Aerobic granulation in a mechanical stirred SBR: treatment of low organic loads
A. Mosquera-Corral, B. Arrojo, M. Figueroa, J. L. Campos and R. Méndez

ABSTRACT
Aerobic granular sludge was produced in a sequencing batch reactor (SBR) characterized by a height to diameter ratio of 2.5 and the use of mechanical stirring. Compact and regular aerobic granules of up to 1.75 mm of average diameter were formed in the reactor with an organic loading rate of 1.75 kg COD/(m$^3$ d). Settling properties of the obtained aggregates were: sludge volumetric index of 30–40 mL/g VSS and settling velocity higher than 8 m/h. The effects of different carbon to nitrogen ratios (TOC/N) in the feeding on the organic matter oxidation and nitrification process were studied. The concentration of organic matter in the feeding was stepwise reduced (from 190.0 to 37.5 mg TOC/L) and ammonium increased (from 25 to 50 mg NH$_4^+$-N/L). TOC/N ratios of 7.50, 3.00, 1.50 and 0.75 g/g in the feeding were tested. The TOC removal percentage was around 80–95% during the whole operational period and the N removal percentages obtained in the reactor were up to 40%, however, physical properties of the granules were not maintained.

Key words | feeding composition, granular biomass, organic load, reactor configuration, SBR

INTRODUCTION
Aerobic granulation is a process of cell autoaggregation through cell-to-cell immobilization to form a stable, contiguous, multicellular association. These aggregated granules have a compact structure as compared to suspended sludge flocs (Tay et al. 2002). Studies showed that aerobic granulation is a gradual process from seed sludge to compact aggregates, further to granular sludge and finally to mature granules (Liu & Liu 2006).

Several factors can affect the process of cell aggregation such as the feeding composition and the reactor design, which affects the hydrodynamic shear forces and the extracellular polymeric substances secreted by cells (Tay et al. 2000a, b, 2005; Liu et al. 2004). To achieve aerobic granulation of the biomass, reactors are operated in cycles comprising short feeding and settling periods and long aerated periods. Selection of granular biomass is achieved by the feast and famine regime and the well settling biomass selection by the use of short settling periods.

Usually aerobic granulation is achieved in SBR reactors with a high height to diameter (H/D) ratio mixed by the application of air (Figueroa et al. 2008). The hydrodynamic conditions that are created by the air depend on the aeration flow and are described by the upflow air velocity. In these systems the reactor configuration has an impact on the flow patterns of liquid and microbial aggregates inside the reactor. The upflow pattern of the air favours the motion of biomass granules which are constantly subjected to circular hydraulic attrition (Liu & Tay 2002).

The feasibility and efficiency of other types of bio-reactors, such as completely stirred tank reactor, applied to the development of aerobic granular sludge have not been sufficiently demonstrated. The different features of these systems rely not only on the hydrodynamics, in terms of interactive patterns between flow and microbial aggregates, but also on the active selection of the biomass during the settling period of the operational cycle, which allows for different minimum settling velocities for the biomass to be retained inside the reactor (Liu & Tay 2002). In this aspect, the column-type upflow reactor with high H/D can provide an optimal interactive pattern between flow and microbial aggregates for granulation while systems with low H/D ratio and mechanical stirring provide different hydrodynamic conditions. Furthermore a high H/D results in a reactor with a small footprint (Beun et al. 2002; de Bruin et al. 2004). This may be a major reason for granular sludge formation in column-type upflow reactors.
In an engineering sense, the desirable interactive pattern between flow and aggregates might be achieved by controlling reactor configurations and operation strategy. Consequently, it is interesting to research new types of reactors to study their feasibility to produce granular sludge.

Studying the possibility of forming granules on wastewater with low organic matter composition in a SBR was a logical step in the scaling-up process and development of this technology (Ni et al. 2009). de Kreuk & van Loosdrecht (2006) studied the formation of aerobic granules with domestic sewage and they found that the chemical oxygen demand (COD) load could be a critical factor for granulation. Domestic sewage typically has much lower content than industrial wastewater and this will influence the granule formation process (Moy et al. 2003). de Kreuk & van Loosdrecht (2006) demonstrated that the organic loading rate (OLR) affected the formation of aerobic granules. They found that there were no granules formed under the OLR of 1 kg COD/m³ d. Instead, flocs with rather loose structure dominated reactor mixed-liquor. The most frequent cause of sludge inactivation is oxygen deficiency, which can cause a reduction in the rate of biodegradation. As a result, too high or too low OLR appeared to be unfavourable for the formation of a compact sludge bed, and further, for maintaining the stability of the reactor.

The objective of the present work is to study the formation of aerobic granules in a mechanical stirred SBR characterized by a relatively low H/D ratio and where low OLRs were treated. The selection of the type of reactor was done in order to evaluate the possibility of transformation of existing activated sludge systems into SBR systems where biomass is grown as aerobic granules.

**MATERIALS AND METHODS**

**Experimental set-up**

A SBR with a total volume of 5 L and a working volume of 3 L was used (Figure 1). Dimensions of the unit were: total height of 0.6 m and inner diameter of 0.12 m. The H/D was of 2.5. Oxygen was supplied to the reactor by using air spargers. The air flow was controlled by means of electrovalves at 11 L/min corresponding to an upflow air velocity of 1.58 cm/s. The mixing by mechanical stirring was performed with a Rushton turbine, comprising a standard 6-blade disk impeller (impeller diameter and height of 0.06 and 0.09 m, respectively). Rotating speed was fixed at 100 rpm. A set of two peristaltic pumps was used to feed the influent and to discharge the effluent. The influent was introduced through a port located at the top of the reactor. The effluent was discharged through a sampling port placed at the middle height of the column reactor. A programmable logic controller, Siemens model S7-224CPU, controlled the actuations over the pumps and valves, and thus the length of every operational phase in the operational cycle of the SBR (Figure 1). The reactor was operated at room temperature (15–20 °C) and without pH control, which varied between 7.4 and 8.5. The dissolved oxygen concentration was maintained around 5 mg O₂/L and 9 mg O₂/L during the feast and famine periods, respectively.

**Inoculum and feeding media**

The SBR was inoculated with flocculent activated sludge (Figure 2) characterized by the sludge volumetric index (SVI) of 100 mL/g VSS and the settling velocity of 0.25 m/h. The reactor was fed with a synthetic wastewater, with sodium acetate as carbon source, prepared according to Beun et al. (1999) and mixed with the trace solution from Smolders et al. (1995). The synthetic wastewater contained soluble COD as the sole organic matter and ammonium as nitrogen source.

**Operational strategy**

The SBR was operated during 220 days in four different stages (Table 1). Different TOC/N ratios of 7.5, 3.0, 1.5 and 0.75 g/g in the feeding were used. The system was operated in cycles of 3 h distributed between filling (3 min), aeration...
(171 min), settling (1 min), effluent withdrawal (3 min) and idle time (2 min). The volume exchange ratio was fixed at 50%. The hydraulic retention time was always 0.25 days.

**Analytical procedures**

The pH, dissolved oxygen, nitrate, ammonia, TOC, volatile suspended solids (VSS), total suspended solids (TSS) and SVI were determined according to Standard Methods for the Examination of Water and Wastewater APHA/AWWA/WEF (2005). The settling velocity \( (V_s) \) was measured by recording the time taken for individual granules to fall from a certain height in a measuring cylinder. The morphology and size distribution of the granules was measured regularly by using an Image Analysis procedure proposed by Tijhuis et al. (1994) counting a sample of more than 200 granules. Biomass density, in terms of grams VSS per litre of granules, was determined with dextran blue, following the methodology proposed by Beun et al. (1999). The presence of nitrifying bacteria in granules was followed by fluorescence in situ hybridization. This analysis was performed with a set of fluorescent labelled 16S rRNA-targeted DNA probes according to the procedure described by Amann (1995). The used probes for in-situ hybridization were labelled with the dyes FITC or Cy3 and in all cases the reagent DAPI was also applied to detect the DNA present in the samples. Details on oligonucleotide probes are available at probeBase (Loy et al. 2007). Fluorescence signals of disaggregated samples were observed under an Axioskop 2 epifluorescence microscope (Zeiss, Germany) provided with a digital camera (Coolsnap, Roper Scientific Photometrics) and images were collected in a computer.

**RESULTS AND DISCUSSION**

**Aerobic granular biomass**

The reactor was operated during 220 days in four operational stages (Table 1). During the first seven experimental days an almost complete suspended biomass washout from the system occurred. Thus, either flocs or aggregates of biomass with settling velocities slower than 9 m/h were removed from the system. Two weeks after the start up of the reactor the formation of small aggregates with an average diameter of 0.5 mm was observed in the system. Suspended flocs gradually disappeared from the reactor and settling properties of the obtained aggregates were: SVI 50 mL/g VSS and \( V_s \) of 10 m/h. Microscopic examination of the sludge showed that the morphology of the granular biomass was completely different from the flocculent sludge that was used as inoculum. The shape of the granules was round with a cauliflower like aspect and very clear outline (Figure 2).

Table 1 | Operational conditions during the different operational stages

<table>
<thead>
<tr>
<th>Stage</th>
<th>Days (d)</th>
<th>TOC (mg/L)</th>
<th>NH(_4)-N (mg/L)</th>
<th>TOC/N (g/g)</th>
<th>OLR (kg COD/m(^3) d)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>0–120</td>
<td>190.0</td>
<td>25</td>
<td>7.5</td>
<td>1.75</td>
</tr>
<tr>
<td>II</td>
<td>121–163</td>
<td>75.0</td>
<td>25</td>
<td>3.0</td>
<td>0.7</td>
</tr>
<tr>
<td>III</td>
<td>164–190</td>
<td>38.7</td>
<td>25</td>
<td>1.5</td>
<td>0.38</td>
</tr>
<tr>
<td>IV(^a)</td>
<td>191–220</td>
<td>34.6</td>
<td>50</td>
<td>0.75</td>
<td>0.35</td>
</tr>
</tbody>
</table>

\(^a\)Inoculation with nitrifying sludge.
to granular sludge of 0.75 mm in diameter and finally to mature granules of 1.75 mm (Figure 3(a)).

The aerobic granular biomass formed in the reactor presented very good settling properties. The SVI was around 30–40 mL/g VSS during most of the steady-state period (Figure 3(b)) reaching, at the end of the experiment, values of 25 mL/g VSS. The $V_s$ was in the range 8–14 m/h during almost the whole operational time (Figure 3(b)). This value decreased down to 4 m/h at the beginning and at the end of the experiment due to the worsening of the settling properties of the sludge in these periods. Biomass density was in the range from 25 to 45 g VSS/(L granules) during most of the experimental period (Figure 3(c)). This value was similar to the values reported for aerobic granules or biofilms formed in airlift reactors of 25–60 g VSS/(L granules) (Kwok et al. 1998) and higher than those reported for other works using acetate, with values of 10–20 g VSS/(L granules) (Tijhuis et al. 1994; Beun et al. 1999; Arrojo et al. 2004; Mosquera-Corral et al. 2005). Therefore it seems that mechanical stirring did not negatively affect the granules.

Biomass concentration in the reactor at the beginning of the experiment was around 0.2 g VSS/L (Figure 4). It increased up to 3 g VSS/L after 45 days and reached a stable value around 6 g VSS/L. From the slope of the curve describing the biomass growth between days 30 and 79 the biomass yield could be estimated as 0.33 g VSS/g COD, a value similar to those estimated by Tay et al. (2001b) for an aerobic granular reactor, and lower than that reported for activated sludge of 0.42 g VSS/g COD (Garrido et al. 2001). As a consequence of the decrease of the TOC concentration in the feeding (Table 1) the biomass concentration decreased down to 4 g VSS/L in Stage III and to 2 g VSS/L (Stage IV). The TSS concentration in the effluent was during most of the experimental period between 0.04 and 0.09 g TSS/L and the VSS concentration was between 0.05 and 0.07 g VSS/L. Only at the beginning of the experiment were these values higher as a consequence of the inoculated sludge wash-out which provoked, solids concentration up to 0.20 and 0.15 g TSS/L and g VSS/L, respectively. After day 190 (Stage IV), the concentration of solids increased up to 0.30 g TSS/L, presumably as a result of the decrease of the TOC concentration applied to the system, which could provoke the breaking up and wash-out of the granules because of the lack of organic matter fed to the present biomass. The biomass concentrations obtained during this study were similar to those obtained in other granular SBRs, between 3 and 7 g TSS/L, and also the VSS/TSS ratio, from 0.8 to 0.9 g/g (Arrojo et al. 2004).

The low TOC concentration in the influent during period IV, corresponded to 0.35 kg COD/(m³ d) and led to granule instability and biomass wash-out due to progressive deterioration of the physical properties of the biomass. During this period, biomass had a fluffy, irregular and loose morphology. Also small particles were observed in this period probably coming from the breakage of the big original granules. These results were similar to those found by de Kreuk (de Kreuk & van Loosdrecht 2006) who demonstrated that granule formation with domestic sewage was only possible if the reactor was operated at OLR higher than 1.6 kg COD/(m³ d) (in the present case only during Stage I). They found that COD load is crucial during startup, which might be hampered if the sewage is too diluted or cycle times are too long. Similar results were observed by
Tay et al. (2005), who studied the influence of OLR on the formation of aerobic granules. They did not obtain granules formed under the OLR of 1 and 1.5 kg COD/(m³ d). So, COD load will be an important process parameter at larger scale operation for the start up of the process and should be taken into account for the stable operation of the reactor once aerobic granules are formed. Although in a previous work, Mosquera-Corral et al. (2005) obtained a nitrifying granular sludge from heterotrophic sludge by decreasing the COD/N until COD was completely eliminated, the difference between each was the presence of nitrifiers in the granules, which maintained the structure.

The FISH technique was used to identify the microbial populations from granules mechanically disrupted. Samples of granules were collected from the reactor in operating days 35, 58 and 170. Positive results were obtained in both samples with probe Ntspa712, which targets most members of phylum Nitrospirae (nitrite oxidizing bacteria) and Nsm156 for Nitrosomonas spp. and Nitrosococcus mobilis (ammonia oxidizing bacteria). In both samples the abundance of ammonia and nitrite oxidizing bacteria was scarce in comparison to the rest of bacteria in the sample (Figures 5(a) and (b)). The presence of these populations was indicative of the imminent appearance of the nitrification process in the reactor which was not fully developed and at the same time, the low percentage of autotrophic bacteria (also on day 170) demonstrates that there was not enough population after the reduction of organic matter in the influent.

The use of a H/D ratio of the reactor smaller than those usually used in aerobic granular reactors did not exert a negative effect on the biomass granulation since the imposed settling velocity was higher than 9 m/h. The geometry of a reactor has to be considered in order to impose proper settling velocities and appropriate hydrodynamic conditions for aerobic granulation (Liu et al. 2005). In the present study, the use of the mechanical stirrer could provoke higher shear stress in the system and also help the granulation process.

**Carbon and nitrogen removal**

The applied OLR decreased stepwise from 1.75 to 0.35 kg COD/(m³ d) and the applied nitrogen loading rate (NLR) increased from 0.1 to 0.2 kg NH₄⁺-N/(m³ · d), from Stages I to IV. During the four operational stages the TOC removal was higher than 80% and most of the time close to 95% with TOC concentrations in the effluent lower than 10 mg TOC/L (Figure 6(a)). Applied OLR decreased stepwise from 0.8 to 0.14 g TOC/(L d) and the applied NLR increased from 0.1 to 0.2 g NH₄⁺-N/(L d), from Stage I to IV. The reactor was operated with decreasing TOC/N ratios from 7.5 to 0.75 g/g (Table 1). The ammonia concentration in the effluent ranged from 10 to 20 mg N/L in Stages I to III and around 55 in Stage IV (Figure 6(b)). N removal efficiencies were around 20–40% during the whole operational time. From the nitrogen balance it was estimated that 90% of the nitrogen removal was nitrogen for biomass growth. The amount of nitrogen consumed for biomass production was estimated considering a general composition of the biomass as C₅H₇NO₂ and using the same equations shown in Mosquera-Corral et al. (2005). Nitrification was almost absent during Stages I–III without nitrite production and the presence of 1–3 mg NO₃⁻/N/L. Incipient nitrification was detected only during Stage IV when 15 mg NO₂⁻-N/L and 8 mg NO₃⁻-N/L were measured in the effluent, 1 day after the inoculation of the system with nitrifying sludge and coinciding with the increase of the fed nitrogen concentration. This process might be related to the biomass washout which could select for nitrifiers to the detriment of
heterotrophs due to the lack of organic matter content in the feeding media.

In order to determine which processes were occurring, measurements during operational cycles were performed on days 62, 140, 175 and 209. Results indicated that the organic matter was consumed after 30 min from the beginning of the cycle. The 150 min left of the cycle could be used, if it were the case, by the nitrifying organisms to oxidize ammonia. Taking into account that the environmental conditions inside the reactor were appropriate for the nitrification to occur, the most probable factor affecting this process could be that the maximum achieved SRT was too short. The fact that at this point the granular biomass experienced a breakage event, owing to the lack of organic matter in the feeding could also contribute to this effect.

CONCLUSIONS

- Granulation of sludge in aerobic conditions has been produced in a SBR reactor with mixture induced by mechanical stirring and an H/D ratio of 2.5. Granules obtained presented good settling properties: SVI of 30–40 mL/g VSS and V<sub>s</sub> higher than 8 m/h. These results open the possibility to transform some already constructed systems into aerobic granular reactors.
- The operation of the aerobic granular SBR at different TOC/N ratios allowed the achievement of removal percentages of 90% for the organic matter and up to 40% for the ammonia nitrogen treating loads up to 2 kg COD/(m<sup>3</sup> d) and 0.1 kg NH<sub>4</sub>+-N/(m<sup>3</sup> d).
- The formation of stable granules was not possible for OLR under 1 kg COD/(m<sup>3</sup> d) (TOC concentrations of 75 mg/L and lower) meaning that a minimum gradient concentration is needed to be able to generate granular biomass.
- The TSS concentration in the effluent was between 40 and 90 mg TSS/L during the different operational stages except for the last one where TSS increased to 0.3 g TSS/L due to the breakage of the granules.

ACKNOWLEDGEMENTS

This work was funded by the Spanish Government (TOGRANSYS CTQ2008-06792-C02-01 and NOVEDAR_Consolider project CSD2007-00055). Authors want to thank Mar Orge, Mónica Dosil and Miriam Vieites for their support with the analytical techniques.

REFERENCES
