Integrated side-stream reactor for biological nutrient removal and minimization of sludge production

M. Coma, S. Rovira, J. Canals and J. Colprim

ABSTRACT

Integrated processes to reduce in situ the sludge production in wastewater treatment plants are gaining attention in order to facilitate excess sludge management. In contrast to post-treatments, such as anaerobic digestion which is placed between the activated sludge system and dewatering processes, integrated technologies are placed in the sludge return line. This study evaluates the application of an anoxic side-stream reactor (SSR) which creates a physiological shock and uncouples the biomass metabolism and diverts the activity from assimilation for biosynthesis to non-growth activities. The effect of this system in biological nutrient removal for both nitrogen and phosphorus was evaluated for the anaerobic, anoxic and aerobic reactors. The RedOx potential within the SSR was maintained at −150 mV while the sludge loading rate was modified by increasing the percentage of recycled activated sludge feed to the SSR (0 and 40% at laboratory scale and 0, 10, 50 and 100% at pilot scale). The use of the SSR presented a slight reduction of phosphorus removal but maintained the effluent quality to the required discharge values. Nitrogen removal efficiency increased from 75 to 86% while reducing the sludge production rate by 18.3%.

Key words | activated sludge, biomass production, energy uncoupling, side-stream reactor (SSR), sludge reduction, yield

INTRODUCTION

Excess sludge generated in wastewater treatment is one of the main costs in wastewater treatment plants (WWTPs). Post-treatment and sludge management accounts for 50–60% of the total operational expenses (Yang et al. 2011) and legislation on sludge disposal is becoming stricter.

Most of the technologies applied nowadays are based on sludge reduction and stabilization for final disposal such as anaerobic mesophilic digestion or aerobic digestion at ambient conditions (Borowski & Szopa 2007). The former, aerobic digestion, is mainly used in small treatment facilities (20,000 m³ d⁻¹) as it is more flexible in operation and less prone to process failure, while the latter is more suitable for large WWTPs where biogas co-production can recover the energy used (Bernard & Gray 2000). While both technologies are placed between the activated sludge and dewatering processes as they are still capital intensive and process-wise complex (Khursheed & Kazmi 2011), some technologies have emerged in order to reduce in situ the sludge production within the activated sludge line. To reduce the biomass production, wastewater processes must be engineered so that the substrate is diverted from assimilation for biosynthesis to fuel exothermic, non-growth activities (Wei et al. 2005). Existing physico-chemical and mechanical methods might be applied for this purpose when making the overall mass more biodegradable by oxidation or lysis (Khursheed & Kazmi 2011), but they have high capital and operational expenses associated with them. Biological strategies, such as uncoupling metabolism, will also lead to effective reduction of excess sludge production, but with no important change in the configuration of the conventional activated sludge (CAS) process (Ye & Li 2010).

Uncoupled metabolism can be achieved when microorganisms are subjected to a physiological shock created by lack of oxygen and substrate (i.e., oxic, anoxic or anaerobic cycling) forcing the system to use adenosine triphosphate (ATP), which is produced during catabolism, as a source of energy instead of biomass synthesis (anabolism). After a lack of oxygen or substrate, when microorganisms are returned to aerobiosis, they rebuild their
energy reserves at the expense of growth. Based on this principle, the oxic-settling-anaerobic (OSA) process reduces the excess sludge production by 40–50% by inserting an anaerobic side-stream reactor (SSR) within the returned activated sludge (RAS) circuit after starvation conditions (settling tank) (Chudoba et al. 1992). Sludge reduction in the OSA system can be explained by sludge decay, which is accelerated effectively under low oxidation-reduction potentials (ORP) achieved by high hydraulic retention times (HRT) in the anaerobic tank (Saby et al. 2005). However, it has been found that neither completely anaerobic nor completely aerobic conditions reduced sludge production (Troiani et al. 2014). Sludge reduction mechanisms in OSA-like processes are attributed to enhanced endogenous decay, destruction of extracellular polymeric substances (EPS), biomass feasting/fasting and energy uncoupling among others, but they cannot be isolated due to sludge cycling, substrate deprivation in the SSR and in some cases extended solids retention time (SRT), which is inversely proportional to sludge yield (Semblante et al. 2014).

OSA-like processes have been applied to aerobic systems where organic matter removal was the only target of the process. Stricter regulations are also applied for nutrients such as nitrogen and phosphorus. While the first is most commonly and economically achieved in a two-stage system through nitrification (under aerobic conditions) and denitrification (under anoxic conditions), biological phosphorus removal requires an extra anaerobic period (Puig et al. 2007). While nitrification and denitrification may occur to a certain degree in biological systems, phosphorus is only removed to a substantial extent with the aid of chemical precipitation (Abegglen et al. 2008). Thus, biological nutrient removal is strongly dependent on the plant layout and the operating conditions. The BIMINEX® process presented in this study integrates all conditions for nutrient removal in a University of Cape Town (UCT) configuration with sludge reduction production by an anoxic SSR (not as restrictive as anaerobic conditions) situated in the sludge return. The main objective of this study is to evaluate the effect of an anoxic ORP (−150 mV) and sludge loads in the SSR for sludge reduction production while maintaining the performance of nitrogen and phosphorus removal. A set-point at −150 mV creates an ORP cycle when values rise rapidly until zero by air pulses and are progressively recovered to more anoxic conditions. This creates ORP shocks that affect the sludge growth. Experiments were carried out in laboratory scale (Girona, Catalonia, Spain) and further scaled up to an industrial pilot plant in Garriga WWTP (La Garriga, Catalonia, Spain).

MATERIALS AND METHODS

Laboratory-scale plant configuration

The laboratory-scale plant consisted of a methacrylate reactor based on a UCT configuration with a total volume of 36.7 L. Anaerobic, anoxic, aerobic and settler reactor volumes were 18.5, 10.4, 7.8 and 6.7 L, respectively. The RAS circuit was divided into two lines which allowed either direct recirculation of the sludge into the anoxic tank or treatment of the sludge in the anoxic SSR before being returned to the anaerobic reactor (Figure 1). The volume of the SSR was 6 L. The pilot plant was equipped with pH, ORP and dissolved oxygen (DO) probes (Crison®) and monitoring and control of the system was carried out by a Memograph (Endress–Hauser®). Room temperature was controlled at 20 ± 2°C.

During the whole experimental period the HRT and the SRT of the water line were maintained for 24 hours and 21 days, respectively. Waste activated sludge (WAS) was modified on a weekly basis to achieve the desired operational SRT. The internal recycle sludge lines were defined as four times the influent flow. The flow of the RAS was defined...
to be 1.2 times the influent flow. Two configurations were tested: (i) without SSR, where RAS was completely returned to the anoxic tank; and (ii) with SSR at 20%, where 20% of the RAS flow was treated in the SSR before being returned to the anaerobic tank. ORP in the SSR was regulated by set-point at $-150 \text{ mV}$ by means of air pulses with an on/off control. Influent for the pilot plant was taken up from Quart WWTP (Girona, Catalonia, Spain) using a grinder pump. Mean concentrations during the experimental study were $499 \pm 274 \text{ mg COD L}^{-1}$; $68 \pm 26 \text{ mg N-TN L}^{-1}$; $59 \pm 10 \text{ mg N-NH}_4^+ \text{ L}^{-1}$; $5 \pm 2 \text{ mg P-PO}_4^{3-} \text{ L}^{-1}$ and $359 \pm 170 \text{ mg TSS L}^{-1}$.

### Pilot plant configuration

The pilot plant was based on a UCT configuration with a total volume of 6 m$^3$. Anaerobic, anoxic, aerobic and settler reactor volumes were 1, 2, 3 and 0.7 m$^3$, respectively. The RAS circuit was divided as in the laboratory-scale plant. The volume of the SSR was 1.4 m$^3$. The pilot plant was equipped with pH-temperature probes in the anaerobic and SSR tanks, ORP probes in the anaerobic, anoxic and SSR tanks and a DO probe in the aerobic tank. Solids probes were installed in the aerobic and SSR tanks and in the effluent flow. The pilot plant was also equipped with flow meters to monitor the influent, waste, external and internal recirculations and SSR inlet and recirculation flows. A programmable logic controller recorded all values from probes and flow meters and, according to their values, regulated the aeration of the aerobic tank and SSR by on/off control governed by set-point, and regulated the system flows by modifying the frequency of the pumps. Temperature was monitored but not controlled, so seasonal variations affected the pilot plant during the entire study.

During the whole experimental period the HRT and the SRT of the water line were maintained for 24 hours and 17 days, respectively. WAS was modified on a weekly basis to achieve the desired operational SRT. The internal recycle sludge lines were defined as four times the influent flow. The flow of the RAS was defined to be the same as the influent flow; however, different percentages of the sludge line were deviated into the SSR to be treated while the rest of the sludge was directly returned to the anoxic tank. Four configurations were tested: (i) 0% SSR, where RAS was completely returned to the anoxic tank; (ii) 10% SSR, where 10% of the RAS flow was treated in the SSR; (iii) 50% SSR, where half of the RAS was treated in the SSR; and (iv) 100% SSR, where RAS was completely treated in the SSR before being returned to the anaerobic tank. HRT in the SSR was fixed according to the recycled flow at 35 hours for 10% SSR, 12 hours for 50% SSR, and 6 hours for 50% SSR. ORP in the SSR was regulated by set-point at $-150 \text{ mV}$ by means of air pulses with an on/off control. Influent for the pilot plant was taken up from La Garriga WWTP after the grids and the sand trap chamber and before the primary settler. Mean concentrations during the experimental study were $464 \pm 300 \text{ mg COD L}^{-1}$; $53 \pm 20 \text{ mg N-TN L}^{-1}$; $31 \pm 9 \text{ mg N-NH}_4^+ \text{ L}^{-1}$; $3 \pm 1 \text{ mg P-PO}_4^{3-} \text{ L}^{-1}$ and $250 \pm 192 \text{ mg TSS L}^{-1}$.

### Analysis and calculations

Chemical oxygen demand (COD), total suspended solids (TSS), volatile suspended solids (VSS) ammonium and Kjeldahl nitrogen were measured according to **Standard Methods** (APHA 2005). Nitrites, nitrates and phosphates were analyzed by ionic chromatography (Metrohm® 761-Compact; Metrosep A Supp 5; 250/4.0 mm). Total nitrogen (TN) was calculated as the sum of Kjeldahl nitrogen, nitrites and nitrates. The SRT was calculated with mean values of VSS and reactor volume and waste and effluent flows. In this study, the observed sludge yield ($Y_{obs}$) was calculated to evaluate the sludge production, taking into account the growth and death of the biomass. Since solids concentrations in both the UCT and SSR systems changed, cumulative terms during the period of study were used to quantify changes in both solids and substrates. Influent, effluent, recirculation and waste flows were taken into account for the sludge production. For this reason, COD and biomass concentrations (VSS) were quantified on a daily basis. $Y_{obs}$ accounted for the slope of the regression line of the cumulative biomass production versus the cumulative organic removal and it is considered the mean experimental value for the observed biomass yield (Coma et al. 2013).

Fluorescent in situ hybridization (FISH) was performed as specified by Amann (1995). Phosphorus accumulating organisms (PAOs) were stained with a PAO mix probe (Crocetti et al. 2000), competitors of phosphorus removal were detected with the glycogen accumulating organism (GAO) mix probe (Crocetti et al. 2002) and ammonium and nitrite oxidizing bacteria were stained with probes defined in Mobarry et al. (1996) and Daims et al. (2000). The probed sludge was examined using a Leica® confocal laser microscope. The area containing specific labeled probe cells was quantified as a percentage of the area of the entire bacterial population (EUBMIX; Daims et al. 2000). Qualitative or semi-quantitative information was extracted using mathematical software for image analysis developed in Matlab®.
Quantitative analysis was only considered when more than 30 images per sample were analyzed. Standard deviation of the mean (standard deviation divided by the square root of the number of images) was evaluated for all cases.

RESULTS AND DISCUSSION

Proof of concept of anoxic uncoupling metabolism

The laboratory-scale plant with a UCT configuration was run for 273 days at an HRT of 24 hours and 21 days of SRT. Afterwards, 20% of the RAS was deviated to an anoxic SSR where the ORP was maintained between −7 and −153 mV (−96 ± 35 mV) by means of aeration pulses at a fixed set-point of −150 mV. The BIMINEX® system (UCT + SSR) ran for 494 days. The overall HRT and SRT in the water line were maintained at the same values as the UCT configuration. Cumulative organic matter removal and biomass production (as VSS, taking into account reactor and waste sludge) were monitored during both periods (Figure 2).

The slope of the regression lines obtained from the cumulative biomass and organic matter removal represent the experimental or observed yield (Yobs). A poor regression was observed during the first experimental points due to process stabilization, but the overall linear regression presented values for r2 of 0.99 for both systems. The obtained Yobs values in the laboratory-scale set-up were far lower compared to the ones reported in the literature (0.4–0.6 kg VSS kg−1 COD (Tchobanoglous et al. 2003)). This fact was attributed to laboratory conditions where environmental conditions and reactor operation are more easily controlled and monitored. However, nearly a 30% decrease in biomass growth was observed when applying shocks of anoxic conditions in the SSR. This improvement was obtained by not even treating the whole RAS (only 20% was treated in the SSR). The accumulation of solids in the SSR (5.7±1.4 g TSS L−1) increased the total SRT of the system (32 days, Table 1), although the SRT in the water line was fixed at 21 days by modifying the waste flow. Sludge yields are strongly affected by SRT and temperature (Figure 3), but even taking into account the theoretical SRT effect in the laboratory-scale reactor, only 15% sludge yield reduction should be observed. Therefore, the uncoupling metabolism of the sludge was achieved under anoxic conditions, which were less strict than the anaerobic conditions applied in

![Figure 2](https://iwaponline.com/wst/article-pdf/71/7/1056/469084/wst071071056.pdf)

**Figure 2** | Observed yields from cumulative biomass production and organic matter removal with and without SSR in the laboratory-scale plant. Linear regressions of r² > 0.99.

<table>
<thead>
<tr>
<th>Sludge loading</th>
<th>Kg VSS Kg COD</th>
<th>Yobs</th>
<th>Kg VSS Kg COD</th>
<th>Sludge reduction %</th>
<th>Type</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>0% SSR 11.5</td>
<td>–</td>
<td>0</td>
<td>0.513</td>
<td>0.403</td>
<td>–</td>
<td>PS</td>
</tr>
<tr>
<td>0% SSR 20</td>
<td>–</td>
<td>12</td>
<td>0.235</td>
<td>0.235</td>
<td>–</td>
<td>LS</td>
</tr>
<tr>
<td>10% SSR 17.3</td>
<td>–</td>
<td>34.5</td>
<td>0.434</td>
<td>0.402</td>
<td>0.2</td>
<td>PS</td>
</tr>
<tr>
<td>20% SSR 20</td>
<td>–</td>
<td>32</td>
<td>0.165</td>
<td>0.165</td>
<td>29.7</td>
<td>LS</td>
</tr>
<tr>
<td>50% SSR 23.5</td>
<td>–</td>
<td>11.8</td>
<td>0.332</td>
<td>0.367</td>
<td>8.9</td>
<td>PS</td>
</tr>
<tr>
<td>100% SSR 20.2</td>
<td>–</td>
<td>5.9</td>
<td>0.327</td>
<td>0.329</td>
<td>18.3</td>
<td>PS</td>
</tr>
<tr>
<td>100% OSA 20</td>
<td>–</td>
<td>10.4</td>
<td>0.112</td>
<td>0.112</td>
<td>58</td>
<td>LS</td>
</tr>
<tr>
<td>100% OSA 25</td>
<td>–</td>
<td>11.6</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>LS</td>
</tr>
<tr>
<td>100% ACLS Real</td>
<td>+ 50 to – 400</td>
<td>240</td>
<td>0.43</td>
<td>–</td>
<td>17</td>
<td>FS</td>
</tr>
</tbody>
</table>

*Type of study as LS: laboratory scale; PS: pilot scale; FS: full scale.

*Sludge loading calculated assuming that no solids from the settler are returned to the reactor. Values are given in kg TSS instead of VSS (VSS not reported).
OSA-like systems. Such systems obtain anaerobic conditions by increasing the retention of sludge in the SSR even to 93 days (Table 1), thus increasing the overall SRT which is inversely proportional to \( Y_{\text{obs}} \). In the presented study, anoxic conditions were fixed by air pulses. This new approach with air pulses at low ORP (minimum of \(-150\) mV) could enhance the conditions obtained during sludge cycling where cell lysis-cryptic growth is repeated and may increment the sludge decrease (Semblante et al. 2014).

**Scale-up and operational conditions for sludge minimization**

Positive results regarding the use of anoxic SSR at \(-150\) mV in the RAS line were achieved in laboratory controlled studies. To achieve a more realistic value in terms of sludge reduction, the BIMINEX® system was scaled-up to pilot plant and run on-site in a WWTP. Different percentages of the RAS flow were treated in the SSR in order to evaluate the effect of applied sludge loading rate on the observed yields (Table 1). The ORP was maintained between \(-75\) and \(-190\) mV by means of aeration pulses at a fixed set-point of \(-150\) mV. Mean values for the three periods were \(-152 \pm 19\), \(-141 \pm 20\) and \(-131 \pm 23\) mV for 0%, 50% and 100% SSR, respectively. ORP was better controlled in the pilot plant reactor compared to the laboratory-scale reactor due to the sludge volume treated.

The conventional configuration (0% SSR) presented the highest observed yield (0.513 kg VSS kg\(^{-1}\) COD) in accordance with the theoretical values reported in the literature (Tchobanoglous et al. 2005). When treating 10% of the RAS to the SSR (10% SSR) the \( Y_{\text{obs}} \) was reduced from 0.513 to 0.434 kg VSS kg\(^{-1}\) COD. The increase to half of the volume of sludge treated with the SSR (50% SSR) decreased the \( Y_{\text{obs}} \) even more to 0.352 kg VSS kg\(^{-1}\) COD. However, the experimental values obtained from the treatment of the entire RAS into the SSR (100% SSR) did not differ significantly from the 50% SSR. The fact that the whole experimental period was carried out in an on-site plant must be taken into account as biomass yield is highly affected by sludge age and temperature (Tchobanoglous et al. 2003). The former parameter was controlled by daily calculations and modification of the wasted sludge to avoid nutrient removal deterioration, but seasonal variations affected the temperature of the water line, ranging from 7 to 27 °C. Higher temperatures may influence the auto-digestion of the sludge giving lower values in observed yields, while, on the contrary, lower temperatures would increase the experimental yield. Therefore, the experimental yield was normalized to 20 °C (\( Y_{\text{obs}}^{20} \)) by an Arrhenius term as the ones used in activated sludge models (\( U_1 = U_0 e^{\theta(20-T)} \) where \( \theta = 1.029 \) (Gujer et al. 1999)). Normalized observed yields together with the sludge load applied in the SSR are summarized in Table 1.

When taking into account the normalized observed yields, a 10% SSR did not significantly improve the significantly reduction on biomass production. However, when increasing the percentage of RAS treated in the SSR, this decreased the biomass production by more than 18% compared to the conventional system (0% SSR). Although the same increase of RAS treated was applied from 0 to 50% than from 50 to 100% SSR, a higher reduction was obtained in the latter case. Again, when taking into account the accumulation of solids in the SSR the total SRT was increased even though the values of the water line were fixed to 17 days (Table 1). However, the theoretical decrease according to SRT and temperature would have accounted for a 10% reduction, slightly lower than the experimental values obtained. When calculating the sludge loading treated within the anoxic reactor according to the biomass concentration analyzed in the reactor, it was observed that the higher the applied sludge loading, the lower the observed yield. Therefore, the quantity of sludge receiving the anoxic shock for uncoupling metabolism is the key parameter for sludge reduction.

**Effect of SSR in nutrient removal**

The UCT configuration is one arrangement that allows both nitrogen and phosphorus removal. This process involves...
recirculating the sludge through anaerobic, anoxic and aerobic zones, which already reduces the biomass production due to sludge cycling. In the anaerobic stage, biodegradable organic matter such as volatile fatty acids (VFA) is taken up and stored in biopolymers (i.e., PHA) by PAOs, which is accompanied by P release. The biomass is then exposed to anoxic conditions if denitrifying P removal is desired. Finally, the production of the necessary nitrate takes place by oxidation of the ammonia in aerobic conditions, where uptake also takes place (Oehmen et al. 2007). Inserting an SSR may affect the nutrient removal process in two different ways. The movement of recycled biomass from aerobic to anaerobic, and also the anoxic shock in the SSR, is key to solubilize organic matter (Semblante et al. 2014) which would enhance the P removal. In contrast, uncoupling metabolism during anoxic shock conditions may reduce the available ATP used as an energy pool, thus also reducing the P removal activity and increasing the concentration in competitor organisms (Zhou et al. 2008). To study the effects of an anoxic SSR discharging into the anaerobic stage together with the raw wastewater, efficiencies (Table 2) and concentration profiles in each compartment (Figure 4) were monitored during the whole performance of the pilot plant.

The BIMINEX® process did not have any negative outcome in terms of organic matter removal and the efficiencies were not significantly different, as already reported by OSA-like processes (Chudoba et al. 1992). However, contradictory results were obtained in terms of nitrogen and phosphorus removal, although absolute concentrations presented lower discharge values (Figure 4, 15 mg N L⁻¹; 2 mg P L⁻¹) when using the BIMINEX® process. Nitrogen presented higher efficiencies when applying the anoxic shock treatment in the SSR to the sludge. Higher removals were unlikely related to the discharge of treated sludge in the anaerobic phase as nitrification, the key process in nitrogen removal, was occurring in the aerobic stage which is the furthest from the SSR (Figure 1). The efficiency improvement in the pilot plant setup was attributed to an increase of temperature in the water line, as the minimum aerobic SRT for nitrification (half of the total SRT in the studied system) must be 4–6 days at 20 °C and higher at lower temperatures (Tchobanoglous et al. 2003). This fact can also be observed in Figure 4 where the lowest concentrations of nitrogen were observed

### Table 2

<table>
<thead>
<tr>
<th>Set-up</th>
<th>COD (%)</th>
<th>Nitrogen (%)</th>
<th>Phosphorus (%)</th>
<th>Mean temperature (°C)</th>
<th>SRT (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Without SSR Lab</td>
<td>90 ± 5</td>
<td>74 ± 18</td>
<td>7 ± 42</td>
<td>20</td>
<td>21</td>
</tr>
<tr>
<td>With SSR Lab</td>
<td>92 ± 6</td>
<td>82 ± 10</td>
<td>18 ± 78</td>
<td>20</td>
<td>21</td>
</tr>
<tr>
<td>Without SSR Pilot</td>
<td>89 ± 5</td>
<td>75 ± 25</td>
<td>92 ± 9</td>
<td>12</td>
<td>16.5</td>
</tr>
<tr>
<td>With SSR Pilot</td>
<td>87 ± 8</td>
<td>86 ± 11</td>
<td>67 ± 18</td>
<td>22</td>
<td>17.5</td>
</tr>
</tbody>
</table>

![Figure 4](https://iwaponline.com/wst/article-pdf/71/7/1056/469084/wst071071056.pdf)

**Figure 4** Nitrogen and phosphorus concentrations profiles in the influent, SSR, anaerobic (ANA), anoxic (ANOX), aerobic (AER) stages and effluent. Dashed lines correspond to the discharge limits (91/271/EC). Error bars stand for standard deviations of the set of data collected during 50 days (n ≥ 15 samples).
in the aerobic phase when SSR was applied, while for the conventional system nitrogen concentration was stable through all compartments. However, TN removal was improved in both laboratory scale and pilot scale. Figure 4 shows slightly lower concentrations for both nitrogen and phosphorus concentrations with SSR compared with the conventional UCT configuration. This fact may be attributed to a higher denitrifying PAO activity which would enhance the final nitrogen removal.

To evaluate the influence of the SSR reactor in the microbial community FISH was performed from the UCT and BIMINEX® configurations, at 0 and 100% SSR (Figure 5). Ammonium and nitrite oxidizing bacteria (AOB and NOB, respectively) were semi-quantified by this technique. In terms of AOB, no effect of the SSR was observed as the nitritation microbial population remained stable (27 ± 2% at 0% SSR and 22 ± 8% at 100% SSR). However, NOB were reduced from 13 ± 3 to 3 ± 2%. This might be in accordance with the presence of denitrifying PAOs which are able to uptake phosphate directly from nitrite, thus increasing the final nitrogen removal.

Regarding phosphorus removal, higher efficiencies were obtained with the conventional system compared with the BIMINEX® system (Table 2). Negative removal values were even obtained in the laboratory-scale set-up due to an increase of phosphates in the effluent when compared to the influent. Phosphate was mainly released within the SSR reaching similar concentrations to those for the anaerobic stage. Therefore, the anoxic shocks in the SSR were reducing the available ATP in the cells (Zhou et al. 2008) before the sludge was returned to the anaerobic tank. As a consequence, not enough energy remained for fatty acids uptake and replenishment of the energy pools, thus phosphate was not further released in anaerobic conditions. When the sludge was submitted to

Figure 5 | FISH confocal micrographs from PAO and GAO population (stained in red and green, respectively; images A and C) and NOB and AOB (stained in red and green, respectively; images B and D). Samples for images A and B are for 0% SSR and images C and D are for 100% SSR from the pilot plant set-up. Scale bar at 10 μm. The full color version of this figure is available online at http://www.iwaponline.com/wst/toc.htm.
anoxic conditions with the presence of an electron donor (nitrite or nitrate), phosphate was taken up in order to replenish the polyphosphate pools. Phosphate uptake was probably not enhanced in aerobic conditions, although higher removal rates were usually obtained in these conditions, due to intracellular energy limitations. For this reason, denitrifying P removal (uptake in anoxic phase) was probably higher than aerobic P removal (Figure 4).

The analysis of the phosphorus removal community showed high enrichment of PAOs in the UCT configuration when compared to conventional systems. At 0% SSR the presence of PAO was of 26 ± 2% while their competitors GAO only accounted for 2 ± 0.5%. Energy limitations when implementing the side-stream reactor in the sludge line decreased the PAO concentration to 9 ± 3% at 100% SSR. However, even though competitors presence increase in low phosphorus content wastewater (Zhou et al. 2008), as shown for the one treated in the pilot set-up, the GAO community was maintained at 3 ± 2%.

Therefore, the implementation of an anoxic SSR caused a depletion of intracellular energy pools which decreased the overall performance of phosphate removal but, controversially, increased the denitrifying P removal capacity.

**Benefits of the anoxic sludge treatment**

The application of a SSR in a CAS plant does not present an important modification of the treatment plant and, as reported, may lead to both beneficial results in terms of sludge reduction and nutrient removal when temperature and SRT are not the key influencing parameters. Sludge reduction has been attributed to different mechanisms caused by alternating bacterial growth conditions such as energy uncoupling or feasting/fasting conditions (Semblante et al. 2014). The latter condition is also applied for biological phosphorus removal, but further research is required to improve the overall phosphorus removal performance by taking advantage of the enhanced anoxic P removal when compared to the aerobic one. Similar results were obtained for nitrogen removal. The enhancement of both anoxic nitrogen and phosphorus removal might be attributed to denitrifying phosphorus accumulating organisms (DPAOs) but further studies are required to determine if this is the case.

The actual reduction on biomass production is positively affecting the sludge post-treatment processes. Figure 6 presents the economical evaluation of La Garriga WWTP where the pilot plant was located if the BIMINEX® process would be implemented. The post-treatment cost was calculated as 2.71 € m⁻³ sludge in terms of dewatering energy and reagents. As the quantity of sludge would be reduced by 18% when applying an anoxic SSR, dewatering and reagents used for the same purpose would observe an annual reduction cost which, if maintained, would decrease the revenue of the technology compared to the most proposed aerobic digestion or anaerobic mesophilic digestion post-treatment processes.

**CONCLUSIONS**

The BIMINEX® process consisting of a SSR integrated in the RAS of a UCT configuration reduces the biomass production of the system without a complex system, monitoring and control. An ORP set-point of -150 mV in the SSR is enough to uncouple the metabolism of the micro-organisms and reduce the observed yields of the sludge. The higher the quantity of sludge treated in the SSR, the lower the observed yield. Furthermore, when maintaining temperature and SRT in the water line, the SSR also improves the stability of the nitrogen and anoxic phosphorus removal processes, although further research is required in terms of competition with other organisms and overall P removal improvement. The system was tested at laboratory scale and pilot scale, which proved the viability of the process for further scale-up.

**ACKNOWLEDGEMENTS**

This research was financially supported by the Spanish Government (CSD2007-00055, CTQ2011-23632, CTQ2011-
REFERENCES


APHA. 2005 Standard Methods for the Examination of Water and Wastewater, American Public Health Association, Washington, DC, USA.


Ye, F. & Li, Y. 2010 Oxic-settling-anoxic (OSA) process combined with 3,3′,4,4′-tetrachlorosalicylanilide (TCS) to reduce excess sludge production in the activated sludge system. Biochem. Eng. J. 49 (2), 229–234.


First received 13 September 2014; accepted in revised form 29 January 2015. Available online 13 February 2015