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Effects of Inoculum Type and Aeration Flowrate on the Performance of Aerobic Granular SBRs

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Abstract: Aerobic granular sequencing batch reactors (SBRs) are usually inoculated with activated sludge which implies sometimes long start-up periods and high solids concentrations in the effluent due to the initial wash-out of the inoculum. In this work, the use of aerobic mature granules as inoculum in order to improve the start-up period was tested, but no clear differences were observed compared to a reactor inoculated with activated sludge. The effect of the aeration rate on both physical properties of granules and reactor performance was also studied in a stable aerobic granular SBR. The increase of the aeration flow rate caused the decrease of the average diameter of the granules. This fact enhanced the COD and ammonia consumption rates due to the increase of the DO level and the aerobic fraction of the biomass. However, it provoked a loss of the nitrogen removal efficiency due to the worsening of the denitrification capacity as a consequence of a higher aerobic fraction.

Keywords: aeration flowrate; aerobic granules; inoculum; sequencing batch reactor; wastewater

1. Introduction

Activated sludge process is the most used technology to remove organic matter, nitrogen, and phosphorous from wastewaters. However, because of the poor settling properties of the flocculent biomass, activated sludge systems require a relatively large implementation surface. A possible option to avoid this drawback is the use of aerobic granular biomass since this type of biomass presents structures more compact and stronger than those of the flocculent biomass and, therefore, allows a better retention of the biomass [1,2].

The formation of aerobic granular biomass is promoted by applying a periodic feast-famine regime and short settling times which can be accomplished with a Sequencing Batch Reactor (SBR) [3,4]. There are several factors that influence the granulation process and the structure of the aggregates, such as the feed composition, organic loading rate (ORL), hydrodynamic shear force caused by aeration flowrate, and type of inoculum [5–7]. Aerobic granular biomass has been successfully obtained using different types of industrial and municipal wastewaters [8–12]. However, in some cases the formation of granules can take several weeks depending on the composition but also on the kind of inoculum used [13]. In fact, most of the aerobic granular reactors used in other research works were inoculated with biomass from activated sludge systems treating urban or industrial wastewater, which caused the loss of biomass during the start-up period leading to poor effluent quality. Today, more attention is

being paid to selecting a suitable inoculum to shorten this period and/or obtain granules with better physical properties [7,14,15].

Pijuan et al. [14] optimized the start-up period using as inoculum a mixture of floccular and crushed aerobic granular biomass (50/50). This strategy maintained the nitrogen removal capacity and avoided biomass losses during the granulation process. Xu et al. [16] proposed the use of pellets obtained from activated sludge flocs as inoculum to develop aerobic granular biomass. These authors found that this type of inoculum had a shorter start-up time compared with that obtained using activated sludge as inoculum. Moreover, the granules obtained had better settling properties which were attributed to the different bacterial populations observed and could be stored without deterioration of their structure for long time [13].

Besides the type of inoculum, another factor that has a strong influence on granule formation is the aeration rate applied. The shear forces caused by aeration promote the production of polysaccharides, which impacts the physical properties of granules [2,17,18]. More compact, stable and denser aerobic granules are obtained by applying high hydrodynamic shear force [19,20]. However, a higher aeration rate implies higher operational costs and, therefore, an optimal aeration rate should be determined [21].

For these reasons, the objective of this work is to determine the influence of the inoculum on the start-up of an aerobic granular system and to optimize the aeration rate applied.

2. Results and Discussion

2.1. Granules Formation and Properties with Different Type of Inoculum

Two SBRs were operated with different type of inoculum: activated sludge (SBR I) and mature aerobic granules (SBR II). The development of the average diameter of the aggregates was different in both reactors. In SBR I, the granule diameters ranged from 0.2 to 2.8 mm at day 10, with an average diameter of 1.8 mm (42% of the aggregates observed) (Figure 1a). Granules size increased up to 9 mm (day 18) (Figure 1b) and, then, granule size decreased. The average diameter of granules used to inoculate SBR II was of 2.5 mm and increased up to 6.6 mm on day 18. At this moment, small filamentous were observed on the granules surface and, finally, they broke up. After that, the solids were washed out from the SBR and the average diameter of the granules decreased down to 5.4 mm at day 29 (Figure 2).

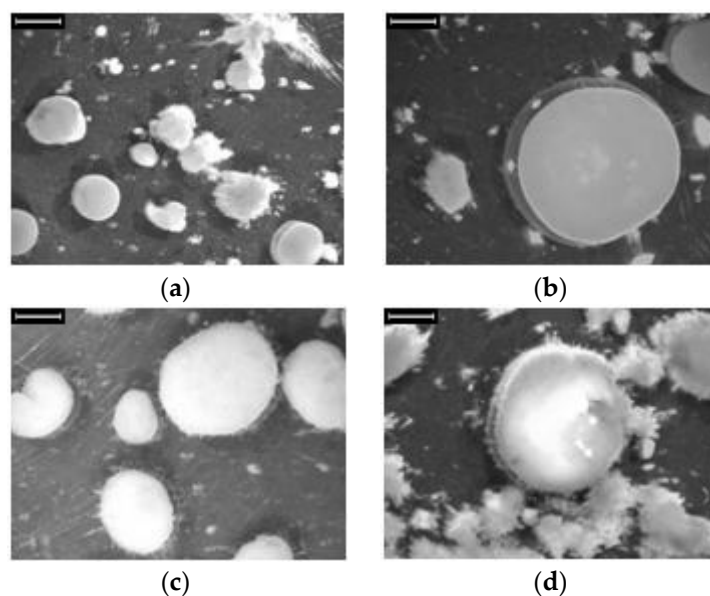


Figure 1. Images of biomass at different operational days: (a) SBR I (10 days); (b) SBR I (18 days); (c) SBR II (9 days); (d) SBR II (18 days). The bar represents 2 mm.

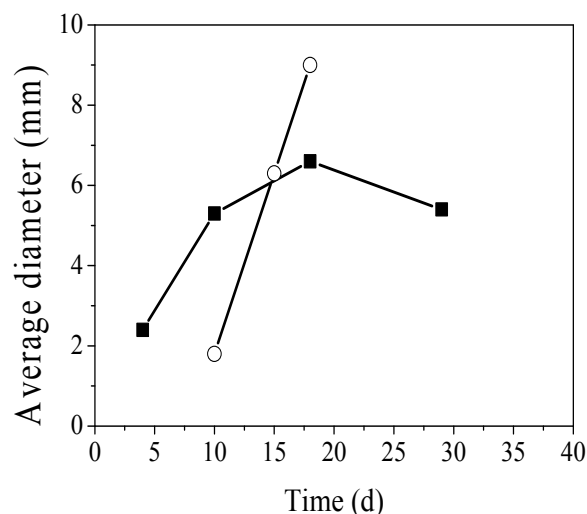


Figure 2. Average diameter of biomass in SBR I (○) and SBR II (■).

The initial volatile suspended solids (VSS) concentration of SBR I was of 0.7 g VSS L^{-1} and increased up to 3.4 g VSS L^{-1} on day 19 (Figure 3a). In SBR II, the solids concentration decreased from 8.5 g VSS L^{-1} to 3.4 g VSS L^{-1} on day 9 (Figure 3b). The biomass concentration increased slowly up to 4.0 g VSS L^{-1} and finally, diminished down to 1.8 g VSS L^{-1} on day 32.

In both cases, granules formed have similar SVI ($52\text{--}58 \text{ mL (g TSS)}^{-1}$), density ($23.9\text{--}29.3 \text{ g VSS L}_{\text{granule}}^{-1}$) and organic fraction (VSS/TSS) ($0.60\text{--}0.65$). These values are in the range of those reported for aerobic granular biomass [22].

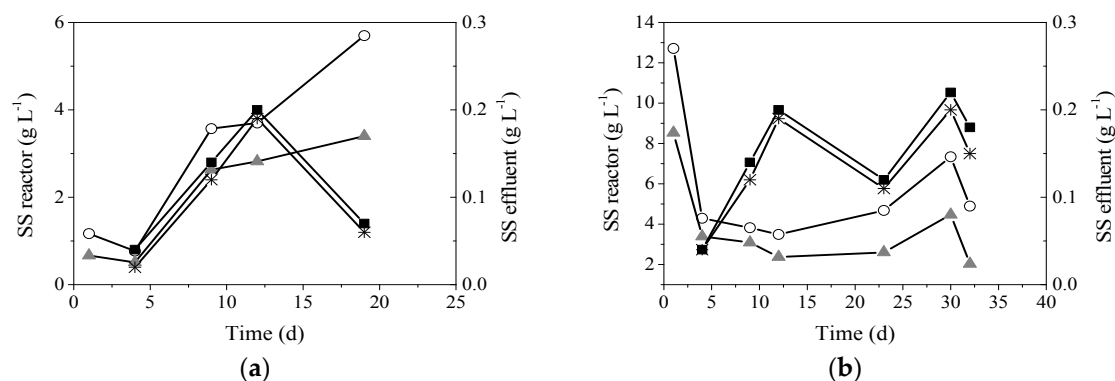


Figure 3. Total suspended solids (TSS) and Volatile suspended solids (VSS) in the reactors and effluent concentrations: (○) TSS reactor; (▲) VSS reactor; (■) TSS effluent; (*) VSS effluent. (a) SBR I and (b) SBR II.

2.2. Organic Matter and Nitrogen Removal with Different Type of Inoculum

In SBR I, the COD removal was maintained around 96% except between days 8–12 of operation when punctual values around $140 \text{ mg COD L}^{-1}$ were measured in the effluent due to the wash-out of biomass (Figure 4a). For SBR II, a similar COD removal efficiency (97%) was observed during whole operational period (Figure 4b). Nitrite and nitrate were detected only at low concentrations in both reactors (Figure 4c,d), nitrogen being removed mainly by biomass assimilation (Table 1). The low development of the nitrification process could be attributed to the biomass wash-out episodes observed in both reactors, which would cause the solids retention times to be lower than those needed to develop nitrifying biomass [23].

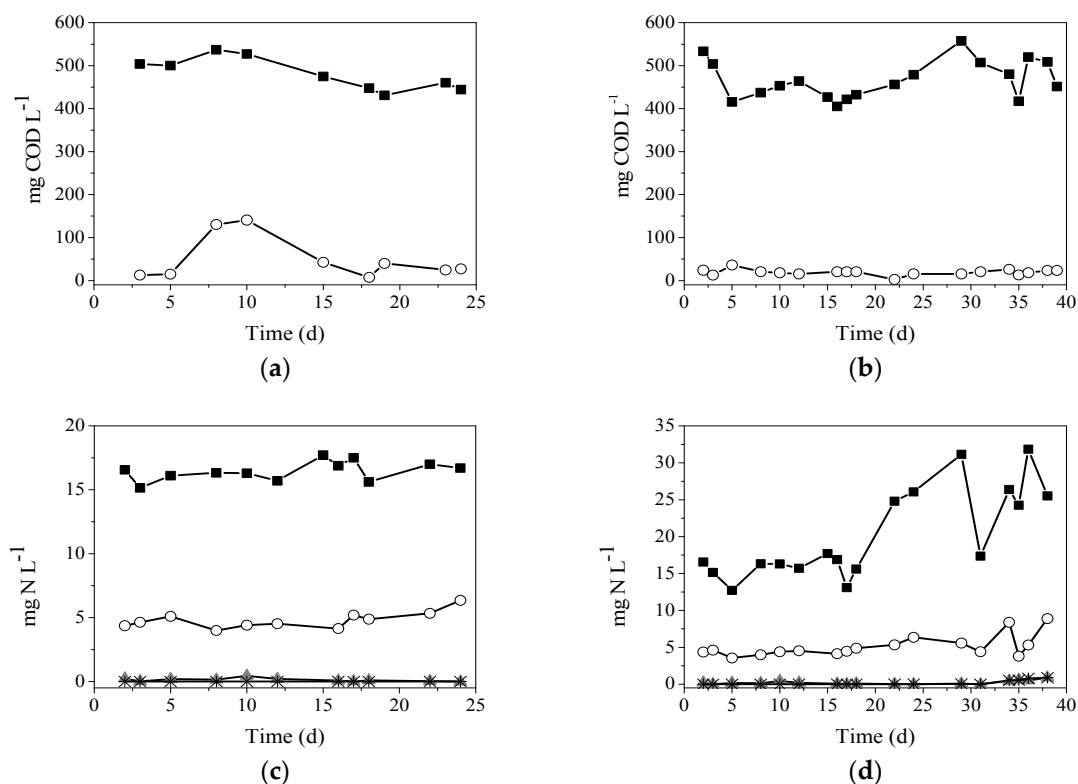


Figure 4. (a,b) COD concentrations in the influent (■) and effluent (○) in SBR I and SBR II, respectively. (c,d) Influent NH_4^+ -N concentrations (■), effluent NH_4^+ -N (○), NO_2^- -N (*), and NO_3^- -N (▲) in SBR I and SBR II, respectively.

Table 1. Nitrogen removal efficiencies in SBR I and SBR II.

Parameter	SBR I	SBR II
NH_4^+ oxidation (%)	74.4	70.4
TN removal (%)	33.4	30.4
$\text{N}_{\text{assimilated}}$ (%) ^a	93.1	82.2
$\text{N}_{\text{denitrified}}$ (%) ^a	6.9	17.8

^a Calculated as the percentage with regard to TN removal.

2.3. Effects of Aeration Flowrates

A SBR reactor containing mature granules (SBR III) was operated at three different aeration flowrates to study their effect on the granules performance. For this propose, oxygen, COD, ammonia, nitrite and nitrate concentrations, and pH profiles were tracked along the cycle for each aeration rate applied (Figures 5–7). From these profiles, COD and ammonia consumption rates and also the generation rates of nitrite and nitrate were calculated (Table 2).

Table 2. Specific activity rates in SBR III operated with three different upflow gas velocities.

Cycle	Upflow gas Velocity (cm s^{-1})	Specific Activities ($\text{g g}^{-1} \text{VSS d}^{-1}$)				Granule Diameter (mm)
Day		COD	N-NH_4^+	N-NO_2^-	N-NO_3^-	
140	1.04	2.77	0.22	0.07	0.003	2.4
152	1.26	2.66	0.34	0.09	0.02	1.9
162	1.40	3.35	0.35	0.12	0.02	1.5

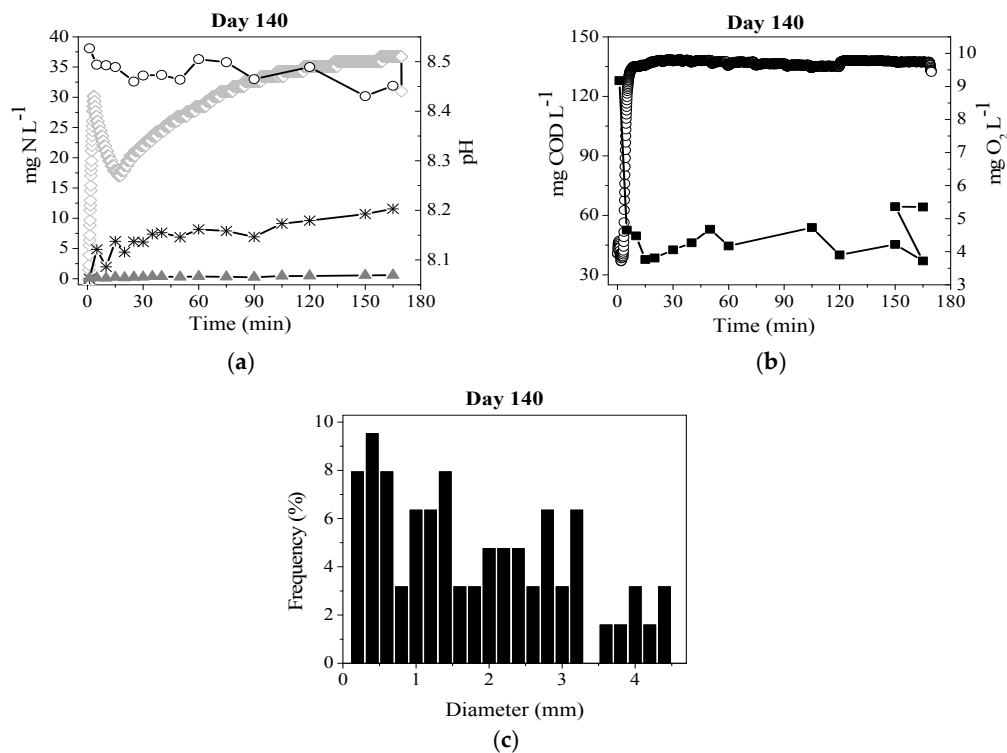


Figure 5. Cycle profile on day 140 with aeration rate of 1.04 cm s^{-1} . (a) Nitrogen compounds concentrations and pH: N-NH_4^+ (○), N-NO_2^- (*), N-NO_3^- (▲), and pH (◇); (b) O_2 concentration (○) and soluble COD (■); (c) Granule size distribution.

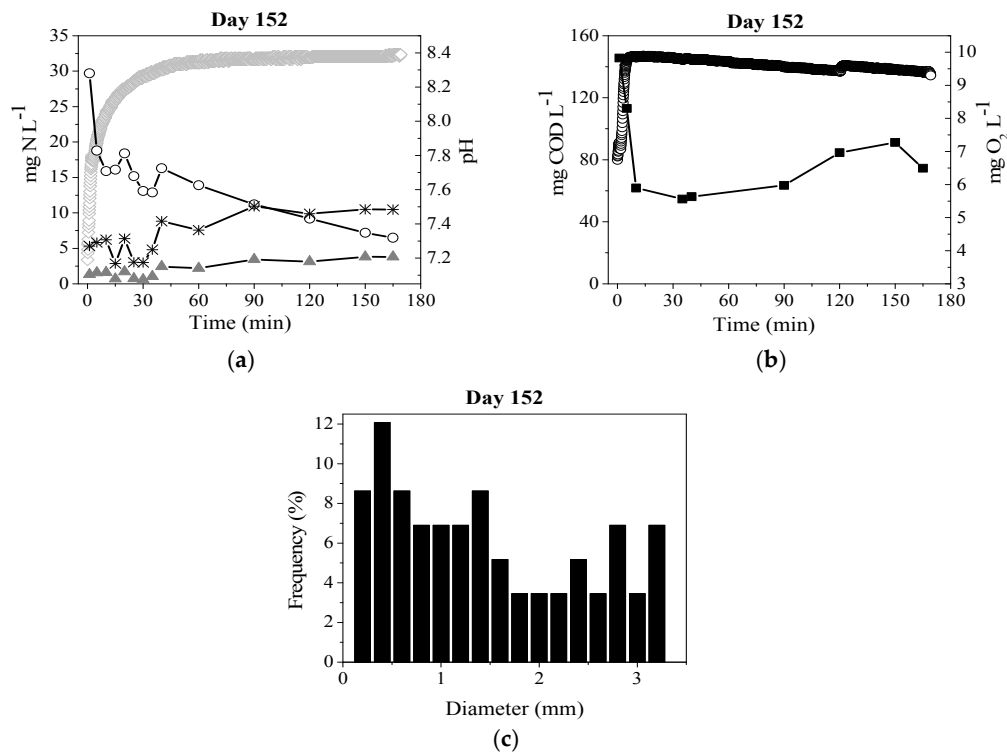


Figure 6. Cycle profile on day 152 with aeration rate of 1.26 cm s^{-1} . (a) Nitrogen compound concentrations and pH: N-NH_4^+ (○), N-NO_2^- (*), N-NO_3^- (▲), and pH (◇); (b) O_2 concentration (○) and soluble COD (■); (c) Granule size distribution.

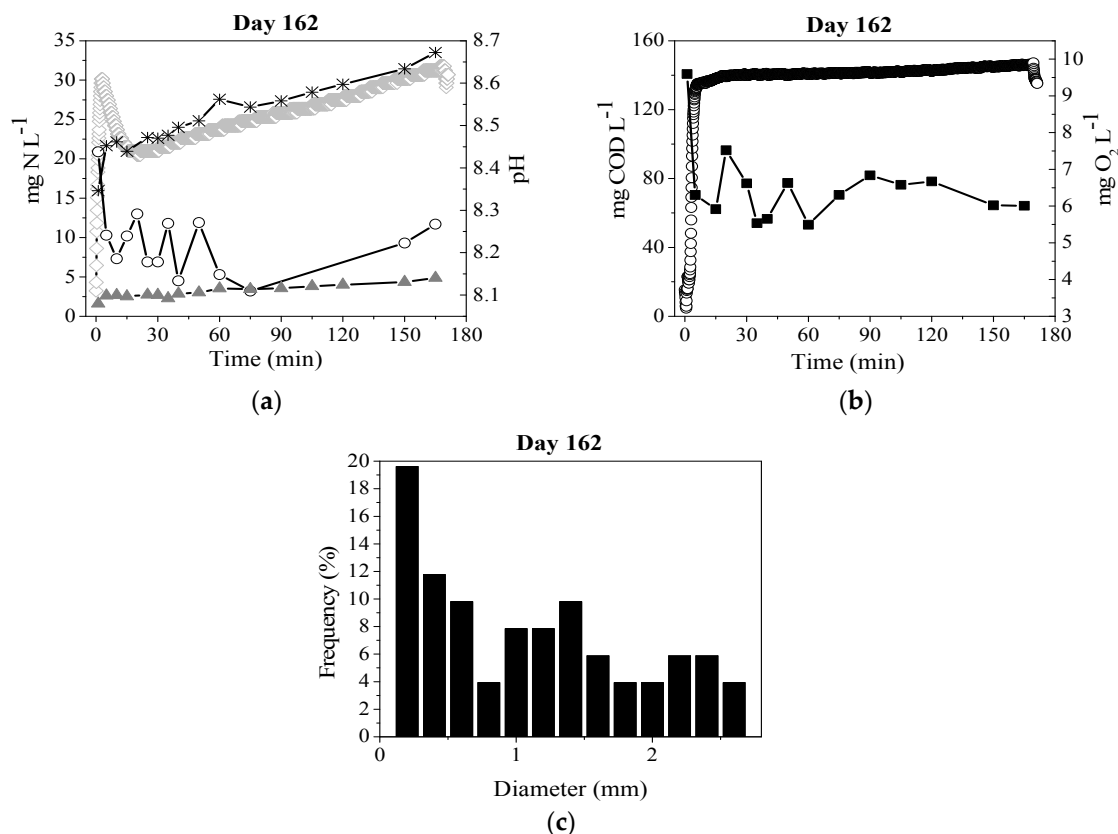


Figure 7. Cycle profile on day 162 with aeration rate of 1.4 cm s^{-1} . (a) Nitrogen compound concentrations and pH: N-NH_4^+ ($\circ$), N-NO_2^- (*), N-NO_3^- ($\blacktriangle$), and pH ($\diamond$); (b) O_2 concentration ($\circ$) and soluble COD ($\blacksquare$); (c) Granule size distribution.

The biodegradable COD was consumed in the first several minutes (less than 15 min) of the aeration phase, which is known as the ‘feast period’. During this period, the DO concentration was low due to the organic matter oxidation. Then, the DO concentration increased near to the saturation level, because the low ammonia oxidation rate and the fraction of non-biodegradable COD remained unchanged. This phase is known as the ‘famine period’. Ammonia was oxidized during the whole cycle, consequently the concentrations of nitrate and nitrite increased inside the reactor.

COD and ammonia consumption rates tended to increase as a higher upflow air velocity was applied. This can be mainly attributed to the increase of the aerobic fraction of the granules, which promoted higher specific activities of the overall biomass. The higher aerobic fraction and higher DO concentration were a consequence of the average granule diameter decrease (Figures 5–7).

The size of the aerobic granules is given a balance between biomass growth and detachment rates. In this case, the biomass growth rate should be constant since the applied OLR is constant but the detachment rates should get bigger due to the increase of the shear forces provoked by the flowrate increase. This fact would explain the drop of the average granules diameter with the increase of the aeration flowrate as it was already observed by Tay et al. [18].

However, higher COD and ammonia consumption rates do not imply higher nitrogen removal. As can be observed in Table 3, with the increase of the upflow gas velocity from 1.26 to 1.40 cm s^{-1} the nitrogen removal percentage decreased from 55.0% to 48.9%. This fact could be explained by the increase of the aerobic fraction as a consequence of the average granule diameter reduction. Therefore, the granules’ anoxic zone decreased, causing a negative impact on denitrification process. A similar effect was found when granules with a constant diameter are operated at different dissolved oxygen levels [24,25]. The anoxic volume of granules relatively decreased at high DO levels, decreasing their

capacity for denitrification. Isanta et al. [25] found there is an optimum value for the DO level which allows maintaining a compromise between denitrification and nitrification and COD removal capacities and obtaining a maximum nitrogen removal percentage. In the present work, the shear stress caused by the aeration flowrate can be used to control the granules size and, therefore, to optimize the nitrogen removal capacity of the reactor.

Table 3. Nitrogen removal efficiencies in SBR III operated with three different upflow gas velocities.

Parameter	Aeration Rate 1.04 (cm s ^{−1}) Day 140	Aeration Rate 1.26 (cm s ^{−1}) Day 152	Aeration Rate 1.40 (cm s ^{−1}) Day 162
TN removal (%)	18.4	55.0	48.9
N _{assimilated} (%) ^a	100	44.5	56.4
N _{denitrified} (%) ^a	0	55.5	43.6

^a Calculated as the percentage with regard to TN removal.

3. Materials and Methods

3.1. Reactors Setups and Operational Strategies

To study the influence of the inoculum type two sequencing batch reactors (SBR I and SBR II) with total and working volumes of 2.7 L and 1.8, respectively, were operated at room temperature (20–25 °C). The SBRs had a height of 480 mm and a inner diameter of 85 mm. The aeration was supplied at the bottom of the reactors by an air diffuser to provide the oxygen necessary and maintain the agitation inside the SBRs. To add the feeding and discharge of the effluent, two peristaltic pumps were used. The volume exchange ratio and the hydraulic retention time (HRT) were of 50% and 0.25 days, respectively.

The operational cycles in the reactors were of 3 h distributed as: 3 min to feed, 171 min to aerate, 1 min to settle, and 5 min for effluent discharge. A programmable logic controller was used to monitor the different operational phases. The SBRs were fed with a simulated wastewater containing (g L^{−1}): 7.1 NaAc·3H₂O, 0.64 NH₄Cl, 0.12 K₂HPO₄, 0.29 MgSO₄, 0.47 CaCl₂·2H₂O, 0.63 KCl, and 1 mL L^{−1} of a trace solution (Smolders et al. [26]). The applied organic (OLR) and nitrogen (NLR) loading rates for both reactors were around 2.0 g COD (L·d)^{−1} and 0.1 g N (L·d)^{−1}, respectively.

For SBR I the inoculum used was 0.5 L of activated sludge (Figure 8a) from the urban wastewater treatment plant of Santiago de Compostela (42°52′11″ N 8°35′55″ W), while SBR II was inoculated with 0.2 L of mature aerobic granules (Figure 8b) collected from a lab scale reactor [23].

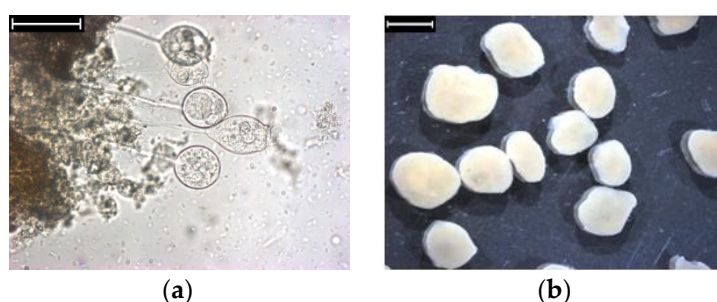


Figure 8. (a) Inoculum by activated sludge in SBR I. The bar represents 50 μm. (b) Inoculum by aerobic granules in SBR II. The bar represents 2 mm.

To study the influence of the aeration flowrate applied another SBR (SBR III, total volume of 9 L) was operated at room temperature (20–25 °C). This reactor had a height and a inner diameter of 1900 and 90 mm, respectively. The simultaneous aeration and agitation was by using an air diffuser. To add the feeding and discharge of the effluent, two peristaltic pumps were used. The volume exchange ratio

and the HRT were of 50% and 0.25 days, respectively. The operational cycles of SBR III were of 3 h comprising: 1 min of feeding, 169 min of aeration, 10 min of settling, and 1 min of effluent discharge. A programmable logic controller was used to monitor the length of the phases. The aeration flowrate was step wisely increased along the operational period in order to obtain aerations rates of 1.04, 1.26, and 1.4 cm s⁻¹.

SBR III was fed with municipal wastewater which was supplemented with acetate to have an inlet COD concentration around 200 mg COD L⁻¹ (Table 4). The applied OLR and NLR were around 2.0 g COD (L·d)⁻¹ and 0.1 g N (L·d)⁻¹, respectively.

Table 4. Municipal wastewater composition and synthetic medium.

Compounds	Concentration g L ⁻¹
Synthetic medium	
CH ₃ COONa ₃ H ₂ O	2.000
Municipal wastewater	
NH ₄ ⁺	0.040
PO ₄ ⁻³	0.014
NaCl	0.080
SO ₄ ²⁻	0.012

Adapted from Jungles et al. (2014).

The SBR III inoculum consisted in activated sludge from the municipal wastewater treatment plant of Florianópolis, Santa Catarina, Brazil (27°33'13.08" S 48°26'15.42" O) [9]. To evaluate the performance of SBR III, we tracked COD, ammonia, nitrite, nitrate, and DO concentrations, as well as pH at an operational cycle for different operational days. Nitrogen balances were performed according to Mosquera-Corral et al. [27] to know the nitrogen removal mechanism.

3.2. Analytical Methods

The pH values and DO, nitrite, nitrate, ammonia, total suspended solids (TSS), volatile suspended solids (VSS), and sludge volumetric index (SVI) were determined according to Standard Methods (APHA, 2005). Chemical oxygen demand (COD) concentrations were determined by the modified Standard Method [28]. DO concentration was measured with a multi-parameter electrode (YSI 6820, YSI, Yellow Springs, Ohio 45387-1107 USA). The settling velocity was determined by measuring the time taken for an individual aggregate to go over a certain height in a measuring cylinder. The granule size and morphology were measured by image analysis procedure [29], considering a sample of more than 200 granules and using a microscope ZEISS 2000-C provided with a light source KL 200. Biomass density in terms of g VSS L_{granules}⁻¹, was determined by the methodology of Beun et al. [3] using dextran blue.

4. Conclusions

No clear differences were observed between the start-up and performance of aerobic granular systems inoculated with activated sludge or with mature aerobic granules. The properties of the granules obtained were similar in terms of SVI (52–58 mL (g TSS)⁻¹), density (24–29 g VSS L_{granule}⁻¹) and VSS/TSS ratio (0.60–0.65). The organic matter removal and ammonia oxidation efficiencies were good (97% and 70%, respectively). However, the total nitrogen removal efficiency (around 30%) was low and mainly by assimilation.

The increase of the aeration flowrate caused the decrease of the average granule diameter. This fact increased the aerobic fraction of granules and, therefore, their COD and ammonia oxidation rates. However, an excessive aerobic fraction could compromise the denitrification activity, decreasing the nitrogen removal capacity.

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Author Contributions: José Luis Campos and Anuska Mosquera-Corral conceived and designed the experiments; Mariele M.K. Jungles performed the experiments; Ramón Méndez and Rejane H.R. Costa analyzed the data; Ramón Méndez contributed reagents/materials/analysis tools; Ángeles Val del Río and Mariele M.K. Jungles wrote the paper.

Conflicts of Interest: The authors declare no conflict of interest.

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