Start-up of an aerobic granular sequencing batch reactor for the treatment of winery wastewater

S. López–Palau, J. Dosta and J. Mata-Alvarez

ABSTRACT

Aerobic granular sludge was cultivated in a sequencing batch reactor (SBR) in order to remove the organic matter present in winery wastewater. The formation of granules was performed using a synthetic substrate. The selection parameter was the settling time, as well as the alternation of feast-famine periods, the air velocity and the height/diameter ratio of the reactor. After 10 days of operation under these conditions, the first aggregates could be observed. Filamentous bacteria were still present in the reactor but they disappeared progressively. During the start-up, COD loading was increased from 2.7 to 22.5 kg COD/(m$^3$ day) in order to obtain a feast period between 30 and 60 minutes. At this point, granules were quite round, with a particle diameter between 3.0 and 4.0 mm and an average density of 6 g L$^{-1}$. After 120 days of operation, synthetic media was replaced by real winery wastewater, with a COD loading of 6 kg COD/(m$^3$ day). The decrease of the organic load implied a reduction of the aggregate diameter and a density increase up to 13.2 g L$^{-1}$. The effluent was free of organic matter and the solids concentration in the reactor reached 6 g VSS L$^{-1}$.

Key words | aerobic granulation, SBR, winery wastewater

INTRODUCTION

More than the 65% of the world production of wine is produced in the European Union. Italy, France and Spain are the largest world wine producers with more than 80% of the European production. During the wine production process, large volumes of wastewater are generated from various washing operations. The discharges of these effluents represent a complex issue due to their particular characteristics: high organic load associated to a high biodegradability (80% of the COD is soluble and the ratio of BOD$_5$/COD ranges between 0.5 and 0.6), a COD ten to hundred times higher than domestic effluents and an acidic pH (sometimes, it may display basic values, on the occasion of the cleaning operations). Some years ago, these effluents were removed in public sewers or disposed off into evaporation ponds causing bad odours and the possibility of the pollution of surface waters and underground aquifers. With the establishment of stringent regulations concerning public waste disposal, there is a growing interest in the development of new technologies and procedures for the decontamination of this wastewater. Aerobic granulation appears to be one of the most suitable techniques to treat this kind of wastewater due to their multiple advantages: an excellent settleability, strong and organized microbial structure, high biomass retention and good ability to handle high organic loading rates and inhibitory and toxic wastes (Liu & Tay 2004). The growth of aerobic granules is sometimes regarded as a special case of biofilm development (Liu & Tay 2002). In fact, microbial granulation is quite fundamental in biology and cell aggregation can be defined as the gathering together of cells to form a fairly stable, contiguous, multicellular association under physiological conditions. Each aerobic...
granule is an enormous metropolis of microbes containing millions of individual bacteria. Almost all aerobic granules have been cultivated in Sequencing Batch Reactors (Morgenroth et al. 1997; Beun et al. 1999; Peng et al. 1999; Etterer & Wilderer 2003; Tay et al. 2001; Liu & Tay 2002; Arrojo et al. 2004). The SBR system is a modified design of the conventional activated sludge process and it has been widely used in municipal and industrial wastewater treatment (Mace & Mata-Alvarez 2002). It seems certain that aerobic granulation is a gradual process involving the progression from seed sludge to compact aggregates, further to granular sludge and finally to mature granules (Dangcong et al. 1999).

MATERIALS AND METHODS

Experimental set-up

A sequencing batch reactor with a working volume of 3.0 l and a height to diameter relation of 3 to 1 was used. The HRT was fixed at 8 hours. The reactor was operated at room temperature and it was equipped with a pH electrode and a dissolved oxygen probe. These parameters were monitored continuously. Air was introduced at the bottom of the reactor using a porous stone, connected to several air pumps.

The reactor was operated in successive cycles of 4 hours length, consisting of four different phases (Arrojo et al. 2004): filling (5 min), aeration (230 min), settling (1 min) and effluent withdrawal (4 min). Aerobic starvation is one of the most important parameters to take into account. Under starvation conditions, bacteria become more hydrophobic, what facilitates microbial adhesion or aggregation (Bossier & Verstraete 1996; Tay et al. 2001; Liu et al. 2003). McSwain et al. (2003) proved that a feast-famine cycle or pulse feeding of the SBR favoured the formation of compact and dense aerobic granules. Thus, the substrate was added in the reactor with only 5 minutes at the beginning of each operational cycle. Apart from this, the short settling time in reactor with only 5 minutes at the beginning of each dense aerobic granules. Thus, the substrate was added in the feeding of the SBR favoured the formation of compact and

Media

During the first stage, a synthetic wastewater was used. The composition of this media was modified along the experimentation, increasing progressively the organic load treated and the nutrients concentration in order to avoid bacteria inhibition. At the end of the experimentation with synthetic wastewater, the influent composition was as follows: NaCH3COO 7.5 g L⁻¹, NH4Cl 1.34 g L⁻¹, K2HPO4 4.82 g L⁻¹, KH2PO4 1.95 g L⁻¹, MgSO4·7 H2O 0.75 g L⁻¹, CaCl2·2H2O 0.58 g L⁻¹, EDTA 0.17 g L⁻¹, trace solution 1 mL L⁻¹. Trace solution composition was the one reported by Beun et al. (2002): FeCl3·6H2O 1.5 g L⁻¹, H3BO3 0.15 g L⁻¹, CuSO4·5H2O 0.03 g L⁻¹, KI 0.03 g L⁻¹, MnCl2·4H2O 0.12 g L⁻¹, Na2MoO4·2H2O 0.06 g L⁻¹, ZnSO4·7H2O 0.12 g L⁻¹, CoCl2·6H2O 0.15 g L⁻¹.

During the second stage, real winery wastewater was treated in the reactor. The main characteristics of this effluent are summarised in Table 1. In each cycle, 1.5 L of water was added to the reactor. It is important to highlight the high organic content as well as the lack of nutrients of this effluent.

Analytical procedures

The DO concentration and the pH in the reactor were monitored with a DO probe WTWoxi232 and a Crison pH 25 electrode. Solids concentration (total solids, total suspended solids, volatile solids and volatile suspended solids), chemical oxygen demand, ammonium nitrogen and

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
<th>Range</th>
<th>Unit of measurement</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soluble COD</td>
<td>3.10</td>
<td>2.76–3.35</td>
<td>g COD L⁻¹</td>
</tr>
<tr>
<td>NH4-N</td>
<td>6.26</td>
<td>6.18–6.43</td>
<td>mg NH4-N L⁻¹</td>
</tr>
<tr>
<td>NO2-N</td>
<td>N.D.</td>
<td>N.D.</td>
<td>MgNO2-N L⁻¹</td>
</tr>
<tr>
<td>NO3-N</td>
<td>N.D.</td>
<td>N.D.</td>
<td>MgNO3-N L⁻¹</td>
</tr>
<tr>
<td>pH</td>
<td>6.5</td>
<td>5.6–7.4</td>
<td>–</td>
</tr>
<tr>
<td>TS</td>
<td>3.85</td>
<td>3.81–3.92</td>
<td>g TS L⁻¹</td>
</tr>
<tr>
<td>VTS</td>
<td>2.19</td>
<td>2.13–2.22</td>
<td>g VTS L⁻¹</td>
</tr>
<tr>
<td>SS</td>
<td>0.34</td>
<td>0.32–0.35</td>
<td>g SS L⁻¹</td>
</tr>
<tr>
<td>VSS</td>
<td>0.12</td>
<td>0.01–0.03</td>
<td>g VSS L⁻¹</td>
</tr>
<tr>
<td>Alkalinity</td>
<td>520–542</td>
<td>529.1</td>
<td>mg CaCO3 L⁻¹</td>
</tr>
<tr>
<td>Temperature</td>
<td>20</td>
<td>18–22</td>
<td>°C</td>
</tr>
</tbody>
</table>

Table 1 | Winery wastewater characterisation
alkalinity were performed following the proceeding described in the Standard Methods for the Examination of Water and Wastewater (APHA 1998). \( \text{NO}_3 \) concentrations were analysed by ionic chromatography. Biomass density was determined with dextran blue as described by Beun et al. (2002). Changes in morphology of granules were followed by flow citometry and by Scanning Electron Microscopy (SEM).

RESULTS

Start-up with synthetic wastewater

The reactor was started up using seed sludge from a municipal wastewater treatment plant as inoculum. In the first stage, the reactor was fed with synthetic wastewater with the composition described above. The settling time is the most important selection parameter (Qin et al. 2004). First of all, it was fixed in 5 min. After 10 days of operation, it decreased to 2 min and finally, on the day 20th, it was fixed in 1 min and maintained during the remaining of the experimental periods. The solids concentration in the effluent reached very low values in very few days each time the settling time was changed (see Figure 1). This means that the biomass with poor settleability was easily washed out of the reactor. Therefore, very high biomass retention and the consequent reduction of sludge produced, one of the advantages of this process, has been demonstrated. In period IV (see Table 2), when the OLR was 22.5 g \( \text{O}_2 \) (L day\(^{-1} \)), the solids concentration in the effluent were slightly increased, as a result of the high growing rate of the biomass.

Filamentous bacteria predominated in the seed sludge used to inoculate the reactor but after 20 days of operation under these conditions, the first aggregates could be observed. Filamentous bacteria were still present in the reactor but their concentration decreased progressively. Simultaneously, the aggregates size and roundness were increased. Figure 2 compares the microstructure of the seed sludge with the one of the granules present in day 40. Aerobic granules have a very compact bacterial structure, in which round species are clearly predominant. However, low levels of filamentous bacteria can still be observed and they likely serve as a backbone that strengthens the structure of aerobic granule.

In order to enhance the growth of biomass the COD concentration in the synthetic wastewater was increased progressively. Several periods could be identified regarding to the COD concentration, as shown in Table 2.

At the end of the start-up, an OLR of 22.5 g \( \text{O}_2 \) (L day\(^{-1} \)) was treated. At this point, the growth of biomass was extremely high due to the high content of sodium acetate, the organic source present in the influent. Consequently, some problems of aeration appeared. The air velocity used until that moment was not enough to maintain a good level of dissolved oxygen concentration. So, some granules were attached and fixed as a mass at the bottom of the reactor. In order to solve this problem, the air supply was increased from 13.5 L min\(^{-1} \) to 20 L min\(^{-1} \), and most of the problems disappeared.

Figure 3 shows the evolution of COD concentration in the influent and in the effluent during the operation with synthetic wastewater. During all this first stage, the concentration of COD in the effluent was almost always

![Figure 1](https://iwaponline.com/wst/article-pdf/60/4/1049/449022/1049.pdf)  
**Figure 1** Solids concentration in the effluent (SS: - - - - ; VSS: - - - - ). (Settling time variation: - - - - , organic load variation: - - - - ).

<table>
<thead>
<tr>
<th>Period</th>
<th>OLR [g ( \text{O}_2 ) (L day(^{-1} ))]</th>
<th>Days</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>2.7</td>
<td>1–40</td>
</tr>
<tr>
<td>I</td>
<td>4.2</td>
<td>41–48</td>
</tr>
<tr>
<td>II</td>
<td>8.4</td>
<td>49–59</td>
</tr>
<tr>
<td>III</td>
<td>15.0</td>
<td>60–76</td>
</tr>
<tr>
<td>IV</td>
<td>22.5</td>
<td>77–120</td>
</tr>
</tbody>
</table>
undetectable, in spite of the first days after increasing the organic loading rate. Nitrogen concentration in the influent was less than 40 mg NH$_4^+$-N L$^{-1}$, which was almost all consumed during metabolic processes of the microorganisms.

Aggregate diameter increased from 2 to 4 mm with increasing the organic load in the reactor but at the same time, biomass density slightly decreased.

At the end of the experimentation with synthetic wastewater, the solids concentration in the reactor was 10.7 g SS L$^{-1}$ and 3.4 g VSS L$^{-1}$. The granule density reached 6.5 g L$^{-1}$.

**Operation with winery wastewater**

After 120 days working with synthetic media, it was replaced by real winery wastewater, whose main characteristics are summarised in Table 1. The lack of nutrients of this effluent made it necessary to add some extra nutrients in the reactor. The nutrients concentration in the influent was calculated following the same proportion ratios used with the synthetic media. NO$_3^-$ were not detected in the effluent due to the low content of ammonia in the influent.

The change of substrate caused no trouble in the system. The mixed liquor got darker due to the winery wastewater colour. Figure 4 shows the appearance of the granules after 10 days of operation with the real effluent.

Since HRT was maintained, the addition of real wastewater implied a decrease of organic load in the reactor and a consequent reduction of the aggregate size was observed. Particle diameter was about 2 mm. On the other hand, granule density increased and reached a value of 13.5 g L$^{-1}$. It has been confirmed that granule density decreases with increasing the aggregate size (Etterer & Wilderer 2001).

COD was also totally consumed in only few minutes, so feast-famine strategy was successfully achieved. Figure 5 shows COD and DO profiles during an operational cycle after 12 days of operation with winery wastewater.

The consumption of COD took place during the first hour of each cycle. It can also be observed that there is a
part of COD (100 mg O₂ L⁻¹ approximately), which was not degraded. This may be recalcitrant matter. The dissolved oxygen profile inside the reactor fits with the COD one. Biomass activity involves an oxygen consumption. Therefore, DO concentration decreases during the first minutes of the operational cycle due to the organic matter degradation. As soon as the biomass activity stopped, dissolved oxygen concentration increased until reaching the initial value. pH profile maintained quite constant during all the operational cycle.

**DISCUSSION**

It was shown that it is possible to cultivate aerobic granular sludge in a sequencing batch reactor with a high height-diameter relation, which makes the selection of granules easier by the difference in settling velocity. Settling time is the parameter that allows only particles with a good settleability to remain in the reactor. Slow settling particles with filamentous bacteria will be washed out with the effluent. To start-up the reactor it is important to decrease this parameter progressively in order to get the biomass used to settle quickly and to avoid the complete wash out of biomass.

**Organic matter degradation**

Several references show successful results of nitrogen removal through nitrification and denitrification processes by granular sludge (Mosquerra-Corral et al. 2005; Qin & Liu 2006; Tsuneda et al. 2006). In this study, nutrients are present in the system only to allow biomass metabolic processes. The main objective of microorganisms is to degrade organic matter. In the first stage of the study, synthetic wastewater with a high organic content was used. It has been demonstrated that aerobic granules are able to treat an organic load of 22.5 g COD (L day⁻¹). Nevertheless, when the organic load was so high, biomass grew really fast and some problems of aeration appeared. Thus, it is possible to treat effluents with an elevated organic content but aeration must be proportional to the COD load. Winery wastewater, an effluent characterized by their high organic content, has been successfully treated with this technology, adding some extra nutrients in the reactor.

**CONCLUSIONS**

The main objectives of this project were the investigation of degradation of organic matter by aerobic granular sludge to treat winery wastewater, and the following conclusions were reached.

1. A COD load of 22.5 g COD (L day⁻¹) of synthetic wastewater has been successfully treated.
2. When COD was higher, some aeration problems appeared. It was required to increase the air supply in the reactor.
3. Degradation of organic matter present in a winery wastewater was successfully performed with the addition of extra nutrients in the system.
4. Aggregate diameter depends on the COD content in substrate. Density of aerobic granules decrease with increasing aggregate diameter.

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