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ANAEROBIC CO-DIGESTION OF SUGARCANE VINASSE AND ELEPHANT GRASS JUICE FOR BIOMETHAN PRODUCTION

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Abstract
In the present study, biogas production was investigated by co-digesting elephant grass juice (EGJ) and sugarcane vinasse using batch reactor. Some factors that influence biomethane production were observed, including initial pH, inoculum concentration and proportions of each substrate. Two tests were carried out. In Experiment I, the following proportions (%v/v sugarcane vinasse/elephant grass juice) were tested: A - 25/75, B - 50/50, C - 75/25, D - 100/0, E - 0/100. In Experiment II, the effect of adding alkalizer to the condition that showed the highest methane production (in Experiment I) was also evaluated. In the Experiment I, the highest accumulated production was observed for the proportion 1:1 corresponding to 50% of EGJ and 50% of vinasse (370.94 $mLCH_4/g_{VS}$). Experiment II showed the higher values of accumulated methane production of 1,364.1 $mLCH_4/g_{VS}$. Regarding addition of alkalizing, a maximum production of 836.18 $mLCH_4/g_{VS}$ was obtained in the experimental condition with 50% EGJ and 50% vinasse, but with the lowest addition of alkalizer tested (0.05 g HCO_3/gCOD). In general, the use of elephant grass caused the acidification of the reactors and was unfavorable for biogas production.

Keywords: anaerobic co-digestion, methane, batch, methanogenic potential.

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Introduction

In the face of a constant increase in global energy demand, different types of residues have been widely studied as a potential source of supplementary energy to substitute fossil fuels. In this way, biological reactors that use agro-industrial waste, such as vinasse, stand out as prominent technologies to generate renewable biofuels (Damrongsak *et al.* 2017, Gao *et al.* 2019).

Vinasse is a subproduct of alcohol processing. Because its high content in nutrients and organic carbon, vinasse is usually used as a fertilizer in sugarcane crops, being the basic economically viable reuse process. However, it is one of the most polluting residues generated in ethanol production, mainly because of its composition with high organic load and acidity. Therefore, the use of vinasse for fertilization is harmful to the soil and water resources, causing problems such as percolation and leaching, possible pollution of groundwater, and emission of greenhouse gases. Besides the environmental impacts, the use for fertilization wastes the potential of vinasse for other applications, such as the generation of biogas (Moraes *et al.* 2015).

The incentives of the Brazilian National Biofuels Policy – RENOVABIO (Law 13,576 of December 26, 2017) tend to encourage the expansion of sugarcane ethanol production in the coming years. Consequently, the production of sugarcane vinasse will also increase since each liter of ethanol produces about 12 liters of this industrial effluent. Thus, a suitable alternative to treat vinasse and deal with the large volumes of this subproduct is the use of anaerobic bioreactors to produce biogas (Mauad *et al.* 2017).

The biogas production in anaerobic digestion utilizes predominantly substrates derived from agro-industrial waste, animal manure, or organic urban solid residues. Studies reported that the process is more efficient when it uses more than one substrate (Scarlat *et al.* 2018, Siddique and Wahid 2018). Advantages of anaerobic co-digestion, which combine the treatment of different feedstocks wastes, include the improvement of process stabilization, nutrient balance, and synergetic effects of microorganisms (Hagos *et al.* 2017).

Pinto *et al.* (2018) evaluated the co-digestion of coffee residues and sugarcane vinasse. They obtained high volumetric proportions of biomethane and biohydrogen, achieving a biogas with a maximum volumetric methane content of 44% and maximum methane yields of 0.14 $mlCH_4/gVS_{added}$. Lovato *et al.* (2019) used an AnSBBR (anaerobic sequencing batch biofilm reactor) to realize the co-digestion of vinasse and cheese whey. The authors stated that increasing vinasse concentration in the influent was unfavorable to methane molar productivity and yield. Borges *et al.* (2021) analyzed the methane production from co-digestion of sugarcane vinasse and distilled glycerol. They found the highest yield of 352 ± 17 $NmLCH_4$ $g^{-1}COD_{rem}$ in a vinasse-to-glycerol proportion of 50:50(%) on a COD basis and stated that adding glycerol to vinasse enhanced methane yield and biogas production rate.

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The cited research reinforces the benefits of anaerobic co-digestion of vinasse with another substrate to produce biogas. An interesting material to employ in this process along with the vinasse is the elephant grass (*Pennisetum purpureum* synonym *Cenchrus purpureus*), a semi-perennial grass with prominent productive potential in tropical and subtropical areas. That species has a rapid growth and a high photosynthetic efficiency that result in a substantial amount of dry matter accumulation, which often exceeds 40 tons/ha, with excellent energy quality attributes (Marafon *et al.* 2020).

Elephant grass is a lignocellulosic biomass that has emerged as a promising energy raw material, applied in various energy conversion methods, including biological processes to produce biofuels and value-added co-products. However, the major problem in the handling forage materials such as elephant grass is the high cost, mainly due to its low apparent density, which demands large volumes for its transport and storage. In this sense, extracting juice from elephant grass can significantly increase the energy density of its bagasse, enabling the transport of this material, besides producing juice abundant in nutrients applicable to be used in industry.

Extracting juice from the elephant grass is similar to extracting sugarcane juice, producing bagasse with much-reduced moisture compared to fresh material. This process maintains the continuous supply and quality of the biomass since dehydration reduces the risks of fermentation and decomposition.

The use of elephant grass juice as a substrate for co-digestion can be advantageous given the high biomass productivity and locational feasibility, with wide distribution and good adaptation throughout the Brazilian territory. This characteristic makes it an excellent alternative for expanding the potential for producing biogas together with sugarcane vinasse (Favare *et al*., 2019).

The sugar-energy industrial plants operate around 200 days a year with a break between harvests when the thermoelectric energy cogeneration process that uses sugarcane bagasse for direct combustion in boilers is interrupted. However, the alternative supplement with dried elephant grass could increase the energy efficiency of direct combustion in the sugarcane industry. In addition, there is the possibility of using elephant grass juice as a co-substrate in anaerobic vinasse digestion processes for biogas production.

Through the production of biogas, sugarcane plants can obtain financial returns and contribute to improving the sustainability of the environment. While the biogas produced can be used in boilers (complementary to sugarcane bagasse) or in stationary engines to generate electricity, biomethane can be used as biofuel to supply the fleet of trucks and tractors to replace diesel oil.

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In this context, this work investigated the potential for biogas production using different proportions of vinasse and elephant grass juice in the anaerobic co-digestion process, analyzing the effect of dilution of substrates and the addition of an alkalizing. The novelty of this study is in the use of elephant grass juice as a component of the method to improve biogas obtaining.

Methodology

The anaerobic co-digestion was realized in laboratory scale using batch reactors fed with two substrates: sugarcane vinasse (SV) and elephant grass juice (EGJ).

The elephant grass juice was obtained in the Madeira clone (BAGCE 145), from the Elephant Grass Active Germplasm Bank of *Embrapa Gado de Leite*, in an experimental area installed at the Campus of Engineering and Agricultural Sciences (CECA) of the Federal University of Alagoas (UFAL), Rio Largo/Alagoas. The EGJ presented a COD of 8,534.21 mg/L, initial pH of 4.3, total volatile solids (TVS) of 21,400 mg/L and yield of 1 liter of juice for every 5.44 kg of stalks pressed in the mill.

Sugarcane vinasse was collected at an industrial plant located in the municipality of Marechal Deodoro-AL, Brazil, with a COD of 31,955.34 mg/L, pH baseline of 3.76 and TVS of 7,400 mg/L. Both EGJ and SV were frozen until the time of the tests.

The inoculum used was sludge obtained from a UASB reactor from the treatment of sanitary sewage in a residential condominium located in Maceió-AL, Brazil, with TVS of 24,920 mg/L. The material was used *in natura*, without previous treatment.

Physical-chemical analyses were conducted for characterization of the substrates and the inoculum (Table 1).

Table 1. Physical-chemical analysis.

Analysis	Equipment or method	Frequency	Reference
pH, COD, Solids, Alkalinity	Standard methods for the examination of water and wastewater	Initial and final	(APHA 2017)
Total carbohydrate	Colorimetric Method for Determination of Sugars and Related Substances	Initial and final	(DuBois *et al.* 1956)
Biogas composition	GC-2010, Shimadzu, Japan[b]	5 times/week	(Maintinguer *et al.* 2008)

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The experimental conditions were divided into two distinct and subsequent phases. Experiment I consisted of using SV and EGJ in different proportions of the mixture. Experiment II evaluated the addition of Sodium Bicarbonate ($NaHCO_3$) (in different concentrations) and the dilution of the substrates with water, considering the best biogas production performance of Experiment I. All procedures performed for the start-up, operation and conclusion of the experiments were standardized for both series of experiments.

<u>Experiment I</u>

Experiment I used batch biodigesters of glass flasks with 106 mL of total volume, 60 mL of reactional volume (with 10% - 6 mL - for the inoculum), and 46 mL of headspace (gaseous volume).

The pH adjustment was carried out to values around 7.5 using a 0.1 N NaOH solution. Argon gas was fluxed in the flasks containing the mixture for one minute to create an anaerobic condition. The flasks were then sealed and placed in the shaker chamber, with a rotation of about 100 rpm and a controlled temperature of 30 ºC.

The study employed 16 digesters distributed as follows: Triplicates of the mixture with different proportions (A, B, C, D and E – 15 units) and one Control containing only inoculum (F). The mixture of EGJ and SV in different volumes is presented in Table 2.

Table 2. Proportion of elephant grass juice (EGJ) and sugarcane vinasse (SV) in percentage (V/J) for each test in Experiment I

Reactor	Proportion (V/J)*	Useful volume (mL)	Reactional volume (mL)	SV (mL)	EGJ (mL)	Inoculum (mL)	Replicas
A	25/75	106	60	13.5	40.5	6	3
B	50/50	106	60	27	27	6	3
C	75/25	106	60	40.5	13.5	6	3
D	100/0	106	60	54	0	6	3
E	0/100	106	60	0	54	6	3
F	Control	106	60	0	0	6	1

**Based on volume/volume ratio (v/v)*

<u>Experiment II</u>

Experiment II was defined from the reactor with the best biogas production obtained in Experiment I. This phase analyzed the effect of dilution using water and the addition of alkalizing agent.

The tests employed batch reactors using glass flasks with useful volume of 106 mL, reactional volume of 40 mL (with 10% - 4 mL - for the inoculum) and headspace volume of 66 mL.

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In this phase, 13 digesters were used, distributed as follows: Duplicates of the mixture with different proportions of SV, EGJ and water (experimental conditions 1, 2, 3, 4, 5, 6 – 12 units) and one Control containing only inoculum (7) (Table 3).

Table 3. Proportion of vinasse, juice, and water in percentage (V/J/W) for each test in Experiment II.

Reactor	Proportion (V/J/W) % - (Experimental condition)	Sodium bicarbonate ($gHCO_3/gCOD$)	Reactional volume (mL)	SV (mL)	EGJ (mL)	Water (mL)	Inoculum (mL)	Replicas
1	50/0/50	0	40	18	0	18	4	2
2	75/0/25	0	40	27	0	5	4	2
3	0/50/50	0	40	0	18	18	4	2
4	50/50/0	0.05	40	18	18	0	4	2
5	50/50/0	0.10	40	18	18	0	4	2
6	50/50/0	0.25	40	18	18	0	4	2
7	Control	0	40	0	0	36	4	1

Experimental conditions 1, 2 and 3 were designed to evaluate the contribution of each substrate to methane production. Conditions 4, 5 and 6 included the addition of sodium bicarbonate to the mixture to evaluate the effect of biogas production in the face of the addition of alkalizing agent.

Monitoring started 24 hours after the startup of the biodigesters. Gas collection occurred by removing 0.1mL of sample from the headspace (gas phase) in the reactors and manually injecting into the chromatograph. Chromatography was performed once a day for the first 7 days. After this period, the biogas production rate and the stabilization of the reactors were analyzed and, as there was a lower production rate for the initial period (24 h), the analysis interval was spaced for a maximum of 48 h. The chromatographic method for the determination of methane production and biogas composition was performed using a gas chromatograph, Shimadzu GC-2010-Plus, equipped with a thermal conductivity detector, according to the methodology developed by Maintinguer *et al.* (2008). A Supelco Carboxen 1010 Plot column (30 m long and 0.53 mm internal diameter) was used.

At the end of the experiments, the final characterization was carried out by means of the analyzes that consist in the determination of the results of the physical-chemical evaluation (Table 1).

The accumulated volume of methane in the headspace in mmol of each reactor was converted into mL-CH_4 through the General Gas Equation. For the triplicates of reactors used, the standard deviation (σ) and the coefficient of variation (CV) were used for the composition of the means.

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For the presentation of data related to methane production, it was applied to the Gompertz sigmoid. The sigmoid was numerically derived, with the help of the same software, to determine the maximum methane production rates in each reactor (experimental condition) and maximum methane production rate.

Results

Experiment I

Table 4 presents the initial characterization of the compositions before the pH adjustment. Reactors fed with greater proportions of vinasse (D and C) had elevated COD concentrations, and those containing more EGJ presented less COD (A and E). Similarly, as more vinasse is added to the mixture, the lower the pH.

Reactor E, having only EGJ and inoculum in the mixture, presented higher total solids (TS = 24046.00 mg/L) and total volatile solids (TVS = 21521.00 mg/L), while reactor D (vinasse + inoculum), had the lower number of solids (TS = 9004.00 mg/L; TVS= 8276.00 mg/L).

Table 4. Initial physical-chemical results of the fractions used in the experiment I.

Parameter	A (25/75)	B (50/50)	C (75/25)	D (100/0)	E (0/100)
pH	4.80	4.35	4.07	3.75	6.22
COD (mg/L)	14375.63	20316.98	26258.33	30601.91	8024.78
Total solids (mg/L)	19900.00	15800.00	11700.00	9004.00	24046.00
Total volatile solids (mg/L)	17900.00	14400.00	10900.00	8276.00	21521.00
Total fixed solids (mg/L)	2000.00	1400.00	800.00	728.00	2525.00

Figure 1 shows the variations of pH for the Experiment 1. Reactor E had the lowest final pH of 3.48, followed by reactor A (V/J = 25/75), with final pH of 3.61. These reactors contained the higher proportion of elephant grass juice. Reactors C and D, with higher amount of vinasse, presented maximum final pH of 5.11 and 5.34, respectively.

All conditions that operated with a higher concentration of elephant grass juice showed a greater pH decay. This fact may represent the expressive behavior of EGJ in the acidification of the reactors. Reduction in pH can be associated with accumulation of volatile acids in the system which tends to be unsuitable for methane production. In general, methanogenic microorganisms' activity is favored at neutral pH of about 7.0 (Kiani Deh Kiani *et al.* 2022).

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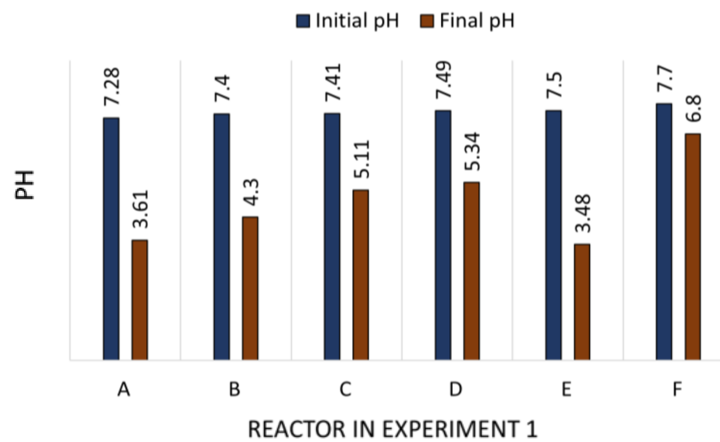

Figure 1. Variation of pH in the reactors of the Experiment 1.

Figure 2 presents total volatile solids removal and COD removal observed in Experiment 1. Minimum TVS removal occurred in reactors E (29.29%) and C (29.66%), while reactor D presented the highest value of 41.60%. The results were smaller than that found by Carvalho *et al.* (2016). The authors realized the anaerobic co-digestion of sewage sludge and elephant grass hydrolysate, achieving TVS reductions of 48, 59 and 65%.

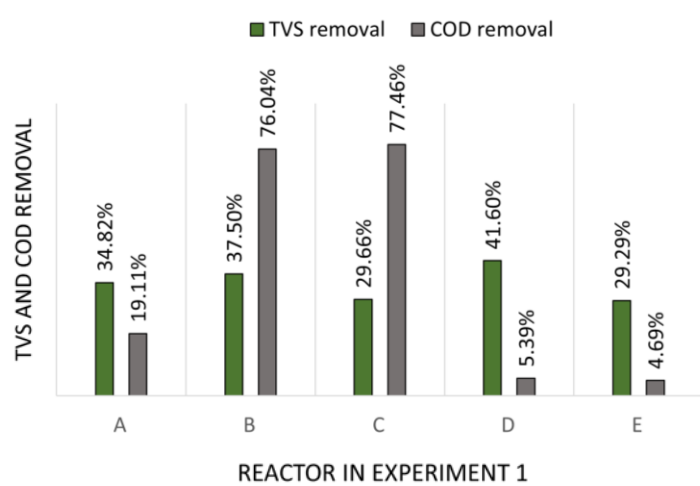

Figure 2. Variation of TVS and COD removal in the reactors of the Experiment 1.

The lowest COD removal occurred in reactor E (4.69%). Reactors B and C presented the maximum COD removal of 76.04 and 77.46%, respectively. Reactor A also achieved a small COD removal of

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19.11%. The reactors with higher amounts of elephant grass juice probably were adverse for TVS and COD removal. However, the low COD decrease in reactor D (5.39%), containing only vinasse and inoculum, may have occurred due to the high load of organic matter since elevated substrate concentrations can cause kinetic limitations that inhibit the capacity of microorganisms to consume carbohydrates (Gois *et al.* 2021).

Figure 3 presents the behavior of the accumulated methane production (the adjustment coefficients for the Gompertz model were higher than 0.99). Reactors D and E, containing only one of the substrates and inoculum, obtained low biogas production. It indicates the combination of substrates in co-digestion proved to be more effective. There was a correlation between COD removal and methane production. The conditions with the lowest accumulated methane production value, D and E, also presented the lowest COD consumption of 5.39% and 4.69% respectively.

The experimental conditions with the best performances in the accumulated production of biogas (B and C) showed the highest COD removal efficiencies of 76.04% and 77.46%, respectively. Experimental condition B presented the highest accumulated methane production, reaching 370.94 $mLCH_4/g_{VS}$, followed by the experimental type C which produced 231.6 $mLCH_4/g_{VS}$. Reactors A, D and E achieved methane productions of 204.96, 81.30 and 6.65 $mLCH_4/g_{VS}$.

The maximum production rates were: Reactor A = 14.57 $mLCH_4$/day; Reactor B = 14.12 $mLCH_4$/day; Reactor C = 26.25 $mLCH_4$/day; Reactor D = 1.55 $mLCH_4$/day; Reactor E = 0.065 $mLCH_4$/day.

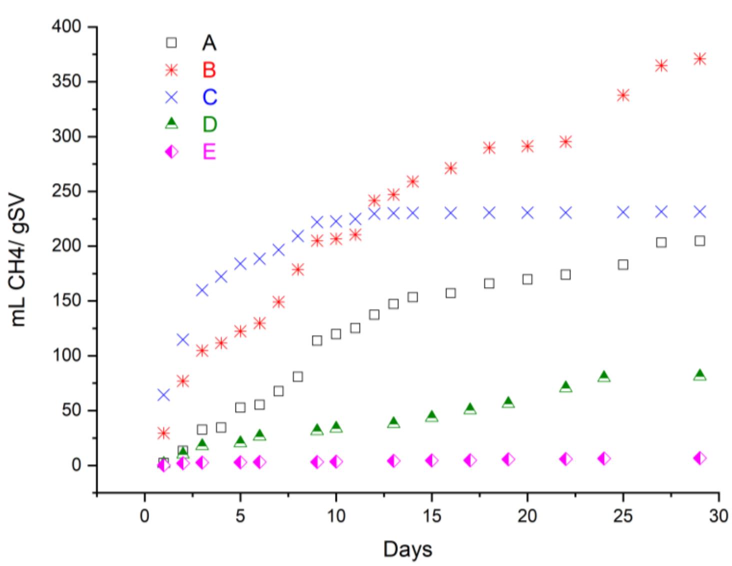

Figure 3. Accumulated methane production adjusted to the Gompertz (Experiment I). % V/C: A (25/75), B (50/50), C (75/25), D (0/100), E (100/0).

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Experiment II

The maximum methane production occurred in reactor B. Therefore, Experiment II was conducted considering its condition. A new characterization was carried out encompassing the parameters presented in Table 5.

Table 5. Physicochemical characteristics of the substrates (Experiment II).%V/C/A (alkalizer): 50/0/50, 75/0/25, 0/50/50, 50/50/0 (0,05), 50/50/0 (0,11), 50/50/0 (0,25).

Parameter	Unity	Vinasse	Elephant grass juice
COD	mg/L	68053.1	81175.41
Total solids	mg/L	16744	86168
Total volatile solids	mg/L	12316	53668
Total fixed solids	mg/L	4428	14500

Reactors 1, 2 and 3 were used to analyze the effect of dilution using water in proportions of V/W = 50/50, V/W = 75/25 and J/W = 50/50, respectively (Table 3).

Reactors 4, 5 and 6 were employed to verify the effect of addition of alkalizing, considering the condition B of Experiment 1. Thus, the mixture was composed of 50% SV and 50% EGJ with concentrations of sodium bicarbonate of 0.05 (reactor 4), 0.10 (reactor 5) and 0.25 $gHCO_3/gCOD$ (reactor 6).

Figure 4 displays initial and final pH for the reactors in Experiment II. Minimum final pH occurred in reactor 3 (EGJ + water + inoculum), reinforcing that elephant grass juice tends to acidify the system. Reactor 1 (SV 50% + water 50% + inoculum) and reactor 2 (SV 70% + water 20% + inoculum) presented higher final pH of 5.82 and 5.61, respectively. Reactors without EGJ achieved better results for final pH.

Reactors 4, 5 and 6 (containing the mixture of SV, EGJ and sodium bicarbonate) had final pH of 5.37, 5.32 and 5.06, respectively. These values were higher than that found for reactor B in Experiment I of 4.3. It indicates that the addition of an alkalizing diminished the acidification.

Figure 5 shows percentages of TVS and COD removal. Maximum TVS removal occurred in reactors 1 (31.99%) and 3 (33.43%), and minimum values were found in reactors 2 (15.45%) and 5 (17.55%). Percentages of COD removal were close, in the range of 50.07-55.44%, with a small standard deviation of 0.021.

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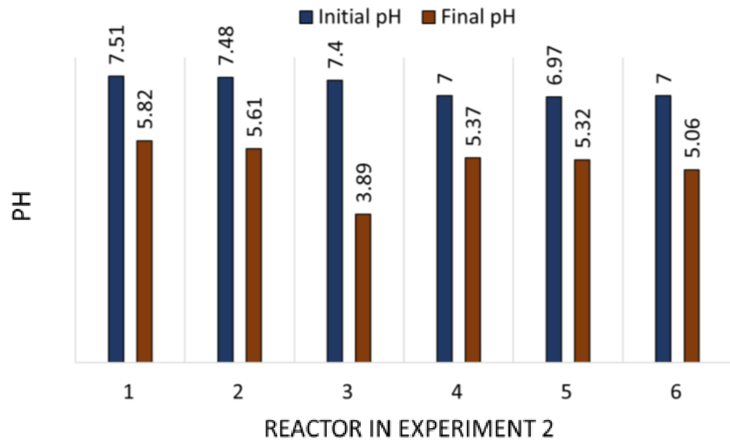

Figure 4. Variation of pH in Experiment II.

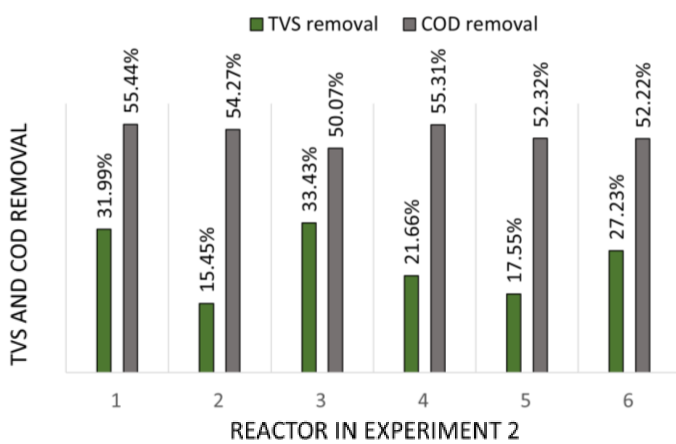

Figure 5. Variation of TVS and COD removal in Experiment II.

Figure 6 displays the accumulated methane production (the adjustment coefficients for the Gompertz model were higher than 0.99). Experimental conditions 1 and 2 showed an increasing accumulated methane production of during about 10 days, ending with condition 2 showing the highest accumulated production of methane of 1,364.1 $mLCH_4/g_{VS}$, followed by reactor 1 (1,100.1 $mLCH_4/g_{VS}$). The accumulated production in Experiment I, using vinasse with 100% of the reaction volume (D), presented a value of 81.30 $mLCH_4/g_{VS}$.

The addition of an alkalizing favored the biogas production Figure 2(b). However, the higher amount of methane was obtained at lower sodium bicarbonate concentration of 0.05 $gHCO_3/gCOD$, in reactor 4, which achieved 836.18 $mLCH_4/g_{VS}$.

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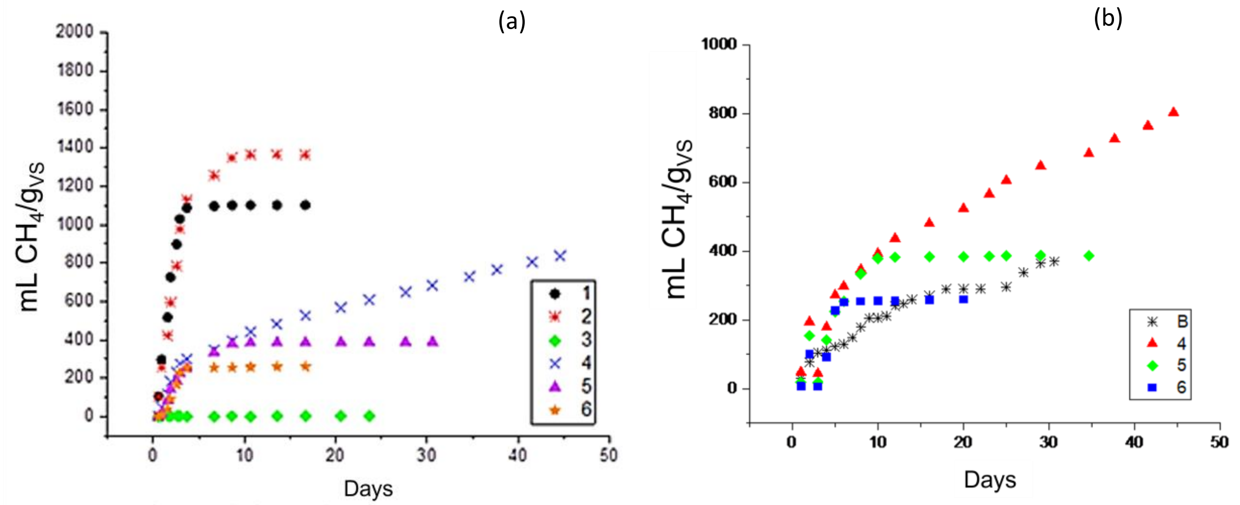

Figure 6. **(a)** Comparison of accumulated methane production under experimental conditions in Experiment II. **(b)** Comparison of accumulated production under experimental conditions with 50% vinasse and 50% elephant grass broth, from assays I and II, with and without addition of sodium bicarbonate. %V/C/A (alkalizer): 1 (50/0/50), 2 (75/0/25), 3 (0/50/50), 4 (50/50/0 - 0.05), 5 (50/50/0 – 0.11), 6 (50/50/0 – 0.25) e %V/C: B (50/50).

The maximum production rates were: Reactor 1 = 158.30 mLCH_4/day; Reactor 2 = 160.02 mLCH_4/day; Reactor 3 = 0.22 mLCH_4/day; Reactor 4 = 30.21 mLCH_4/day; Reactor 5 = 126.01 mLCH_4/day; Reactor 6 = 210.00 mLCH_4/day.

In general, the use of EGJ was adverse for anaerobic digestion. The results presented by Haryanto *et al.* (2018) can corroborate this statement. In the co-digestion of cow dung and elephant grass, the authors achieved the best results of methane production for the reactor without elephant grass (422.58 mLCH_4/g_{VS}). Reactors containing the mixture and diluted with water presented lower methane production of 7.35, 16.75 and 111.72 mLCH_4/g_{VS}.

Conclusion

Anaerobic co-digestion, using vinasse and elephant grass juice as substrates, operated under mesophilic conditions, proved to be efficient.

In Experiment I, reactor with higher proportions of elephant grass juice (A and E) presented lower final pH, indicating that this substrate may be related to the accumulation of acids. Reactor B (V/J = 50/50) and C (V/J = 75/25) had the highest accumulated methane production of 370.94 mLCH_4/g_{VS} and 231.6 mLCH_4/g_{VS}, respectively. They also presented the best COD removal of 76.04% (reactor B) and 77.04% (reactor C).

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In Experiment II, reactors 1 (SV 50% + water 50%) and 2 (SV 70% + water 20%) achieved the higher final pH (5.82 and 5.61), maximum COD removal (55.44 and 54.27%) and the greater accumulated methane production of 1,100.1 and 1,364.1 $mLCH_4/g_{VS}$, respectively.

The addition of sodium bicarbonate (reactors 4, 5 and 6) was favorable to methane production in comparison with the condition B in the Experiment I, which contained the same substrate concentrations. However, the best result was obtained for the lower alkalizing concentration of 0.05 $gHCO_3/gCOD$ in reactor 4.

The experiments conducted in this work showed that the use of elephant grass juice in the anaerobic co-digestion with vinasse was adverse for biogas production. The best results were achieved when this substrate was used in the minor concentrations or even in its absence.

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