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Post-treatment of swine wastewater using aerobic granular sludge: Granulation, microbiota development, and performance

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ABSTRACT

We studied the applicability of aerobic granular sludge (AGS) technology in association with upflow anaerobic sludge blanket (UASB) reactors for swine wastewater (SWW) treatment. Granule formation, long-term operational evaluation, and the system performance for simultaneous C, N, and P removals were assessed. Finally, the AGS microbial changes were evaluated by metagenomic analysis. Aerobic granulation occurred after 54 days of operation, and 81% of the granules had a diameter above 1.0 mm after 96 days of the experiment. The granules formed were spherical, had cohesive structure and good sedimentation capacity, although they were classified as non-resistant. After the sludge granulation (steady-state), high COD, BOD and ammonia removals were found, although the removals of TN and TP were considered moderate. Nonetheless, overall, a good operational stability was achieved. The metagenomic analysis revealed that the microbial groups suffered great influence from the substrate characteristics, but they are commonly found in AGS systems.

1. Introduction

Swine Wastewater (SWW) is a polluting waste resulting from the pork production process. It is estimated that each pig can generate around 160 to 541 L of SWW throughout its production process. This residue has high organic matter content in terms of chemical oxygen demand (COD, 3–30 g L^{-1}) and biochemical oxygen demand (BOD, 1.5–8.7 g L^{-1}), high concentrations of nitrogen (0.6–6 g L^{-1}) and phosphorus (0.1–1.4 g L^{-1}) (Silva et al., 2020), high concentration of suspended solids (SS, 3.3–4.8 g L^{-1}), and a low C/N ratio (4.8–5.4) (Zhang et al., 2021).

The anaerobic digestion (AD) of SWW can convert part of the organic matter into bioenergy by producing methane or hydrogen and by-products such as high added value carboxylic acids (Silva et al., 2020). The upflow anaerobic sludge blanket (UASB) reactors represent an AD technology widely used in treating high organic loads agro-industrial wastewaters, such as those produced in pig farming (Oliveira et al., 2020a). According to Oliveira et al. (2021), UASB reactors can be considered an interesting treatment technology for SWW, as they have low cost and easy operation, can be implemented for different types of scales and production units, in addition to allowing the use of biogas with high levels of methane, generated due to the high concentration of organic matter present in the wastewater. Sometimes two-stage anaerobic processes are applied, the first hydrolytic/acidogenic and the second acetogenic/methanogenic, to better remove organic matter. Nonetheless, the AD process can only remove part of the influent COD, and the nutrients are not removed, therefore requiring a post-treatment to accomplish standards for reuse or surface water discharge.

In this regard, aerobic technologies with nitrification and denitrification are the most promising for the SWW post-treatment (Nagarajan et al., 2019). However, the carbon and ammonia concentrations are still high, requiring high aeration levels and the subsequent impact on the investment and operational costs. Furthermore, Zhang et al. (2021) report that ammonia nitrogen concentrations can negatively influence the development of nitrifying microorganisms. Additionally, a low C/N ratio can impair nutrient removal (Yu et al., 2020). Another factor that negatively affects the SWW aerobic post-treatment is the wastewater characteristics, which may cause medium acidification through the alkalinity consumption during nitrification. This imbalance is due to the inefficient denitrification process caused by the lack of readily available substrate in the anoxic medium that does not compensate the alkalinity consumed during nitrification (S. Wang et al., 2018b).

Aerobic Granular Sludge (AGS) technology has called attention to

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allowing the simultaneous removal of organic matter, nitrogen, and phosphorus in a single reactor. Such behavior is due to the characteristic of biomass auto-immobilization in the form of granules and the coexistence of different redox zones in the granule structure. In addition, it presents a lower sludge production when compared to traditional aerobic technologies such as activated sludge (AS) and its variants. Compared to AS, AGS has operating costs of 20 to 25% lower, 50 to 75% footprint reduction since this technology does not require secondary clarifiers. Furthermore, it can have an electricity consumption of 23 to 40% lower (Rollemberg et al., 2018).

According to Bassin et al. (2019), the aerobic biomass granulation process and the stabilization of the generated granules are affected by factors such as the type of wastewater to be treated, applied organic load, shear stress, microbial community existing in the sludge, among other factors. Cetin et al. (2018) evaluated the effect of SS concentration on the performance of an AGS used for sewage treatment and observed that the presence of a high SS concentration could negatively influence granule structure, decreasing its mature size and impairing its stability. Yu et al. (2020) investigated the influence of the C/N ratio on AGS performance for sewage treatment. They found a loss in nitrogen removal efficiency as the C/N ratio decreased, which varied between 30 and 7.5.

According to the authors mentioned above, achieving a stable aerobic biomass during the treatment and/or post-treatment of wastewater rich in particulate organic matter and low C/N ratio, such as SWW, represents a major challenge for the AGS technology consolidation. In case of success, it could minimize the negative environmental aspects observed in pork production and make this agribusiness sector environmentally sustainable.

Due to the different redox zones in the granules' structure and diverse microbiome, the AGS technology becomes quite versatile from an operational point of view. According to Iorhemen et al. (2020), an operational strategy with an anaerobic/anoxic phase at the beginning of the cycle can guarantee the readily available substrate utilization for nutrients removal and development of slow-growing microorganisms, essential for granules' stability. This same strategy can provide, in the aerobic period, only the particulate substrate (rich in SS), promoting the development of hydrolytic microorganisms. In this sense, the latter strategy, which will be adopted in this study, can facilitate the treatment of wastewater with high SS concentrations and low C/N ratios.

To date, as far as we are concerned, there are no published studies that have evaluated the association of UASB reactors with AGS for SWW post-treatment. Moreover, discussions that address the difficulty of AGS systems in treating wastewater with the characteristics of the present research, which include a low C/N ratio, where most of the organic matter is constituted by SS, were also not observed. Given the above, this work aimed to evaluate the applicability of AGS technology in association with UASB reactors for SWW treatment. Granule formation, long-term operational evaluation, and the system performance in terms of simultaneous C, N, and P removals were evaluated. Finally, the AGS microbial changes were assessed by metagenomic analysis.

2. Materials and methods

2.1. Experimental set-up

The raw SWW was produced by washing the stalls of the breeding, gestation, maternity, daycare, and finishing sectors that contained waste, urine food remains, and other excreta from a traditional confined intensive system pig, located in the Zootechnics Department from the Federal University of Ceará (UFC). Raw SWW collection was made weekly, gathering all the content produced on the day. Afterwards, the raw SWW went through a preliminary treatment in a static sieve with a mesh of 2 mm aperture, simulating a full-scale SWW treatment plant. Then it was homogenized, characterized, and conducted for the lab-scale experiments, which consisted of UASB-AGS reactors (Supplementary Data, Fig. A.1).

Raw SWW was placed in a mechanically stirred equalization tank (ET1). Then, the UASB reactor's feeding took place using a peristaltic pump (MasterFlex, USA). The UASB effluent was sent by gravity to a second equalization tank (ET2), also mechanically stirred. In the sequence, a diaphragm pump (pulse) pumped the anaerobic effluent to the AGS reactor. Both equalization tanks were kept refrigerated at 4 °C in order to avoid the natural SWW biodegradation. The mechanical agitation inserted in both ET1 and ET2 provided a constant homogenization to avoid solids sedimentation and uniform composition.

The AGS system was operated as a sequencing batch reactor (SBR). It was made of acrylic with an internal diameter (D) of 10 cm and a useful height (H) of 100 cm, which resulted in a H/D ratio of 10. The SBR had a working volume of 6.6 L and operated with a 50% volumetric exchange ratio with a 12 h cycle time. The mixing inside the SBR was promoted by the aeration process carried out by an air compressor connected, through a silicone hose, to a porous diffuser located at the bottom of the SBR to distribute the air bubbles. The average operating temperature throughout the cycle was 28 ± 6 °C (room temperature). At the end of each cycle, the effluent was stored in a tank for analysis and discharge. However, for AGS effluent analyzes, always a fresh wastewater sample was collected.

2.2. Start-up and experimental procedure

For the reactor start-up, the SBR was inoculated with 3.3 L (50% of the total reactor volume) of flocculent sludge (Volatile Suspended Solids: 3.4 g L^{-1}; sludge volumetric index at 30 min (SVI_{30}): 190.4 mL g^{-1}) from a conventional activated sludge system used in the treatment of sewage from a textile industry located in the city of Fortaleza, state of Ceará, Brazil.

The AGS cycle consisted of the phases anaerobic (120 min), aerobic (559 min), sedimentation (20 min), and withdrawal (1 min), totaling a cycle time of 12 h. The airflow was kept constant throughout the experiment, providing a surface air velocity of 1.2 cm s^{-1}, which is reported to guarantee the formation of granules with a larger diameter (Zhu et al., 2015). The dissolved oxygen (DO) concentration varied from 4.3 mg L^{-1} (beginning of the aerobic period) to 7.0 mg L^{-1} (end of the aerobic period).

2.3. Granulation Evaluation: physical and microbiological aspects

The granulation process was evaluated in terms of physical aspects (granulometry, sludge sedimentation capacity, and granule resistance) and microbiological aspects such as the production of extracellular polymeric substances (EPS), biomass morphology, and microbial diversity.

The granulometry was performed based on the sieve methodology described by Bin et al. (2011), using sieves with 0.2, 0.6, and 1.0 mm mesh opening diameters and a volume of mixed liquor of 40 mL. The sedimentation capacity was determined using the SVI_{30}, in which 1 L of mixed liquor was subjected to a sedimentation period of 30 min in a graduated cylinder (Schwarzenbeck et al., 2005). Granules' physical resistance analysis followed the methodology described by Nor-Anuar et al. (2012); the granular biomass present in 1 L of mixed liquor was subjected to a shear force promoted by a propeller with rotation of 200 rpm for 10 min. The defragmented fraction was expressed in terms of the stability coefficient (S).

Granules' formation and morphology were evaluated using Scanning Electron Microscopy (SEM), combined with Dispersive Energy Spectroscopy (EDS) (Inspect S50 - FEI). Samples preparation followed the methodology described by Rollemberg et al. (2019). SEM and granule resistance analyses were performed at the end of the stage, i.e., after 96 days of operation.

EPS was analyzed through an extraction process proposed by Tay et al. (2001). The quantification was determined in terms of PN (total proteins) and PS (total polysaccharides) according to elsewhere (Long et al., 2014). EPS, granulometry, and SVI analyses were performed weekly.

Inoculum (flocculent sludge) and granular sludge after the experimental period (96 days) were collected, after which genetic sequencing was carried out where the DNA was extracted using the PowerSoil® DNA isolation kit (MoBio Laboratories Inc., USA), following the manufacturer's instructions. The procedures for sequencing and amplifying the 16S rRNA gene and processing the data obtained to analyze microbial diversity followed the procedure described by Rollemberg et al. (2019).

2.4. Chemical analysis

For 96 days, the AGS reactor was monitored twice a week by samples collected from ET2 (AGS influent) and the supernatant discarded at the end of the cycle (AGS effluent). The physical-chemical parameters Total, particulate and soluble COD and $BOD^{5,\ 20^{\circ}C}$, solids, pH, total ammoniacal nitrogen (NH_4^+-N), total Kjeldahl nitrogen (TKN), organic nitrogen (ON), nitrite (NO_2^--N), nitrate (NO_3^--N), phosphate (PO_4^{3-}-P) and total phosphorus (TP) were determined according to APHA (2012). Total alkalinity (TA) was determined by the titrimetric method of Kapp (1992) apud Buchauer (1998). DO concentration in the reactor was monitored using a multiparametric probe (YSI, Model Pro 1020).

Ion chromatography was performed using a Dionex ™ $ICS^{-1}100$, equipped with a Dionex ™ IonPac ™ AG23 pre-column (2 × 50 mm), a Dionex ™ IonPac ™ AS23 column (2 × 250 mm), and a suppressor Dionex ™ AERS ™ 500 (2 mm) (Thermo Scientific, USA). 5 μL of each filtered sample (0.45 μm) was injected and then eluted by an aqueous solution containing 4.5 mM sodium carbonate and 0.8 mM sodium bicarbonate at a constant flow of 0.25 mL min^{-1}. The oven temperature was 30 °C, the current applied was 7 mA, and the run time was 30 min.

2.5. Calculation methods and statistical analysis

With the results obtained for each evaluated parameter, the identification and exclusion of outliers were made. Then, descriptive statistics were used using Microsoft Excel software to express the value ranges obtained for the influent and effluent during acclimatization (12 data) and steady-state (11 data), and biomass characteristics.

The TCOD, TP, and TN removal efficiency values were subjected to a statistical test to compare means between two independent samples, called Student's *t*-test with a 5% significance level, for the acclimatization and steady-state periods. The results obtained were expressed in error bars next to the mean and with lower case letters to show the difference or statistical equality between the means. The different letters indicate a statistically significant difference between the means considering 95% confidence interval ($p \leq 0.05$). Equal letters indicate that the statistical difference was not significant ($p > 0.05$). The *t*-test was performed using the Sisvar software version 5.6.

3. Results and discussion

3.1. Granulation process

The acclimatization stage started with the flocculent sludge inoculation and lasted 47 days, ending when the predominance of granules was observed in the AGS system. The concept of granule adopted in this work was the same established by Liu et al. (2010), where more than 50% of the biomass had a diameter greater than 0.2 mm. The stage

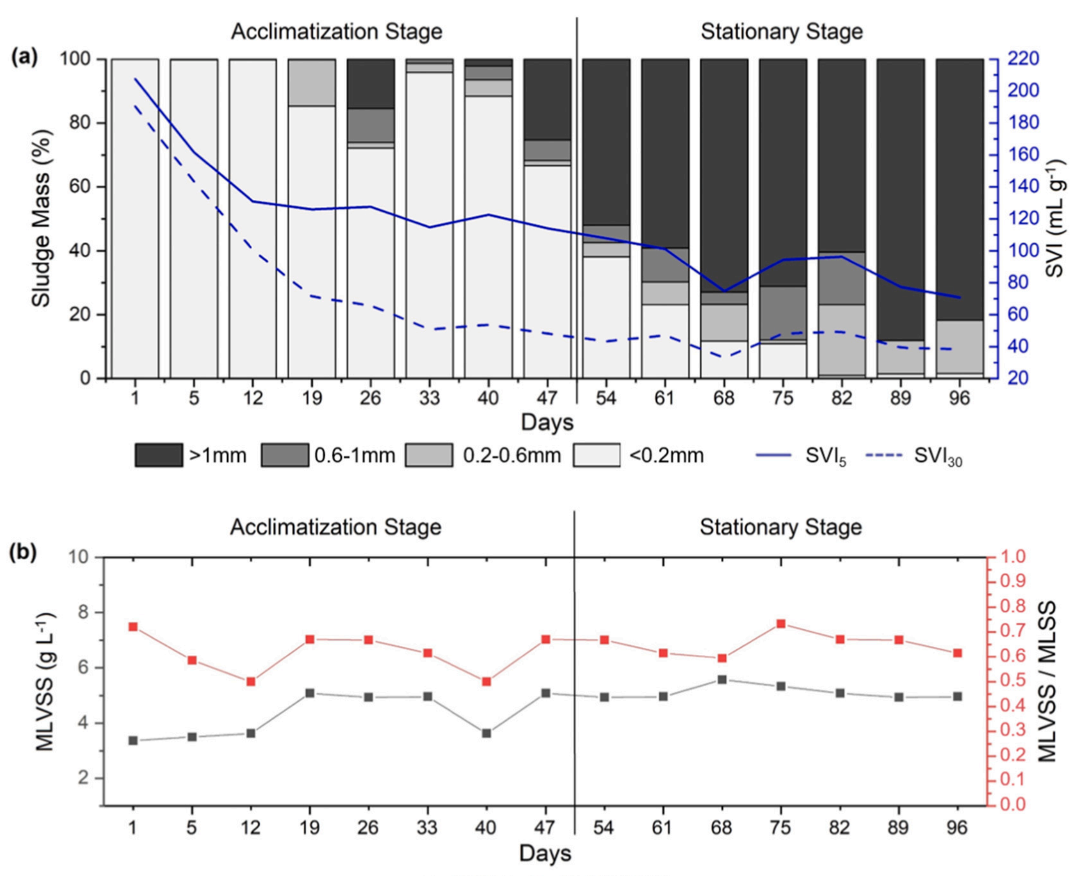

Fig. 1. Granulation process and biomass evolution. (a) granulometric profile and SVI, (b) volatile suspended solids and MLVSS/MLSS ratio in the reactor. Legend: MLVSS: Mixed Liquor Volatile Suspended Solids; MLSS: Mixed Liquor Suspended Solids; SVI: Sludge Volumetric Index in 30 min (SVI_{30}) and 5 min (SVI_5).

called steady-state was related to the post-granulation monitoring period and lasted 49 days. Granules formation and evolution (de Kreuk et al., 2007) over the 96 days of operation are shown in Fig. 1.

It can be seen that until the 47th day (94th operating cycle), the biomass was not yet considered granular (more than 50% of the biomass had a diameter lower than 0.2 mm), characterizing the acclimatization phase. However, for the sample collected from the 54th day (108th operating cycle), the biomass diameter varied between 0.2 and 0.6, 0.6 and 1.0, and >1.0 mm. Therefore, the full granulation of SWW pre-treated in the UASB reactor occurred between days 47 and 54 in the AGS SBR system.

Cetin et al. (2018) evaluated the granulation process in an SBR operating with cycles of 6 h and fed with sewage containing different SS concentrations. They obtained a granulation in 25 days with a medium SS concentration (200 mg L^{-1}) and 44 days with high SS concentration (700 mg L^{-1}). The authors state that the SS influence in the granulation may be related to the shear process, where the high SS concentration facilitates the fragmentation of the still immature granules. These results are in line with the present investigation since the SS concentrations in the AGS influent are higher than 800 mg L^{-1} during the acclimatization (Table 1).

Table 1
Operational performance of the AGS system during the acclimatization (1–47 days) and steady-state (54–96 days) periods.

Parameter	Acclimatization			Stead-state		
	Influent	Effluent	%	Influent	Effluent	%
TCOD (mg L^{-1})	1036 ± 586	452 ± 207	54 ± 25	1710 ± 256	441 ± 276	74 ± 17
PCOD (mg L^{-1})	587 ± 598	251 ± 145	49 ± 29	1324 ± 251	329 ± 290	74 ± 25
SCOD (mg L^{-1})	449 ± 153	201 ± 96	54 ± 16	385 ± 90	112 ± 24	69 ± 10
TBOD (mg L^{-1})	709 ± 356	59 ± 32	64 ± 18	1122 ± 259	139 ± 89	87 ± 9
PBOD (mg L^{-1})	422 ± 288	169 ± 87	50 ± 33	976 ± 212	124 ± 97	86 ± 10
SBOD (mg L^{-1})	287 ± 152	59 ± 32	78 ± 8	147 ± 90	15 ± 13	89 ± 7
SS (mg L^{-1})	879 ± 518	299 ± 134	58 ± 22	1769 ± 1053	339 ± 255	75 ± 23
NTK (mg L^{-1})	189.3 ± 89.9	98.6 ± 56.3	57 ± 18	306.3 ± 222.7	57.8 ± 65.9	90 ± 4
NH_4^+-N (mg L^{-1})	134.7 ± 68.9	59.9 ± 56.8	64 ± 28	130.6 ± 46.5	9.1 ± 3.3	93 ± 3
NO_2^--N (mg L^{-1})	0 ± 0	14.5 ± 20.0	–	0.8 ± 1.4	1.5 ± 4.2	–
NO_3^--N (mg L^{-1})	0.5 ± 0.7	6.1 ± 10.0	–	2.3 ± 5.3	90.8 ± 70.8	–
TN (mg L^{-1})	189.8 ± 89.4	119.2 ± 34.1	43 ± 21	309.3 ± 221.4	150.1 ± 70.3	57 ± 22
TP (mg L^{-1})	98.7 ± 32.8	76.0 ± 44.2	30 ± 29	66.5 ± 37.5	25.5 ± 12.4	57 ± 23
PO_4^{3-}-P (mg L^{-1})	33.7 ± 18.6	21.5 ± 13.3	–	20.2 ± 10.0	28.8 ± 6.4	–
pH	7.5 ± 0.2	7.4 ± 0.5	–	8.0 ± 0.3	7.2 ± 0.7	–
Alkalinity (mg $CaCO_3$ L^{-1})	897.6 ± 390.8	441.8 ± 321.6	–	1071.9 ± 403.2	274.6 ± 307.7	–

Legend: T,P,S COD: Total (T), Particulate (P) and Soluble (S) Chemical Oxygen Demand; T,P,S BOD: Total (T), Particulate (P) and Soluble (S) Biochemical Oxygen Demand; SS: Total Suspended Solids; TKN: Total Kjeldahl Nitrogen; NH_4^+-N: Ammoniacal Nitrogen; NO_2^--N: Nitrite; NO_3^--N: Nitrate; TN: Total Nitrogen; TP: Total Phosphorus; PO_4^{3-}-P: Phosphate.

Sludge sedimentation capacity was evaluated in terms of SVI_5 and SVI_{30}. The sharp drop in SVI_5 and SVI_{30} values (Fig. 1a) indicated that the operation mode improved the biomass sedimentability characteristics. After the acclimatization period, it was possible to verify a stability in the SVI_{30} value, always keeping in the range of 30–50 mL g^{-1}, which indicates an excellent capacity for granules' sedimentation and achieving their maturation stage. Even so, it was possible to verify a slight drop in the SVI_{30} value when the percentage of granules larger than 1 mm in diameter was increased. Thus, the granulometry influence in SVI_5 and SVI_{30} is much more significant during steady-state than in the acclimatization period, where there was a sharp drop in SVI_5 and SVI_{30} even without granules' formation. After 54 days, almost 62% of the biomass was considered granular (diameter > 0.2 mm). After 96 days, the granular sludge corresponded to more than 98% of the biomass, in which 81% of the granules had a diameter above 1.0 mm.

It can also be noted that there was a gradual SVI_{30} drop, from 190 to 39 mL g^{-1} (Fig. 1a), being observed in other granulation studies, and representing the evolution from flocculent sludge to granular sludge. From day 33, the sludge SVI_{30} varied from 55 to 33 mL g^{-1}, staying within the range of 30 to 80 mL g^{-1} found in other studies using real effluents (Derlon et al., 2016). The SVI_{30} remained stable from day 54, varying between 49 and 33 mL g^{-1}.

It was also possible to observe that, except for the first 12 days of operation, where the SVI_{30} values were greater than 100 mL g^{-1}, the greater the presence of granules in the biomass, the more the SVI_5 and SVI_{30} values approached, reaching a mean ratio (SVI_{30}/SVI_5) for the steady-state of 0.48 ± 0.05. However, the SVI_{30}/SVI_5 ratio was below the expectations since Cetin et al. (2018) obtained values above 0.9 operating an AGS SBR fed with sewage with high SS concentrations. Another aspect of being considered is the effect of the high SS concentration with a low sedimentation velocity in the AGS influent, interfering in the SVI test, especially the SVI_5 (high value), which results in a low SVI_{30}/SVI_5 ratio.

According to the classification given by Nor-Anuar et al. (2012), where granules with a stability coefficient of less than 5% are considered very resistant granules, between 5 and 20% resistant, and greater than 20% non-resistant granules, the granules formed in the reactor were considered non-resistant, presenting a stability coefficient of 58 ± 9%. The EPS production can influence the granular biomass stability. The total EPS content given in terms of PN (proteins) and PS (polysaccharides) for this study was 668 ± 153 mg g^{-1} VSS, much higher than that identified by Rollemberg et al. (2019). However, the PS/PN ratio was 0.2, showing a low PS production in relation to the PN production. Zhang et al. (2015) point out the PS as the main responsible for promoting stable granules. Thus, the low PS/PN ratio may explain the instability in the granules formed.

In the sample collected at 96 days of operation, the SEM images show that the aerobic biomass had a regular spherical shape and compact appearance (Supplementary Data, Fig. A.2a). Furthermore, although the granule has a cohesive and well-designed structure, there were numerous channels in its surface structure (Supplementary Data, Fig. A.2b), contributing to the substrate permeability since the treated SWW is composed predominantly of particulate COD. Cetin et al. (2018) observed the same channels in the biomass structure that operated with a real substrate with a high SS concentration, which was not observed in studies that used soluble substrate, such as those conducted by Mendes Barros et al. (2021). This result suggests that these channels are an adaptive granule feature that facilitates the particulate substrate entry to the innermost regions of the granule.

The Dispersive Energy Spectroscopy (DES) carried out together with the SEM showed that the EPS matrix (granule surface region) was composed mainly of Oxygen (42.6%), Carbon (36.8%), Potassium (6.7%), Phosphorus (6.2%), Calcium (2.3%) and Sodium (2.2%). The concentrations of PO_4^{3-}-P and calcium in the system influent varied around 74 ± 43 mg L^{-1} and 58 ± 21 mg L^{-1}, respectively, and the operational pH remained in the range of 6.4–7.8. The low MLVSS/MLSS ratio around 0.7 ± 0.1 at steady-state (Supplementary Data, Fig. A.2b) suggests an accumulation of inert material in the biomass.

3.2. Operating performance

The general performance of AGS SBR in terms of removal efficiency and operational stability is shown in Table 1, emphasizing the distinction between the acclimatization (1–47 days) and steady-state (54–96 days) periods.

During the experiment, the AGS was subjected to average values of volumetric organic load of 1.7 kgCOD m^{-3} d^{-1}; biological load of 0.03 kgCOD $kgVSS^{-1}$ d^{-1}; hydraulic retention time (HRT) of 1 d; solids retention time (SRT) of 12.1 d; food to microorganisms (F/M) ratio of 0.2 kgCOD $kgVSS^{-1}$ and Carbon/Nitrogen Ratio (C/N) of 5.5.

The influent and effluent pH showed little variation throughout the AGS operation, having remained close to neutrality. pH maintenance indicates good buffering capacity, likely due to the high alkalinity of the anaerobically treated SWW, which values were around 897.6 and 1071.9 mg $CaCO_3$ L^{-1} for the acclimatization and steady-state periods, respectively.

As the UASB reactor was receiving raw SWW, which presented large variations, the UASB effluent also presented high fluctuations, evidenced by the high standard deviation identified for each constituent. Concerning organic matter, about 70% of its fraction was in the particulate form, representing a challenge for AGS technology. The removal of organic matter, in terms of TCOD removal and real COD removal (considering the soluble effluent fraction), effluent SS concentrations, and VSS/SS ratio are shown in Fig. 2.

During the entire operation period, it is possible to observe a reduction in the TCOD removal efficiency and an increase in the effluent SS concentration to values around 339 ± 255 mg L^{-1} in the stationary state. Both reductions can be considered apparent since a large part of the effluent SS concentration was composed of VSS (active sludge washed out), as shown in Fig. 2 through the VSS/SS ratio. Thus, the real COD best represents the COD removal efficiency. Fig. 3 shows the efficiency of removing TCOD, TN, and TP throughout acclimatization and steady-state periods.

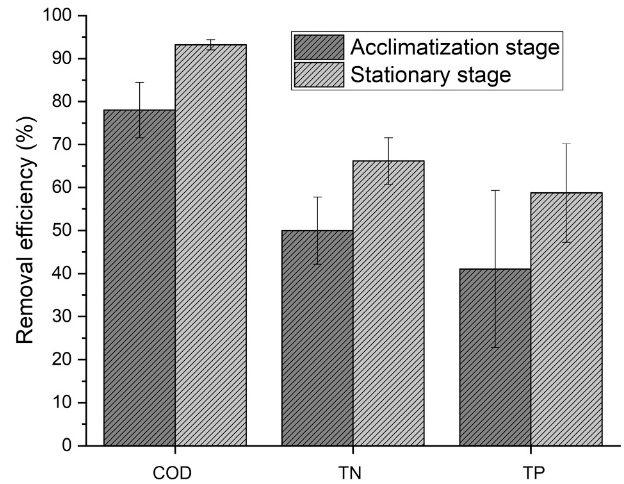

Fig. 3. Mean and confidence intervals (bars) of the organic matter and nutrients (N and P) removals throughout acclimatization and steady-state periods in the AGS reactor.
Legend: COD: Chemical Oxygen Demand; TN: Total Nitrogen; TP: Total Phosphorus.

For the organic matter removal in the form of COD, the *t*-test for independent samples indicates a statistically significant difference between the means ($p \leq 0.05$). Based on the average value, it was possible to observe that the COD removal efficiency at steady-state was higher than that of the acclimatization period, indicating that the AGS has a better performance for removing organic matter than the flocculent sludge.

Regarding nutrients removal in terms of TN, the *t*-test for independent samples showed a statistically significant difference between the means ($p \leq 0.05$). Nitrogen removal by the AGS (steady-state) was also greater than that of flocculent sludge (acclimatization), making clear the importance of mature granules for removing pollutants. However, the t-test for independent samples suggests no statistically significant difference ($p > 0.05$) regarding TP removal, although the mean was greater at steady-state than at acclimatization.

The SS removal was about 75%. However, the solids concentration in

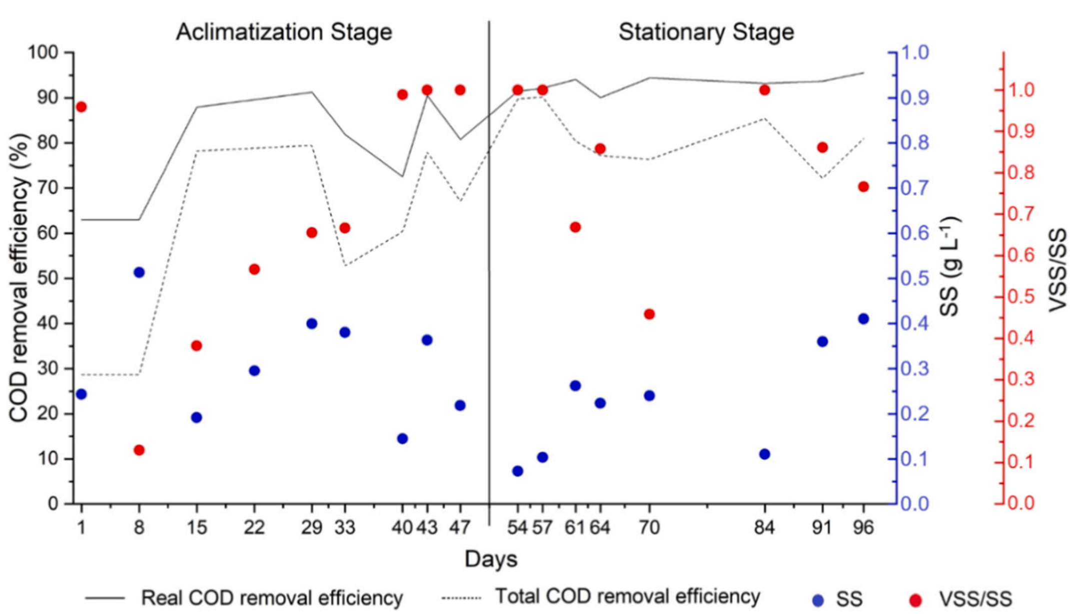

Fig. 2. Real COD removal efficiency and TCOD removal efficiency, effluent SS profile, and SSV/SS ratio throughout the AGS operation.
Legend: COD: Chemical Oxygen Demand; SS: Total Suspended Solids; SSV/SS: Volatile Suspended Solids/Total Suspended Solids ratio.

the effluent was still high due to biomass that was washed out from the AGS and the high SS concentration in the SWW influent. For the acclimatization period and the period defined as steady-state, the COD removal efficiency was 78% and 93%, respectively, being considered high for the steady-state. The system is highly efficient for removing biodegradable organic matter in terms of BOD, reaching almost 99% efficiency. Therefore, even though a large part of the biodegradable organic matter has already been removed in the anaerobic treatment, the AGS influent still shows high biodegradability.

BOD/COD ratios of the AGS effluent during the acclimatization and steady-state periods were 0.44 and 0.34, respectively. This result indicates that the treated SWW still has biodegradable characteristics, and another biological polishing step can be used. The treated effluent biodegradability may be related to the presence of complex compounds in the SWW that, during the aerobic treatment, were transformed into simpler substances, liable to biological degradation. It would be ideal that the polishing step also performs N and P removals (Table 1). Oliveira et al. (2020b), in a situation similar to that observed in this work, used constructed wetlands as polishing step of SWW treated in a UASB reactor and post-treated in a trickling filter, contributing to the final effluent quality improvement.

For nitrogen removal, ammonia oxidation by autotrophic nitrifying bacteria (AOBs) achieved 93% efficiency. Such removal was similar to that found in the work of Layer et al. (2020), who used different types of substrates in since at the beginning of aeration the available DO is preferably used by heterotrophic bacteria the operation of four AGS reactors, including simple and complex synthetic wastewaters, and raw and primary sewage. The authors used synthetic wastewater, where the carbon source was sodium acetate (550 mg COD L^{-1}; 50 mg NH_4^+-N L^{-1}). Authors such as Rollemberg et al. (2019) stated that long aerobic periods, such as the one used in the current research, favored nitrification that oxidize organic matter and at the end of the aeration, by the nitrifying autotrophic bacteria.

In contrast, He et al. (2018) had already achieved efficiencies for removing ammonia nitrogen in the range of 90% in 6 h operating cycles with just 1 h of aeration, using low-concentrated synthetic wastewater (200 mg COD L^{-1} and 20 mg NH_4^+-N L^{-1}). This situation differs from the conditions existing in the present study, which used high influent concentrations of nitrogen from organic matter (Table 1).

The simultaneous nitrification and denitrification process is the main TN removing mechanism, reaching about 56.7%, close to the value observed elsewhere using a synthetic substrate (Rollemberg et al., 2019). Thus, the high efficiency of nitrogen oxidation in the aerobic period results in about 90.8 mg N L^{-1} nitrate accumulation. Accumulation of nitrite is verified in AGS systems operated with simple synthetic wastewater (Layer et al., 2020; Rollemberg et al., 2019), whereas nitrate accumulation is reported in systems operated with sanitary sewage, considered a more complex substrate, such as the one of the present research.

Meng et al. (2015) showed that the anaerobically treated SWW has a low C:N ratio, which can negatively influence the denitrification process in AGS systems. They obtained an ammonia removal in the order of 69.0% and a nitrite accumulation of 94.4%. Wang et al. (2018a) studied the effect of the C:N ratio on the simultaneous removal of C, N and P, using AGS to treat synthetic wastewater (COD: 220 mg L^{-1}, TP: 3 mg L^{-1}, TN: 11–55 mg L^{-1}) with C:N ratios of 20, 10 and 4. The authors identified that the C and P removals remained stable in all situations. However, TN removal efficiency decreased with the C:N ratio reduction to 94.9%, 84.4%, and 74.8%, respectively.

In the present study, the wastewater influent to the AGS reactor has a C:N ratio of 5.5. However, as there is an accumulation of nitrate at the end of the operating cycle, the C:N ratio may be even lower at the beginning of the cycle, indicating unfavorable conditions for nitrogen removal. Even so, the nitrogen removal efficiency reached values equivalent to those found by Rollemberg et al. (2019), using AGS to treat synthetic wastewater.

As for phosphorus removal, the AGS system showed a removal efficiency of 30% and 57% for the acclimatization and steady-state periods, respectively. The removal achieved in the steady-state period was considered moderate since the total phosphorus concentration was much higher than the usual concentrations reported in the AGS literature. Wang et al. (2018a) achieved phosphorus removals of around 98%, using the same operational strategy for synthetic effluent. This efficiency was obtained with an influent TP concentration of 3 mg L^{-1}, which differs from the wastewater characteristics used in this study. However, Cetin et al. (2018) observed phosphorus removal in the range of 50% by investigating an AGS reactor treating sewage with high SS concentrations, where the TP concentration was 12 mg L^{-1}. For Rollemberg et al. (2019), the presence of NO_2^--N and NO_3^--N in the anaerobic phase after feeding or from the previous SBR cycle may promote competition between phosphate-accumulating organisms (PAO) and denitrifying heterotrophic microorganisms (DNB), interfering negatively on phosphorous removals. Thus, the low P removals can also be associated with competition between PAOs and DNBs, as previously reported (Barros et al., 2020).

The reactor performance was considered satisfactory, especially when the UASB/AGS system was evaluated, compared to other technologies to treat SWW. For instance, Oliveira et al. (2021) operated a microaerobic UASB reactor (R2) treating the raw SWW of the current research and obtained mean removals in the stationary phase of 74.5 ± 3.2% (TCOD), 79.4 ± 4.6% (PCOD), 82.6 ± 2.1% (TBOD), and 77.2 ± 2.4% (PBOD). On the other hand, the control UASB reactor R1 achieved removals of 65.4 ± 1.9% (TCOD), 62.4 ± 5.4% (PCOD), 63.3 ± 3.9% (TBOD), and 55.7 ± 7.2% (PBOD). Therefore, a large fraction of the organic matter could be removed under anaerobic conditions and converted into biogas. Such a conversion of organic matter is important to decrease the oxygen demand in the AGS reactor but also to preserve some organic matter to sustain nitrogen and phosphorous removals. The AGS systems are proven to decrease the footprint, aeration demand, and sludge production up to 70% compared to activated sludge systems (Lema and Martinez, 2017). Finally, the biogas produced could be converted into electricity and decrease the costs with aeration in the treatment plant. The present information indicates that both replacing traditional post-treatment technologies by AGS technology and the UASB/AGS association are promising, guaranteeing operational savings without compromising system performance.

3.3. Analysis of the microbiological community

At the end of the experiment, the metagenomic analysis identified 73,672 sequences for the flocculent sludge used as inoculum sludge (IS) and 34,030 sequences for the aerobic granular sludge (AGS) produced in the SBR after 96 days of operation. The richness and diversity indexes for both samples are shown in Table A.1 in the Supplementary Data. It is possible to observe an increase in the diversity in the AGS in relation to the inoculum sludge, as shown by the Inverse Simpson and Shannon indexes. However, the richness indexes (Chao and ACE) point to a reduction.

Barros et al. (2020) worked with simultaneous fill/draw SBR (cycle time: 360 min; sedimentation: 30 to 5 min; filling/drawing: 30 min; anaerobic reaction: 90 min; aerobic reaction: 210 min) for evaluating the granulation process using synthetic wastewater composed of ethanol as a carbon source (800 mg L^{-1}), nitrogen (100 mg L^{-1}) and phosphorus (10 mg L^{-1}). They obtained an increase in both richness and diversity, which was related to the type of SBR and the selection pressure imposed that influences the microbiological community's richness behavior. Thus, high selection pressures, such as the one present in conventional SBRs, can cause a reduction in the community richness in relation to the inoculum sludge.

Liu et al. (2017) obtained a reduction in species richness and diversity using a conventional type SBR-AGS (cycle time: 280 min; filling: 1 min; anaerobic reaction: 99 min; aerobic reaction: 150 to 175 min;

sedimentation: 30 to 5 min), operating with synthetic wastewater composed of acetate as a carbon source (513.0 mg L^{-1}), nitrogen (179.0 mg L^{-1}), phosphate (86.9 mg L^{-1}). Both studies indicate that the microbiology of SBR-AGS systems is subject not only to the selection pressure and the type of reactor but also to factors such as substrate type and operational strategy, making it difficult to predict the microbiota's behavior observed in this study. Fig. 4 shows the relative abundance of the microbial community at the phylum, class, family, and gender level for both the inoculum sludge and the AGS obtained 96 days after start-up.

At the phylum level, Proteobacteria, Planctomycetes, Actinobacteria, Nitrospirae, Bacteroidetes, and Firmicutes were the groups of microorganisms with greater abundance in the samples (Fig. 4a). Fan et al. (2018) showed that these phyla are the most commonly observed in AGS systems. The most abundant groups in the inoculum sludge were Proteobacteria and Actinobacteria. Although there was a reduction in the abundance of these groups in the AGS, the Proteobacteria group continued to be dominant, likely facilitating granulation. Proteobacteria is a group of microorganisms responsible for the denitrification process and favors granulation (Fan et al., 2018). The lack of readily available substrate in the anoxic phase may explain the nitrate accumulation at the end of the cycle and highlight the abundance loss for this group. Compared to the inoculum sludge, the most remarkable growths are verified for the phyla Nitrospirae and Bacteroidetes.

In terms of class, the Gammaproteobacteria group was the most abundant in the inoculum sludge, with a very low presence of the classes Clostridia and Bacteroidia groups (Fig. 4b). However, these two later groups had the most remarkable growths over the experiment, while Nitrospira and Phycisphaerae groups practically disappeared in the AGS. The Gammaproteobacteria class, responsible for organisms of the genus Thauera, can produce amyloid adhesins, an abundant component of the EPS fraction in activated sludge flocs (Larsen et al., 2008). However, there was a significant abundance decrease of this group, from 35.8% to 19.8%. This loss suggests that the low resistance and stability identified for the granules formed are likely associated with EPS production.

At the family level, the groups Chitinophagaceae and Clostridiaceae 1 were the most favored families present in the AGS (Fig. 4c). Chitinophagaceae is a family responsible for polysaccharides consumption and EPS accumulation, being usually present in AGS systems and facilitating the formation of bacterial aggregates (Chen et al., 2019). The favoring of this group shows how the SWW characteristic, rich in polysaccharides,

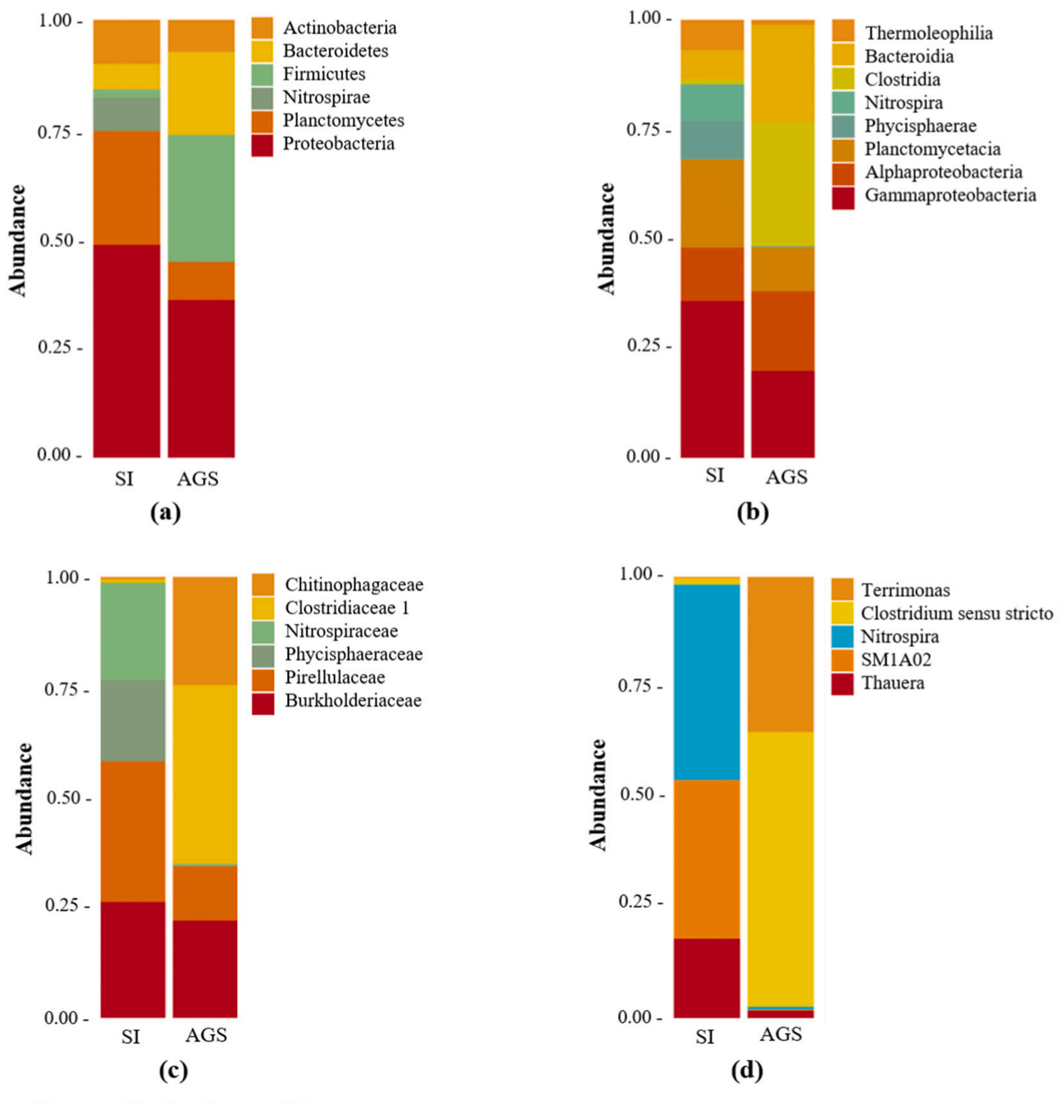

Fig. 4. Microbiological community at the level of phylum (a), class (b), family (c), and genus (d) of the flocculent sludge used as inoculum sludge (IS) and aerobic granular sludge (AGS) after 96 days of operation.
Legend: SI: Inoculum sludge; AGS: Aerobic Granular Sludge in steady-state.

can also influence its microbial selection.

The genus Terrimonas, belonging to the Chitinophagaceae family, was one of the most favored groups, increasing from 0.3% to 35.1% (Fig. 4d). Terrimonas are strictly aerobic and non-filamentous bacteria, which occur as single rods in pure culture and are responsible for hydrolyzing various substrates such as sugars, proteins, amino acids, and fatty acids (Ha et al., 2013). Therefore, the wastewater characteristic favored this microbial group since the SWW is a predominantly particulate substrate (capable of hydrolysis) rich in polymeric compounds such as proteins, lipids, and carbohydrates.

Another important genus verified in the AGS biomass was Clostridium Senso Stricto 1, which increased from 1.4% (inoculum sludge) to 62.3% (Fig. 4d). It is strictly anaerobic fermenting bacteria (Cadoret et al., 2017). Therefore, relative abundance increase is due to the big size of the granules achieved and the existence of an anaerobic zone in their inner part. Fig. 4d shows the relative abundance of groups of microorganisms with an abundance greater than 5%. In a more detailed characterization, with an abundance greater than 1%, a single genus of the Archaea domain, Methanobacterium, was identified, presenting a relative abundance of 0.19% for the inoculum sludge and 2.18% for the AGS reactor sludge.

Taxonomic affiliation at the genus level was used to infer the

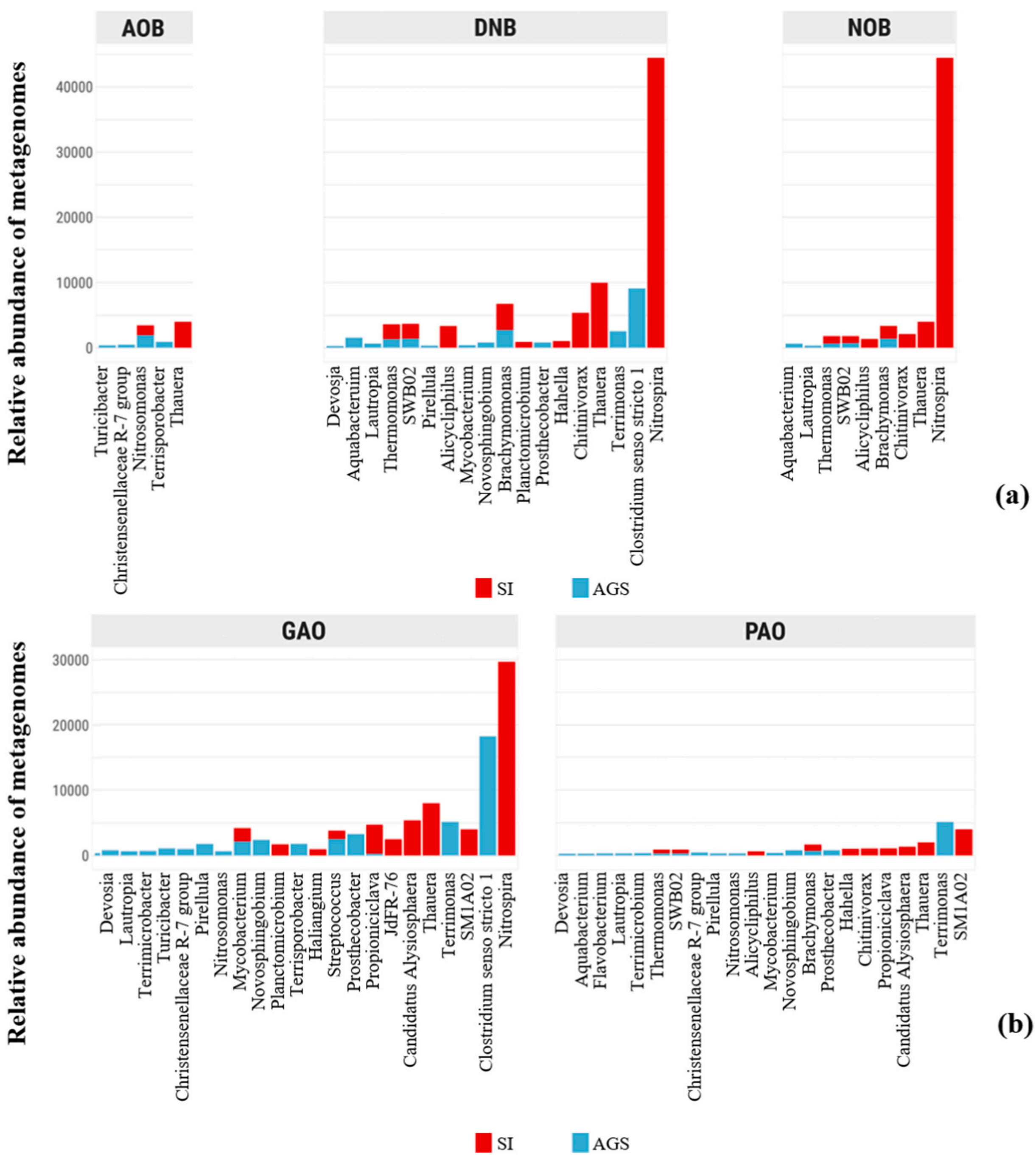

Fig. 5. Functional distribution of the taxonomic classification at the gender level of innocuous sludge (SI) and granular sludge (AGS) after 96 days of operation. (a) AOB, DNB and NOB, (b) GAO and PAO.
Legend: IS: Inoculum sludge; AGS: Aerobic Granular Sludge in steady-state; AOB: Ammonia Oxidizing Bacteria; DNB: Denitrifying Bacteria; GAO: Glycogen Accumulating Organisms; NOB: Nitrite Oxidizing Bacteria; PAO: Phosphate Accumulating Organisms.

functional groups related to the removal of C, N, and P. The groups were divided into AOB, NOB, DNB, GAOs, and PAOs (Fig. 5). Nitrate accumulation and the low nitrite concentrations in the AGS effluent seem to oppose the abundance reduction of NOB microorganisms, especially the Nitrospira genus. Regarding denitrification, there was an abundance decrease for the DNB genera Chitinivorax, Thauera, and Nitrospira, and an increase for the genera Aquabacterium and Terrimonas. However, it is possible that the nitrate accumulation occurred due to the relative lower growth of DNB group compared to the AOB.

The abundance of PAO was significantly lower compared to GAO and DNB. As the abundance of the GAO group was already high in the inoculum sludge, and the operational conditions favored its development, the GAO group suppressed PAO and DNB, contributing to the moderate phosphorus removal and nitrate accumulation in the system (Fig. 5). The GAO group favoring likely occurred through those microorganisms specialized in consuming polysaccharides since this was the main substrate source. On the other hand, microorganisms from the PAO group prefer a more easily assimilated substrate, therefore being suppressed and impairing phosphorus removal.

4. Conclusions

Granulation occurred after 54 days of operation, and a percentage of 81% of the granules with a diameter above 1.0 mm was found after 96 days of the experiment. The granules formed were spherical, had cohesive structure and good sedimentation capacity, although they were classified as non-resistant. After the sludge granulation (steady-state), high COD, BOD, and ammonia removals were found, although TN and TP removals were considered moderate. Nonetheless, overall good operational stability was achieved. The metagenomic analysis revealed that the microbial groups suffered great influence from the substrate characteristics, but they are commonly found in AGS systems.

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.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.biteb.2021.100862.

References

APHA (American Public Health Association), American Water Works Association, Water Environment Federation, 2012. Standard Methods for the Examination of Water and Wastewater, 22th ed. American Public Health Association (APHA), American Water Works Association and Water Environment Federation, Washington, DC.

Barros, A.R.M., Rollemberg, S.L.de S., de Carvalho, C.de A., Moura, I.H.H., Firmino, P.I.M., dos Santos, A.B., 2020. Effect of calcium addition on the formation and maintenance of aerobic granular sludge (AGS) in simultaneous fill/draw mode sequencing batch reactors (SBRs). J. Environ. Manage. 255. https://doi.org/10.1016/j.jenvman.2019.109850.

Bassin, J.P., Tavares, D.C., Borges, R.C., Dezotti, M., 2019. Development of aerobic granular sludge under tropical climate conditions: the key role of inoculum adaptation under reduced sludge washout for stable granulation. J. Environ. Manag. 230, 168–182. https://doi.org/10.1016/j.jenvman.2018.09.072.

Bin, Z., Zhe, C., Zhigang, Q., Min, J., Zhiqiang, C., Zhaoli, C., Junwen, L., Xuan, W., Jingfeng, W., 2011. Dynamic and distribution of ammonia-oxidizing bacteria communities during sludge granulation in an anaerobic-aerobic sequencing batch reactor. Water Res. 45, 6207–6216. https://doi.org/10.1016/j.watres.2011.09.026.

Buchauer, K., 1998. A comparison of two simple titration procedures to determine volatile fatty acids in influents to waste-water and sludge treatment processes. Water SA 24, 49–56.

Cadoret, F., Alou, M.T., Afouda, P., Traore, I.S., Bréchard, L., Michelle, C., Di Pinto, F., Andrieu, C., Delerce, J., Levasseur, A., Fournier, P.E., Raoult, D., 2017. Noncontiguous finished genome sequences and description of bacillus massiliglaciei, bacillus mediterraneensis, bacillus massilinigeriensis, bacillus phocaeensis and bacillus tuaregi, five new species identified by culturomics. New Microbes New Infect. 19, 45–59. https://doi.org/10.1016/j.nmni.2017.04.005.

Cetin, E., Karakas, E., Dulekgurgen, E., Ovez, S., Kolukirik, M., Yilmaz, G., 2018. Effects of high-concentration influent suspended solids on aerobic granulation in pilot-scale sequencing batch reactors treating real domestic wastewater. Water Res. 131, 74–89. https://doi.org/10.1016/j.watres.2017.12.014.

Chen, H., Li, A., Cui, D., Cui, C., Ma, F., 2019. Evolution of microbial community and key genera in the formation and stability of aerobic granular sludge under a high organic loading rate. Bioresour. Technol. Rep. 7, 100280 https://doi.org/10.1016/j.biteb.2019.100280.

Derlon, N., Wagner, J., da Costa, R.H.R., Morgenroth, E., 2016. 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, 341–350. https://doi.org/10.1016/j.watres.2016.09.007.

Fan, X.Y., Gao, J.F., Pan, K.L., Li, D.C., Zhang, L.F., Wang, S.J., 2018. Shifts in bacterial community composition and abundance of nitrifiers during aerobic granulation in two nitrifying sequencing batch reactors. Bioresour. Technol. 251, 99–107. https://doi.org/10.1016/j.biortech.2017.12.038.

Ha, S.J., Galazka, J.M., Joong Oh, E., Kordić, V., Kim, H., Jin, Y.S., Cate, J.H.D., 2013. Energetic benefits and rapid cellobiose fermentation by Saccharomyces cerevisiae expressing cellobiose phosphorylase and mutant cellodextrin transporters. Metab. Eng. 15, 134–143. https://doi.org/10.1016/j.ymben.2012.11.005.

He, Q., Chen, L., Zhang, S., Chen, R., Wang, H., Zhang, W., Song, J., 2018. Natural sunlight induced rapid formation of water-born algal-bacterial granules in an aerobic bacterial granular photo-sequencing batch reactor. J. Hazard. Mater. 359, 222–230. https://doi.org/10.1016/j.jhazmat.2018.07.051.

Iorhemen, O.T., Zaghloul, M.S., Hamza, R.A., Tay, J.H., 2020. Long-term aerobic granular sludge stability through anaerobic slow feeding, fixed feast-famine period ratio, and fixed SRT. J. Environ. Chem. Eng. 8, 103681 https://doi.org/10.1016/j.jece.2020.103681.

de Kreuk, M.K., Kishida, N., van Loosdrecht, M.C.M., 2007. Aerobic granular sludge - state of the art. Water Sci. Technol. 55, 75–81. https://doi.org/10.2166/wst.2007.244.

Larsen, P., Nielsen, J.L., Svendsen, T.C., Nielsen, P.H., 2008. Adhesion characteristics of nitrifying bacteria in activated sludge. Water Res. 42, 2814–2826. https://doi.org/10.1016/j.watres.2008.02.015.

Layer, M., Villodres, M.G., Hernandez, A., Reynaert, E., Morgenroth, E., Derlon, N., 2020. Limited simultaneous nitrification-denitrification (SND) in aerobic granular sludge systems treating municipal wastewater: Mechanisms and practical implications. Water Res. https://doi.org/10.1016/j.wroa.2020.100048.

Lema, J.M., Martinez, S.S., 2017. Innovative Wastewater Treatment \& Resource Recovery Technologies: Impacts on Energy, Economy and Environment. IWA Publishing.

Liu, Y.Q., Moy, B., Kong, Y.H., Tay, J.H., 2010. Formation, physical characteristics and microbial community structure of aerobic granules in a pilot-scale sequencing batch reactor for real wastewater treatment. Enzym. Microb. Technol. 46, 520–525. https://doi.org/10.1016/j.enzmictec.2010.02.001.

Liu, J., Li, J., Wang, X., Zhang, Q., Littleton, H., 2017. Rapid aerobic granulation in an SBR treating piggery wastewater by seeding sludge from a municipal WWTP. J. Environ. Sci. (China) 51, 332–341. https://doi.org/10.1016/j.jes.2016.06.012.

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

Mendes Barros, A.R., Argenta, T.S., de Amorim de Carvalho, C., da Silva Oliveira, F., Milen Firmino, P.I., Bezerra dos Santos, A., 2021. Effects of the antibiotics trimethoprim (TMP) and sulfamethoxazole (SMX) on granulation, microbiology, and performance of aerobic granular sludge systems. Chemosphere 262. https://doi.org/10.1016/j.chemosphere.2020.127840.

Meng, J., Li, Jiuling, Li, Jianzheng, Antwi, P., Deng, K., Wang, C., Buelna, G., 2015. Nitrogen removal from low COD/TN ratio manure-free piggery wastewater within an upflow microaerobic sludge reactor. Bioresour. Technol. 198, 884–890. https://doi.org/10.1016/j.biortech.2015.09.023.

Nagarajan, D., Kusmayadi, A., Yen, H., Dong, C., Lee, D., 2019. Current advances in biological swine wastewater treatment using microalgae-based processes. Bioresour. Technol. 289, 121718 https://doi.org/10.1016/j.biortech.2019.121718.

Nor-Anuar, A., Ujang, Z., Van Loosdrecht, M.C.M., De Kreuk, M.K., Olsson, G., 2012. Strength characteristics of aerobic granular sludge. Water Sci. Technol. 65, 309–316. https://doi.org/10.2166/wst.2012.837.

Oliveira, J.F., Fia, R., Fia, Fátima Resende, Luiz Rodrigues, F.N., de Matos, M.P., Siniscalchi, L.A.B., 2020. Principal component analysis as a criterion for monitoring variable organic load of swine wastewater in integrated biological reactors UASBSABF and, 262. https://doi.org/10.1016/j.jenvman.2020.110386.

Oliveira, J.F., Fia, R., Rodrigues, F.N., Fia, F.R.L., de Matos, M.P., Siniscalchi, L.A.B., Sanson, A.L., 2020. Quantification, removal and potential ecological risk of emerging contaminants in different organic loads of swine wastewater treated by integrated biological reactors. Chemosphere 260. https://doi.org/10.1016/j.chemosphere.2020.127516.

Oliveira, M.G., Marques Mourão, J.M., Souza Silva, F.S., Bezerra dos Santos, A., Lopes Pereira, E., 2021. Effect of microaerophilic treatment on swine wastewater (SWW) treatment: engineering and microbiological aspects. J. Environ. Manag. 299, 113598 https://doi.org/10.1016/j.jenvman.2021.113598.

Rollemberg, S.L.S., Mendes Barros, A.R., Milen Firmino, P.I., Bezerra dos Santos, A., 2018. Aerobic granular sludge: cultivation parameters and removal mechanisms. Bioresour. Technol. 270, 678–688. https://doi.org/10.1016/j.biortech.2018.08.130.

Rollemberg, S.L.de S., de Oliveira, L.Q., Barros, A.R.M., Melo, V.M.M., Firmino, P.I.M., dos Santos, A.B., 2019. Effects of carbon source on the formation, stability,

bioactivity and biodiversity of the aerobic granule sludge. Bioresour. Technol. 278, 195–204. https://doi.org/10.1016/j.biortech.2019.01.071.
Schwarzenbeck, N., Borges, J.M., Wilderer, P.A., 2005. Treatment of dairy effluents in an aerobic granular sludge sequencing batch reactor. Appl. Microbiol. Biotechnol. 66, 711–718. https://doi.org/10.1007/s00253-004-1748-6.
Silva, A.S., Sales Morais, N.W., Maciel Holanda Coelho, M., Lopes Pereira, E., Bezerra dos Santos, A., 2020. Potentialities of biotechnological recovery of methane, hydrogen and carboxylic acids from agro-industrial wastewaters. Bioresour. Technol. Rep. 10, 100406. https://doi.org/10.1016/j.biteb.2020.100406.
Tay, J.H., Liu, Q.S., Liu, Y., 2001. The role of cellular polysaccharides in the formation and stability of aerobic granules. Lett. Appl. Microbiol. 33, 222–226. https://doi.org/10.1046/j.1472-765X.2001.00986.x.
Wang, H., Song, Q., Wang, J., Zhang, H., He, Q., Zhang, W., Song, J., Zhou, J., Li, H., 2018. Simultaneous nitrification, denitrification and phosphorus removal in an aerobic granular sludge sequencing batch reactor with high dissolved oxygen: Effects of carbon to nitrogen ratios. Sci. Total Environ. 642, 1145–1152. https://doi.org/10.1016/j.scitotenv.2018.06.081.
Wang, S., Zheng, D., Wang, Shuang, Wang, L., Lei, Y., Xu, Z., Deng, L., 2018. Remedying acidification and deterioration of aerobic post-treatment of digested effluent by using zero-valent iron. Bioresour. Technol. 247, 477–485. https://doi.org/10.1016/j.biortech.2017.09.078.
Yu, Z., Zhang, Y., Zhang, Z., Dong, J., Fu, J., Xu, X., Zhu, L., 2020. Enhancement of PPCPs removal by shaped microbial community of aerobic granular sludge under condition of low C/N ratio influent. J. Hazard. Mater. 394, 122583 https://doi.org/10.1016/j.jhazmat.2020.122583.
Zhang, C., Zhang, H., Yang, F., 2015. Diameter control and stability maintenance of aerobic granular sludge in an A/O/A SBR. Sep. Purif. Technol. 149, 362–369. https://doi.org/10.1016/j.seppur.2015.06.010.
Zhang, D.M., Teng, Q., Zhang, D., Jilani, G., Ken, W.M., Yang, Z.P., Alam, T., Ikram, M., Iqbal, Z., 2021. Performance and microbial community dynamics in anaerobic continuously stirred tank reactor and sequencing batch reactor (CSTR-SBR) coupled with magnesium-ammonium-phosphate (MAP)-precipitation for treating swine wastewater. Bioresour. Technol. 320, 124336 https://doi.org/10.1016/j.biortech.2020.124336.
Zhu, L., Zhou, J., Yu, H., Xu, X., 2015. Optimization of hydraulic shear parameters and reactor configuration in the aerobic granular sludge process. Environ. Technol. (United Kingdom) 36, 1605–1611. https://doi.org/10.1080/09593330.2014.998717.