Phosphorus removal in a membrane-assisted BNR process with focus on evolutions of PAOs and DPAOs
Z. Z. Wang, J. Li, C. W. Wang and Y. L. Wang

ABSTRACT

A bench-scale UCT (University of Cape Town)-type membrane bioreactor (UCT–MBR) fed with low-strength synthetic wastewater was operated to investigate phosphorus removal with reference to poly-phosphate accumulating organisms (PAOs) and denitrifying poly-phosphate accumulating organisms (DPAOs). A series of kinetic assays of PAOs and DPAOs were also conducted to analyze the metabolic activities of PAOs and DPAOs. Results showed that 93% of chemical oxygen demand (COD) and 77% of total nitrogen could be removed at 0.08 kgCOD kg\(^{-1}\) MLSS d\(^{-1}\) and 0.015 kgN kg\(^{-1}\) MLSS d\(^{-1}\) loading (MLSS: mixed liquor suspended solids). Removal efficiencies of total phosphorus increased during the experimental phase, with an ultimate removal efficiency of 96.1%. K\(_\text{ano}\) and K\(_\text{aer}\) increased from 1.95 and 6.29 mgPO\(_4^{3-}\)g\(^{-1}\) MLSS h\(^{-1}\) to 5.47 and 11.13 mgPO\(_4^{3-}\)g\(^{-1}\) MLSS h\(^{-1}\) for DPAOs and PAOs respectively, with the increased ratio of DPAOs to PAOs from 31 to 49% implying DPAO metabolic activity increased faster than that of PAOs during the DPAO accumulation phase. Pano-uptake increased by 6.6 mg L\(^{-1}\) and the ratio of PTano-uptake to PTupt increased from 58.97 to 91.62%. The ratio of DPAOs to PAOs tended to stabilize at around 50% over time.

Key words | biological nutrients removal, denitrifying poly-phosphate accumulating organisms, enhanced biological phosphorus removal, membrane bioreactor, poly-phosphate accumulating organisms

SYMBOLS AND ABBREVIATIONS

<table>
<thead>
<tr>
<th>BNR–MBRs</th>
<th>Biological nutrients removal–membrane bioreactors</th>
<th>PHB</th>
<th>Poly-(\beta)-hydroxybutyrate</th>
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<tbody>
<tr>
<td>COD</td>
<td>Chemical oxygen demand</td>
<td>PTano-up</td>
<td>Total amount of phosphate uptake in the anoxic tanks, mg d(^{-1})</td>
</tr>
<tr>
<td>(C_{\text{COD}})</td>
<td>Average concentration of chemical oxygen demand</td>
<td>PTrel</td>
<td>Total amount of phosphate release in the anaerobic tanks, mg d(^{-1})</td>
</tr>
<tr>
<td>(C_{\text{TN}})</td>
<td>Average concentration of total nitrogen</td>
<td>PTupt</td>
<td>Total amount of phosphate uptake in the anoxic and aerobic membrane tanks, mg d(^{-1})</td>
</tr>
<tr>
<td>(C_{\text{TP}})</td>
<td>Average concentration of total phosphorus</td>
<td>(r_1)</td>
<td>Recirculation from aerobic-membrane tank to anoxic tank-1</td>
</tr>
<tr>
<td>DO</td>
<td>Dissolved oxygen</td>
<td>(r_2)</td>
<td>Recirculation from anoxic tank-2 to anaerobic tank</td>
</tr>
<tr>
<td>F/M</td>
<td>Food to microorganisms</td>
<td>SPPR</td>
<td>Specific phosphate release rate, mgPO(_4^{3-})g(^{-1}) MLSS h(^{-1})</td>
</tr>
<tr>
<td>HRT</td>
<td>Hydraulic retention time, hours</td>
<td>SPUR</td>
<td>Specific phosphate uptake rate, mgPO(_4^{3-})g(^{-1}) MLSS h(^{-1})</td>
</tr>
<tr>
<td>Kaer</td>
<td>The maximum value of SPUR under aerobic condition</td>
<td>SRT</td>
<td>Sludge retention time, days</td>
</tr>
<tr>
<td>Kana</td>
<td>Maximum value of SPRR under an aerobic condition</td>
<td>TN</td>
<td>Total nitrogen</td>
</tr>
<tr>
<td>Kano</td>
<td>Maximum value of SPUR under anoxic condition</td>
<td>TP</td>
<td>Total phosphorus</td>
</tr>
<tr>
<td>NO(_X)-N</td>
<td>Sum of NO(_3)-N and NO(_2)-N</td>
<td></td>
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</tr>
</tbody>
</table>

TP_{aer} Total phosphorus in the effluent of aerobic tank
TP_{ana} Total phosphorus in the effluent of anaerobic tank
TP_{ano-1} Total phosphorus in the effluent of anoxic tank-1
TP_{ano-2} Total phosphorus in the effluent of anoxic tank-2
TP_{eff} Total phosphorus in the effluent of the membrane
TP_{inf} Total phosphorus in the influent

INTRODUCTION

Membrane bioreactor (MBR) processes are being increasingly used for wastewater reuse and/or as the pretreatment tool for reverse osmosis wastewater treatment due to the excellent effluent quality produce (Itokawa et al. 2008; Santos et al. 2011). Complete nitrification can be easily achieved in aerobic MBRs due to the total retention of nitrifiers and the prolonged cell residence time. However, the high sludge retention time provides the limitation for phosphate being incorporated into new cells and thus limits phosphorus removal. The biological phosphorus removal process requires different operational conditions for removal of carbon and nitrogen, i.e. low sludge age and high biomass production rate.

In the conventional enhanced biological phosphorus removal (EBPR) process, poly-phosphate accumulating organisms (PAOs) having high phosphorus adsorption capacity are selected to become the dominant microbial community under alternating anaerobic–aerobic conditions. PAOs are capable of assimilating volatile fatty acids (VFAs) to synthesize poly-β-hydroxybutyrate (PHB) with the release of phosphate under anaerobic conditions, followed by O₂ being utilized as electron acceptors under aerobic conditions. A high-activity state can be then maintained for the activated sludge, with PAOs favored due to their competitive advantage over non-polyphosphate accumulating microorganisms under starvation conditions (Yilmaz et al. 2007). A particular group of microorganisms called denitrifying poly-phosphate accumulating organisms (DPAOs) plays an important role in carrying out the bioprocess of phosphate removal coupled to denitrification, during which competition of external carbon sources between PAOs and heterotrophic denitrifying bacteria can be eased as PHB is consumed as the carbon source (Barnard et al. 1999). Thus operation at low food to microorganism (F/M) ratios may enhance denitrifying phosphorus removal for biological nutrients removal (BNR)–MBRs.

Unlike conventional activated sludge processes, where sedimentation is used to retain the solids, MBRs achieve complete solids retention under aerobic conditions, avoiding the release of phosphate which otherwise frequently takes place in sedimentation tanks. MBRs are thus capable of generating an effluent with total phosphorus (TP) levels lower than a conventional EBPR process (Monti et al. 2006), and so offer great potential for phosphorus removal if operated under appropriate conditions. This study aimed to evaluate the phosphorus removal performance in a University of Cape Town (UCT)–MBR process, with focus on the evolutions of PAOs and DPAOs, to provide more insights to the dynamic community successions of PAOs and DPAOs.

MATERIALS AND METHODS

Experimental set-up and operational conditions

The bench-scale plant (Figure 1) comprised a bioreactor (total volume 28 L) with UCT configuration: anaerobic tank (20% of the total volume), anoxic tank-1 (20%) and anoxic tank-2 (20%) with mechanical mixtures, followed by an aerobic tank (40%) with one submerged flat-sheet membrane (Kubota corporation: chlorinated polyethylene; pore size: 0.4 μm; surface area: 0.1 m²). A pH-dissolved oxygen (DO) sensor (WTW Multi 340i) was fitted to monitor the aerobic membrane tank dissolved oxygen levels. Temperature was controlled at 20–23 °C by a heater. The plant was provided with a programmable logic controller (PLC) and data acquisition system that controlled flows in all the streams. The membrane was operated at a flux of 20 L m⁻² H⁻¹ with intermittent suction (9 min suction and 1 min relaxation). The wastewater from the storage tank was pumped to the anaerobic tank followed by the anoxic tank-1 and anoxic tank-2, and then aerobic membrane tank and finally pumped out through the membrane module. The mixed liquor suspended solids (MLSS) concentration in the aerobic membrane tank was maintained at around 6,500 mg L⁻¹, and the MLSS concentration ratio of anaerobic tank to anoxic tank-1 to anoxic tank-2 to aerobic-membrane tank was 1:1.3:1.3:1.6. Other operating conditions are provided in Table 1.

Characteristics of synthetic wastewater and seed sludge

The synthetic wastewater used contained: CH₃COOH, 0.25–0.3 mL L⁻¹ (C_{COD}: 285.6 mg L⁻¹); NH₄Cl, 190–195 mg L⁻¹;
C\textsubscript{NH4\textsuperscript{+}}-N: 51.9 mg L\textsuperscript{-1}; KH\textsubscript{2}PO\textsubscript{4}, 25–27 mg L\textsuperscript{-1} (C\textsubscript{TP}: 6.6 mg L\textsuperscript{-1}; NaHCO\textsubscript{3}, 340–350 mg L\textsuperscript{-1}; FeCl\textsubscript{3}-6H\textsubscript{2}O, 4 mg L\textsuperscript{-1}; CaCl\textsubscript{2}, 11 mg L\textsuperscript{-1}; MgSO\textsubscript{4}, 11 mg L\textsuperscript{-1}; KCl, 8 mg L\textsuperscript{-1}; NaCl, 8 mg L\textsuperscript{-1}. The pH was controlled at 7.2–7.4 by adding NaOH and HCl solution. The seed sludge was taken from Gao bei dian wastewater treatment plant in Beijing, which employs a typical anoxic–aerobic process treating municipal wastewater and achieves satisfactory biological nitrogen removal. After 1 month of sludge acclimation, a stable performance was achieved and experiments commenced according to the operational procedures shown in Table 2.

**Analytical methods**

Chemical oxygen demand (COD), NH\textsubscript{4}\textsuperscript{+}-N, NO\textsubscript{3}\textsuperscript{-}-N, NO\textsubscript{2}\textsuperscript{-}-N, TP, PO\textsubscript{4}\textsuperscript{3-}-P, MLSS were measured according to Standard Methods (APHA 1998). The concentration of anoxic P-removal (P\textsubscript{ano-uptake}, mg L\textsuperscript{-1}) and the efficiency of anoxic P-removal (T, %) were calculated according to the following equations:

\[
P_{\text{ano-uptake}} = \frac{r_1 c_{\text{aer}} + (1 + r_2) c_{\text{ana}}}{1 + r_1 + r_2} - c_{\text{ano2}} \tag{1}
\]

\[
T = 1 - \frac{(1 + r_1 + r_2) c_{\text{ano2}}}{(1 + r_2) c_{\text{ana}} + r_1 c_{\text{aer}}} \tag{2}
\]

where \(c_{\text{ana}}, c_{\text{ano2}}, c_{\text{aer}}\) refer to the effluent phosphate concentrations from the anaerobic, anoxic (tank-2) and aerobic membrane tanks; \(P_{\text{ano-uptake}}\) and \(T\) indicate that the phosphate in both effluent of anaerobic tank and the internal recycle \((r_1)\) was removed in the anoxic tanks.

The metabolic kinetic activities of the PAOs under aerobic conditions and the DPAOs under anoxic conditions were determined using the method proposed by Wachtmeister (Wachtmeister et al. 1997). The batch tests were conducted in a 1 L sequencing batch reactor (SBR) with temperature \((21 \pm 1 ^\circ \text{C})\) controller. pH was kept around 7.2 by adding hydrochloric acid. Eight hundred milliliters of waste sludge was washed for 5 min by the distilled water to remove the ortho-phosphate in the bulk liquid.

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**Table 1** Operating conditions of the UCT-MBR plant

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Units</th>
<th>Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>HRT</td>
<td>hours</td>
<td>15.5</td>
</tr>
<tr>
<td>SRT</td>
<td>days</td>
<td>20–25</td>
</tr>
<tr>
<td>Flux</td>
<td>L m\textsuperscript{-2} H\textsuperscript{-1}</td>
<td>20</td>
</tr>
<tr>
<td>(r_1)</td>
<td>% of the inflow</td>
<td>400%</td>
</tr>
<tr>
<td>(r_2)</td>
<td>% of the inflow</td>
<td>100%</td>
</tr>
<tr>
<td>DO</td>
<td>mg L\textsuperscript{-1}</td>
<td>1.5–2.5</td>
</tr>
</tbody>
</table>

\(r_1\), \(r_2\) was adjusted according to the NO\textsubscript{x}-N concentration until 400% was reached.

**Table 2** Operational procedures of the UCT-MBR plant

<table>
<thead>
<tr>
<th>Operation</th>
<th>Procedures</th>
<th>Days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phase I</td>
<td>The start of PAO accumulation</td>
<td>0–20</td>
</tr>
<tr>
<td>Phase II</td>
<td>Characterization of PAO, DPAO</td>
<td>21–60</td>
</tr>
<tr>
<td>Phase III</td>
<td>Dynamic community evolutions of PAOs and DPAOs</td>
<td>61–90</td>
</tr>
</tbody>
</table>

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Figure 1 | Schematic diagram of the bench-scale UCT-MBR. 1. storage tank; 2. influent pump; 3. anaerobic tank; 4. anoxic tank-1; 5. anoxic tank-2; 6. recirculation pump; 7. sludge lift pump; 8. aerobic membrane tank; 9. permeate pump; 10. air compressor; 11. sludge discharge pump; 12. DO/pH sensor; 13. pressure gauge; 14. water level controller; 15. heater.
After that, the sludge was incubated under anaerobic condition with sodium acetate for 4 h. Then the anaerobic sludge was divided into two parts. One part was exposed to aerobic conditions with DO (1.5–2.5 mg L\(^{-1}\)), and the other was exposed to anoxic conditions with NO\(_3\)-N (50 mg L\(^{-1}\)) for 4 h respectively. The specific phosphate release rate (SPRR) and specific phosphate uptake rate (SPUR) were measured and compared at different operational phases.

**RESULTS AND DISCUSSION**

Characteristics of phosphorus removal at different phases

Figure 2 shows phosphorus removal over all operational phases. Phosphate release in the anaerobic tank gradually increased with time, during which inhibition impacts on the phosphate release were not apparent due to oxidized forms of nitrogen (mainly nitrate). At the beginning of the experiment, phosphorus reduction in the anoxic tanks was evident. The pH value in the anoxic tanks was controlled by dosing with HCl during the whole running phases since a high value results in chemical phosphorus removal. Pano-uptake was kept in the range of 2–3 mg L\(^{-1}\) based on mass balance, providing a \(T\) value of 35% on average. It was observed that Pano-uptake increased by 6.6 mg L\(^{-1}\) and \(T\) to 85.3% by the end of Phase II compared with Phase I. The concentration of phosphate release in the anaerobic tank was up to 30 mg L\(^{-1}\) and Pano-uptake reached 10.2 mg L\(^{-1}\) at the end of Phase III, with a TP removal efficiency of 96.1% being accomplished on average, somewhat higher than the 80% removal reported by Hanmin *et al.* (2009). The ultimate concentration of TP in the effluent decreased to 0.26 mg L\(^{-1}\) due to the complete retention of the colloidal phosphorus by the membrane.

\(P_{\text{Tupt}}\) was found to be greater than \(P_{\text{Rel}}\) from the beginning of the experiment, and also increased with the increasing of \(P_{\text{Trel}}\). The ratio of \(P_{\text{Tupt}}\) to \(P_{\text{Trel}}\) was maintained at 1.15 on average through Phases I–III, implying that the capacity of the phosphate uptake increased with phosphate released, indicating significant phosphate transformation by PAOs from the dissolved to solid state to achieve phosphorus removal.

Tests to measure kinetic activities of PAOs and DPAOs

Figure 3 presents the metabolic kinetic activities of PAOs and DPAOs at the end of Phases I and II. Kana at Day 60 reached 9.49 mg PO\(_4\)^3-\(P\) g\(^{-1}\) MLSS h\(^{-1}\), rising from 4.2 mg PO\(_4\)^3-\(P\) g\(^{-1}\) MLSS h\(^{-1}\) at Day 21. Over the same period, P levels in the effluent of the anaerobic tank increased to 35.58 mg L\(^{-1}\) from 19.16 mg L\(^{-1}\) due to the enrichment of PAOs by the anaerobic–anoxic–aerobic bioprocess. Moreover, Kano increased from 1.95 to 5.47 mg PO\(_4\)^3-\(P\) g\(^{-1}\) MLSS h\(^{-1}\) during the same period and the DPAO activity increased faster than that of the PAOs, with the ratio of DPAOs to PAOs increasing from 31 to 49%.

DPAO activity did not always increase faster than that of PAOs. Results for DPAO and PAO activity during Phase III showed that although Kano increased from 5.47 to 6.68 mg PO\(_4\)^3-\(P\) g\(^{-1}\) MLSS h\(^{-1}\) and Kae increased from 11.13 to 13.13 mg PO\(_4\)^3-\(P\) g\(^{-1}\) MLSS h\(^{-1}\), the ratio of DPAOs to PAOs tended to stabilize at 50%, suggesting that DPAOs and PAOs develop at the same rate under
these operating conditions. Similar research has shown the ratio of DPAOs to PAOs to be sustained at around 40% despite both aerobic and anoxic SPURs increasing constantly over 150 d of operation in a pilot BNR–MBR process treating municipal wastewater (Monclús et al. 2010).

Figure 4 shows the metabolic activity of DPAOs for different electron accepters at the end of Phase III based on purely aerobic conditions compared to anoxic conditions created by the addition of 50 mg L⁻¹ of NO₃⁻-N or NO₂⁻-N. Results indicate that the maximum value of 13.13, 6.68 and 3.25 mgPO₄³⁻-P g⁻¹ MLSS h⁻¹ were respectively obtained from the trials when O₂, NO₃⁻-N, NO₂⁻-N represented the electron acceptor. Although NO₃⁻-N was the dominant existing form of nitrogen in the aerobic membrane tank as well as the main electron acceptor for denitrifying phosphorus removal in the anoxic tanks, these results showed the capability of NO₂⁻-N as the electron acceptor for DPAOs. The testing results probably proved the existence of another particular group of PAOs, which could take up phosphorus using nitrite as the electron acceptor besides the nitrate and oxygen. The fraction of DPAOs (NO₃⁻-N) and DPAOs (NO₂⁻-N) among populations of PAOs was 50 and 25% respectively.

This has also been reported by Flowers et al. (2009), who claimed that DPAOs are a subgroup of PAOs which are able to use nitrate or nitrite as electron acceptors for anoxic P-uptake. Reduction capabilities of different electron acceptors normally suggest differences in the metabolism of clades of Accumulibacter lineage. PAOs I and PAOs II were classified according to the use of nitrate or nitrite only as electron acceptors, whereas both of them were capable of using oxygen as electron acceptor under aerobic conditions. Furthermore, PAOs II are responsible for reducing nitrite to nitrogen gas concomitant with anoxic phosphate uptake, while the conversion of nitrate to nitrite was conducted by denitrifying glycogen-accumulating organisms (DGAOs) (Bassin et al. 2012).

**COD, NH₄⁺-N and total nitrogen (TN) removal**

The UCT–MBR demonstrated robustness to organic loading shocks for influent COD fluctuating between 230.6 and 342 mg L⁻¹ over the all phases. During the anaerobic/anoxic/aerobic bioprocesses, the concentration of COD decreased to 35.6 mg L⁻¹ in the aerobic membrane tank and 87.5% of COD could be removed without membrane retention. It has been proved that the microbial metabolic chemicals generated (i.e. protein, carbohydrate, etc.) formed the main component of soluble COD under aerobic conditions, also contributing to irreversible membrane fouling (Chalor & Gary 2007). A 14.8 mg L⁻¹ reduction in soluble COD levels resulted from membrane filtration in the current study.

Sufficient electron acceptors could be supplied to DPAOs as a result of complete nitrification achieved in the aerobic membrane tank. 40.51 mg L⁻¹ of TN was removed on average with a removal efficiency of 77%, mainly due to the anoxic denitrification process. In addition, the concentration of NOₓ⁻-N decreased from 4.4 mg L⁻¹ in the anoxic tank-1 to 2.64 mg L⁻¹ in the anoxic tank-2, reflecting that NOₓ⁻-N could further be utilized by DPAOs to achieve denitrifying dephosphate. The anoxic tank-2 effluent NOₓ⁻-N concentration of 2.64 mg L⁻¹ was sufficiently low to avoid the phenomenon of secondary phosphorus release.

**CONCLUSIONS**

The UCT–MBR process provided significant biological nutrient removal, i.e. 93% COD combined with 77% TN at loading conditions of 0.08 kgCOD kg⁻¹ MLSS d⁻¹ and 0.015 kgTN kg⁻¹ MLSS d⁻¹. Denitrifying phosphorus removal was enhanced with Kano increasing from 1.95 at Day 21 to 5.47 mgPO₄³⁻-P g⁻¹ MLSS h⁻¹ at Day 60. DPAO metabolic activity increased faster than PAO metabolic activity with the ratio of DPAOs to PAOs increasing from 51 to 49%. Furthermore, synchronized dynamic succession of DPAOs among the populations of PAOs was observed over extended periods.

Membrane-assisted BNR process can be demonstrated as an efficient tool to strengthen denitrifying dephosphate by means of accumulating DPAOs rapidly. Further studies
are required to unravel community evolutions of PAOs and DPAOs by employing Fluorescent In-Situ Hybridization (FISH) and Polymerase Chain Reaction Denaturing Gradient Gel Electrophoresis (PCR–DGGE) analyses.

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REFERENCES


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