Long-term study on the impact of temperature on enhanced biological phosphorus and nitrogen removal in membrane bioreactor

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A B S T R A C T

The study involved experimental observation and performance evaluation of a membrane bioreactor system treating municipal wastewater for nutrient removal for a period 500 days, emphasizing the impact of high temperature on enhanced biological phosphorus removal (EBPR). The MBR system was operated at relatively high temperatures (24–41 °C). During the operational period, the total phosphorus (TP) removal gradually increased from 50% up to 95% while the temperature descended from 41 to 24 °C. At high temperatures, anaerobic volatile fatty acid (VFA) uptake occurred with low phosphorus release implying the competition of glycogen accumulating organisms (GAOs) with polyphosphate accumulating organisms (PAOs). Low dissolved oxygen conditions associated with high wastewater temperatures did not appreciably affect nitrification but enhanced nitrogen removal. Dissolved oxygen levels around 1.0 mgO2/L in membrane tank provided additional denitrification capacity of 6–7 mgN/L by activating simultaneous nitrification and denitrification. As a result, nearly complete removal of nitrogen could be achieved in the MBR system, generating a permeate with no appreciable nitrogen content. The gross membrane flux was 43 LMH corresponding to the specific permeability (K) of 413 LMH/bar at 39 °C and above, indicating nearly complete removal of nitrogen could be achieved in the MBR system.

1. Introduction

The wastewater temperature is of great importance in biological treatment since it influences not only the physical state of certain substances (i.e. solubility of oxygen, ammonia) but also the reaction rates of biochemical processes for nutrient removal. There is a limited literature body on nutrient removal processes in countries in the tropical climate zones, especially with respect to the impact of the high temperature on enhanced biological phosphorus removal (EBPR).

In general, the rate of biological processes increase at higher temperatures. This was tested by Krishna and Van Loosdrecht (1999), specifically for substrate storage and settleability, in a temperature range including the tropical level above 30 °C; the accumulation of storage polymers was found to increase with temperature, together with observable rates of related biochemical reactions. Temperature dependency of EBPR was also explored by Brdjanovic et al. (1997) using an anaerobic-aerobic, acetate-fed, sequencing batch reactor sustained at 20 °C. Conversion of relevant compounds for biological phosphorous removal was investigated at 5, 10, 20 and 30 °C in separate batch tests; while a continuous increase was observed in the interval of 5–30 °C for the conversion rates under aerobic conditions, the rate of anaerobic phosphorous-release (or acetate-uptake) mechanism reached a maximum at 20 °C with a steady increase between 5 and 20 °C that could be defined in terms of temperature coefficient, θ of 1.078. This was not in agreement with earlier studies (Barnard et al., 1985; Ekama et al., 1984) reporting that EBPR efficiency was higher at lower temperatures in the range from 5 to 24 °C.

Only a few studies, mostly carried out at laboratory-scale with synthetic substrates, were devoted to the fate of the EBPR process in the tropical temperature range. Baetens (2001) reported declining rates of phosphate release and uptake at temperatures of 35 °C and higher, with a significant inhibition at 42.5 °C and above.
while no phosphate release or uptake was observed at 45 °C. Undoubtedly, temperature dependency of EBPR efficiency closely depends upon the metabolic competition between phosphorus accumulating organisms (PAOs) and glycogen accumulating organisms (GAOs). In this context, Lopez-Vazquez et al. (2008) investigated the short-term temperature effects on the aerobic metabolism of glycogen-accumulating organisms within a temperature range from 10 to 40 °C, concluding that GAOs were not observed to have metabolic advantages over PAOs concerning the effects of temperature on their aerobic metabolism; At high temperatures, competitive advantages favoring GAOs were associated with anaerobic processes (Lopez-Vazquez et al., 2009a) also evaluated the influence of temperature together with different carbon sources and pH on the competition between PAOs and GAOs in pure culture studies; they found that at low and moderate temperatures PAOs remained dominant and sustained effective EBPR, whereas at high temperature (30 °C), GAOs tended to be the dominant species. While these studies provided valuable clues, the impact of tropical temperature still needs to be assessed, based on long-term observations of treatment systems operating with real sewage.

Aside from direct metabolic impact, high temperature may also impair and reduce in the reactor the level of dissolved oxygen in systems where aeration cannot be adjusted. The effect of low dissolved oxygen conditions induced by high temperature is definitely worth investigating for all aerobic processes related to nutrient removal, especially on nitrogen removal mechanisms involving simultaneous nitrification-denitrification (SND) process (Hocaoglu et al., 2011; Insel et al., 2011; Münch et al., 1996). The filtered COD experiments showed that nearly all soluble biodegradable COD fractions were consumed. However, remaining slowly biodegradable COD together with endogenous heterotrophic biomass could serve as carbon source for SND in the aerobic and MBR tanks.

In this study, the effect of long-term temperature variation on enhanced biological phosphorus removal process was investigated for a membrane bioreactor system treating a high strength municipal wastewater in Saudi Arabia. The EBPR performance of a pilot MBR was evaluated together with biological nitrogen removal under varying process temperatures ranging from 25 to 40 °C during 500 days of operation. It should be noted that the pilot MBR selected for the study also served for testing the feasibility of effluent recovery and reuse, which was defined as the other major objective of the study. The MBR system proved to be useful in the sense that it eliminated all settling problems enabling uninterrupted survey and evaluation of the nutrient removal performance under different operating conditions.

2. Material and methods

2.1. Pilot plant information

The experimental study was carried out using a pilot-scale membrane bioreactor, which was installed at the head works of a sewage treatment plant to enable easy intake of raw wastewater of Dubai Municipality at Al Aweer (Fig. 1). Degritted sewage was fed to the MBR pilot plant having a 1 mm screen prior to the bioreactors. The pilot plant used in this study was a flat-sheet membrane bioreactor with a cut-off size of 0.2 μm based on microfiltration. The membrane module (total area of 18 m²) was supplied from Hitachi, Japan. It included a pumping station, anaerobic (selector) tank (0.9 m³), anoxic tank (1.26 m³), aerobic (1.8 m³) and a membrane tank (2.5 m³) where the membrane cassettes were immersed. Treated effluent was collected in a permeate tank (0.3 m³). The process flow diagram of the MBR pilot was illustrated in Fig. 2.

The pilot plant was setup in a very flexible way to test the system under all biological nutrients removal (BNR) options that are commonly applied in full scale systems (University of Cape Town (UCT), Virginia initiative plant (VIP), Bardenpho, Anaerobic-Anoxic-Oxic (A²O) etc.). This also enabled to investigate the effect of high dissolved oxygen (DO) on EBPR and denitrification in case membrane return activated sludge (RAS) is returned directly to the head of the plant without being initially returned to the aeration tank. The pilot plant was equipped with online oxidation reduction potential (ORP), pH, DO measurement for controlling the process together with differential pressure (PIT) and flow controllers (FIT) for membrane filtration (Fig. 2). The membrane tank also enables sludge wastage (WAS) for sludge retention time (SRT) control.

In this study, the airflow rate was set to a constant level to investigate the impact of DO variability on the system performance under dynamic loadings. It was possible to observe the direct and indirect effects of temperature on the physical and bioprocesses. In this regard, the combined effect of GAO-PAO competition together with DO oscillations in aeration and MBR tank were experimented. It is important to note that the aeration intensity of MBR module had been limited by the membrane producer.

2.2. MBR operation

During this study, the MBR operation was carried out based on UCT configuration. The average influent flow rate (Q) was adjusted to 25.3 ± 4.2 m³/day. The “A” and “S” flow rates were set to 4 Q and Q, respectively. The return activated sludge was adjusted to 45 m³/day to convey activated sludge back to the aerobic reactor. The hydraulic retention time of the system was 9 h. The hydraulic retention time for anaerobic, anoxic and aerobic tanks corresponds to those temperatures of 25, 30 and 37 °C, respectively. Based on average mixed liquor suspended solids (MLSS) concentration in MBR tank was maintained in the range of 11,000–13,000 mg/L together with a scouring airflow rate of 8 Nm³ per hour. The aerobic reactor was bubbled with blower/diffuser system having 30 Nm³/hour airflow rate. The filtration was provided with external peristaltic permeate pumps. The filtration and relaxation periods of the membranes were adjusted to 20 and 2 min, respectively by programmable logic controller (PLC) system. The prolonged air scouring was applied and it continued during relaxation period. The excess sludge was wasted from RAS stream in order to adjust sludge age. Average daily sludge wastage rates were 550, 440 and 320 L/day for the corresponding temperatures of 25, 30 and 37 °C, respectively. Based on mass balance over TP, the actual total SRTs corresponding to those temperatures can be calculated as 10, 11 and 13 days. The SRT of the
The process temperature increased from 24°C to 36°C starting from day 201 to day 350, accordingly (Fig. 3). The DO in aerobic and membrane tanks was measured as 3.5 mg/L and 1.0 mg/L, respectively. Depending upon influent loads, a large extent of DO variation was reported within this period. The average nitrate (NO₃⁻) levels in permeate were recorded as 3.8 ± 2.5 mgN/L. Similarly, a complete oxidation of ammonia nitrogen (NH₄⁺) was achieved, as well. In addition, the average effluent phosphorus (PO₄³⁻) concentration of 1.1 ± 0.5 mgP/L slightly increased to 1.4 ± 0.7 mgP/L and leveled off as shown in Fig. 5. The ratio of influent average COD/TKN ratio was 11.6 ± 1.7 gCOD/gN in period P4.

• Fifth Period (P5: Day 351 to 500): Starting from day 351, the process temperature increased up to 37.5°C within 150 days as indicated in Fig. 3. The influent COD/TKN ratio was 11 ± 1.2 gCOD/gN. The average dissolved oxygen (DO) concentration in aerobic and membrane tanks was around 1.8 ± 1 mgO₂/L and 1.0 ± 0.5 mgO₂/L, respectively (Fig. 5). The average effluent phosphorus (PO₄³⁻) concentration was reported as 3.0 ± 1.4 mgP/L, as summarized in Table 2.

3.2. Influent wastewater characterization

The plant performance was monitored during 500 days of operation starting from date 12.06.2011 until 24.10.2012. Yearly
The variation of process temperature was illustrated in Fig. 3. The minimum and maximum measured temperatures were measured as 23.6 and 41.7 °C, yielding an average value of 33.6 ± 4.3 °C.

Respectively, Table 1 shows the summary of average (x̄) and standard deviation (σ) of influent parameters corresponding to the different experimental periods. It should be noted that a one-week rain event was reported starting from day 230. The rest of the time, no rain event occurred in the region. The yearly average influent wastewater characterization of the pilot plant was also included in Table 1.

The average COD, TKN and TP concentrations were 785 ± 228 mg/L, 65 ± 9 mgN/L and 9.9 ± 2.1 mgP/L, respectively. The average influent COD/TKN ratio was 12 ± 2 which is suitable for efficient nitrogen removal (Henze et al., 2008; Randall et al., 1992). Fig. 3 below shows the influent COD/TKN profile during MBR operation. It is important to note that influent wastewater contains high VFA levels (around 100 mgCOD/L) due to solubilization and fermentation of biodegradable organics in sewer system at elevated temperatures. The results during the different experimental periods showed good EBPR seems to be possible because of high VFA.

Table 1
Influent wastewater characterization and temperature during the different operational periods.

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<td>740</td>
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<td>1010</td>
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<td>25</td>
<td>205</td>
<td>21</td>
<td>196</td>
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<tr>
<td>BOD₅</td>
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<td>VFA⁴</td>
<td>mgO₂/L</td>
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<td>118</td>
<td>14</td>
<td>111</td>
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<tr>
<td>TSS</td>
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<td>356</td>
<td>128</td>
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<td>71.7</td>
<td>10</td>
<td>75.1</td>
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<tr>
<td>NH₄-N</td>
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<td>3.2</td>
<td>41.9</td>
<td>2.4</td>
<td>43.3</td>
<td>3.2</td>
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<tr>
<td>Total P</td>
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<td>2.2</td>
<td>10.2</td>
<td>2.1</td>
<td>10.7</td>
<td>2.5</td>
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<td>PO₄-P</td>
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<td>Temp. °C</td>
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<td>4.3</td>
<td>37.4</td>
<td>0.8</td>
<td>31.7</td>
<td>3.3</td>
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Table 2
Process performance of membrane bioreactor system during periods.

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<td>Effluent conc.</td>
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<td>mgO₂/L</td>
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<td>NH₄</td>
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<td>4.1</td>
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<td></td>
<td>NO₃</td>
<td>mgN/L</td>
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<td>2.1</td>
<td>6.1</td>
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<td>mg/L</td>
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<td>3.3</td>
<td>6.2</td>
<td>4.0</td>
<td>10.0</td>
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<td></td>
<td>TP</td>
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<td>4.3</td>
<td>1.5</td>
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<td>Removal performance</td>
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<td>0.6</td>
<td>97</td>
<td>0.8</td>
<td>98</td>
<td>0.4</td>
<td>98</td>
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<td></td>
<td>NH₄ %</td>
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<td>90</td>
<td>6</td>
<td>86</td>
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<td>91</td>
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<td></td>
<td>TP %</td>
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<td>22.1</td>
<td>47</td>
<td>13</td>
<td>53</td>
<td>18</td>
<td>91</td>
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<tr>
<td>Operation</td>
<td>Temp. °C</td>
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<td>4.3</td>
<td>37.4</td>
<td>0.8</td>
<td>31.7</td>
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<td></td>
<td>SRT days</td>
<td>12</td>
<td>4.1</td>
<td>13</td>
<td>3.0</td>
<td>11</td>
<td>2.3</td>
<td>10</td>
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<tr>
<td></td>
<td>DO in aer mgO₂/L</td>
<td>3.1</td>
<td>1.2</td>
<td>3.2</td>
<td>0.7</td>
<td>4.1</td>
<td>0.5</td>
<td>3.9</td>
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<tr>
<td></td>
<td>DO in MBR mgO₂/L</td>
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<td>0.8</td>
<td>0.9</td>
<td>0.8</td>
<td>2.5</td>
<td>0.7</td>
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<td>Anox. ORP mV</td>
<td>−226</td>
<td>69</td>
<td>−217</td>
<td>67</td>
<td>−183</td>
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<td></td>
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<td>70</td>
<td>−471</td>
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<td>−490</td>
<td>51</td>
<td>−468</td>
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</table>

Fig. 3. Yearly variation of process temperature and influent COD/TKN ratio.
levels in raw sewage (Metcalf et al., 2003; Randall et al., 1992; Wentzel et al., 1992). The influent pH of raw wastewater was 7.4 ± 0.3 during the experimental period. The alkalinity of the inlet wastewater was measured as 5.6 ± 0.1 mmol/L.

3.3. Operational parameters

The influent flow rate of the system was adjusted to 17 m³/day via pressure and flow control in the pilot MBR (Fig. 2). The net flux can be calculated as 43 LMH. The hydraulic permeability (K) was calculated as 288 LMH/bar and 413 LMH/bar at 25 °C and 39 °C, respectively. The Specific Air Demand (SAD) was 0.59 Nm³/m² which corresponds to 15 Nm³ air/m³ permeate. The average mixed liquor suspended solids (MLSS) concentration in anaerobic, anoxic, aerobic and MBR tank were kept at 4000 ± 1375, 7150 ± 1530, 8250 ± 1375 and 12400 ± 2035 mgSS/L, respectively by excess sludge wasting from RAS (Fig. 2). The sludge wastage rates were adjusted to 540 and 350 L/day during winter and summer periods. In order to calculate the actual SRT (Table 2), phosphorus balance was provided considering the inlet, permeate and sludge wastage streams.

As summarized in Table 2, the SRT of the system ranged from 9.0 to 13.0 days pertaining to the winter and summer periods. In order to calculate SRT precisely, the phosphorus balance in the system was summarized in Table A.1 (Appendix 1) together with the equation used for SRT calculation. The relative errors reflect the % difference between inlet and outlet loads. The error in calculation of TP balance was below 3% for both summer and winter periods.

Regarding the performance, the system exhibited full

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Fig. 4. Air Flow and Dissolved Oxygen profiles in aeration and membrane tank.

Fig. 5. PO₄-P profiles in anaerobic tank and membrane bioreactor permeate.
nitrification during the course of operation despite the SRT variation. The highest performance with respect TN was reported in winter periods at SRT of 9 days. On the other hand, no correlation between denitrification and EBPR performance with different SRTs ranging from 9 to 13 days (In Table 2). The literature studies related to the SRT impact on EBPR and nitrogen removal also supported that finding (Onnis-Hayden et al., 2013; Orhon and Artan, 1994).

The airflow rate ($Q_{Air}$) in aeration tank was adjusted to 30 m$^3$/hour until day 300 (Fig. 4). Higher aeration intensity yielded an average dissolved oxygen 4–5 mgO$_2$/L in the aeration basin for the periods of P3 and P4. Fig. 4, below reflects the dissolved oxygen (DO) concentrations in aerobic and MBR tanks during 500 days of operation. A direct impact of temperature on DO levels can be seen in Fig. 4. For instance, the increasing trend in DO profile from P1 to P3 can be attributed to the higher solubility of dissolved oxygen (DO) as a result of low process temperature (Perry et al., 1997). The variations in DO resulted from the variations in conversion rate and oxygen solubility while the aeration flow rate was kept constant. Period P3 has the highest DO level that corresponds to the coldest process temperature of 25°C at a constant air supply rate of 30 Nm$^3$/hour. Short-term oscillation of DO dynamics can be attributed to the change of incoming COD and N loads causing temporal changes of oxygen uptake rate. In the following phase, the airflow rate was reduced to 20–25 m$^3$/h starting from day 300 until the end of period P5 due to diffuser fouling in the aeration tank. The impact of airflow reduction can also be observed in aeration and MBR tank by analyzing the DO profiles. As a result, the reduction of aeration intensity ($Q_{Air}$) and high temperatures lowered the DO concentrations below 1 mgO$_2$/L in aeration tank and 0.5 mgO$_2$/L in membrane tank within period P5.

3.4. Phosphorus removal performance

The effluent phosphorus profile can be evaluated on the basis of long and short-term operation of the pilot system (Fig. 5). Temperature dependent competition between GAOs and PAOs impacted on the temporal variation of EBPR. Under anaerobic conditions, the competition of substrate uptake kinetics of those microorganisms determined the abundances (PAOs or GAOs) in the system. This condition can also be validated from the P release (in anaerobic tank) capability of biomass (around 20 mgP/L) at high temperatures (Fig. 5). The P release over VFA uptake ($P_{rel}$/$VFA_{uptake}$) was around 0.2 mgP/gCOD/VFA at high temperature periods of P1–P5 where the TP removal level was around 45%. However, the value of this parameter increased to 0.4–0.45 mgP/gCOD when the process temperature dropped to 24°C, securing a high EBPR efficiency of 89% of MBR system. It can be expected that the GAO impaired the activity of PAOs via utilizing the VFA pools much faster in the anaerobic tank at higher temperatures. The performance of MBR system together with operational parameters was summarized in Table 2, below.

The lowest effluent P concentration was reported after the first half of the period P3 by securing complete removal of phosphorus at 230th day at 24°C. In Period 4, the effluent PO$_4^{3-}$ concentration exhibited an increasing trend with temperature increase from 24°C to 35°C. As a result, a gradual decline of phosphorus removal performance from 95% to 85% was experienced in period P4. Finally, the phosphorus removal efficiency dropped to 50% at the end of the final segment (P5) just after the increase in process temperature. In this study, the VFA was completely utilized, however, the phosphorus was not released especially at the temperatures over 30°C.
This evaluation relied upon the latest findings in the literature (Lopez-Vazquez et al., 2008; 2009a; 2009b). The filtered COD in the anaerobic tank was monitored around 35 mg/L during the course of operation. The ortho phosphate in anaerobic tank was also measured in parallel to filtered COD samples. At high temperatures, the COD could be taken up by biomass corresponding to less P release in the bulk compared to low temperature operation. So, this observation was attributed to the presence of GAOs that were able to store soluble substrates (VFAs). The proliferation of other microorganisms (i.e. SRBs, Methanogens) were not possible since their growth rate ($\mu_{\text{max}} = 0.1 - 0.2 \text{ d}$) is too low to outcompete other heterotrophic microorganism under anaerobic conditions (the anaerobic SRT of the system can be calculated as $\text{SRT}_{\text{anaer}} = \text{Anaerobic Mass Fraction} \times \text{SRT} = 15\% \times 10 \text{ days} = 1.5 \text{ days}$).

Many studies proved that the temperature was regarded as a key factor for the dominance of glycogen accumulating bacteria (GAOs) in activated sludge systems (Filipe et al., 2001; Lopez-Vazquez et al., 2008; 2009a; 2009b; Yagci et al., 2003).

The average release P concentration was measured as $42 \pm 13 \text{ mgP/L}$ in anaerobic tank with the influent VFA level of $105 \pm 15 \text{ mgCOD/L}$ at the first period (P1). During this period, the lowest effluent concentration was maintained around $0.5 \text{ mgP/L}$ at $25^\circ C$ (in period P2).

The ratio of P release over VFA uptake can be calculated as $0.40 \text{ gP/gCOD}$ under anaerobic condition is in agreement with the suggested values in the range of $0.35 - 0.50$ (Comeau et al., 1986; Mino et al., 1995; Smolders et al., 1994; Wentzel et al., 1991). It should be noted that this ratio was calculated on the basis of VFA consumed and $\text{PO}_4^{3-}$ – P released in the anaerobic tank. Since the system received real wastewater, additional VFA could be generated from interactive hydrolysis and fermentation processes mediated by the heterotrophs. The reduction for anaerobic hydrolysis ($\eta_h$) was reported to be in the range of $0.1 - 0.4$ in Activated Sludge Models (Henze et al., 2000). So, the effect of anaerobic hydrolysis on readily biodegradable COD generation under anaerobic condition was expectedly low (Ekama and Wentzel, 1999) by considering the $0.4 \text{ gP/L}$ released per gCOD taken up by PAOs. It was also suggested that the phosphorus release/uptake primarily relied upon utilization of influent VFA for the MBR system under study (Insel et al., 2012).

The filtered (0.45 $\mu$m) COD measurements conducted for anaerobic and MBR permeate were reported as $35 \pm 20$ and $21 \pm 4 \text{ mg/L}$, respectively. The filtered COD in anaerobic tank was higher than the MBR permeate. The reasons of having higher filtered COD in anaerobic tank could be (a) the presence of soluble hydrolysable COD in anaerobic tank remained after bioconversion and/or (b) physical barrier of cake filtration in membrane module that filtered out soluble COD (Hocaoglu et al., 2011).

In Fig. 6, the P content of active sludge together with process temperature was shown on daily basis throughout 500 days of operation. The P content of sludge was measured as 1.4% (w/w) in periods P1 and P5 at lowest EBPR activity. On the other hand, it increased up to 3.1% when the maximum phosphorus removal efficiency was achieved in P3 and P4. These values fit well with the P-balance over the total system. The maximum level of P content corresponds to the coldest weather conditions at 25$^\circ C$ process temperature of MBR operation. In this study, no considerable impact of dissolved oxygen levels in aerated tanks on enhanced biological phosphorus performance was observed.

The off-line measurements carried out for the anoxic reactor showed that nitrate was completely consumed, as validated by related stoichiometric calculations (Appendix 2). The complete removal of nitrate would be partly attributed to DnPAO and DnGAO activity, together with ordinary heterotrophs (Ekama and Wentzel, 1999). The available data only indicated overall activity and did not allow ascertaining the relative role of GAOs and PAOs, which would be better visualized by process modeling. During high temperature period, low dissolved oxygen in the internal recycle stream presumably supported the nitrate removal in the anoxic tank. The effect of high temperature on PAOs would also be expected to exert a similar adverse effect on DnPAO activity. This argument seems to be supported by a few off-line $\text{PO}_4^{3-}$ – P measurements performed in the anoxic tanks during summer periods, indicating no appreciable difference in $\text{PO}_4^{3-}$ – P levels under anaerobic and anoxic conditions ($T = 38^\circ C$).

### 3.5. Nitrogen removal performance

The on-line measured oxidation reduction potential during all phases of the pilot plant operation was given in Fig. 7. The ORP signal ranged from $-250 \text{ mV to } -100 \text{ mV}$ in the anoxic tank for the periods with a lower temperature (P2 – P4). In the periods with higher temperatures (P1, P5). The ORP was lower ranging from $-250 \text{ mV to } -450 \text{ mV}$. These redox values indicate anaerobic conditions. The nitrate recirculation (A-Reycle in Fig. 2) was limiting the supply of nitrate for denitrification. The process temperatures above $35^\circ C$ increased the denitrification rates as well as the hydrolysis rate for the particulate substrates. During the full experimental period no nitrite build-up was observed in aerobic and membrane tank. It is important to note that DO levels in the aerobic tank directly correlate with the DO level in the membrane tank. The oxygen transfer during membrane scouring together with oxygen utilization resulted in $0.5 - 1.0 \text{ mgO}_2/L$ in the membrane tank. As a consequence, it is expected to have variations in nitrate levels in the effluent in parallel to the DO in membrane tank. The lowest NO$_3$ concentrations (2 – 3 $\text{mgN/L}$) were reported on days 240, 300 and 420 at dissolved oxygen concentrations around $3 \text{ mgO}_2/L$ and $1 \text{ mgO}_2/L$ in the aerobic and membrane tank, respectively. The temperature had a noticable impact on total nitrogen removal due to dissolved oxygen variation in aeration and MBR tanks. This was also validated by the stoichiometric calculation for nitrogen removal (Appendix 2).

The MBR system were operated with an anoxic mass fraction of $(V_D/V_N = 0.40)$ by taking into account the actual reactor volumes of the pilot. The average nitrate concentrations in the effluent was measured as $10 \text{ mgN/L}$ (Fig. 8). This level of effluent nitrate can be calculated (Appendix 2) based upon the stoichiometric balance between denitrification potential, $N_D$ and oxidized nitrogen, NO$_3$ (Orhon and Artan, 1994). Enhanced nitrogen removal was observed by indirect activation of the SNDn process when the dissolved oxygen concentration in aerobic and MBR reactor below 2.0 and $1.0 \text{ mgO}_2/L$. The effluent nitrate concentration in permeate was calculated to be $10 \text{ mg/L}$ based upon stoichiometric relation between oxidized nitrogen and denitrification potential. However, lowering the dissolved oxygen set point below those concentrations adds extra $7 - 8 \text{ mg/L}$ additional nitrogen removal (Fig. 8).

Lower DO concentrations in aerobic and membrane tanks (days 100, 230, 310, 360) yielded nearly complete removal of total nitrogen. During those periods the DO levels in aerobic tank were $<2.5 \text{ mgO}_2/L$ and $<1.5 \text{ mgO}_2/L$ in aerobic and membrane tanks, respectively. Few filtered samples were taken from aerobic and MBR tanks for nitrate measurements. Under low DO conditions the MBR tank has $2 - 4 \text{ mgN/L}$ lower nitrate concentrations compared to the aerobic tank (results not shown). This was experimented where the DO levels in aerobic and MBR tanks were around 3 and $1 \text{ mgO}_2/L$, respectively. It should be noted that the soluble COD (0.45 $\mu$m filtered) did not differ a lot from one tank to the others. The average soluble COD concentrations in anaerobic, anoxic, aerobic and MBR tanks were measured as $35 \pm 20$; $25 \pm 6$; $20 \pm 5$ and $21 \pm 4 \text{ mg/L}$, respectively (using 350 data). However this does not directly reflect
the utilization of biodegradable COD since nearly 75% of organic matter is in the particulate form that contains mostly slowly biodegradable COD ($X_{sp}$). In addition to endogenous biomass, this COD pool can serve as a denitrification potential in the aerobic and MBR tanks at low DO levels. Several studies have indicated that additional nitrogen removal (8–9 mgN/L) could be possible due to simultaneous nitrification denitrification at lower DO levels in the membrane tank (Insel et al., 2011, 2014; Münch et al., 1996; Sarioglu et al., 2009, 2010).

It is noteworthy to mention that short-term nitrate peaks in the effluent correlated directly with the effluent $PO_4^{3-}$ concentrations and the maximum TP removal corresponded to the periods with complete nitrogen removal. This is due to the fact that there was no nitrate recirculated back to anaerobic tank, leading to less substrate competition with denitrifying heterotrophs. But also the low redox potential in the anoxic tank will have favored further selection of PAOs. During the course of operation, concomitant $NO_3^-$ and $PO_4^{3-}$ effluent peaks were reported on days of 210, 260, 315 and 380. These days were selected for evaluation since they correspond to the period covering the maximum EBPR performance periods of the system. For instance, in Period P3 (day 210), a sudden increase in nitrate concentration (7 mgN/L) resulted in an instantaneous build-up of 2.7 mgP/L effluent phosphate concentration. Similarly, sudden peak of nitrate around 10 mg/L triggered 4 mgP/L of phosphate in the MBR permeate. This can be attributed to the fact that the nitrate recycle back to the anaerobic reactor partially consumes the VFA by the denitrifying activity of ordinary heterotrophic organisms (OHOS) (Henze et al., 2008). As a result, the activity of PAOs was hindered due to the consumption of VFA by OHOS (Henze et al., 2008; Smolders et al., 1994). However, a detailed model evaluation is required to unravel the interaction of bioprocesses on systems performance including the dynamics of dissolved oxygen and nitrate. Better interpretation of the observed results will be possible by model evaluation of the system, which will be elaborated in the next phase of this study.

Recently, the effect of sulfur metabolism on EBPR performance has been emphasized by Wu et al. (2013, 2014). The activation of sulfur metabolism added competitive advantage to the PAO for replenishing internal PHA pools especially at hot climate regions. In the last period of operation (P5), a set of sulfate analyses were carried out on raw wastewater together with grab filtered samples collected from the anaerobic, anoxic, aerobic and MBR tanks. The sulfate concentrations were measured as 200, 120, 143, 151 and 158 mg/L, respectively. By comparing the $SO_4^{2-}$ concentrations in anaerobic and aerobic conditions; 31 mg/L higher sulfate levels in aerobic condition indicated a sulfur cycle was active in the system. Whether this was related to sulfur metabolism by PAOs as similar to the findings of Wu et al. (2014) would need further investigation. It was suggested that sulfur metabolism can contribute to EBPR activity especially during high temperatures. However; in this study, the GAO activity impaired the EBPR performance in spite of the sulfate metabolism of PAOs at elevated temperatures (40°C).

As a future work, the impact of sulfur metabolism on EBPR performance needs to be elaborated in detail in order to envisage the long-term interactions between GAO and PAO on long run operation of sewage plants.

4. Conclusion

Long-term survey of the pilot MBR treating sewage indicated that phosphorus release potential of the biomass was significantly reduced in the range of 40–50% during periods of high wastewater temperatures above 35°C; this induced a parallel impairment of the EBPR efficiency of the system, presumably due to competitive metabolic edge of GAOs under anaerobic conditions.

Lower dissolved oxygen concentrations of 0.5–1.5 mgO$_2$/L, established during periods of high wastewater temperature did not exert a negative impact on the rate and extent of nitrification. Enhanced nitrogen removal was observed by indirect activation of the SNDN process when the dissolved oxygen concentration remained below 2 mg/L and 1 mgO$_2$/L, in aerobic and MBR tank, respectively.

A comprehensive analysis by system modeling will further support experimental observations by providing the kinetic basis of the competition between PAOs and GAOs at high temperatures and the interactions of dissolved oxygen dynamics with biochemical reactions mediating nitrogen removal and enhanced biological phosphorus removal processes under varying process temperatures.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.watres.2015.06.054.

Appendix 1

By using daily MLSS measurements the total SRT of the system

Fig. 8. Effluent nitrogen fractions in membrane bioreactor permeate.
was calculated by using the expression as given below:

\[
SRT = \frac{V_{ana} \cdot X_{ana} + V_{anx} \cdot X_{anx} + V_{aer} \cdot X_{aer} + V_{MBR} \cdot X_{MBR}}{Q_{WAS} \cdot X_{MBR}}
\]

where:

- \(V_{ana}\): anaerobic reactor volume [0.90 m³]
- \(V_{anx}\): anoxic reactor volume [1.26 m³]
- \(V_{aer}\): aerobic reactor volume [1.80 m³]
- \(V_{MBR}\): MBR reactor volume [2.50 m³]
- \(X_{ana}\): MLSS concentration in anaerobic reactor [g/m³]
- \(X_{anx}\): MLSS concentration in anoxic reactor [g/m³]
- \(X_{aer}\): MLSS concentration in aerobic reactor [g/m³]
- \(X_{MBR}\): MLSS concentration in MBR reactor [g/m³]
- \(Q_{WAS}\): Daily flowrate for wastewater [m³/day]

The phosphorus balance for the MBR system was given in Table A1.

### Table A1
The TP balance over MBR system for SRT calculation

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Unit</th>
<th>Winter (25 °C)</th>
<th>Summer (38 °C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Influent flowrate</td>
<td>m³/day</td>
<td>17</td>
<td>17</td>
</tr>
<tr>
<td>Influent TP concentration</td>
<td>mgP/L</td>
<td>10.9</td>
<td>9.2</td>
</tr>
<tr>
<td>Effluent TP concentration</td>
<td>mgP/L</td>
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<td>6.2</td>
</tr>
<tr>
<td>Sludge Wastage Rate</td>
<td>L/day</td>
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<td>350</td>
</tr>
<tr>
<td>TP content of sludge</td>
<td>%</td>
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<td>1.3</td>
</tr>
<tr>
<td>MLSS in MBR</td>
<td>g/L</td>
<td>10.5</td>
<td>10.2</td>
</tr>
<tr>
<td>P loads in streams</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Influent P load</td>
<td>gP/day</td>
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<td>156</td>
</tr>
<tr>
<td>Effluent P load</td>
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<td>105</td>
</tr>
<tr>
<td>P load in waste sludge</td>
<td>gP/day</td>
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<td>46</td>
</tr>
<tr>
<td>Relative Error</td>
<td>%</td>
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<td>2.93</td>
</tr>
<tr>
<td>SRT</td>
<td>days</td>
<td>9</td>
<td>13</td>
</tr>
</tbody>
</table>

### Appendix 2

The average nitrogen removal performance analysis of MBR system was calculated by using the average influent wastewater characterization given in Table 1. In BNR systems, the efficiency of nitrogen removal process relies upon the balance between denitrification potential, \(N_{DP}\) and oxidized nitrogen, \(N_{OX}\). The \(N_{DP}\) basically refers to the organic matter utilization potential of the system under anoxic conditions, therefore, it is closely related to the biodegradable COD content \(\left(C_{S1}\right)\) of the influent wastewater. Assuming that the influent biodegradable COD is 80% fraction of influent COD, the denitrification potential can be calculated as:

\[
N_{DP} = \frac{V_D \cdot \left(1 - Y_{NH}\right) \cdot C_{S1}}{V} = 0.4 \cdot (1 - 0.28) \cdot 0.8 \cdot 785 = 63.2 \text{mgN/L}
\]

where

- \(V_D/V\): anoxic volume fraction [40%]
- \(Y_{NH}\): net heterotrophic yield [0.28 gcellCOD/gCOD]
- \(C_{S1}\): influent biodegradable COD [80% of \(C_{T1}\)]

A portion of influent (biodegradable) TKN is utilized by the heterotrophs under anoxic/aerobic conditions for their metabolic requirement. This amount of nitrogen is known as incorporated nitrogen, \(N_X\).

\[
N_X = i_{XB} \left(1 - Y_{NH}\right) C_{S1} = i_{XB} \left[1 - \frac{Y_H (1 + f_E b_H \theta_X)}{(1 + b_I \theta_X)}\right] C_{S1} = 0.07 \left[1 - \frac{0.6 (1 + 0.2 \cdot 0.2 \cdot 10)}{(1 + 0.2 \cdot 10)}\right] = 12.3 \text{mgN/L (A2)}
\]

where

- \(i_{XB}\): nitrogen fraction of heterotrophic biomass [0.07 gN/gcellCOD]
- \(Y_H\): true heterotrophic yield [0.60 gcellCOD/gCOD]
- \(Y_{NH}\): net heterotrophic yield
- \(f_E\): inert fraction of endogenous biomass [0.15]
- \(\theta_X\): sludge age of the system [10 days]

The NO\(_X\) governs the magnitude of oxidized nitrogen that was nitrified, and known as the remaining part of influent TKN (Eq. (A3)).

\[
N_{OX} = N_{TKN} - N_X = 65 - 12.5 = 52.5 \text{mgN/L (A3)}
\]

Then, the effluent nitrate (SNO) concentration can be calculated from the stoichiometric balance using oxidized nitrogen (\(N_{OX}\)) and denitrification potential (\(N_{DP}\)) as follows:

\[
S_{NO} = N_{OX} - N_{DP} = 52.5 - 63.2 < 0 \quad (A4)
\]

However, for the calculation of effluent nitrate, \(S_{NO}\) with the use of Eq. (A4) is not possible when \(N_{OX}\) is higher than \(N_{DP}\). This practically means that all recycled nitrate is completely consumed in anoxic volume \(V_O\) and \(S_{NO}\) solely depends upon the recycle ratio \(R\) as formulated in Eq. (A5). Thus, expected effluent nitrate, \(S_{NO}\) in MBR permeate can be calculated by incorporating \(N_{OX}\) and nitrate recirculation \(R\) in Eq (A5).

\[
S_{OX} = \frac{N_{OX}}{1 + R} = \frac{52.5}{1 + 4} = 10.5 \text{mgN/L (A5)}
\]

### References


Insel, G., Erol, S., Ovez, S., 2014. Effect of simultaneous nitrification and