Journal of Water Process Engineering 44 (2021) 102378

Contents lists available at ScienceDirect

Journal of Water Process Engineering

journal homepage: www.elsevier.com/locate/jwpe

Impact of feeding strategy on the performance and operational stability of aerobic granular sludge treating high-strength ammonium concentrations

Vicente Elício P.S.G. da Silva, Silvio Luiz de Sousa Rollemberg, André Bezerra dos Santos *

Department of Hydraulic and Environmental Engineering, Federal University of Ceará, Fortaleza, Ceará, Brazil

A R T I C L E I N F O

Keywords:
Aerobic granular sludge
Concentrated-wastewaters
Leachate treatment
Step-feeding

A B S T R A C T

We evaluated the impact of feeding strategy on the performance and operational stability of aerobic granular sludge (AGS) treating high-strength ammonium concentrations. Synthetic wastewater with characteristics close to those found in sanitary landfill leachate was applied in sequential batch reactors (SBR) for biomass cultivation. In this sense, differing only in the feeding method, three identical 7.6 L (working volume) reactors were operated with the same total cycle time of 12 h duration. In R1 and R2, it was adopted feeding in the anaerobic period with a duration of 20 min (fast) and 40 min (slow), respectively. In R3, feeding was distributed throughout the cycle (step-feeding), half of which was introduced initially, and the other half divided equally with 40 and 60% of the cycle. Substrate distribution throughout the cycle (R3) minimized three of the biggest problems faced when treating leachate in AGS systems: granules' stability, biomass retention, and nitrite accumulation. Besides, compared to fast (R1) and slow (R3) feeding, this mode of operation obtained the best total phosphorus (TP, 53%) and total nitrogen (TN, 92%) removals, without any nitrite or nitrate accumulations. COD removals were very similar in R2 and R3, but TN and TP removals were significantly greater in R3. Therefore, the feeding method directly interferes with the performance, granules' characteristics, and system stability. The results obtained in this research can be used in future works applying the AGS technology for sanitary landfill leachate and other complex wastewaters treatment.

1. Introduction

Leachate treatment from sanitary landfills has been carried out mainly by classic biological processes. However, landfill age ends up limiting the application of these processes due to toxicity by free ammonia (FA) and free nitrous acid (FNA) in old landfills (>10 years) [1,2]. This complex wastewater is characterized by high concentrations of total nitrogen (TN, especially ammonia), recalcitrant organic matter and low biodegradability ($BOD_5/COD < 0.1$), heavy metals, inorganic salts, and xenobiotic organic compounds [3–5].

New alternatives to conventional biological treatments have been sought to cover all landfills leachate characteristics, and the aerobic granular sludge (AGS) system technology has been considered promising, especially for its capacity of carrying out almost all biological conversions in a single system, i.e., organic matter, nitrification, denitrification, and phosphorus removals [6,7]. However, operational strategies must be adopted to minimize the problems reported by several authors Lee et al. [39], Lin et al. [40], such as biomass instability, long period of granule formation when using real sewage, nitrite accumulation, low phosphorus removal in some cases, among others. Advances in the use of the AGS technology for sanitary landfill leachate treatment are still incipient and include the pre-treatment study and optimization of the aeration system [8,9], the comparison with activated sludge process regarding different raw leachate dilutions [2,5,10], and the co-treatment with domestic sewage [3,10]. Therefore, there are still many gaps to be filled, especially concerning optimizing operational conditions for the formation and maintenance of aerobic granules.

The feeding mode is one of the key factors in the selection, formation, and stability of aerobic granules [11,12]. The anaerobic filling in sequential batch reactors (SBR) has been widely accepted, in which the duration and influent load are considered efficiency determining factors.

Little studied, but with great potential to favor granulation in effluents with high refractory loads, such as sanitary landfill leachate, step-feeding can be an efficient strategy to remove organic matter and nitrogen simultaneously. It is known that during biological nitrogen removal, denitrification is usually the limiting step due to the lack of a proper carbon source in the leachate to support denitrification [3,5]. Consequently, a high concentration of nitrite (NO_2^-) or nitrate (NO_3^-)

* Corresponding author: Department of Hydraulic and Environmental Engineering, Campus do Pici, Bloco 713, Pici, CEP: 60455-900 Fortaleza, Ceará, Brazil.
E-mail address: andre23@ufc.br (A. Bezerra dos Santos).

https://doi.org/10.1016/j.jwpe.2021.102378
Received 26 August 2021; Received in revised form 5 October 2021; Accepted 12 October 2021
Available online 20 October 2021

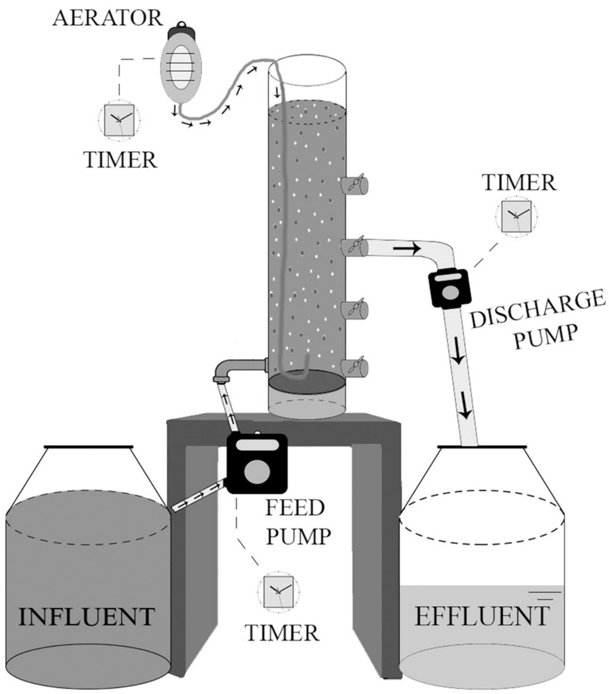

Fig. 1. Schematic of an experimental SBR-AGS system in this study.

can be found in the effluent, reducing nitrogen removal efficiency. In AGS, denitrification can occur in the anoxic zone of the granules or anoxic phases of the cycle. However, even before denitrification occurs, the influent carbon has already been depleted. Therefore, the substrate distributed throughout the SBR operating cycle can make good use of the influent organic matter as the carbon source, allowing nitrification to occur with a lower organic load in the aerobic phase, and favoring denitrification [13,14].

To date, the number of investigations applying the AGS technology with step-feeding is still limited. Chen et al. [15] obtained TN removals of 93% for granules of 0.7 mm and 95.9% for granules of 1.5 mm, without nitrite accumulations. However, Wang et al. [14] observed nitrite accumulation in the order of 93 ± 5%, reducing TN removal to 70%. TN removals were greater than 90% and more significant in C:N ratios of 5:1 than 3:1 [13]. Zhong et al. [16] achieved TN removals of 89.7–92.4% in step-feeding mode and 48.1–59.5% in single feeding mode. However, all these investigations alternated anoxic phases with oxide phases and worked with low-medium influent loads.

In this context, the present research evaluated the feeding impact on the performance and operational stability of AGS systems treating high-strength ammonium concentrations. For this, three AGS reactors were fed with synthetic effluent with characteristics similar to the leachate from a Brazilian sanitary landfill. Each reactor had a different feeding strategy (conventional fast, conventional slow, and step), mainly aiming at increasing biomass retention, minimizing nitrite accumulation, and favoring phosphorous removal. As far as we are concerned, step-feeding has not been previously investigated for leachate treatment in AGS systems.

2. Material and methods

2.1. Experimental set-up

The experiments were carried out in three identical SBRs inoculated with the same biomass and operated under the same conditions, changing only the feeding method. The reactors had 7.85 L, with working volume of 7.6 L, internal diameter of 10 cm, height of 100 cm, and height-to-diameter ratio (H/D) of 10, with a 50% exchange volume.

Table 1
Composition of the raw sanitary landfill leachate.

Parameter	Value
pH	7.45 ± 0.5
Conductivity (μS/cm)	15,330 ± 181.6
BOD total (mg/L)	852 ± 236
COD total (mg/L)	3743 ± 453
NH_4^+-N (mg/L)	650.7 ± 165
NO_2^--N (mg/L)	4.7 ± 2.6
NO_3^--N (mg/L)	5.1 ± 3.3
TKN (mg/L)	752 ± 126
Total phosphorous (mg/L)	72.6 ± 12.2
Chloride (mg/L)	8.5 ± 0.5
Sulfate (mg/L)	43.3 ± 32.6
Fluoride (mg/L)	30.3 ± 9.3
Bromide (mg/L)	5.4 ± 2.5
Sulfide (mg/L)	111.6 ± 46.1

The total experiment duration was 120 days for R1 and 134 days for R2 and R3, both divided into two periods by changing the sedimentation time (Ts). In the first 40 days of operation, the Ts was 20 min (period I), being reduced to 10 min (period II) until the experiment completion. The reduction in Ts favors biomass selection and increases efficiency and long-term stability, benefiting aerobic granulation [6].

The duration of each cycle was 12 h, which consisted of feeding (20–40 min), aerobic reaction (659–679 min), sedimentation (20–10 min), and discharge (1 min). In the aerobic phase, the air was injected by porous fine bubble diffusers through the reactor bottom using an air compressor Yuting SUN, China, ensuring a dissolved oxygen (DO) concentration between 2 and 5 mg/L. Systems' operation was automated using synchronized timers (Fig. 1).

The feeding differentiation in the reactors followed the description below:

- R1: conventional feeding (piston flow) in anaerobic/anoxic phase lasting 20 min (fast);
- R2: conventional feeding (piston flow) in anaerobic/anoxic phase lasting 40 min (slow);
- R3: step-feeding over the cycle in three moments, with 50% of the influent volume being introduced at the beginning of the cycle and the other half divided equally with 40 and 60% of the cycle.

The reactors were operated at room temperature, and the reaction temperature was around 28 ± 2 °C. During the experiment, there was no programmed sludge discharge, resulting in different sludge retention times (SRTs).

2.2. Sludge source and synthetic wastewater composition

The reactors were inoculated with the same volume of flocculent aerobic sludge from an activated sludge plant treatment domestic sewage (Fortaleza, Ceará, Brazil). A synthetic wastewater was used, presenting the C:N:P ratio of 50:10:1 found in the raw leachate from the Municipal Sanitary Landfill West of Caucaia — ASMOC (Ceará, Brazil) (Table 1). It is important to mention that heavy metals concentration in the sanitary landfill leachate is usually very low, not causing toxicity to the biological treatment [3,10]. Furthermore, heavy metals solubility is reduced due to the higher pH found in old leachate, which allows metal precipitates formation, along with sorption processes on the colloidal matter surface, decreasing the leachate toxicity [17,18].

Thus, the influent synthetic wastewater was composed of 1000 mg COD/L of sodium acetate as a carbon source, 200 mg/L of NH_4^+-N (NH_4Cl) as nitrogen source, 20 mg/L of PO_4^{3-}-P (from KH_2PO_4) as a phosphorus source, and 1 mL/L of trace element solution as described by Rollemberg et al. [19]. Like the ASMOC leachate and the ones investigated by Ren et al. [2,10], the pH was kept close to neutrality, being adjusted with sodium bicarbonate.

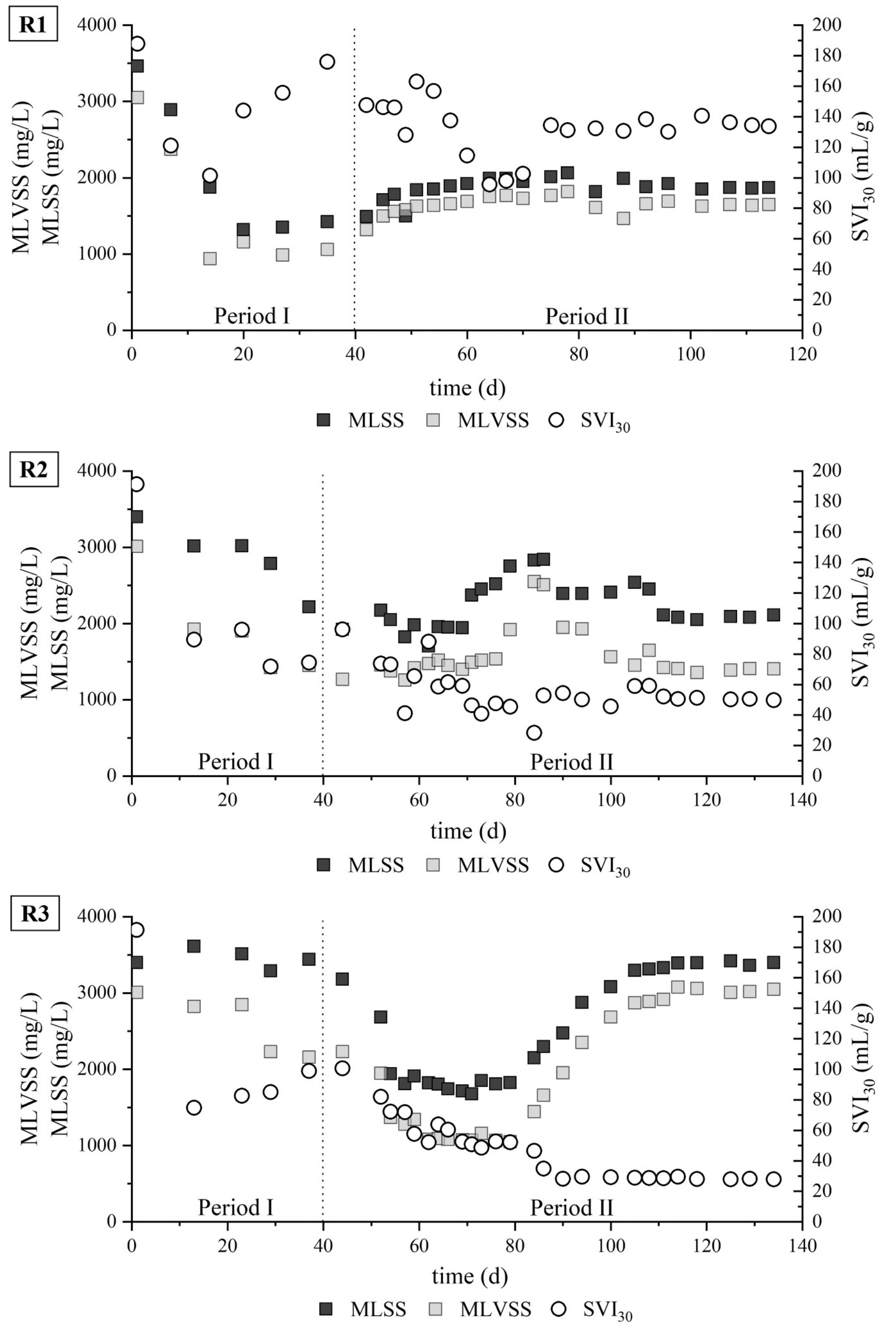

Fig. 2. Stability in terms of SS, VSS, and SVI_{30} of AGS systems with fast feeding (20 min, R1), slow feeding (40 min, R2), and step-feeding (R3) for the sedimentation times of 20 min (Period I) and 10 min (Period II).

2.3. Analytical methods

System influent and effluent COD, pH, NH_4^+-N, NO_2^--N, NO_3^--N, PO_4^{3-}-P, mixed liquor suspended solids (MLSS), mixed liquor volatile suspended solids (MLVSS), and sludge volumetric index (SVI) in 10 e 30 min (SVI_{10} and SVI_{30}) were analyzed two to three times a week and determined according to APHA [41]. Dissolved oxygen (DO) was measured by a YSI 5000 probe (YSI Incorporated, USA). Total inorganic nitrogen (TIN) was considered as the sum of NH_4^+-N, NO_2^--N, and NO_3^--N [20]. The content of EPS (extracellular polymeric substances) was also quantified by the modified heat extraction method according to Rollemberg et al. [19]. Particle size was measured to determine the granulation process and granules' stability using Image-Pro Plus software from microscopic images. The reactor reaches the aerobic granulation stage only when more than 80% of the biomass had a diameter greater than 0.2 mm [21].

The cycle tests were carried out at the end of the experiment (period II) when the reactors reached complete stability to understand the SNDPR mechanisms (simultaneous nitrification, denitrification, and phosphorus removal). Concentrations of NH_4^+-N, NO_2^--N, and NO_3^--N, PO_4^{3-}-P, and DO were determined as described by Li et al. [22].

2.4. Morphology of the granules

The structure of the mature granules was analyzed by scanning electron microscopy (SEM) combined with spectrum energy dispersive X-rays (Inspect S50, FEI Company, USA). The pretreated consisted of fixing, washing, and lyophilization, according to the methodology described by Motteran et al. [23].

2.5. Statistical methods

Statistical analyses were performed with the Origin 2018 computer software using the Mann-Whitney Rank Sum test to compare the reactor performance at a 95% confidence level. The data groups were statistically different when $p < 0.05$.

3. Results and discussion

3.1. Start-up, formation, and stabilization of the granules

The three reactors were initially operated with the same sludge source, presenting about 3.4 g/L of MLSS, MLVSS/MLSS ratio of 88% and SVI_{30} of 190 mL/g. The evolution of these parameters throughout the experiment is shown in Fig. 2. After start-up, the MLSS concentration gradually decreased in both R1 (20 min feeding) and R2 (40 min feeding). Sedimentation time reduction was a key strategy for promoting granulation. However, even after biomass stabilization, a constant sludge loss (washout) was observed in these reactors, which is very common in AGS systems operated with high-load wastewaters [24–26]. It may indicate the formation of a biomass with high growth of filamentous microorganisms at high rates of substrate transport, making them more flocculent.

As the high influent organic load is not biodegradable, there will be impacts on the carbon supply for denitrification, which might also result in biomass washout [3,5,8]. Thus, an external addition of soluble COD becomes necessary for AGS cultivation when it is intended to treat effluents not favorable to slow-growing bacteria development. Besides, distributing the organic load throughout the cycle to reduce toxicity and favor denitrification seems to be an efficient strategy.

Disintegration of the granules was frequent in R2, and MLSS concentration was very unstable, failing to achieve a consistent regranulation. As previously reported for AGS systems treating leachate from sanitary landfills, granule disintegration also occurred after 50 days of operation and excessive biomass loss was found [3,10]. These authors also pointed out that loads above 200 mg/L NH_3-N favored granules' disintegration.

Therefore, it becomes evident that the influent COD/N ratio is preponderant for the formation and maintenance of stable granules. When this ratio is high, there is a growth of filamentous microorganisms that can cause granule disintegration [27,28]. On the other hand, reducing this ratio generates great changes in the microbial community. It decreases the EPS content, impacting nitrification, and resistance, size, and sedimentation capacity of the granules, and subsequent biomass loss. Thus, the instability and disintegration of aerobic granules in high influent loads can be attributed to the increase in granule size due to the inability of carbon penetration, to the hydrolysis and protein degradation of the granule nucleus, and to the loss of microorganisms' ability to self-aggregate due to reduction of EPS protein content [29,30].

In R3 (step-feeding), during period I (sedimentation time of 20 min), despite MLSS concentration has increased, the SVI also increased, which indicates a sludge of low sedimentability, possibly dispersed or flocculent. After reducing the sedimentation time, this poor-quality sludge was washed out. After 30 days of stability, there was again a biomass growth, significantly improving sedimentability and reaching a MLSS concentration similar to the inoculum. Therefore, as in the experiments by Wang et al. [14] with two feeds throughout the cycle, the MLSS first decreased and then increased and stabilized. These results are also in line with those of Wei et al. [9], treating leachate without dilution (3.2 g/L MLSS), and Bueno et al. [3], with 5% leachate diluted in synthetic domestic sewage (3.3 g/L MLSS).

Therefore, R3 had greater solids retention (3.4 g/L MLSS), followed by R2 (2.1 g/L MLSS) and R1 (1.9 g/L MLSS). Also, at the end of the operation, MLVSS proportion in relation to MLSS was 90% in R3, 88% in R1, and 67% in R2. Retention of solids in AGS reactors has been one of the difficulties encountered when treating leachate, with controversial results and without a defined tendency. Wei et al. [9] and Bella and Torregrossa [8] obtained a decrease in MLSS concentration when they started with 4 and 11 g/L MLSS, respectively, and ended the experiments with 3 and 5 g/L MLSS. Ren et al. [5,10] practically achieved twice the initial MLSS concentration, while Ren et al. [10] did not obtain any change. Apparently, the only pattern found is that the higher the leachate concentration, the greater the solids loss, agreeing with some previous studies [2,3]. Wang et al. [14] point out that the MLSS maintenance is mainly linked to the inoculum quality, as the high concentration of inoculum sludge causes stronger and more frequent collisions and friction among microorganisms, resulting in the microbial self-aggregation improvement. Other causes are high influent carbon and nitrogen loads, system operation, and dilution factors.

In terms of sedimentability, in all reactors, the first falls in the SVIs were due to the initial biomass washout. However, except R1, the SVI improved a lot after the adaptation period, reaching a good stability. Thus, R3 had the best SVI_{30} result (<30 mL/g), while R1 had the worst result with SVI_{30} greater than 120 mL/g. As in R1 the aeration phase was greater, it was expected to present better sedimentability, which did not occur. However, the SVI_{30} was greater than 160 mL/g during period I, being improved with sedimentation time reduction (period II). Therefore, to improve the sedimentability in R1, lower sedimentation time would be necessary to select the biomass better. In addition, between 60 and 70 days of operation, the sludge from R2 and R3 reached $SVI_{30} < 60$ mL/g, while in R1, it was above 100 mL/g. Therefore, the granulation process was better in R3 and R2, respectively, since normally mature granules have SVI_{30} between 30 and 80 mL/g [31]. Even with higher carbon and nitrogen loads, the results for R3 were similar to those that adopted the same feeding configuration ($SVI_{30} < 30$ mL/g) [13,14] and those that used dilutions that varied between 10 and 100% ($SVI_{30} < 25$ mL/g) [2,10].

These results showed that, compared with reactors with fast single feeding (R1) and slow single feeding (R2), the step-feeding distributed throughout the cycle is an excellent strategy to retain biomass and improve sedimentability. This configuration inhibits the excessive proliferation of fast-growing heterotrophic bacteria. Through the succession of feast/famine conditions, it promotes the development of granules of good sedimentation with reinforced structure, contributing greatly to the system stability [13,32]. Besides, it has become evident that fast

Table 2
Granules' characteristics throughout the experimental periods for AGS systems with fast feeding (20 min, R1), slow feeding (40 min, R2), and step-feeding (R3).

Characteristics	Period I			Period II		
	R1	R2	R3	R1	R2	R3
SVI_{30} (mL/g)	139.6 ± 29.2	83.0 ± 11.7	85.3 ± 10.0	132.3 ± 17.4	55.8 ± 14.5	46.7 ± 19.9
SVI_{10} (mL/g)	156.1 ± 31.4	105.2 ± 21.0	99.4 ± 8.9	172.6 ± 35.5	59.6 ± 15.2	49.8 ± 23.4
SVI_5 (mL/g)	194.2 ± 41.4	139.0 ± 28.3	124.4 ± 18.6	220.4 ± 52.0	74.5 ± 25.0	56.7 ± 30.1
SVI_{10}/SVI_{30}	1.1 ± 0.2	1.3 ± 0.1	1.2 ± 0.2	1.3 ± 0.2	1.1 ± 0.1	1.1 ± 0.1
SVI_5/SVI_{30}	1.4 ± 0.3	1.7 ± 0.2	1.5 ± 0.3	1.7 ± 0.3	1.3 ± 0.2	1.2 ± 0.2
Mean diameter (mm)	0.1 ± 0.1	0.2 ± 0.1	0.3 ± 0.1	0.5 ± 0.2	0.8 ± 0.1	1.0 ± 0.3
SRT (d)	–	5 ± 3	6 ± 4	–	11 ± 4	11 ± 5
PS (mg/g MLVSS)	141.1 ± 108.5	46.1 ± 9.2	46.7 ± 2.1	54.9 ± 12.5	50.7 ± 14.2	60.1 ± 8.7
PN (mg/g MLVSS)	385.3 ± 203.7	217.4 ± 17.2	182.8 ± 11.7	285.0 ± 58.9	236.0 ± 23.0	233.6 ± 25.6
PN/PS	4.1 ± 2.7	4.8 ± 0.6	3.9 ± 0.3	5.2 ± 0.5	4.9 ± 1.1	3.9 ± 0.4

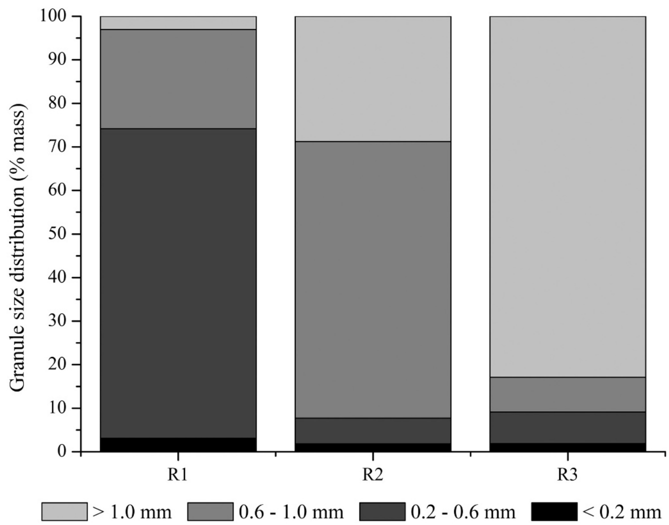

Fig. 3. Granule size distribution (% mass) at the end of Period II for AGS systems with fast feeding (20 min, R1), slow feeding (40 min, R2), and step-feeding (R3).

feeding imposes a strong selection pressure, making biomass retention and granulation difficult. As the COD is not readily oxidized in the anaerobic period and in the first hours of the aerobic reaction, ordinary heterotrophic organisms (OHO) begin to develop, mainly in filaments, being eliminated in the frequent washouts. Therefore, if a large part of the COD is not oxidized at the beginning of the cycle, problems with biomass may be more significant. As the organic matter present in the leachate is recalcitrant and of low biodegradability, a longer time is required for hydrolysis. Thus, an anaerobic feeding with a longer duration favors granulation, and studies with longer times than those used in this research are recommended. In the case of step-feeding, the COD toxicity is minimized as it is distributed throughout the cycle, favoring the development of beneficial microorganisms for the granulation without being eliminated since the washouts are much less frequent and biomass growth is greater than its loss.

3.2. Characteristics of the granules

The aerobic granules showed some different physical and chemical characteristics (Table 2). Therefore, how the reactors were fed affected granules' characteristics, probably due to the different microbial groups that were favored with each strategy adopted. In reactors R2 and R3, it can be seen that the values of SVI_5, SVI_{10}, and SVI_{30} became lower with the sedimentation time decrease. This result demonstrates that biomass sedimentability has improved over time, being a typical evolution behavior from flocculent sludge to granular sludge. The opposite occurred with R1, in which SVI_5 and SVI_{10} increased with reduced sedimentation time, indicating poor sedimentability and filamentous biomass.

Several authors point out that the SVI_8/SVI_{30} or SVI_5/SVI_{30} ratios can be considered good predictors of granulation, meaning that a value closer to 1.0 indicates that the sludge consists mainly of mature granules [19,33,34]. For effluents with high loads, they observed that there is a predominance of aerobic granules when this ratio is between 1.2 and 1.8. Values above 1.8 characterize an AGS thickening. Thus, the results indicate that the granulation in R2 and R3 was better than in R1, whose SVI_5/SVI_{30} ratio of 1.7 ± 0.3 suggests biomass thickening. In addition, the higher the leachate proportion, the closer to 1.0 will be the SVI_5/SVI_{30} ratio [3,5,10]. With this regard, R3 was the best strategy to achieve such a profile.

The literature also reports that the reactor is considered granular when more than 80% of the biomass has a diameter greater than 0.2 mm [34]. Therefore, the three reactors fit as aerobic granular systems since more than 80% of the granules are larger than 0.2 mm (Fig. 3).

In R3, more than 80% of the granules were not only larger than 0.2 mm but larger than 1.0 mm, with an average diameter in period II of 1.0 mm and 1.3 mm at the end of the experiment. Thus, the average diameter of the granules in R3 after 134 days of operation was greater than those obtained in all existing AGS studies so far on leachate treatment: 0.36–0.60 mm [9]; 0.80–0.90 mm [8]; >0.31 mm [10]; 1.1 mm [5]; 0.21–0.48 mm [2,10]; 0.61 mm [3] (however, some granules with a diameter of 1.5 mm were observed). Furthermore, they were also superior to the granules reported in the studies by Wang et al. [14] with feeding distributed in two stages of the cycle (~1.1 mm) and similar to those of Chen et al. [13] with alternating feeding in 3 times (~1.3 mm).

Furthermore, as shown in Fig. 4, only R3 presented a granule with a more stable and uniform surface, making it possible to verify the dominance of coccus over bacillus and filamentous bacteria. R1 and R2 did not exactly present a uniform granular structure, being observed a tangle of filaments. However, in R2, a more granular structure that tends to uniformity is verified, despite not showing dominance of coccus.

Concerning EPS, these substances are biopolymers consisting of polysaccharides, proteins, and other substances, which play a fundamental role in the granules' structure, formation, and stability. In other words, they act as a "biological glue" in which PS and PN are responsible, respectively, for granule aggregation and mechanical stabilization [6].

As expected, R1 had a higher total EPS content, which agrees with Rusanowska et al. [35], who reported that smaller granules have a higher amount of EPS. Besides, the longer aeration phase duration also influences the EPS content, confirming that EPS production is stimulated by the stress caused by the aeration condition [6]. However, this high EPS production in R1 did not result in a better sedimentation capacity of the granules.

The reported EPS results for R2 and R3 were lower than R1 and similar to each other, indicating a balance between EPS production and consumption. As is known, EPS production occurs mainly during the feast period, and its consumption occurs during the famine period. So, it was expected that in step-feeding, EPS production tended to balance, being lower than in the other reactors, since the operation produces successive periods of feast/famine distributed throughout the cycle.

In most studies, aerobic granules that are stable have a higher protein portion (PN) than polysaccharides (PS), being correlated to hydrophobicity. Therefore, because PN promotes AGS stability, PN/PS ratio is a way of characterizing its stability [6]. Thus, the granules in R1 also showed better results (PN/PS = 5.2) than those in R2 (PN/PS = 4.9) and R3 (PN/PS = 3.9).

Retention of solids has generated inconsistent results among the leachate studies. It seems that EPS production does not follow a trend as well. For instance, PN/PS ratio was 4.8 [9], while it did not exceed 0.6 in other studies [5,10]. EPS production is influenced by several factors such as aeration time, cycle time, shear stress, reactor settings, type of inoculum, among others. Therefore, the set of configurations adopted in this study favored EPS production and the PN/PS ratio, possibly improving granules' stability and structure.

3.3. Performance of the reactors during the granulation process

The performance of the reactors was evaluated in terms of COD, nitrogen, and phosphorus (Table 3). In all reactors, the COD removal was high, but nitrogen and phosphorus removals had different behaviors and were better with the sedimentation time reduction.

The fast anaerobic feeding (R1) showed total and soluble COD removals statistically different and lower than in R2 with slow anaerobic feeding ($p < 0.001$) and the one achieved in R3 with step-feeding ($p < 0.001$). R3 showed a greater and significantly different total COD removal compared to R2 ($p < 0.001$). However, there were no statistical differences between R2 and R3 regarding soluble COD removals ($p =$

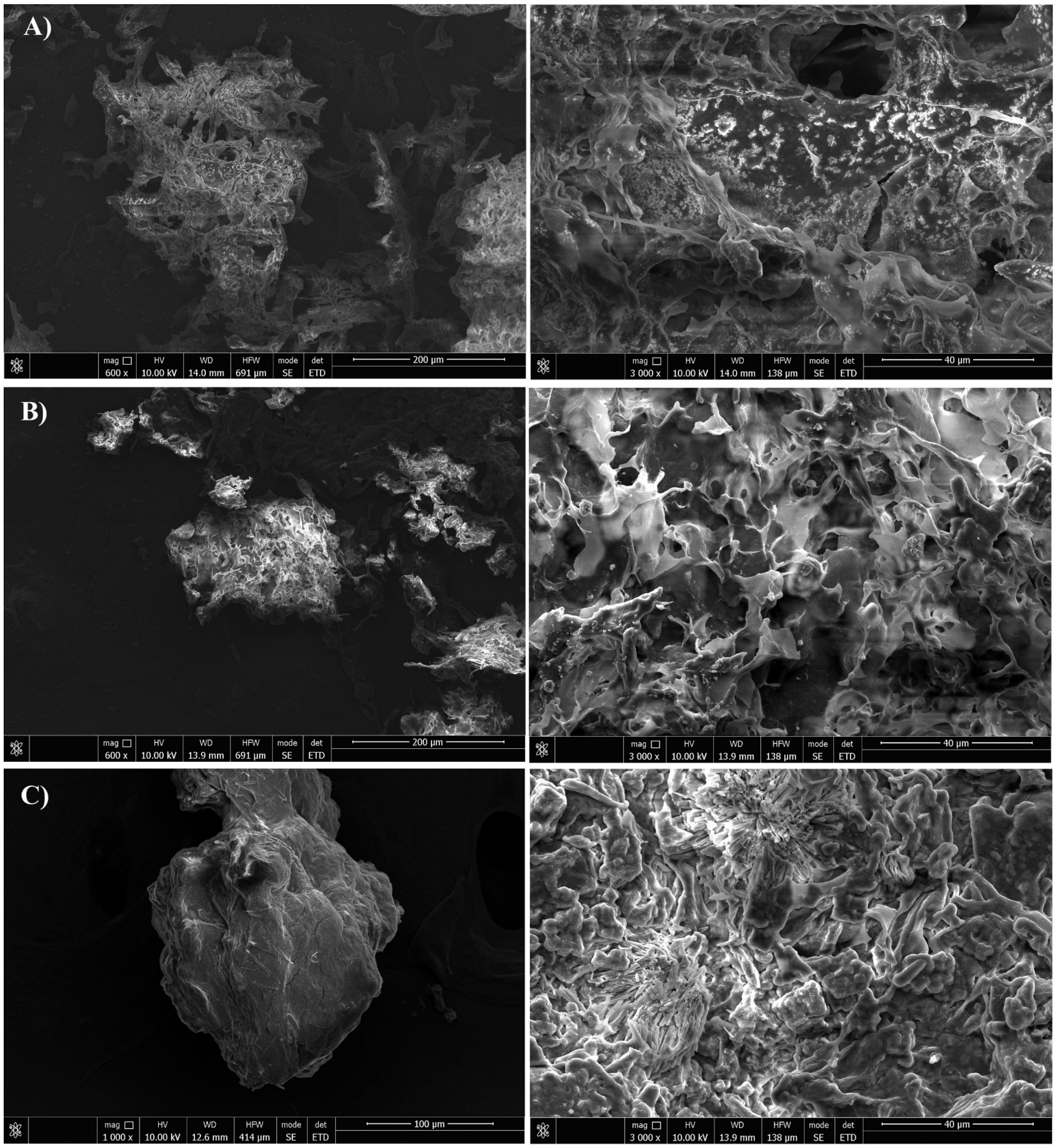

Fig. 4. Granule scanning electron micrograph of the reactors R1 (a), R2 (b) and R3 (c) at the end of period II.

0.604), which may once again emphasize that the constant washouts in R2 may have influenced the total effluent COD concentration.

Regarding total nitrogen (TN) removal, mean values above 50% were observed during the entire operation (except R1 in period I), and significant statistical differences between the three systems were found ($p < 0.001$). As the profile of nitrogenous fractions was different, it is worth mentioning that the removal mechanisms were also different. There was nitrite accumulation in R1 and R2, being significantly lower in R2 ($p = 0.004$). In R3, low nitrite and nitrate concentrations were observed, resulting in higher TN removals (92%). TN removals in R3 were superior to the values of 75.4% [9] and <50% [10] reported with real sanitary leachate.

During the two periods, the nitrification process was observed in both R1 and R2 systems, with values greater than 70%, and increased when the sedimentation time was reduced. However, in period II, it was possible to verify significant differences between R1 and R2 ($p < 0.001$) and between R2 and R3 ($p = 0.008$). Thus, the largest removal of ammonia occurred in R3 (99%), followed by R2 (98%) and R1 (97%).

As the MLVSS concentration decreased (R1 and R2) and remained unchanged (R3) after system stability, the demand for DO did not increase. Since the aeration flow rate was kept unchanged during the operation, nitrifying bacteria activity was not affected, favoring nitrification efficiency. In addition, in R2, during granules' disintegration and recurrent washouts, ammonia removal was reduced (although, on some days, ammonia removal was restored), possibly due to the loss of nitrifying bacteria that were present in the broken granules. When washouts occur at higher frequencies, the sludge age is reduced, and nitrification will be affected if the sludge age is too low. According to Rollemberg et al. [6], several studies have shown that the sludge age is an important parameter for granules' stabilization and reactors' performance since it is directly related to the maintenance of slow-growing bacteria.

It has been reported that the step-feeding mode is effective for making good use of the influent carbon source, increasing the denitrification rate and TN removal [15]. Thus, nitrification occurs with a lower organic load in the aerobic phase, accelerating the nitrification rate and saving DO consumption to oxidize the influent organic matter. This feeding mode benefits ammonia-oxidizing bacteria (AOB) growth and inhibits nitrate-oxidizing bacteria (NOB), accelerating nitrite accumulation [13,14]. However, in this study, no accumulation of nitrite was

Table 3
COD, nitrogen, and phosphorous removals in AGS systems with fast feeding (20 min, R1), slow feeding (40 min, R2), and step-feeding (R3). COD T: Total Chemical Oxygen Demand, COD S: Soluble Chemical Oxygen Demand.

Parameters	Period I			Period II		
	R1	R2	R3	R1	R2	R3
COD T_{inf} (mg/L)	1029 ± 44	1019 ± 35	1019 ± 34	1005 ± 23	1022 ± 38	1014 ± 29
COD T_{eff} (mg/L)	695 ± 57	239 ± 54	176 ± 37	180 ± 37	143 ± 31	91 ± 18
COD S_{inf} (mg/L)	976 ± 41	975 ± 33	979 ± 35	983 ± 32	988 ± 20	986 ± 26
COD S_{eff} (mg/L)	639 ± 58	152 ± 35	108 ± 40	160 ± 31	42 ± 15	34 ± 13
COD T removal (%)	32 ± 9	78 ± 5	84 ± 7	81 ± 9	86 ± 3	91 ± 1
COD S removal (%)	31 ± 9	85 ± 3	89 ± 5	81 ± 10	95 ± 5	97 ± 1
NH_4^+-N_{inf} (mg/L)	196 ± 4	194 ± 4	193 ± 5	198 ± 7	197 ± 3	198 ± 2
NH_4^+-N_{eff} (mg/L)	97 ± 11	54 ± 16	47 ± 15	15 ± 8	2 ± 1	1 ± 1
NO_2^--N_{eff} (mg/L)	76 ± 14	25 ± 9	27 ± 11	99 ± 23	30 ± 20	10 ± 8
NO_3^--N_{eff} (mg/L)	2 ± 1	4 ± 2	10 ± 4	4 ± 3	9 ± 7	4 ± 3
NH_4^+ removal (%)	70 ± 12	71 ± 10	74 ± 9	97 ± 1	98 ± 2	99 ± 1
TN removal (%)	21 ± 14	67 ± 29	56 ± 18	56 ± 9	87 ± 6	92 ± 5
PO_4^{3-}-P_{inf} (mg/L)	20 ± 1	21 ± 1	20 ± 1	20 ± 1	20 ± 1	20 ± 1
PO_4^{3-}-P_{eff} (mg/L)	19 ± 1	15 ± 5	10 ± 5	19 ± 1	15 ± 1	9 ± 2
TP removal (%)	4 ± 1	30 ± 5	54 ± 2	6 ± 2	22 ± 4	53 ± 3

observed. It is important to note that in the later works, in addition to the low influent loads, the reaction phase was not totally aerobic, interspersed with anaerobic/anoxic phase, which may have contributed to failures in the simultaneous nitrification and denitrification (SND). Besides, in R3, there was no solids loss, favoring AOB and NOB maintenance in the system. The larger granules of R3 may also have favored SND since this process occurs mainly in granules of larger size, in which nitrification occurs in the outer layer and denitrification in the innermost layer (anoxic). As is known, the proportion of denitrified nitrate in relation to the nitrate produced increases with the average granule diameter, i.e., with a greater anoxic layer [19].

Concerning total phosphorus (TP) removals, the three systems showed significantly different values ($p < 0.001$) and were practically unchanged by decreasing the sedimentation time. As expected, R1 had the lowest TP removals due to the rapid anaerobic feeding and the absence of anaerobic/anoxic phases during the cycle. R2 presented TP removals similar to traditional AGS cycles for low-load effluents and smaller than in R3. Probably, in R2, there may have been competition between phosphate accumulating organisms (PAOs) and denitrifying microorganisms, in which denitrifying ones may have been favored. Another probable cause is the presence of glycogen accumulating organisms (GAOs), which have a similar metabolism to PAOs but do not accumulate phosphorus. Besides, substrate complexity must be considered, which may not have favored phosphorus removals in both reactors.

The best TP removals were in R3 (53%), in which the three feast moments during the cycle favored the selection of PAOs. It is important to mention that from the 20th to the 35th, TP removals in R3 were greater than 80%; however, there was a reduction to stabilize then. Probably, the bacteria saturated, and the efficiency decreased, requiring sludge age control. Zhu et al. [36] demonstrated that aerobic granule deterioration occurred more easily in AGS systems with high SRT of granular sludge, and an adequate selective sludge discharge favors process stability. Bassin et al. [37] and Rollemberg et al. [6] also suggest controlled sludge removal (bed or bottom) to remove these saturated bacteria and improve phosphorus removal. Also, TP removals in R3 were slightly higher than those by Bueno et al. [3] when treating higher leachate concentrations. In both low and high leachate dilutions, Ren et al. [2] did not obtain phosphorus removal, being sometimes even reported “negative” values.

Therefore, it was found that the anaerobic feeding with a longer duration had the best TN and TP removals (Table 4). For COD removals, feeding duration does not seem to interfere with efficiency. However, the influent load increase negatively impacts COD removal. However, the step-feeding investigated in this work showed a higher COD removal efficiency than all previous studies.

Except for [10], who used influent concentrations of phosphorus much lower than the current investigation, TP removal through step-feeding was also the highest observed. Regarding TN removal, step-feeding showed efficiency greater than 90%, also being better than the values reported elsewhere, likely because it provides carbon for denitrification to occur throughout the cycle. Therefore, these COD, TN, and TP removal results clearly demonstrated that the step-feeding mode in

Table 4
Comparisons between the AGS systems in this study with related works.

Reference	Influent (mg/L)				Reactor			Removal (%)		
	COD	NH_4^+-N	C:N	TP	Type	Feed	Cycle (h)	COD	TN	TP
R1 (fast feeding)	1000	200	5	20	O/A	20	12	81	56	6
R2 (slow feeding)	1000	200	5	20	O/A	40	12	95	87	22
R3 (step-feeding)	1000	200	5	20	O/A	40	12	97	92	53
Wei et al. [9]	4298–5547	72–374	18	–	O/A	60	12	84.4	75.4	–
Wei et al. [9]	4502–5992	602–1168	5	–	O/A	60	12	82.8	35–58.1	–
Bella and Torregrossa [8]	9738	1960	3	–	O	5	24	40–50	Low	–
Bella and Torregrossa [8]	4560	945	2	–	O	5	12	50–60	Low	–
Ren et al. [10]*	448–654	120–500	2	32.5	A2O	30	8	66–73	39	34–54
Ren et al. [5]*	448–654	120–500	2	32.5	A2O	30	8	67–87	44–48	49
Ren et al. [10]	1080	340	–	2–6	O/A	90	8	65	40	80
Ren et al. [10]	1194	580	–	4–6	O/A	90	8	43	25	40
Ren et al. [10]	1539	900	–	5–6	O/A	90	8	20	<10	40
Ren et al. [2]	550–1000	130–785	–	3–6	A2O	30	8	43–65	24–37	0
Ren et al. [2]	1000–1100	785–1085	–	3–6	A2O	30	8	31–40	23–24	0
Ren et al. [2]	1100–1200	1085–1209	–	3–6	A2O	30	8	7–31	21–23	0
Bueno et al. [3]	650	88	6	13.1	O/A	60	8	87	99	36
Bueno et al. [3]	863	136	5	15.2	O/A	60	8	89	99	42
Bueno et al. [3]	1421	281	5	17.5	O/A	60	8	88	98	45

O (oxic), O/A (oxic, anoxic), A2/O (anaerobic, anoxic, oxic).
* Studies with synthetic leachates.

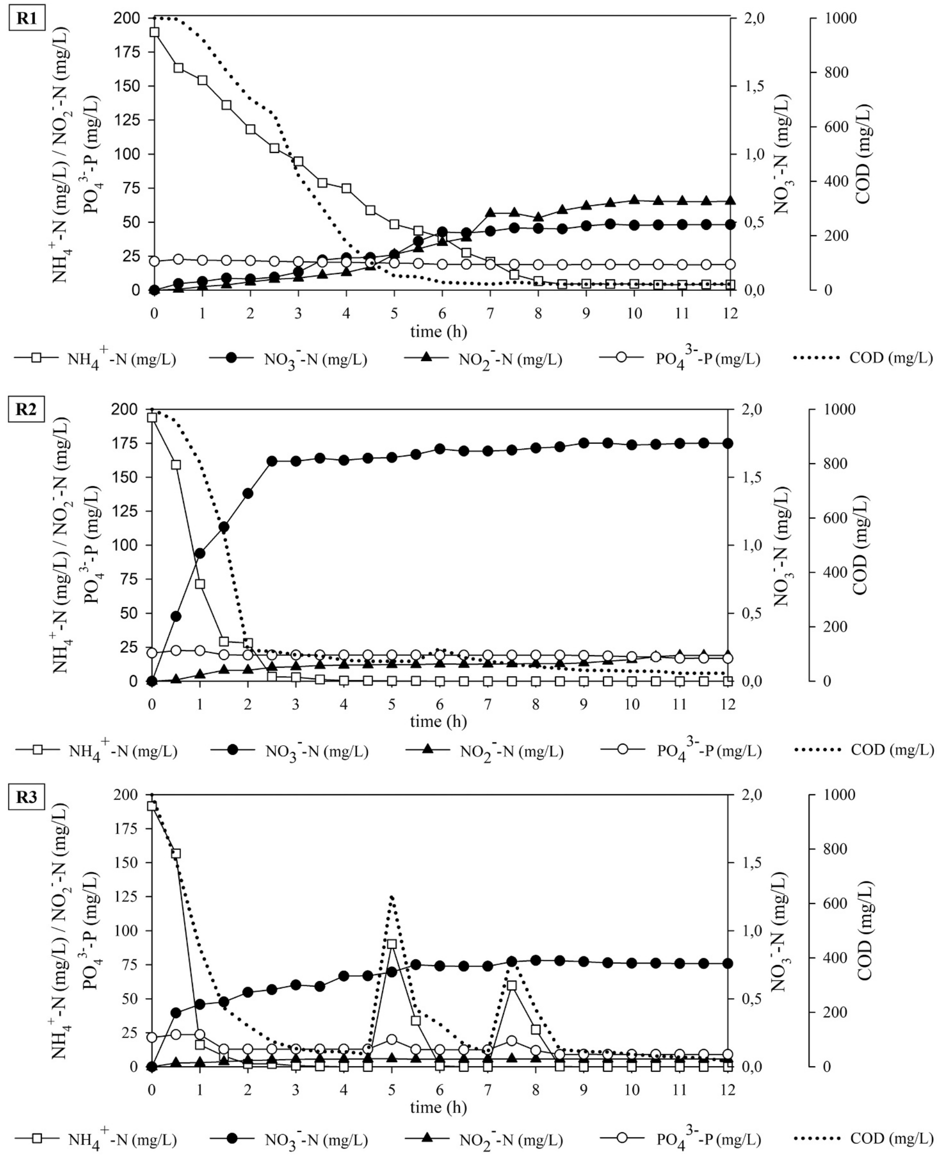

Fig. 5. Performance profile of AGS systems with fast feeding (20 min, R1), slow feeding (40 min, R2), and step-feeding (R3) distributed over a cycle.

AGS-SBR can be applied in high-load wastewater and/or with a C:N ratio similar to that of leachate, favoring granules' stability and treatment performance.

3.4. Cycle experiments

The simultaneous conversions and removals of organic, nitrogenous, and phosphorus constituents were investigated over a complete cycle (Fig. 5).

From oxygen analysis during the cycle, it was observed that the DO was between 2 and 4 mg/L during the first 4 h of aeration of R1 and, during the first 2 h of aeration of R2 and R3, being in all cases greater than 5 mg/L at the end of the aeration period. These times coincided with the famine period, i.e., when the available COD is in very low concentrations. Therefore, the famine period coincided with the DO increase since the microorganisms enter in the endogenous phase and require lower oxygen concentrations for their metabolism.

In R1, as soon as COD is practically consumed, nitrite begins to accumulate significantly, and the nitrate concentration increases slightly, simultaneously with ammonia oxidation. Although R2 had the same profile, nitrite accumulation was much lower. The low denitrification in R1 and R2 during the oxic period may be associated with the rapid carbon source consumption rate and the absence of an anoxic condition. Polyhydroxyalkanoates (PHA) accumulation during a short period of COD depletion in the aerobic phase may not be sufficient for the subsequent denitrification. In addition, the carbon source in the feeding was not fully utilized by denitrification due to microorganisms' growth and maintenance, which also led to incomplete denitrification and, therefore, decreasing TN removal.

In R3, ammonia was completely oxidized without significant nitrite and nitrate accumulations. Therefore, SND during the oxic period was the main mechanism of removing the nitrogen fractions. When complete nitrification occurred, there was still enough time in the oxic phase for the remaining nitrite to be converted to nitrate by NOBs since, in this reactor configuration, free ammonia did not cause toxicity to NOBs.

According to Wang et al. [14], NOB was much more sensitive to FA than AOB. It is important to mention that heterotrophic denitrification can also occur using EPS as an electron donor during the starvation period. It is possible that at the end of the oxic phase, the extracellular content initially produced was used as an electron donor to remove nitrogenous fractions endogenously. In addition, from the data obtained, it is possible to point out that a fraction of the partial nitrification product was denitrified, and the remaining fraction underwent complete nitrification to be subsequently denitrified. Such results were similar to those of Chen et al. [15], in which a step-feeding strategy created exclusive and ideal conditions for denitrification right after the total ammonia oxidation without relying solely on the anoxic zone within the granules.

During the cycle, a low pH variation was also observed in R1 (7.0–7.1), and in R2 and R3 (7.1–7.9), probably due to the balance in alkalinity consumption and production during nitrification and denitrification, respectively (SND).

This profile is also in line with other investigations that have observed that step-feeding positively influences the distribution of the main functional groups of microorganisms, and the microbiota responsible for the denitrification process may change positively [13,14,32]. Thus, microorganisms that remove phosphorus can also use the nitrogen products from nitrification as electron acceptors, which favors the high TP removal in R3 [38].

Therefore, it is possible to verify that the removal of nitrogenous constituents may have occurred from different processes. The accumulated nitrogen fraction was immediately converted using the influent organic matter as an electron donor during the feeding period, performing exogenous denitrification. In the oxic periods, the SND process prevailed for ammonia conversion. During the step-feeding (R3), other nitrogenous fractions were removed endogenously, using the intracellular organic constituents as electron donors.

Therefore, the cycle that showed the best performance was the one used in R3, since, without affecting granules' sedimentation and stability, the three feast periods interspersed with famine periods were sufficient for nitrification, facilitating denitrification due to the availability of COD in the feast periods distributed throughout the cycle and obtaining high phosphorus removals. This is excellent for treating more complex wastewater because the nitrification and denitrification processes generally depend on the amount of influent/available organic matter.

4. Conclusions

Step-feeding (R3) minimized three great problems of leachate treatment in AGS systems: granules' stability, biomass retention, and nitrite accumulation. Compared to fast (R1) and slow (R2) feeding, step-feeding achieved the best TP (53%) and TN (92%) removals. It also kept low carbon concentrations during the oxic period, which accelerated the ammonia conversion process, favored denitrification, and reduced the oxygen demand to remove organic matter. This is notable because the operation mode can reduce extra carbon addition for denitrification, expanding its practical application, especially for wastewater with high recalcitrant loads, such as sanitary landfill leachate.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

[1] R.B. Brennan, E. Clifford, C. Devroedt, et al., Treatment of landfill leachate in municipal wastewater treatment plants and impacts on effluent ammonium concentrations, J. Environ. Manag. 188 (2017) 64–72, https://doi.org/10.1016/j.jenvman.2016.11.055.

[2] Y. Ren, F. Ferraz, A.J. Kang, et al., Treatment of old landfill leachate with high ammonium content using aerobic granular sludge, J. Biol. Eng. 11 (1) (2017) 1–8, https://doi.org/10.1186/s13036-017-0085-0.

[3] R.F. Bueno, J.K. Faria, D.P. Uliana, et al., Simultaneous removal of organic matter and nitrogen compounds from landfill leachate by aerobic granular sludge, Environ. Technol. (U.K.) (2020) 1–15, https://doi.org/10.1080/09593330.2020.1740798.

[4] C. Pastore, E. Barca, G. Moro, C. Iaconi, M. Loos, H.P. Singer, G. Mascolo, Comparison of different types of landfill leachate treatments by employment of nontarget screening to identify residual refractory organics and principal component analysis, Sci. Total Environ. 635 (2018) 984–994, https://doi.org/10.1016/j.scitotenv.2018.04.135.

[5] Y. Ren, F. Ferraz, M. Lashkarizadeh, et al., Comparing young landfill leachate treatment efficiency and process stability using aerobic granular sludge and suspended growth activated sludge, J. Water Process Eng. 17 (February) (2017) 161–167, https://doi.org/10.1016/j.jwpe.2017.04.006.

[6] S.L.D. Rollemberg, A.R.M. Barros, P.I.M. Firmino, et al., Aerobic granular sludge: cultivation parameters and removal mechanisms, Bioresour. Technol. 270 (2018) 678–688, https://doi.org/10.1016/j.biortech.2018.08.130.

[7] M. Sarvajith, G.K.K. Reddy, Y.V. Nancharaiah, Aerobic granular sludge for high-strength ammonium wastewater treatment: effect of COD/N ratios, long-term stability and nitrogen removal pathways, Bioresour. Technol. 306 (January) (2020), 123150, https://doi.org/10.1016/j.biortech.2020.123150.

[8] G.D. Bella, M. Torregrossa, Aerobic granular sludge for leachate treatment, Chem. Eng. Trans. 38 (2014) 493–498, https://doi.org/10.3303/CET1438083.

[9] D. Wei, L. Shi, T. Yan, et al., Aerobic granules formation and simultaneous nitrogen and phosphorus removal treating high strength ammonia wastewater in sequencing batch reactor, Bioresour. Technol. 171 (1) (2012) 211–216, https://doi.org/10.1016/j.biortech.2014.08.001.

[10] Y. Ren, F.M. Ferraz, Q. Yuan, Landfill leachate treatment using aerobic granular sludge, J. Environ. Eng. (United States) 143 (9) (2017) 1–7, https://doi.org/10.1061/(ASCE)EE.1943-7870.0001236.

[11] M.K. De Kreuk, J.J. Heijnen, M. Van Loosdrecht, CM simultaneous COD, nitrogen, and phosphate removal by aerobic granular sludge, Biotechnol. Bioeng. 90 (6) (2005) 761–769, https://doi.org/10.1002/bit.20470.

[12] R.A. Hamza, Z. Sheng, O.T. Iorhemen, et al., Impact of food-to-microorganisms ratio on the stability of aerobic granular sludge treating high-strength organic wastewater, Water Res. 147 (2018) 287–298, https://doi.org/10.1016/j.watres.2018.09.061.

[13] F. Chen, Y. Liu, J. Tay, P. Ning, Alternating anoxic/oxic condition combined with step-feeding mode for nitrogen removal in granular sequencing batch reactors (GSBRs), Sep. Purif. Technol. 105 (2013) 63–68, https://doi.org/10.1016/j.seppur.2012.12.015.

[14] X. Wang, L. Jiang, Y. Shi, M. Gao, S. Yang, S. Wang, Effects of step-feed on granulation processes and nitrogen removal performances of partial nitrifying granules, Bioresour. Technol. 123 (2012) 375–381, https://doi.org/10.1016/j.biortech.2012.07.080.

[15] F. Chen, Y. Liu, J. Tay, P. Ning, Operational strategies for nitrogen removal in granular sequencing batch reactor, J. Hazard. Mater. 189 (1–2) (2011) 342–348, https://doi.org/10.1016/j.jhazmat.2011.02.041.

[16] C. Zhong, Y. Wang, Y. Wang, J. Lv, Y. Li, J. Zhu, High-rate nitrogen removal and its behavior of granular sequence batch reactor under step-feed operational strategy, Bioresour. Technol. 134 (2013) 101–106, https://doi.org/10.1016/j.biortech.2013.01.155.

[17] S.M. Iskander, R. Zhao, A. Pathak, A. Gupta, A. Pruden, J.T. Novak, Z. He, A review of landfill leachate induced ultraviolet quenching substances: sources, characteristics, and treatment, Water Res. 145 (2018) 297–311, https://doi.org/10.1016/j.watres.2018.08.035.

[18] J. Wiszniowski, D. Robert, J. Surmacz-Gorska, K. Miksch, J.V. Weber, Landfill leachate treatment methods: a review, Environ. Chem. Lett. 4 (1) (2006) 51–61, https://doi.org/10.1007/s10311-005-0016-z.

[19] S.L.S. Rollemberg, L.Q. De Oliveira, A.R. Barros, V.M.M. Melo, P.I.M. Firmino, A.B. dos Santos, Effects of carbon source on the formation, stability, bioactivity and biodiversity of the aerobic granule sludge, Bioresour. Technol. 278 (2019) 195–204, https://doi.org/10.1016/j.biortech.2019.01.071.

[20] B. Long, C. Yang, W. Pu, J. Yang, G. Jiang, J. Dan, C. Li, F. Liu, Rapid cultivation of aerobic granular sludge in a pilot scale sequencing batch reactor, Bioresour. Technol. 166 (2014) 57–63, https://doi.org/10.1016/j.biortech.2014.05.039.

[21] M.K. De Kreuk, N. Kishida, M.C.M. Van Loosdrecht, Aerobic granular sludge — state of the art, Water Sci. Technol. 55 (8–9) (2007) 75–81, https://doi.org/10.2166/wst.2007.244.

[22] J. Li, B. Wu, Q. Li, Y. Zou, Z. Cheng, X. Sun, B. XI, Ex situ simultaneous nitrification-denitrification and in situ denitrification process for the treatment of landfill leachates, Waste Manag. 88 (2019) 301–308, https://doi.org/10.1016/j.wasman.2019.03.057.

[23] F. Motteran, E.L. Pereira, C.M.M. Campos, The behaviour of an anaerobic baffled reactor (ABR) as the first stage in the biological treatment of hog farming effluents, Braz. J. Chem. Eng. 30 (2) (2014) 299–310, https://doi.org/10.1590/S0104-66322013000200008.

[24] C. Leal, A. Val Del Río, D.P. Mesquita, et al., Sludge volume index and suspended solids estimation of mature aerobic granular sludge by quantitative image analysis and chemometric tools, Sep. Purif. Technol. 234 (September 2019) (2020), 116049, https://doi.org/10.1016/j.seppur.2019.116049.

[25] B. Long, C. Yang, W. Pu, et al., Tolerance to organic loading rate by aerobic granular sludge in a cyclic aerobic granular reactor, Bioresour. Technol. 182 (2015) 314–322, https://doi.org/10.1016/j.biortech.2015.02.029.

[26] C. Zhang, H. Zhang, F. Yang, Diameter control and stability maintenance of aerobic granular sludge in an A/O/A SBR, Sep. Purif. Technol. 149 (2015) 362–369, https://doi.org/10.1016/j.seppur.2015.06.010.

[27] J. Carrera, T. Vicent, J. Lafuente, Effect of influent COD/N ratio on biological nitrogen removal (BNR) from high-strength ammonium industrial wastewater, Process Biochem. 39 (12) (2004) 2035–2041, https://doi.org/10.1016/j.procbio.2003.10.005.

[28] J. Luo, T. Hao, L. Wei, H.R. Mackey, Z. Lin, G.H. Chen, Impact of influent COD/N ratio on disintegration of aerobic granular sludge, Water Res. 62 (2014) 127–135, https://doi.org/10.1016/j.watres.2014.05.037.
[29] S.S. Adav, D.J. Lee, Potential cause of aerobic granular sludge breakdown at high organic loading rates, Appl. Microbiol. Biotechnol. 85 (5) (2010) 1601–1610, https://doi.org/10.1007/s00253-009-2317-9.
[30] Y. Liu, Q.S. Liu, Causes and control of filamentous growth in aerobic granular sludge sequencing batch reactors, Biotechnol. Adv. 24 (1) (2006) 115–127, https://doi.org/10.1016/j.biotechadv.2005.08.001.
[31] N. Derlon, J. Wagner, R.H.R. Costa, E. Morgenroth, Formation of aerobic granules for the treatment of real and low-strength municipal wastewater using a sequencing batch reactor operated at constant volume, Water Res. 105 (2016) 341–350, https://doi.org/10.1016/j.watres.2016.09.007.
[32] S.F. Corsino, R. Campo, G.D. Bella, M. Torregrossa, G. Viviani, Study of aerobic granular sludge stability in a continuous-flow membrane bioreactor, Bioresour. Technol. 200 (2016) 1055–1059, https://doi.org/10.1016/j.biortech.2015.10.065.
[33] S.F. Corsino, D. Di Trapani, M. Torregrossa, et al., Aerobic granular sludge treating high strength citrus wastewater: analysis of pH and organic loading rate effect on kinetics, performance and stability, J. Environ. Manag. 214 (2018) 23–35, https://doi.org/10.1016/j.jenvman.2018.02.087.
[34] X. Liu, G. Sheng, H. Luo, F. Zhang, S. Yuan, J. Xu, R.J. Zeng, J. Wu, H. Yu, Contribution of extracellular polymeric substances (EPS) to the sludge aggregation, Environ. Sci. Technol. 44 (11) (2010) 4355–4360, https://doi.org/10.1021/es9016766.
[35] P. Rusanowska, A. Cydzik-Kwiatkowska, P. Świątczak, I. Wojnowska-Baryła, Changes in extracellular polymeric substances (EPS) content and composition in aerobic granule size-fractions during reactor cycles at different organic loads, Bioresour. Technol. 272 (2019) 188–193, https://doi.org/10.1016/j.biortech.2018.10.022.
[36] L. Zhu, Y. Yu, X. Dai, X. Xu, H. Qi, Optimization of selective sludge discharge mode for enhancing the stability of aerobic granular sludge process, Chem. Eng. J. 213 (2013) 442–446, https://doi.org/10.1016/j.cej.2012.11.132.
[37] J.P. Bassin, M.K.H. Winkler, R. Kleerebezem, M. Dezotti, M.C.M. Van Loosdrecht, Improved phosphate removal by selective sludge discharge in aerobic granular sludge reactors, Biotechnol. Bioeng. 109 (8) (2012) 1919–1928, https://doi.org/10.1002/bit.24457.
[38] Q. He, L. Chen, S. Zhang, L. Wang, J. Liang, W. Xia, H. Wang, J. Zhou, Simultaneous nitrification, denitrification and phosphorus removal in aerobic granular sequencing batch reactors with high aeration intensity: impact of aeration time, Bioresour. Technol. 263 (2018) 214–222, https://doi.org/10.1016/j.biortech.2018.05.007.
[39] K.Y. Show, D.J. Lee, J.H. Tay, Aerobic granulation: Advances and Challenges, Appl. Biochem. Biotechnol. 167 (6) (2012) 1622–1640, https://doi.org/10.1007/s12010-012-9609-8.
[40] H. Lin, R. Ma, Y. Hu, J. Lin, S. Sun, J. Jiang, Q. Lian, J. Luo, Reviewing bottlenecks in aerobic granular sludge technology: Slow granulation and low granular stability, Environ. Pollut. 263 (2021) 1–11, https://doi.org/10.1016/j.envpol.2020.114638.
[41] APHA, Standard Methods for the Examination of Water and Wastewater, 22 ed., American Public Health Association, American Water Works Association, Water Environment Federation, Washington, DC, 2012.