Biological nutrient removal by applying SBR technology in small wastewater treatment plants: carbon source and C/N/P ratio effects

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Abstract SBR technology is considered an alternative to conventional processes such as Phoredox, Five-stage Bardenpho, among the others for treating nutrients in wastewaters. It is especially applicable to small communities of a just few people to a population equivalent (p.e) up to 4000. In this paper, biological nutrient removal using SBR technology in a single reactor is presented. Biological nutrient removal requires a sequence of anaerobic–anoxic–aerobic phases with multiple feeding events over one cycle. This filling strategy was adapted to enhance denitrification and phosphate release, using the easily biodegradable organic matter from the wastewater. In spite of using this feeding strategy, the organic matter concentration can be insufficient. The results show that biological nutrient removal was successfully achieved by using only one reactor, working with a low organic matter concentration in the influent (C/N/P ratio of 100:12:1.8). Nevertheless, when the C/P ratio was lower than 36 g COD·g⁻¹ P-PO₄, an accumulation of phosphate was observed. After that, the system responded quickly and returned to ideal conditions (C/P ratio of 67 g COD·g⁻¹ P-PO₄), taking only 15 days to achieve the complete nutrient removal. Furthermore, the operational conditions and the synthetic wastewater used conferred a selective advantage to polyphosphate accumulating organisms (PAOs) over glycogen accumulating non-poly-P organisms (GAOs) as shown by the FISH analysis performed.

Keywords C/P and C/N/P ratios; nutrient removal; PAOs; SBRs; step-feed strategy

Introduction

Treatment of contaminated wastewater with biological processes has been widely used for classical urban and industrial wastewaters (Metcalf and Eddy, 2003). Since stricter legislation is being applied, more attention to phosphorus removal is needed. The traditional method for removing phosphate from wastewater is adding precipitating chemicals (iron or aluminium salts) to the wastewater. Besides the fact that using chemicals in waste treatment should be minimized, there are several negative aspects of this practice (Van Loosdrecht et al., 1997). However, although there are many biological nutrient removal processes to deal with nutrient removal, some of their drawbacks must be considered, such as moderate levels of nitrogen removal (i.e. A²/O™, VIP, UCT(University of Cape Town) processes), moderate to poor phosphorus removal (i.e. Five-stage Bardenpho process), large reactor volumes required (i.e. A²/O™ (Grady et al., 1999)) or large investments in extra infrastructure (i.e. Phostrip process (Van Loosdrecht et al., 1997)).

SBR technology is considered an alternative to these conventional processes for removing nutrients from wastewaters. It is especially applicable to small communities with a few inhabitants up to 4000 p.e. SBR technology has successfully been applied in small wastewater treatment plants treating urban (Lee et al., 2004; Puig et al., 2005) and industrial (Torrijos et al., 2001; Vives et al., 2003) wastewaters. It is especially
Nutrient removal in SBRs requires a combination of anaerobic–anoxic–aerobic phases. During the anaerobic phase, the carbon source in the form of volatile fatty acids (VFAs) is taken up by polyphosphate-accumulating organisms (PAOs) releasing phosphate into the liquid phase. Within the cells, VFAs are stored basically as polyhydroxybutyrate (PHB) while intracellular glycogen is converted to polyhydroxyalcaonate (PHA). In the anoxic phases, if a soluble and biodegradable carbon source is present, a classic heterotrophic denitrification process takes place. Finally, under aerobic conditions, PAOs grow and accumulate phosphate in the cells, while autotrophic biomass is responsible for the nitrification process. Phosphorus removal is achieved through the wastage of excess sludge with high poly-P content.

Glycogen-Accumulating Organisms (GAOs) are a group of bacteria capable of competing with PAOs during the anaerobic VFA uptake. Like PAOs, GAOs take up VFAs anaerobically and convert them into PHAs via hydrolysis of glycogen as their sole source of energy for this process (Mino et al., 1998). Nevertheless, PAOs have a clear competitive advantage in that they can accumulate VFAs in the cells without the necessity of an external electron acceptor because, under aerobic or anoxic conditions, PAOs can grow at the expense of their stored substrate.

When operating SBRs for nutrient removal, special attention has to be given to the availability and use of the easily biodegradable substrate. A step-feed strategy for nutrient removal means that the influent filling phases must be carried out under anaerobic or anoxic conditions in order to increase the denitrification and phosphate release efficiencies (Puig et al., 2004). In spite of using this feeding strategy, the organic matter concentration can be insufficient. When an excessive C/P ratio is provided, PAOs can accumulate a high content of polyphosphate coupled with a higher and faster substrate uptake ability, successfully out-competing GAOs. In contrast, reducing the C/P ratio causes the depletion of the polyphosphate content in PAOs, eventually leading to them being replaced by GAOs as the majority (Liu et al., 1997).

Puig et al. (2004) described a successful implementation in SBRs using 4 to 6 filling events with a sequence of anoxic–aerobic phases treating synthetic and urban wastewater for carbon and nitrogen removal. In this paper, the SBR cycle was adapted for biological nutrient removal (designed to treat 90 p.e.) in a single reactor. A filling strategy was adapted to enhance denitrification and phosphate release. Furthermore, the influence of C/P and C/N feeding ratios on the efficiency of nutrient removal using an SBR was investigated. In addition, biological phosphorus removal has been assessed by monitoring the evolution of PAOs versus GAOs.

Materials and methods

Synthetic feed

The synthetic wastewater was basically composed of a mixed carbon source, an ammonium solution, a phosphate buffer, an alkalinity control (NaHCO₃) and a microelements solution (adapted from Dangcong et al., 2000). The synthetic wastewater was prepared twice a week with a concentration of 502 ± 175 mg L⁻¹ COD, 61.5 ± 10.1 mg L⁻¹ N-TKN, 47.9 ± 7.3 mg L⁻¹ N-NH₃ and 9.1 ± 3.8 mg P-PO₄ L⁻¹ during stable-state operation, giving C/N and C/P ratios of 8 ± 2 mg COD mg⁻¹ N-TKN and 52 ± 16 mg COD mg⁻¹ P-PO₄, respectively. The composition contained per L: 0.27 ml EtOH, 0.56 g DME (Dehydrated meat extract), 0.4 mL milk, 13.3 mL leachate, 280 mg NaHCO₃, 183 mg NH₄Cl, a microelement solution (described in Vives, 2004) and a phosphate buffer (7.0 mg KH₂PO₄, 18 mg K₂HPO₄, 14 mg Na₂HPO₄·7H₂O).
The SBR was composed of a cylindrical reactor with maximum and minimum working volumes of 30 L and 20 L, respectively. To achieve nutrient removal in the SBR, the hydraulic retention time (HRT) and solids retention time (SRT) were maintained at 1 day and 20 days, respectively. The SBR plant was seeded with sludge from the Sils-Vidreres wastewater treatment plant (Girona, Spain) and it treated synthetic wastewater for nutrient removal.

An 8 hour cycle (Figure 1) with 3 feeding steps (the first one double the volume of the others) was implemented for nutrient removal. The wastewater was always introduced under anaerobic or anoxic conditions in order to enhance phosphate release and denitrification, using the easily biodegradable organic matter from the wastewater. The cycle was divided into two parts, one focussed on phosphorus removal and the other on nitrogen removal. The first anaerobic–aerobic pair phase was related to phosphorus removal. The anaerobic phase improved the readily biodegradable organic matter uptake by PAOs and was followed by an aerobic phase for the phosphate uptake. Phosphorus removal was achieved through the wastage of excess sludge with high poly-P content. The second part consisted of a sequence of anoxic–aerobic phases for nitrogen removal. The cycle was divided into a reaction phase (426 minutes; 48.8% and 20.2% under anaerobic and anoxic conditions respectively), a settling phase (39 minutes) and a draw phase (15 minutes).

Fluorescence in situ hybridization (FISH) analysis was performed as specified by Amman (1995) using the Cy5-labeled-EUBMIX probe to target the entire bacterial community (Daims et al., 1999), and the Accumulibacter and Competibacter probes were used to target Cy3-labelled PAOMIX (consisting of PAO462, PAO651, and PAO846) for Accumulibacter, the only currently identified PAO and Fluos-labelled GAOMIX (consisting of GAO431 and GAO989) was used to target Competibacter, the only currently identified GAO (Crocetti et al., 2002). The probed sludge was examined using an Epifluorescence microscope. The area containing specific labelled probe cells (Cy3 and Fluos for PAOMIX and GAOMIX, respectively) was quantified as a percentage of the area of the entire Cy5-labeled bacterial population probe (EUBMIX).

Total suspended solids (TSS), volatile suspended solids (VSS), total chemical oxygen demand (COD), ammonium (N-NH₄⁺), total Kjeldahl nitrogen (N-TKN), nitrates (N-NO₃⁻), nitrates (N-NO₂⁻) and phosphate (P-PO₄³⁻) were all measured according to Standard Methods (APHA, 1995). Total nitrogen (TN) was calculated as the sum of N-TKN, and nitrite and nitrate concentrations as mg N-TN·L⁻¹.

The SBR plant operated for three months using an 8-hour cycle (Figure 1) for nutrient removal. A filling strategy was adopted to enhance denitrification and phosphate release.
using the easily biodegradable organic matter from the wastewater. This cycle operated with different load conditions divided into three periods according to the C/N and C/P ratios (Table 1).

Figure 2 shows the evolution of carbon, nitrogen and phosphorus components and the C/N and C/P feeding ratios during the experimental study.

**Period 1: Start-up of the SBR.** The reactor was seeded with sludge from urban wastewater (Sils-Vidreres WWTP) and fed with synthetic wastewater. A stable state (Figure 2) was reached after 2 weeks of operation achieving average COD, TN and phosphate effluent concentrations of 44, 2.2 and 0.15 mg·L\(^{-1}\), respectively, and leading to removal efficiencies of 94%, 95% and 98% for carbon, nitrogen and phosphorus, respectively.

In spite of the competition for the carbon source between PAOs and denitrifiers, C/P (55 g COD·g\(^{-1}\) P-PO\(_4\)) and C/N (8 g COD·g\(^{-1}\) N-TKN) ratios were enough to achieve complete nutrient removal.

**Period 2: C/P ratio decrease.** After achieving complete nutrient removal in period 1, an increase in phosphate concentration in the influent (17 mg P-PO\(_4\)·L\(^{-1}\), on average) was applied in period 2 the carbon and nitrogen concentrations remained the same. Despite the small amount of carbon source available (Figure 2B), the nitrogen effluent concentrations (0.3 mg N-NH\(_4^+\)·L\(^{-1}\) and 2.5 mg N-NO\(_3^-\)·L\(^{-1}\)) were not affected and the nitrogen concentration was always lower than the European Directive values (D. level, Figure 2). However, the lower amount of available organic matter (C/P ratio of 36 g COD·g\(^{-1}\) P-PO\(_4\)) clearly affected the phosphorus removal performance (Figure 2D) as phosphate started to accumulate in the reactor.

**Period 3: Recuperation time for the complete nutrient removal.** The aim was to discover the time needed to achieve complete nutrient removal after a period of phosphate accumulation. For this reason, the C/P was kept higher (67 g COD·g\(^{-1}\) P-PO\(_4\)) than in period 2.

In spite of the high COD variability in the influent (Figure 2B, range between 102 and 596 mg COD·L\(^{-1}\)), complete nutrient removal was achieved. Thus, the nitrogen removal efficiencies remained high in spite of the low influent COD concentration on days 66 and 69. Nevertheless, recovering the phosphorus removal took more time. The PAOs’ response was slower and, after 15 days of operation, complete phosphorus removal was achieved again.

Table 2 compares the variation of C/N and C/P ratios and of nitrogen and phosphorus removal efficiencies in this study with the results reported by other researchers.

Comparing the data shown in Table 2, some variability can be observed between the C/N and C/P feeding ratios and nitrogen and phosphorus removal efficiencies in the different studies. The nitrogen efficiencies presented (periods 1 and 3) are higher than those obtained by other researchers working with the same or a higher C/N ratio (C/N of 7–10 g COD·g\(^{-1}\) TKN) and applying the step-feed strategy. In single-feed or continuous feeding strategies (Yu et al., 1997; Akin and Ugurlu, 2004; Ma et al., 2005), substantial

**Table 1** Definition of the different periods of study according to the C/N and C/P ratios

<table>
<thead>
<tr>
<th>Period</th>
<th>Length days</th>
<th>C/N g COD/g N-TKN</th>
<th>C/P g COD/g P-PO(_4)</th>
<th>C load g C/d</th>
<th>N Load g N/d</th>
<th>P Load g P/d</th>
</tr>
</thead>
<tbody>
<tr>
<td>Period 1</td>
<td>31</td>
<td>8</td>
<td>55</td>
<td>8.8</td>
<td>1.1</td>
<td>0.16</td>
</tr>
<tr>
<td>Period 2</td>
<td>20</td>
<td>9</td>
<td>36</td>
<td>9.6</td>
<td>1.1</td>
<td>0.27</td>
</tr>
<tr>
<td>Period 3</td>
<td>37</td>
<td>7</td>
<td>67</td>
<td>6.2</td>
<td>0.9</td>
<td>0.09</td>
</tr>
</tbody>
</table>
amounts of nitrate might remain in the effluent because endogenous denitrification cannot reduce nitrate efficiently in the anoxic reaction and settle period due to the lack of an organic electron donor (Chang and Hao, 1996).

However, the step-feed strategy also improved phosphorus removal in the experimental study working with the same C/P ratio (55–58 g COD·g⁻¹ P-PO₄), as other researchers that used continuous feeding and feeding during the settle phase strategies have also reported (Yu et al., 1997; Keller et al., 2001). Akin and Ugurlu, 2004 showed that when the C/P ratio was too low (19 g COD·g⁻¹ P-PO₄), the phosphorus removal efficiency decreased.

**Figure 2** Evolution of carbon, nitrogen and phosphorus components during the experimental period applying an 8-hour cycle in the SBR pilot plant (D.level: Maximum discharge concentration admissible)

**Table 2** Comparison of C/N and C/P ratios and of nitrogen and phosphorus removal efficiencies in different studies of nutrient removal

<table>
<thead>
<tr>
<th>Publication</th>
<th>Kind of wastewater</th>
<th>Reactor configuration</th>
<th>C/N</th>
<th>C/P</th>
<th>C/N/P</th>
<th>% N removal</th>
<th>% P removal</th>
</tr>
</thead>
<tbody>
<tr>
<td>This study (Period 1)</td>
<td>Synthetic</td>
<td>SBR</td>
<td>8</td>
<td>55</td>
<td>100:12:1.8</td>
<td>95</td>
<td>98</td>
</tr>
<tr>
<td>This study (Period 3)</td>
<td>Synthetic</td>
<td>SBR</td>
<td>7</td>
<td>67</td>
<td>100:14:1.5</td>
<td>96</td>
<td>90</td>
</tr>
<tr>
<td>Yu et al., 1997</td>
<td>Synthetic</td>
<td>SBR</td>
<td>7</td>
<td>55</td>
<td>100:14:1.8</td>
<td>81</td>
<td>61</td>
</tr>
<tr>
<td>Keller et al., 2001</td>
<td>Urban</td>
<td>SBR</td>
<td>10</td>
<td>58</td>
<td>100:10:1.7</td>
<td>96</td>
<td>84</td>
</tr>
<tr>
<td>Kargi and Uygur, 2003</td>
<td>Synthetic</td>
<td>SBR</td>
<td>10–50</td>
<td>40–250</td>
<td>100:2·0.54</td>
<td>94</td>
<td>99</td>
</tr>
<tr>
<td>Akin and Ugulu, 2004</td>
<td>Synthetic</td>
<td>A²/O</td>
<td>8</td>
<td>19</td>
<td>100:13:5.3</td>
<td>37</td>
<td>80</td>
</tr>
<tr>
<td>Ma et al., 2005</td>
<td>Synthetic</td>
<td>A²/O</td>
<td>6</td>
<td>18 to 61</td>
<td>100:17:</td>
<td>75–84</td>
<td>90–98</td>
</tr>
</tbody>
</table>

(1.6 to 5.6)
FISH analysis of the biomass

The FISH analysis for the PAO and GAO communities was done to assess the population dynamics. FISH analysis was done for the initial evolution of sludge during phase 1, presenting the initial and final sludge after one month of operation.

Comparing initial and final phase data, the PAOs’ population increased from 9.4% to 17.5% in spite of the sludge presenting a significant change in wastewater quality (from the real plant to the synthetic wastewater). Nevertheless, influent wastewater was composed of ethanol and DME as the synthetic carbon source, without any of the conventional carbon sources used for phosphorus removal (acetate (Smolders et al., 1994), propionate (Oehmen et al., 2004) or glucose (Pala and Bölükbaş, 2005), among others). The slight increase in the GAOs population (from 3.1% to 3.5%) shows that the operational conditions and the synthetic wastewater used are a favourable substrate for enhanced biological phosphorus removal (EBPR), probably providing a selective advantage to PAOs over their competitors as reported by Oehmen et al. (2004) who used propionate.

Conclusions

Biological nutrient removal has been successfully achieved (seeding sludge from an urban wastewater treatment plant without phosphorus activity and a low presence of PAOs) by using only one reactor and working with a low organic matter concentration in the influent (C/N/P ratio of 100:12:1.8). The high nutrient efficiencies achieved (94%, 95% and 98% for carbon, nitrogen and phosphorus, respectively) proved that the sequence of anaerobic–anoxic–aerobic phases with multiple feeding events over one cycle is a promising strategy for removing nutrients from wastewater.

However, when the C/P ratio was below 36 g COD·g\(^{-1}\) PO\(_4\) it affected the phosphorus performance, increasing the phosphate accumulation in the reactor. After a period of phosphate accumulation applying a C/P ratio of 67 g COD·g\(^{-1}\) PO\(_4\), the PAOs were observed to respond quickly (15 days).

The selective enrichment of PAOs, working with a carbon source without any VFAs, shows that the operational conditions and the synthetic wastewater used are a favourable substrate for EBPR that probably provide a selective advantage to PAOs over their competitors. This topic will be further developed in future work.

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