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Influence of sequencing batch reactor configuration on aerobic granules growth: Engineering and microbiological aspects

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A B S T R A C T

This work evaluated the effect of two sequencing batch reactor (SBR) configurations used for aerobic granular sludge (AGS) cultivation (conventional and constant-volume) on the physical and microbiological characteristics of the granules, as well as on the systems' performance. In the conventional SBR (R1), the filling, reaction, settling and decanting phases occurred sequentially, while, in the SBR operated at constant volume (R2), the filling and decanting phases occurred simultaneously followed by the reaction and settling phases. A faster formation of granules (about 30 days) with a larger size (⌀ > 1 mm) and better settleability ($SVI_{30} \approx 44.8$ mL/g) was observed in R1. On the other hand, R2 presented a slower formation of granules (about 50 days) with a smaller diameter (⌀ ≈ 0.8 mm) and a worse settleability ($SVI_{30} \approx 70.7$ mL/g). Although R2 presented smaller granules (and longer time for biomass formation), this configuration presented several advantages, such as better system stability during the entire operation (125 days), higher solids retention, and granules with better physical resistance. In terms of performance, both systems presented high values of COD (>90%), NH_4^+ (>90%) and TN (>50%) removals after stabilization. However, the phosphorus removal in R2 was higher (≈50%) than in R1 (≈25%). The results justify the use of SBRs operated at constant volume in most full-scale AGS wastewater treatment plants, such as Nereda®. Therefore, the SBR configuration has a direct influence on the granule formation and its physical and microbiological characteristics as well as on the system performance in terms of efficiency and operational stability.

1. Introduction

The aerobic granular sludge (AGS) technology is an upcoming treatment process for domestic and industrial wastewaters. The aerobic granules are characterized by a compact structure, without the need for carrier material, resulting in high settling velocities and low sludge volume index (SVI). In addition, they are also characterized by their layered structure (an aerobic outer layer and an anaerobic or anoxic core), which allows the simultaneous removal of organic matter, nitrogen and phosphorus (Rollemberg et al., 2018). Compared with conventional activated sludge systems, AGS systems have a better settleability, smaller footprint, higher nutrient removal performance, and lower energy consumption (de Kreuk et al., 2007; Rollemberg et al., 2018). Therefore, the AGS technology has been extensively investigated in lab- and pilot systems, and even in full-scale wastewater treatment plants (Xie et al., 2019).

Although some recent investigations reported granule formation in continuous-flow systems (Kent et al., 2018), AGS cultivation has been preferentially carried out in sequencing batch reactors (SBRs). In these systems, all phases (filling, reaction, settling, and decanting) take place in the same tank. Therefore, a secondary clarifier is not needed as in activated sludge systems (Rollemberg et al., 2018). There are two main SBR configurations for the cultivation of aerobic granules: conventional SBR (filling, reaction, settling, and decanting) and constant-volume SBR (simultaneous filling and decanting, reaction, and settling). The latter is also known as simultaneous fill-and-draw SBR (Derlon et al., 2016).

Most of the lab-scale studies on AGS are carried out in

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conventional SBRs. However, most of the pilot- and full-scale systems use constant-volume SBRs since they have a simpler operation than conventional SBRs, whose filling and decanting phases must be performed in a short time, requiring a large pumping system (Derlon et al., 2016; Li et al., 2014). Therefore, the integration of the filling and decanting phases into a single step in constant-volume SBRs simplifies the operation (Pronk et al., 2015).

Many questions have been clarified in studies which used either conventional or constant-volume SBRs, such as: (i) anaerobic filling supports granulation because this strategy selects microorganisms, such as polyphosphate-accumulating organisms (PAOs) and glycogen-accumulating organisms (GAOs); (ii) high upflow velocities (>10 m/h) form large and round granules (d > 0.63 mm) with a fluffy surface, whereas, at 1 m/h, the granular sludge was smaller (0.25 < d < 0.63 mm); and (iii) a plug-flow regime during the anaerobic filling results in a lower storage of organic carbon and a general destabilization of the granulation process (Derlon et al., 2016; Franca et al., 2018). However, to the best of authors' knowledge, there is no investigation into the differences between the AGS formed in these two SBR configurations when the reactors were operated under the same conditions. As it is known, the wastewater composition, organic loading rate (OLR), hydrodynamic shear force, feast-famine regime and feeding strategy are the main parameters which influence granules formation (Rollemberg et al., 2018).

Therefore, this work aimed to evaluate the impact of these two SBR configurations (conventional and simultaneous fill-and-draw) on the engineering (formation, stability, activity and characteristics of the granule) and microbiological aspects of AGS cultivated in SBRs operated under the same conditions.

2. Material and methods

2.1. Experimental set-up

The aerobic granules were cultivated in two different SBR configurations: conventional (R1) and constant-volume (simultaneous fill-and-draw) (R2). Both reactors had diameter of 100 mm and total height of 1 m (working volume of 7.2 L), i.e. a ratio of height to diameter (H/D) of 10. A column-type upflow reactor with a high H/D ratio (close to 10) is suggested to provide a longer circular flowing trajectory, which creates an effective hydraulic friction for microbial aggregation (Liu and Tay, 2002). The hydraulic retention time (HRT) was 12 h, and the volume exchange ratio was 50%. The dimensions and operational parameters were defined according to a previous work in which stable granules were obtained (Rollemberg et al., 2019).

The reactors were inoculated with aerobic sludge of a domestic wastewater treatment plant (WWTP) (Fortaleza, Ceará, Brazil) at an initial concentration of mixed liquor volatile suspended solids (MLVSS) of approximately 2 g/L, whose sludge volume index at 30 min (SVI_{30}) was 110 mL/g.

The SBRs were fed with a synthetic wastewater composed of ethanol (700 mg COD/L, 95% purity, Neon Comercial), NH_4Cl (100 mg N/L, 97% purity, Neon Comercial), KH_2PO_4 (10 mg P/L, 97% purity, Neon Comercial), $NaHCO_3$ (1 g/L, 99% purity, Synth), and 1 mL/L of a trace elements solution (Rollemberg et al., 2019). Ethanol was used as the carbon source due to promising results for AGS cultivation (Muda et al., 2011; Nancharaiah and Reddy, 2018; Rollemberg et al., 2019), mainly attributed to the production of acetate during the anaerobic phase, which is reported as a good substrate for PAOs and other beneficial AGS microorganisms (Kragelund et al., 2006; Mino et al., 1998).

The total operational cycle of the SBRs was the same (6 h), which consisted of anoxic filling (30 min, upflow velocity of 0.92 m/h), anaerobic reaction (90 min), aerobic reaction (220-230 min, aeration rate of 10.0 L/min, superficial gas velocity of 2.1 cm/s), settling (20-10 min). In R1, the decanting lasted 1 min (the last minute of the settling phase), in which the effluent was withdrawn from the half of its working height, whereas, in R2, such operation occurred simultaneously with the filling (30 min) from its bottom, and the effluent was withdrawn from its top (Fig. 1).

Both reactors were operated (at 28 ± 2 °C) in two periods, with settling times of 20 min (period I) and 10 min (period II). In order to keep a 6-h operational cycle in period II, 10 min were added to the aerobic reaction phase.

2.2. Methods

Chemical oxygen demand (COD), pH, ammonium, nitrite, nitrate, total phosphorus (TP), total and volatile suspended solids (TSS and VSS), and sludge volume index at 10 and 30 min (SVI_{10} and SVI_{30}) were determined according to APHA (2012). Dissolved oxygen (DO), extracellular polymeric substances (EPS), i.e. protein (PN) and polysaccharides (PS) contents were determined as described elsewhere (Rollemberg et al., 2019). The resistance of aerobic granules was determined in terms of the stability coefficient (S), which represents the change in the granule diameter after a shear test (rotation of 200 rpm for 10 min). The lower the values of S (%), the higher the stability of aerobic granules (Nor-Anuar et al., 2012).

The sludge settling velocity was determined according to Wang et al. (2018) using an acrylic column with a working height of 0.4 m and a diameter of 75 mm, filled with a liquid with the same density of the synthetic wastewater. The granules collected from the SBRs were introduced on the top of the column, and, then, the time for their complete settling was measured in triplicate.

Also at the end of the maturation period, samples from the mixed liquor of both reactors were collected (at the end of the aeration reaction phase) in order to assess the differences between their microbial communities. The DNA extraction, 16S rRNA gene amplicon sequencing and data processing were carried out as described elsewhere (Rollemberg et al., 2019).

The non-parametric Mann-Whitney test was used to compare the performance of the reactors at a confidence level of 95.0%.

3. Results and discussion

3.1. Start-up and stabilization

Period I (acclimation) started with SVI_{30} of about 110 mL/g and 2 g/L of MLVSS. However, the initial behavior of R1 and R2 was very different (Fig. 2). In R1, a decrease in the solids concentration in the first days was observed. In contrast, a MLVSS increase was verified in R2 during start-up.

R1 presented mean values of MLVSS close to 2.2 g/L in period I. After the reduction of the settling time from 20 to 10 min (period II), most of the filamentous solids were washed out, and a reduction of the MLVSS was observed (1.4 g/L in period II). On the other hand, R2 presented MLVSS mean values of 5.5 g/L and 7 g/L in periods I and II, respectively. Differently from R1, solids washout after the settling time reduction was not observed, resulting in a MLVSS concentration increase.

Regarding the SVI_{30} values, R1 had mean values below 38 mL/g in all periods. On the other hand, in R2, mean values of 124.3 mL/g (period I) and 75.3 mL/g (period II) were observed. Concerning the SVI_{30}/SVI_{10} ratio, the closer the values of SVI_{30} and SVI_{10} (i.e. $SVI_{30}/SVI_{10} \approx 1$), the better the sludge settleability (de Kreuk et al., 2007; Bassin et al., 2012). SVI_{30}/SVI_{10} ratios of 0.98 and 0.87 were observed in R1 and R2, respectively, indicating a better sludge settleability for R1. Thus, as occurred for the solids concentration, the type of SBR configuration influenced the SVI values.

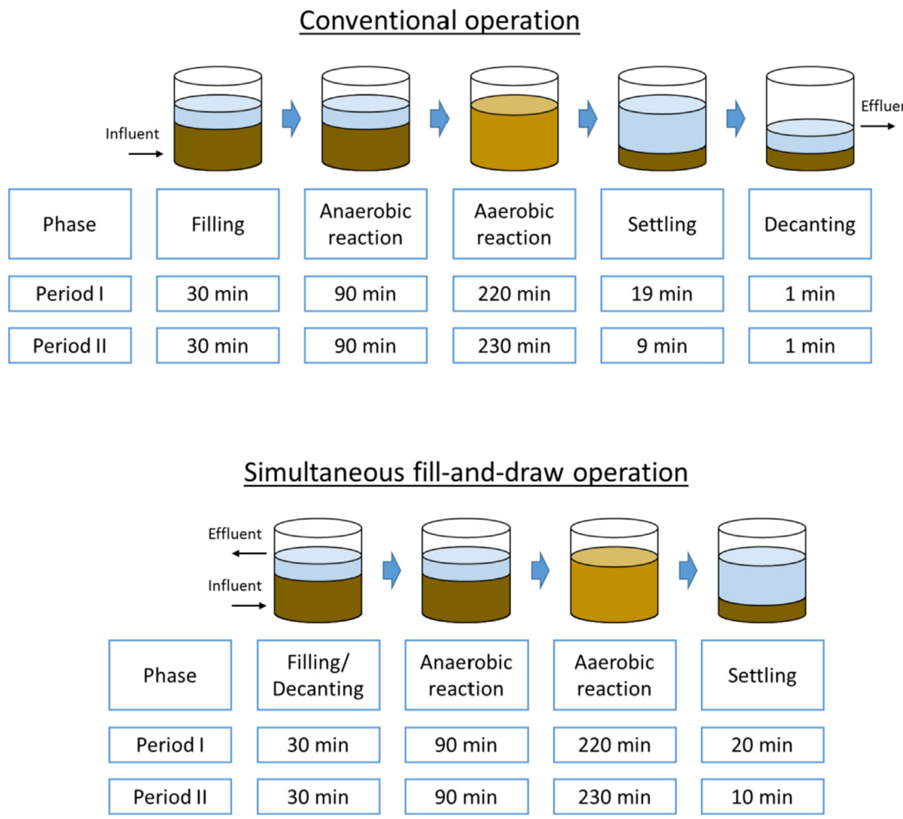

Fig. 1. Schematic of the conventional operation (R1) and simultaneous fill-and-draw operation (R2).

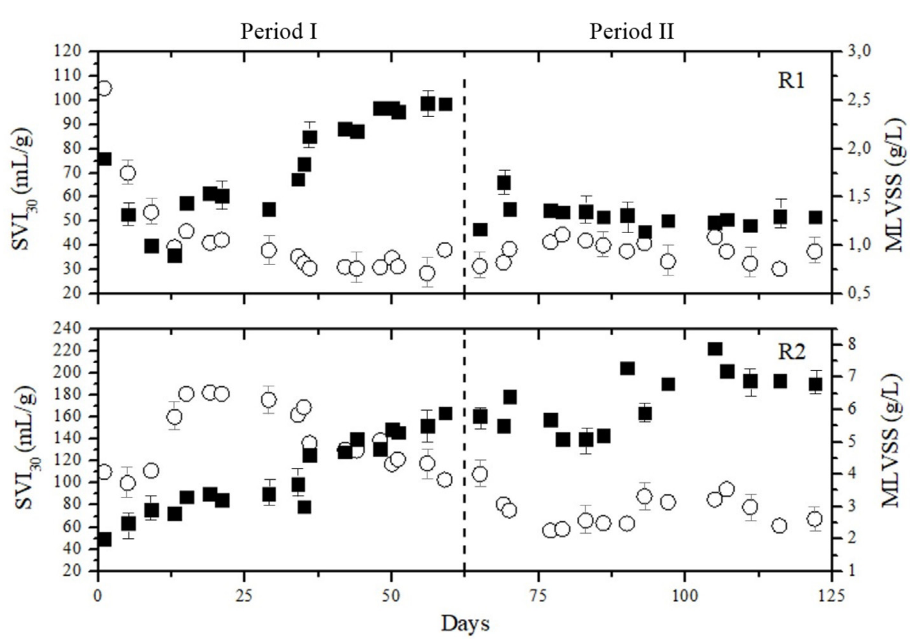

Fig. 2. AGS formation and stability in terms of MLVSS (■) and SVI_{30} (○). R1, conventional SBR; R2, simultaneous fill-and-draw SBR.

These different behaviors observed in the reactors might be due to the different selection pressures imposed on the sludge. The physical mechanism of granule selection in R1 was simple. Considering the volumetric exchange of 50% and working reactor height of 0.92 m, minimum settling velocities of 1.38 and 2.76 m/h were required to keep the sludge in R1 in periods I and II, respectively. On the other hand, in R2, the physical mechanism of selection pressure was different. In this configuration, the solids only needed a settling velocity higher than the upflow velocity during the filling (0.92 m/h). Therefore, it might not be directly related to the settling time.

The understanding of the different physical mechanisms

Table 1
Different values of settling time and reactor upflow velocity of some SBRs which successfully cultivated stable AGS.

SBR configuration	Scale	Substrate	Settling time (min)	Upflow velocity (m/h)	Granule characteristics	Reference
Conventional	Lab-scale	Domestic wastewater (200–600 mg COD/L)	10	30	$SVI_{30} = 38$ mL/g ⌀ > 0.3 mm MLVSS < 3 g/L	Li et al. (2014)
Conventional	Lab-scale	Synthetic wastewater - sodium acetate (1–2 g/L)	2	20	$SVI_2 = 37$ mL/g ⌀ > 1 mm MLVSS < 4 g/L	He et al. (2018)
Conventional	Lab-scale	Synthetic wastewater - sodium acetate (1–2 g/L)	5	20	$SVI_5 = 55$ mL/g ⌀ > 0.5 mm MLVSS <4 g/L	Wu et al. (2018)
Simultaneous fill-and-draw	Full-scale	Domestic wastewater (200–600 mg COD/L)	60	2	$SVI_{30} = 60$ mL/g ⌀ > 0.5 mm MLVSS > 5 g/L	Li et al. (2014)
Simultaneous fill-and-draw	Full-scale	Domestic wastewater (200–700 mg COD/L)	90	2.7	$SVI_{30} = 40$ mL/g ⌀ > 1 mm MLVSS > 8 g/L	Pronk et al. (2015)
Simultaneous fill-and-draw	Full-scale	Domestic wastewater (450–1000 mg COD/L)	60	2–4	$SVI_{30} = 33$ mL/g ⌀ > 1 mm MLVSS > 10/L	van Dijk et al. (2018)

explained the following observations: (i) the period change in R1 caused solids washout, as the minimum settling velocity increased from 1.38 to 2.76 m/h. However, the SVI_{30} presented improvements because the system selected the granules of better settleability; (ii) in R2, the period change did not affect the solids loss. On the contrary, an increase in the MLVSS concentration was observed because, although the settling time decreased, the filling velocity remained constant.

Therefore, in simultaneous fill-and-draw SBRs, which are operated at constant volume, the change in the settling time might not be a key factor for the removal of filamentous bacteria (floating sludge). The main selection pressure in these systems was found to be the liquid upflow velocity.

Other studies also observed that the upflow velocity is an important parameter for granules selection in simultaneous fill-and-draw SBRs. Derlon et al. (2016) reported that an upflow velocity higher than 5 m/h caused flocs washout and favored predominance of granules. However, this strategy caused low sludge retention/concentration in the reactor. On the other hand, when the upflow velocity was close to 1 m/h, the sludge granules were smaller ($0.25 < d < 0.63$ mm), but high solids concentration was observed. The authors concluded that, in a simultaneous fill-and-draw system, the upflow velocity is one of the most important parameter of selection pressure (Derlon et al., 2016).

Similarly, van Dijk et al. (2018) report that, for Nereda® wastewater treatment technology, it is important to have an upflow velocity between 2 and 4 m/h. The relatively higher upflow velocities in Nereda® reactors might be responsible for the washout of floating sludge and fat-like particles. Therefore, this can justify the fact that many full-scale WWTPs (with simultaneous fill-and-draw SBRs) use high settling time (>40 min) and still obtain stable granules (mature granules capable of remaining in the reactor for long periods without disintegrating) since the main selection parameter for this configuration is most likely the liquid upflow velocity.

In this context, Table 1 presents the different values of settling time and upflow velocity of some systems which had success in the cultivation of stable AGS. Accordingly, a short settling time (<15 min) might be the key in conventional SBRs, and the upflow velocity might be negligible. On the other hand, for constant-volume SBRs, the upflow velocity might be the main parameter since the settling time is generally high (e.g. 40 min) and might play a secondary role in the process.

3.2. Characteristics of the granules

After the maturation of the granules (period II), the biomass growth in the two reactors showed different colors (brown in R1 and dark brown in R2) and mean sizes (⌀ = 1.2 mm in R1 and ⌀ = 0.8 mm in R2). The values of the main parameters of the AGS characteristics obtained in the two systems are presented in Table 2.

Considering that the granulation process is achieved when more than 80% of the solids have a diameter greater than 0.2 mm (de Kreuk et al., 2007), it is observed that R1 presented complete granulation with approximately 30 days, and R2 with about 50 days.

After AGS maturation (end of period II), about 94.1% of the granules in R1 were above 1 mm. On the other hand, in R2, less than 65% of the granules formed had a diameter greater than 1 mm. Regarding the settling velocity, the granules cultivated in R1 showed an average velocity of 31.5 m/h, whereas, in R2, velocities close to 14.0 m/h were observed.

In the granules resistance evaluation (Table 2), the comparison was done through the stability coefficient (S). The test evaluates the change in granule diameter using a shear force. High values of S (%) indicate low stability of aerobic granules (Nor-Anuar et al., 2012). Thus, granules cultivated in R2 presented lower values of S (28.5%) compared to those found for R1 (41.8%), indicating that simultaneous fill-and-draw SBRs form granules with better resistance indices, probably because of their lower diameter.

Another parameter strongly influenced by the SBR configuration was the EPS composition. As it is known, they are biopolymers

Table 2
Granules characteristics of the SBRs after maturation (end of period II).

Characteristics	R1	R2
SVI_{30} (mL/g)	44.8	70.7
SVI_{10} (mL/g)	45.6	81.2
SVI_{30}/SVI_{10}	0.98	0.87
TSS > 1 mm (%)	94.1	64.9
Mean diameter (mm)	1.2	0.8
Stability indicator - S (%)	41.8	28.5
SRT (d)	6	35
PS (mg/g MLVSS)	51.2	86.8
PN (mg/g MLVSS)	49.6	50.3
PN/PS	1.00	0.58
Settling velocity (m/h)	31.5	14.0

made of polysaccharides, proteins and others substances, which are important to granule formation and stability because they act as a glue (Rollemberg et al., 2018). During the cultivation process, good granule structure, stability and settleability are kept by EPS (Kocaturk and Erguder, 2016). The granules grown in both systems had similar values of proteins (PN). However, the values of polysaccharides (PS) in R2 were almost the double of R1. Therefore, R2 presented a higher total EPS content, which is in accordance with Rusanowska et al. (2019), who reported that smaller granules have a larger amount of EPS.

Normally, stable aerobic granules have a PN content greater than the PS one, and its increase is directly related to hydrophobicity. Therefore, PN promotes the stability of AGS, and the PN/PS ratio is a way to characterize its stability (Kocaturk and Erguder, 2016). Hence, the granules in R1 presented better results (PN/PS = 1.00) than those in R2 (PN/PS = 0.58) (Table 2).

3.3. Reactor performance

COD, nitrogen and phosphorus removal efficiencies are shown in Table 3. The systems had high COD removals during the entire period of operation (>90%), and no significant differences were observed in R1 and R2 in both periods ($p = 0.09$), showing that the different SBR configurations did not affect the organic matter removal.

Regarding total nitrogen (TN) removal, mean values over 50% were observed during the entire operation in both reactors, and no significant differences were observed in both periods ($p = 0.08$). Although the removal values were similar, it is noteworthy that the removal mechanisms were different. In R1, low nitrite and nitrate concentrations were observed in both periods, although only a fraction of the influent ammonium was oxidized (about 60%). On the other hand, in R2, the influent ammonium was almost completely oxidized to nitrate, which accumulated in the system.

Some explanations for such observations are: (i) a higher loss of solids was verified in R1, likely decreasing the abundance of nitrifying bacteria (ammonia-oxidizing bacteria, AOB, and nitrite-oxidizing bacteria, NOB) compared to R2, which resulted in a lower nitrification efficiency (about 60% ammonium oxidation), and, (ii) as the systems had an anaerobic reaction phase followed by an aerobic one in the cycles, nitrogen removal occurred mainly in the aerobic phase by the simultaneous nitrification and denitrification (SND). However, this process occurs mostly in granules of larger size, in which nitrification occurs in the outer layer, and denitrification takes place in the inner (anoxic) layer. Because R1 formed larger granules, possibly the SND might have be favored. As it is known, the ratio of denitrified nitrate to the produced nitrate increases with the mean granule diameter, i.e. with a larger anoxic volume (Rollemberg et al., 2019). Similarly, Chen et al. (2011) reported an increase of the total nitrogen removal efficiency from 67.9 to 71.5% with the increase of the granules size from 0.7 to 1.5 mm, showing a direct relationship between the SND process and the diameter of the sludge granule.

Still concerning nitrogen removal, results obtained in R2 are similar to those found by Derlon et al. (2016). The authors observed that the granules formed in a SBR operated at constant volume with low upflow velocity (1 m/h) formed small granules, and, consequently, the SND was not favored. Therefore, it was observed that denitrification took place especially in the anaerobic phase, and the residual nitrate was denitrified in the next cycle.

With regard to phosphorus removal, the systems presented similar removal values in period I (R1 ≈ 48% and R2 ≈ 47%) ($p = 0.08$), but, in period II, R2 presented higher values (48 ± 10%) than those found in R1 (22 ± 8%), which were statistically different ($p = 0.04$). Probably, the decrease of phosphorus removal in the R1 in period II might have been due to the reduction of solids concentration and phosphorus accumulating bacteria (PAOs) caused by the settling time reduction from 20 to 10 min.

Table 3
Mean values of COD, nitrogen and phosphorus removal efficiencies in the SBRs.

Parameters	Period I		Period II	
	R1	R2	R1	R2
COD_{inf} (mg/L)	652 ± 35	626 ± 57	621 ± 58	694 ± 86
COD_{eff} (mg/L)	41 ± 15	37 ± 20	63 ± 49	31 ± 23
COD removal (%)	92 ± 2	94 ± 4	90 ± 4	96 ± 3
$N\text{-}NH_4^+{}_{inf}$ (mg/L)	92 ± 8	91 ± 5	95 ± 9	93 ± 6
$N\text{-}NH_4^+{}_{eff}$ (mg/L)	36 ± 15	5 ± 1	35 ± 11	7 ± 3
$N\text{-}NO_2^-{}_{eff}$ (mg/L)	7 ± 4	4 ± 3	9 ± 3	10 ± 8
$N\text{-}NO_3^-{}_{eff}$ (mg/L)	2 ± 1	32 ± 14	2 ± 1	24 ± 9
TN removal (%)	52 ± 9	54 ± 7	53 ± 8	56 ± 4
$P\text{-}PO_4^{3-}{}_{inf}$ (mg/L)	10 ± 1	10 ± 2	10 ± 1	10 ± 2
$P\text{-}PO_4^{3-}{}_{eff}$ (mg/L)	4 ± 1	5 ± 2	7 ± 1	5 ± 2
TP removal (%)	48 ± 2	47 ± 2	25 ± 9	48 ± 10

3.4. Microbial community

3.4.1. Overall taxonomic populations

The identified bacterial structures and relative abundances of the aerobic granules cultivated in the conventional (R1) and simultaneous fill-and-draw (R2) SBRs are shown in Fig. 3.

The most abundant phyla in R1 and R2 were, respectively, Proteobacteria, Bacteroidetes, Planctomycetes, Chloroflexi, Verrucomicrobiae and Actinobacteria. Such phyla are present in previous works on AGS (Zhang et al., 2017). Studies have observed that the abundance of these groups may vary according to the type of substrate used (Rollemberg et al., 2019), operational conditions (He et al., 2018) and many others parameters (Wang et al., 2018). In this study, it was also observed that the configuration of SBR directly affected the abundance of microbial groups.

There was a greater abundance of Proteobacteria in R2 than in R1. This group usually presents a wide diversity and metabolic capacity, acting on important environmental functions such as the cycles of C, N, S and P (Meyer et al., 2016). The families that presented the greatest differences of abundance in this phylum were the Deltaproteobacteria and Alphaproteobacteria. Literature reports that these families have a huge amount of bacteria that can secrete EPS (Ding et al., 2015), which may justify the higher content of total EPS in the granules cultivated in R2 compared to R1, as discussed in section 3.2.

The phylum Bacteroidetes was also present in abundance in both reactors, although slightly higher in R1. These microorganisms are known for their fermentative properties and also play an important role in the degradation of complex polymers, as they hydrolyze some substrates (such as polysaccharides, proteins and lipids) into acetate, long-chain fatty acids, CO_2, formate and hydrogen (Jabari et al., 2016).

Because of the higher abundance of Bacteroidetes in R1 and the higher size of the cultivated granule (section 3.2), the SND process might have been favored (section 3.3). Indeed, some studies have shown that practically all organic matter is no longer readily available in the reactor after a few minutes of aeration (He et al., 2018), which hinders the process of heterotrophic denitrification. However, this process occurs in another metabolic pathway since EPS (produced in the feast phase) can be used as an electron donor if there are microbial groups (related to Bacteriodetes) capable of degrading these polymers. The lower content of total EPS in the granules cultivated in reactor R1 may be related to its consumption via SND.

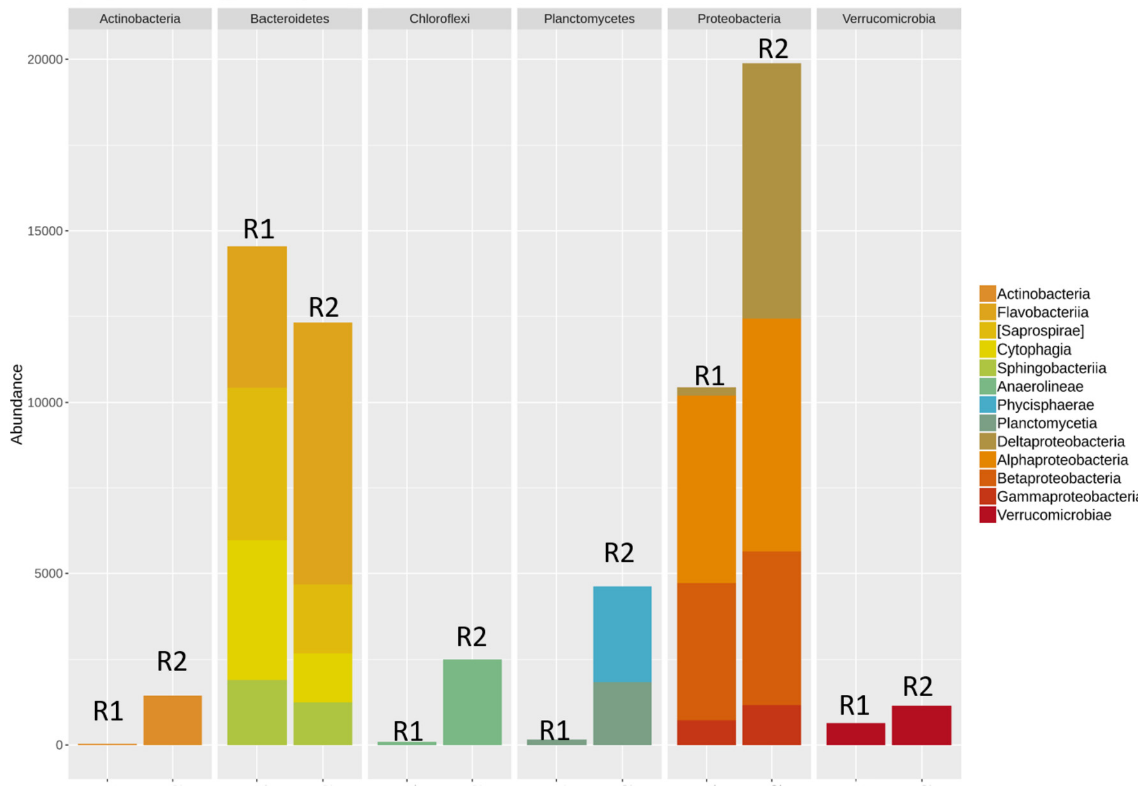

Fig. 3. Taxonomic affiliation of the aerobic granules. R1, conventional SBR; R2, simultaneous fill-and-draw SBR.

It is important to note the higher presence of Actinobacteria and Chloroflexi in R2. These phyla are related to the presence of filamentous bacteria, which play an important role in the stability of granules, especially under relatively high hydrodynamic shear forces (Zhou et al., 2015). The higher values of SVI_{30} (greater abundance of filamentous bacteria) in R2 were likely related to the presence of microorganisms of those phyla. It is worth mentioning that the abundance of filamentous bacteria might cause operational problems, such as sludge bulking and foaming (Seviour et al., 2008).

Regarding the Planctomycetes, it was the third most abundant phylum in R2 and was practically absent in R1 (Fig. 3). Special emphasis is given to this phylum, as they are comparatively slow-growing organisms with low demand for carbon (favoring granulation). Additionally, some species are anaerobic ammonium oxidation bacteria (ANAMMOX).

3.4.2. Key functional groups

Besides the taxonomic study, the presence of specific microbial groups involved in the removal of carbon, nitrogen and phosphorus (also known as key functional groups) was evaluated. Taxonomic affiliation of each OTU (operational taxonomic unit) was used to infer functional content related to the key functions, such as ammonia-oxidizing bacteria (AOB), nitrite-oxidizing bacteria (NOB), glycogen-accumulating organisms (GAOs), polyphosphate-accumulating organisms (PAOs) and denitrifying bacteria (DNB) (Fig. 4).

The abundance of AOB was similar in both reactors, although there was a higher ammonia oxidation in R2 (about 92% compared to 63% in R1, in period II) (Table 3). However, there was a greater abundance of NOB in R2. Consequently, low nitrate accumulation in R1 was found (partial nitrification), and high concentrations of this compound were found in R2 (complete nitrification) (section 3.3 and Table 3). Such a behavior may be related to the solids retention time (SRT) or sludge age since the values found for both reactors are completely different, i.e. 6 days in R1 and 35 days in R2 (Table 2). Literature reports that high temperature with short SRT could inhibit NOB and lead to nitrite accumulation for SND (Lemaire et al., 2008).

The abundance of denitrifying bacteria was higher in R1 than in R2. However, the latter system showed a greater diversity. Comamonadaceae was the only family found in R1, whereas Hyphomanadaceae, Comamonadaceae, and Demabacteraceae were the families found in R2 (Fig. 4). Ginige et al. (2005) reported that members of the family Comamonadaceae can use nitrite as electron acceptor. Since the SND may be favored in R1 because of the cultivated granule size (section 3.3), likely, in this system, denitrification via either nitrite or nitrate might have occurred. In addition, some works on AGS have shown the importance of this family for the maintenance of granule structure (Sun et al., 2017). The higher abundance of DNB bacteria in R1 explains the low effluent concentrations of nitrite and nitrate.

Concerning the PAOs, under aerobic/anoxic conditions, these microorganisms can utilize the energy of their intracellular polyhydroxyalkanoates (PHAs) to uptake phosphorus from wastewater. PAOs can compete with GAOs because they have similar metabolism and consume the same organic carbon substrates, such as volatile fatty acids (VFAs). However, GAOs cannot accumulate phosphorus, therefore decreasing the efficiency of the system to remove this compound (Bassin et al., 2012).

High abundances of PAOs and GAOs were observed in both reactors, explaining the low P removals found (Table 3) in either conventional SBR (R1) ($25 \pm 9\%$, period II) or simultaneous fill-and-draw SBR (R2) ($48 \pm 10\%$, period II) probably due to the high competition for the organic substrates.

The microorganisms of the Saprospiracae and Rhodospirillaceae families were the main PAOs found in R1 and R2, respectively. On

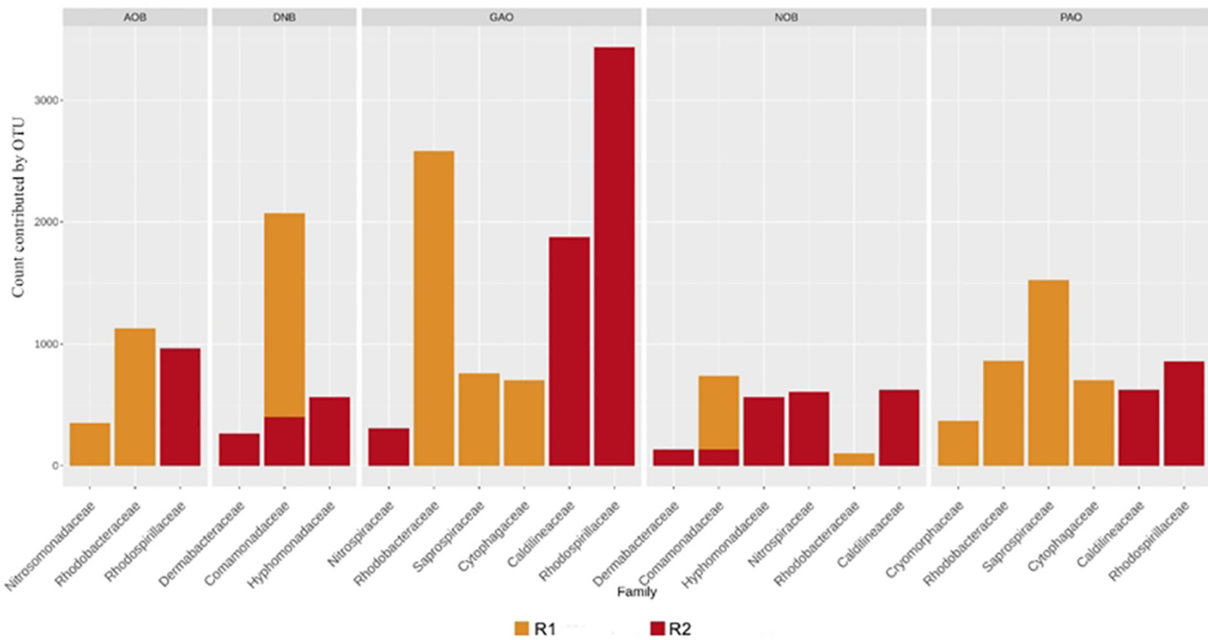

Fig. 4. Functional distribution of the taxonomical classification at family level of the microorganisms involved in nitrogen and phosphorus removals. R1, conventional SBR; R2, simultaneous fill-and-draw SBR.

the other hand, the main GAOs families found in the reactors were Rhodospirillaceae and Caldilineaceae in R1, and Rhodobacteraceae, Saprospiraceae, and Cytophagaceae in R2. The main PAOs and GAOs found in the current work are usually reported in other investigations into AGS. Some microorganisms of the Rhodospirillaceae family are reported as denitrifying PAOs (DPAOs), therefore having the ability of simultaneously remove nitrogen and phosphorous (He et al., 2019).

The greater abundance of denitrifying bacteria in R1 might also be related to the low removal of phosphorus in this system since these microorganisms also compete with PAOs for the organic substrates during the anoxic phase (filling phase of 30 min) of the SBR cycle (Rollemberg et al., 2018).

Pronk et al. (2015) found low phosphorus removal in AGS systems when readily biodegradable substrates were used because they were not converted anaerobically into storage polymers such as polyhydroxyalkanoates (PHA), leading to the formation of unstable granular sludge. They also reported that polymers generated with ethanol, as carbon source did not favor PAOs.

4. Conclusion

In the conventional SBR (R1), granulation was faster (30 days), and granules with larger diameters (1.2 mm) were obtained. On the other hand, the SBR operated at constant volume (R2) showed better stability (especially in terms of phosphorus removal), higher solids concentration (7 g MLVSS/L), and easier operation, justifying the use of this configuration in full-scale AGS systems, such as Nereda®.

Significant differences between the microbial communities of both reactors were also observed, reinforcing the better performance achieved by R2. The reduction in the settling time (from 20 to 10 min) in R2 did not play a key role in the filaments removal as in R1. Thus, the main selection pressure in simultaneous fill-and-draw SBRs might be the upflow velocity, whereas, in conventional SBRs, low settling times (<15 min) might be required for an effective AGS cultivation.

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