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Temperature and pH Effects on High-Methane Biogas Production

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ABSTRACT:
Objective: Biomass is a renewable, widely available, and economically viable energy source for use in anaerobic digestion processes to produce biogas and methane (CH_4). To harness this potential, various technologies have been improved to better reuse organic waste, such as those from the agroindustry, to generate renewable energy and also to contribute to minimizing the environmental risks caused by the improper disposal of these materials. The objective of this study was to define the best operational conditions for the production of biogas and CH_4 from the anaerobic co-digestion of bovine manure, cassava wastewater, and coffee husk.
Methods: All tests showed significant biogas production with at least 80% methane. The statistical tool of experimental design made it possible to identify an inversely proportional relationship between pH and temperature, within the conditions analyzed for these factors.
Results: Thus, it was defined that the combination of an initial pH above 9.5 and a temperature below 35 °C is capable of resulting in a biogas volume > 600 cm³ and CH_4 > 500 cm³. For example, the best experimental performance was obtained with an initial pH of 10.0 and a temperature of 30 °C, which resulted in 798.72 cm³ of biogas and 638.98 cm³ of CH_4 accumulated after 15 days of hydraulic retention time. These best experimental conditions enabled 58.53% removal of chemical oxygen demand and a final pH close to neutrality (6.3).
Conclusion: This represents good fermentation conditions for methanogenic bacteria and confirms the feasibility of using co-digestion of the three evaluated residues.

KEYWORDS: Anaerobic co-digestion. Agroindustrial waste. Renewable energy.

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I. INTRODUCTION

The search for renewable energy sources has grown significantly in recent years due to the environmental impacts caused by the use of fossil fuels (Kabeyi & Olanrewaju, 2022). The majority of the world's energy supply still comes from polluting and non-renewable sources such as oil, coal, and natural gas, which accounted for 85% of primary energy consumption in 2019 alone, with a projected increase of 9% by 2030 (Qadir et al., 2020; Rashedi et al., 2020). Public policies have been formulated to reduce the use of fossil fuels, improve energy security, protect the environment, and promote economic growth, encouraging the search for renewable energy sources (Altoé et al., 2017).

Energy from biomass is a candidate to be one of the most widely used renewable energy resources due to the guarantee of continuous generation and high availability worldwide (Forster-Carneiro et al., 2013; Liso & Mark, 2020). The Brazilian agro-industrial chain generates approximately 291 million tons of waste per year and this waste is considered a by-product with little or no market value (Siqueira et al., 2022). This includes animal confinement waste, grain cleaning waste, products that rot in warehouses, and which, in the end, become significant environmental liabilities, improperly discarded, contaminating soil and water, and emitting gases generated by their decomposition (Peres et al., 2019).

One of the biggest problems in the intensive cattle confinement model is the large amount of manure produced daily in a reduced area. In Brazil, this confinement period is short, with an average of 38.5 kg of manure produced per head per day (Senés-Guerrero et al., 2019). In 2021, Brazil reached a herd of 224.6 million head of cattle, considering that 6.5 million were finished cattle in the intensive confinement model with 90 days, generating approximately 22.5 million tons of bovine manure (IBGE, 2021;

Herrera et al., 2021). Consequently, the disposal of this waste becomes a challenge for farmers and specialists, as it involves technical, sanitary, and economic aspects (Dotto & Wolff, 2012).

In addition to livestock, Brazil is a global leader in cassava production, with an output of 18.4 million tons in 2021 (CONAB, 2022a; IBGE, 2022). In terms of cassava by-products, cassava flour production from root processing stands out, generating a significant amount of liquid residue (cassava wastewater). This residue contains a high concentration of hydrocyanic acid (HCN; average of 3.5 mg/L^{-1}), resulting from the hydrolysis of cyanogenic glycosides (Hasan et al., 2015), as well as a high organic load expressed in Chemical Oxygen Demand (COD) of 14,043 - 141,030 mg/L^{-1} and Biochemical Oxygen Demand (BOD) of 1,968 - 44,624 mg/L^{-1} (Costa et al., 2022). These attributes make cassava wastewater extremely harmful to human health and the environment if not properly treated or disposed of in natural resources such as soil and water. It is estimated that for every ton of processed cassava root, an average of 300 liters of cassava wastewater is obtained, which is equivalent to the pollution caused by a population of 150 to 200 inhabitants per day (Peres et al., 2019).

Brazil also holds a prominent position in coffee production, leading the world in bean production, with an estimated 53.4 million bags in 2022, contributing approximately one-third of global production (OIC, 2021; CONAB, 2022b). In Brazil, coffee cherries are typically processed using the dry method, resulting in the accumulation of coffee husk, which can account for up to 50% of the dry coffee cherry obtained. In other words, for every ton of coffee bean produced, one ton of husks is generated (Du F et al., 2021). Various uses for coffee husk have been suggested, such as animal feed and soil cover (Oliveira & Franca, 2015). However, considering the vast amount of waste generated, there is still a need to explore better alternatives and more profitable and viable uses for this residue (Santos et al., 2018).

Given that Brazil is a potential producer of biomass suitable for renewable energy generation such as biogas, new technological routes have been proposed to enable the reuse of agro-industrial residues while achieving high biogas yields. Regarding biogas, Brazil's theoretical production potential is 84.6 billion cubic meters (m³) per year, which would be sufficient to supply 40% of the domestic demand for electricity and 70% of diesel consumption, but the country currently exploits only 3% of this potential (CIBiogás, 2022). The main constituent of biogas is methane (CH_4, 60-70%) and carbon dioxide (CO_2, 30-40%), along with small amounts of other gases considered contaminants, such as hydrogen sulfide (H_2S) and ammonia (NH_3) (Rasi et al., 2007). It is important to evaluate the combination of substrates (co-digestion) and system parameters for process optimization, such as temperature and pH, which can influence the maximization of quality (CH_4 content) and biogas yield (Sarker et al., 2019).

For the study of the combination of different process parameters, the statistical tool of Experimental Design is well-established (Rodrigues & Iemma, 2005). The Central Composite Design (CCD) matrix is one example of the application of this tool to determine the optimal process conditions for maximizing methane yields (Dima et al., 2020). For instance, the effect of swine manure concentration (250 gSV) and the ratio of cassava residual pulp to water (1:1.22 kg/L) was optimized using the tool, resulting in a maximum biogas yield of 7.43 ± 0.58 L/kg of cassava residual pulp (Jaro et al., 2021). Similarly, the anaerobic co-digestion of *Prosopis juliflora*, water hyacinth, dry leaves, and bovine manure, under conditions of pH (7) and temperature (35.5°C), achieved a maximum production of 396.0 ± 6 L/kg-SV of CH_4 and anaerobic biodegradability of 76.6% (Prabhu et al., 2021).

In light of the foregoing, the objective of this study was to enhance the production of biogas and methane from bovine manure, coffee husk, and cassava wastewater by investigating the influence of key parameters such as pH and temperature using the statistical tool of experimental design.

II. MATERIAL AND METHODS

Obtaining agro-industrial residues

Cassava wastewater samples were collected from cassava flour production facilities located in the microregion of Vitória da Conquista/BA and transported to the laboratory in decontaminated and properly sealed plastic containers. To ensure the evaporation of HCN (boiling point 25.6 °C) from the cassava samples until their use, they were exposed to uncontrolled ambient conditions with solar incidence and air circulation for a period of 15 days in an open container (Bradbury et al., 1999) and then used in the fermentation process. Coffee husk samples (*Coffea arabica L.*) were obtained from rural producers in the municipality of Barra do Choça/BA. The material was dried in a microprocessor-controlled BOD incubator BT 62 from Biothec® at 50 °C until it reached a stable weight, and then it was ground, sieved, and stored at room temperature. Bovine manure samples were obtained from experimental animals at the State University of Southwest Bahia that were confined and fed only forages. The samples were used in the anaerobic co-digestion processes in their fresh form, and thus were collected with a sterile plastic bag.

Assembly and feeding of benchtop bioreactors

Benchtop bioreactors were constructed using 250 ml kitasato flasks, connected by silicone tubing to their respective gasometers, a floating dome type, consisting of PVC tubes (100 and 75 mm) 20 cm long. The inner tube had 1 cm vertical markings for biogas measurement and could hold a maximum volume of 880 cm³ of biogas (Figure 1). The bioreactors were operated in batch mode, and the organic load volume was defined as 43.3 ml of each residue (coffee husk, cassava wastewater, and bovine manure) for 40 ml of distilled water (Leite et al., 2020). A benchtop microprocessor pH meter MPA-210 was used to adjust the pH with a 10

M sodium hydroxide (NaOH) solution. The bioreactors were maintained in a BOD incubator for temperature control, with a hydraulic retention time (HRT) of 15 days.

Figure 1. Benchtop bioreactors constructed using 250 mL kitasato flasks, connected to their respective gasometers via silicone tubing.

Study of process conditions

A 2^2 central composite design (CCD) with 4 experiments under varying conditions and triplicate of the central points, totaling 7 experiments, was conducted to evaluate the influence of pH and temperature (T, °C) on biogas production (cm³) and methane gas (CH_4, cm³), with the aim of studying the best fermentation conditions. The initial pH (pH) and temperature (T) variables were evaluated at the highest coded level (+1) as: 10.0 and 50 °C, and at the lowest coded level (-1) as: 8.0 and 30 °C, respectively. The responses evaluated were biogas production (cm³) and methane production (cm³). With the obtained values, Coefficient Analysis was performed with 85% reliability, in order to include a larger number of significant coefficients in the model (Rodrigues & Iemma, 2005). Subsequently, for the simplified model, analysis of variance (ANOVA) was performed with 95% reliability, and contour curves were generated to assist in determining the best process conditions (Rodrigues & Iemma, 2005). The analyses and contour curves were performed using Statistica® v. 10.0 software.

Quantification and analysis of biogas

Biogas was quantified at time zero (first day of incubation) and subsequently, at 48-hour intervals, over 15 days, measured using a water displacement system (Figure 1). The obtained values were converted to cm³ according to standard temperature and pressure (STP) conditions, using the local pressure of the municipality of Vitória da Conquista-BA (763.56 mmHg) (Equation 01) (Barana & Cereda, 2000). To evaluate the quality of the biogas produced, the concentrations of methane (CH_4), carbon dioxide (CO_2), and ammonia (NH_3) were quantified using the Alfakit® biogas analysis kit, according to the manufacturer's recommendations.

$$\frac{P_0 \times V_0}{T_0} = \frac{P_1 \times V_1 \times F}{T_1}$$

Equation 1. Where: P_0 is the pressure at standard temperature and pressure (STP), V_0 is the volume at STP, T_0 is the temperature at STP, P_1 is the pressure in Vitória da Conquista - BA, V_1 is the measured volume, T_1 is the measured temperature, and *F* is the humidity correction factor.

Effluent analysis

Chemical Oxygen Demand (COD) analysis of the digestate was performed at the end of 15 days of hydraulic retention time (HRT) to quantify the efficiency of organic matter removal (Equation 2), and the pH was measured.

$$Removal\ (\%) = \frac{COD_i - COD_f}{COD_f} \times 100$$

Equation *2.* Where: COD_i it is initial chemical oxygen demand, COD_f it is the final COD.

For COD analysis, the Alfakit® kit was used, following the manufacturer's instructions. The sample was filtered and then placed in test tubes with potassium dichromate (1.5 ml) and sulfuric acid (2.5 ml), with the aid of the microprocessor-controlled digester AT 509, the temperature was raised to 150 °C for two hours. The amount of organic matter susceptible to oxidation was quantified using a microprocessor-controlled biophotometer at 600 nm.

III. RESULTS

The co-digestion process was conducted according to the parameters observed in Table 1. From the 2^2 CCD matrix, it can be observed that all tests allowed for the production of biogas with at least 80% CH_4. Among the obtained results (Table 1), test 3 (pH = 10 and 30 °C) showed the best performance in biogas production (798.72 cm^3) and CH_4 (638.98 cm^3). In second place was test 2, with 27.57% less biogas and 23.05% less CH_4 compared to test 3, under different pH (8) and temperature (50 °C) conditions. On the other hand, the lowest production of biogas and CH_4 was obtained with test 1, which showed 82.05% less biogas and 80.92% less CH_4 compared to test 3. In this test, the initial pH was the same as test 2, combined with the temperature of test 3. The central points (Table 1) showed a mean value of 341.33 ± 9.65, which indicated a good deviation between the replicates.

Table 1. Encoded design matrix DCC 22 for factors pH (pH) and temperature (T, °C), and for the responses biogas volume produced (cm^3) and methane (CH_4, cm^3) quantified over 15 days of HRT. The actual values of each factor are presented in parentheses.

	Factors		*Response*	
Essay	***pH***	***T (°C)***	***Biogas (cm^3)***	***CH_4 (cm^3)***
1	-1 (8)	-1 (30)	143,36	121,86
2	-1 (8)	+1 (50)	578,45	491,68
3	+1 (10)	-1 (30)	798,72	638,98
4	+1 (10)	+1 (50)	327,68	286,72
5 (C)	0 (9)	0 (40)	327,68	278,53
6 (C)	0 (9)	0 (40)	348,16	295,94
7 (C)	0 (9)	0 (40)	348,16	295,94

The Coefficient Analysis for fitting a quadratic model to each evaluated response was performed as shown in Table 2 for the selection of the model coefficients that were statistically significant ($p < 0.15$).

Table 2. Analysis of significant coefficients for biogas and methane (CH_4) responses, considering pH and temperature.

Biogas (cm^3)				
Variables	***Effect***	***Pure error***	***t- value***	***p- value***
Average	410,316	34,684	11,830	*0,001
pH	101,148	45,882	2,204	*0,115
Temperature (T)	- 8,988	45,882	-0,196	0,857
pH e T	- 226,533	45,882	-4,937	*0,016
CH_4 (cm^3)				
Average	344,236	27,227	12,643	*0,001
pH	78,040	36,018	2,166	*0,119
Temperature (T)	4,390	36,018	0,121	0,911
pH e T	-180,520	36,018	- 5,011	*0,015

* Statistically significant values ($p < 0.15$)

Subsequently, simplified models were obtained for the biogas (Equation 3) and methane (Equation 4) responses with R^2 of 0.9059 and 0.9082, respectively. These R^2 values are not ideal, however, it should be noted that co-digestion is a complex process that can naturally present variability, both in its execution and in the analytical methodologies applied, however, this does not diminish the importance of the data obtained. Furthermore, since a smaller number of experimental tests were performed (a characteristic of the statistical tool), it is more interesting to reduce the rigor of the statistical analysis so as not to waste data and to be able to obtain a more realistic view of the system under analysis.

$$Y\%_{Biogas} = 410{,}316 + 101{,}148\,(pH) - 226{,}533\,(pH).(T)$$

Equation 3

$$Y\%_{Metano} = 344{,}236 + 78{,}040\,(pH) - 180{,}520\,(pH).(T)$$

Equation 4

The performance of the ANOVA (Table 3) indicated, for both the biogas and methane responses, that both the Regression and Lack of Fit terms were statistically significant. This means that the simplified models (Eqs.3 and 4) are capable of describing the responses, however, they have a low predictive capacity when comparing theoretical and experimental values (which was already indicated by the R^2 values). In this case, it was chosen to perform an analysis of the Contour Curves generated in comparison with the experiments performed (Table 1), in order to define good conditions for the anaerobic co-digestion of bovine manure, cassava wastewater, and coffee husk.

Table 3. Analysis of variance (ANOVA) for the simplified mathematical models obtained for the responses biogas and methane (CH_4) production.

	Sum of squares	Degrees of freedom	Mean square	F-Cal	F-Tab	p- value
Biogas (cm^3)						
Regression	246191,2	2	123095,6	19,245	6,944	*0,00886
Residual	25585,2	4	6396,3			
Lack of fit	25305,6	2	12652,8	90,506	19,000	*0,01088
Pure error	279,6	2	139,8			
Total	271776,4	6				
CH_4 (cm^3)						
Regression	154710,8	2	77355,4	19,778	6,944	*0,00843
Residual	15644,4	4	3911,1			
Lack of fit	15442,3	2	7721,15	76,409	19,000	*0,01292
Pure error	202,1	2	101,05			
Total	170355,2	6				

* Statistically significant values ($p < 0.15$)

The Contour Curves (Figure 2) obtained do not indicate optimization conditions for the two evaluated responses but provide important data on the factors. In general, it can be observed for both the biogas response (Figure 2a) and methane (Figure 2b) that the regions in red indicate the combinations of pH and T that result in the highest responses, that is, in the two extreme regions with a combination of higher T and lower pH and lower T and higher pH. These results reflect the behavior observed by the experimental values of tests 2 and 3, with factor conditions at opposite levels (Table 2), as mentioned earlier. In general, considering the experimental values selected for the pH and T factors, the Contour Curves suggest obtaining biogas volumes above 600 cm³ and methane above 500 cm³ under conditions around pH = 8.0 and 50 °C (close to test 2) and around pH = 9.5 – 10.0 and T = 35 – 40 °C (close to test 3). Among these two areas of better responses, test 3 stands out as mentioned earlier.

The biogas quantified, over 15 days of biodigestion, is represented in Figure 3, in which a production peak (614.41 cm³)

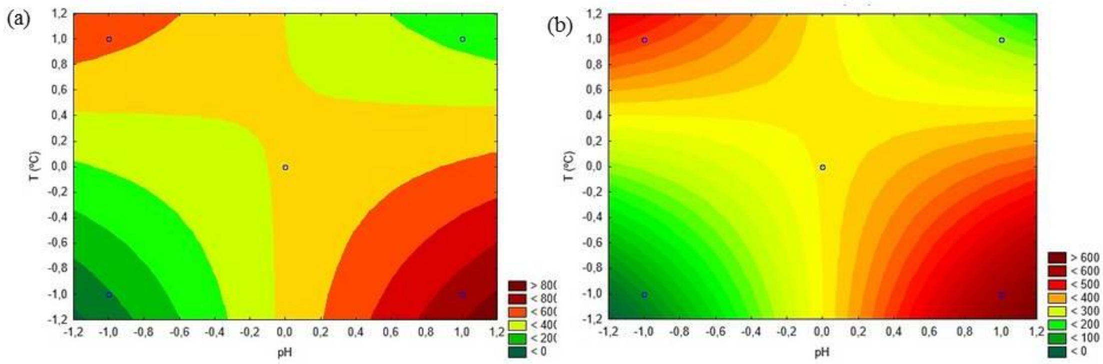

Figure 2. Contour plots for biogas (a) and methane (b) production (cm³) considering pH-temperature interactions.

can be observed for test 3 on the 3rd day. From the 5th day on, a decrease in the accumulated biogas volume (122.89 cm³) was observed followed by an increase in biogas production (184.33 cm³) on the 9th day. After this period, the biogas values remained stable until the end of the biodigestion process (Figure 3). Test 2, which despite having achieved the second-best biogas production, did not maintain the accumulation over 15 days, with a decline in production after the 3rd day. Test 4 showed a behavior similar to the central points of the design (5, 6 and 7), with an increase in biogas production in the first 7 days and subsequent decline. Test 1 showed an increase in biogas production up to the 3rd day (143.36 cm³), with a subsequent decrease in accumulated biogas on the 9th day (20.48 cm³) which remained until the end of the 15th day.

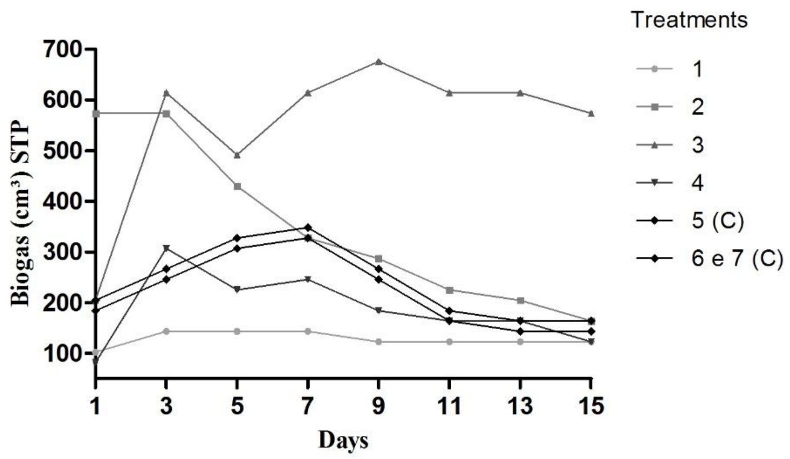

Figure 3. Average cumulative biogas output from biodigesters co-digesting bovine manure, cassava wastewater, and coffee husk for 15 days HRT.

After 15 days of biodigestion, the ammonia (NH_3) concentration in the biogas composition was evaluated (Table 4). It was observed that test 2 presented the highest concentration of NH3 (85 ppmV), all other tests presented a concentration of 15 ppmV. The digestate (remaining material from biodigestion) was evaluated in terms of percentage of chemical oxygen demand (COD) removal and final pH (Table 4). The Pearson correlation between the variables (COD removal and final pH), and biogas production, were significant ($p < 0.05$) and positive ($r = 0.97$ and 0.99, respectively). The 3rd test, which obtained the best experimental performance, presented 58.53% COD removal with pH = 6.3. The percentage of COD removal was directly proportional to the biogas production of all tests, that is, with the increase in the amount of COD removal, the amount of gas produced in the system also increased. The COD removal efficiency was less than 60% for all tests, probably due to the difficult biodegradability of the substrates used, which are rich in lignocellulose. The most acidic final pH (4.8) is observed in test 1, which obtained the lowest production of biogas and CH_4.

Table 4. Physicochemical characteristics of digestate (COD and pH) and biogas (NH_3) after 15 days of hydraulic retention time (HRT) of co-digestion of bovine manure, cassava wastewater, and coffee husk in equal proportions, with variations in temperature.

Assay	*Digestate*			*Biogas*
	DQO		*pH*	*NH₃ (ppmV)*
	Final	*Removal* (%)*		
1	249,82	19,83	4.8	15
2	170,37	45,33	5.9	85
3	129,22	58,53	6.3	15
4	212,14	31,92	5.3	15
5 (C)	224,67	27,90	5.3	15
6 (C)	195,51	37,26	5.5	15
7 (C)	195,51	37,26	5.5	15

*Based on an initial COD of 311.61 $mgL^{-1}O_2$ for all tests prior to pH adjustment.

IV. DISCUSSION

Biogas technology offers various environmental, economic, and social benefits. Through anaerobic digestion, it is possible to treat and reuse various organic residues, reducing greenhouse gas (GHG) emissions, odors, and potential pathogens (Kasinath et al., 2021). Biogas can be distributed through existing natural gas infrastructure and used in the same applications as natural gas. In addition to its use for renewable electricity and heat production, biogas can replace fossil fuels in the transportation sector (Holm-Nielsen et al., 2009).

Considering the importance of biogas and CH_4, the present study achieved efficient production after 15 days of HRT, when compared to other studies involving mono digestion and co-digestion of bovine manure, cassava wastewater, and coffee residues.

The monodigestion of bovine manure (250 mL) at ambient temperature (25-35 °C) with 30 days of HRT produced 625 cm³ of biogas (Nasir et al., 2015). On the other hand, the monodigestion of cassava did not produce biogas; only when co-digested with 25% bovine manure was 446 and 1929.6 cm³ of biogas obtained in 15 and 30 days of HRT, respectively (Madeira et al., 2020). Coffee pulp, pre-treated with NaOH, and bovine manure (1:3) were co-digested for 90 h of HRT, at pH = 8 and 40 °C, reaching a production of 144 mL/kg of CH_4 (Selvankumar et al., 2017). In this present study, the co-digestion of cassava wastewater, bovine manure, and coffee husk associated with the experimental design tool (CCD) made it possible to define the best operational conditions as an initial pH of 10.0 and a temperature of 30 °C, which resulted in a biogas production of 798.72 cm³ and CH_4 of 638.98 cm³, which is equivalent to, respectively, 7975.07 mL/kg and 5900.09 mL/kg (considering the mass of total residues used with the addition of cassava wastewater).

The definition of the best process conditions (fermentation) for biogas production is crucial and involves a series of physicochemical parameters, such as pH and temperature, which are important to ensure the metabolism of different bacterial species under satisfactory conditions (Guendouz et al., 2021). pH is important in the hydrolysis phase of the OM, especially for substrates where acidification occurs easily at the beginning of digestion, such as cassava (Zhai et al., 2015; Madeira et al., 2020; Du N et al., 2021). Studies report inhibition of microbial activity and volatile fatty acid (VFA) production at pH $\leq$ 4, affecting the efficiency of biogas and CH_4 production (Ma et al., 2013; Feng et al., 2018; Wang et al., 2021). The ideal pH range in the AD process is 6-8, that is, values below those recorded in the present study (pH 8, 9 and 10) (Yang et al., 2015). The pH range for biogas production is relatively wide and the initial pH value varies according to the substrate used and the biodigestion techniques (Zhai et al., 2015).

Temperature control is fundamental for the efficiency of anaerobic digestion and influences the quantity and quality of the biogas produced, in terms of methane content (Dobre et al., 2014). The temperature range used in this study (30, 40 and 50 °C) is generally applied in anaerobic digestion, demonstrating good operational performance (Risberg et al., 2013). A study with coffee husk, pulp, and mucilage at different fermentation temperatures (21, 30 and 37 °C) observed that, although CH_4 production occurred rapidly at 37 °C, the CH_4 yield was practically the same for fermentation at 30 and 37 °C, both mesophilic temperatures (Chala et al., 2019). Although most biodigesters are operated at mesophilic temperature, the transition to thermophilic temperature can be promising by shortening the biodigestion time and increasing the degradation of lignocellulosic residues (Yang et al., 2015), in addition to promoting higher degradation rates, higher methane yields, and greater pathogen inactivation are also reported (Risberg et al., 2013). However, it should be evaluated, as it is also related to an increase in volatile fatty acids (VFAs) and a decrease in pH, which can cause inhibition of biogas production (Yang et al., 2015).

V. CONCLUSION

Agro-industrial residues represent a significant source of renewable energy, given their abundant availability worldwide, particularly in Brazil. When subjected to anaerobic digestion, these residues exhibit a high potential for biogas and methane production. This study demonstrated that a stable operation can be achieved when co-digesting coffee husk, cassava wastewater, and bovine manure under varying pH and temperature conditions. Efficient biogas and methane production was attained within 15 days of hydraulic retention time, highlighting the suitability of the lignocellulosic coffee husk, which was merely ground. These findings underscore the viability and promise of these residues as feedstocks for bioenergy production. Future research could focus on scaling up production to pilot scale and investigating the influence of additional operational factors.

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