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Effect of microaerophilic treatment on swine wastewater (SWW) treatment: Engineering and microbiological aspects

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ABSTRACT

The microaerobic process on swine wastewater (SWW) treatment was investigated, evaluating its effect on organic matter hydrolysis and removal, biogas production, operational stability, and microbial community structure. UASB reactors operating under higher organic loading rates (OLRs) and lower hydraulic retention times (HRTs) than those found in the SWW treatment literature were also assessed. The microaerophilic reactor R2 presented a higher total and particulate organic matter removals and operational stability than the anaerobic reactor R1, reaching COD_P removals of 79.4 ± 4.6%. In the specific methanogenic activity (SMA) tests, the microaerobic sludge (R2) showed hydrolytic and acetogenic/methanogenic activity superior to inoculum and anaerobic sludge (R1). The microbiological evaluation of R2 revealed the high presence of hydrolytic microorganisms, therefore justifying the higher hydrolytic activity found in the SMA tests and higher particulate organic matter removal found in the microaerobic reactor.

1. Introduction

Pig farming has a fundamental role in Brazilian and global agriculture. According to the United Nations Food and Agriculture Organization (FAO), pork is the most consumed meat globally, corresponding to 32% of animal consumption protein worldwide, which resulted in a production of 109×10^6 tons in 2019 (FAO, 2020). The pig production increase results in a proportional increase in swine wastewater (SWW) since each pig generates 4.0–8.0 L of SWW per day (Nagarajan et al., 2019).

According to Morais et al. (2020), SWW is characterized by high concentrations of organic matter in the form of Total (COD_T) and Particulate (COD_P) Chemical Oxygen Demand (COD), with average values of 18.7 and 15 $gO_2\ L^{-1}$, respectively. The COD_P/COD_T ratio indicates that approximately 80% of the organic matter is in particulate form, constituting a complex substrate, and demanding a high hydrolysis activity (Li et al., 2019; Oliveira et al., 2020; Chen et al., 2021). Studies report that SWW is also rich in nutrients (nitrogen and phosphorus) and alkalinity, not requiring supplementation upon the anaerobic treatment (Cheng et al., 2020).

The anaerobic digestion (AD) follows a series of interdependent biochemical steps (hydrolysis, acidogenesis, acetogenesis, and methanogenesis/sulfetogenesis), in which a certain product is fundamental to sustain the following stage (Cremonez et al., 2021). Anaerobic technologies are already recognized as of great importance among pig farmers due to the low cost and the production of methane-rich biogas that has been used to generate electricity and heat in pig farms (Cheng et al., 2018). 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., 2020).

However, a major problem when using AD in treating complex substrates such as SWW is linked to the hydrolysis stage, which involves the transformation of complex organic particulate compounds into simple soluble compounds. Usually, this is the limiting step of AD when treating particulate substrates, which affects the production of fatty acids (propionate, butyrate etc.), acetate, hydrogen, and, consequently, methane (Jin et al., 2021). Thus, strategies aimed at increasing the hydraulic retention time (HRT) and, consequently, reducing the organic loading rate (OLR) applied to anaerobic reactors have been carried out in an attempt to facilitate hydrolysis during SWW anaerobic treatment (Ruan et al., 2019). In this context, some studies on SWW treatment in

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UASB reactors were conducted considering two-stage processes, values of HRT above 16 h and OLR below 10 kgCOD m^{-3} d^{-1}, aiming to avoid overloads and promoting an efficient COD_P hydrolysis (Ramires and Oliveira, 2014; Oliveira et al., 2020). However, from the economic point of view, strategies to operate anaerobic processes with lower HRT and higher OLR are necessary (Lim et al., 2016; Pramanik et al., 2019).

Microaeration has already been used in studies to increase particulate organic matter hydrolysis during wastewater treatment (Lim et al., 2014; Xu et al., 2014; Zhou et al., 2021), characterized by injecting small doses of oxygen into anaerobic processes, such as UASB reactors. Therefore, it does not require an additional processing unit, avoiding additional installation and operating costs. The oxygen sources are atmospheric air (usually) or pure oxygen, with doses varying from 0.005 to 5 LO_2 L_{feed}^{-1} d^{-1} depending on purpose treatment and substrate composition (Nguyen and Khanal, 2018). The microaerobic environment can also be assessed through the system's redox potential monitoring, ranging from 0 to −300 mV (Cheng et al., 2020). However, to date, no published studies have evaluated the microaerobic process to increase the rate of hydrolysis and methane production of UASB reactors treating SWW.

Therefore, this work aimed to investigate the microaerobic process on swine wastewater treatment, evaluating its effect on organic matter hydrolysis and removal, biogas production, operational stability, and microbial community structure. UASB reactors operating under higher OLRs and lower HRTs than those found in the SWW treatment literature were also assessed.

2. Materials and methods

2.1. Experimental set-up

The raw SWW used throughout the experiment came from the pig bays cleaning. The pigs were in many development stages throughout the experiment and were fed with corn and soybean-based food. All the zootechnical control was performed by the Zootechny Department (DZO) of the Federal University of Ceará (UFC) in Fortaleza, Brazil. The raw SWW was subjected to a preliminary treatment in a 2 mm square mesh sieve to separate coarse solids, simulating the condition found in full-scale treatment plants. After the preliminary treatment, the wastewater was sent to the Sanitation Laboratory (Labosan), also at the UFC, where the experiments were located.

The SWW was placed in an equalization tank (ET) with mechanical agitation to avoid solids sedimentation, kept under refrigeration at 4 °C to avoid natural biodegradation of organic matter, which impacts the loading rates. The reactors were fed with the SWW by two peristaltic pumps (ColeParmer MasterFlex L/S 7522-30, USA).

Reactor 1 (R1) operated as a traditional UASB reactor. Reactor 2 (R2) was built with the same dimensions and material as R1 but was microaerated with synthetic air (80% N_2:20% O_2, White Martins, Brazil) at its basis through a mass flow controller (GFC17, Cole-Parmer, USA).

Reactors R1 (UASB) and R2 (upflow microaerobic sludge blanket, UMSB) were inoculated with sludge from a full-scale UASB reactor used in a sewage treatment plant located in Fortaleza, Brazil. Both reactors were inoculated with 1.6 L of sludge, representing about 50% of each reactor's useful volume. The concentrations of total solids (TS), total volatile solids (TVS), and total fixed solids (TFS) in the inoculum sludge were 44.3 ± 2.5, 29.5 ± 1.4, and 14.7 ± 1.1 g L^{-1}, respectively.

2.2. Start-up and experimental procedure

The start-up occurred with a SWW average COD of 5 g L^{-1} and flow rate (Q) of 4.5 mL min^{-1}, resulting in: biological organic rate (BOR) of 0.7 kgCOD $kgVS^{-1}$ d^{-1}, OLR of 10.4 ± 0.9 kgCOD m^{-3} d^{-1}, volumetric hydraulic load (VHL) of 2 m^3 m^{-3} d^{-1}, and HRT of 12 h, identical for both reactors. The values of the influent COD, Q, OLR, VHL, and HRT were kept constant during the 95 days of the experiment.

The air dose of the micro-aeration used in R2 was approximately 2.0 L_{air} $feed^{-1}$ d^{-1}, corresponding to 0.5 LO_2 L_{feed}^{-1} d^{-1}, maintained constant during the experimental period. The microaeration dose was chosen according to the range for increased hydrolysis established by Nguyen and Khanal (2018).

2.3. Chemical analysis

COD (total, particulate, and soluble), $DBO_5^{20°C}$ (total, particulate, and soluble), pH, total solids (TS), fixed solids (FS), total suspended solids (TSS), volatile solids (VS), volatile suspended solids (VSS), total Kjeldahl nitrogen (TKN), $N–NH_4^+$, total phosphorus (TP), PO_4^{3-}, SO_4^{2-} and S^{2-} were determined by APHA (2012). Total alkalinity (TA) and volatile fatty acids (VFA) were determined by the Kapp titrimetric method (Buchauer, 1998).

The quantification of CH_4, CO_2, H_2, and H_2S in the biogas were determined by gas chromatography with ionization detection by dielectric barrier discharge (GC BID-2010 Plus, Shimadzu Corporation, Japan), equipped with GS-GASPRO column (60 m × 0.32 mm) (Agilent Technologies Inc., USA). Helium gas was used as the carrier gas (White Martins LTDA, Brazil) at a flow rate of 2 mL min^{-1}, with a run time of 9 min. Oven, injector, and detector temperatures were 50, 100, and 250 °C, respectively. O_2 and N_2 were quantified by gas chromatography with thermal conductivity detection (GC-TCD) (GC-17A, Shimadzu Corporation, Japan). The biogas sample (1.0 mL) was injected in splitless mode, and chromatographic separation was performed on a Mol Sieve 5A PLOT column (30 m, 0.32 mm ID) (Restek Corporation, USA). Oven, injector, and detector temperatures were 35, 40, and 230 °C, respectively. Helium (White Martins, Brazil) was used as the carrier gas at a flow rate of 7 mL min^{-1} and the run time was 5 min.

2.4. Quantitative monitoring of sludge at R1 and R2 and of the biological activity of inoculum, R1, and R2 sludge

The sludge monitoring of reactors R1 and R2 was carried out by comparing the seed sludge with the biomass (40 mL) collected at the end of the experimental period (95 days) in each sampler installed. Part of the sample was used to evaluate VS concentration profile along R1 and R2 heights, allowing the differentiation between the sludge blanket and sludge bed and achieving the solids production (P) and solids yield (Y) in the system. The rest of the sludge collected was homogenized and used in specific methanogenic activity (SMA) tests, carried out using three substrates (starch, glucose, and VFA mixture) individually.

Starch was used as a representative of complex substrates reflecting the sludge's hydrolytic activity based on the amount of methane produced. Glucose was used as an intermediate substrate allowing the metabolic activity of fermenting microorganisms (acidogenic), syntrophic (acetogenic), and methane producers (methanogenic). Thus, both the use of starch and glucose allowed evaluating the anaerobic consortium activity more deeply. The volatile fatty acids (VFA) solution used was composed of acetic (C2), propionic (C3), and butyric (C4) acids, resulting in a proportion of 24.3: 34.4: 41.3%, respectively, in terms of COD. This VFA mixture was used to evaluate the methanogenic archaea's activity and the system's syntrophic capacity.

The SMA assay was performed following the methodology described by (Angelidaki et al., 2009). Batch bioreactors (borosilicate vials) were used, with an internal volume of 110 mL, 50 mL of reactional volume, and 60 mL of headspace. A substrate/microorganism ratio (S/M) of 0.5 gCOD gVS^{-1} was used, obtained using 2.5 gCOD L^{-1} as substrate (starch, glucose, or AGV mixture) and 5.0 gVS L^{-1} of sludge concentration. Macro and micronutrients, and sodium bicarbonate (1 g L^{-1}) were added to the substrate to compose the basal medium. Each sludge (inoculum, R1, and R2) was tested separately with the selected substrates, and three repetitions were performed. Endogenous control (sludge and basal medium, without carbon source) was tested for each sludge (inoculum, R1, and R2). All bioreactors were sealed with butyl

rubber stoppers and purged with nitrogen (N_2) for approximately 1 min to establish an anaerobic atmosphere inside the flasks. Then, they were placed in a shaker-type incubator (MA420, Marconi LTDA, Brazil) under orbital agitation of 150 rpm and temperature of 35 ± 0.3 °C for 28 days, a period necessary to observe stabilization in biogas production.

The reactors' volumetric biogas production monitoring was performed through the headspace pressures using a gauge pressure transmitter (Warme LTDA, Brazil). The pressures were verified on days 1, 2, 4, 7, 14, 21, and 28, and these values were converted into volumetric biogas production (in mL). At the end of the test, the biogas produced inside the flasks were submitted to gas chromatography analysis to quantify CH_4, CO_2, N_2, H_2, and H_2S gases by the methods described in item 2.3. The calculation of SMA in terms of $kgCODCH_4\ kgVS^{-1}\ d^{-1}$ followed the procedures described in Angelidaki et al. (2009).

2.5. Microbial diversity

At the beginning and end of the experiment, sludge samples of inoculum, R1, and R2 were collected after 95 days of operation to analyze the microbial changes. For this purpose, 40 mL of sludge was collected in sterile flasks. For R1 and R2, four volumes of 10 mL were collected from different reactor heights, characterizing a biomass composite sample. The identification of the present microorganisms took place according to the species diversity index methodology in which the DNA sequencing of the gene 16S rRNA was performed. The DNA extraction was done in triplicate for each sludge sample and then sequenced by an Illumina MiSeq Desktop Sequencer. The molecular biology analyses were performed at the Center for Genomics and Bioinformatics (CeGenBio) of the Center for Research and Development of Drugs (NPDM) of the Federal University of Ceará, Brazil. All procedures were based on the methodology used by Rollemberg et al. (2019).

2.6. Calculation methods and statistical analysis

The results were divided into two stages called acclimatization and stationary state. It was considered a stationary state the date on which the R1 (statistical control) presented COD's removal efficiency with consecutive variations of less than 5%. The whole period that preceded the stationary state was denominated as acclimatization. The data obtained in the parameters' monitoring for the influent and effluent of R1 and R2 in the acclimatization stage (12 data) and the stationary state (11 data) were treated with descriptive statistics using Microsoft Excel.

Therefore, 23 samples were collected over the 95 days experimental period, resulting in an average of 1 sample for every 4.2 days. It is worth mentioning that this value is in the range of many reports in the literature for continuous-flow experiments (Oliveira et al., 2020; Jiang et al., 2019), and the amount of data was enough for the statistical tests performed.

The removal efficiency values of COD_T, COD_P, BOD_T, DBO_P, and VSS were submitted to a statistical test to compare means between two independent samples, called Student's T-test at a 5% significance level, considering R1 as the control group and R2 as the experimental group. The results were expressed with lowercase letters next to the means, with different letters indicating a statistically significant difference with a 95% confidence interval ($p \leq 0.05$). In contrast, equal letters indicate that the statistical difference was not significant ($p > 0.05$). The T-test was performed using Sisvar software version 5.6 (Ferreira, 2014).

An Analysis of Variance (ANOVA) followed by a statistical test to compare means (Tukey's test) at a 5% significance level, comparing within the same substrate (starch, glucose, and VFA mixture), the SMA of each sludge. The three repetitions performed for each sludge within the same substrate were used to calculate the mean. The results were expressed with lowercase letters next to the means, with different letters indicating a statistically significant difference with a 95% confidence interval ($p \leq 0.05$). In contrast, equal letters indicate that the statistical difference was not significant ($p > 0.05$). The ANOVA and Tukey test were performed using Sisvar software version 5.6 (Ferreira, 2014).

3. Results and discussion

3.1. Operating performance

The nutrients present in SWW were quantified during the experiment (Table 1) to assess whether SWW offered the nutritional requirements for anaerobic treatment. P_T removal efficiencies were 60.3 ± 16.4% and 70.0 ± 12.1% for R1 and R2, respectively. According to Stazi and Tomei (2018), these values are considered high for anaerobic processes, whose efficiencies are mainly linked to TSS removal (Table 2). Therefore, physical removal was the most important process for reducing P_T concentrations, as also occurred in the study of Urbinati and Oliveira (2014). It should be noted that the greater P_T removal in the microaerobic reactor R2 was mainly due to organic phosphorus removal, as can be seen in the higher percentage of $P\text{-}PO_4^{3-}$ in relation to P_T. This fact should possibly be linked to the higher hydrolysis of the SWW particulate fraction.

The AD and the biogas' quality depend directly on the substrate C/N ratio, in which a high value can lead to the accumulation of organic acids and a consequent significant decrease in pH, making the environment unsuitable for methanogenic archaea. On the other hand, the low C/N ratio leads to nitrogen accumulation, especially ammonia, increasing the effluent's pH and exerting a toxic effect on the methanogenic microorganisms (Wang et al., 2012). The effluent C/N ratio was on average 20.4 ± 8.0, in which an optimal ratio between organic matter and nitrogen of 20–35:1 for anaerobic digestion and methane production is reported in the literature (Kainthola et al., 2019; Pang et al., 2017). Unlike the P_T, there were no significant variations in the forms of nitrogen present in the raw SWW and reactors' effluent. Despite the ammonification in both reactors, the average concentration of ammoniacal nitrogen was below 1700 mg L^{-1}, the threshold value for methanogenesis inhibition (Chen et al., 2008).

Sulfate found in SWW comes mostly from the degradation of proteins used in animal feed, soybean meal in the present work. This SO_4^{2-} during the SWW can be reduced to sulfide via sulfate reduction. Sulfide can stay in the dissolved (S^{2-}) or gaseous (H_2S) forms, depending on the pH, pressure inside the reactor, temperature, amongst others. Sulfate reduction is a competitive process of methanogenesis that can lead to reactor acidification (Li et al., 2019). The biogas analysis did not show H_2S, and dissolved sulfide (S^{2-}) was never detected in the reactors' effluent. Therefore, sulfate reduction was not remarkable in the reactors, as low or negative values of efficiencies were found, likely due to the low sulfate concentration in the influent. According to Cruz-Salomón et al. (2017), sulfate reduction is favored when the COD/SO_4^{2-} ratio in the wastewater is lower than 10 but with strong pH dependence. In this study, the COD/SO_4^{2-} ratio was 313, which justifies the low sulfate reduction achieved.

From day 0–53, consecutive variations between COD_T removal efficiency values greater than 5% were observed, characterizing the acclimatization phase, with 12 monitoring data for R1 and R2. From day 56th to 95th, consecutive variations of less than 5% were observed, characterizing the stationary-state phase, with 11 monitoring data. Besides minor variations in the parameters' concentrations, only after 53 days of operation did methane begin to be detected in biogas analyses. The

Table 1
Average concentrations of nutrients in the influent and reactors effluent.

Parameter	SWW	R1 effluent	R2 effluent
P_T (mg L^{-1})	189.0 ± 96.6	76.0 ± 30.9	57.0 ± 33.9
$P\text{-}PO_4^{3-}$ (mg L^{-1})	26.3 ± 12.0	23.0 ± 12.1	25.4 ± 8.8
TKN (mg L^{-1})	260.1 ± 121.4	220.1 ± 126.9	157.9 ± 53.5
$N\text{–}NH_4^+$ (mg L^{-1})	101.7 ± 63.8	143.9 ± 48.9	122.3 ± 43.6
SO_4^{2-} (mg L^{-1})	16.1 ± 16.4	21.0 ± 16.5	22.1 ± 8.0

Table 2
Parameters analyzed in the acclimatization and stationary phases.

Parameters	SWW	Acclimatization		Stationary	
		R1	R2	R1	R2
pH	7.2 ± 0.2	7.6 ± 0.2	7.6 ± 0.3	8.0 ± 0.2	7.9 ± 0.1
TA ($mgCaCO_3$ L^{-1})	924.1 ± 430.5	886.6 ± 354.7	838.1 ± 416.4	1112.7 ± 238.6	992.6 ± 186.0
VFA ($mgCH_3COOH$ L^{-1})	1056.6 ± 501.7	441.5 ± 392.3	319.8 ± 227.5	394.3 ± 127.8	398.0 ± 76.7
COD_T (mgO_2 L^{-1})	5151.3 ± 255.6	1015.4 ± 257.1	851.3 ± 220.9	1755.7 ± 150.9	1295.9 ± 173.1
COD_P (mgO_2 L^{-1})	3977.7 ± 504.6	566.9 ± 283.2	517.7 ± 208.7	1406.8 ± 160.4	766.9 ± 151.8
COD_S (mgO_2 L^{-1})	1173.6 ± 434.1	448.4 ± 103.2	333.6 ± 126.0	348.9 ± 48.7	528.9 ± 164.7
COD_T (mgO_2 L^{-1})	2903.2 ± 638.6	728.2 ± 379.7	776.4 ± 322.4	1095.4 ± 225.5	701.2 ± 329.4
COD_P (mgO_2 L^{-1})	2231.3 ± 581.8	430.3 ± 310.0	567.6 ± 261.2	933.8 ± 204.3	472.7 ± 321.2
COD_S (mgO_2 L^{-1})	671.8 ± 261.8	297.9 ± 160.7	208.8 ± 108.9	161.2 ± 65.5	228.6 ± 60.1
TS (mg L^{-1})	4643.6 ± 636.9	1995.1 ± 632.9	1821.2 ± 591.2	2751.8 ± 557.7	2025.5 ± 285.0
TSS (mg L^{-1})	3185.7 ± 719.9	971.1 ± 519.2	918.7 ± 526.9	1681.8 ± 600.4	715.9 ± 338.8
VSS (mg L^{-1})	2387.9 ± 512.7	625.8 ± 333.7	584.0 ± 356.8	1069.1 ± 411.9	394.1 ± 189.5

monitoring data achieved in each phase for R1 and R2 are presented in Table 2.

Both in the acclimatization and stationary phases, the pH of the two reactors' effluent remained alkaline (above 7), with values that did not harm the anaerobic digestion and indicating that there was no VFA accumulation. A high VFA consumption was verified for both phases, resulting in 394.3 ± 127.8 and 398.0 ± 76.7 $mgCH_3COOH$ L^{-1} for R1 and R2 effluents, respectively. This VFA consumption, especially in the stationary phase, may also indicate a good balance between methanogenesis and the previous AD steps (Vrieze et al., 2012). As a result of anaerobic processes with high efficiency, as in the present experiment, there was a TA maintenance, which is important for operational stability.

The values of TA and VFA presented in Table 3 are within the range found in other studies that used anaerobic reactors on SWW treatment (Oliveira et al., 2020; Pereira et al., 2015). The maintenance of effluent pH of R1 and R2 above 7 probably occurred due to the excess of TA in relation to VFA, with VFA/TA ratios of 0.5 and 0.4 for R1 and R2, respectively, during the acclimatization phase; during the stationary phase, a VFA/TA ratio value of 0.4 was found for both reactors. The same effluent VFA/TA ratio values for both acclimatization and stationary phases suggest that the microaeration process can contribute to the biological system's buffering and stability. In both reactors, a greater stability (smaller standard deviations) in terms of pH, VFA, and TA was observed in the stationary phase. The stability of organic matter removal in this stage was also verified (Fig. 1).

As already exposed, the main challenge for SWW AD is the high concentration of complex organic matter in the form of COD_P, BOD_P, and VSS, which makes hydrolysis a limiting step. Fig. 1 shows the result of using the statistical test to compare means in two groups R1 and R2, using the data obtained during the stationary phase in relation to the removal efficiency of complex organic matter (VSS, COD_T, COD_P, BOD_T, and BOD_P).

Table 3
Sludge production in anaerobic and microaerobic reactors.

Sludge	P (gSS d^{-1})	Y (gSS $kgCOD_{apl}^{-1}$)	VS/TS
Inoculum	–	–	0.67
R1	0.209	6.32	0.68
R2	0.259	7.84	0.74

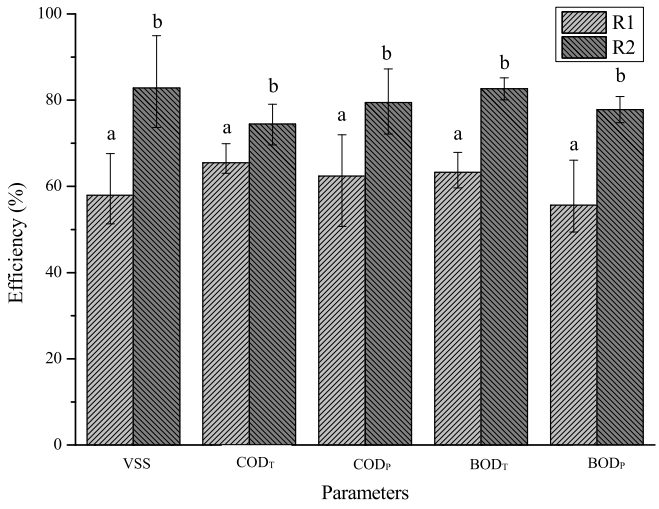

Fig. 1. Comparative analysis during stationary phase between R1 and R2 for complex organic matter removal.

The removal efficiency results of complex organic matter in the form of VSS, COD_P, and BOD_P reflect the process's efficiency (anaerobic or microaerobic) in hydrolyzing complex organic matter. As shown in Fig. 1 and from Supplementary Material (Table S1), with the analysis of variance (ANOVA), when comparing the removal efficiencies of these parameters obtained in R1 and R2, the results differ statistically ($p < 0.05$), confirming that the sludge hydrolytic activity adapted to anaerobic conditions is different from that adapted to microaerobic conditions. As the mean values of VSS, COD_P, and BOD_P removal efficiencies were higher at R2 than at R1, it can be stated that microaeration was the factor that increased the sludge hydrolytic activity.

During the stationary phase, R1 obtained a VSS removal efficiency of 57.9 ± 5.6%, while R2 removed 82.8 ± 6.2%. Another important point to highlight regarding the hydrolytic capacity is the effluent SS/TS ratio. The lower is this value, the higher is the amount of soluble compounds. The SS/TS ratios in R1 and R2 effluents were 0.60 ± 0.1 and 0.35 ± 0.1, indicating a higher fraction of soluble compounds in R2, corroborating for its higher hydrolysis. According to Lim et al. (2014), the increase in extracellular hydrolytic enzyme production from more abundant hydrolytic bacterial communities under microaerobic conditions improves the hydrolysis of carbohydrates, proteins, and other complex organic substrates.

Similar to solids removal, R2 obtained better results regarding organic matter removal and solubilization. The microaerobic reactor (R2) had mean removals in the stationary phase of 74.5 ± 3.2% (COD_T), 79.4 ± 4.6% (COD_P), 82.6 ± 2.1% (BOD_T), and 77.2 ± 2.4% (BOD_P). On the other hand, R1 achieved removals of 65.4 ± 1.9% (COD_T), 62.4 ± 5.4% (COD_P), 63.3 ± 3.9% (BOD_T), and 55.7 ± 7.2% (BOD_P). Therefore, R2 performance for organic matter removal was better, showing a higher particulate fraction removal. This can be confirmed by the effluent COD_S/COD_T ratios of 0.2 ± 0.0 and 0.4 ± 0.1 for R1 and R2, respectively. Therefore, the UMSB reactor enhanced 2-fold the dissolved organic fraction in the effluent.

Jeníček et al. (2017), when analyzing in a wastewater treatment plant in Central Europe the microaeration effect on biogas desulphurization, observed a greater presence of soluble COD and dissolved solids in the treated effluent. In another study with microaerobic treatment, Diak et al. (2013) reported greater COD solubilization due to the higher rate of hydrolysis of carbohydrates and proteins. Xu et al. (2014) also reported increased solubilization of digested organic waste in a leaching bed reactor (LBR) by applying a microaeration dose of 258 L_{air} $kgTS^{-1}$ d^{-1}. Finally, Lim and Wang (2013) applied a microaeration dose of 37.5 mLO_2 $L_{reactor}$ d^{-1} in the pre-treatment of co-digestion of brown water

and food waste, which increased the solubilization of the pre-digested, resulting in a 21% higher methane production.

When comparing the removal efficiencies shown in Fig. 2 with other reactors used in the anaerobic treatment of SWW, the R2 had a better organic matter removal efficiency. Duda et al. (2015), in an experiment with a horizontal anaerobic reactor operating under OLR of 12 kgCOD m^{-3} d^{-1}, obtained 47% COD removal. Yang et al. (2019) operated an upflow anaerobic sludge bed-filter (UBF) under an OLR 7.8 kgCOD m^{-3} d^{-1} with 75% COD removal.

Another relevant factor is that the environmental legislations usually use COD_T and BOD_T as discharge standards. Therefore, when comparing the removal efficiencies of COD_T and BOD_T obtained in the anaerobic reactor R1 (Fig. 2) with those obtained in UMSB reactor R2, the results differ statistically at 5% ($p < 0.05$). The average values found for R2 were higher than those found for R1, suggesting that the microaerophilic treatment was a more efficient process in removing all organic matter fractions.

3.2. Sludge and methane production

It is important to study biomass development to understand the evolution of reactors' performance. Table 3 shows the VS/TS ratio results and parameters that express the sludge's evolution: sludge production (P) and solids yield (Y).

As shown in Table 3, there was a greater increase in the VS/TS ratio of microaerobic sludge than anaerobic sludge. A higher VS/TS ratio indicates a higher sludge organic content, while an increase of the inorganic fraction may stop the organic matter removal (Zinare et al., 2019). Such fact can also explain the higher efficiency of organic matter removal of R2 compared to R1. Another important parameter to be analyzed is the sludge granules' formation. Besides the granulometry test, an indirect way to obtain the granule production rate is the sludge profile analysis (Fig. 2).

After 95 days of operation, an increase in VS concentration of both reactors' sludge was observed along with the reactors' height in all four sample points (Fig. 3), with emphasis on the highest concentrations of VS in the region of the first sample point (9 cm from the base). This increase in VS concentration indicates biomass growth and SS removal from the influent.

Fig. 3 shows the biogas volumetric production and composition obtained in the UASB (Fig. 3a) and UMSB (Fig. 3b) reactors. In R1, it took approximately 21 days to start methane production and 73 days to stabilize the biogas volume production with 70.1 ± 0.2% methane (Fig. 3a). Based on the UASB reactor's monitoring (Fig. 3a), it is possible to state that during the stabilization step, the R1 had an average daily production of 5.6 ± 3.0 L of methane and an average production rate of 1.1 $L CH_4$ $gCOD_{app}^{-1}$. From the 53rd day, the UASB reactor started to produce a biogas with methane content to be used as an energy source (Fig. 3a), as it must have at least 45% methane in its composition (Cruz-Salomón et al., 2017). Methane production in R1 started only in the stationary phase, reinforcing the hypothesis that in the first half of

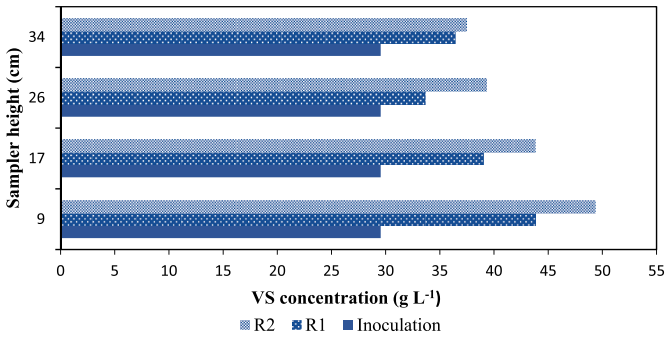

Fig. 2. Reactor profile evolution.

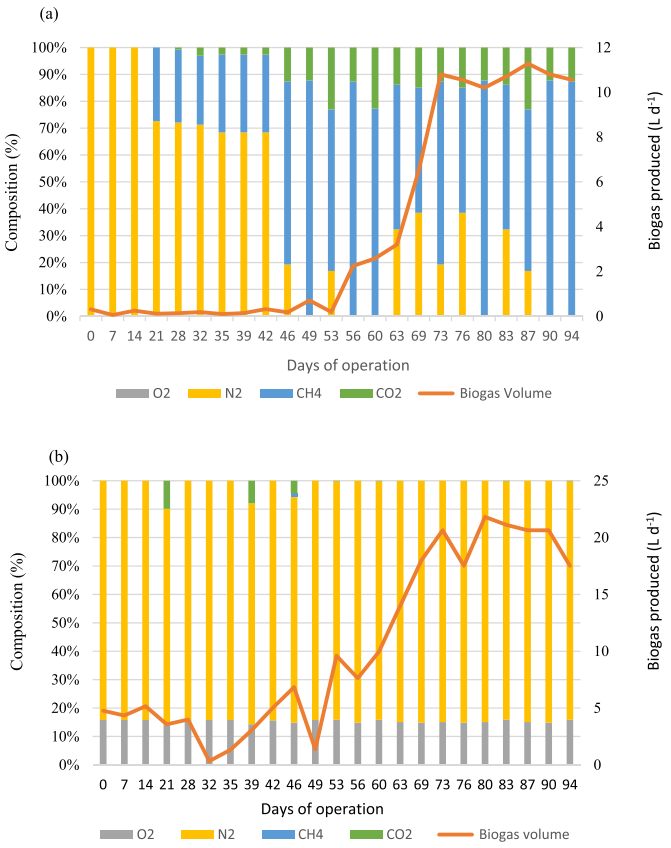

Fig. 3. Composition and volumetric biogas production from (a) R1 and (b) R2.

the experiment, most of the organic matter removal was occurring by solids retention and not microbial conversion (Fig. 3a).

The USMB reactor showed biogas productions of 4.12 ± 2.5 L d^{-1} and 17.2 ± 4.8 L d^{-1} during acclimatization and stationary phases, respectively (Fig. 3b). However, no methane gas was detected, which raised two hypotheses. One, a methanogenic archaea inhibition, in which VFA would accumulate in the system. However, such a hypothesis could not be sustained based on the results in Table 2 that indicated low VFA concentrations in the effluent and also based on the results found in Figs. 4–7 that proved the presence of methanogenic archaea and methanogenic activity in the USMB reactor sludge.

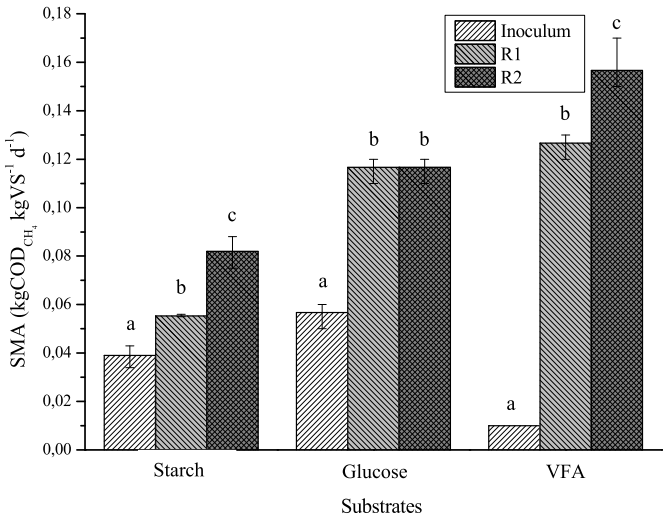

Fig. 4. Sludge specific mathanogenic activity (SMA) tests.

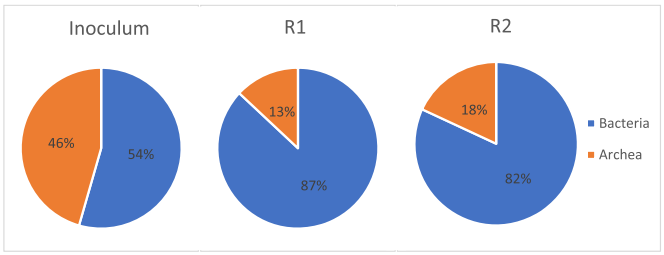

Fig. 5. Taxonomic distribution of DNA sequences (phylum level) found in inoculum (I) and reactors (R1 and R2) sludges.

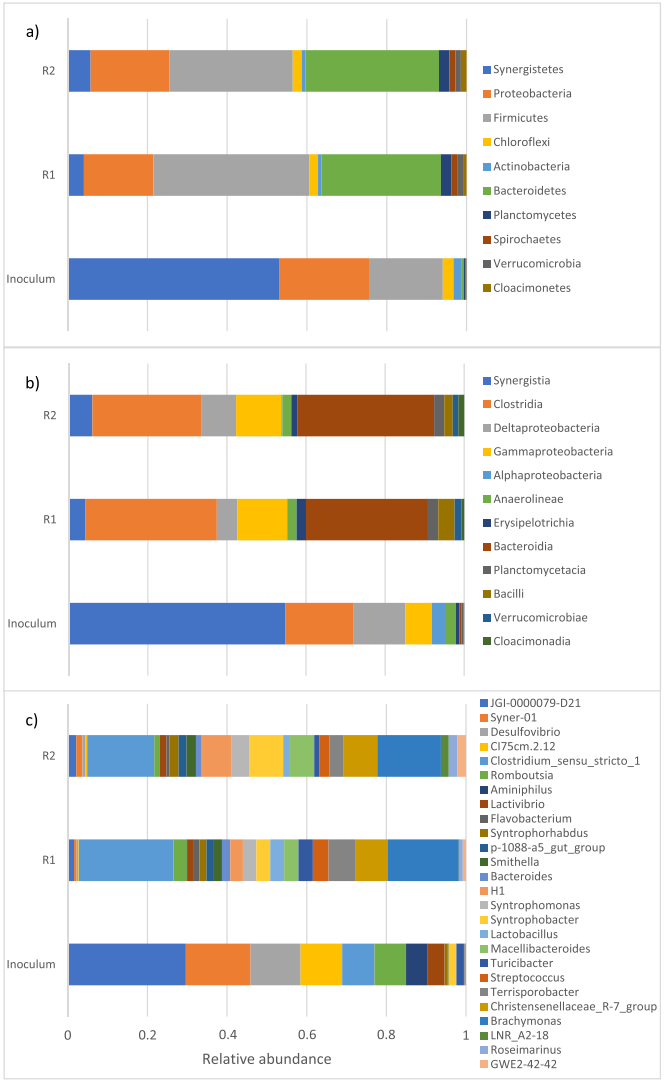

Fig. 6. Variation of bacterial community structure found in inoculum (I) and reactors (R1 and R2) sludges at the phylum (a), class (b) and genus level (c).

The second hypothesis would be the methane dilution by other gases since microaeration was being done with atmospheric air. Therefore, N_2 and O_2 concentrations in biogas were monitored (Fig. 4b). It was clear that indeed the CH_4 was not detected due to the dilution caused by microaeration. Therefore, studies evaluating other microaeration doses with atmospheric air must be carried out to combine the system's efficiency and methane content in the biogas.

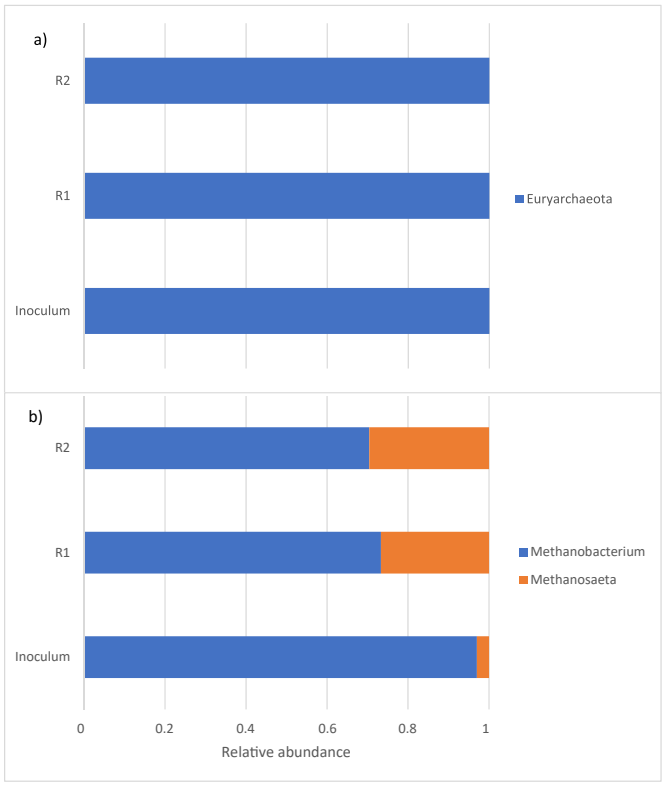

Fig. 7. Variation of archaeal community structure with reactor depth at the phylum (a) and genus (c) levels.

3.3. Biological activities of inoculum, anaerobic and microaerobic sludges

Analyzing the endogenous controls, methane concentration was below the method quantification limit, thus indicating that only the productions found in the bioreactors (Fig. 4) were referring to each substrate's conversions.

The SMA results with starch indicated a hydrolytic activity increase compared to the inoculum, for both anaerobic (R1) and microaeration (R2) conditions. The SMA value for R2 was 110% and 48% higher than the inoculum and anaerobic sludge R1, respectively. More specifically, the hydrolytic activity increased on average from 0.04 to 0.05 (R1) and to 0.08 $gCODCH_4$ (R2) gVS^{-1} d^{-1}.

The SMA results with glucose (Fig. 5) also showed an increase in the acidogenic activity of both anaerobic (R1) and microaerobic (R2) sludges compared to the inoculum. However, their SMA did not differ statistically.

In the presence of a VFA mixture, the SMA results showed that the activity increased drastically from 0.01 (inoculum) to 0.13 (R1) and 0.16 (R2) $gCODCH_4$ gVS^{-1} d^{-1}. Therefore, microaerobic treatment positively affected the syntrophic conversions and formation of the methanogenic substrate precursors, such as acetate, H_2/CO_2, formate etc., as further discussed in item 3.4. The increase in the bacteria diversity and activity is important for keeping VFA concentration low, promoting the DA process's overall stability (Nguyen and Khanal, 2018).

3.4. Diversity of microbiological communities

The relative abundance of bacteria and archaea at the phylum and genus levels of the inoculum, R1, and R2 sludges collected at the end of the experiment are shown in Figs. 5–7. The depth of sequencing of the three samples was all greater than 0.99, indicating that the samples effectively characterized microbial communities.

Fig. 5 illustrates the proportion of microorganisms belonging to the

Bacteria and Archaea domains. As shown, 54% of the microbiological community of the inoculum sludge was made up of phylum belonging to the Bacteria domain. In the samples from reactors R1 and R2, this presence increased to 87% and 82%, respectively.

This increase in bacteria presence probably occurs because bacteria perform the hydrolysis, acidogenesis, and acetogenesis of complex and simple compounds present in wastewater, requiring a variety of species. The amount of organic suspended solids in SWW further promoted the favoring of fermentative bacteria in reactors, justifying the low rate of hydrolysis and methane production at the beginning of the experiment. Duda et al. (2015) observed similar behavior when operating four horizontal anaerobic reactors for SWW treatment, with sludge samples being made up of 83.7–89.7% of microorganisms from the Bacteria domain.

3.4.1. Bacterial community structure

At the phylum level (Fig. 6a), Synergistetes, Proteobacteria, and Firmicutes were predominant in the inoculum sludge, representing 53.2%, 22.4%, and 18.5% of the bacterial gene sequences, respectively. These microbial groups are very common in sludge from UASB reactors treating domestic sewage, like the one used in the present investigation. In R1, there was a sudden decrease in the presence of Synergistetes, accompanied by an increase in the number of Firmicutes (39.1%), Bacteroidetes (30.0%), and Proteobacteria (17.4%). Bacteroidetes, Firmicutes, and Proteobacteria were the most abundant found in R2, with 33.5%, 31.0%, and 19.6%, respectively. The latter groups are recognized as important microorganisms for the anaerobic degradation of complex substrates, such as those present in swine wastewater (Ruan et al., 2019). The phylum Firmicutes includes many bacteria in the class Clostridia that are involved in the hydrolysis step, cellulose and organic compounds degradation, sometimes performing syntrophic reactions (Hao et al., 2016) with several different hydrogenotrophic methanogens (Wang et al., 2021; Song et al., 2010).

Proteobacteria is involved in the hydrolysis and acidogens steps and is usually present in the SWW because it is the most common group of microorganisms in the pig gastrointestinal tract and the farm environment. As such, the microbial community is usually composed of a high abundance of fermentative bacteria and a relatively low abundance of syntrophic bacteria and methanogens (Zeng et al., 2019). Bacteroidetes have a strong metabolic capacity to decompose protein, lipids, and other macromolecules into simple compounds (Yao et al., 2019). At the phylum level, Zhang et al. (2021) investigated a continuously stirred tank reactor and sequencing batch reactor (CSTR-SBR) on SWW treatment. They found Firmicutes (32.9%), followed by Bacteroidetes (26.5%), Proteobacteria (16.0%), Cloacimonetes (9.1%), and Spirochaetae (3.6%), as the main microbial groups, which agrees with our findings.

At the class level (Fig. 6b), Synergistia showed a relative abundance of 54.8% in the inoculum. In R1 sludge, there is a predominance of Clostridia (Firmicutes), Bacteroidia, and Gammaproteobacteria, with 33.2%, 30.8%, and 12.6% of species, respectively. These classes are related to sulfate-reducing bacteria and a variety of fermentative bacteria that metabolize short-chain fatty acids, sugars, and proteins, to form acetic acid (Hahnke et al., 2016). For R2, the classes Bacteroidia and Clostridia were the most abundant, with 34.6% and 33.2%, respectively. Among their representatives, there are sugar and protein fermenting microorganisms, of which many are facultative anaerobes (Hyun et al., 2014). Zhang et al. (2021) found that the dominant bacteria at the class level included Clostridia, Bacteroidia, Cloacimonetes, Bacteroidetes, Deltaproterobacteria, Gammaproteobacteria, Spirochaetes, and Sphingobacteria, which also agrees with our findings. Jiang et al. (2020), while investigating a high-rate anaerobic digestion of swine wastewater in an anaerobic membrane bioreactor, found Clostridia was the most abundant (44% at 2 days HRT and 61% at 1 day HRT). They are also reported to be involved in the oxidation of fatty acids in association with hydrogenotrophic methanogens (Song et al., 2010).

In terms of genus level (Fig. 6c), JGI-0000079-D21 (Synergistetes bacterium) and Syner-01 (fermentative bacteria Propionimicrobium) demonstrate an advantage in inoculum sludge, accounting, respectively, for 29.6% and 16.3% of the bacterial gene sequences. For the R1 and R2 sludge, the species belonging to *Clostridium sensu stricto 1* (class Clostridia) were the most abundant, representing 23.8% and 16.8% of the sequences, respectively. Members of the genus *Clostridium sensu stricto 1* can convert saccharides (such as glucose) as energy sources into acetate, butyrate, lactate, ethanol, H_2, and CO_2 (Bauchart-Thevret et al., 2009). *Clostridium sensu stricto* is always found in pig guts and is associated with dietary protein (Fan et al., 2017). Jiang et al. (2019) found that *Clostridium sensu stricto* was the dominant bacterium while studying SWW treatment on anaerobic conditions, which is in line with our results. Yang et al. (2019), while investigating the anaerobic digestion of SWW in a UBF reactor, also found the *Clostridium sensu scricto* as the most abundant, accounting for 20.0% of the sequences.

Another dominant genus was Brachymonas (class Gamaproteobacteria), presenting very close values of relative abundance for reactors R1 and R2, 17.8% and 16.0%, respectively. The genus Brachymonas denitrificans was reported in thermophilic anaerobic pre-treatment (TP) of primary sludge, although it is known as a mesophilic (30–35 °C) aerobic chemoorganotroph, capable of anaerobic denitrification and iron reduction. However, nitrate and iron levels in the anaerobic pre-treatment reactor were negligible, and oxygen was never detected in the reactor biogas (results not shown), suggesting other energy conservation reactions not characterized in B. denitrificans (Pervin et al., 2013).

Previous studies detected core anaerobic digestion (AD) populations related to known aerobic and facultative microorganisms, including Thauera, Brachymonas, and Rhodobacter (Wang et al., 2021; Pervin et al., 2013), which are also found in high relative abundance in activated sludge (Zhang et al., 2012) or aerobic granular sludge (Rollemberg et al., 2019). Their appearance on anaerobic microbiomes is likely due to incomplete digestion, in contrast to other core populations such as methanogens, syntrophs, and fermenters (Mei et al., 2017).

It was also observed the growth of syntrophic genera in the reactors, such as Syntrophobacter, Syntrophomonas, Syntrophorhabdus, and Smithella. Zeng et al. (2019) noticed, while studying the anaerobic treatment of SWW with exogenous granular sludge (EGS), at the genus level, the members of syntrophic bacteria displayed higher abundance after the acclimation. However, while they add up only 10.7% of the bacteria present in the sludge of R1, these genera represent 17.7% of the bacterial community of R2. They are essential to overcome the thermodynamic barriers in the anaerobic oxidation and degradation of various VFAs into acetate and hydrogen used by methanogenic archaea (Pramanik et al., 2019; Xu et al., 2018). This may also justify the greater removal of organic matter from R2 and the greater methane production during SMA tests with microaerobic sludge.

3.4.2. Archaea community structure

At the phylum level (Fig. 7a), Euryarchaeota, microorganisms known in biogas production, represented 100% of the archaeal community in the inoculum, R1, and R2 sludge. Yang et al. (2019) reported a minimum relative abundance of 92% of Euyarcheota in three different anaerobic reactors during the SWW treatment. Zhang et al. (2021) investigated a continuously stirred tank reactor and sequencing batch reactor (CSTR-SBR) on SWW treatment and found that Euryarchaeota (46.3%) and Cloacimonetes (40.0%) represented the most abundant phyla. As methanogenic archaea, Euryarchaeota has been reported as the most dominant phylum in wastewater treatment processes. On the other hand, Jiang et al. (2020), while investigating a high-rate anaerobic digestion of SWW in an anaerobic membrane bioreactor, found that the methanogenic community was dominated by the phyla Methanomicrobia and Methanobacteria.

The archaea distribution at the genera level (Fig. 7b) showed that

Methanobacterium represented 97.0% of the genera totality in the inoculum. It decreased along the experimental period to values of 73.3% and 70.4% in the reactors R1 and R2, respectively. The species belonging to the genus Methanobacterium are hydrogenotrophic methanogens, which use H_2/CO_2 and sometimes formate and alcohols as substrates for growth and methane production. Growth occurs under strictly anaerobic conditions, and most species are capable of autotrophic growth (Kern et al., 2015). Zeng et al. (2019), while studying the anaerobic treatment of SWW with indigenous granular sludge (IGS) and EGS at the genus level, also noticed a predominance of Methanobacterium, with a minimum relative abundance of 37.3%.

On the other hand, there was an increase of Methanosaeta in both reactors, to values of 26.7% (R1) and 29.6% (R2). Methanosaeta is a typical acetoclastic methanogen (Cheng et al., 2018). The increase in its presence in the reactor sludge may be due to the higher production of acetate, SWW characteristics, and the greater presence and activity of fermentative and syntrophic bacteria. Several studies about the anaerobic treatment of SWW reported Methanosaeta as a prevalent genus in the archaea community (Duda et al., 2015; Jiang et al., 2019, 2020; Yang et al., 2019; Zhang et al., 2021).

4. Conclusions

The microaerophilic reactor R2 presented a higher total and particulate organic matter removals and operational stability than the anaerobic reactor R1, reaching COD_P removals of 79.4 ± 4.6%. In the SMA tests, the microaerobic sludge (R2) showed hydrolytic and acetogenic/methanogenic activity superior to inoculum and anaerobic sludge (R1). The microbiological evaluation of R2 revealed the high presence of hydrolytic microorganisms, therefore justifying the higher hydrolytic activity found in the SMA tests and higher particulate organic matter removal found in the microaerobic reactor. This research highlights the possibility of success in the microaeration in SWW treatment plants that use one-stage (methanogenic) or two-stages (hydrolytic followed by methanogenic) anaerobic reactors.

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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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jenvman.2021.113598.

Sample credit author statement

Maurício Guimarães de Oliveira: Conceptualization, Investigation, Formal analysis, Writing, José Marcos Marques Mourão: Conceptualization, Investigation, Francisco Schiavon Souza Silva: Investigation, André Bezerra dos Santos: Writing – review & editing; Funding acquisition, Erlon Lopes Pereira: Supervision, Conceptualization, Investigation, Formal analysis, Writing

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