each zone, as well as the whole process, were calculated using their respective hydraulic flows and concentrations.

**P release and uptake batch test**

Ex-situ batch tests to measure specific P release and P uptake activity, and to study denitrifying phosphorus accumulation organism (DPAO) activity, were carried out mainly according to Neethling et al. (2005). The 5 L working volume beaker was used as reactor. A heater controlled the temperature inside the reactor at 50 ± 2 °C. In ex-situ specific P release and uptake batch test, the anaerobic phase lasted 45 min and the aerobic phase 140 to 260 min. For DPAO study, anaerobic phase lasted 180 min with PE as carbon sources and 75 min with final effluent amended by HAc as carbon sources; anoxic phase lasted 90 min and NO3-N, NO2-N and both NO3-N and NO2-N as electron acceptor were added at the beginning of the anoxic phase with an initial concentration of either 5 mg NO2-N/L or 5 mg NO3-N/L or both 5 mg NO2-N/L and 5 mg NO3-N/L; aerobic phase lasted 30 to 260 min. During the anaerobic and anoxic phase, dissolved oxygen (DO) was controlled to below 0.05 mg/L by bubbling nitrogen gas into mixed liquor; during the aerobic phase, DO was controlled to above 1.5 mg/L by using air diffusers to bubble air into the liquid. NaOH and H2SO4 were used to control the pH similar to the site conditions (pH: 6.5–7.2). Sludge, including RAS and MLSS at the end of the last aerobic zone, was taken from the site prior to testing in the laboratory. The volume ratio of sludge and liquor was 1.5:2.5 and MLSS in the reactor was maintained around 2,000 mg/L. A sample was taken from the reactor regularly (every 10–30 min). Liner regression on the first 50 min data was used to calculate the specific activity of P release and VFA-COD uptake during the anaerobic phase. Similarly, liner regression on the first 60 min data was used to calculate specific PO4-P, NO2-N and NO3-N uptake activity during the anoxic phase, and the data...
from the first 30 or 60 min were used for calculating specific P uptake activity during the aerobic phase.

Sample analysis

Standard methods (APHA 1998) were adopted in the analyses of NH$_4$$_+$$, NO$_3$-, COD, TP PO$_4$-P, ALK, and MLSS. NO$_2$ was analysed according to USEPA (1987). DO and temperature were measured by portable meter (YSI 85D). For all the liquid composition analyses the samples were filtered (0.45 μm, Whatman) immediately after sampling on site, then stored within 1 h at a temperature below 4°C prior to analysis. The samples for VFA analysis were filtered prior to acetate, propionate, butyrate and valerate analyses using gas chromatography (Prominence, Shimadzu) equipped with a flame ionization detector fitted with a DB-FFAP (30 m length, 0.25 m diameter, and 0.25 μm film) column. Specific oxygen uptake rate (SOUR) and nitrate uptake rate (NUR) measurements were according to Melcer et al. (2003). For fluorescent in-situ hybridization (FISH) analysis of PAO and GAO including the probes and procedures, etc.; see Winkler et al. (2011).

RESULTS AND DISCUSSION

PE characterization and treatment efficiency

As shown in Table 1, the average TN removal was 86% owing to mainstream PN/A and conventional denitrification (Cao et al. 2014b), and the average TP removal efficiency was 66%. Both were achieved under an influent COD/N ratio of 8.2 (BOD$_5$/N of 3.4). The average VFA concentration was 38.0 (±3.1) mg VFA/L (45.0 mg COD/L), which was calculated according to diurnal sampling and analysis of the PE (Figure 2) and was 13% of the influent COD. Of the 38.0 mg/L of VFA, 31.0 (±2.4) mg/L was HAc, 5.4 (±1.2) mg/L propionates, 1.0 (±0.2) mg/L butyrate and 0.6 (±0.1) mg/L valerate. The ratios of VFA/HAc (1.2) and HAc/HPr (5.7) indicated acetate was the dominant VFA.

The average PO$_4$-P in liquid phase of the RAS was 10 mg PO$_4$-P/L, most likely due to secondary release in the final settling tanks (FST). The PE hydraulic flow based phosphorus input from the RAS and thickener supernatant to the activated sludge process was equivalent to 5.2 mg PO$_4$-P/L, which was mainly contributed by the RAS. With
this additional PO₄ load, the PO₄-P concentration at the inlet of the activated sludge process was 10.2 mg PO₄/P/L. Given 10.2 mg PO₄-P/L at the inlet and 1.2 mg PO₄-P/L at the outlet, the average PO₄-P removal efficiency of the activated sludge process was 88% (Figure 3).

EBPR process kinetics

As shown in Figures 4 and 5, the release and uptake were most significant in the first step feed pass, then become weaker further downstream. The average P release of 12.4 mg P/L (Figure 4) in the first anoxic zone is in the same range as that reported for the EBPR process in moderate or cold climates in the USA (Neethling et al. 2005; Gu et al. 2008) and for warm climates reported by Ginestet et al. (2014), but lower than that in warm climates reported by Sayi-Ucar et al. (2015), where the VFA in the feed (>100 mg VFA/L) was two times higher than the case reported here. The uptake (removed) concentrations were higher than those released in the preceding anoxic zone (Figure 5). According to Figure 5, which shows the PE flow corrected PO₄-P release and uptake profiles, the PO₄-P removal efficiency was 86%, almost the same as that (88%) calculated according to PO₄-P at the inlet and the outlet of activated sludge process.

Three factors contributed to the higher P release and uptake in the initial step feed pass and the subsequent
decrease in the following passes. (i) The real anaerobic environment of the first ‘anoxic’ zone because of little NO$_3^-$ and NO$_2^-$ (<1.0 N mg/L) present in the PE and RAS. This resulted in little competition for carbon between PAO and heterotrophic denitrifiers, while the other four anoxic zones received higher NO$_2^-$ and NO$_3^-$ (>4 mg N/L) from the preceding aerobic zones, resulting in less carbon for PAO due to the competition with heterotrophic denitrifiers. As a consequence, the intracellular stored PHA/glycogen for PAO decreased, impairing P uptake activity in the aerobic zone (Neethling et al. 2005). (ii) P taken up by DPAO (see section Denitrifying PAO) occurring in the second to fifth anoxic zones, which reduced the apparent P release activity observed. (iii) The intracellular PHA/PHV storage of PAO increased after FST as reported by Yang et al. (2014). The batch tests also showed that following a pre-anoxic phase with little sCOD in the liquid, the P release and sCOD uptake rate increased during the anaerobic phase (data not shown). More investigations on the mechanism(s) involved are needed.

The average P release activity of 2.6 mg P/g VSS.h in the first anoxic zone was higher than those in the downstream anoxic zones (Figure 6). But it was lower than that (4–12 mg P/g VSS.h) reported by Ginestet et al. (2014). The low F/M ratio of step-feed process is most likely the main underlying cause. In fact, the sum of release activity of 6.0 mg P/g VSS.h for the five anoxic zones was in the same range as that reported by Ong et al. (2014) and Ginestet et al. (2014). The average specific P uptake activity of 2.7 mg P/g VSS.h in the first aerobic zone (Figure 5) was close to that (2.5 mg P/g VSS.h) reported by Ong et al. (2014). The sum of the uptake activity of 9.7 mg P/g VSS.h was higher than the sum of the release activity (6.0 mg P/g VSS.h).

The ratio of P release/sCOD uptake in the first anoxic zone varied from 0.28 to 0.60, with an average of 0.45, which was close to 0.50 mg P/mg VFA-COD, the stoichiometric ratio for PAO at a temperature of 25°C and pH 7 (Smolders et al. 1994) and was in the range (>0.2 mg P/mg VFA-COD) indicating PAO dominance according to Schuler & Jenkins (2005).

Figure 7 shows that pH reduction occurred in the five aerobic zones, owing to partial nitritation with concomitant ALK reduction. pH increase was observed in the last four anoxic zones, most likely due to denitrification but not in the first anoxic zone where little denitrification occurred.
ALK increase was observed in the second, third and fourth anoxic zones but not in the first and fifth anoxic zones. The underlying causes for the irregular pattern of ALK were not clear, although the release of K⁺, Mg²⁺, Ca²⁺ concomitantly with PO₄ in the anoxic phase (Neethling et al. 2005) and the resultant interaction of the different buffer systems present (bicarbonate and phosphate) may be one of the factors.

**Ex-situ EBPR specific activity**

The ex-situ specific activities (Table 2) calculated from the profiles in Figure 8 were all higher than those of the in-situ activities and exhibited the same tendency of decreasing in downstream process due to: (i) the higher F/M ratio in batch tests; (ii) little denitrification in batch reaction fed by PE; and (iii) ideal plug flow pattern in the batch reactor experiment. The specific P release activity (25.1 mg P/g VSS.h) of RAS with the carbon of PE as electron donor was approximately the same as the maximum activity (25.6 mg P/g VSS.h, with the initial concentration of 60 mg HAc/L). The ex-situ specific P release activities of sludge from the first to fifth anoxic zones were in the similar range (5–30 mg P/g VSS.h) of plants with effective EBPR in moderate and cold temperature in the USA (Gu et al. 2008; Neethling et al. 2005) and Europe (4.0–20.6 mg P/g VSS.h) (Lopez-Vazquez et al. 2008; Fenu et al. 2013) and those (maximum activity, between 13 and 17 mg P/g VSS.h) in the full-scale nitrogen short-cut activated sludge process in the city of St Petersburg South-west WRF under warm climates (Jimenez et al. 2014) and higher than that (11 mg P/g VSS.h) of the full-scale 3 stage Bardenpho process under high temperature in the Middle East (Ginestet et al. 2014). Similar observations applied to comparisons between the specific P uptake activities (between 12.5 and 6.6 mg P/g VSS.d) in this study and those by Neethling et al. (2005), Jimenez et al. (2014), and Ginestet et al. (2014). The ratio of P-release/P-uptake activity varied between 0.8 and 4, in the same range (1.3–5) as those reported by Neethling et al. (2005). These comparisons demonstrated the strong EBPR activity of the sludge in the SFAS process at the Changi WRP. The P release/VFA-COD removal ratio of 0.61, measured from the batch test with RAS as sludge and PE as carbon substrate, and 0.58 with RAS as sludge and final effluent amended by HAc as carbon substrate, were in the same range as that (0.45 mg P/mg sCOD) measured in-situ, and further suggest the dominance of PAO in the P removal microbial community. However, the underlying causes on the almost double aerated P uptake rate of RAS fed with PE compared to that of PLE amended by HAc is not clear.

**Denitrifying PAO**

Figure 9(a) and 9(b) show that PO₄-P uptake seemed to halt when NO₃ or NO₂ was almost depleted during the anoxic phase with PE carbon as electron donor. Figure 9(c) and 9(d) show that both PO₄ uptake, along with NO₃/NO₂ and NH₄ reduction, occurred concomitantly during the anoxic phase with HAc as electron donor, indicating the co-existence of Anammox and DPAO (possibly also heterotrophic denitrification). The potential competition between DPAO and Anammox (and heterotrophic denitrifiers) for NO₂ needs further investigation. Figure 9(a)–9(d) illustrate that PAO were able to use both NO₂ and NO₃ as electron acceptors for P uptake, thus, are categorised as DPAO I according to Oehmen et al. (2010). Table 3 complies the specific activities of P uptake during the anoxic and aerobic phase and the specific activities of NO₃ and NO₂ uptake during the anoxic phase. The relative contributions of DPAO using NO₃ as electron acceptor with three different types of carbon substrates were estimated (Table 4) by NUR and SOUR measurement and a theoretical 2.86 conversion factor (dissimilated oxygen demand of per unit NO₃-N denitrified). Estimations suggested that the DPAO activity contribution was between 13% and 18% of the anoxic electron acceptor demand.

<table>
<thead>
<tr>
<th>Sludge/carbon sources</th>
<th>RAS/PE</th>
<th>RAS/final eff.amended with HAc</th>
<th>AE1/PE</th>
<th>AE3/PE</th>
<th>AE5/PE</th>
<th>RAS/final effluent, endogenous</th>
</tr>
</thead>
<tbody>
<tr>
<td>Release activity, mg PO₄-P/g VSS.h</td>
<td>25.1</td>
<td>25.6</td>
<td>17.1</td>
<td>14.2</td>
<td>6.8</td>
<td>1.8</td>
</tr>
<tr>
<td>P released/COD removed, mg PO₄-P released/mg COD up-taken</td>
<td>0.61</td>
<td>0.58</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Up-take activity, mg PO₄-P/g VSS.h</td>
<td>12.5</td>
<td>6.6</td>
<td>12.2</td>
<td>6.9</td>
<td>9.2</td>
<td>4.3</td>
</tr>
</tbody>
</table>
Phosphorus removal microbial community

Figure 10(a) and 10(b) show the dominant abundance of PAO population, and small population of GAO in the sludge, which was consistent with the P-release/VFA-COD removal ratio measured in the in-situ and ex-situ batch tests. Another study using FISH on the microbial population in the same activated sludge process reported *Accumulibacter* PAO accounted for 6.0% of total EUBmix stained area, and *Tetrasphaera* PAO accounted for 4.0% of total EUBmix stained area (Yang et al. 2016), which were comparable with those found in EBPR processes in the USA (Gu et al. 2008) and Europe (Mielczarek et al. 2013); while GAO were rarely detected in the sludge (Yang et al. 2016), which was consistent with our FISH images presented here.

In addition to stable supply of VFA-COD of the feed, plug-flow anoxic-aerobic reactor configuration, short aerobic SRT (2.5 d) and effective PN/A process contribute to the successful EBPR co-existence with PN/A in the SFAS process at the Changi WRP. The effective PN/A process plays a determining role in little NO₃⁻/NO₂⁻ in RAS, thus generating an anaerobic environment in the first anoxic zone favouring P release and VFA-COD uptake. The essential role of PN/A process in providing ‘additional’ VFA-COD utilization to PAO compared to heterotrophic denitrification is illustrated in the following section of fate of COD. The short aerobic SRT favours proliferation of

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**Figure 8** | PO₄-P release and uptake profiles measured by batch tests. ANA: anaerobic phase, AE: aerobic phase. Number: sequence of the step feed.

**Figure 9** | (a) P release and uptake in the presence of NO₂⁻ and oxygen with initial presence of electron donor from PE carbon. (b) P release and uptake in the presence of NO₃⁻ and oxygen with initial presence of electron donor from PE carbon. (c) P release and uptake in the presence of NO₂⁻, NH₄⁺ and oxygen with an initial concentration of 60 mg HAc/L. (d) P release and uptake in the presence of NO₂⁻, NO₃⁻, NH₄⁺ and oxygen with an initial concentration of 60 mg HAc/L.
PAO and wash-out of GAO (Daigger et al. 1988; Whang & Park 2006; Whang et al. 2007; Barnard & Comeau 2014). As a consequence, efficient EBPR under warm climates can be achieved under limited supply of VFA-COD as demonstrated in this study and other full-scale processes (Onnis-Hayden et al. 2013; Ginestet et al. 2014). Furthermore, recent reports (Cao et al. 2013; Ong et al. 2013, 2014; Sayi-Ucar et al. 2015) showed even under a longer SRT, high efficient EBPR can be achieved under warm climates when a high influent bCOD/N ratio (e.g. >10) is maintained or additional carbon input is provided. In this situation, PAO are able to share VFA-COD, co-exist with GAO, and to be active in the process. In summary, effective EBPR is achievable in warm climates under several conditions. This paper also illustrates stable EBPR with dominance of PAO population can be achieved and maintained in warm climates when acetate, rather than propionate, is predominantly in the feed. This may provide an opportunity to re-think the popular opinion, which forms the basis of modelling EBPR in high temperatures, that propionate supports sustainable PAO population, while acetate favours GAO (Whang et al. 2007; Lopez-Vazqueza et al. 2009b; Oehmen et al. 2010).

To reduce the final effluent P concentration, it is necessary to explore alternate operational strategy for the FST to reduce the high returning PO4-P loading from the RAS to the activated sludge process. Also, the centrifuge thickening operation needs attention.

### Fate of carbon

A COD mass balance for the activated sludge process was conducted using plant operational data (Table 5). Heterotrophic denitrification (NOx-N ≤ 1.5 mg N/L denitrified) carbon usage in the FSTs, carbon input from the thickening supernatant and the variation in carbon contents of RAS were negligible relative to the PE carbon. Carbon usage

#### Table 3 | Specific NO3, NO2 uptake activities during the anoxic phase and PO4-P uptake rate during the anoxic and aerobic phase (all tests using RAS)

<table>
<thead>
<tr>
<th>Electron donor (carbon source)</th>
<th>PE</th>
<th>HAc added to final effluent</th>
<th>Endogenous</th>
</tr>
</thead>
<tbody>
<tr>
<td>Electron acceptor</td>
<td>NO2</td>
<td>NO3</td>
<td>NO2</td>
</tr>
<tr>
<td>Anoxic PO4-P uptake, mg P/g VSS.h</td>
<td>1.7</td>
<td>2.1</td>
<td>2.9</td>
</tr>
<tr>
<td>Anoxic NOx-N uptake, mg N/g VSS.h</td>
<td>2.3</td>
<td>4.4</td>
<td>1.5</td>
</tr>
<tr>
<td>Anoxic NH4-N removal, mg N/g VSS.h</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Aerobic PO4-P uptake, mg P/g VSS.h</td>
<td>5.1</td>
<td>3.1</td>
<td>3.6</td>
</tr>
</tbody>
</table>

#### Table 4 | Oxygen and nitrate uptake activities during the anoxic phase

<table>
<thead>
<tr>
<th>Electron donor</th>
<th>PE</th>
<th>Acetate</th>
<th>Endogenous</th>
</tr>
</thead>
<tbody>
<tr>
<td>Electron acceptor</td>
<td>NUR, mg NO3-N/g VSS.h</td>
<td>2.3</td>
<td>1.5</td>
</tr>
<tr>
<td>SOUR, mg O2/g VSS.h</td>
<td>36</td>
<td>30</td>
<td>22</td>
</tr>
<tr>
<td>SOUR/NUR</td>
<td>5.4</td>
<td>7.0</td>
<td>5.5</td>
</tr>
<tr>
<td>DPAO contribution, %</td>
<td>18</td>
<td>13</td>
<td>18</td>
</tr>
</tbody>
</table>

#### Table 5 | COD mass balance for the activated sludge process

<table>
<thead>
<tr>
<th>Carbon source</th>
<th>PE</th>
<th>HAc added to final effluent</th>
<th>Endogenous</th>
</tr>
</thead>
<tbody>
<tr>
<td>Electron donor (carbon source)</td>
<td>NO2</td>
<td>NO3</td>
<td>NO2</td>
</tr>
<tr>
<td>Electron acceptor</td>
<td>Anoxic PO4-P uptake, mg P/g VSS.h</td>
<td>1.7</td>
<td>2.1</td>
</tr>
<tr>
<td>Anoxic NOx-N uptake, mg N/g VSS.h</td>
<td>2.3</td>
<td>4.4</td>
<td>1.5</td>
</tr>
<tr>
<td>Anoxic NH4-N removal, mg N/g VSS.h</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Aerobic PO4-P uptake, mg P/g VSS.h</td>
<td>5.1</td>
<td>3.1</td>
<td>3.6</td>
</tr>
</tbody>
</table>

Figure 10 | (a) FISH picture showing PAO (green, mix Cy3) and GAO (dark pink, Mix Fluos). (b) All bacteria (blue, Mix 338 Cy5). Please refer to the online version of this paper to see this figure in colour: http://dx.doi.org/10.2166/wst.2016.565.
for heterotrophic denitrification was calculated based on the nitrogen balance (refer to Table 5 ‘Heterotrophic dissimilation’), which illustrated the significant contribution of PN/A to nitrogen removal. The COD usage of 75 mg VFA-COD/L EBPR was calculated according to the stoichiometric coefficient of 10 mg VFA-COD for 1 mg PO₄-P removal (Wentzel et al. 1988; Henze et al. 1997; Grady et al. 1999) and 7.5 mg P/L removed by the EBPR process calculated from 10.2 mg P/L of the influent, 1.2 mg P/L in the effluent of the activated sludge process, and 1.5 mg P/L for normal growth of bacteria. Given the waste sludge being assimilating biomass of all bacteria, and 0.5 kg COD biomass/kg COD removed as the observed yield coefficient (Henze et al. 1997), the influent based COD used for EBPR dissimilation was 38 mg/L (7.5/2 × 10 mg COD/L). As illustrated in Figure 11, 11% of the input COD from the PE was used for EBPR dissimilation, which was same as that (36 mg COD/L) for heterotrophic denitrification dissimilation.

For an MLE process with a similar influent C/N ratio as in this study in Singapore, 20 mg NO₃-N/L was denitrified (Cao et al. 2014b). Assuming 5.7 mg COD/L used per mg NO₃-N/L heterotrophically denitrified (Henze et al. 1997), the COD used for conventional denitrification dissimilation will be 57 mg COD/L (accounting for 17% of influent COD). This suggests that PN/A provided an additional 21 mg COD/L for EBPR dissimilation, which can remove about 4 mg PO₄-P/L. Kinetically, fast uptake of VFA-COD by PAO in the anaerobic/anoxic zone largely limited the carbon available for heterotrophic denitrification (Onnis-Hayden et al. 2013). It has the same effect as a reduction in the influent BOD₅/N ratio from 3.4 to 1.6 favouring the Anammox process. The case reported in this paper and the EBPR in full-scale nitrogen short cut process in the city of St Petersburg Southwest WRF, USA (Jimenez et al. 2014) demonstrate the feasibility to accommodate EBPR into the mainstream PN and PN/A process. The local conditions, typically temperature, etc., should be taken into account when considering this feasibility.

CONCLUSIONS

High efficiency EBPR was achieved concomitantly with excellent nitrogen removal in a 200,000 m³/d mainstream PN/A activated sludge process in Singapore. Ex-situ specific P release and uptake activities were comparable with those of effective EBPR in the USA and Europe. PAO populations
were dominant and able to use NO₃ and NO₂ as electron acceptors to perform PO₄-P uptake in anoxic conditions. Few GAO were observed as compared to PAO. COD oxidation (11% of input COD) by EBPR dissimilatation was the same as that of heterotrophic denitrification. Compared to conventional biological nitrogen removal, much lower carbon demand for nitrogen removal owing to autotrophic PN/A conversions made effective EBPR possible. This paper demonstrated for the first time the occurrence of EBPR co-existing with PN/A in mainstream process, the feasibility of accommodating EBPR into mainstream PN/A, and the interactions between C, N- and P Cycle forming an energy and resource – efficient BNR in a large full-scale activated sludge process. It also showed that EBPR can work under warm climates.

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