Anaerobic co-digestion of acerola (Malphigia emarginata) agro-industry effluent with domestic sewage at mesophilic and thermophilic conditions

Co-digestão anaeróbia de efluente da agroindústria da acerola (Malphigia emarginata) com esgoto doméstico em condições mesófilas e termófilas

Andreza Carla Lopes André[1]; Eduardo Souza Costa Barros[2];
Paula Tereza de Souza e Silva[3]; Dian Lourençoni[4];
Miriam Cleide Cavalcante de Amorim[5]

Abstract

This study evaluated the effect of temperature on the anaerobic co-digestion of acerola agro-industry effluent (EAV) and domestic sewage (EDT). The assays were performed in triplicates of reactor flasks using treatments with different mixture compositions (T_1= 5% EDT + 95% EAV; T_2= 20% EDT + 80% EAV; T_3= 30% EDT + 70% EAV) and anaerobic sludge as inoculum (5 g.L^{-1}), at mesophilic (35 °C) and thermophilic (55 °C) temperatures. The analyses of soluble chemical oxygen demand (COD_s) and volatile fatty acids (VFA) were performed by determining the removal efficiency of the CODs, the decay rate constant of the COD (K_d), and the percentages of anaerobic biodegradability (%BD) and methanation (%M). The inoculum biomass of the treatments was observed through scanning electron microscopy (SEM) at the end of the degradation process (12 days). Regardless of the temperature, the anaerobic digestion was considered efficient, with biodegradability above 60%. The mesophilic temperature favored the anaerobic co-digestion for all mixture compositions, presenting more diversified and structured biomass at the end of the assays, as well as higher removal efficiencies of the COD_s and methanization, especially for T_3 at 35 °C (63% and 51%, respectively). Furthermore, the kinetics of the degradation process proved to be more accelerated at mesophilic conditions (K_d 0.1d^{-1}) and in the treatments with a higher percentage of sewage (T_{2M} and T_{3M}).

Keywords: Biodegradation. Methanation. Temperature.

Resumo

Este estudo avaliou o efeito da temperatura na co-digestão anaeróbica de efluente da agroindústria aceroleira (EAV) e esgoto doméstico tratado (EDT). Os ensaios foram realizados em frascos reatores em triplicata utilizando tratamentos com diferentes composições de mistura (T_1 = 5% EDT + 95% EAV; T_2 = 20% EDT + 80% EAV; T_3 = 30% EDT + 70% EAV) e lodo anaeróbico como inóculo (5 g.L^{-1}), em temperaturas mesófilas (35 °C) e termófilas (55 °C). Foram realizadas análises de demanda química de oxigênio solúvel (DQO_s) e ácidos graxos voláteis (AGV) determinando-se a eficiência de remoção de DQO_s, a taxa de decaimento de DQO (K_d), e os percentuais de biodegradabilidade anaeróbica (%BD) e metanização (%M). A biomassa do inóculo dos tratamentos foi observada através de microspia eletrônica de varredura (MEV) no final do processo de degradação (12 dias). Independente da temperatura, a digestão anaeróbia foi considerada eficiente, com biodegradabilidade superior a 60 %. A temperatura mesófila favoreceu a co-digestão anaeróbia para todas as composições de mistura, apresentando biomassa mais diversificada e estruturada ao final dos ensaios, bem como maiores eficiências de remoção de DQOs e metanização, especialmente para T_3 a 35 °C (63% e 51%, respectivamente). Além disso, a cinética do processo de degradação revelou-se mais acelerada em condições mesófilas (K_d 0.1d^{-1}) e nos tratamentos com maior percentual de esgoto (T_{2M} e T_{3M}).

Palavras-chave: Biodegradação. Metanização. Temperatura.

[1] Agri. and Environ. Engineering, UNIVASF, Juazeiro, Bahia, Brazil, E-mail: andreza_carlalopes@hotmail.com
[2] Master in Agricultural Engineer, UNIVASF, Juazeiro, Bahia, Brazil. E-mail: barros-eduardo2005@hotmail.com
[3] Researcher at EMBRAPA semiarid, Petrolina, Pernambuco, Brazil. E-mail: paula.silva@embrapa.br
[4] Prof. Dr., Dept of Agri. and Environ. Engineering, UNIVASF, Juazeiro, Bahia, Brazil. E-mail: dian.lourenconi@univasf.edu.br
[5] Prof. Dr., Dept of Agri. and Environ. Engineering, UNIVASF, Juazeiro, Bahia, Brazil. E-mail: miriam.cleide@univasf.edu.br

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Introduction

Given its high perishability, the processing of acerola (*Malpighia emarginata* D.C.) became an important alternative for the maintenance of the productive chain of this fruit, ensuring its quality and commercialization (MALEGORI *et al*., 2017). However, during fruit processing, residual waters are generated, which may represent a source of environmental pollution if not subjected to some sort of treatment (BOLZONELLA *et al*., 2019).

The agroindustry uses ultrafiltration in fruit processing, which consists in the separation by semipermeable membranes, used for the clarification of fruit juices (STOFFEL; MOREIRA, 2013). The fraction that does not permeate the membrane is called retentate or residue of the clarification process, which, due to its composition and amount generated, may constitute an environmental impact (PENHA *et al*., 2001). It is especially characterized by the high content of organic matter in terms of chemical oxygen demand (COD) and proteins, with lower contents of sugars and salts, conferring it a characteristic of hard biological degradation, or recalcitrant.

In this sense, studies point to anaerobic digestion as an effective process for the treatment of agro-industry effluents (CORREIA; DEL BIANCHI, 2008; PUKASIEWICZ *et al*., 2017). It use for the biologic treatment of residual waters has revealed to be highly attractive due to the lower operational costs for implantation, savings with aeration, low sludge production, and especially the obtainment of clean energy in the form of methane and value-added by-products, such as volatile fatty acids and bioproducts (DE CLERCQ *et al*., 2017; ESPOSITO *et al*., 2012). Review papers have reported excellent results of anaerobic digestion processes in agro-industrial effluents through the co-digestion of different combinations of municipal, industrial, and agricultural residues (HAGOS *et al*., 2017), although few of them approach the recalcitrant effluents of wastewaters in the fruit farming agroindustry (SHEN *et al*., 2019). In this particular case, according to the studies performed by Montefusco *et al*. (2019), the effluent from the ultrafiltration in the acerola processing presents this characteristic. Zhang *et al*. (2013) affirm that anaerobic digesters that use mono-digestion are more susceptible to greater instabilities in the process, presenting undesirable drops in performance (due to imbalanced nutritional profiles), accumulation of volatile fatty acids, and consequent inhibition of methanogenesis.

When employing different effluents, the anaerobic co-digestion can overcome difficulties related to the recalcitrant character of one of the effluents, as well as the deficiencies of mono-digestion (ANDRÉ *et al*., 2020; SIDDIQUE; WAHID, 2018), since it promotes greater stability of the process, resulting in a more balanced and diversified substrate in terms of nutrients, provides the dilution of eventual toxic compounds present in one of the co-substrates, increases the content of biodegradable compounds, besides promoting the increase of bacterial strains that will take part in the process, and improvement of the methane production rate (ATANDI; RAHMAN, 2012; CHOW *et al*., 2020; ESPOSITO *et al*., 2012).

However, it is worth noting that several parameters can affect anaerobic digestion, such as the type of substrate, volumetric organic load, and temperature (AMANI; NOSRATI; SREEKRISHNAN, 2010; XU *et al*., 2019). Among these, temperature is considered one of the most important parameters in anaerobic digestion, as they affect microbial growth and species selection, because it can influence the process in several ways, affecting from the solubility of substrates to the speed of metabolism of microorganisms (RITMANN; MCCARTY, 2001).

According to Metcalf and Eddy (2003), microorganisms can be classified according to the temperature range in which they develop best into: psychrophiles (12-18 °C), mesophiles (25-40 °C), or thermophiles (55-65 °C). Although anaerobic digestion is commonly performed with an operating temperature within the mesophilic range (30 to 35 °C), studies suggest that the thermophilic range (50 to 55 °C) can potentialize the process, resulting in greater removal efficiency of organic matter (WIJAYA *et al*., 2020).

Within this context, and considering that the operational parameters interfere with the biomethanation process and that the domestic sewage, according to Bertolino, Carvalho and Aquino (2008), represents a significant source of organic matter, thus being potentially able to be included as a co-substrate for co-digestion, the present study aimed to evaluate the efficiency of the anaerobic co-digestion of the effluent from green acerola processing and from treated domestic sewage in batch flask reactors with different mixture compositions at mesophilic (35 ºC) and thermophilic conditions (55 °C), obtaining the mass balance and the rate of the organic load removal process in terms of chemical oxygen demand and analyzing the biomass morphology of the inoculum.

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Material and methods

Substrates: collection and characterization

The anaerobic co-digestion assays were performed by the association of the effluent from the ultrafiltration stage of acerola processing with the treated domestic sewage, originated from the NIAGRO (Nichirei do Brasil Agrícola Ltda, agroindustry located in the Industrial District of Petrolina–PE, Brazil) and from COMPESA (State Sanitation Company of Pernambuco, Petrolina–PE, Brazil), respectively. Individual samples were collected and these stored at 4 °C until the moment of the analytical characterization and use in the experiment.

The substrates were characterized by the determination of the COD, proteins, carbohydrates, nitrogen, total phosphorus, pH, and VFA, according to the *Standard Methods for the Examination of Water and Wastewater* (APHA, 2012), as shown in Table 1.

Inoculum

The anaerobic sludge from a UASB reactor (*Upflow Anaerobic Sludge Blanket*) was used as inoculum, originated from a sewage treatment plant (ETE) of the State Sanitation Company of Pernambuco (COMPESA), located in Petrolina-PE, Brazil, with specific methanogenic activity of 0.223 mL CH_4. g TVS d^{-1}. The initial microbial concentration in terms of total volatile solids (TVS) in the mixture was 5 g $TVS.L^{-1}$ (BERTOLINO; CARVALHO; AQUINO, 2008), with nutritional supplementation according to Florêncio (1994).

### Anaerobic Co-digestion Assays: temperatures and mixing ratios

In order to evaluate the effect of temperature on the anaerobic co-digestion process through the association of the above-mentioned effluents, batch assays were conducted in reactor flasks with 0.89 L of useful volume and 0.22 L of headspace, as recommended by Holliger *et al.* (2016).

For the mesophilic and thermophilic conditions, the temperatures of 35 °C (T_M) and 55 °C (T_T) were evaluated, respectively, with all treatments in triplicate plus the blank of each temperature, totaling 20 reactor flasks.

The flasks were placed in a room on a bench and with the aid of a Comfort® PR 1500 W electric heater, the room temperature was kept at 35°C. For the thermophilic assays, the reactor flasks were immersed in water within a thermal box equipped with a heating system by means of an immersion heater and a temperature sensor, both connected to an Arduino, which was previously programmed to maintain the temperature at 55 °C $\pm$ 0.5 °C. The homogenization of temperature in the thermal box was ensured by means of a submersible filter pump (flow of 350 L.h^{-1}) connected continuously to promote water circulation, avoiding the formation of cold-water pockets, illustrated in Figure 1.

Figure 1 – Scheme of the treatments conducted at 55 °C.
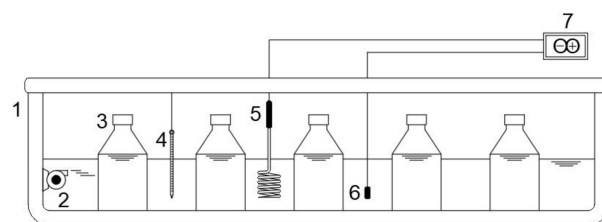
1 – Thermal box;	2 – submersible pump;
3 – Reactor flasks;	4 – Thermometer;
5 – Immersion heater;	6 – Thermostat;
7 – Arduino.	

Source: The authors.

A completely randomized design was adopted, and the co-digestion mixing ratios were defined by adopting a higher percentage of the green acerola processing effluent in relation to the domestic sewage, for the mesophilic and thermophilic conditions, according to Table 2.

The pH of all mixtures in the reactor flasks was measured with a Tecnopon® pH meter, model MPA210, and corrected at the beginning of the assays (day zero) to ensure values close to neutrality using sodium bicarbonate. Afterward, the reactor flasks were isolated from light, closed, and properly sealed to maintain anaerobic conditions.

The assays lasted for 288 h, in which 30 mL aliquots were removed every 48 h from the reactor flasks in order to perform the analyses of VFA (APHA, 2012) and COD_S, closed reflux method, as described in APHA (2012).

Evaluation and performance of the process: Mass balance in terms of COD and kinetics of the reaction

For the mass balance, the mass removal efficiency (COD_{rem}) of the COD_S, the COD effectively transformed into methane (COD_{CH4}) and the COD present in the effluent in the form of volatile fatty acids not converted to methane (COD_{VFA}) were determined.

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Table 1 – Composition of the green acerola processing effluent (EAV) and treated domestic sewage (EDT).

	COD (mg.L^{-1})	Proteins (mg.L^{-1})	Carbohydrates) (mg.L^{-1})	Total Nitrogen (mg.L^{-1})	Total Phosphorus (mg.L^{-1})	pH
EAV	79.078	52.536	54.950	230	1.402	3.2
EDT	278	154	0	23	24.36	7.3

EAV: Green acerola processing effluent;
EDT: Treated domestic sewage.
Source: The authors.

Table 2 – Mixing ratios and initial COD.

Treatments	Mixing ratio in relation to the useful volume of the reactor	Initial COD (g.L^{-1})
T$_{1M}$	5%of EDT + 95%of EAV	40.5
T$_{1T}$		44.3
T$_{2M}$	20%of EDT + 80%of EAV	36.0
T$_{2T}$		39.2
T$_{3M}$	30%of EDT + 70%of EAV	28.8
T$_{3T}$		25.5

EDT: Treated domestic sewage;
EAV: Green acerola processing effluent;
T$_{1M}$: Treatment 1 at 35 °C; T$_{2M}$: Treatment 2 at 35 °C; T$_{3M}$: Treatment 3 at 35 °C;
T$_{1T}$: Treatment 1 at 55 °C; T$_{2T}$: Treatment 2 at 55 °C; T3T: Treatment 3 at 55 °C.
Source: The authors.

The COD$_{CH4}$ was calculated according to Metcalf and Eddy (2003), in which COD$_{CH4}$ is the COD of thecumulative methane production based on 0.395L CH4.(g COD)$^{-1}$. The COD$_{VFA}$ was calculated by the difference between the initial COD mass (COD$_{initial}$) and the COD$_{CH4}$, assuming as zero the COD for the formation of new cells, due to the degradation period.

The percentage of %BD and the %M were calculated according to Elbeshbishy and Nakhla (2012), given by equations (1) and (2), respectively

$$\%BD = \left(\frac{COD_{initial} - COD_{final}}{COD_{initial}} \right) \times 100 +$$
$$+ \left(\frac{COD_{VFA}}{COD_{initial}} \right) \times 100 \qquad (1)$$

$$\%M = \left(\frac{COD_{CH4}}{COD_{initial}} \right) \times 100 \qquad (2)$$

The co-digestion kinetics was evaluated using as a parameter the organic load removal rate in terms of COD applied per liter of the reactor (COD$_{Apl.}$ L$_R^{-1}$), calculating the degradation rate constant K$_d$ considering that in a batch reactor the rate of change of reagent concentration is proportional to the concentration of this reagent in a given instant, admitting, therefore, a first-order reaction (METCALF; EDDY, 2003; NIKOLAEVA *et al*. 2009), given by equation (3)

$$r = \frac{dS}{dt} = -K_d S, \qquad (3)$$

where r is the reaction speed (g.L^{-1} days), S is the load of the limiting reagent COD (g), t is the hydraulic detention time (days), and K$_d$ is the rate constant for the first-order reaction (d^{-1}).

Morphological analysis of inoculum biomass

At the end of the assays, the microbiota that constitutes the inoculum sludge of the treatments that presented the best methanization efficiencies at each temperature was analyzed by SEM, following the methodology by Araújo *et al*. (2003), in which 0.5 mL samples of the inoculum were fixed with phosphate buffer (pH 7.3) containing 25% glutaraldehyde for 2 h at 4 °C, dehydrated with ethanol solutions by washing the samples 6 times, at 10-minute intervals, with growing concentrations (45, 70, 80, 95, and 100% v.v^{-1}), placed on aluminum stubs with double-sided carbon ribbon, and dried at 30 °C in a forced-air oven, for 2 h. Afterward, the samples were covered with a 20 nm thick gold layer, in a Quorum®

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sputter coater, model Q 150R ES, with a 15 mA current and metallization time of 90 s, with the observations performed in a VEGA3 TESCAN scanning electron microscope.

Statistical Analyses

The results of COD removal and %M were subjected to ANOVA, and the means were compared by Tukey's test at 5% of probability using the SISVAR statistical software (FERREIRA, 2011).

Results and discussion

Removal efficiency of organic matter (COD_S)

It is noted that there was a significant difference between the treatments subjected to mesophilic and thermophilic temperatures ($p < 0.05$, F- test), in which the co-digestion at 35 °C obtained the highest COD_S removal efficiencies, Figure 2. Similar results were obtained in studies by Xiao, Shi and Ruan (2019), who compared the performance of anaerobic digestion at mesophilic (39 °C) and thermophilic temperatures (55 °C) using leachate from kitchen residues as a substrate, and observed that the anaerobic digestion in the mesophilic range presented better digestive performance, supporting a higher organic loading rate, presenting greater stability, and better COD_S removal efficiency (98%) when compared to thermophilic digestion. The authors attributed these results to the fact that the thermophilic anaerobic biomass is more sensitive to the accumulation of VFAs, compared to the mesophilic biomass.

Considering that according to KIM; KIM; YUN (2017), the thermophilic anaerobic digestion process, when compared to the mesophilic, is much more sensitive to load shocks, the addition of inhibiting materials, and variations in temperature, and admitting a maximum variation of 2 °C per day, the accumulation of VFA may have also been caused by variations in temperature, during co-digestion (of $\pm$ 2 °C), since during the development of the experiment there were unforeseen power outages in the laboratory.

In this manner, all treatments at 35 °C obtained COD_S removals above 50%, in which T_{1M}, T_{2M}, and T_{3M} reached maximum removals of 58, 53, and 63%, respectively. Although T_{1M} did not present the highest COD_S removal, the removal verified is seen as satisfactory, considering the application of 95% of EAV in the mixture composition of this treatment and the difficulty

in the treatment of recalcitrant agro-industrial effluents. Furthermore, although there was no statistically significant difference between treatments in mesophilic temperature ($p > 0.05$, F-test), T_{3M} presented the highest efficiency in organic matter removal. This result is attributed to the lower COD concentration applied to this treatment, a result of the higher percentage of domestic sewage present in this treatment, since the domestic sewage promoted the dilution of the agro-industrial effluent studied, increasing the water content, and also favoring the hydrolysis process, which is the initial stage of the digestion process.
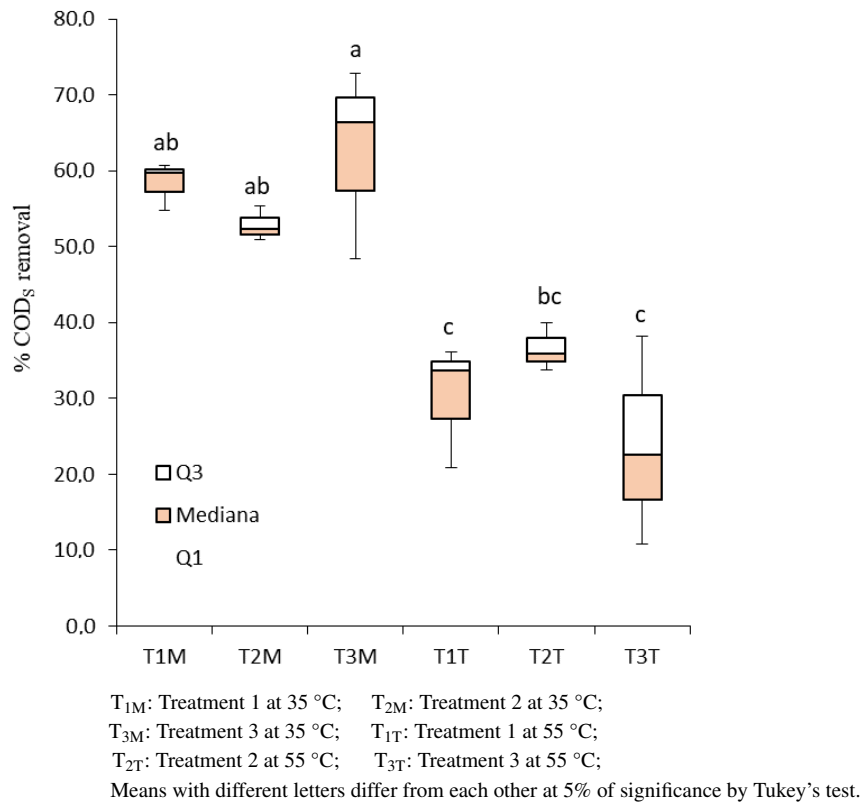
However, it is worth noting that the removal efficiencies verified for all treatments at 35 °C were considered satisfactory when compared to other works present in the literature, employing the anaerobic co-digestion of agro-industrial and agricultural effluents at mesophilic temperatures. Cremonez *et al*. (2015) conducted anaerobic co-digestion studies with swine wastewater and vinasse in a UASB reactor at 35 °C and obtained a total COD removal around 68%. Although this removal was superior to those obtained here, it is highlighted that the present study used the COD_S as a parameter, not comprising, therefore, the insoluble COD parcel (particulate), which probably will not be stabilized by microorganisms. Náthia-Neves, Forster-Carneiro and Berni (2015), also in anaerobic co-digestion assays with food residues and mesophilic sludge from vinasse treatment, at mesophilic temperature conditions (37 °C), using a system of three sequential reactors operating semi-continuously, obtained 48% of total COD removal in the methanogenic reactor.

Evaluation and performance of the process: Mass balance in terms of COD and kinetics of the reaction

For both temperatures, the percentage of anaerobic biodegradability of all treatments was above 69%, indicating that the application of co-digestion constitutes a viable and efficient alternative for the removal of organic matter, either at 35 °C or 55 °C, see Table 3.

Considering the studies performed by André *et al*. (2019), which consider the existence of a relevant inorganic fraction and recalcitrant organic fraction in the green acerola processing effluent, the verified results demonstrate that the association of this effluent with domestic sewage contributed to a high biodegradability, since, according to Bertolino, Carvalho and Aquino (2008), the domestic sewage is essentially organic.

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Figure 2 – COD_S removal efficiency (%) at 35 and 55 °C.

T_{1M}: Treatment 1 at 35 °C; T_{2M}: Treatment 2 at 35 °C;
T_{3M}: Treatment 3 at 35 °C; T_{1T}: Treatment 1 at 55 °C;
T_{2T}: Treatment 2 at 55 °C; T_{3T}: Treatment 3 at 55 °C;
Means with different letters differ from each other at 5% of significance by Tukey's test.

Source: The authors.

Table 3 – Composition of green acerola processing effluent (EAV) and treated domestic sewage (EDT).

Treatments	T_{1M}	T_{2M}	T_{3M}	T_{1T}	T_{2T}	T_{3T}
%BD	90 ± 3.7	71 ± 1.8	76 ± 0.2	69 ± 1.2	69 ± 3.1	69 ± 15.0
%M	48 a ± 2.6	47 a ± 5.2	51 a ± 10.7	26 b ± 4.7	32 ab ± 2.2	20 b ± 11.4
COD_{VFA} (g)	15 ± 1.4	11 ± 1.5	8 ± 1.7	22 b ± 1.3	18 ± 0.5	14 ± 3.1
K_d (d^{-1})	0.09 ± 0.001	0.10 ± 0.004	0.10 ± 0.013	0.04 ± 0.002	0.06 ± 0.004	0.06 ± 0.013

T_{1M}: Treatment 1 at 35 °C; T_{2M}: Treatment 2 at 35 °C; T_{3M}: Treatment 3 at 35 °C
T_{1T}: Treatment 1 at 55 °C; T_{2T}: Treatment 2 at 55 °C; T_{3T}: Treatment 3 at 55 °C;
%BD – Anaerobic biodegradability;
%M – Parcel of the COD converted into methane;
COD_{VFA} – Portion of the COD present in the effluent in the form of VFA's not converted to methane;
K_d – Decay rate constant of the COD.
Values of %M with different letters differ from each other at 5% of significance by Tukey's test.

Source: The authors.

It is observed that among the treatments at 35 °C, T_{1M} – treatment with the highest percentage of agro-industrial effluent – presented the highest percentage of transformed soluble organic matter, that is, %BD, which indicates that this was the most biodegradable treatment. However, part of this transformed organic matter (biode-graded and not converted into methane) in T_{1M} remained in the reactor flasks in the form of VFA, as the results of the COD_{VFA} demonstrated, Table 3. On the other hand, although T_{3M} has presented a lower %BD compared to the T_{1M}, it presented higher COD_S removal, Figure 2, and higher conversion of this into methane (51%).

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The same could not be observed in the treatments at 55 °C, which presented the same %BD, probably due to the instabilities observed at this operating temperature, such as greater sensitivity of the thermophilic anaerobic biomass to accumulation of VFA.

When comparing the operating temperatures, it is observed that the highest VFAs accumulations occurred in the treatments at 55 °C. These results relate directly to the removal efficiency of the COD_S and to the percentage of %M, in which the treatments with the highest accumulations of VFAs presented the lowest COD_S removal efficiencies and conversion of this into methane. These results corroborate studies performed by Xiao, Shi and Ruan (2019), in which the thermophilic anaerobic digestion reinforces the toxicity of the VFAs, a fact that implies on the decrease of microbial activity, damaging the aerobic digestion process.

In this manner, the mesophilic treatments (35 °C) also presented the highest percentages of %M compared to the thermophilic range, with methanogenic bacteria being the most affected in this process (55 °C).

However, it is worth noting that, in all treatments, there were percentage differences between %BD and %M above 5%, a circumstance that according to Elbeshbishy and Nakhla (2012) suggests that there was inhibition of methanogenesis during the stabilization process of the organic matter in all treatments and both temperatures studied. Such a fact is also justified by the COD_{VFA}, that is, the highest values occurred for the treatments with the highest percentages of recalcitrant effluent, those with the highest trends toward the acidification or accumulation of VFAs since their chemical composition presents higher percentages of insoluble matters, thus evidencing the positive influence of domestic sewage as a co-substrate.

However, the results verified for the COD_S removal in the treatments at 35 °C did not indicate inhibition problems of biodigestion, thus indicating that the microbial communities in these reactor flasks showed to be adapted to environments with high concentrations of COD and VFAs.

As can be seen in Table 3, there was no statistically significant difference (p > 0.05, F-test) among the treatments at 35 °C with respect to the %M, however, it is noteworthy that among these treatments T_{3M} presented the highest percentage of methanization (%M) even being the treatment with the lowest concentration of COD applied (Table 3), besides also presenting the highest percentage of COD effectively removed, Figure 2, and lower

mass of COD present in the effluent in the form of VFA, COD_{VFA}, Table 3. Thus, treated domestic sewage is a potential co-substrate to improve the process of anaerobic digestion in agro-industrial effluents.

The treatments under mesophilic temperature presented the highest K_d values when compared to the thermophilic temperature range, Table 3. This result relates to the highest values of %BD and %M observed in these treatments, in which the removal of COD and its conversion into methane occurred more efficiently.

It is worth noting that the mixture composition of the treatments was directly related to the K_d, in which treatments with a higher percentage of domestic sewage, regardless of temperature, presented the highest K_d values. These results are probably associated to the higher degradability of the organic material present in the domestic sewage, since this – unlike the green acerola processing effluent – is mostly found in the soluble form, and to a lesser extent in particulate form.

Furthermore, considering that according to Metcalf and Eddy (2003), the kinetics of anaerobic degradation is ruled by the slowest stage of effluent conversion (usually the methane formation stage), the lowest values found for the treatments at 55 °C corroborate with a possible negative interference under methanogenic microorganisms when presenting the lowest K_d values.

The K_d values obtained in all treatments, regardless of the operating temperature, are considered low when compared to the values obtained for domestic wastewaters, K_d 1.62 d^{-1} (BRASIL et al., 2007) and K_d 1.72 d^{-1} (CHAGAS et al., 2011), and for slaughterhouse wastewater, K_d 0.2203 d^{-1} (LOPES et al., 2015).

*Morphological analysis of the microbial biomass*

At the end of the assays, the morphology of the microorganisms present in the biomass of the treatments was observed, presenting the highest %M at 35 and 55 °C (T_{3M} and T_{2T}, respectively), shown in Figure 3.

In the biomass of T_{3M}, the presence of short bacilli is verified, whose morphology resembles that of methanogenic archaea of the species *Methanobrevibacter smithii*, Figure 3a), filamentous with flattened ends, like bamboo sticks, resembling *Methanosaeta*, Figure 3b), and short and long rod-shaped individuals that resemble *Methanobacterium*, Figure 3c).
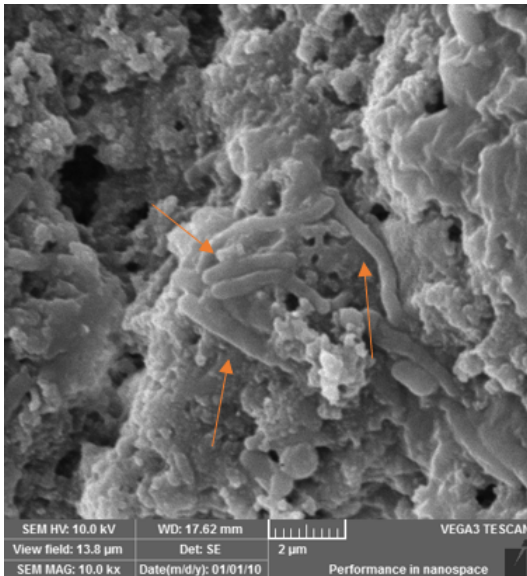
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Figure 3 – Micrographs of the biomass samples present in: a)-c): T_{3M} and d) T_{2T}
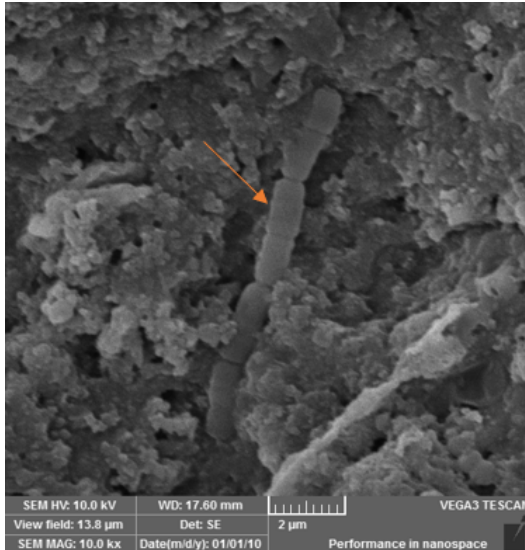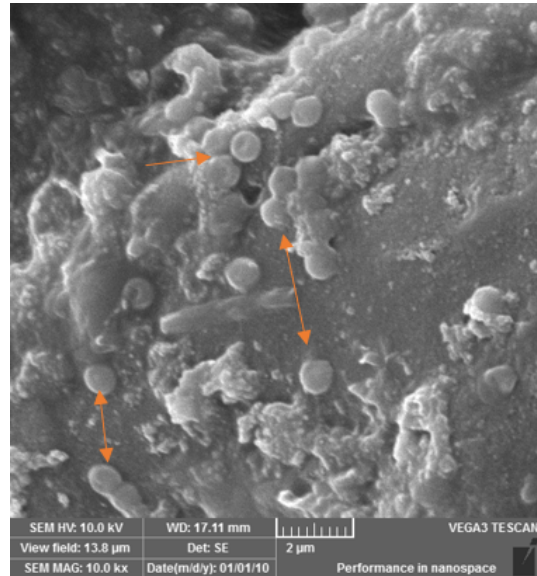

a) Short bacilli similar to Methanobrevibacter smithii (T_{3M});
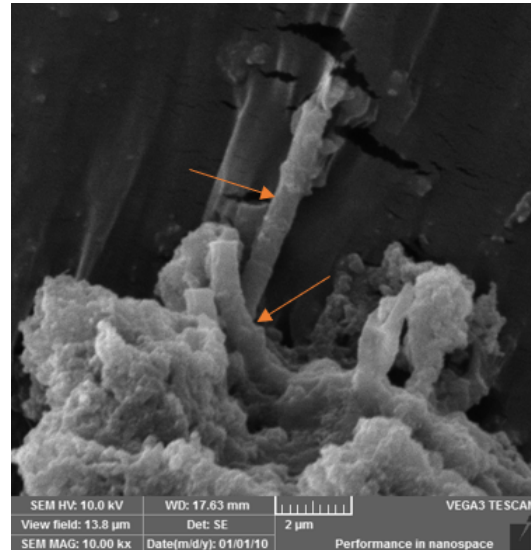
b) Bamboo bud-like filaments similar to Methanosaeta (T_{3M});

c) Long rod-like rods similar to Methanobacterium (T_{3M});

d) Cocci similar to Methanosarcina thermophila (T_{2T});

Source: The authors.

Such morphologies demonstrate a greater biomass structuration in this treatment, evidencing the balanced presence of groups responsible for methanation. In fact, it was the treatment that presented the lowest COD_{VFA} mass and the highest methanation percentage, 8 g and 51%, respectively. The presence of *Methanosaeta* is associated with low concentrations of volatile fatty acids (VRIEZE *et al*., 2012). *Methanosaeta* is, along with *Methanosarcina*, the two only acetoclastic genera able to convert acetate into methane, thus explaining the higher methanation percentage found in this treatment. In the biomass of T_{2T}, only similar morphologies to methanogenic archaea of the genus *Methanosarcina thermophila* are observed, justifying the fact that this is the thermophilic treatment with the highest methanation (32 %), Figure 3d).

The lower microbial diversity found at 55 °C is also associated with the higher accumulation of VFAs in the treatments subjected to this temperature since some volatile fatty acids, according to Mussati *et al*., (2005) present a toxic effect at low concentrations on some genera of methanogenic archaea, such as *Methanobrevibacter*, thus inhibiting their growth and causing the decrease of methane production.

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In spite of the existence of methanogenic archaea at both temperatures, the presence of similar morphologies is not observed, which indicates that this operating parameter of the reactor flasks exerts influence on the microbial communities that act in the process of anaerobic co-digestion, corroborating the studies performed by Zhang *et al*. (2019), who, when comparing the mesophilic and thermophilic digestion of soybean processing by-products, concluded that the temperature and the organic loading rate are variable and that they change the taxonomic diversity of genera of methanogenic archaea.

Conclusion

The present study evaluated the effects of temperature on the anaerobic co-digestion of acerola agro-industry effluent and treated domestic sewage. The results showed that co-digestion proved to be an efficient alternative for all treatments, with biodegradability above 69%. Therefore, it is an effective alternative to minimize the recalcitrant effects of this type of effluent.

The mesophilic condition favored the process of anaerobic co-digestion with all treatments, presenting COD_S removals and its conversion into methane above 50% and 45%, respectively. Among the treatments at 35 °C, the one with the highest percentage of sewage applied presented the highest COD_S removal efficiency and its conversion into methane (63% and 51%, respectively). This condition also presented the highest values of K_d.

The biomass of the mesophilic treatments exhibited greater microbial diversity, with the predominance of methanogenic archaea of the genera *Methanobrevibacter*, *Methanosaeta*, and *Methanobacterium*, resulting in better percentages of biodegradation and methanization.

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