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Pilot-scale aerobic granular sludge in the treatment of municipal wastewater: Optimizations in the start-up, methodology of sludge discharge, and evaluation of resource recovery

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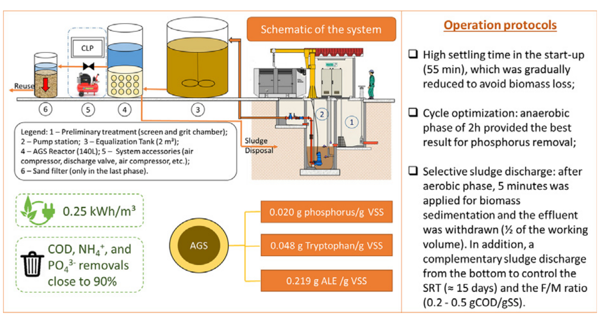
GRAPHICAL ABSTRACT



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ABSTRACT

This work evaluated the formation, maintenance, performance, and microbiology of a pilot-scale aerobic granular sludge reactor treating low-strength municipal wastewater under tropical climate conditions. Additionally, different resource recovery possibilities (phosphorous, tryptophan, and alginate-like exopolysaccharides) were investigated from the produced sludge. Granulation occurred after 35 days without external carbon source supplementation (COD_{inf} ≈ 461 mg/L; COD/DBO_5 ≈ 3.2). Some protocols were implemented: (i) fat separation to decrease granule flotation; (ii) high exchange rates (60%) during rainy periods to increase the organic load; (iii) selective sludge discharge methodology. After granules formation, optimizations were done to improve reactor performance (COD, BOD, NH_4^+, and PO_4^{3-} removals close to 90%), and energy demand reduced from 0.43 (start-up) to 0.25 kWh/m^3 (after optimizations). The produced sludge had a high concentration of phosphorus (0.020 g P/g VSS), tryptophan (0.048 g tryptophan/g VSS), and alginate-like exopolysaccharides (0.219 g ALE/g VSS), indicating a good resource recovery possibility.

1. Introduction

Aerobic granular sludge (AGS) is considered one of the most promising biological wastewater treatment technology of the 21st century (de Kreuk and van Loosdrecht, 2004) due to the high capacity of pollutants removal, good settling ability of the developed biomass, strong and compact microbial structure, resource recovery possibilities etc. (Rollemberg et al., 2018). In lab-scale tests, especially when the

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granules are cultivated with synthetic wastewater, it is common to report removals of COD, TN, and TP above 90% (Nancharaiah et al., 2018).

The AGS technology is patented as Nereda®, and currently, there are more than 20 wastewater treatment plants (WWTP) in operation worldwide (Nereda, 2020). However, some recurring problems are reported (mainly when domestic sewage is used), which cause adverse implications for granulation, and the efficiencies are usually lower than those found in lab-scale experiments (Pronk et al., 2015; Derlon et al., 2016; Wagner et al., 2015; Zhang et al., 2011). The main problems are: (i) granule disintegration in long-term operation; (ii) low removal of nitrogen (low/absence of denitrification or high nitrite accumulation); (iii) long start-up period (approximately six months) to achieve good pollutant removal and system stability; (iv) small granules with irregular structure; and (v) flotation of the granules (washout) due to suspended solids and the presence of fat in the influent (Pronk et al., 2015; Liu et al., 2010; Wagner et al., 2015; Cetin et al., 2018).

To solve these issues, preliminary studies in pilot-scale systems are essential for the development of the AGS technology. Significant differences in wastewater characteristics and climate conditions should be considered in the design and process operation (van Haandel and van der Lubbe, 2012). According to Pishgar et al. (2019), a pilot study is a crucial testing/optimization step to scale up AGS reactors. A typical case is the EPE WWTP in the Netherlands, where initially, the system operation was studied in a pilot-scale, which helped the full-scale design (Giesen et al., 2013).

Over the last few years, some strategies to decrease the formation time of granules and improve their stability in long-term operation have been proposed: (i) addition of external carbon sources to accelerate the granulation process and form more stable granules during treatment of low-strength wastewaters (Coma et al., 2012); (ii) use of calcium to speed up granulation (Li et al., 2009); (iii) AGS cultivation using synthetic wastewater and use as inoculum in full-scale WWTP after achieving the maturation period (Pijuan et al., 2011; Li et al., 2014); and (iv) mix of low-strength domestic sewage with industrial wastewaters (Giesen et al., 2013; Liu et al., 2010).

Although these strategies have provided improvements in the formation and stability of the granules, some of them are not feasible for large-scale WWTP. Thus, there is need for studies of new strategies for the formation and maintenance of aerobic granules, especially in pilot-scale with low-strength wastewaters, for which just a few studies are reported in the literature (Cetin et al., 2018; Derlon et al., 2016; Giesen et al., 2013; Liu et al., 2010; Ni et al., 2009; Wagner et al., 2015).

In this context, this work evaluated the formation, maintenance, performance, and microbiology of a pilot-scale aerobic granular sludge (AGS) reactor treating low-strength municipal wastewater under tropical climate conditions. Different resource recovery possibilities (phosphorous, tryptophan, and alginate-like exopolysaccharides – ALE) were also investigated from the produced sludge.

2. Material and methods

The experiment was divided into four stages: (I) reactor start-up with granules formation (0–90 day); (II) cycle optimization (90–120 day); (III) selective sludge discharge (120–150 day); and (IV) wastewater reuse (effluent polishing using a sand filter) and resource recovery from sludge (150–210 day).

During stage I, the reactor started with 55 min of settling time, which was reduced to 45, 30 and 15 min, respectively, resulting in minimum settling velocities of 1.1, 2.2, 2.4 and 3.6 m/h, respectively. The settling time reduction was used for the selection of biomass with fast settleability (granules retention, removing filamentous microorganisms). It occurred in a controlled way, considering the solids concentration stability in the effluent. Therefore, the settling time and settling velocity used on the remaining stages II, III, and IV were 15 min and 3.6 m/h, respectively.

At stage II, the cycle configuration and aeration rates were optimized to improve system performance and energy demand. For this, lab-scale tests were performed to evaluate the best duration of the cycle phases (oxic, anaerobic, and anoxic). In parallel, the best airflow in the aerobic period was also evaluated.

During stage III, a new methodology for selective sludge discharge was implemented, also using some recommendations of literature (Wu et al., 2018; Zhu et al., 2013). For this, the sludge retention time (SRT) and F/M (food/microorganisms) ratio were used as control parameters for sludge discharge. In the last phase of the experiment (stage IV), a sand filter for effluent polishing was implemented, aiming solids reduction in the effluent for reuse. The characteristics of the produced sludge were also studied to evaluate the possibility of resource recovery.

2.1. Set-up

The pilot-scale reactor was installed in a Wastewater Treatment Plant (WWTP) located under a tropical climate condition in Fortaleza, Ceará, Brazil, which receives more than 50% of the sewage collected in the city, representing about 3 m^3/s. The WWTP is operated by the local sanitation company (Cagece) and consists of a preliminary treatment followed by ocean disposal. Because the sewage system receives a contribution of rainwater and infiltration, the sewage has a low organic concentration (average COD < 400 mg/L).

The experiments were carried out in a column-type sequencing batch reactor (SBR) with a working volume of 140 L, internal diameter (D) of 0.3 m and height of 2.0 m (H). The air was injected (in the aerobic period) into the bottom of the reactor by an air compressor through a fine bubble porous diffuser. The cycle duration was 6 h during stage I, which consisted of filling (1 min), anaerobic reaction (60 min), aerobic reaction (240/250/255/280 min), settling (55/45/30/15 min), decanting (1 min), and idle (3 min).

Synchronized timers automated the SBR operation. In all phases, the volumetric exchange ratio was from 40% (most of the time) to 60% (during the rainy season), and the hydraulic retention time (HRT) was 12 h. The temperature was not controlled during the experiment, and the wastewater temperature inside the reactor was 31 ± 2 °C.

During the period of reactor start-up and formation of the granules (stage I), the dissolved oxygen (DO) concentration was kept high, with values greater than 4 mg/L. However, from stage II onwards, DO limits were implemented (between 2 and 3 mg/L) in order to optimize the energetic costs with aeration and improve the mechanism of simultaneous nitrification and denitrification (SND).

2.2. Seed sludge and wastewater

The reactor was inoculated with an activated sludge biomass from a full-scale reactor, resulting in an initial concentration of mixed liquor suspended solids (MLSS) of ∼ 2.5 g/L. The sludge volume index at 30 min (SVI_{30}) during the start-up was 185 mL/g. The reactor was fed at the bottom during the anaerobic phase. The main characteristics of the municipal wastewater used are shown in Table 1.

2.3. Workflow process

After coarse screening and grit removal, the wastewater was pumped to a mixing equalization tank, to ensure fresh sewage to be pumped into the AGS reactor. At stage III, when the sludge discharge was started, the biomass produced was recirculated to the pump station. The tertiary treatment (sand polishing filter for reuse of the treated sewage) was implemented just at stage IV.

The average sludge loading rate (SLR) ranged from 0.2 to 1.0 kg COD/kg TSS·d. This parameter was calculated by dividing the influent organic load (kg COD/d) by the total biomass present in the AGS reactor (kg TSS).

Table 1
Characteristics of municipal wastewater (influent).

Parameter	Min (mg/L)	Max (mg/L)	Mean (mg/L)	Load (kg/day)
COD_{total}	35	662	461	0.18
$COD_{soluble}$	19	457	172	0.05
BOD_5	5	210	148	0.04
COD_{total}/BOD_5 ratio	1.3	11	3.2	–
Suspended solids	97.4	228.3	172.5	0.05
Volatile suspended solids	64.5	131.7	119.2	0.03
Total fat	1.3	31	11.5	0.003
NH_4^+-N	22.5	57.1	36.9	0.01
NO_2^--N	0	0.7	< 0.1	< 0.001
NO_3^--N	0	1.1	< 0.1	< 0.001
Total nitrogen	23.4	70.4	43.0	0.012
PO_4^{3-}-P (dissolved)	0.05	10.2	4.8	0.001
Total phosphorus	0.1	11.6	5.1	0.001

2.4. Analytical methods

Influent and effluent samples were collected three times a week for the determination of COD, biochemical oxygen demand (BOD), total Kjeldahl nitrogen (TKN), sulfide (S^{-2}), ammonium (NH_4^+-N), nitrite (NO_2^--N), nitrate (NO_3^--N), phosphate (PO_4^{3-}-P), and total phosphorus (TP). Mixed liquor samples during the aerobic period of the SBR were collected once a week for analyzing mixed liquor suspended solids (MLSS), mixed liquor volatile suspended solids (MLVSS), and sludge volumetric index (SVI).

COD, BOD, sulfide, pH, TKN, NH_4^+-N, NO_3^--N, NO_2^--N, TP, solids, and SVI at 10 and 30 min (SVI_{10} and SVI_{30}) were determined according to APHA (2012), whereas DO was measured by a YSI 5000 m. Total inorganic nitrogen (TIN) was regarded as the sum of NH_4^+-N, NO_3^--N, and NO_2^--N (Long et al., 2014). The extracellular polymeric substances (EPS) were extracted (weekly) by a modified heat extraction method proposed by Yang et al. (2014). The protein (PN) content was determined by a modified Lowry method, and the polysaccharides (PS) content was analyzed using a phenol–sulfuric acid method (Long et al., 2014). The EPS was regarded as the sum of PN and PS.

The physical resistance (shear test) analysis of the granules followed the methodology described by Nor-Anuar et al. (2012). Therefore, a shear force caused by a rotation of approximately 200 rpm for 10 min was applied to the granules. The defragmented fraction identified was expressed in terms of stability coefficient (S). This coefficient was classified into three categories: very resistant (S < 5%), resistant (5% ≤ S ≤ 20%), and non-resistant (S > 20%). Therefore, the lower the values of S (%), the higher the stability of the aerobic granules. The granulometric distribution of the biomass was performed using the screening method proposed by Bin et al. (2011).

A modified method was used to isolate alginate-like exopolysaccharides from the aerobic granular sludge (Lin et al., 2008). Regarding tryptophan determination, a high-performance liquid chromatography (HPLC) method was used (Rigol L 3000, Beijing, China) (Wang et al., 2014; Peltre et al., 2017).

2.5. DNA extraction, 16S rRNA gene amplicon sequencing and data processing

Biomass samples at different reactor stages were collected for microbiological analysis as following: sludge in the reactor start-up (stage I, 55 min settling time), granulated sludge in the maturation phase (stage I, 15 min settling n time), granular sludge after system optimization (end of stage III). They were collected at the end of the aeration period, and the PowerSoil® DNA isolation kit (MoBio Laboratories Inc., USA) was used according to the manufacturer's instructions to extract DNA from the sludge. The other procedures for 16S rRNA gene amplicon sequencing and data processing are described elsewhere (Rollemberg et al., 2019).

2.6. Statistical methods

The non-parametric Mann-Whitney test was used to compare the performance of the reactors at a confidence level of 95%, in which the data groups were statistically different when p ≤ 0.05.

3. Results and discussion

3.1. Start-up and formation of the granules – Stage I

After the system inoculation (using biomass from an activated sludge system), the reactor presented a MLSS of 2.5 g/L and SVI_{30} of 185 mL/g on the first day. A high biomass washout was observed in the early days due to the selection pressure imposed on the AGS system. In parallel, there was also an increase in MLVSS/MLSS ratio, reduction of SVI, and an increase of SVI_5/SVI_{30} ratio. As it is known, granulation starts when the SVI_5 is closer to the SVI_{30} (Corsino et al., 2016). Usually, mature granules show SVI_{30} between 30 and 80 mL/g (Derlon et al., 2016). In this context, Fig. 1a shows the evolution of solids concentration (MLSS and MLVSS) and SVI_{30}. It was observed that, after granules formation, the system had SVI_{30} values about 60 mL/g. After this period, the system presented excellent stability, with little oscillation, indicating maturation of the granules.

Literature reports that biomass is considered an aerobic granule when its diameter is greater than 0.2 mm and the system is considered complete granular when more than 80% of the biomass is in the form of aerobic granules with an SVI_{30}/SVI_5 ratio greater than 90% (Liu et al., 2010). In this context, based on the results present in Fig. 1b, the system was already considered granular after 35 days even for a low-strength wastewater, showing that the applied operational conditions and the tropical climate conditions favored the fast granulation time. The short granulation time reported in the current investigation shows the evolution of AGS operation during the treatment of municipal wastewater, as the first studies observed that more than 300 days were required to the granules become dominant in the reactor (Ni et al., 2009). Li et al. (2014), while using the AGS technology for municipal wastewater treatment, obtained a granulation in about 50 days after start-up. Cetin et al. (2018) reported a granulation time of about 30 days, but the municipal wastewater was much more concentrated (average COD of 1100 mg/L) than the used in the present investigation (COD of 409 ± 81 mg/L).

In this work, the initial settling time was 55 min, which is considered relatively high compared to 20 min used frequently in lab-scale studies (He et al., 2018). This choice was based on the fact that some studies have shown that the application of strong selection pressure by applying short sedimentation time was inadequate when treating wastewater containing particulate organic matter (Wagner et al., 2015).

The presence of particulate organic matter favors the growth of filamentous bacteria, which are continuously washout from the reactor under short settling times, thus affecting granule formation, causing instability and, in some cases, total biomass washout (Wagner et al., 2015). An alternative strategy proposed in the current work to avoid biomass loss was to decrease the hydraulic selection pressure at the reactor start-up, starting with a long sedimentation time (55 min) with a gradual reduction to reach 15 min finally. In the study reported by Pronk et al. (2015) in a full-scale AGS system also treating municipal wastewater, the minimum settling time used was 30 min.

3.2. Reactor stability and performance in terms of water quality

During the initial 90 days of operation, a rainy period was observed, which decreased the influent COD (≈ 400 mg/L). Several studies have reported instability and even disintegration of granules when they were cultivated with diluted wastewater (Peyong et al., 2012). A strategy of

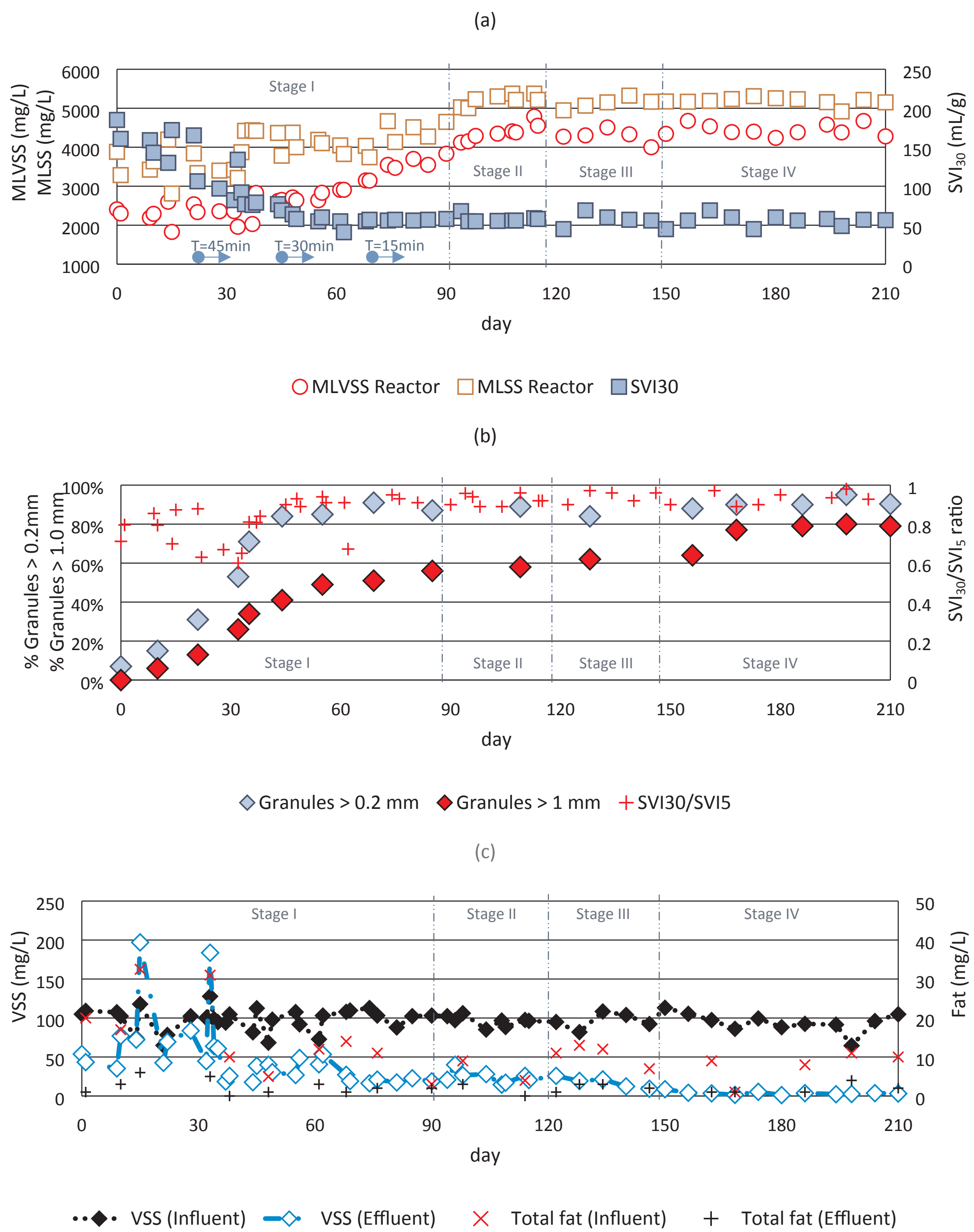

Fig. 1. Performance evaluation of the AGS reactor. (a) Solids concentration; (b) solids sedimentation; (c) solids in the influent and effluent.

increasing the volumetric exchange rate (VER) to 60% was used during the rainy season to overcome this problem, aiming to operate the reactor with the minimum organic loading rate (OLR) of 0.5 kg COD/m^3·d reported to obtain stable granules (Franca et al., 2017).

Other different strategies have been proposed in the literature to increase OLR when using low-strength wastewater in AGS cultivation. Sguanci et al. (2019) proposed the dosage of acetate to increase the OLR in municipal wastewater; however, it is not economically feasible for full-scale sewage treatment plants. The strategy to mix low-strength domestic sewage with industrial wastewater to increase the OLR (Giesen et al., 2013; Liu et al., 2010) is not feasible in most of the cases, such as in the present investigation. Another possibility was presented by Pronk et al. (2015) by applying cycles of 3 h in rainy seasons (diluted sewage) and cycles of 6.5 h in dry seasons (more concentrated sewage). While many activated sludge plants can operate relatively well under low organic loads, in AGS systems, it is essential to control a minimum OLR, since low OLR lead to a disintegration of the granules in a relatively short time of operation (2 months), causing system collapse (Zhang et al., 2011).

Although the instability due to low OLR was resolved, there were some situations (16th and 33rd days) where a loss of biomass was observed (Fig. 1c). During these periods of instability, there was a higher concentration of suspended solids in the influent, especially fat (greater than 30 g/L). Thus, the presence of oils and greases affected the aerobic granules, as they became fluffy and floatable, causing biomass washout. A fat retention barrier was adapted in the equalization tank (approximately on day 40), which allowed a higher biomass accumulation (MLVSS greater than 4 g/L) and AGS immobilization in the reactor (Fig. 1a).

Previous studies that evaluated the cultivation of AGS fed with

Table 2
Removal of COD, BOD, nitrogen, phosphorus, and solids.

Parameters	Stage			
	I	II	III	IV
COD_{inf} (mg/L)	409 ± 81	478 ± 59	464 ± 39	453 ± 47
COD_{eff} (mg/L)	52 ± 50	31 ± 28	19 ± 15	20 ± 13
COD removal (%)	85 ± 7	92 ± 5	94 ± 4	92 ± 4
BOD_{inf} (mg/L)	129 ± 17	153 ± 25	146 ± 19	144 ± 22
BOD_{eff} (mg/L)	14 ± 9	8 ± 6	4 ± 3	4 ± 2
BOD removal (%)	89 ± 3	97 ± 8	99 ± 8	99 ± 5
NH_4^+-N_{inf} (mg/L)	41 ± 6	49 ± 5	47 ± 10	46 ± 9
NH_4^+-N_{eff} (mg/L)	8 ± 7	6 ± 5	4 ± 2	4 ± 2
NO_2^--N_{eff} (mg/L)	14 ± 11	7 ± 4	5 ± 4	4 ± 3
NO_3^--N_{eff} (mg/L)	5 ± 3	3 ± 3	3 ± 3	2 ± 1
TN removal (%)	38 ± 15	71 ± 16	75 ± 13	77 ± 8
PO_4^{3-}-P_{inf} (mg/L)	4 ± 2	5 ± 3	5 ± 4	5 ± 4
PO_4^{3-}-P_{eff} (mg/L)	2 ± 1	1 ± 1	1 ± 5	1 ± 2
TP removal (%)	62 ± 27	79 ± 12	88 ± 7	91 ± 3
TSS_{inf} (mg/L)	119 ± 15	111 ± 4	113 ± 8	108 ± 4
TSS_{eff} (mg/L)	92 ± 44	41 ± 23	29 ± 5	11 ± 3
TSS removal (%)	24 ± 11	56 ± 9	72 ± 4	89 ± 4

municipal wastewater rich in particulate matter also observed that oils and greases could adhere to the granule surface and cause instability (de Kreuk et al., 2010).

Regarding the removal of organic matter and nutrients (Table 2), high removals of COD (≈ 85%), BOD (≈ 89%), and NH_4^+ (≈ 80%) were found during stage I. However, nitrite accumulation was observed, which compromised the total nitrogen (TN) removal (< 40%). Some AGS studies reported problems with nitrite accumulation (partial nitrification) (Coma et al., 2012), mainly in pilot-scale systems with low COD for denitrification (Derlon et al., 2016). In addition to the low TN removal, phosphorus removal below 65% was also verified, which is relatively low compared to literature (Nancharaiah et al., 2018). In this context, some optimizations in the system (already granulated) were implemented to ensure both granules' maintenance and high pollutants' removals.

3.3. Cycle optimization – Stage II

Although the reactor was fully granular at stage I, it was noted that some operational problems observed could be improved by cycle modification (90th − 120th day), such as: (i) long aerobic phase (greater than80% of the total cycle duration) and (ii) high aeration rate (Q_{air} = 1.8 L/s) causing a high energy consumption (0.43 kWh/m^3); (iii) nitrite accumulation (NO_2^--N ≈ 15 mg/L) in the final effluent; and (iv) relatively low phosphorus removal (≈ 60%).

3.3.1. Optimizing the aeration rate

As it is well known for AGS reactors, the aeration has a fundamental role not only in providing dissolved oxygen for metabolic processes (Tay et al., 2001), but also for promoting shear stress, controlling filaments growth on granule surface, which results in biomass stability over long periods of operation (Adav et al., 2008).

In addition, the shear forces promote the increase of extracellular polymeric substances – EPS, which acts as a "biological glue" to aggregate cells and then form granules (Nancharaiah et al., 2018). In the design of AGS reactors, the shear stress is represented by the upflow air velocity. Therefore, the higher the reactor H/D ratio, the lower the airflow demand, because the air bubbles will have a longer circular flow path, allowing microorganism aggregates to be constantly subjected to hydraulic friction and high turbulence, thus forming aerobic granules (Rollemberg et al., 2018).

Some authors proposed minimum values of upflow air velocity of 1.2 cm/s (Tay et al., 2001). However, these tests have been evaluated in lab-scale experiments, usually using synthetic effluents. In the present study, the air velocity at stage I was approximately 2.5 cm/s, which caused a fast saturation of dissolved oxygen. The optimum value of Q_{air} was 0.8 L/s, referring to an upflow air velocity of approximately 1.0 cm/s.

Considering the influent sewage characteristics (Table 1), the upflow air velocity used was lower than those presented in lab-scale studies with synthetic wastewater (Rollemberg et al., 2019; He et al., 2018). However, these values can be justified by the research conducted by Devlin et al. (2017). These authors observed that the upflow air velocity of 0.41 cm/s was enough for the formation and maintenance of granules when the COD was below 300 mg/L. In spite of that, the same velocity was not suitable for granulation when the COD was above 600 mg/L. Therefore, the characteristics of the wastewater directly impact the optimal system airflow, demanding optimization.

3.3.2. Optimization of cycle distribution

In addition to reducing aeration intensity and subsequent aeration demand, the cycle distribution was also optimized. The total cycle of 360 min consisted of only 60 min for the anaerobic period. The duration of the anaerobic period affects the metabolism of phosphorus-accumulating bacteria (PAOs) as they initially require fermentative bacteria to hydrolyze the organic material into organic acids (especially acetate and propionate) aiming the mechanisms of phosphorus release and subsequent sequestration in the aerobic period (Rollemberg et al., 2018).

In this sense, other anaerobic periods of 90, 120, and 150 min were tested, keeping the same total cycle (360 min), in which 120 min provided the best result for phosphorus removal. He et al. (2018) proposed that AGS reactors aiming at enhanced biological phosphorus removal (EBPR) should have anaerobic periods between 2 and 3 h, therefore in accordance with the present findings. For the designed Nereda® AGS, the anaerobic period accounts for about 25% of the total cycle, i.e., about 150 min considering the usual total cycle of 6 h (Van Haandel and Van der Lubbe, 2012).

A short anoxic phase (10 min) was also included at the end of the cycle, so the total cycle was changed to an anaerobic-oxic-anoxic (A/O/A) setting, distributed as follows: filling (1 min), anaerobic reaction (120 min), aerobic reaction (210 min), anoxic reaction (10 min), settling (15 min), decanting (1 min) and idle (3 min). A short duration of the anoxic period was used as suggested in literature because high anoxic periods can make the granules unstable and fluffy (Pishgar et al., 2019). During this period, the effluent was recirculated internally to prevent biomass sedimentation, similarly to what occurs in some Nereda® reactors (Pronk et al., 2015).

The results before and after the cycle optimizations are shown in Fig. 2. In the initial cycle (Fig. 2a), approximately 20% of the initial COD was removed in the anaerobic phase. With the cycle optimization (Fig. 2b), about 45% of the initial COD was removed in the anaerobic phase, possibly due to a greater COD accumulation in the form of polymers (polyhydroxybutyrate) (He et al., 2018). In terms of energy demand, there was a significant reduction from 0.43 (reactor start-up – stage I) to 0.25 kWh/m^3 (after optimizations – stage II). This value was still higher than those reported by Pronk et al. (2015) evaluating a full-scale WWTP. However, this difference can be attributed to the difference in scale, effluent characteristics, and degree of system modernization.

Cycle optimization provided considerable improvement in total nitrogen removal (Table 2). The concentrations of the oxidized nitrogen forms (NO_2^--N and NO_3^--N) decreased from 20 mg/L at stage I to 10 mg/L at stage II. Therefore, the inclusion of an anoxic period (Fig. 2b) helped to reduce the accumulated nitrite and nitrate. Considering that, at the end of the cycle, there was no COD available (famine period), endogenous denitrification likely occurred, and the carbon used as an electron donor could be the stored intracellular polymers (polyhydroxybutyrate) or the excreted EPS (He et al., 2018; Rollemberg et al., 2019). It is also possible to observe that the N

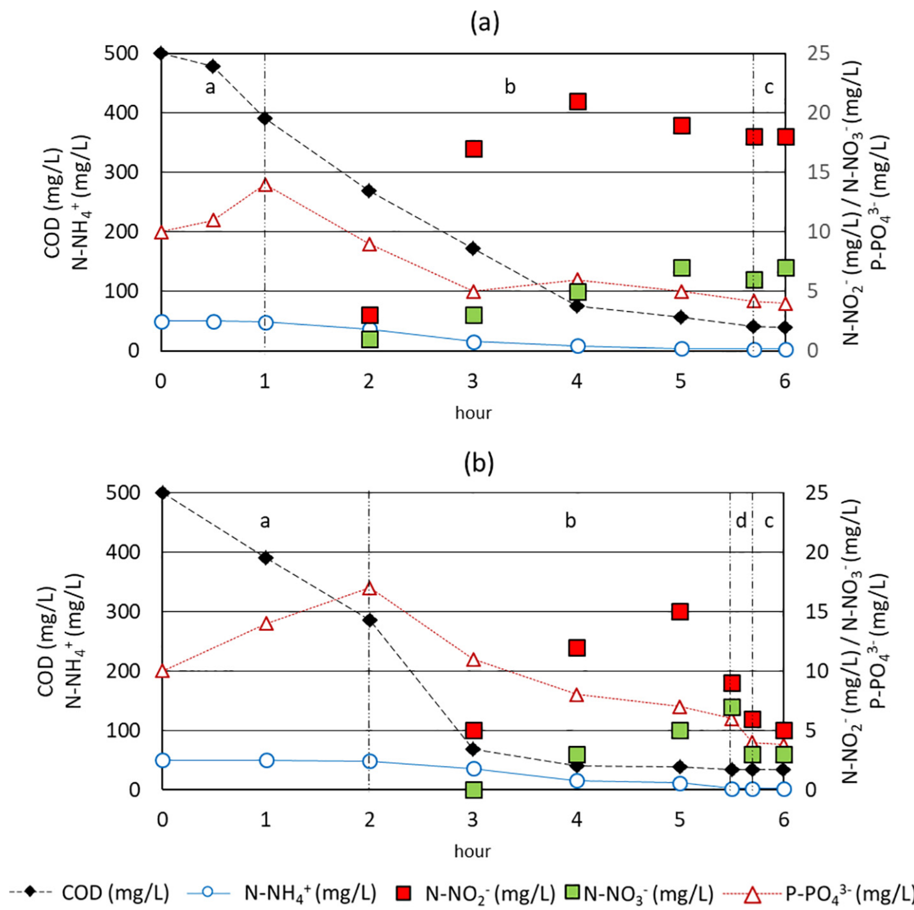

Fig. 2. Cycle analysis before (a) and after (b) optimization.

removal occurred mainly in the aerobic period (Fig. 2b), indicating the mechanism of simultaneous nitrification and denitrification (SND). Yu et al. (2014) found in an AGS system that the SND mechanism was responsible for approximately 80% of N removal, in which the remaining nitrogen was removed by assimilation or biosorption.

The phosphorus removal was also favored after cycle changes, increasing from 60% at stage I to approximately 80% at stage II. This improvement was attributed to both the anaerobic phase increase and the inclusion of a short anoxic phase after the aerobic phase, changing the operation for A/O/A cycles. The better performance of A/O/A cycles in AGS reactors in comparison with A/O cycles was also found in lab-scale experiments with synthetic wastewaters (Rollemberg et al., 2019). A/O/A cycles (i.e., adding an anoxic phase at the end of the cycle) can favor the growth of DPAOs (phosphorus-accumulating denitrifying organisms), which can simultaneously remove nitrogen and phosphorus (He et al., 2018). An important point is that DPAOs are reported to have low growth rates compared to conventional denitrifying organisms and can reduce sludge generation in about 30–40% (Zeng et al., 2004).

Regarding EPS production, the amount of these substances increased after optimizations (Table 3), possibly due to the shorter aerobic period. Literature reports that long aerobic periods may cause long famine periods, thus stimulating the consumption of EPS and reducing the amount of EPS at the end of the cycle (He et al., 2018).

Furthermore, it is observed that the optimizations provided higher solids retention (Table 3), likely due to the enrichment of a biomass with better settleability, i.e., polymer-accumulating bacteria, which are reported to have high settling velocity (Wang et al., 2018). An increase in the granule settling rate was also observed after modifications. Other parameters, such as granule percentage greater than 1 mm and the stability indicator, also improved. However, it is observed that, at all stages, the granules were characterized as non-resistant ($S > 20\%$) according to the classification proposed by Nor-Anuar et al. (2012). As in other studies, it is observed that this parameter was not conclusive regarding the stability of the granules (Barros et al., 2020). Even for values of $S > 20\%$, there was no rupture of the granules or instability in the reactor.

3.4. Selective sludge discharge implementation – Stage III

At stage II, the cycle optimizations were important for increasing nutrients removal (Table 2), improving granule characteristics (Table 3), and reducing the energy demand. At stage III, a controlled sludge discharge was applied, also focusing on optimization, aiming to control some parameters that affect the maintenance of granules over long periods of operation, such as solids retention time (SRT) and F/M ratio (food/microorganisms). Besides, sludge discharge may increase phosphorus removal and help in the control of effluent solids (van Dijk et al., 2018; Nancharaiah et al., 2018).

The process of sludge discharge in AGS systems is still not very well explored in literature, especially in pilot or full-scale systems, and many investigations did not reveal details concerning sludge age control or sludge discharge (Li et al., 2014; Pronk et al., 2015; van Dijk et al., 2018).

On the other hand, some sludge discharge methodologies have been suggested. Winkler et al. (2011) proposed to remove sludge from the middle of the reactor, where is located the sludge with the worst settling capacity. Other authors suggest that the removal must be a mix of the sludge from the bottom and from the top, thereby achieving a more stable system operation (Bassin et al., 2012). The removal of sludge

Table 3
Granules characteristics of the AGS system at all stages.

Sludge characteristics	Stage I	Stage II	Stage III	Stage IV
MLVSS (g/L)	3.7 ± 1.9	4.7 ± 0.8	4.2 ± 0.6	4.2 ± 0.5
SVI_{30}/SVI_5	~0.9	~0.92	~0.95	~0.95
TSS greater than 1 mm (%)	58.1 ± 22.3	64.3 ± 8.7	73.6 ± 7.1	73.4 ± 2.9
Mean diameter (mm)	0.9	1.2	1.3	1.3
Stability indicator (%)	56.2 ± 8.3	34.6 ± 9.2	34.7 ± 5.1	35.0 ± 2.3
SRT (days)	8 – 25	16 – 23	12 – 15	12 – 15
PS (mg/g VSS)	318.2 ± 15.9	144.0 ± 9.2	139.4 ± 7.0	136.0 ± 3.9
PN (mg/g VSS)	430.8 ± 13.5	496.6 ± 6.8	501.1 ± 5.8	513.7 ± 4.3
PN/PS	1.4	3.5	3.5	3.5
Settling velocity (m/h)	15.7 ± 5.1	21.3 ± 4.4	21.0 ± 5.3	21.2 ± 3.1
Color	Gray-yellow	Yellow-brown	Yellow-brown	Yellow-brown

from the mixed liquor during the aeration phase is also reported (Li and Li, 2009).

In this research, a new methodology for sludge discharge was done based on SRT control and the removal of solids with low settleability. For this, a "selective sludge discharge cycle" was created with the following procedure: once a week, after the aerobic phase, a short period of 5 min was applied for biomass settling, and the effluent was withdrawn through a register located at the height of ½ of the working volume. Therefore, all non-settleable solids present in the effluent above this register were removed from the reactor, which returned to the sewage pump station that fed the AGS reactor. Also, a complementary sludge discharge from the bottom part of the reactor was implemented to control the SRT (≈ 15 days) and the F/M ratio (between 0.2 and 0.5 gCOD/gSS). F/M ratio in this range is reported to be necessary to obtain stable aerobic granules (Tay et al., 2004; Wu et al., 2018).

The applied protocol for sludge discharge improved phosphorus removal (Table 3). While at stage II, the mean removal was 79% ± 12, at stage III, there was an average removal of 88% ± 7, and they were statistically different ($p = 0.04$). This result was expected because the sludge discharge reduces the SRT, besides removing PAO bacteria that may be saturated with phosphorus, thereby promoting a "renewal" of sludge (Bassin et al., 2012). Besides, selective sludge discharge provided a reduction of suspended solids in the effluent from about 40 mg/L at stage II to 30 mg/L at stage III, which were statistically different ($p = 0.04$).

On the other hand, total nitrogen removal was little affected by the sludge discharge methodology, and there was no statistical difference between stages II and III ($p = 0.09$). Improved phosphorus removal without compromising nitrification shows that the controlled SRT between 12 and 15 days was adequate for phosphorus removal (stage III). However, very low values of SRT (< 5 days) may inhibit nitrification, thus compromising the removal of total nitrogen (Metcalf and Eddy, 2003).

Regarding the characteristics of the granules, it was observed that the used protocol for sludge discharge did not significantly change the mean diameter and settling velocity of the biomass. Besides, no significant changes were observed in the stability indicator and EPS composition (Table 3). Therefore, new studies must be conducted at full-scale application aiming at the optimization of the operational procedure and a better understanding of the effects in long-term operation.

3.5. Wastewater reuse and resource recovery from the produced sludge – Stage IV

After all optimizations, although the effluent showed great quality for some parameters (COD < 20 mg/L, NH_4^+-N < 5 mg/L and PO_4^{3-}-P < 1 mg/L), the concentration of total/suspended solids was still considered high (≈ 30 mg/L).

Secondary clarifiers are reported as not needed in treatment plants with the AGS technology. However, many full-scale systems face the same problem of high concentration of TS or SS in the effluent (Liu et al., 2005). Suspended solids values of 20 mg/L have been reported in the WWTP of Garmerwolde (Pronk et al., 2015), Dinxperlo, and Epe (van der Roest et al., 2011). Literature report as possible reasons: flotation of granules by the presence of fat (Cetin et al., 2018), degasification due to denitrification (van Dijk et al., 2018), and the presence of filaments with low sedimentation velocity (Franca et al., 2017).

After the tertiary filtration implemented at stage IV, the final effluent improved in terms of quality (TS below 15 mg/L and turbidity below 3.5 NTU). Van Dijk et al. (2018) installed vertical baffles in front of the AGS effluent weirs and obtained a final effluent with very good quality, i.e., SS near 8 mg/L and turbidity close to 5.0 NTU. Nonetheless, there is a need for more studies on suspended solids control in AGS systems, not only in terms of standard discharge accomplishment but also when the reuse is considered.

Regarding the evaluation of resource recovery, approximately 0.020 g P/g VSS, 0.048 g tryptophan/g VSS and 0.219 g ALE/g VSS were obtained, which were lower than the concentrations reported in other studies (Liu et al., 2016; Zhang et al., 2018), although these granules were cultivated with synthetic effluent. To the best of the authors' knowledge, this is the first report that evaluates the possibility of resource recovery in pilot-scale systems treating low-strength municipal wastewater.

Finally, it was observed that during the whole stage IV, the AGS remained stable, and the MLVSS concentration was between 4 and 5 g/L, with SVI_{30} < 60 mg/L and SVI_{30}/SVI_5 very close to 1. The average granule diameter remained close to 1.3 mm, and over 70% of the reactor TSS had a diameter greater than 1.0 mm. Therefore, the system was able to operate for long periods with low oscillation in terms of quality and keeping a granular biomass.

3.6. Bacterial community analysis

Three samples of AGS were collected for molecular biology analysis (Fig. 3): sludge in the reactor start-up (SI, stage I, 55 min sedimentation time), granulated sludge in the maturation phase (SII, stage I, 15 min sedimentation time), and granular sludge after system optimization (SIII, end of stage III). A total of 36014–55644 sequence tags were retrieved from the aerobic granules that were assigned to 1213–1709 OTUs.

The most abundant classes found at the seed sludge (stage I) were Planctomycetacia, Anaerolineae, and Bacteroidia. These classes are typical components of activated sludge biomass (associated with heterotrophic bacteria) and are usually found in AGS studies, being associated with the oxidation of organic matter (Serviour et al., 2009; Li et al., 2014).

Regarding Alphaproteobacteria, the relative abundance was close to 25% at stage I, 15% at stage II, and 5% at stage III. Literature reports that this class has a huge number of bacteria that can secrete EPS (Ramos et al., 2015), which may justify the higher content of total EPS

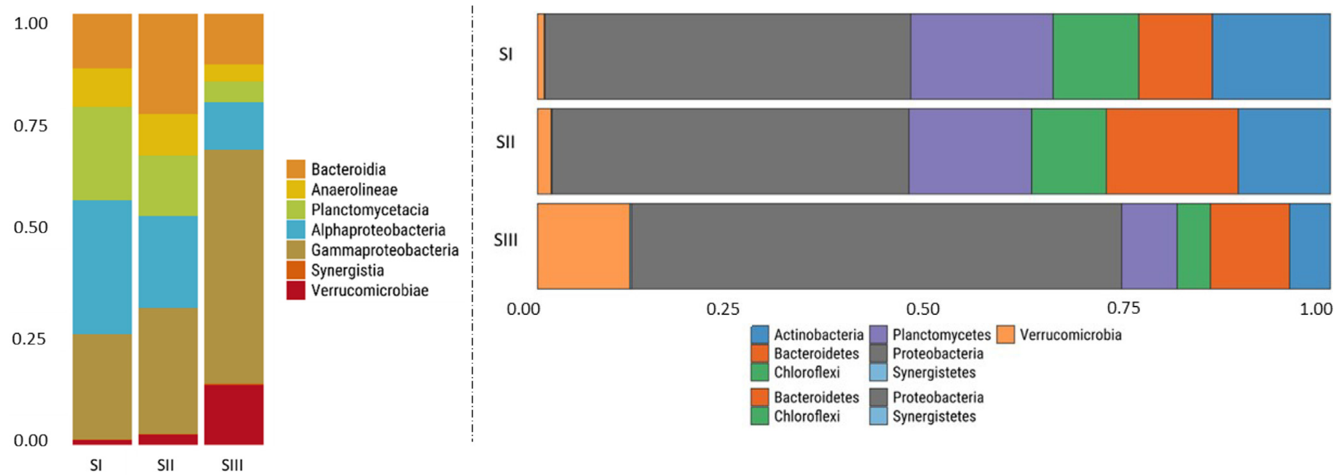

Fig. 3. Relative abundance in terms of class and phylum for the AGS samples collected: SI (sludge in the reactor start-up, stage I, 55 min sedimentation time), SII (granulated sludge in the maturation phase, stage I, 15 min sedimentation time), and SIII (granular sludge after system optimization, end of stage III).

in the granules at stage I (specifically PS). The EPS reduction at stage II may be related to the shorter aeration period. It is clear from the literature that aeration is directly related to shear stress, which in turn increases EPS production (Tay et al. 2004). Thus, possibly reduced aeration caused a decrease in total EPS (reducing the Alphaproteobacteria class), which may be beneficial for the granule as it is reported that the excess of EPS may cause clogging of the granule pores (Rollemberg et al., 2018). As the total cycle and phase time distribution at stages II and III were the same, the decreasing tendency observed in the relative abundance of this class must have continued.

Gammaproteobacteria class showed an increase in the relative abundance throughout the stages, being greater than 50% at stage III. Literature reports that the microbial groups present in this class are heterotrophic, usually related to denitrifying bacteria, PHA-producing bacteria, PAOs and GAOs bacteria (Rollemberg et al., 2019; He et al., 2018). The increase of Gammaproteobacteria class from stages I to II may be related to the favoring of DPAO and DGAO bacteria, because the reduction of aeration time and the creation of an anoxic phase favors these microbial groups (He et al., 2017). On the other hand, the relative abundance increase verified at stage III is possibly due to PAOs bacteria increase because the sludge discharge has shown to assist phosphorus removal, thus favoring this microbial group (Bassin et al., 2012).

Concerning phylum distribution, during the stages, there was a reduction of the Actinobacteria and Chloroflexi, similarly to that reported by Świątczak and Cydzik-Kwiatkowska (2018). Throughout the granulation process, the reduction of these phyla indicates biomass stability, as literature reports problems with bulking sludge and foaming in activated sludge plants due to their excessive growth.

The relative abundance of Verrucomicrobia phylum also increased throughout the stages, especially at stage III, possibly due to the sludge discharge, which can positively influence the microbial growth of some bacterial groups (Bassin et al., 2012; Zhu et al., 2013). The importance of this phylum is related to its crucial role in the degradation of organic compounds (aerobic oxidation and denitrification, i.e., ordinary heterotrophic organisms (OHOs), and denitrifying OHOs (DOHOs)) and floc-forming structure of activated sludge (McIlroy et al., 2017). Besides, this class is reported to be essential in the formation and maintenance of granules. Literature reports that strains in Verrucomicrobia had high correlations with the detected AHL (acyl-homoserine lactone), a substance that induces biomass formation. Because of this, the bacteria of this phylum have been reported as quorum-sensing (QS) bacteria (Panchavinin et al., 2019). In the study published by Yuan et al. (2017), granule disintegration was observed when the Verrucomicrobia phylum decreased from 5.21% (before disintegration) to 0.90% (after disintegration). Therefore, the presence of this phylum, and even the increase of the relative abundance observed, may be an indicator of granule stability because QS bacteria assist in granule maintenance (Ren et al., 2010).

The results obtained in the microbiological analysis showed that the increase of phylum Bacteroidetes, Proteobacteria, and Verrucomicrobia justify the improvement of the biomass settling and greater removal of nutrients over the stages (Świątczak and Cydzik-Kwiatkowska, 2018).

4. Conclusion

The use of variable exchange volumes from 40 to 60% (during the rainy season) proved to be an important strategy for AGS formation, especially in rainy periods where the COD is low, requiring a larger influent volume to maintain the minimum OLR for granule maintenance. The optimizations allowed COD, BOD, NH_4^+, and PO_4^{3-} removals close to 90%, and the energy demand reduced from 0.43 (start-up period) to 0.25 kWh/m^3 (after optimizations). The produced sludge had a high concentration of value-added products (0.020 g phosphorus/g VSS; 0.048 g tryptophan/g VSS and 0.219 g ALE/g VSS), indicating a good resource recovery possibility.

CRediT authorship contribution statement

Silvio Luiz Sousa Rollemberg: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Writing - original draft, Writing - review & editing. **Lorayne Queiroz Oliveira:** Investigation, Methodology. **Amanda Nascimento Barros:** Investigation, Methodology. **Paulo Igor Milen Firmino:** Conceptualization, Funding acquisition, Investigation, Methodology, Project administration, Supervision, Writing - original draft, Writing - review & editing. **André Bezerra Santos:** Conceptualization, Funding acquisition, Investigation, Methodology, Project administration, Supervision, Writing - original draft, Writing - review & editing.

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.

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