A novel wastewater treatment process: simultaneous nitrification, denitrification and phosphorus removal

R.J. Zeng, R. Lemaire, Z. Yuan and J. Keller
Advanced Wastewater Management Centre, The University of Queensland, St Lucia, Brisbane 4072, Australia (E-mail: raymond@awmc.uq.edu.au)

Abstract Simultaneous nitrification and denitrification (SND) via the nitrite pathway and anaerobic–anoxic enhanced biological phosphorus removal (EBPR) are two processes that can significantly reduce the COD demand for nitrogen and phosphorus removal. The combination of these two processes has the potential of achieving simultaneous nitrogen and phosphorus removal with a minimal requirement for COD. A lab-scale sequencing batch reactor (SBR) was operated in alternating anaerobic–aerobic mode with a low dissolved oxygen concentration (DO, 0.5 mg/L) during the aerobic period, and was demonstrated to accomplish nitrification, denitrification and phosphorus removal. Under anaerobic conditions, COD was taken up and converted to polyhydroxyalkanoates (PHA), accompanied with phosphorus release. In the subsequent aerobic stage, PHA was oxidized and phosphorus was taken up to less than 0.5 mg/L at the end of the cycle. Ammonia was also oxidised during the aerobic period, but without accumulation of nitrite or nitrate in the system, indicating the occurrence of simultaneous nitrification and denitrification. However, off-gas analysis found that the final denitrification product was mainly nitrous oxide (N₂O) not N₂. Further experimental results demonstrated that nitrogen removal was via nitrite, not nitrate. These experiments also showed that denitrifying glycogen-accumulating organisms rather than denitrifying polyphosphate-accumulating organisms were responsible for the denitrification activity.

Keywords Denitrification and phosphorus removal; denitrifying glycogen-accumulating organisms; nitrite pathway; nitrous oxide; simultaneous nitrification; TOGA

Introduction
Nitrogen (N) and phosphorus (P) are the key nutrients causing eutrophication in waterways. Therefore, they are compulsorily removed from wastewater sources in most developed countries. In conventional biological nutrient removal (BNR) systems, nitrogen removal is accomplished by a two-stage treatment, aerobic nitrification and anoxic denitrification (Metcalf and Eddy, 1991), while phosphorus removal is achieved through enhanced biological phosphorus removal (EBPR) under alternating anaerobic–aerobic conditions using polyphosphate-accumulating organisms (PAOs) (Comeau et al., 1986; Wentzel et al., 1988). Both N and P removal processes require COD, which is often the limiting substrate in the incoming wastewater. Making best use of the available COD for N and P removal is one of the objectives of current R&D efforts in BNR design and operation, and is also the aim of this study.

More studies have increasingly shown that nitrification and denitrification can occur concurrently in one reactor under aerobic conditions with low dissolved oxygen (DO), through the so-called simultaneous nitrification and denitrification (SND) process (von Münch et al., 1996; Bertanza, 1997). Additionally, it has been reported that nitrogen removal can be achieved by partial oxidation of ammonium to nitrite, which is then directly reduced to nitrogen gas (Surmacz-Gorska et al., 1997; Yoo et al., 1999). This process, termed SND via nitrite, saves 40% on the COD requirement compared to the conventional denitrification via nitrate. It has also been reported to achieve higher denitrification rates (Turk and Mavinic, 1986).

It has also been found that denitrification can be accomplished by the so-called
denitrifying PAOs (DPAOs) in anaerobic–anoxic EBPR systems, allowing simultaneous nitrate/nitrite reduction and phosphorus uptake using the same COD (Kuba et al., 1993; Kerrn-Jespersen et al., 1994). In addition, compared to PAOs, DPAOs are 40% less efficient in generating energy and thus have a 20–30% lower cell yield (Murnleitner et al., 1997). Therefore, the use of DPAOs in BNR systems is highly beneficial in terms of a lower COD demand, reduced aeration cost and less sludge production.

Ideally, if SND via nitrite could be accomplished with the DPAOs, even more COD could be saved because the soluble COD in the domestic wastewater is typically limiting. This would also enable a complete BNR process with just anaerobic and aerobic conditions. To demonstrate such a process was the objective of this study. Biomass was enriched in a lab-scale sequencing batch reactor (SBR) running with alternating anaerobic and low DO aerobic conditions to promote SND and P removal concurrently. Experiments were designed and performed to investigate this process, in particular to determine the nitrogen removal pathway and the organisms involved in denitrification and P removal.

Materials and methods

Reactor setup and operation
Biomass was enriched in a lab-scale anaerobic–aerobic sequencing batch reactor (SBR). The SBR, with a working volume of 4 L, was seeded with sludge from the Caboolture Sewage Treatment Plant, Queensland, Australia, a biological nutrient removal process with intermittent (SBR-type) operation. The lab-scale SBR was operated with a cycle time of 4.8 h in a temperature-controlled room (18–22°C). Each cycle consisted of a 1 h anaerobic and a 3 h aerobic period, followed by 43 min settling and 5 min decant. Two litres of synthetic wastewater (composition given below) was pumped into the reactor in the first 10 min of the anaerobic stage. During the aerobic stage, air was provided intermittently using an on/off control system to keep the DO level at between 0.45–0.55 mg/L. During an “on” period, the air flow rate was maintained at 0.5 L/min. After the settling period, 2 L of supernatant was removed, resulting in a hydraulic retention time (HRT) of 9.6 h. The wasting rate was 265 mL per day to keep the solids retention time (SRT) at about 15 days. The reactor was constantly mixed with a magnetic stirrer (250 rpm) except for the settling and decanting periods. The pH in the system was recorded but not controlled, and fluctuated between 7.0 and 7.5.

Synthetic feed
Volume of 2 L synthetic wastewater feed contained 100 mL solution A and 1.9 L solution B. Solution A contained per litre: 17.53 g NaAc·3H2O, 1 g MgSO4·7H2O, 0.45 g CaCl2·H2O, 3.3 g NH4Cl, 0.5 g Peptone (Difco) and 6 mL nutrient solution (Smolders et al., 1994). It was adjusted to pH 5.5 with 2 M HCl and autoclaved. Solution B contained per litre: 28.5 mg KH2PO4 and 32.5 mg K2HPO4, which was adjusted to pH 10 with 2 M NaOH. The complete influent contained 400 mg COD/L, 40 mg NH4·N/L and 15 mg PO4-P/L.

Batch experiments on TOGA sensor
The titration and off-gas analysis (TOGA) sensor was used for batch experiments. The sensor is a reactor-based instrument, consisting of a bioreactor, a pH control system and an off-gas measurement arrangement (Pratt et al., 2003). In this study, the dinitrogen and nitrous oxide concentrations (both products of denitrification) in inlet and outlet gases were measured, allowing the calculation of N2 and N2O transfer rates (Zeng et al., 2003b).

Analyses
Ammonia nitrogen (NH4·N), nitrate nitrogen (NO3-N), nitrite nitrogen (NO2-N) and
orthophosphate (PO₄-P) were analysed using a Lachat QuikChem8000 Flow Injection Analyser. MLSS and MLVSS were analysed according to the standard methods for the examination of water and wastewater (APHA, 1995). The measurements of acetate, glycogen, polyhydroxybutyrate (PHB) and polyhydroxyvalerate (PHV) have been described in detail by Zeng et al. (2003b).

**Results and discussion**

**Cyclic studies in TOGA**

The parent SBR reached quasi steady-state within 90 days after start-up. The MLSS and MLVSS were measured as 4.32 and 3.34 g/L, respectively. Cyclic studies were carried out in the TOGA sensor with pH controlled at 7.3, a pH that is similar to that in the parent SBR. Figure 1 shows the results from a typical cyclic study with the measured nutrient/carbon profiles in (1a) and gas profiles in (1b). During the anaerobic stage, acetate was completely consumed, which was accompanied by the consumption of glycogen, production of PHA (PHB+PHV) and release of phosphorus. In the subsequent aerobic stage, ammonium was nitrified but without nitrite or nitrate accumulation. Meanwhile, PHA was oxidized, glycogen was replenished and phosphorus was taken up. Nitrogen gas (N₂) was produced only at the beginning and at the end of the aerobic stage and in small amounts. The major final product of denitrification was nitrous oxide (N₂O). Figure 1 clearly shows that

**Figure 1** Results of a cyclic study in the TOGA sensor with nutrient/carbon profiles: (a) for the entire cycle and gas profiles; (b) for the aerated period. NOₓ-N is the combination of NO₂-N and NO₃-N

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simultaneous nitrification, denitrification and P removal (SNDPR) is possible in this system with just anaerobic and aerobic (with low DO) conditions.

**Nitrogen balance and N₂O production**

The nitrogen conversions in Figure 1 are summarised in Table 1. The amounts of N₂O and N₂ produced were obtained via the integration of the N₂O and N₂ transfer rates shown in Figure 1b. In this process, ammonia was consumed not only for nitrification/denitrification, but also for bacteria growth. The amount of ammonia for growth was calculated as follows: in the parent SBR, the SRT was 15 days, which means that 45.9 mg/L VSS was wasted per cycle based on the VSS concentration in the reactor of 3.34 g/L. Glycogen and PHA stored in the biomass contributed 21% w/w of VSS (from direct analysis). Therefore, the active biomass (excluding storage products) that was wasted per cycle was 36.2 mg/L. Assuming an active biomass formula of CH₂O₀.₅N₀.₂, 0.15 mmol N (36.2 mg/L / 24.4 mg/C-mmol * 0.2 N-mmol/C-mmol * 0.5 L) would have been used for growth. The nitrogen balance shown in Table 1 closes to 101%, indicating that the measurements are accurate.

The surprising result is that N₂O, not N₂, was the major denitrification gas product. This is a major concern since N₂O is a very strong greenhouse gas, having 310 times the greenhouse warming potential of CO₂. von Schulthess et al. (1994) reported that under low oxygen concentrations, N₂O was produced when the nitrite concentration was higher than 2 mgN/L. However, at very low nitrite concentrations, the N₂O production was almost stopped. In our system, the measurable nitrite concentration was very low (< 1 mgN/L) but there was still significant N₂O emission. The reason for it needs to be further investigated.

**Nitrogen removal pathway**

As indicated in Figure 1, there was no nitrite or nitrate accumulation during the aerobic periods. Therefore, the pathway of nitrogen removal (via nitrite or nitrate) in SNDPR is not obvious. To evaluate the nitrogen removal pathway, mixed liquor (250 mL) was transferred from the parent SBR at the end of the aerobic stage to the 500 mL TOGA bioreactor. This sludge was mixed with 247 mL RO water. 3.2 mL solution A without acetate was added to the reactor to achieve an initial NH₄-N concentration of 10 mg/L. Oxygen was controlled at 0.5 mg/L. Figure 2 shows that nitrite instead of nitrate was the major nitrification product, suggesting the pathway of nitrogen removal in the SBDPR system is via nitrite.

**Existence of glycogen-accumulating organisms (GAOs) and their activity**

The consumption of acetate and glycogen and the formation of PHB and PHV during the anaerobic phase in Figure 1a are summarised in Table 2 and compared with predictions from a well established model for polyphosphate-accumulating organisms (PAOs) (Smolders et al., 1994). Table 2 shows that the measured results do not match the PAO model predictions. Compared to the model values, less P was released and more glycogen was utilised. Furthermore, PHV was produced in significant quantities as well, which is not expected for PAOs (Mino et al., 1998) when acetate is used as the carbon source. All of

<table>
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<tr>
<th>Measured compound</th>
<th>Cyclic study in TOGA</th>
<th>Unit</th>
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<tr>
<td>NH₄-N consumed</td>
<td>-0.76</td>
<td>N-mmol</td>
</tr>
<tr>
<td>N₂O production</td>
<td>0.49</td>
<td>N-mmol</td>
</tr>
<tr>
<td>N₂ production</td>
<td>0.13</td>
<td>N-mmol</td>
</tr>
<tr>
<td>Calculated active biomass growth</td>
<td>0.15</td>
<td>N-mmol</td>
</tr>
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</table>
these findings strongly indicate that glycogen-accumulating organisms (GAOs), the competitor of PAOs in EBPR, might exist in this system. The metabolism of GAOs is similar to that of PAOs except that no phosphorus transformations are taking place (Satoh et al., 1994; Liu et al., 1996).

Recently, Zeng et al. (2003c) developed a method to predict the activity of PAOs and GAOs under anaerobic conditions. The method is based on the different stoichiometries of PAOs and GAOs models (Smolders et al., 1994; Zeng et al., 2003a). Using the total consumption of acetate and glycogen under anaerobic conditions, it can be estimated how much acetate is taken up by either PAO or GAO. This can be described as (C-mol based):

\[
a = \frac{(1 + 2\alpha_{GAO})HAc - Gly}{0.5 + 2\alpha_{GAO}}
\]

\[
b = \frac{Gly - 0.5HAc}{0.5 + 2\alpha_{GAO}}
\]

where \(a\) and \(b\) are the amounts of acetate taken by PAO and GAO, respectively, \(\alpha_{GAO}\) represents the energy required to transport 1 C-mol acetate across the GAO cell membrane and is pH dependent. At pH 7.3, which is the operating pH in the cyclic study, \(\alpha_{GAO}\) is approximately 0.075 mol ATP/C-mol HAc (Filipe et al., 2001).

Substituting \(HAc = 6.83\), \(Gly = 5.55\) from Table 2 into Eqs. (1) and (2), we obtain:

\[
a = 3.55 \quad b = 3.28
\]

Meanwhile, PHB and PHV can be found using the PAO and GAO models as (Zeng et al., 2003a):

\[
PHB = 1.33a + \frac{(9/6 + 2\alpha/3)^2}{(5/3 + 4\alpha/3)}b = 9.22
\]

\[
PHV = \frac{2.5*(9/6 + 2\alpha/3)(1/6 + 2\alpha/3)}{(5/3 + 4\alpha/3)}b = 1.56
\]

Based on the PAO model of Smolders et al. (1994), the anaerobic P release to acetate uptake ratio is: \(Y_{P/HAc} = 0.19*PHop - 0.85\), where \(PHop\) is the operating pH, which is 7.3 in this case. Therefore, the total amount of P released can be calculated as \(0.19*7.3 - 0.85)*a = 1.91\) P-mmol/L.

**Figure 2** Batch experiment without COD feeding at dissolved oxygen concentration of 0.5 mg/L showing more nitrite accumulation
These calculated values (PHB, PHV and P released) are very close to the measured values given in Table 2, which is a further confirmation of the existence and activity of GAOs in this system. From these calculations, it can be concluded that PAOs and GAOs each take up approximately half of the COD in the feed during the anaerobic period.

Which group of organisms is responsible for denitrification in the SNDPR system?

From the cyclic study data, it is still not obvious which organisms were responsible for the denitrification process in our SNDPR system. It is known that many heterotrophs can use nitrate or nitrite as electron acceptors and therefore carry out denitrification. In a recent study of ordinary heterotrophs, Drysdale et al. (2001) found that some bacteria are able to reduce NO$_3$ to N$_2$ (via NO$_2$), others are only able to reduce NO$_3$ to NO$_2$ or only NO$_2$ to N$_2$, while the rest are not able to denitriﬁcate. In our SNDPR system, we primarily need the nitrite reducing organisms and, ideally, denitrifying PAOs (DPAOs) would be selected for denitrification. However, the existence of GAOs in this SNDPR system suggests that they may also play a role in denitrification. Denitrifying GAOs (DGAOs) have recently been found in our work and described by Zeng et al. (2003b). In our SNDPR system, the denitrifying PAOs and GAOs were the two likely candidates responsible for denitrification since all soluble COD supplied to the system was completely consumed during the anaerobic period, leaving very little COD available for other heterotrophs to use under the aerobic/anoxic conditions.

In order to determine whether DPAOs or DGAOs are responsible for the denitrification, mixed liquor (250 mL) was transferred from the parent SBR at the end of the aerobic stage to the 500 mL TOGA bioreactor. This sludge was mixed with 242 mL RO water. 3.2 mL solution A was added to the reactor to achieve an initial COD concentration of 100 mg/L. After 1 h anaerobic period, 4.8 mL nitrite (2 g/L NO$_2$-N) was added at a slow rate of 0.05 mL/min. Figure 3 shows the nutrient and off-gas profiles during the anoxic stage. N$_2$ was produced for the first half hour only and declined rapidly after an initial fast increase in production rate. At the same time, the N$_2$O production rate increased and remained steady for most of the experiment, declining once the nitrite concentration decreased. The nitrite concentration was also nearly steady at around 2 mg/L. Phosphorus uptake was 5 mg/L over the 2 h experimental period.

A comparison of the phosphorus uptake rates in the cyclic study (Figure 1a) and in the batch study with nitrite addition suggests that DGAOs were likely the main organisms reducing nitrite in the system. In this experiment, only a low phosphorus uptake rate of about 2.5 mg/L/h was observed (Figure 3). This contrasts strongly with the high P uptake rates of 15–19 mg/L/h seen during the normal aerobic operation (Figure 1a). While it is known that P uptake under anoxic conditions is slower, this large discrepancy would strongly suggest that DPAO activity was very low in this system. The reason why DGAOs seemingly outcompete DPAOs under anoxic conditions needs to be further investigated.

### Table 2

<table>
<thead>
<tr>
<th></th>
<th>Measured change</th>
<th>PAO model prediction</th>
<th>PAO/GAO model prediction</th>
<th>Unit</th>
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<tbody>
<tr>
<td>Acetate consumption</td>
<td>–6.83</td>
<td>–6.83 (basis)</td>
<td>–6.83 (basis)</td>
<td>C-mmol/L</td>
</tr>
<tr>
<td>Glycogen hydrolysis</td>
<td>–5.55</td>
<td>–3.42</td>
<td>–5.55 (basis)</td>
<td>C-mmol/L</td>
</tr>
<tr>
<td>PHB formation</td>
<td>9.14</td>
<td>9.08</td>
<td>9.22</td>
<td>C-mmol/L</td>
</tr>
<tr>
<td>PHV formation</td>
<td>1.53</td>
<td>0</td>
<td>1.56</td>
<td>C-mmol/L</td>
</tr>
<tr>
<td>P release</td>
<td>1.90</td>
<td>3.28</td>
<td>1.91</td>
<td>P-mmol</td>
</tr>
</tbody>
</table>
Meanwhile, the pattern of $N_2$ and $N_2O$ production in both the cyclic study (Figure 1b) and this batch test with nitrite addition (Figure 3) is quite similar. This further supports that the main pathway of nitrogen removal in SNDPR system is via nitrite.

**Conclusions**

This research aimed at investigating the possibility and characteristics of simultaneous nitrification, denitrification and phosphorus removal (SNDPR), with alternating anaerobic and low DO aerobic stages. The main outcomes from this study are:

- Nitrogen and phosphorus could be removed simultaneously in the SNDPR system. No nitrite or nitrate was accumulated in the aerobic period. However, $N_2O$ rather than $N_2$ was the major denitrification end-product, which is a significant environmental concern.
- Nitrogen removal was found to be via nitrite rather than nitrate: ammonium was oxidized to nitrite, which was directly denitrified.
- PAOs and GAOs were found to coexist in this particular process. Their relative activities can be predicted well by the previously developed method using the acetate and glycogen consumption.
- Denitrifying GAOs seemed to be mainly responsible for the denitrification while phosphorus uptake was likely accomplished by PAOs utilising oxygen as the electron acceptor for its PHA oxidation.

**References**


