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Microaerophilic treatment enhanced organic matter removal and methane production rates during swine wastewater treatment: A long-term engineering evaluation

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ABSTRACT

Anaerobic biotechnology has been widely used for swine wastewater (SWW) treatment. However, its organic loading rate (OLR) is far lower than expected, mainly because of the low rate of hydrolysis. In this study, the comparative process performance and efficiency of an up-flow anaerobic sludge blanket (UASB) reactor (R1) and an up-flow microaerobic sludge blanket (UMSB) reactor (R2) were evaluated for SWW treatment, operating for 264 days under higher OLRs and lower hydraulic retention times than those found in the literature. The R2 was subjected to the three different air doses: 0.09 (stage I), 0.17 (stage II), and 0.25 (stage III) $L_{O2}\ L_{feed}^{-1}\ d^{-1}$ aiming at enhancing the hydrolysis step of the anaerobic digestion (AD). The overall results showed that 0.17 $L_{O2}\ L_{feed}^{-1}\ d^{-1}$ was the best experimental condition evaluated, which provided volatile suspended solids, total chemical oxygen demand, and particulate chemical oxygen demand removal efficiencies of 85.0 ± 1.9%, 83.8 ± 2.5%, and 82.1 ± 4.8%, respectively. This performance was due to the higher organic matter hydrolysis, resulting in higher methane production. Therefore, the UMSB reactor treatment was demonstrated to be a feasible alternative for SWW, although some strategies to control biomass washout must be investigated.

1. Introduction

Pork is the most consumed meat globally, accounting for 32% of animal consumption protein worldwide, which resulted in a production of 109 × 10^6 tons in 2019 [1]. Swine wastewater (SWW), resulting from washing the animals' confinement bays to remove feces, urine, and food scraps, is rich in suspended and dissolved organic matter, solids, and nutrients [2,3].

The anaerobic treatment process is one of the most commonly used technologies for treating SWW, especially anaerobic lagoons and high-rate reactors such as the up-flow anaerobic sludge blanket (UASB) reactors. Compared to the efficiencies observed in sewage treatment, the UASB reactor usually has a lower performance when treating effluents with high concentrations of particulate material, as is the case with SWW [4,5]. Thus, sometimes two-stage anaerobic processes are applied to remove organic matter more effectively: the first being hydrolytic/acidogenic, and the second being acetogenic/methanogenic. This is because hydrolysis improvement is fundamental to solubilize these complex substrates in simple organic substrates for downstream conversion [6,7,8].

Microaeration is already considered a consolidated technology for hydrogen sulfide removal in anaerobic reactors [9]. 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 the purpose and type of substrate to be removed [10]. Such a process has also shown promising results for BTEX (benzene, toluene, ethylbenzene, and xylenes) compounds [11,12] and even organic micropollutants [13] removals. Depending on the objective, such as increasing the hydrolysis of complex substrates with a high content of solids, increased volatile fatty acids (VFA) production, prevented VFA accumulation, removed hydrogen sulfide from biogas, or improved methane yield, different microaeration doses are necessary [19,29].

However, no studies published to date have evaluated microaerobic treatment to increase the hydrolysis rate and methane production of UASB reactors treating SWW. The present study

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aimed to evaluate the feasibility of an up-flow microaerobic sludge blanket (UMSB) reactor subjected to different air doses to enhance the hydrolysis step in SWW treatment and subsequent increase in solids and organic matter removal and methane production.

2. Materials and methods

2.1. Experimental set-up

The raw SWW used throughout the experiment was provided by cleaning the pig bays. The pigs were fed corn- and soybean-based food. 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 for solid separation, simulating the conditions found in full-scale treatment plants. After preliminary treatment, the wastewater was sent to the Sanitation Laboratory (Labosan) at the UFC, where the experiments were conducted.

The SWW was placed in an equalization tank (ET) of 50 L with mechanical agitation, providing constant influent homogenization to avoid solid sedimentation. The ET was kept in a refrigerator at 4 °C to prevent natural biodegradation of organic matter, which affects the loading rates. The reactors were fed with SWW using two peristaltic pumps (ColeParmer MasterFlex L/S 7522-30, USA).

Reactor 1 (R1) was operated as a traditional UASB reactor. It was built with polyvinyl chloride (PVC) pipes and had an effective volume of 3.25 L. The digestion compartment had a sectional area of 19.6 cm^2 and a height of 29.6 cm, whereas the settling compartment had a sectional area of 78.5 cm^2 and a height of 34.0 cm. Four sludge samplers were placed equidistant at heights of 9, 17, 26, and 34 cm from the base. The up-flow microaerobic sludge blanket (USMB) reactor R2 was built with the same dimensions and material as R1. However, it was microaerated with synthetic air (80% N_2 + 20% O_2, White Martins, Brazil) using a mass flow controller (GFC17, Cole-Parmer, USA).

2.2. Start-up and experimental procedure

The start-up occurred with an SWW average chemical oxygen demand (COD) of 5 g L^{-1} and flow rate (Q) of 4.5 mL min^{-1}, OLR of 10.4 ± 0.9 kg COD 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 influent COD, Q, OLR, VHL, and HRT values were kept constant during the 246 d of the experiment.

The sludge sources were from a previous study with two reactors treating SWW, one anaerobic and another microaerobic. The sludge used in R1 was from the anaerobic reactor, with concentrations of total solids (TS), total volatile solids (TVS), and total fixed solids (TFS) in the inoculum sludge were 51.3 ± 0.6, 16.3 ± 2.6, and 35.0 ± 1.9 g L^{-1}, respectively. The sludge used in R2 was from the microaerobic reactor, and the concentrations of TS, TVS, and TFS in the inoculum sludge were 52.0 ± 1.1, 13.6 ± 0.3, and 38.4 ± 1.3 g L^{-1}, respectively. Both reactors were inoculated with 1.6 L of sludge, representing about 50% of each reactor's effective volume.

The R2 was operated under progressive microaeration doses (Table 1) based on the interval suggested by Ref. [10] to increase particulate organic matter removal and methane production in biogas without causing remarkable biogas dilution.

Table 1
Microaeration doses during the experiment.

Stages	I	II	III
Duration (d)	77	89	80
Microaeration (mL_{air} min^{-1})	2	3.8	5.6
Dose of oxygen (L_{O2} L_{feed}^{-1} d^{-1})	0.09	0.17	0.25

2.3. Chemical analysis

COD (total [CODT], particulate [CODP], and soluble [CODS]), $BOD_5^{20°C}$ (total, particulate, and soluble), pH, total solids (TS), total fixed solids (TFS), 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 Ref. [14]. Total alkalinity (TA) and volatile fatty acids (VFA) were determined using the Kapp titrimetric method [15].

The quantification of CH_4, CO_2, H_2, and H_2S in the biogas was determined by gas chromatography with ionization detection by dielectric barrier discharge (GC BID-2010 Plus, Shimadzu Corporation, Japan), equipped with a 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. The oven, injector, and detector temperatures were 50 °C, 100 °C, 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). The oven, injector, and detector temperatures were 35 °C, 40 °C, and 230 °C, respectively. Helium (White Martins, Brazil) was used as the carrier gas at a flow rate of 7 mL min^{-1} with a run time of 5 min.

2.4. Sludge profile and biological activity monitoring

Sludge monitoring in the reactors was carried out on days 0, 77, 166, and 246, and 40 mL of sludge was collected from each sampler installed in the reactors. Part of the sample (20 mL from each sampler) was used to evaluate the sludge profile describing the TVS concentration and the reactor height, thus allowing discrimination between the sludge blanket and bed. The rest of the collected sludge (20 mL from each sampler) was homogenized and used in the specific methanogenic activity (SMA) tests carried out to monitor the sludge biological activity adapted to anaerobic (R1) and microaerobic (R2) conditions. Two substrates (glucose and VFA mixture) were evaluated individually for each type of sludge collected in R2 at the end of stages I, II, and III and in R1 on the same day that the collections were made in R2. For the SMA test, 80 mL of sludge from the reactors was used (20 mL from each sampler) and then homogenized.

Glucose was used as an intermediate substrate, allowing the metabolic activity of fermenting microorganisms (acidogenic), syntrophic (acetogenic), and methane producers (methanogenic). Thus, the use of glucose allowed the evaluation of anaerobic consortium activity as a whole. The VFA solution used was composed of acetic (C2), propionic (C3), and butyric (C4) acids, resulting in proportions of 24.3%, 34.4%, and 41.3%, respectively, in terms of COD. This VFA mixture was used to evaluate the activity of methanogenic archaea and the syntrophic capacity of the system.

The SMA assay was performed following the methodology described by Ref. [16]. Batch bioreactors (borosilicate vials) with an internal volume of 110 mL, 50 mL of reaction volume, and 60 mL of headspace. A substrate/microorganism ratio (S/M) of 0.5 g COD g VS^{-1} was selected, obtained using 2.5 g COD L^{-1} as substrate (glucose or VFA mixture) and 5.0 g TVS L^{-1} of sludge concentration. Macro- and micronutrients and sodium bicarbonate (1 g L^{-1}) were added to the substrate to form 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 a 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. They were then 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 reactor volumetric biogas production monitoring was performed through headspace pressures using a gauge pressure transmitter (Warme LTDA, Brazil). They were verified on days 1, 2, 4, 7, 14, 21, and 28, whose values were converted into volumetric biogas production (in mL). After completion of the test, the biogas produced inside the flasks was subjected to gas chromatography analysis to quantify CH_4, CO_2, N_2, H_2, and H_2S gases using the methods described in Section 2.2. SMA calculation in terms of kg COD_{CH4} kg VS^{-1} d^{-1} followed the procedures described by Ref. [16].

2.5. Calculation methods and statistical analyses

The monitoring data for R1 and R2 were treated with descriptive statistics using Microsoft Excel. The removal efficiency values of CODT, CODP, 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, the same letters indicate that the statistical difference was not significant ($p > 0.05$). The *t*-test was performed using Sisvar software version 5.6 [17].

An analysis of variance (ANOVA) followed by a statistical test for comparing averages (Tukey's test) at the 5% significance level were conducted using the AgroEstat software. Sludge SMA average calculation was performed with three repetitions performed for each sludge within the same substrate. The results are expressed as uppercase and lowercase letters. The capital letters compare the results obtained between the reactors (R1 and R2) within the same stage (I, II, or III) considering the same substrate. The lowercase letters reflect the results obtained for stages I, II, and III for the same reactor (R1 or R2), considering the same substrate. For both conditions (upper or lower case), different letters indicate a statistically significant difference with a 95% confidence interval ($p \leq 0.05$). In contrast, the same letters indicate that the statistical difference was not significant ($p > 0.05$).

3. Results and discussion

3.1. Operational performance in stages I, II, and III

Table 2 shows the physicochemical characterization of influent SWW during the experiment. It is important to highlight that there was no supplementation with either alkalizing agents or nutrients, considering only the natural composition.

The average CODT applied to the reactors during stages I, III, and III was 4996, 5369, and 5133 mg L^{-1}, respectively. The influent CODT remained at a level similar to that previously described by Refs. [18] and Yang et al., 2015; 5868 and 5692 mg L^{-1}, respectively. The CODP/CODT ratios in the SWW were 0.7 ± 0.1, 0.8 ± 0.1, 0.9 ± 0.0 in stages I, II, and III, respectively. The high organic matter concentration in CODP is due to the high VSS content, as observed in earlier studies on SWW [19,20].

The influent SWW contained, on average, 1390.8 ± 503.4, 897.4 ± 391.3, and 1830.0 ± 570.0 mg L^{-1} of oils and greases (O&G) in stages I, II, and III, respectively. According to Ref. [21]; high concentrations of O&G can damage sludge granulation in anaerobic reactors. These compounds involve the granules, reducing their density with consequent flotation and loss with the effluent. O&G concentrations greater than 65 mg L^{-1} can cause operational problems in treatment plants, mainly in primary and secondary treatments [22].

Table 2
Mean values and standard deviation of the parameters used to characterize the influent SWW to the UASB and UMSB reactors in stages I, II, and III.

Parameters	Experimental Period		
	I	II	III
CODT (mgO_2 L^{-1})	4996 ± 198	5369 ± 92	5133 ± 423
O_2			
CODP (mgO_2 L^{-1})	3442 ± 298	4283 ± 503	4371 ± 434
TS (mg L^{-1})	3936 ± 564	3788 ± 622	5129 ± 219
TSS (mg L^{-1})	2519 ± 527	2575 ± 492	3604 ± 199
TVS (mg L^{-1})	1920 ± 535	1856 ± 392	2146 ± 401
TKN (mg L^{-1})	212.9 ± 45.5	205.8 ± 49.0	229.6 ± 47.5
NH_4^+-N (mg L^{-1})	98.3 ± 33.1	49.6 ± 22.5	45.0 ± 8.0
TP (mg L^{-1})	140.2 ± 50.3	432.0 ± 111.8	93.7 ± 35.1
pH	6.43 ± 0.5	7.33 ± 0.4	7.32 ± 0.3
Alkalinity ($mgCaCO_3$ L^{-1})	422.0 ± 123.7	606.4 ± 34.4	721.5 ± 71.5
(mg CaCO3 L^{-1})			
VFA ($mgCH_3COOH$ L^{-1})	792 ± 152.2	561.2 ± 79.4	512.3 ± 89.4
O&G (mg L^{-1})	1390.8 ± 503.4	897.4 ± 391.3	1830.0 ± 570.0
SO_4^{2-} (mg L^{-1})	26.9 ± 11.8	9.4 ± 5.4	16.4 ± 5.5

Biogas quality and process efficiency are influenced by the optimal relationship between organic matter (C) and nitrogen (N). The influent C/N ratio was, on average, 24.4 ± 6.3; 26.3 ± 2.4; 22.4 ± 1.8 for stages I, II, and III, respectively. An optimal C/N ratio of 20–35:1 has been reported for anaerobic digestion and methane production [23,24,25]. A high C/N ratio can lead to VFA (organic acids) accumulation and consequent excessive decrease in pH, making the environment unsuitable for methanogenic archaea. On the other hand, the low C/N ratio stimulates the ammonification of organically bound nitrogen and its medium accumulation as NH_4^+-N/NH_3, increasing the effluent pH and sometimes exerting a toxic effect [26]. The effluent NH_4^+-N/NH_3 concentrations (Table 3) were below the threshold value of 1700 mg L^{-1} to cause toxicity to anaerobic microorganisms [27], and together with the low effluent VFA and pH close to 7 (Table 4), justifies the good operational performance of both reactors in all experimental stages. The effluent pH maintenance of R1 and R2 probably occurred because of the excess of TA compared to the VFA concentration.

Another parameter to be analyzed in the influent is the relationship between COD and SO_4^{2-}. The SO_4^{2-} found in SWW comes mostly from the degradation of proteins used in animal feed. In this study, the major protein source was the soybean meal used in pig feed. During AD, SO_4^{2-} can be reduced to dissolved sulfide (S^{2-}) and then reduced to hydrogen sulfide (H_2S) by sulfate-reducing bacteria (SRB) through the sulfetogenesis process, which is a competing process of methanogenesis [28]. Low sulfate concentrations were found in the SWW influent, as well as a low sulfate reduction (Table 3).

According to Ref. [29]; the sulfetogenesis process stands out methanogenesis for influents with COD/SO_4^{2-} ratios lower than 10. SWW had an average COD/SO_4^{2-} ratio greater than 200 during the entire experiment. In addition, the effluent dissolved sulfide (S^{2-}) analysis from the UASB and UMSB reactors in stages I, II, and III indicated its absence. Therefore, the removed organic matter was due to methanogenesis, and the competition between methanogenesis and sulfate reduction was not an issue.

The nutrients present in the UASB (R1) and UMSB (R2) reactor

Table 3
Mean values and standard deviation of nutrient concentrations in the effluent from reactors R1 and R2.

Nutrient	Stage I		Stage II		Stage III	
	R1	R2	R1	R2	R1	R2
TP (mg L^{-1})	46.1 ± 20.9	51.4 ± 20.7	87.1 ± 31.0	67.4 ± 15.5	42.6 ± 18.7	39.2 ± 16.4
TKN (mg L^{-1})	201.6 ± 23.7	187.5 ± 21.0	117.6 ± 11.2	92.4 ± 36.4	67.2 ± 25.5	95.2 ± 39.7
$N-NH_4^+$ (mg L^{-1})	156.9 ± 31.8	140.2 ± 26.0	63.1 ± 25.0	76.4 ± 29.2	78.1 ± 16.2	76.3 ± 12.0
SO_4^{2-} (mg L^{-1})	25.4 ± 8.0	24.7 ± 8.4	10.5 ± 4.3	10.6 ± 3.2	6.59 ± 1.4	10.43 ± 4.2

Table 4
Average values and standard deviation of the parameters analyzed in the effluent of R1 and R2 throughout stages I, II, and III.

Parameter	Stage I		Stage II		Stage III	
	R1	R2	R1	R2	R1	R2
pH	7.8 ± 0.2	7.6 ± 0.2	7.7 ± 0.2	7.8 ± 0.2	7.7 ± 0.2	7.8 ± 0.2
TA ($mgCaCO_3$ L^{-1})	862.5 ± 129.0	822.8 ± 97.1	643.9 ± 63.7	589.1 ± 76.7	687.9 ± 61.5	706.4 ± 42.7
VFA ($mgCH_3COOH$ L^{-1})	259.5 ± 53.5	270.3 ± 37.2	151.6 ± 36.3	130.8 ± 56.7	122.5 ± 11.1	153.1 ± 25.6
CODT (mgO_2 L^{-1})	1223 ± 230	1194 ± 124	1098 ± 337	858 ± 207	1004 ± 300	1483 ± 314
CODP (mgO_2 L^{-1})	962 ± 252	779 ± 219	761 ± 299	546 ± 213	638 ± 285	1089 ± 210
TS (mg L^{-1})	1903 ± 433	1966 ± 446	1592 ± 383	1398 ± 203	2408 ± 619	3494 ± 326
TSS (mg L^{-1})	894 ± 434	969 ± 413	511 ± 200	447 ± 151	1130 ± 396	2135 ± 365
VSS (mg L^{-1})	624 ± 282	540 ± 268	313 ± 123	281 ± 99	466 ± 150	1124 ± 244
O&G	638 ± 403	752 ± 496	698 ± 350	538 ± 300	700 ± 395	806 ± 629

effluents were quantified during the experiment (Table 3) to assess their influence on the reactor's performance and stability, organic matter removal, and methane production. TP efficiencies for R1 were 63.9 ± 18.7%, 76.8 ± 6.8%, and 54.5 ± 6.1% for stages I, II, and III, respectively. For R2, the removals were 58.1 ± 24.1%, 79.9 ± 6.2%, and 58.1 ± 5.9% for stages I, II, and III, respectively. According to Ref. [30]; these values are considered high for an anaerobic process, and these TP removals are mainly linked to suspended solids removal, indicating that physical removal was the most important process for reducing TP concentrations, as also observed in the study by Ref. [31].

As already mentioned, the main challenge for SWW treatment is the high concentration of complex organic matter in the form of CODT, CODP, and VSS, which limits AD hydrolysis. Fig. 1 shows the effect of the microaeration dose on the removal efficiency of complex organic matter (VSS, CODP, and CODT). Throughout the experiment, on average, the CODT, CODP, and VSS removal efficiencies in R1 were 76.1 ± 2.0%, 78.0 ± 2.4%, and 79.7 ± 3.0%, respectively. In stage I, the microaerobic reactor achieved similar removal rates in terms of organic matter removal (VSS = 71.7 ± 5.6%; CODT = 76.0 ± 3.1%; CODP = 77.3 ± 5.8%). During this period, R2 operated under microaeration of 0.09 L_{O2} L_{feed} d^{-1}.

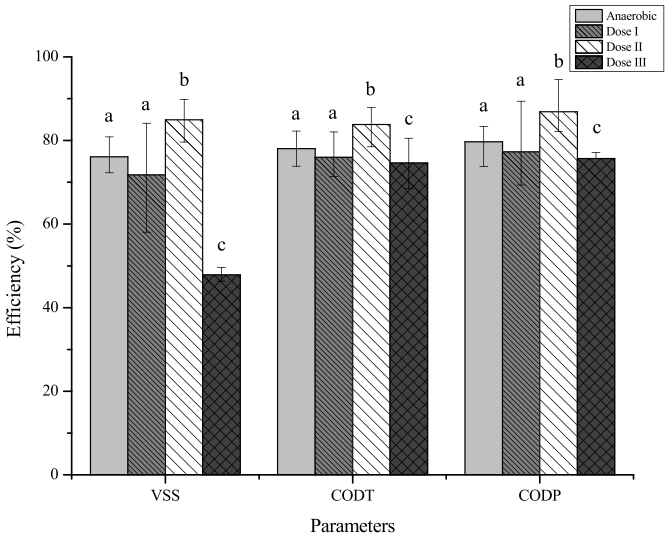

Fig. 1. Comparative analysis between the average efficiencies of removing organic matter from the SWW when subjected to microaeration doses: 0 (anaerobic), 0.09 (Stage I), 0.17 (Stage II), and 0.25 (Stage III) L_{O2} L_{feed} d^{-1}.

According to Ref. [10]; this is a low flow for wastewater treatment such as SWW, when the objective is to enhance the hydrolysis and provide a redox potential close to that of the anaerobic medium. This may justify the R2 organic matter removal, which was statistically equal to the values found in R1. It is important to highlight that the sludge from R2 came from an experiment where the microaeration dose was five times higher. Therefore, the system required an adaptation period, which justifies the close performance values between R1 and R2 during stage I.

However, during stage II, the microaerobic treatment showed a higher performance compared to the anaerobic reactor, statistically different (Fig. 1), resulting in high VSS (85.0 ± 1.9%), CODT (83.8 ± 2.5%), and CODP (82.1 ± 4.8%) removals. This behavior was due to biomass adaptation and the positive effect of microaeration in the hydrolysis step, resulting in higher methane production, as discussed later.

According to Ref. [32]; the increased production of extracellular hydrolytic enzymes by the hydrolytic bacterial communities more abundant under microaerobic conditions improves the hydrolysis of carbohydrates, proteins, and other complex organic substrates [33]. investigated a continuously stirred tank reactor and sequencing batch reactor (CSTR-SBR) for swine wastewater treatment. They reported that with a favorable HRT of 7 days, an average COD removal of approximately 73% was achieved. Other studies have shown efficiencies of above 70% in anaerobic reactors. For instance Ref. [34], found for SWW treatment over 90% COD removal in a system composed of a UASB reactor, submerged aerated biological filters (SABF), and horizontal subsurface flow constructed wetland (HSSF-CW) under an organic loading rate of 4 kg COD m^{-3} d^{-1}.

During stage III, when the highest microaeration flow was applied in R2, 0.25 L_{O2} L_{feed} d^{-1}, a lower efficiency removal was observed compared to the previous stage and the control reactor R1

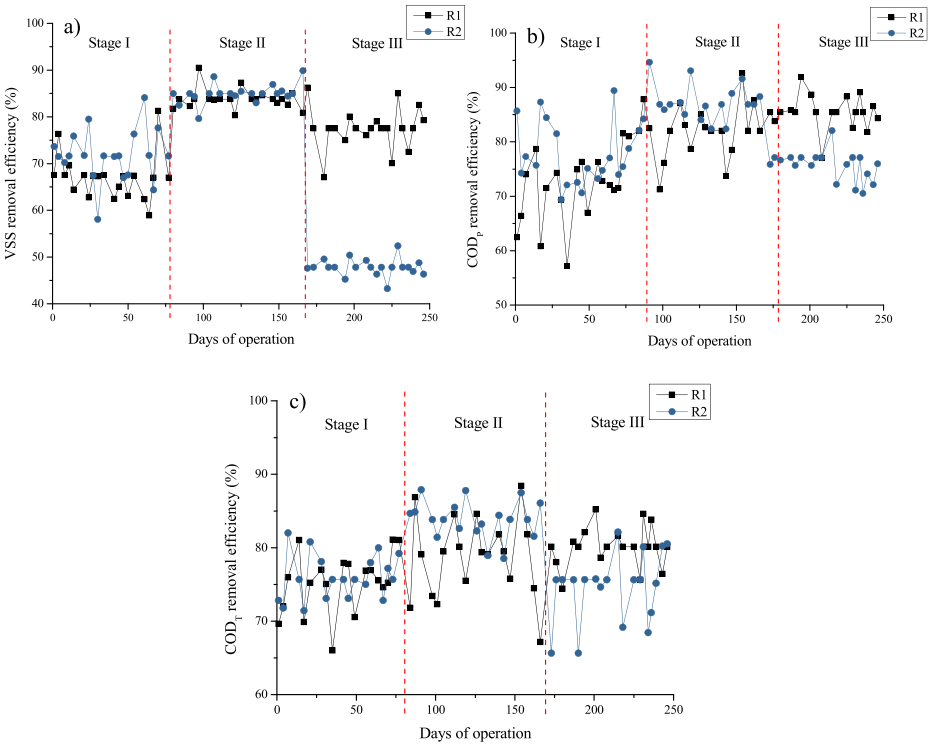

Fig. 2. Time-course removal profile of VSS (a), CODP (b), and CODT (c) during the experiment.

(Fig. 2) due to biomass washout. Nevertheless, the CODT and CODP removals were still high, i.e., 74.6 ± 3.9% and 75.7 ± 2.1%, respectively. Microaeration, even in a minimal airflow, together with fat accumulation in the anaerobic granules, facilitated biomass detachment and loss in the effluent.

However, the values of CODT removal at stage III for reactors R1 and R2 were similar or higher than those of several SWW anaerobic treatment studies [35]. reported a 65% removal efficiency of CODT when treating SWW with an anaerobic baffled reactor (ABR) operated at an HRT of 15 h [19]. evaluated the self-agitation anaerobic reactor (SaABR) treating SWW and reported a close CODT efficiency (69%), but the HRT used was three days. However [36], only found a CODT removal efficiency of 47% in a CSTR treating SWW, even with a 10-days HRT.

3.2. Sludge concentration and methane production

To understand reactor performance evolution, it is important to study their biomass development. Sludge profile analysis was carried out on day 0 (beginning of stage I), 77 (end of stage I), 166 (end of stage II), and 264 (end of stage III), as shown in Fig. 3. After stages I and II in R1 (Fig. 3a), sludge growth was observed along with the UASB reactor height, as indicated by the increase in the TVS concentration in the samplers. At the end of stage III in the same reactor, the concentrations in samplers 1 and 4 remained equivalent, and in samplers 2 and 3 remained lower than those found in stage II. For R2 (Fig. 3b), the highest sludge TVS concentrations were observed after 166 days of operation (end of stage II).

Emphasis was given to the highest TVS concentrations in the first sampler (9 cm from the base) region, with an average sludge concentration in terms of TVS of 5.3% v/v (Figs. 3a) and 4.7% v/v (Fig. 3b) for R1 and R2, respectively. The TVS concentration increase in the base indicates a denser sludge in this region, which is a characteristic of the granules formed in the UASB reactors [37]. observed a significant influence of granule formation on organic matter removal and biogas production in a UASB reactor treating sewage. This can help justify the best removal efficiency values found for stage II, as shown in Fig. 2.

In stage III, a decrease in TVS concentration was observed over the entire height of R2, mainly in the two samplers closest to the base, suggesting a biomass washout. Although the washout coincides with the highest microaeration dose, biomass loss should not be associated only with the increase in airflow in the reactor. During the last stage, a higher influent O&G concentration was observed. Compared to R1, a higher R2 effluent O&G concentration was verified, which contributed to the biomass washout in this reactor. Another reason may be the long experiment time and the changes in the R2 biomass characteristics.

Other researchers have indicated that biomass physically adsorbs fat/lipids, causing biomass flotation and washout [38,39]. [40] investigated continuous and intermittent UASB reactors treating dairy wastewater and subjected them to fat, hydraulic, and temperature shocks and reported a heavy TSS washout in a continuous system. They attributed this to the combined effect of the high up-flow velocity and the presence of accumulated substrates on the biomass surface, a typical result of the continuous operation of UASB reactors [5]. investigated an up-flow solid reactor (USR) for swine wastewater treatment. They observed serious biomass washout, likely due to the short solids retention time (SRT) applied, resulting in low efficiency values [41]. evaluated the treatment of mixed long-chain fatty acid-containing synthetic dairy wastewater with expanded granular sludge bed (EGSB) and reported granular sludge flotation and washout after two months.

There are also no previous studies that indicate washout due to any microaeration intensity. Therefore, strategies to remove fat from SWW must be considered to promote long-term operational stability. Another possibility is to evaluate other anaerobic reactors with specific three-phase separators for high-fat-content

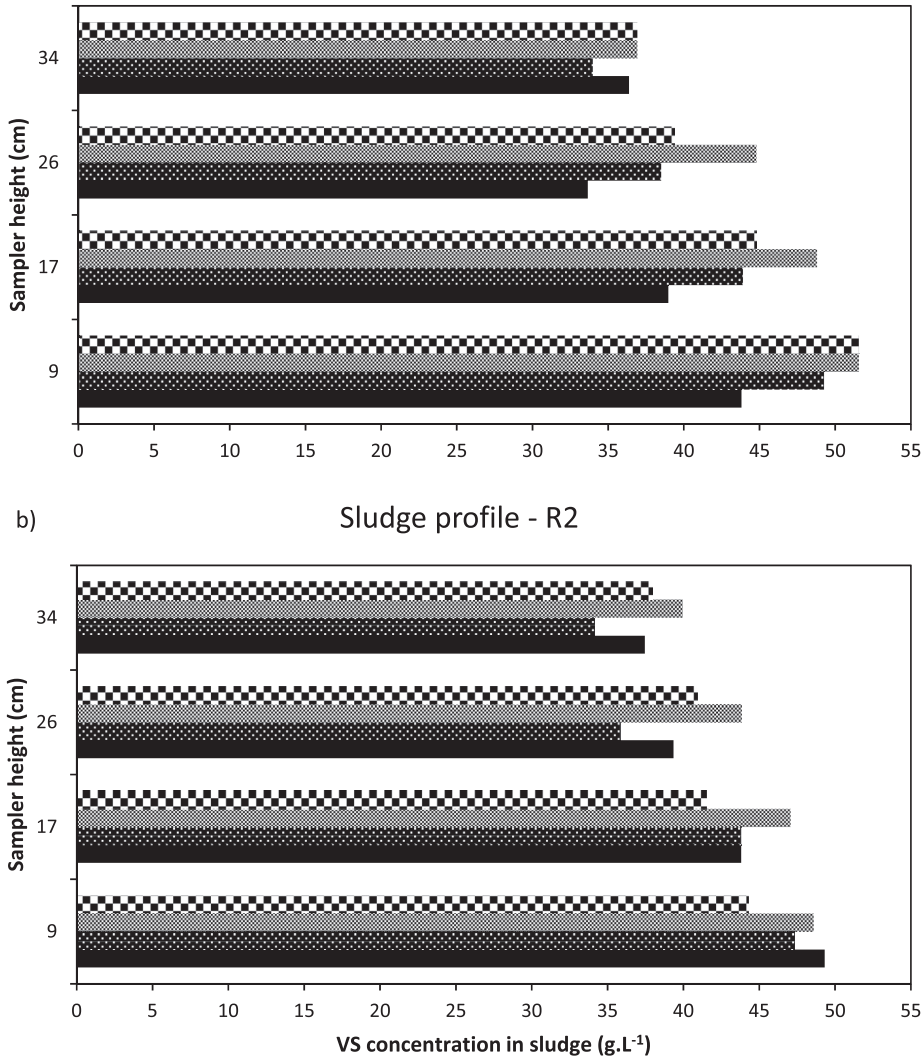

Fig. 3. Sludge profile evolution of reactors R1 (a) and R2 (b) during the experimental period.

wastewaters subjected to microaerobic processes.

Fig. 4 shows the volumetric production of biogas and its composition during the three stages of the UASB (Fig. 4a) and UMSB (Fig. 4b) reactors. Despite the decreased organic matter removal in stage II, methane production was higher in stage III for both reactors. In the last stage, the higher alkalinity and the higher TA/VFA ratio in the influent and the high acid consumption might have provided greater stability for the methanogenesis step. In stage III, R1 had an average concentration of 82% methane in the biogas, with a specific production rate of 178.5 ± 28.4 L_{CH4} kg $COD_{rem.}^{-1}$ d^{-1}. On the other hand, R2 had a lower average methane concentration (80.3%) due to O_2 and N_2 in the biogas (Fig. 4), owing to the higher dose of air applied in the reactor. Nevertheless, R2 obtained a higher specific production rate of 281.2 ± 47.5 L_{CH4} kg COD_{rem}^{-1} d^{-1}. Both reactors produced biogas with a high energy value, with methane content superior to the minimum value of 45% required for its combustion [29].

The values of specific methane production of R1 and R2 were similar to those reported in the literature for the anaerobic treatment of SWW. [18]; in an experiment with a horizontal anaerobic reactor operating under an OLR of 12 kg COD m^{-3} d^{-1}, obtained a specific production of 250 L_{CH4} kg COD_{rem}^{-1} d^{-1} [42]. treated swine wastewater in a UASB reactor and obtained 280, 330, 310, and 290 L_{CH4} kg COD_{rem}^{-1} d^{-1}, with higher HRTs than those used in this study: 7.0, 6.4, 5, 0, and 3.5 d, respectively.

As already mentioned, the UMSB reactor had a higher absolute and relative methane production than the UASB reactor. Several studies have used microaeration as a strategy to increase methane production during AD. Lim and Wang, 2013 studied the effects of microaeration on the co-digestion of brown water and food residues and obtained accumulated methane production of 318 ± 8 and 258 ± 15 L_{CH4} kg VS_{apl}^{-1}, for the microaerobic and anaerobic treatments, respectively.

Ruan et al., 2019; when comparing the performance of two tank reactors with continuous agitation, one with anaerobic sludge and the other with previously micro-aerated sludge, found that microaeration not only increased the biogas production but also improved the methane content in the biogas until a certain point.

Microaeration enhances extracellular hydrolytic enzyme production from more abundant and diverse hydrolytic bacterial communities, which accelerates the hydrolysis of particulate and complex organic substrates. In addition, the integration of aerobic VFA oxidation by facultative heterotrophs with anaerobic methanogenesis facilitates the energetic conversion of intermediates [10]. Furthermore [43], observed that the hydrogenotrophic methanogenic pathway was promoted under microaerobic

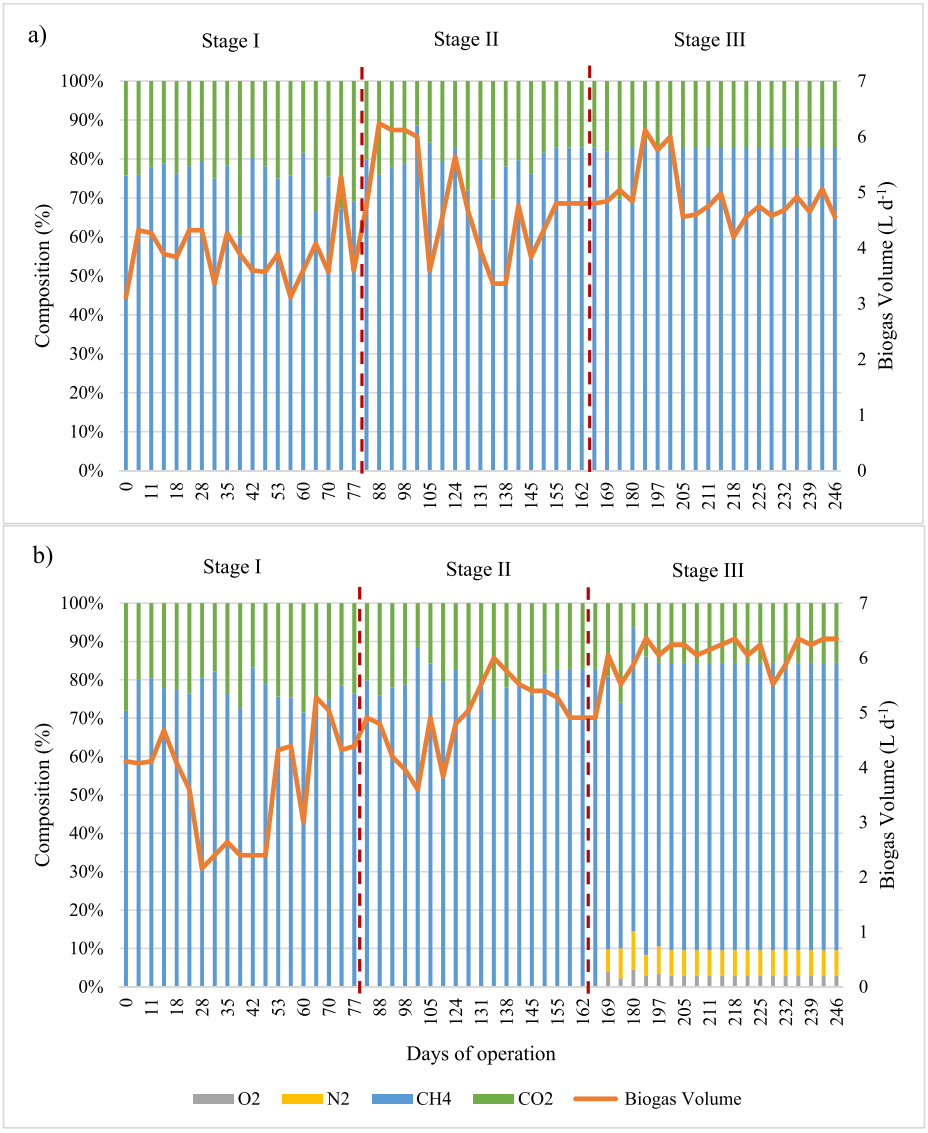

Fig. 4. Composition and volumetric production of biogas produced from R1 (a) and R2 (b) in stages I, II, and III.

conditions, which maintained a low hydrogen partial pressure while efficiently producing methane. Therefore, microaeration improves AD stability and performance and increases methane yield [44].

3.3. Biological activities of anaerobic and microaerobic sludge

Upon analyzing the endogenous control, it was found that the methane concentration was below the method limit of quantification, indicating that the production found in the bioreactors (Fig. 5) was due to the substrates used. ANOVA analysis comparing each sludge to the same substrate indicated statistically significant differences between the sludges. Tukey's test at 5% probability was used to compare the sludge SMA values for the same substrate (Fig. 5).

When comparing the R1 and R2 SMAs (capital letters) in stages I and II for the glucose substrate, it was found that the SMA values do not differ statistically, suggesting that the fermentative (acidogenic), syntrophic (acetogenic), and methanogenic activities of anaerobic sludge was statistically similar to that of microaerobic sludge. However, when performing the same comparison (R1 with R2 for the glucose substrate) in stage III, it is observed that the activity of fermentative (acidogenic) microorganisms from anaerobic sludge is slightly higher (0.23 kg COD_{CH4} kg VS^{-1} d^{-1}) than that of microaerobic sludge (0.18 kg COD_{CH4} kg VS^{-1} d^{-1}).

Comparing R1 and R2 SMAs (capital letters) in stages I and II for the VFA substrate, a statistical difference was noted, suggesting that the activity of acetogenic and methanogenic microorganisms of anaerobic sludge is greater than that of microaerobic sludge. However, in stage III, the activity of acetogenic and methanogenic microorganisms of the anaerobic sludge (0.26 kg COD_{CH4} kg VS^{-1} d^{-1}) do not differ statistically from the microaerobic sludge (0.28 kg COD_{CH4} kg VS^{-1} d^{-1}).

Although the SMA values of the anaerobic sludge were higher than those of the microaerobic sludge throughout the stages, to the detriment of the organic matter removal values and volumetric methane production during continuous-flow experiments, it is important to note that the SMA tests aimed to evaluate indirectly, only the presence of acidogenic and syntrophic/methanogenic groups in the sludge.

Furthermore, it is expected that the microaerobic sludge results will be lower than that of the anaerobic sludge due to the medium

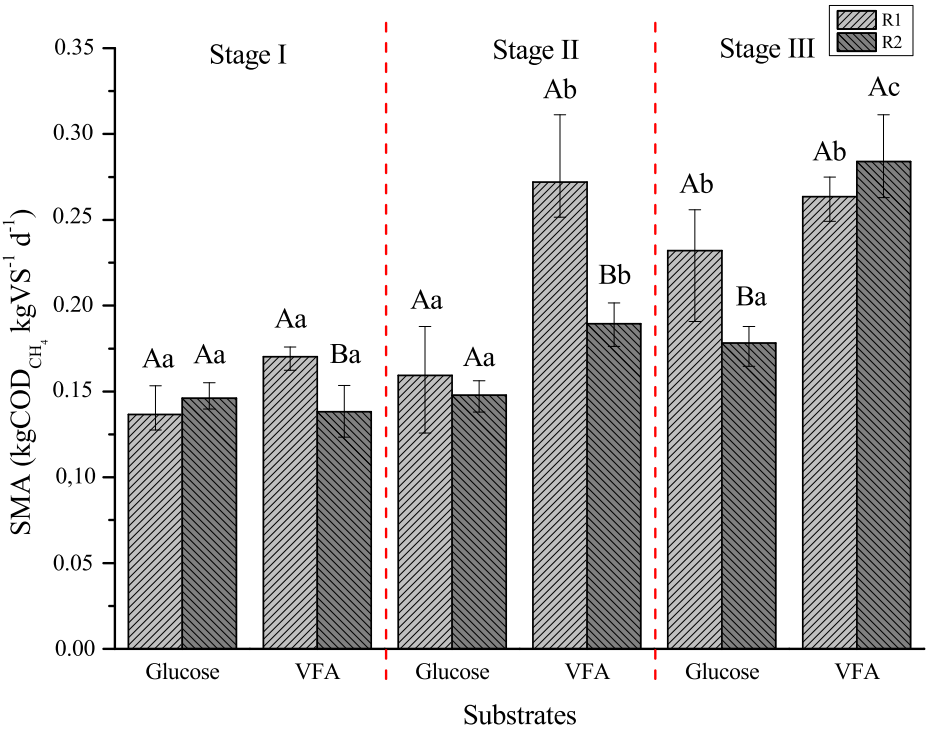

Fig. 5. Results of the Specific Methanogenic Activity (SMA) test of anaerobic and microaerobic sludge collected at the end of stages I, II, and III.
Legend: * The capital letters (A, B, C) reflect the comparison of the results obtained between the reactors (R1 and R2) within the same stage (stage I, stage II, or stage III) considering the same substrate. ** The letters lower case (a, b, c) reflect the comparison of the results obtained among the Stages I, II, and III for the same reactor (R1 or R2) considering the same substrate.

characteristics change, affecting the microbial consortium, from a medium with small amounts of oxygen in the reactor to a strictly anaerobic medium in the bioreactors used during the tests.

Therefore, analyzing only the microaerobic sludge results along the stages, it is possible to observe an increase in specific activities as long as the microaeration dose is enhanced. Although there is no significant difference between the SMA values in the presence of glucose, the highest absolute value of acidogenic activity from the microaerobic sludge was observed at stage III (0.18 kg COD_{CH4} kg VS^{-1} d^{-1}). With the VFA mixed solution as substrate, the SMA values for the microaeration doses applied showed statistically significant differences, indicating that the dose applied in stage III promoted the highest acetogenic/methanogenic activity among the microaerobic sludges (0.28 kg COD_{CH4} kg VS^{-1} d^{-1}) [19]. performed an SMA test at 25 °C with sodium acetate as the substrate and inoculum sludge collected from a SaABR treating SWW and obtained 0.16 g COD $_{CH4}$ g VSS^{-1} d^{-1}.

These results demonstrated an increase in the syntrophic and methanogenic groups with an increase in the microaeration dose. Microaeration also directly affects the methane production step by modifying the dominant methanogenic pathway. With the ability to use both acetate and hydrogen to produce methane, along with aerotolerance, *Methanosarcina* was found to be the dominant archaea in the microaerobic system [45]. Overall, it is believed that microaeration can increase the biomass methanization capacity because of higher methanogenic substrates production [10].

4. Conclusions

The microaerobic process resulted in higher total and particulate organic matter removal and methane production during SWW treatment. The UMSB reactor, when operated under a microaeration of 0.17 L_{O2} L_{feed}^{-1} d^{-1}, showed the highest organic matter removal, reaching 83.8 ± 2.5% in terms of CODT. Under a microaeration dose of 0.25 L_{O2} L_{feed}^{-1} d^{-1}, the highest specific volumetric production rates were found, although biomass washout also occurred. Therefore, strategies to remove fat from SWW must be considered to promote long-term operational stability. Another possibility is to evaluate other anaerobic reactors with specific three-phase separators for high-fat-content wastewaters subjected to microaerobic processes.

CRediT authorship contribution statement

Maurício Guimarães de Oliveira: Conceptualization, Investigation, Formal analysis, Writing – review & editing. **José Marcos Marques Mourão:** Conceptualization, Investigation. **Ana Katherinne Marques de Oliveira:** Investigation. **André Bezerra dos Santos:** Writing – review & editing, Funding acquisition. **Erlon Lopes Pereira:** Supervision, Conceptualization, Investigation, Formal analysis, Writing – review & editing.

Declaration of competing interest

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

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