Development and optimization of a sequencing batch reactor for nitrogen and phosphorus removal from abattoir wastewater to meet irrigation standards

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ABSTRACT

A sequencing batch reactor (SBR) was used for the treatment of abattoir wastewater to produce effluent with desirable nitrogen and phosphorus levels for irrigation. The SBR cycle consisted of an anaerobic phase with wastewater feeding, a relatively short aerobic period (allowing full ammonium oxidation), a second anoxic period with feeding, followed by settling and decanting. This design of operation allowed biological nitrification and denitrification via nitrite, and therefore with reduced demand for aeration and COD for nitrogen removal. The design also allowed ammonium, rather than oxidized nitrogen, being the primary nitrogen species in the effluent. Biological phosphorus removal was also achieved, with an effluent level desirable for irrigation. A high-level of nitrite accumulation (40 mg N/L) in the reactor caused inhibition to the biological P uptake. This problem was solved through process optimization. The cycle time of the SBR was reduced, with the wastewater load per cycle also reduced, while the daily hydraulic loading maintained. This modification proved to be an effective method to ensure reliable N and P removal. N₂O accumulation was measured in two experiments simulating the anoxic phase of the SBR and using nitrite and nitrate respectively as electron donors. The estimated N₂O emissions for both experiments were very low.

Key words | abattoir wastewater, biological phosphorus removal, irrigation, nitrite, N₂O, sequencing batch reactor

INTRODUCTION

The meat processing industries use large amounts of water during processing and cleaning operations. It has been estimated that a modern abattoir would use 2.5 m³ of water per animal processed, much of which will be discharged as wastewater heavily contaminated with organic matter and nutrients (Meat and Livestock Australia 1998). Irrigation is by far the most common method used for effluent disposal by Australian abattoirs. However, before irrigation can be applied, the raw abattoir wastewater is typically pre-treated with a primary and a secondary treatment. Primary treatment is used to remove fats, oil and grease which can cause fouling of irrigation equipment and the soil. The secondary treatment aims to reduce the organic mater as measured by biological oxygen demand which can lead to odorous conditions during storage, in soil and even kill crops. This is most often achieved using biological systems like anaerobic ponds with a hydraulic residence time (HRT) ranging between 7 and 14 days followed by aerobic ponds. However, the discharge of large amounts of nutrients (total nitrogen: 200–300 mg N/L; total phosphorus: 35–40 mg P/L), which are not removed in the ponds, into the soil can cause over fertilization of soil and may also cause groundwater pollution. Therefore, activated sludge systems for C and N removal are gaining application to reduce nutrient loads (Beccari et al. 1984; Willers et al. 1993). Lemaire et al. (2009) developed a sequencing batch reactor.
reactor (SBR)-based technology to also remove phosphorus, along with nitrogen and organic carbon achieving an effluent quality suitable for river discharge. However, such high-levels of N and P removal are not required for irrigation. Indeed, it is beneficial to have certain levels of N (30–50 mg N/L, dependent on the type of soil or the type of crop) and P (5–15 mg P/L) in the irrigated water, in order to provide nutrients to the soil. The lower level of nutrient removal required also provides opportunities for saving operational costs.

The aim of this study was to develop a technology for cost-effective treatment of abattoir wastewater to achieve an effluent quality suitable for irrigation. The operation of an SBR was designed such that it achieves the desirable levels of N and P removal with minimum requirement for aeration and organic carbon. The production of N\textsubscript{2}O, a powerful green house gas, during anoxic conditions was assessed.

**MATERIALS AND METHODS**

**SBR configuration**

The SBR was inoculated with a non-EBPR (enhanced biological phosphorus removal) sludge from a full-scale SBR treating abattoir wastewater in Queensland. It had a working volume of 8 L and was operated in a temperature controlled room (20–22°C). In the first 10 month of reactor operation, a cycle time of 8 h was applied. Each cycle began with a 10 min feeding period, where 1 L of abattoir wastewater was supplied, followed by a 50 min anaerobic period, 125 min aeration, 3 min anoxic conditions, 10 min of a second feeding period, when another 1 L of abattoir wastewater was added, 227 min anoxic period and 50 min settling. During the last 5 minutes of the cycle 2 L of effluent was discharged, providing an HRT of 32 h. SRT was maintained at 12 days by wasting sludge at the end of the anoxic period.

The SBR cycle was reduced to 4 h on day 323 of operation, keeping the HRT at 32 h by reducing the wastewater load to 0.5 L in each feeding period. The new cycle was as follows: 5 min of feeding period followed by 30 min of anaerobic conditions, 65 min aeration, 3 min anoxic conditions, 5 min second feed, 119 min anoxic conditions, 10 min settling and 3 min decant.

During aerated periods, air was provided intermittently using an on/off control system to keep the dissolved oxygen level between 1.5 and 2 mg O\textsubscript{2}/L. The pH was monitored, but not controlled ranging between 6.9 and 7.8.

**Starvation period**

The abattoir closed down for 5 weeks over a Christmas break (Days 215–261 of the reactor operation) and no wastewater was available. During this period, the operational strategy described in Yilmaz et al. (2007) was implemented. The sludge was aerated and mixed for 15 min in each 8 h cycle and was allowed to settle for the rest of the cycle.

Six batch experiments were conducted on weekly basis, being the first one on the first day without wastewater feed and the last one after 5 weeks without feeding. 250 mL of mixed liquor was withdrawn from the SBR at the end of the aeration period. After washing the sludge with effluent from another SBR treating the same wastewater with very low levels of P and N to remove any NH\textsubscript{4} plus NO\textsubscript{x} residuals, a pulse of NH\textsubscript{4} plus was given, resulting in an initial concentration in the batch reactor of 15 mg N-NH\textsubscript{4}/L. Aeration was started immediately after the addition. Liquid phase samples were collected every 10 min for a period of 1 h for the analysis of ammonium concentration. After 1 h, when ammonia was already depleted, a pulse of nitrite was added to reach a concentration of 20 mg N-NO\textsubscript{2}/L in the reactor. Samples were taken every 10 min for another hour for the measurement of nitrite. The rates of ammonium and nitrite oxidation were calculated through linear regression.

**N\textsubscript{2}O batch experiments**

Two batch experiments with the addition of nitrate and nitrite respectively, were conducted to determine N\textsubscript{2}O production during denitrification. 500 mL of mixed liquor was withdrawn from the SBR at the end of an aerobic period and transferred to a 500 mL batch reactor. The sludge was washed with effluent from the same reactor to remove the NO\textsubscript{x} present in the mixed liquor. The reactor...
was not sealed in this test in order to mimic the operational conditions of the SBR. Nitrite or nitrate stock solution was added as a pulse into the reactor. The initial nitrite or nitrate concentration was 18 mg N/L. Abattoir wastewater was added at the same time in both cases, simulating the second feed of the SBR. An N₂O microsensor (N₂O25, Unisense A/S, Aarhus, Denmark) was inserted in the reactor with the sensor tip submerged in the liquid phase to measure the dissolved N₂O concentration.

The loss of nitrous oxide through the reactor surface \(r_{N_2O}\) was calculated according to Equation (1) (Schulthess & Gujer 1996):

\[
r_{N_2O} = K_{LN_2O}a \times \left( S_{N_2O} - \frac{C_{N_2O_{air}}}{H_{N_2O}} \right)
\]

For the \(K_{LN_2O}a\) a value of 2 d\(^{-1}\) was chosen, being a typical \(K_{LN_2O}a\) value for anoxic tanks (Siegrist & Gujer 1994). \(C_{N_2O_{air}}\) corresponds to the N₂O concentration in the air and the value of 0.0003 g N/m\(^3\) was chosen (Lyon et al. 1989). \(H_{N_2O}\) corresponds to the Henry coefficient for nitrous oxide \((H_{N_2O} = 1.6,\) Liss & Slater 1974).

**Abattoir wastewater**

The wastewater used in this study was collected from a local abattoir in Queensland. At this site, the raw effluent passes through four anaerobic ponds before being further treated. The four anaerobic ponds produced different concentrations of VFAs due to different organic loading rates. The effluent from anaerobic pond A, which was the only easily accessible pond for wastewater collection, was collected on a weekly basis and stored at 4°C. Compared to the others ponds, pond A contains similar levels of total nitrogen (TN) and total phosphorus (TP) but much lower levels of biodegradable COD and VFAs, which are essential for the nutrient removal process. Therefore, in this study modified wastewater with externally added VFAs that simulated the VFA levels in pond B was used from day 180 onwards to improve the phosphorus removal. The characteristics of the anaerobic pond A effluent and pond B are detailed in Table 1. Both COD/N and COD/P ratios are low resulting from the COD but not N and P removal in the anaerobic pond.

**Table 1 | Abattoir wastewater characteristics**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Anaerobic pond A effluent</th>
<th>Anaerobic pond B effluent</th>
</tr>
</thead>
<tbody>
<tr>
<td>COD(_t) (mg/L)</td>
<td>639–1,116</td>
<td>740–950</td>
</tr>
<tr>
<td>COD(_s) (mg/L)</td>
<td>269–776</td>
<td>440–531</td>
</tr>
<tr>
<td>VFA (mg COD/L)</td>
<td>20–240</td>
<td>272–358</td>
</tr>
<tr>
<td>TN (mg/L)</td>
<td>235–275</td>
<td>240–262</td>
</tr>
<tr>
<td>N-NH(_4^+) (mg/L)</td>
<td>210–254</td>
<td>220–226</td>
</tr>
<tr>
<td>TP (mg/L)</td>
<td>36–47</td>
<td>37–40</td>
</tr>
<tr>
<td>P-PO(_4^{3-}) (mg/L)</td>
<td>31–40</td>
<td>33–36</td>
</tr>
</tbody>
</table>

**Analyses**

The ammonia (NH\(_4^+\)), nitrate (NO\(_3^-\)), nitrite (NO\(_2^-\)) and orthophosphate (PO\(_4^{3-}\)) were analysed using a Lachat QuikChem8000 Flow Injection Analyser (Lachat Instrument, Milwaukee). Total and soluble chemical oxygen demand (COD\(_t\) and COD\(_s\), respectively), total Kjeldahl nitrogen (TKN), total phosphorus, mixed liquor suspended solid (MLSS) and volatile MLSS (MLVSS) were analysed according to the standard methods (APHA 1995). VFAs were measured by Perkin-Elmer gas chromatography.

Fluorescence in situ hybridization (FISH) was performed as described in Amann (1995) with Cy5-labelled EUBMIX probes (for most Bacteria; Daims et al. 1999), Cy3-labelled PAOMIX probes (for Candidatus Accumulibacter phosphatis, Crocetti et al. 2000).

**RESULTS AND DISCUSSION**

The SBR was operated for a period of 500 days during which several modifications in the operation of the reactor had to be implemented. This led to stable operation during the last 175 days demonstrating the robustness of the strategy implemented. Figure 1 shows the N and P present in the influent and effluent of the reactor during the operational period as well as the VFA concentration in the wastewater.

After the first 3 weeks of operation, an effluent N level desirable for land irrigation was achieved. NH\(_4^+\) was preferred as a final N species present in effluent instead of NO\(_x\) because (1) less demand for aeration was required as...
less N was oxidized (around 15% less oxygen needed); (2) the presence of NO\textsubscript{x} in the effluent would imply its presence in the anaerobic phase of the subsequent cycle, negatively affecting the biological phosphorus removal (Comeau et al. 1986; Furumai et al. 1999). Moreover, NH\textsubscript{4}\textsuperscript{+} has better ion exchange characteristics and is less mobile in the soil. P removal took almost two months to be detected in the system. This was due to two reasons. The sludge used to seed the reactor was a non-EBPR sludge and FISH demonstrated that Accumulibacter (a well known PAO) was hardly detected in the seeding sludge. Also, during the first weeks of operation, a significant level of NO\textsubscript{x} (up to 50 mg N/L) was detected in the effluent and therefore carried over into the subsequent cycle. Under such conditions and due to the limitation of biodegradable C in the wastewater, EBPR could not be developed. However, around day 50 some P removal was detected, coinciding with very low levels of NO\textsubscript{x} in the effluent and an increase of VFA concentration in the wastewater. From then until the starvation period (Day 215), EBPR kept improving in the reactor.

VFA concentration in the wastewater was very variable reaching very low levels in some stages of the operation (Figure 1(A)) probably due to a non-optimum functioning of the pond where the wastewater was collected. This variability in the VFA and also COD present in the wastewater was affecting the reactor operation, especially the bio-P process. In order to overcome this problem, the VFA in the wastewater was corrected from day 150. Acetate was added after measuring the acetate in the wastewater collected in order to achieve a stable concentration of around 220 mg acetate/L. With these levels, the wastewater simulated the effluent of pond B (see Materials and Methods), which was non-accessible for wastewater collection.

There was an interruption of the wastewater supply due to closure of the abattoir for a period of 5 weeks due to annual maintenance. During that time the biomass was maintained through the use of an alternating anoxic/anaerobic and aerobic starvation strategy that has been demonstrated to successfully preserve the activity of the biomass (Yilmaz et al. 2007). After 2 days of resuming the wastewater supply the reactor performance had recovered to a level similar to that before the starvation period.

**Achieving the nitrite pathway**

On Day 279, two weeks after the starvation period, nitrogen removal via nitrite was achieved without significant nitrate formation. Figure 2 shows two cycle studies carried out in...
the SBR just before the starvation and after two weeks of resuming the wastewater supply. Before the starvation period, both nitrate and nitrite accumulated at the end of the aerobic period. However, nitrite was the primary product of nitrification two weeks after the starvation period, indicating the full establishment of the nitrite pathway. Nitrogen removal via nitrite results in two significant benefits. Firstly, it reduces the oxygen consumption for nitrification by 25%. Secondly, it reduces the carbon requirement for denitrification by 40%. As shown in Table 1, the anaerobically pre-treated abattoir wastewater has relatively low COD to N and P ratios.

Indeed, Lemaire et al. (2009) had to incorporate a prefermenter, where raw abattoir wastewater was fermented, to provide COD to facilitate P removal and denitrification. The nitrite pathway and also the ammonium (instead of oxidized nitrogen) discharge, both achieved through innovative operational design, allowed N removal with minimum requirement for aeration and COD.

The establishment of the nitrite pathway is hypothesized to be a result of applying a relatively short aerobic period followed by an anoxic period with feeding. Since early stages of operation, nitrite was always present at the

![Figure 2](https://iwaponline.com/wst/article-pdf/61/8/2105/448460/2105.pdf)

Figure 2 | Cycle studies profiles before (left) and 2 weeks after starvation conditions (right): ○, N-NH₄⁺; ●, P-PO₄³⁻; ▲, N-NO₂⁻; ▼, N-NO₃⁻.

![Figure 3](https://iwaponline.com/wst/article-pdf/61/8/2105/448460/2105.pdf)

Figure 3 | Variation of the NH₄⁺ and NO₂⁻ oxidation rates over the starvation period and the best fits produced by the first order decay model: ●, NH₄⁺; ○, NO₂⁻.

![Figure 4](https://iwaponline.com/wst/article-pdf/61/8/2105/448460/2105.pdf)

Figure 4 | Cycle study profile with 1 L of wastewater load and 4 h cycle time: ○, N-NH₄⁺; ●, P-PO₄³⁻; ▲, N-NO₂⁻; ▼, N-NO₃⁻.

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end of the aerobic period. Although ammonium was completely depleted, the aeration length was not enough to oxidize all the nitrite to nitrate. In an SBR also treating abattoir wastewater, Lemaire et al. (2008) showed that nitrite pathway could be completely achieved by switching off aeration when ammonium oxidation is completed. In our case, no aeration phase length control was implemented, but we have chosen a relatively short aerobic time so that the aeration period was long enough for ammonium oxidation but not for full conversion of nitrite to nitrate. In this way, the nitrite remaining in the reactor at the end of each cycle is reduced by denitrifiers (with carbon supplied through the second feeding), and thus is not available as an energy source for nitrite oxidizing bacteria (NOBs). This reduces the growth of NOBs and over cycles, will result in further increases of nitrite accumulation in the subsequent cycles. Over time, nitrite oxidizers would be eliminated.

The 5-week starvation period likely contributed to the elimination of nitrite oxidizers. During starvation conditions, ammonia oxidation and nitrite oxidation rates were followed through weekly batch experiments. The results are shown in Figure 3.

The decay rates determined during starvation conditions were 0.0109 d$^{-1}$ for the Ammonia Oxidizing Bacteria (AOB) and 0.0335 d$^{-1}$ for the NOB. The faster decay rate could have helped to achieve the nitrite pathway.

**Nitrite inhibition on P-uptake**

P removal drastically stopped when concentrations of nitrite reached 40 mg N/L at the end of the aeration period (Figure 2, right). Nitrite in very low concentrations has been reported to inhibit aerobic (Meinhold et al. 1999; Saito et al. 2004) and anoxic P-uptake (Zhou et al. 2007). In order to overcome this problem in the SBR the level of nitrite at the end of the aerobic period was reduced by reducing the wastewater load to 1 L per cycle, providing 0.5 L in each feeding period. Also, the cycle time was reduced to 4 h, so the HRT in the reactor was kept the same. Figure 4 shows a cycle study profile obtained in the SBR, 1 week after implementing this strategy.

The levels of nitrite in the SBR were halved and the EBPR activity was recovered. The reactor was kept under these conditions until the end of its operation. A summary of the influent and effluent nutrient concentrations during the last 2.5 months of operation is presented in Table 2. During this period the system was able to achieve 76.8% COD removal (the remaining COD was not biodegradable, as confirmed through an independent test), 86.4% N-NH$_4^+$ removal and 67.8% P-PO$_4^{3-}$ removal from the abattoir wastewater, producing an effluent suitable for land irrigation.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Influent average (SD/NS)</th>
<th>Effluent average (SD/NS)</th>
<th>Nutrient removal efficiency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CODt</td>
<td>600 (65.9)</td>
<td>139.4 (15.2;9)</td>
<td>76.8</td>
</tr>
<tr>
<td>CODs</td>
<td>470 (78.9)</td>
<td>112.0 (14.2;9)</td>
<td>76.2</td>
</tr>
<tr>
<td>TKN</td>
<td>251.0 (7.9;9)</td>
<td>43.1 (10.9)</td>
<td>82.8</td>
</tr>
<tr>
<td>N-NH$_4^+$</td>
<td>236.2 (5.2;9)</td>
<td>32.0 (12.9)</td>
<td>84.6</td>
</tr>
<tr>
<td>N-NO$_x$</td>
<td>–</td>
<td>2.7 (2.5;9)</td>
<td>–</td>
</tr>
<tr>
<td>TP</td>
<td>41.4 (2.8;9)</td>
<td>14.2 (2.9)</td>
<td>65.7</td>
</tr>
<tr>
<td>P-PO$_4^{3-}$</td>
<td>34.5 (1;9)</td>
<td>11.1 (2.5;9)</td>
<td>67.8</td>
</tr>
</tbody>
</table>

SD: Standard deviation; NS: Number of samples.

Figure 5 | N$_2$O accumulation in the anoxic period when nitrite was added (left) and when nitrate was added (right).
This system provides a high degree of flexibility. By adjusting the feed this process could provide different N and P concentrations depending on the requirements.

**N₂O accumulation during anoxic conditions**

Several studies have suggested that the presence of nitrite in the anoxic period can cause N₂O accumulation and emission (Zhou et al. 2008). Also, Kishida et al. (2004) found that N₂O production from the anoxic phase was extremely affected by the low influent C/N ratio. Since both conditions were occurring in the SBR used, the production of N₂O in the anoxic phase of our system was measured. A nitrite pulse of 18 mg N/L was added at the beginning of anoxic test (Figure 5-left). It was found that N₂O concentration in the bulk increased rapidly to 10 mg N-N₂O/L in the first 20 minutes and decreased also rapidly after the complete depletion of nitrite. Nitrate was used in another experiment to compare with nitrite the N₂O accumulation (Figure 5-right). Although no nitrite was added in this experiment, nitrite concentration increased to approximately 10 mg N/L after 30 min due to the reduction of nitrate to nitrite. There was a sharp increase in N₂O accumulation (up to 8 mg N/L) as nitrite accumulation was observed.

Nitrite reduction rate was calculated in both experiments resulting in a very similar rate (0.224 mg N-NO₂⁻/L min when nitrate was added and 0.225 mg N-NO₂⁻/L min when nitrite was added). Also, N₂O reduction rate calculated through linear regression when nitrite was depleted in both experiments was very similar (0.132 mg N-N₂O/L in the nitrite experiment and 0.126 mg N-N₂O/L in the nitrate experiment).

The N₂O accumulation for both experiments was very similar and when Equation (1) (Materials & Methods) was used to estimate the potential amount of N₂O emitted, that resulted in a very low value for both cases (1.72 × 10⁻⁶ mg N-N₂O/L during the experiment with nitrite and 1.29 × 10⁻⁶ mg N-N₂O/L for the experiment with nitrate).

**CONCLUSIONS**

The following conclusions can be drawn from this study:

- An SBR-based process was developed for the treatment of abattoir wastewater to achieve an effluent standard suitable for irrigation. The demand for aeration and organic carbon for nitrogen removal is reduced through achieving nitrogen removal via nitrite and discharging ammonium as the primary nitrogen species in the effluent. The process, with adaptation, is likely suitable for the treatment of other types of wastewaters.
- High level of nitrite accumulation inhibits aerobic P uptake, and should be avoided for the benefit of stable P removal. This was achieved by reducing the cycle time and the wastewater load per cycle while maintaining the hydraulic retention time.
- There is not a substantial difference between N₂O production under anoxic conditions when nitrite or nitrate is the electron donor under the conditions used in this study.

**ACKNOWLEDGEMENTS**

This work was funded by the Environmental Biotechnology Cooperative Research Center (EBCRC), Australia.

**REFERENCES**


