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Short Communication

Study of aerobic granular sludge stability in a continuous-flow membrane bioreactor

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HIGHLIGHTS

- The potentiality of continuous AGS is confirmed by experimental data.
- The feast/famine alternation is a key issue in continuous flow for AGS maintenance.
- EPSs had a key role in granules strength.
- Hydraulic selection pressure is necessary to ensure flocculent sludge washout.
- A correct management of granular sludge withdrawal should improve membrane fouling.

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ABSTRACT

A granular continuous-flow membrane bioreactor with a novel hydrodynamic configuration was developed to evaluate the stability of aerobic granular sludge (AGS). Under continuous-flow operation (Period I), AGS rapidly lost their structural integrity resulting in loose and fluffy microbial aggregates in which filamentous bacteria were dominant. The intermittent feeding (Period II) allowed obtaining the succession of feast and famine conditions that favored the increase in AGS stability. Although no further breakage occurred, the formation of new granules was very limited, owing to the absence of the hydraulic selection pressure. These results noted the necessity to ensure, on the one hand the succession of feast/famine conditions, and on the other, the hydraulic selection pressure that allows flocculent sludge washout. This preliminary study shows that the proposed configuration could meet the first aspect; in contrast, biomass selection needs to be improved.

1. Introduction

Compared with the conventional wastewater treatment plants, aerobic granular sludge (AGS) offers several advantages, among others lower volume necessity because of greater biomass concentration (up to 20 g TSS L^{-1}), the possibility to degrade simultaneously organic carbon and nutrients, and finally their remarkable settling capability. Nowadays, AGS has been widely investigated in sequencing batch reactors (SBRs). Indeed, it is believed that the ideal conditions for aerobic granulation like the succession of feast/famine conditions (Val del Río et al., 2012), the hydraulic selection pressure (Adav et al., 2009) and the hydraulic shear forces (Zhou et al., 2014), occur or could be easily controlled in SBR reactors. Nevertheless, SBRs are difficult to implement for a large sewage treatment, where continuous-flow reactors are normally favorable due to the lower installation costs and easier operation, maintenance and control (Juang et al., 2010). Moreover, up to date the most of biological granular sludge systems have been studied in column-type reactors. These reactors are characterized by a height to diameter ratio (H/D) higher than 6–8, which helps to maximize the hydraulic shear forces, enhancing the formation of the aerobic granules. Therefore, column-type reactors had a predominant in height development, and this characteristic could limit their application in a full-scale plant, since reactors might result higher than 8–10 m.

Although in some studies is reported that stable aerobic granular sludge can be achieved in a continuous flow reactor, others demonstrated that the aerobic granules lost their stability more rapidly compared with a SBR in the long-run (Zhou et al., 2014). Nevertheless, there is not unanimity about the causes of granules breakage, because in a continuous-flow reactor, many of crucial parameters for the aerobic granulation simultaneously fail. To

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increase the knowledge about the aforementioned issues, a continuous-flow reactor with a novel geometric configuration was developed, with the aim of analyzing the stability of AGS in terms of structural characteristics and biological performances. The first results reported in this brief discussion, confirm the interest about the topic and support further investigations to gain knowledge about this topic.

2. Methods

2.1. Experimental set-up

A continuous flow reactor with a working volume of 7.5 L, operating with a flow rate of 0.7 L h^{-1} was used. The granular continuous flow membrane bioreactor (GCFMBR) has been designed with the aim of recreating hydraulic conditions as similar as possible to those that generally occurred in the column-type reactor operating in batch mode. The bench scale plant (Fig. 1) was constituted by two reactions and a final compartment (8 × 8 cm) where an ultrafiltration (UF) hollow fiber GE Zenon ZW10 membrane (nominal surface: 0.093 m^2; pores dimension 0.04 μm) in submerged configuration was located. The membrane flux was maintained equal to 14 L m^{-2} h^{-1}. The membrane was periodically backwashed (every 4.5 min for a period of 1.5 min) by pumping a fraction of the permeate back through the membrane modules. From the hydrodynamic point of view, the reactor was constituted by two complete mixing sub-reactors in series (2.8 L each), the first of which close to the filling zone, worked as a high load reactor, whereas the second, close to the extraction zone, worked with a lower organic load. Therefore, feast and famine conditions, that in SBR reactors occur in time, successed in the space.

Five identical compartments, operating as risers and downcomers, ensured the high hydraulic shear forces and the hydraulic connection of the reaction compartments.

In detail, the authors supported that the continuous flux inversion that occurred in the risers and downcomers sectors, helped to maximize the hydraulic shear forces. In contrast to the column-type reactors, in this case the hydraulic shear forces were distributed longitudinally and this could limit the height of the granular continuous flow reactors.

The GCFMBR was operating for 72 days, divided into two periods. During the "Period I" (42 days) the reactor was continuously fed. Unfortunately, the alternation of feast and famine conditions partially occurred, but not as desired, because the volume of the first compartment would have been smaller, so that the organic load rate would result higher. Subsequently, with the aim of enhancing the feast and famine succession, during the "Period II" (30 days), the GCFMBR was intermittently filled. In detail, the effluent discharge occurred in continuous mode, whereas the influent wastewater was intermittently fed, with a flow greater than the effluent withdrawal (4 L h^{-1} vs 0.7 L h^{-1}). Consequently, the reactor operated with variable volume (7.5–9.5 L). In this way, the organic load rate was increased by increasing the influent flow rate. Two level controllers, placed at a minimum and a maximum filling level, commanded the switching on/off of the feeding pump. The reactor was seeded with aerobic granular sludge previously cultivated in a SBR column-type reactor, and fed with acetate-based synthetic wastewater (Beun et al., 2002). The influent wastewater had the following composition: COD of 1200 ± 20 mg L^{-1}, NH_4-N of 60 ± 5 mg L^{-1} and PO_4-P of 15 ± 2 mg L^{-1}. During both periods the "hydraulic retention time" (HRT) was maintained at 7.5 h (on average). Contrarily, the "sludge retention time" (SRT), was not fixed a priori, but it has been adjusted depending on the biomass growth within the reactor. This choice caused the "complete sludge retention" during the Period I, where the biomass grew very slowly (prevalent growth in starvation condition), while during the Period II, due to a slightly higher biomass growth, the SRT was

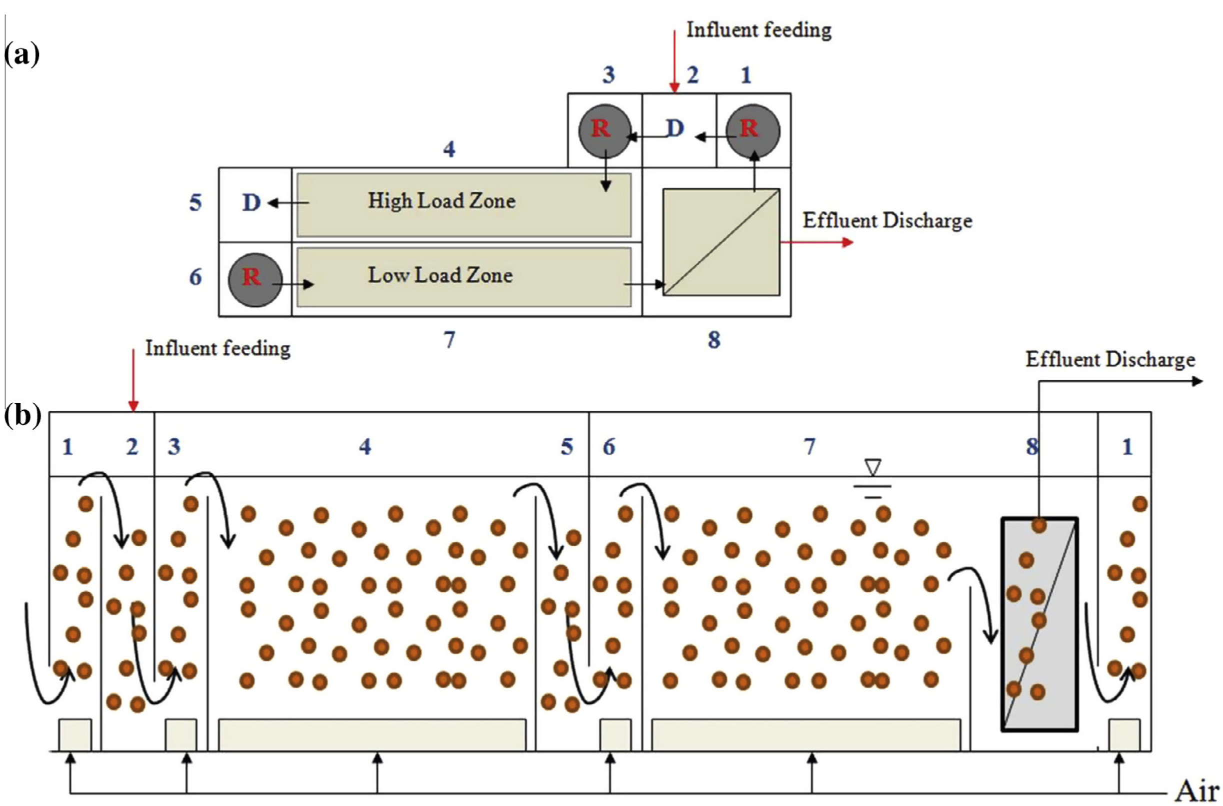

Fig. 1. Bench-scale plant aerial projection (a) and longitudinal flow development (b).

kept at approximately 50 days by daily purging a known amount of mixed liquor volume.

2.2. Analytical methods

All of the chemical-physical analyses (COD, NH_4-N, NO_3-N, NO_2-N, PO_4-P, Total and Volatile Suspended Solids (TSS, VSS) were performed according to standard methods (APHA, 2005). The size of the granules was measured by digital image capture using a stereomicroscope. The granule size distribution was represented in terms of three main statistical size parameters: the d_{10}, d_{60} and d_{90} (mean the size of granules in which 10%, 60% or 90% of the particles are smaller, respectively). The granulation rate and the water content were measured in accordance with Liu et al. (2012). The density and hydrophobicity of the granular sludge was determined using the Dextran Blue method described by Beun et al. (2002) and according with the method described by Rosenberg et al. (1980), respectively. The extraction of the extracellular polymeric substances (EPSs) was carried out according to the Heating Method as described by Le-Clech et al. (2006). Afterwards, carbohydrates (PS) and proteins (PN) were determined in accordance with the methods described by Dubois et al. (1956) and Lowry et al. (1951), respectively. The membrane fouling was evaluated by daily measuring the trans-membrane pressure (TMP) and the permeate flux. Then, the specific resistances to filtration were evaluated applying a resistance in series (RIS) model (Cosenza et al., 2013).

3. Results and discussions

3.1. Analyses of granules stability

As aforementioned, the reactor was seeded with stabilized aerobic granules collected from a column-type SBR reactor. During the first experimental period, feast and famine conditions did not alternate as feasible. The granules rapidly lost their structural integrity, resulting in loose and unstable aggregates. Both the TSS and VSS concentrations showed a slightly increased trend (Fig. 2a). Instead, in less than 20 days their ratio significantly increased from 65% up to 90%. In contrast, the density of the granules gradually reduces from 100 g TSS L^{-1} (value of the seed granules) up to 50 g TSS L^{-1} (Fig. 2b). At the same time, the water content increased from 90% up to 94%, whereas the sludge hydrophobicity remained quite similar approximately at the inoculum value. These results indicated a gradual loss in the structural stability of the granules, which rapidly disintegrated, resulting in the increase of the flocculent sludge percentage. After on, the granulation rate significantly reduced below 40% (Fig. 2f). According to these results, the extracellular polymeric substances (EPSs) content significantly decreased from approximately 500 mg EPS g VSS^{-1} up to a value close to 200 mg EPS g VSS^{-1} (Fig. 2c). Specifically, the proteins significantly decreased, whereas the carbohydrates content was almost constant. As a result, the PN/PS ratio decreased (Fig. 2d). Several authors have noted the importance of the proteins for maintaining the structural strength of the aerobic granules (Xiong and Liu, 2013). The reduction of the proteins content confirmed the structural weakening of AGS. Consequently, the average size of the granules gradually decreased from 2 mm up to 1 mm. In more detail, the most unstable fraction was the d_{90}, while the d_{60} showed a slightly decreasing trend. In contrast, the d_{10} fraction increased because most of the large granules disintegrated (Fig. 2e). The results confirmed that under continuous-flow AGS had relatively small diameters. Liu et al. (2012) ascribed this changing to the reduction of the diffusion of the nutrients toward the inner layers of the granules. Indeed, the organic substrate concentration in the GCFMBR bulk was the same with that of the effluent, due to the complete mixing hydrodynamic. Consequently, the driving forces for nutrient diffusion within the granules reduced. Obviously, this was mainly noted in the biggest granules, which thickness opposed a greater barrier to the mass diffusion phenomena. The morphology of the aerobic granules gradually modified in a loose, fluffy and irregular structure in which filamentous bacteria were dominant. These large and fluff granules gradually disintegrated.

The overgrowth of filamentous bacteria was one of the main reasons for the AGS disintegration as observed by Morales et al. (2012). De Kreuk et al. (2010) found that filamentous organisms prevailed on non-filamentous organisms in systems characterized by micro-gradients inside sludge flocs or biofilms. Under continuous flow operation, the organic substrate was always available, and it was likely degraded in the outer layers of the granules (Zhou et al., 2014). This produced a substrate gradient inside the granules that favored the proliferation of filamentous microorganisms. In addition, it is well known that the alternation of feast/famine conditions inhibits the proliferation of filamentous microorganisms, whereas it favors the growth of floc-forming bacteria (Val del Río et al., 2012). Therefore, because no alternation of feast/famine conditions occurred, the floc-forming bacteria had no benefits on filamentous microorganisms. Furthermore, ultrafiltration membranes completely retained the most of microorganisms within the reactor, so filamentous bacteria grew and competed with the other bacteria strains. According to Adav et al. (2009), a great competition between several bacteria strains could reduce the EPSs secretion. These general considerations underlined the importance of providing the alternation of feast and famine conditions, that if not adequately guaranteed in the space, could be improved by intermitting the feeding. According to this consideration, since the 42th day the reactor was intermittently fed.

During the Period II, the aerobic granules stability generally improved. The intermittent feeding allowed obtaining the alternation of feast and famine conditions. Both the density and the hydrophobicity significantly increased, proving a new thickening of bio-aggregates (Fig. 2b). Similarly, the EPS content, especially the protein fraction, rapidly grew up, as well as the PN/PS ratio (Fig. 2c and d). The average size of the granules and granulation rate were constant (Fig. 2f), so no further breakage was noted. The results indicated as the feast/famine alternation was crucial for the maintenance of the AGS, moreover, it seemed to promote a slow but gradual growth in the granule sizes (Fig. 2e). However, the absence of the hydraulic selection pressure, on the one hand limited a significant recovery of granulation process, and on the other, implied a significant worsening of the mixed liquor quality. Indeed, without the hydraulic selection pressure, the mixed liquor modified toward a hybrid system of granular and flocculent sludge. Furthermore, the conspicuous EPS production, if on the one hand, favored the maintenance in granules stability, on the other contributed in worsening of mixed liquor quality. Indeed, during the famine phase, due to the lack of organic substrate, bacteria hydrolyzed the EPSs transforming in soluble forms (SMP) which were mainly retained within the reactor because of their deposition on the membrane surface (Cosenza et al., 2013). Because SMSs were mainly constituted by proteins, which are hydrophobic substances, at the end of the Period II the hydrophobicity of mixed liquor rapidly increased. Consequently, as also observed by Li et al. (2008), the mixed liquor became very viscous like a gelatin. Obviously, in these conditions, the oxygen transfer efficiency dramatically reduced (Germain et al., 2007). Among the possible causes of the failure in the new granulation resume, the role of hydraulic retention time (HRT) is not to be excluded. Morales et al. (2012) suggested to apply short SRT (1–3 h) to washout those microorganisms that are not able of forming biofilms. In the present work, the HRT was 7.5 h, so much higher of the recommended value. The

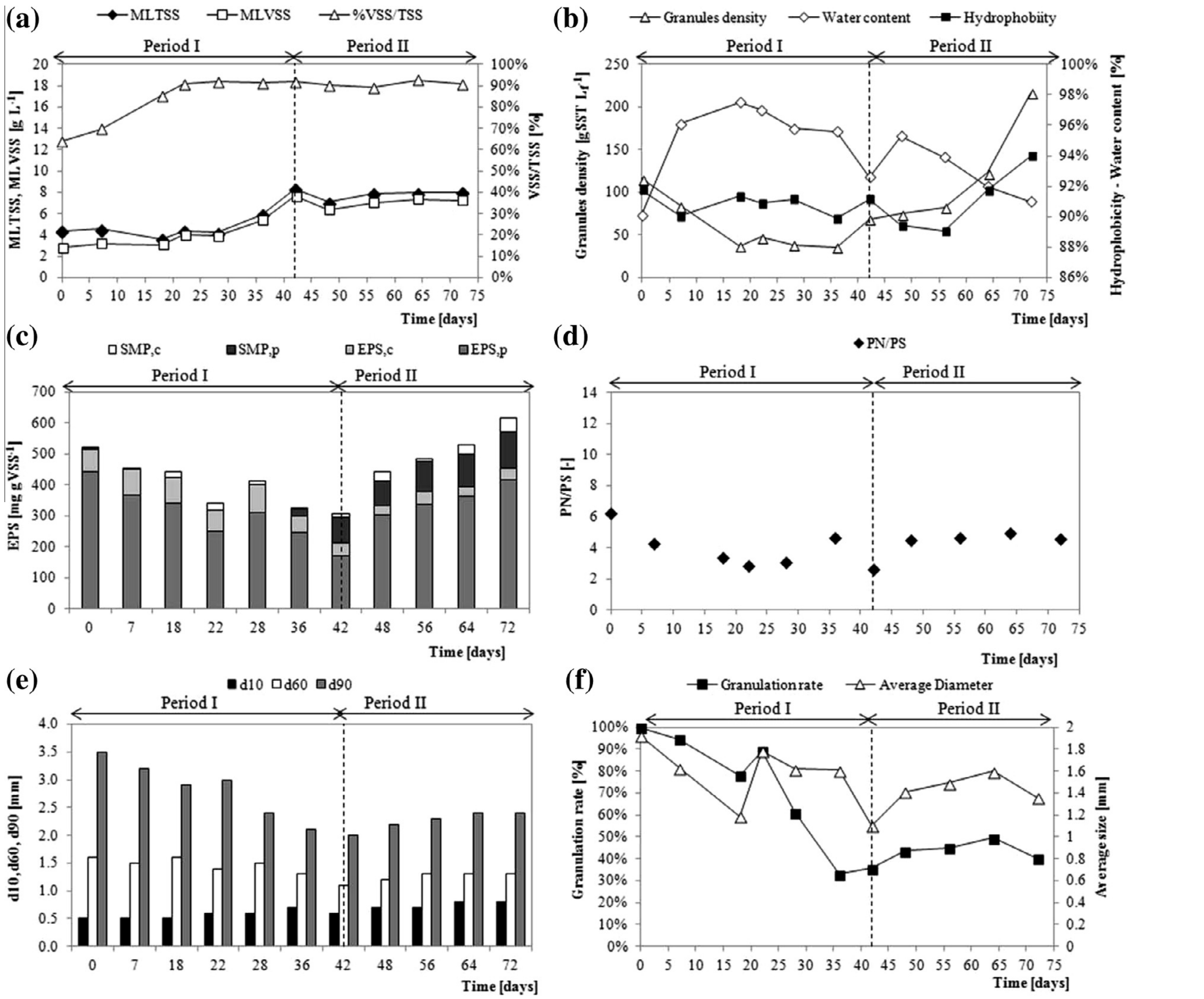

Fig. 2. (A) Trend of TSS, VSS and VSS/TSS ratio; (b) granules density, hydrophobicity and water content; (c) EPS content; (d) ratio between proteins and carbohydrates in aerobic granules; (e) particle size distribution; (f) granulation rate and granules average size.

effect of HRT reduction was not evaluated, because it would have been necessary to increase the influent/effluent flow rate, that meant to exceed the membrane critical flux.

3.2. Bench-scale plant performances

During both periods, the organic matter removal efficiency was excellent, with yields approximately close to 90%. Nevertheless, as aforementioned, during the Period I the succession of feast/famine conditions didn't occur as feasible. The dissolved oxygen concentration in both reaction compartments was about 7 mg L^{-1}, resulting significantly different of what generally occurs in SBRs, where DO concentration is lower during the feast phase (2–4 mg L^{-1}) whereas it is close to the saturation value (7–8 mg L^{-1}) during the famine period. The nutrients removal efficiencies were significantly affected by the aerobic granules breakage. With regard to nitrogen, although the most of the ammonia was oxidized (yielding NH_4-N removal rate close to 94%), the total nitrogen efficiency removal gradually reduced, due to nitrate accumulation. The reduction of the granules sizes, especially in d_{90} fraction, caused a deterioration of the denitrification efficiency, because, as noted by Di Bella and Torregrossa (2013), only in the largest granules nitrate reduction could realize thanks to the presence of the anoxic layers. For the same reason, phosphorous efficiency removal dropped in less than 30 days. During the Period II, after that the operating conditions were changed, the feast and famine succession finally occurred. During the influent loading, the oxygen concentration within the bulk was close to 2 mg L^{-1} and the organic substrate was mainly degraded (residual COD 150 mg L^{-1}). During the following drawn phase, the oxygen concentration rapidly increased up to 8 mg L^{-1}, whereas the residual organic substrate was oxidized up to 60 mg L^{-1}. The nitrogen removal efficiency did not increase because, as aforementioned, the reduction of the oxygen transfer within the mixed liquor caused a significant nitrite accumulation up to 30 mg L^{-1}.

Concerning the hydraulic performances of the system, because they were not the main focus of this work, the operating parameters (such as EPSs, SRT, MLSS) were not managed with the aim of mitigating the membrane fouling. Therefore, physical cleanings were frequently carried out to ensure a constant flow of the effluent permeate (three times a week on average during Period I, while twice during the second one).

Observing the Table 1, the cake deposition was the main fouling mechanism, principally in the irreversible form ($R_{cake,irr}$), in contrast to what was noted by Wang et al. (2013). More specifically, during the Period I the aforementioned operational parameters,

Table 1
Results of fouling analysis.

Period	$R_{cake,rev}$			$R_{cake,irr}$			R_{PB}		
	Min (m^{-1})	Average (m^{-1})	Max (m^{-1})	Min (m^{-1})	Average (m^{-1})	Max (m^{-1})	Min (m^{-1})	Average (m^{-1})	Max (m^{-1})
I	$3.64 \cdot 10^{11}$	$1.66 \cdot 10^{12}$	$3.47 \cdot 10^{12}$	$6.20 \cdot 10^{12}$	$2.95 \cdot 10^{13}$	$7.37 \cdot 10^{13}$	$6.55 \cdot 10^{11}$	$2.57 \cdot 10^{12}$	$4.07 \cdot 10^{12}$
II	$4.96 \cdot 10^{11}$	$1.60 \cdot 10^{12}$	$3.22 \cdot 10^{12}$	$7.19 \cdot 10^{12}$	$1.56 \cdot 10^{13}$	$2.08 \cdot 10^{13}$	$4.32 \cdot 10^{12}$	$5.22 \cdot 10^{12}$	$5.81 \cdot 10^{12}$

together with the significant granules breakage, implied an increase in a compact and irreversible cake, confirmed by an average value of the fouling total resistance ($2.95 \cdot 10^{13}\ m^{-1}$) with a peak of $7.37 \cdot 10^{13}\ m^{-1}$. During the Period II, the modified operational conditions reduced the irreversible cake deposition, which had an average value approximately close to $1.60 \cdot 10^{13}\ m^{-1}$. Regarding to the reversible cake deposition ($R_{cake,rev}$), it was less significant than the irreversible form previously discussed. On the other hand, during both periods, the pore blocking resistance (R_{PB}) was about one order of magnitude lower than the irreversible cake, so no chemical cleanings were necessary. This is a great advantage of AGS in MBR systems compared with the CAS, because the pore-blocking mechanism would require chemical cleanings that could reduce the membrane service time.

The results up to now discussed underline that a better management in the operating parameters, such as the regulation of time-cycles of both membrane filtration and intermittent influent feed, the HRT and SRT, could reduce the membrane fouling. Indeed, a longer SRT might led bacteria to cell lysis due to the biomass ageing (Ersu et al., 2010), and this would involve a significant release of SMPs that have a great impact on the irreversible fouling mechanisms. Therefore, the SRT control could reduce the frequency of the physical membrane cleaning.

4. Conclusions: proposed for future implementations

This preliminary study of the AGS in a continuous flow reactor pointed out undeniable difficulties for both the granules formation and their maintenance. New experimentations are necessary to focus on biological and management issues, like the SRT control to reduce the EPS production by bacteria. SRT lower of those generally applied in granular sequencing batch reactors or MBR systems, would be recommended. Moreover, OD and the feeding timing need to be controlled in order to favor an adequate succession of feast and famine conditions to favor the structural integrity of the granules and their stratification. Furthermore, these suggestions could also reduce the membrane fouling.

Stereo-microscope images of the aerobic granules are provided as Supplementary material (Fig. S1).

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.biortech.2015.10.065.

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