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Evaluation of sludge discharge methodologies in aerobic granular sludge reactors

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ABSTRACT

We performed a comparison of different aerobic granular sludge (AGS) discharge methodologies in sequencing batch reactors (SBRs), evaluating the system efficiency, physical and microbiological characteristics of granules, and resource recovery of the discharged biomass. In R1, there was a selective discharge (30% top sludge and 70% bottom sludge, based on biomass weight) with SRT control (10–20 days). The same selective discharge was applied to R2, but with control of food to microorganisms (F/M) ratio (0.22–0.44 gCOD/gVSS·day). In R3, the discharge was conventional (similar to activated sludge reactors), i.e., mixed liquor during the aerobic period. Additionally, the best protocol of the lab-scale studies was used in a pilot-scale reactor (R4) treating municipal wastewater. R1 protocol was the best strategy for AGS formation, maintenance, and performance, achieving better removal efficiencies of COD (95%), total nitrogen (85%), and phosphorus (80%). Sludge discharge improved the system's performance and resource recovery, especially alginate-like exopolysaccharides (ALE).

1. Introduction

Aerobic granular sludge (AGS) systems are considered a very promising technology of wastewater treatment based on biological processes (de Kreuk and van Loosdrecht, 2004). Currently, there are more than 70 Nereda® systems, commercial name of the AGS technology, in operation or under construction worldwide (Rollemberg et al., 2021). Compared to activated sludge, the main advantages are the capacity to remove organic matter and nutrients (N and P) in the same reactor without a secondary clarifier, lower sludge production, lower demand for area and energy, and others (Pronk et al., 2015).

In the last few decades, some authors studied (Liu et al., 2005; Rollemberg et al., 2018) the best conditions to form and maintain granules (e.g., dissolved oxygen, organic loading rate, concentration, settling time, volume exchange ratio etc.). Most of these experiments aimed to solve problems in AGS reactor, such as: (i) instability and disintegration of granules; (ii) low phosphorus removal (<30%); (iii) solids concentration in the effluent; and (iv) long start-up, in some cases, up to 6 months. Although there are considerable improvements, few questions need answers, for example, the impact of different sludge discharge protocols and their consequences for AGS efficiency and long-term stability (Pronk et al., 2015; Rollemberg et al., 2018).

This process is essential in a high-rate biological system (anaerobic or aerobic reactors) to control sludge retention time (SRT) and keep an effluent with low total suspended solids (TSS) concentration. Despite that, this concern has been little addressed in experiments, and works are developed without presenting a clear sludge discharge methodology/protocol. Most research on AGS did not show a controlled discharge of solids, and there is a subsequent loss in the treated effluent. In the specific case of Nereda® systems, as far as we are concerned, there is no information to date on the methods and protocols for sludge discharge in the operation of full-scale reactors.

AGS systems do not have a secondary clarifier, and the sludge discharge must be done in the sequencing batch reactor (SBR), taking care to remove larger granules at the bottom and smaller granules at the top. The absence of a secondary clarifier hinders the sludge age control process, unlike activated sludge reactors, where this control is usually maintained through sludge recirculation from the secondary clarifier to the aeration tank (Nancharaiah et al., 2018; Rollemberg et al., 2018).

Additionally, calculating SRT in aerobic granules is much more complex than in activated sludge. In this regard, Zhu et al. (2013) studied the selective sludge discharge approach in the AGS reactor to improve the process's long-term stability. They considered that the sludge age could be calculated by splitting the different biomasses inside

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the reactor: SRT_{total}, SRT_{flocs}, and $SRT_{granules}$.

The questions presented above justify why many studies do not investigate the sludge discharge in AGS systems in-depth. Winkler et al. (2011b) carried out one of the first studies on sludge discharge in AGS reactors. The authors evaluated a methodology called "non-selective sludge discharge based on SRT", in which the process takes place during the aeration phase, by withdrawing mixed liquor (sludge and effluent). This methodology caused a significant reduction in aerobic granules, low phosphorus removal (<35%), and high system instability.

Later, Bassin et al. (2012) presented another methodology called "selective sludge discharge based on SRT", in which the sludge discharge takes place after the settling period in the SBR. Most of the sludge was discharged from the top (80%, sludge blanket), while 20% was discharged from the sludge bed bottom, all in a biomass weight basis. The authors observed biomass segregation: in the sludge blanket, *Candidatus Competibacter phosphatis* (glycogen-accumulating organisms – GAOs) were dominant, while at the bottom (sludge bed), *Candidatus Accumulibacter phosphatis* (polyphosphate-accumulating organisms – PAOs) dominated. This method achieved 99% P removal, but aspects, such as chemical oxygen demand (COD), total nitrogen (TN), and TSS removals, as well as maintenance of granules and reactors' stability in long-term operation, were not evaluated.

In addition to these techniques, other methods have been proposed, however, without success. Wu et al. (2018) tested selective sludge discharge by controlling the F/M ratio. Unlike other studies, this work did not use SRT as a control method. Using a food to microorganisms (F/M) ratio of 0.44–0.55 gCOD/gVSS·day resulted in the formation of stable aerobic granules with good settleability. However, this methodology may be difficult to implement in wastewater treatment plants (WWTPs) due to variation in organic load.

Li and Li (2009) tested non-selective sludge discharge (from the mixed liquor) in a fixed proportion of 10% to 33% at different granulation stages, based on the reactor working volume. The authors noted the importance of starting the reactor with a higher sludge discharge percentage, reducing it after stabilization. Also, they concluded that granulation is difficult to occur when the sludge discharged is from the mixed liquor.

Accordingly, it is necessary to study different sludge discharge methodologies, evaluating their effects on system performance, microbiology, and the excess sludge characteristics, aiming at resource recovery. In this sense, this work presents a comparison of different sludge discharge methods (see methodology), evaluating the system efficiency, physical and microbiological characteristics of granules, and resource recovery (phosphorous, tryptophan, and alginate-like exopolysaccharides – ALE) of the discharged biomass. In the end, a sludge discharge protocol was applied to a pilot-scale reactor treating municipal wastewater. It is believed that this investigation can assist in developing a sludge discharge protocol for AGS in WWTPs, aiming to optimize the system in terms of efficiency, long-term operational stability, and resource recovery.

2. Material and methods

The AGS experiments started with lab-scale studies evaluating three sludge discharge protocols. The best protocol was then applied to a pilot-scale reactor treating municipal wastewater. The engineering and microbiological aspects and resource recovery possibilities were analyzed in all cases.

2.1. Seed sludge

The lab- and pilot-scale reactors were inoculated with a biomass from a full-scale activated sludge reactor, resulting in an initial concentration of mixed liquor suspended solids (MLVSS) of ~2.0 g/L. The sludge volume index at 30 min (SVI_{30}) during the start-up was around 180 mL/g.

2.2. Synthetic wastewater for lab-scale experiments

The synthetic wastewater used was composed of (per liter): 500 mg COD (acetate), 50 mg NH_4^+-N, and 10 mg PO_4^{3-}-P. In addition, 1 mL of trace elements solution was added to 50 L of influent, according to Rollemberg et al. (2021).

2.3. Municipal wastewater for pilot-scale experiments

Municipal wastewater was used for pilot-scale experiments. The reactor was installed in the largest WWTP of Fortaleza, Ceará, Brazil, and receives about 3 m^3/s. The treatment consists of a preliminary treatment followed by ocean disposal. 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. The main wastewater characteristics were: COD – 470 mg/L, biochemical oxygen demand (BOD_5) – 191 mg/L, TSS – 169.5 mg/L, NH_4^+-N – 37.9 mg/L, PO_4^{3-}-P (dissolved) – 4.9 mg/L (Table 2).

2.4. Reactors and cycles characteristics

For the lab-scale experiments, three SBRs in acrylic, with a diameter of 100 mm, a height of 1 m, a working volume of 7.2 L, and a height to diameter ratio (H/D) of 10, were used. All reactors had an exchange volume ratio of 50%, and the room temperature was about 28 ± 2 °C. The total cycle of the SBR reactors was 6 h, consisting of the anaerobic filling (65 min), aerobic reaction (270–285 min), settling (20–5 min), and decanting (5 min). The hydraulic retention time (HRT) was around 12 h. The system was operated in three stages, varying the settling time: 20 min (0–20th day), 10 min (20th–30th day), and 5 min (30th day–end of the experiment). Synchronized timers automated the SBR operation.

For the pilot-scale experiments, a reactor was built in acrylic, which had a working volume of 140 L, an internal diameter (D) of 0.3 m, and a height of 2.0 m (H). The air was injected (in the aerobic period) into the reactor bottom by an air compressor (Aco-002, Sunsun, China) through a fine bubble porous diffuser, and the dissolved oxygen (DO) was between 2 and 4 mg/L. The reactor had an exchange volume ratio of 50%, a total cycle of 6 h, resulting in an HRT of 12 h. A centrifugal pump (Centrifugal Pump Schneider, 1 cv) was used to feed the SBR, which provided an ascending velocity of 1 m/h. A synchronized timer (BM002, Gold, Brazil) automated the SBR operation. The cycle duration was 6 h, 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) in stage 1 (0–35 days), stage 2 (35th–60th day), stage 3 (60th–75th day), and stage 4 (75th–90th day).

2.5. Sludge discharge

Three sludge discharge protocols were tested, as follows:

Protocol 1 (R1) – Selective sludge discharge through SRT control (between 10 and 20 days) was applied after the settling period. Granular (bottom, sludge bed) and flocculent (top, sludge blanket) sludge discharges were implemented, constituting 30% and 70% of the total sludge discarded, respectively, on a biomass weight basis. The idea was to discharge both old P-rich/saturated granule present in the bottom and the filamentous/flocculent sludge present at the top;

Protocol 2 (R2) – Selective sludge discharge through F/M ratio control (between 0.22 and 0.44 gCOD/gVSS·day, in which a VSS/TSS ratio of 0.9 was used). Granular (bottom, sludge bed) and flocculent (top, sludge blanket) sludge discharges were implemented, constituting 30% and 70% of the total sludge discarded, respectively, on a biomass weight basis, as described in Protocol 1;

Protocol 3 (R3) – Mixed liquor sludge discharge (i.e., no segregation of the bottom granules and the filamentous/flocculent sludge present at the top) through SRT control (between 10 and 20 days). The sludge discharge was done at the end of the aeration period (mixed liquor), the excess sludge was removed from the bottom since it is a complete mixing-type system. This discharge method is similar to that performed in activated sludge systems with sludge age control from the aeration tanks and not from secondary clarifiers.

Table 1 compares the sludge discharge methods.

2.6. Analytical methods

Influent and effluent samples were collected three times a week for the determination of pH, total COD, ammonium (NH_4^+-N), nitrite (NO_2^--N), nitrate (NO_3^--N), and phosphate (PO_4^{3-}-P). Mixed liquor samples were collected once a week for the analysis of mixed liquor suspended solids (MLSS), mixed liquor volatile suspended solids (MLVSS), and sludge volumetric index (SVI).

COD, pH, NH_4^+-N, NO_3^--N, NO_2^--N, PO_4^{3-}-P, 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 (YSI Incorporated, EUA). Total inorganic nitrogen (TIN) was the sum of NH_4^+-N, NO_3^--N, and NO_2^--N (Long et al., 2014). Nitrite, nitrate, and phosphate were determined by Ion Chromatography (Dionex™ ICS-1100) with a guard column (Dionex™ IonPac™ AG23 - 2 × 50 mm), a column (Dionex™ Ion Pac™ AS23 - 2 × 250 mm), and a suppressor (Dionex™ AERS™ 500; 2 mm; ThermoScientific, EUA). The biomass's granulometry was performed using the screening method proposed by Bin et al. (2011) using sieves of 200 mm (ABNT#70), 600 mm (ABNT#30), and 1000 mm (ABNT#18) diameter, and the physical characteristics (color, size, and settling velocity) were evaluated according to Rollemberg et al. (2019).

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 the sum of PN and PS. A modified method was used to isolate the alginate-like exopolysaccharides (ALE) from the aerobic granular sludge (Lin et al., 2010). Regarding tryptophan determination, it was measured by high-performance liquid chromatography (HPLC CTO-20A, Shimadzu Corporation, Japan). The HPLC was equipped with a Hypersil BDSC-18 column (250 mm × 4.6 mm, 5 mm), UV 280 nm, and UV/VIS detector (Injection volume 20 μL, run of 6 min, isocratic elution). The mobile phase was a molar rate of methanol and water of 1:1, and the flow rate was 1 mL/min.

Table 1
Sludge discharge methods in R1, R2 and R3.

Parameters	Protocol 1 (R1)	Protocol 2 (R2)	Protocol 3 (R3)
Methodology	Selective sludge discharge through SRT control	Selective sludge discharge through F/M ratio control	Non-selective sludge discharge through SRT control
SRT	10–20 days	No control (between 7 and 13 days)	10–20 days
Discharge point	Discharge in 2 points: Granular (bottom, sludge bed) – 30% of the total sludge discarded (weight basis); flocculent (top, sludge blanket) – 70% of the total sludge discarded (weight basis)	Discharge in 2 points: Granular (bottom, sludge bed) – 30% of the total sludge discarded (weight basis); flocculent (top, sludge blanket) – 70% of the total sludge discarded (weight basis)	Mixed liquor sludge discharge (i.e., no segregation of the bottom granules and the filamentous/flocculent sludge present at the top)
Discharge frequency	Daily	Daily	Daily
Total volume of sludge discharged	Approximately 200 mL of MLSS at the top point (≈2.3 g/L TSS) and 20 mL (≈10 g/L TSS) at the bottom point	Approximately 240 mL of MLSS at the top point (≈1.9 g/L TSS) and 20 mL (≈9 g/L TSS) at the bottom point	Approximately 340 mL of MLSS (≈1.8 g/L TSS)
F/M ratio	No control (between 0.11 and 0.33 gCOD/gVSS·day)	0.22–0.44 gCOD/gVSS·day	No control (between 0.11 and 0.22 gCOD/gVSS·day)

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

Four samples of AGS for molecular biology analysis were collected at the end of the experiment (maturation phase) as follows: sludge in reactors R1 (S1), R2 (S2), R3 (S3), and the pilot-scale reactor R4 (S4). Sample collection was at the end of the aeration period, and PowerSoil® DNA isolation kit (MoBio Laboratories Inc., USA) was used according to the manufacturer's instructions to extract DNA from the sludge. Other 16S rRNA gene amplicon sequencing and data processing procedures are described elsewhere (Rollemberg et al., 2019).

2.8. Statistical methods

The reactor's performance at a confidence level of 95% is compared using the non-parametric Mann-Whitney test, in which the data groups were statistically different when $p \leq 0.05$.

3. Results and discussion

3.1. Effect of sludge discharge on stabilization and maintenance of aerobic granules

Controlling sludge discharge is a way to obtain faster granulation and favor the growth of microorganisms that can remove organic matter and nutrients (Bassin et al., 2012; Li & Li, 2009; Zhang et al., 2019; Zhu et al., 2013). The biomass used as inoculum had a flocculent structure, low settling velocity, and low EPS content. However, throughout the experiment, these characteristics changed, which depended on the discharge protocol applied.

Starting with MLVSS, Protocol 1 (R1, SRT of 10–20 days) showed to be the best alternative to increase biomass inside the reactor. After 20 days, biomass concentration increased on average 12% in R1, while in R2 only 4%, and in R3, a reduction of 11% was observed (Fig. 1). R3 had repeated washouts and took around 60 days to be stable, while R1 and R2 only took 17 days. Considering the total experimental period, the MLVSS increased from 2000 to 2578 mg/L in R1, kept constant in R2, and decreased to 1815 mg/L in R3 (Fig. 1). Zhang et al. (2019) showed that with SRT of 6 and 12 days, it was possible to increase MLSS from 3.5 gTSS/L to 4.0 gTSS/L and 6.0 gTSS/L, respectively, in 20 days. Also, controlling SRT, they maintained granules with large and compact structures. The strategy of keeping the same F/M ratio (R2) (0.30 ± 0.04 gCOD/gVSS·day) was the best one to keep MLVSS close to the initial concentration of 2 g/L. Similar results were found by Wu et al. (2018), who, maintaining an F/M around 0.4 gCOD/gTSS·day (≈0.44 gCOD/gVSS·day), obtained MLSS of 4 gTSS/L during all experiments.

Biomass settleability was evaluated in terms of SVI_{30}, SVI_5, and settling velocity. As it is known, the lower the SVI_{30}, the better the settleability of the granules. All protocols applied improved sludge settleability in terms of SVI_{30}. For instance, SVI_{30} values were 52.1, 59.6, 68.3, and 180.0 mL/g for reactors R1, R2, R3, and inoculum sludge, respectively (Table 2). The SVI_{30}/SVI_5 ratio was also evaluated, with values of 0.95, 0.85, and 0.80 being observed for R1, R2, and R3, respectively. Regarding settling velocity, values of 39.1, 30.4, and 19.7 m/h for reactors R1, R2, and R3, respectively, were found, which justifies the successive biomass washouts of this latter (Table 2). It is important to note that the method applied to R1 eliminated small and slow-settling sludge, which impacted sludge settleability.

The loss of solids in the effluent also varied according to the protocol

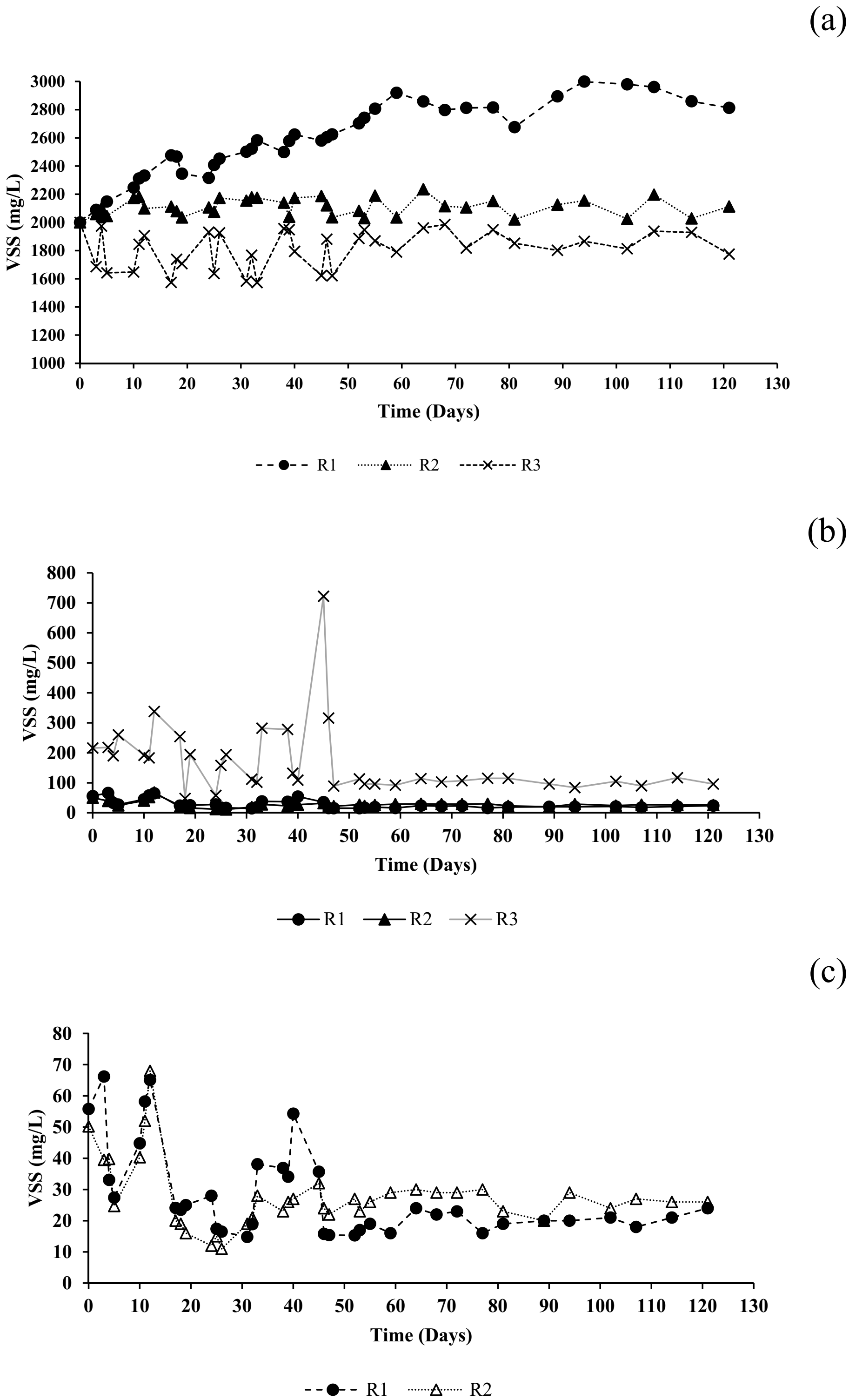

Fig. 1. VSS in the reactors R1, R2 and R3 mixed liquor (a) and effluent (b) during the operation period, and a more detailed comparison of the R1 and R2 effluent VSS concentration (c).

applied (Fig. 1). R1 and R2 had similar behaviors, providing lower VSS concentration during the start-up period (<50 mgVSS/L) and operation (<30 mgVSS/L) compared to R3 (average of 231 mgVSS/L during start-up and 167 mgVSS/L during operation). Therefore, Protocols 1 and 2 favored the maintenance of granules and filamentous microorganisms' control, which could influence the settling properties. The results agree with those obtained by Wu et al. (2018). They showed that aerobic granules cultivated with F/M around 0.4 gCOD/gTSS·day could develop stable biomass without granule disintegration or high loss of biomass in the effluent.

R3 had a higher instability period (until day 46, Fig. 1). After that, the average solids loss in the effluent was 102 mgTSS/L, which was almost 4-fold higher than those obtained in reactors R1 and R2. Therefore, the applied protocol was putting away important microorganisms to granules maintenance. Li and Li (2009) used unselective sludge discharge at a fixed ratio of 10% and obtained a TSS

Table 2
Granules' characteristics of the AGS system in all stages (data obtained at the end of the experiment).

Characteristics	R1	R2	R3	R4
VSS (mg/L)	2578	2110	1805	4200
SVI_{30} (mL/g)	52.1	59.6	68.3	63.7
SVI_{30}/SVI_5	0.95	0.85	0.80	0.90
Settling velocity (m/h)	39.1	30.4	19.7	21.0
Mean diameter (mm)	1.5	1.1	0.7	1.3
% > 0.2 mm	89.6 ± 18.3	87.5 ± 22.6	80.1 ± 36.9	84.7 ± 17.6
% > 1.0 mm	61.5 ± 24.5	69.2 ± 28.1	50.1 ± 34.1	73.6 ± 7.1

Table 3
Content of polysaccharides (PS), proteins (PN), alginate-like exopolysaccharides (ALE), tryptophan (TRY), and total phosphorus (TP) throughout the granulation process (data obtained at the end of the experiment).

Period	Biomass	PS (mg/gVSS)	PN (mg/gVSS)	ALE (mg/gVSS)	TRY (mg/gVSS)	TP (mg/gTSS)
Start-up	Inoculum	41 ± 6	27 ± 3	12 ± 1	7 ± 2	4 ± 1
Formation	R1	148 ± 38	152 ± 27	194 ± 38	39 ± 8	16 ± 7
	R2	151 ± 47	147 ± 24	191 ± 34	40 ± 11	17 ± 6
	R3	121 ± 29	129 ± 19	178 ± 39	33 ± 9	7 ± 3
	R4	145 ± 6	317 ± 12	226 ± 28	45 ± 5	17 ± 4
Maturation	R1	134 ± 15	153 ± 9	189 ± 13	42 ± 4	29 ± 2
	R2	134 ± 17	131 ± 5	187 ± 14	37 ± 7	17 ± 2
	R3	119 ± 4	137 ± 9	152 ± 16	41 ± 3	9 ± 3
	R4	139 ± 7	501 ± 6	219 ± 15	48 ± 7	18 ± 3

concentration of more than 150 mg/L in the effluent, therefore in line with results obtained in the present investigation.

Thereby, Protocol 1 was efficient to increase biomass inside the reactor, Protocol 2 had better application to keep constant the microorganism concentration, i.e., avoiding considerable loss of biomass, and Protocol 3 showed to be the least effective in keeping a high MLVSS concentration in the reactor and a low VSS concentration in the effluent.

3.2. Effect of sludge discharge on EPS production and granule characteristics

Literature reports that biomass is considered an aerobic granule when the diameter is greater than 0.2 mm. The system is completely granular when more than 80% of biomass is greater than 0.2 mm (Liu et al., 2010). All reactors had granules with a mean diameter bigger than 0.2 mm (1.5 mm in R1, 1.1 mm in R2, and 0.7 mm in R3). After system stabilization, reactors R1, R2, and R3 had 89.6%, 87.5%, 80,1% of the granules with a diameter higher than 0.2 mm, respectively (Table 2). Li and Li (2009), who compared the numerical model with the experimental test, obtained similar results. The reactor with selective discharge had bigger granules (5 mm) than that with unselective discharge (0.7 mm).

As known, EPS production acts as a glue, and it is important to granule formation and maintenance. EPS is commonly presented in studies as the sum of polysaccharides (PS) and proteins (PN) (Rollemberg et al., 2018). EPS production is directly related to the shear force, feast/famine period, and the presence of microbial groups responsible for excreting large amounts of this substance (Nancharaiah et al., 2018; Rollemberg et al., 2018). Considering that sludge discharge interferes in the F/M ratio (and therefore influences the feast/famine period) and the selection of microbial groups, it is believed that sludge discharge methodologies can influence the production of this substance vital in granulation (Nancharaiah et al., 2018). For this purpose, the EPS content in the sludge generated in the three systems was evaluated.

The results showed (Table 3) that at the reactors' start-up, there was a significant EPS content increase (3 times greater) of PS and PN in R1, R2, and R3 compared to the inoculum, showing that the aerobic granular biomass excretes much more EPS than activated sludge flocs, as already presented in other studies (He et al., 2017; Rollemberg et al., 2018). It is known that, during granule formation, there is a greater excretion of EPS to accelerate the process nucleation and formation of the first aerobic granules (Nancharaiah et al., 2018; Rollemberg et al., 2018).

After stabilizing the systems, similar PS values were observed in R1 and R2 (≈134 mgPS/gVSS), and there was no statistical difference between the reactors ($p = 0.1$). On the other hand, there was a significant difference in PS content in R3 (≈119 mgPS/gVSS). The lower PS production in R3 may be related to: (i) lower granule content in this system, since this reactor had the lowest percentage of granular biomass among the three systems; (ii) lesser presence of bacteria responsible for the excretion of EPS, as shown in the microbiological evaluation (Section 3.7).

In terms of PN, as it is known, an increase of PN is related to growth in hydrophobicity, and stable granules have more PN than PS (Zhu et al., 2013). Therefore, PN promotes the stability of aerobic granular sludge, and the PN/PS ratio is a way to characterize the stability of granular sludge (Kocaturk and Erguder, 2016). In this work, there was no significant difference in the PN content between the reactors ($p = 0.06$), showing that the discharge methodology did not significantly influence the PN. This was expected because the substrate used was the same and the operating conditions were identical (cycle, shear stress etc.), justifying why the formed granules have similar resistance (no disintegration was observed). PN fraction of EPS is usually correlated to the granule resistance. Moreover, it is important to mention that many studies have shown that the formation of unstable granules is one of the problems to be solved in AGS reactors, leading to disintegration and washout (due to granule breakdown) (Rollemberg et al., 2018; Wagner, 2015).

3.3. Reactor performance in terms of C, N, and P removal

At the reactors' start-up, similar results were observed in all systems concerning total COD removal, 79 ± 8%, 70 ± 5%, and 76 ± 5%, for R1, R2, and R3, respectively (Table 3). There was no statistical difference between the reactors ($p = 0.09$). These values are lower than those obtained before starting the discharge process (92%), likely due to the high VSS concentration in the effluent, especially in R3 (216 mg VSS/L), where biomass washout was even more apparent.

R1 was the system that achieved the fastest stability (25 days). After granulation and reactor stabilization, R1 reached efficiency above 98% and was maintained until the end of the study (Table 3). On the other hand, R2 and R3 achieved stability after 60 and 70 days of operation, respectively. The COD removals in R2 and R3 were similar, with values close to 92% and 90%, respectively, and no statistical difference ($p = 0.06$). The COD efficiency obtained in R1 was statistically different from R2 and R3 ($p = 0.04$).

Regarding NH_4^+-N removal, R1 (SRT of 10–20 days) had the best efficiency (97%), while R2 and R3 had 89% and 87%, respectively. Similar results were for TN removals, 85%, 74%, and 73% for R1, R2, and R3, respectively (Table 3). According to Li et al. (2019), maintaining SRT lower than 20 days is recommended for nitrogen removal. The oxygen gradient inside the granule creates a favorable environment for developing a variety of microorganisms, which have different metabolic functions, including nitrification and denitrification (Gao et al., 2011).

The larger the granule diameter, the greater the depth of these zones. So, the anoxic environment created facilitates the TN removal processes because denitrification is favored. High efficiencies are expected as the granules formed in reactors R1 and R2 are larger (Table 2). However, high removals of ammonium and TN were observed only in R1.

Apply sludge discharge to control solids retention time selects which organisms should remain in the reactor. Previous studies show that systems with high SRT have operational problems, including nutrients removal (Nancharaiah et al., 2018). This control, when properly applied, can remove old PAOs that are probably already saturated with P, allowing obtain organisms with lower phosphorus content and renewal microbiota. Additionally, maintaining a low SRT promotes a competitive advantage of PAOs against filamentous bacteria. Thus, possibly this aspect contributed to the R1 reactor showing better phosphorus removal efficiencies (79%).

Confirming this, Bassin et al. (2012) obtained phosphorus removal close to 100% with the SRT around 30 days by selective sludge discharge. The high removal was due to the discharge of a larger portion of sludge from a region rich in GAOs, reducing the PAOs-GAOs competition, and the discharge of a small amount of sludge rich in PAOs, which allowed obtaining biomass with less poly-P content inside the reactor. Additionally, Winkler et al. (2011a) proved that sludge from the top is dominated by GAO, while from the bottom has more PAO.

On the other hand, the discharge protocols applied in R2 and R3 barely favor the proper development of PAOs. Although in R3 there was discharge with sludge age control (which favors the removal of P and the presence of PAOs), because the discharge is not selective (as in R1 and R2), granules were being removed in the mixed liquor, which may have reduced the abundance of PAOs. Therefore, non-selective discharge can favor GAOs since they have a higher coefficient of cell production than PAOs and can grow associated with filaments, unlike PAOs that generally grow associated with granules.

3.4. Integrated analysis for selection of the best protocol for pilot-scale studies

As previously presented, the sludge discharge protocol influenced the granules' properties and removal efficiencies. Considering all these aspects, Protocol 1, selective sludge discharge with sludge age control, was considered the best approach to be used in the pilot-scale experiments. These results may be related to favoring microbial groups known to improve the granules' settleability, such as PAOs (Bassin et al., 2012). R1 indeed achieved the highest rates of phosphorus removal. Therefore, selective sludge discharge with sludge age control may be the key to accomplish at the same time the retention of PAOs, increase in the P removal, and improvement in the physical characteristics of the granules, including size, SVI, and settling velocity (Rollemberg et al., 2019).

Some studies have shown that the presence of phosphorus and other minerals precipitated at the reactor bottom may assist in the formation of granules (serving as a nucleus for the granule matrix) (He et al., 2018). On the other hand, the excess of these precipitated minerals can harm the system's stability (excess of inorganic fraction in the biological sludge). In addition to this information, the present investigation showed in an unprecedented way that the application of sludge discharge protocol in AGS reactors could influence both system performance and the formation of an ideal granule.

Regarding granules' stability, there was no breakdown or disintegration of the granules throughout the experiment in reactors R1 and R3. This observation shows what was presented by Zhu et al. (2013). According to the authors, when there is no sludge discharge, the high SRT in the biomass can favor granules deterioration and the consequent biomass washout, as described in several studies (Rollemberg et al., 2018; Wagner, 2015). On the other hand, the appropriate sludge discharge can favor the granule formation process and improve biomass stability, avoiding granule breakage, either by the appearance of filaments in the granules' outer layer that cause them to rupture, or by the excessive granules' growth (>5 mm), which makes it difficult for carbon to penetrate, causing cell lysis in the inner layer (Rollemberg et al., 2018; Wagner, 2015). In both cases, when this instability occurs, there is a considerable loss of biomass in the reactor, sometimes requiring a new start-up (Franca et al., 2015; Zhu et al., 2013).

3.5. Selective sludge discharge implementation in pilot-scale experiments

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).

Most studies on aerobic granules have been focused on laboratory reactors on a well-controlled scale with high or medium-strength synthetic effluents (Cetin et al., 2018). However, the operational parameters and the experiences acquired in these studies may be applicable to using the aerobic granular process for sewage treatment (Pronk et al., 2015). In this sense, given the promising results obtained in R1, the same protocol was applied to a pilot-scale reactor (R4) treating municipal wastewater to evaluate the effect of sludge discharge on granulation, system performance, stability etc.

As mentioned, the pilot-scale reactor had a working volume of 140 L, treating low-strength municipal wastewater under tropical conditions. The reactor started with the same inoculum as the lab-scale reactors and a VSS concentration of 2 g/L.

Comparing the granulation time, R1 granulated with 25 days while R4 granulated in 47 days. This result is justified because R1 was fed with acetate (which favors granulation), while R4 was fed with low-strength municipal wastewater (Nancharaiah et al., 2018). The characteristics of the granules formed in R4 were as good as those formed in R1 (Table 2), providing values of $SVI_{30} = 63.7$ mL/g and $SVI_{30}/SVI_5 = 0.9$.

The granules' settling velocity in R4 (21 m/h) was reduced by almost half compared with the values achieved in R1 (39 m/h). It was also observed that the SVI_{30} values in R4 (63.7 mL/g) were slightly higher than those obtained in R1 (52.1 mL/g) (Table 1). These results show that granules' settleability in R1 was better than those obtained in R4, clearly attributed to the effect of the substrate acetate, which favors the formation of granules, making the existence of filaments difficult. On the other hand, when granules are grown with municipal wastewater, there is a greater difficulty in forming compact and dense granules due to the greater wastewater complexity and the presence of suspended solids in the influent (Pronk et al., 2015; Wagner, 2015).

The performance of R4 in the removal of C, N, and P was considered satisfactory after achieving the maturation stage of the granules. Values above 90% were observed for COD and NH_4^+-N, 75% for TN, and 88% for PO_4^{3-}-P (Table 3). These values were higher than other studies that applied AGS reactors on a pilot and full-scale domestic sewage treatment without sludge discharge (Derlon et al., 2016). The applied protocol for sludge discharge improved phosphorus removal, from 79 ± 12% to 88 ± 7%. However, they were not statistically different ($p = 0.06$). Sludge discharge reduces the SRT, besides removing PAOs that may be saturated with phosphorus, thereby promoting a “renewal” of sludge (Bassin et al., 2012). Also, selective sludge discharge reduced the effluent TSS from about 40 to 30 mg/L, which were statistically different ($p = 0.04$).

On the other hand, sludge discharge methodology slightly affected total nitrogen removal ($p = 0.09$). Improved phosphorus removal without compromising nitrification showed that the controlled SRT between 12 and 15 days was adequate for both processes.

Regarding the EPS content, there was no significant difference in the PS content between R1 and R4 ($p = 0.07$) (Table 2). On the other hand, the PN concentration was about 3.5 times higher in R4 (501 mgPN/gVSS) than in R1 (150 mgPN/gVSS). This result may be related to the complexity of municipal wastewater composition compared to synthetic effluents (He et al., 2018; Rollemberg et al., 2018).

3.6. Evaluation of resource recovery from excess sludge of the AGS reactors

Recently, some studies have focused on resource recovery from aerobic granules excess, such as alginate-like exopolysaccharides (ALE), tryptophan (TRY), and phosphorus (P) (Wang et al., 2018; Zhang et al., 2018). In this sense, this work evaluated the presence of ALE, TRY, and P from the reactors' excess sludge.

The concentration of TRY in the four reactors' excess sludge was similar (≈40 mg TRY/gVSS), with no significant differences ($p \approx 0.07$) (Table 3). On the other hand, the phosphorus concentration was significantly different in the systems ($p \approx 0.04$). After maturation, values of 29, 17, 9 mgP/gTSS for reactors R1, R2, and R3, respectively, and 18 mgP/gTSS in the pilot-scale reactor R4, were observed (Table 3).

The results showed that the selective sludge discharge with SRT control and the use of a simpler substrate such as acetate showed a better result regarding the phosphorus content in the excess sludge. For instance, comparing R1 and R4, the reactor fed with acetate (R1) provided a greater P accumulation since PAOs are favored when using acetate or other VFAs as substrate (Rollemberg et al., 2018).

Among all the evaluated by-products, the high presence of ALE in the excess sludge stands out (≈190 mgALE/gVSS in R1 and R2, 150 mgALE/gVSS in R3, 220 mgALE/gVSS in R4). This result suggests that the more granular biomass is formed, the more ALE can be recovered. For instance, while R1 had about 90% granular biomass, R3 had only 80% (Table 3).

The high presence of ALE in the excess sludge shows that the recovery of this by-product might be interesting for applying the biorefinery concept. As it is known, the ALE present in the aerobic granules has chemical and mechanical properties (gel-forming capability) that allow industry applications. The high potential of ALE recovery from AGS provided a new Nereda® project through RoyalHaskoning HDV, called Kaumera Nereda® Gum, aiming to produce bio-based resources to various oil-based materials (RoyalHaskoningDHV, 2020). In this context, this work has shown in an unprecedented way that the discharge method can influence the content of value-added by-products present in the excess sludge.

3.7. Bacterial community analysis

There were four samples of AGS for molecular biology analysis collected at the end of the experiment (maturation phase) (Fig. 2): sludge in reactors R1 (S1), R2 (S2), R3 (S3), and the pilot-scale reactor R4 (S4). 31,767–52,743 sequence tags were retrieved from the aerobic granules and assigned to 1147–1813 OTUs.

The Alphaproteobacteria was an abundant class in S1, S2, and S4. The literature reports that this class has many bacteria that can secrete EPS (Ramos et al., 2015), which may justify the higher content of total EPS in the granules at R1, R2, and R4.

The abundance of Alphaproteobacteria can also justify the results obtained with the removal of ammonia. This class includes the microbial groups of autotrophic nitrifying bacteria. The reactor that showed the lowest percentage of ammonia removal was R3 (Table 4), which had the lowest abundance of Alphaproteobacteria. Therefore, the non-selective sludge discharge can impair the retention of EPS-producing biomass and nitrifying microorganisms.

Planctomycetacia, Anaerolineae, and Bacteroidia were abundant in all systems. They are typical components of activated sludge and AGS biomasses, both associated with heterotrophic bacteria involved in organic matter oxidation (Li et al., 2014; Seviour et al., 2009). The high presence of these groups justifies the high COD removals found in all systems. However, some studies reported that the excess abundance of Bacteroidia can cause system instability due to the high presence of filaments in the granules, and washout may also occur when a short settling time is applied (Zhang et al., 2011).

Gammaproteobacteria is an essential group in aerobic granular sludge. They are heterotrophic and usually related to denitrifying bacteria, PHA-producing bacteria, PAOs, and GAOs (He et al., 2018; Rollemberg et al., 2019). Reactor R3 presented the greatest abundance of Gammaproteobacteria (Fig. 2). However, this system had the lowest phosphorus removal efficiency among all reactors. Therefore, it is likely that Protocol 3 of sludge discharge favored GAOs, unlike the other methodologies (especially Protocol 1), which favored PAOs. As it is known, PAOs compete with GAOs, because they both consume the same substrate, volatile fatty acids (VFAs), and have similar metabolism. However, GAOs cannot accumulate phosphorus, thus decreasing the system efficiency for nutrients removal (Bassin et al., 2012).

Some studies have shown that increased ALE production may be associated with PAOs (Guimarães, 2017). This relationship could be applied because when comparing R1, R2, and R3, in similar conditions, the reactor that showed better removal of P also had a higher ALE content in the excess sludge. Therefore, one of the strategies to be applied in AGS reactors' operation when aiming to produce a higher ALE content in excess sludge would be to favor the development and retention of PAOs. Therefore, selective sludge discharge based on SRT (Protocol 1) could be an effective strategy for this goal. For example, Winkler et al. (2012) showed the predominance of PAOs in granules with SRT of 13 ± 4 days. It is important to note that when there is no sludge removal

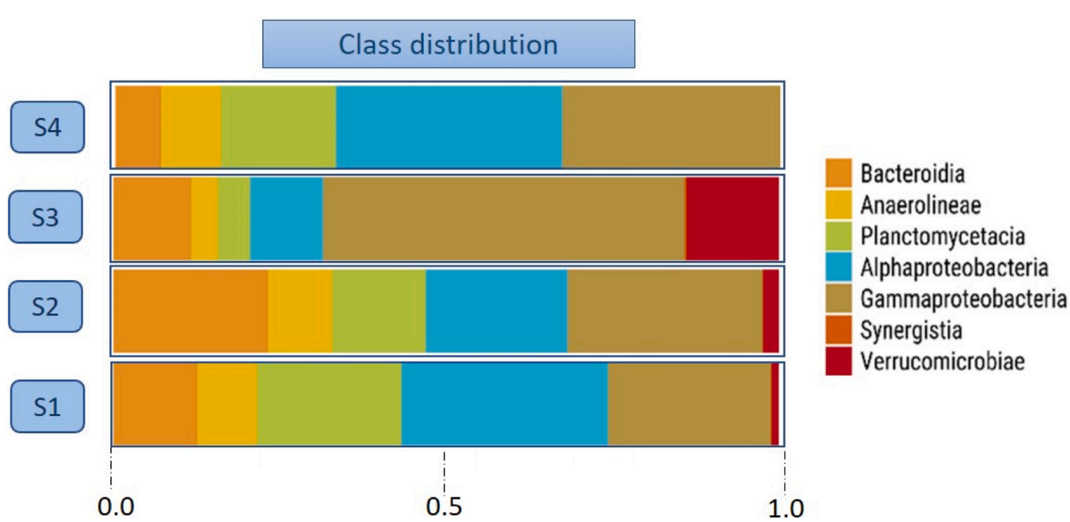

Fig. 2. Relative abundance in terms of class obtained in the lab-scale reactors R1 (S1), R2 (S2), R3 (S3), and the pilot-scale reactor R4 (S4) (data obtained at the end of the experiment).

Table 4
Removal of COD, nitrogen, and phosphorus (data obtained at the end of the experiment).

Reactor	Sample	Average concentration (mg/L)			Average removal (%)			
		COD	NH_4^+-N	PO_4^{3-}-P	COD	NH_4^+-N	TN	PO_4^{3-}-P
R1	Influent	564 ± 13	46 ± 5	11.2 ± 1	95 ± 3	96 ± 4	84 ± 2	79 ± 3
	Effluent	25 ± 6	2 ± 1	2.3 ± 1				
R2	Influent	570 ± 15	48 ± 5	9.7 ± 1	91 ± 2	89 ± 3	74 ± 3	60 ± 2
	Effluent	47 ± 9	5 ± 1	3.8 ± 1				
R3	Influent	592 ± 15	47 ± 5	12.4 ± 1	88 ± 4	86 ± 2	72 ± 3	58 ± 2
	Effluent	64 ± 8	6 ± 1	5.1 ± 2				
R4	Influent	464 ± 21	47 ± 5	5.0 ± 1	94 ± 2	91 ± 3	75 ± 3	88 ± 3
	Effluent	19 ± 6	4 ± 1	1.0 ± 1				

from the bottom in AGS reactors, there will be phosphorus accumulation (in the precipitated form). Over time the sludge will have a high ash content in addition to the release of part of this P in the treated effluent (deteriorating the final effluent quality). On the other hand, the excess discharge of the bottom sludge causes the removal of granules rich in PAOs, which will compromise phosphorus removal and impair biomass settleability (since PAOs are known to assist granules settleability). Therefore, the biomass discharge must be carried out in a controlled way to maintain PAOs in the system (Bassin et al., 2012; Zhu et al., 2013).

Thus, this study showed that the sludge discharge also influences the microbial groups present in the excess sludge, which influences the system's performance in terms of pollutants removal, reactor stability, and the content of by-products present in the excess sludge to be recovered.

4. Conclusions

Different methods of sludge discharge were tested in AGS reactors. The use of selective sludge discharge controlling SRT between 10 and 20 days proved to be an important strategy for AGS formation, maintenance, and performance, with removal efficiencies of organic matter (95%), ammonia (97%), total nitrogen (85%) and phosphorus (80%). Similar results were reproduced when applied in the pilot-scale. Regarding resource recovery, a considerable amount of ALE (219 ± 15 mg/gVSS) and P (18 ± 3 mg/gTSS) were found in the excess sludge. Therefore, it is possible to apply the biorefinery concept in AGS systems, besides producing proper water for reuse.

CRediT authorship contribution statement

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André Bezerra dos Santos: Writing - Review & Editing; Funding acquisition.

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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