Effect of Temperature Variation on Codigestion of Animal Waste and Agricultural Residue for Biogas Production

Rafaela Franqueto [1] · Joel Dias da Silva [1] · Michel Konig [1]

Abstract

Anaerobic digestion and codigestion are processes that may aggregate economic value to the organic waste, not only through the production of biogas, as the main product, but also with a by-product, the digestate. The production of biogas (renewable and sustainable energy source) reduces GEE emissions, as well as the impact caused by waste disposal from the agribusiness sector. The present work aims at the potential optimization biogas production in rice residues (rice straw) in different proportions along with bovine residues (waste), under the effect of temperature increase (from 36 to 60 °C). Preliminary investigation consisted of sampling and drying the residues with analytical tests (TS, VS, COD, TOC, N, P, pH, moisture), which allowed the determination of the proportions to be used in experimental research. Then, anaerobic bench reactors (A, B, C, Control) in different proportions were monitored by means of BMP tests, in order to evaluate the potential of methane production in a period of 60 days. During this period, different temperatures were tested, varying from 36 to 60 °C, gradually increased by 2 to 2 °C, every three or 5 days, in order to adapt the anaerobic microorganisms, present in the waste mass. The three reactors presented different biogas production, which can be explained by the different temperatures proposed. Reactors A (ratio 1:1) and C (ratio 1:10) did not reach the proposed objective as their production level was below the production of the control reactor. The B reactor (3:1 ratio) was the one that presented the highest accumulated biogas production during the test period, with 76.95 NmL and the rice straw contribution of 7.55 NmL. As regarding to temperature, all reactors showed to adapt to the two conditions tested: mesophilic and thermophilic fact that demonstrates synergism among the residues tested. Despite the verified adaptability, the mesophilic condition was defined as the most favorable for biogas production because of its greater stability and lower energy cost. The BMP test has proven them to be a viable, easy-to-use, and inexpensive operational tool to monitor and determine biogas production potential for the waste used.

Keywords Bovine waste · Rice straw · Anaerobic codigestion · BMP · Biogas

Introduction

Anaerobic digestion (AD) is one of the processes that values organic waste [1], as it promotes waste management [2], the main product being biogas [3, 4], and a by-product that can be used as fertilizer, called digestate [5–7]. Another process that has been highlighted in the valorization of biomasses is the anaerobic codigestion (AcoD) [8–11]. AcoD uses two or more residues [12], with different characteristics [13], in the same reactor [14], seeking to improve the performance of AD by means of optimization [15]. The use of biogas in the production of biogas (greater production of methane) [15–17] accelerated the degradation process and reduced solid content. According to [18], when supported by different organic residues, AcoD allows the increase of microbial diversity and can contribute to the stability of biodigesters. Data from the Ministry of Agriculture, Livestock and Supply (MAPA) [19] show that the use of animal waste as a substrate for biogas production is a necessity caused by the inappropriate disposal of wastes [20] resulting from bovine breeding systems [21] and the need to mitigate their environmental impact [22].

Agricultural-related wastes (plant residues, forests, agricultural crops, etc.) follow the general trend of those of animal origin, commonly used to produce biofuels [23]. When it comes to choosing the residue or the biomass to be used in the codigestion process, it is usual to use residues that are

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highly available in the region [24], thus seeking alternatives for their reuse and valorization [25], in addition to contributing to the reduction of their environmental impact if they remain in the field [26, 27]. In this context, the present study uses bovine waste and residues from rice crops (rice straw).

According to reports released by the FAO, Brazil will be the largest animal protein exporter by the year 2025 [28]. However, in order to reach this goal, the country should triple the number of animals in the confined system, producing significant amounts of waste (bovine 10 kg day^{-1}, pigs 2.25 kg day^{-1}, chickens 0.18 kg day^{-1} per head). Therefore, increasing the use of residues to produce biogas will promote rural sanitation [24] and the expansion of the animal herd [29].

Rice is one of the most consumed food by the world population [30, 31] and this is not different in Brazil [32]. According to the Agricultural Research and Rural Extension Company of Santa Catarina (EPAGRI) in 2016, Santa Catarina was considered the second largest producer in Brazil [33] and produced approximately 1051 thousand tons of rice. Such production is concentrated in the South part of the State (61.9%), followed by the Middle/Low Itajaí-Açú Valley and North (25.2%), High Itajaí-Açú Valley (9.04 %) and Coastal Center (3.9%). Among the residues produced, the most significant are straw and bark [34]. The bark of rice may be used as fuel for the parbolization process [35]; however, its burning produces ashes and the emission of greenhouse effect gases [36].

The objective of this work is to investigate the optimization of the biogas production potential from rice residues in different proportions with bovine waste and under the effect of different temperatures (36 to 60 °C).

Material and Methods

Firstly, a theoretical and bibliographical research with topics related to biogas production, bovine farming, and agricultural residues, in Science Direct database and in periodicals with high impact factor, was conducted. Other databases used were Portal Periods of CAPES, SciELO. Official publications from governmental agencies such as EPE (Energy Research Company) and IBGE (Brazilian Institute of Geography and Statistics) were used in order to determine the main residues produced by the agrobusiness in Brazil and Santa Catarina.

The second phase was experimental, according to Fig. 1.

Collection and Preparation of the Residues for Biodigestion Testing

Rice straw was collected in a private property, in the Itajaí-Açú Valley region, in the State of Santa Catarina, Brazil (Fig. 2), dried under the sun, and refrigerated until starting test preparation. In the laboratory, to maintain the samples'

homogeneity, the straw was milled because the smaller the particle size, the larger is the surface area of the samples and consequently the residue degradation rate [37]. In relation to rice straw, it was sun-dried and, in order to maintain the homogeneity of the samples and the increase in the degradation rate of the residues, the rice straw (3–5 cm) was milled using a mill knife in the laboratory [38–40]. In addition, microorganisms can act rapidly in the hydrolysis stage [41]. After milling, the rice straw was stored at room temperature in a sealed plastic container until incubation with the bovine waste.

The animal waste was collected in a private property, in the Itajaí Valley region in the state of Santa Catarina, Brazil (Fig. 2), and immediately transferred to the laboratory for cooling until starting the test. In the laboratory, the bovine waste was manually homogenized with the aid of a shovel and then, small portions were taken to determine the solids content (TS), moisture, nitrogen (N), phosphorus (P), total organic carbon (TOC), and chemical demand of oxygen (COD); the rest of the sample was conditioned in a plastic bag and kept under refrigeration (4 °C) until the beginning of the incubation to avoid fermentation, a procedure also performed by other researchers [1–41]. Twenty-four hours prior to use, the sample was taken off and left at room temperature.

Figure 3 presents the residues studied (rice straw and bovine waste) after laboratory preparation.

### Quantitative Analysis: Analytical Methods

The parameters monitored for each waste were moisture (%), total organic carbon (TOC), nitrogen (N), total phosphorus (P), ionic potential (pH), chemical oxygen demand (COD) obtained by the colorimetric method and total solids (TS), and volatile solids (SV) obtained by the gravimetric method [42], all of them were verified prior to and at the end of the process. Table 1 shows the analytical methods used to determine the characterization parameters.

Mounting and Incubation of Anaerobic Biodigesters—BMP Assay

The experimental phase consisted of the assembly and monitoring of anaerobic bench digesters, through BMP test, to evaluate the potential methane production in agricultural residues and animal waste. The biodigesters (BMP) consisted of 250-mL borosilicate flasks, with a screw cap fitted with a gas outlet valve and manometer (reading range 0 to 2.5 kgf cm^{-2}, scale of 0.20 kgf cm^{-2}) to read the bottles' internal pressure (Fig. 4).

It should be noted that prior to the insertion of the blends into the reactors, the system was checked to identify possible leakage. The verification consisted of applying a pressure of 0.3 kgf cm^{-2} in each reactor and immersing them in a bucket of water to cover its lid and observing for 1 min if there was

Fig. 1. Simplified flowchart of the experimental phase
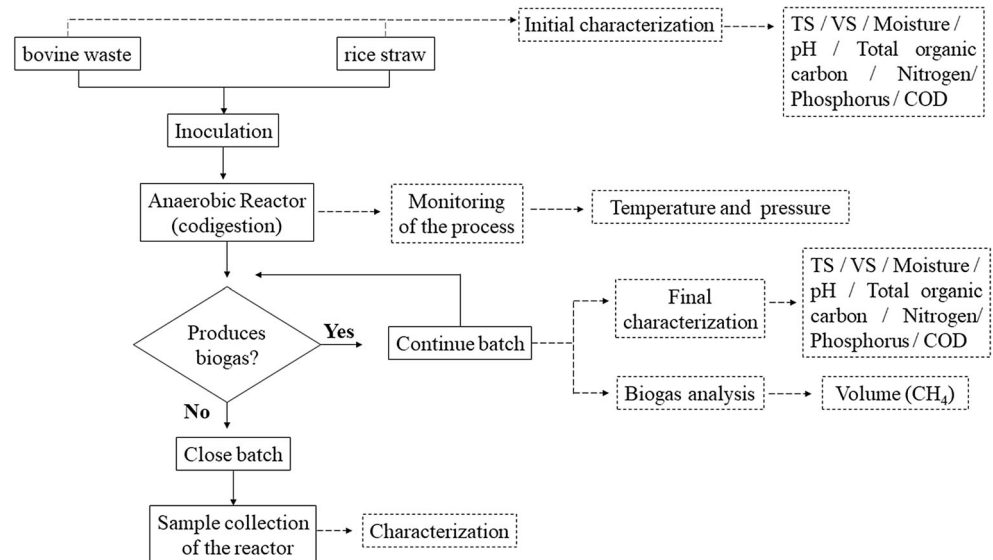
the release of air bubbles coming from the reactor. After observation time, it was also checked if there was a decrease in the pressure indicated by the manometer. When no leaking was detected in the system, the air inside the reactor was sucked by a vacuum pump. In case of leaking, the reactor was opened, and the sealing procedure performed again in order to correct the flaw. In some cases, reactors with significant failures have been replaced.

The ratio of animal waste/agricultural residue was fixed based on total solids' (TS) values. In this way, and as suggested by [43] to have statistical significance, four reactors in triplicate were used:

1. Control reactor: proportion 1:0 (animal waste/agricultural residue), reactor only with bovine waste;

2. Reactor A: 1:1 ratio (animal waste/agricultural residue);
3. Reactor B: 3:1 ratio (animal waste/agricultural residue);
4. Reactor C: ratio 10:1 (animal waste/agricultural residue).

Total solids (TS) was chosen as the initial parameter for determining the proportions. The initial solids concentration in the system was adjusted from 15 to 20% based on previous research [44, 45]. The range of solids used does not imply the need to add water to reduce solids loading or the use of special reactors, adapted to operate with high solids concentrations, since the use of large quantities of water in industrial processes is viewed with caution as it is an increasingly scarce resource. The moisture chosen for this work was 85%, within the recommended range for biogas generation in [46–48]. pH in each reactor was adjusted whenever necessary.

Fig. 2. Location of the State of Santa Catarina in relation to Brazil (a); largest producers of rice in the State of Santa Catarina (b); largest bovine herds in the state of Santa Catarina. Source: [32]
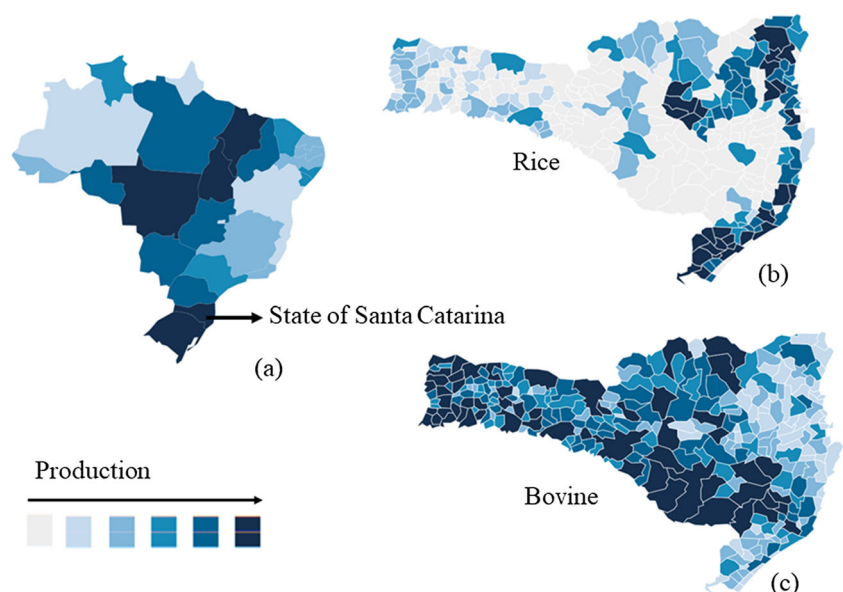
Fig. 3. Rice straw and bovine waste prepared in the laboratory

After sealing the reactor's caps, a current of nitrogen gas (N_2) (99.9% purity) was applied for a period of approximately 5 min, thus ensuring, according to [49], the anaerobic conditions of the medium.

The reactors were incubated in a chilled incubator chamber (MA1415/275), at temperatures ranging from 36 to 60 °C, increasing by 2 °C every three to 5 days in order to adapt to the anaerobic microorganisms present in the waste mass [50]. This procedure aims to determine the ideal temperature for the association of residues, based on what the literature reports: in thermophilic conditions (55 °C), most of the biogas is produced during the first 10 days of the test [51, 52], while in mesophilic conditions (35 °C), the methanogenic activity starts gradually and, consequently, biogas production occurs very slowly [43–53]. It is important to state that the transition temperature from the mesophilic to thermophilic condition should be performed slowly to promote the adaptation of the microbial community present [50]. A control reactor with only a sample of bovine waste was used and incubated in order to verify the amount of biogas produced using only such waste and making it possible to see the difference when using the rice straw mixed in the waste.

Monitoring Biogas Production Potential in BMP Reactors

The method used to determine the volume of biogas produced in the tests was the manometric method [43–55]. To obtain the volume generated, the pressure (daily, except on Saturdays and Sundays) in the bottles was read using manometers coupled to the reactors and the external pressure was obtained through the Epagri website [56]. Then, the pressure values were converted into biogas volume as recommended by Labatut et al. [53].

The net amount of biogas produced was obtained by subtracting the number of control reactors from the amount produced in the reactors with proportions, according to the procedure recommended by Angelidaki et al. [43] and employed by Browne et al. [1].

Statistical Analysis

Averages and standard deviation were submitted for analysis of variance (ANOVA) and after, to the Tukey Test, at 5% of significance, using Excel 2010® software and Past (version 3.21). Principal component analysis (PCA) was also used to check which parameters (time and/or temperature) had the greatest influence on the biogas production of each treatment.

Results and Discussion

Physico-Chemical Characterization

All residues used in the research (bovine waste, rice straw) were previously characterized before incubation. Table 2 shows the detailed physico-chemical characteristics for each proportion referring to the biodegradability test of the binary mixtures of rice straw with bovine waste. The parameters were determined in triplicate.

Table 1. Methods used for characterization of residues

Parameter	Analytical method	Description of method
Moisture (%)	Gravimetric	Drying of the sample in an oven (103 ± 2)° C up to constant weight
TS, VS (%)	Gravimetric	Drying of the sample in an oven (103 ± 2)° C up to constant weight
COD (g L^{-1})	Spectrophotometric	Digestion at 150 °C for 120 min and reading in the spectrophotometer
P (g L^{-1})	Spectrophotometric	Digestion at 150 °C for 30 min and reading in the spectrophotometer
TOC (g L^{-1})	Spectrophotometric	Digestion at 105 °C for 120 min and reading in the spectrophotometer
N (g L^{-1})	Spectrophotometric	Digestion at 105 °C for 30 min and reading in the spectrophotometer
pH	Potentiometric	Direct measurement using a specific electrode
Mass of residues (g)	Weighing	Analytical balance with 0.1 mg precision

Fig. 4. Examples of biodigesters (BMP) containing bovine waste and rice straw
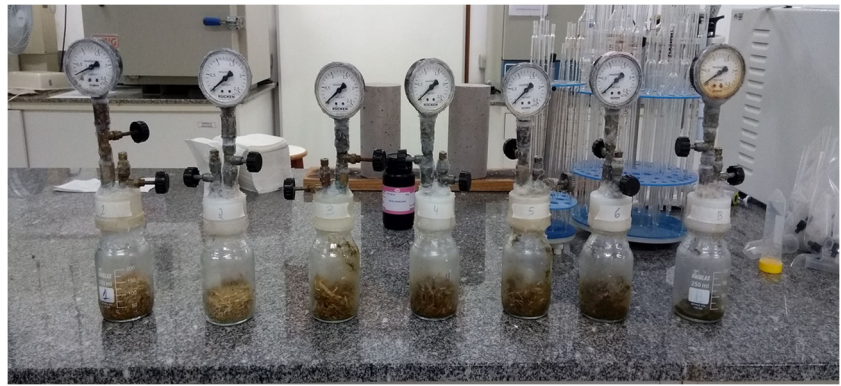
According to Table 2, it could be inferred that there was removal of the initial organic matter, specially because there was a reduction on parameters COD and SV, both considered to be good indicators for biogas production process [57]. The control reactor achieved a 26% reduction in TS and 30% in VS. When compared with the Control reactor, Reactor A presented the largest reductions in TS and VS, 33% and 37%, respectively. As for Reactor B, it presented the largest reduction among all reactors, 45% for TS and 59% for VS. Reactor C showed the smallest reductions, with TS of 14% and VS of 17%. Possibly, the smallest reduction is due to the low availability of soluble organic matter for the activity of microorganisms [58], which ends up limiting biogas production and, consequently, the reduction in the amount of VS. The reduction of TS and VS is an evidence of the efficiency of the anaerobic codigestion process, especially in the reduction of VS concentration associated with higher biogas production. The VS parameter indicates that the organic fraction of biomass was degraded during the codigestion process, signaling the action of methanogenic archaea [12–34, 36–59].

In relation to moisture, the initial values in all reactors come close to the range cited in the literature as ideal (60 to 90%) for the anaerobic codigestion process [46, 47]. As rice straw was very dry, all moisture available in the reactors is due to bovine waste (about 83%).

Based on the results shown in Table 2, the reductions in the COD parameter were significant and the largest reduction happened in Reactor B, 60%, then 30% in Reactor A, and 16% in Reactor C, when compared with the Control reactor, which showed 29% reduction. As for reductions in P, the largest ones were found in Reactor B, 33%, then 22% in Reactor A and 17% in Reactor C; Control reactor showed 20% reduction.

As for N, the largest reduction occurred in Reactor B, 47%, then Reactor A, 27%, and Reactor C, 13%. Control reactor showed a reduction of 38%. Therefore, Reactor C presented the smallest reduction of all. According to Table 2, all reactors presented concentration of N higher than those reported by the authors [60] for non-acclimated inoculum; however, when the concentration in Reactor C was higher, 4.36 g L^{-1} (initial) and 3.78 g L^{-1} (final), this resulted in low biogas production. This may be associated with an inhibition of the anaerobic codigestion process, confirming the fact reported by Yenigun and Demirel [60].

The C/N ratio in the assays ranged from 11 to 58, depending on the concentration of TS in which the reactors are submitted. The initial C/N ratio for Reactor A (C/N 52) showed that the carbon content is dominant because this proportion was the one with the highest quantity of rice. Therefore, the proportion in Reactor A for anaerobic codigestion of rice

Table 2. Physico-chemical characteristics of biodegradability tests

Parameters	Reactor A		Reactor B		Reactor C		Control reactor	
	Initial	End	Initial	End	Initial	End	Initial	End
TS (%)	16.54	11.09	16.87	9.31	16.89	14.49	17.09	12.64
VS (%)	86.34	53.91	88.71	36.17	84.74	70.05	85.01	59.72
Moisture (%)	83.00	89.00	83.00	91.00	83.00	85.00	83.00	87.00
COD (g L^{-1})	112.53	79.17	137.17	54.84	152.39	127.22	176.38	126.11
P (g L^{-1})	4.01	3.12	3.87	2.61	3.47	2.89	4.03	3.21
C (g L^{-1})	159.41	129.24	110.18	51.81	83.01	66.79	55.82	47.86
N (g L^{-1})	3.04	2.23	3.91	2.07	4.36	3.78	4.85	3.02
C/N	52.44	57.96	28.18	48.03	19.04	17.67	11.51	15.85

straw with bovine waste was not considered efficient when compared with bovine waste monodigestion, since biogas production was below the value found in the Control reactor. In this case, the high C/N ratio caused the biogas production process to be unstable thus inhibiting the activity of methanogenic microorganisms [61, 62].

In Reactor B, the initial C/N (C/N 28) was able to ensure a good rate of organic reduction (VS) and the C/N ratio was balanced, allowing enough nutrient supply to the microorganisms present in the process. For Reactor C, the C/N ratio (11) was the lowest of all reactors and it was outside the range reported in the literature (C/N 20–30) [63]. The low C/N ratio may have contributed to the low production of biogas and lower reduction of VS.

Daily Rate and Cumulative Production of Biogas

Biogas production started soon after the start of the tests for all proportions, in variable but significant amount and the maximum daily rate of biogas production was achieved in the early days of monitoring, as can be seen in Fig. 5, except for Reactor C, which showed a biogas peak in around 50 days, a fact that might be related to reactor's overload.

It is also observed that the curves of biogas showed a similar pattern, with an increased production in the early days. The peaks of biogas production in the early days may be related to easily biodegradable substances that are present in rice straw (high solids concentration, carbohydrates, proteins, fats, among others) [64].

It was also observed that, after the conversion of the easily biodegradable fraction, the system needed to start the degradation of more complex compounds, with a greater level of difficulty for biological degradation [65–67], a fact that can be evidenced by lower or even inexistent biogas production. Another possible explanation for these peaks of biogas is the

lack of oxygen at the beginning of the test, caused by N_2 flow in the reactor headspace (ensuring an anaerobic medium) [65].

The earliest production peak in the control reactor (approximately 2nd or 3rd day) may be associated with the capacity for adaptation to the anaerobic codigestion process of the microorganisms already present in the bovine waste, a fact that was not seen in the other reactors since the other ones contained agricultural fractions with lignocellulosic characteristics that create a lignin layer that affects the hydrolysis step of the process [64], thus demanding more time for the microorganisms to initiate access to biological degradation.

The BMP test [68] showed the highest daily biogas production for animal waste and rice straw during the first 15 days (40 days of monitoring altogether), where in the first day, a quick biogas production was observed, reaching 55 NmL. The authors justify the rapid production of biogas in the early days due to the large amount of organic matter available in the system [68].

The cumulative volume of biogas in the reactors followed the same trend shown in Fig. 5. The reactor with the highest biogas volume produced (Reactor B) also presented the highest daily biogas production rates.

According to Fig. 6, it is possible to see that cumulative production in Reactor A increased steadily until it stabilized around the 40th test day, with a cumulative production of 71.33 mL biogas. However, biogas production in Reactor A was below the minimum produced in the Control reactor, thus making this ratio inefficient for biogas production. For the codigestion process using animal waste and agricultural waste to be efficient, it is necessary that the volume of biogas produced in the mixture is greater than in monodigestion (animal waste only) [69]. The low process efficiency in Reactor A may be associated with the existence of a wide variety of toxic substances that may inhibit the anaerobic process, such as organic loading, inhibiting agents [70], and high lignin

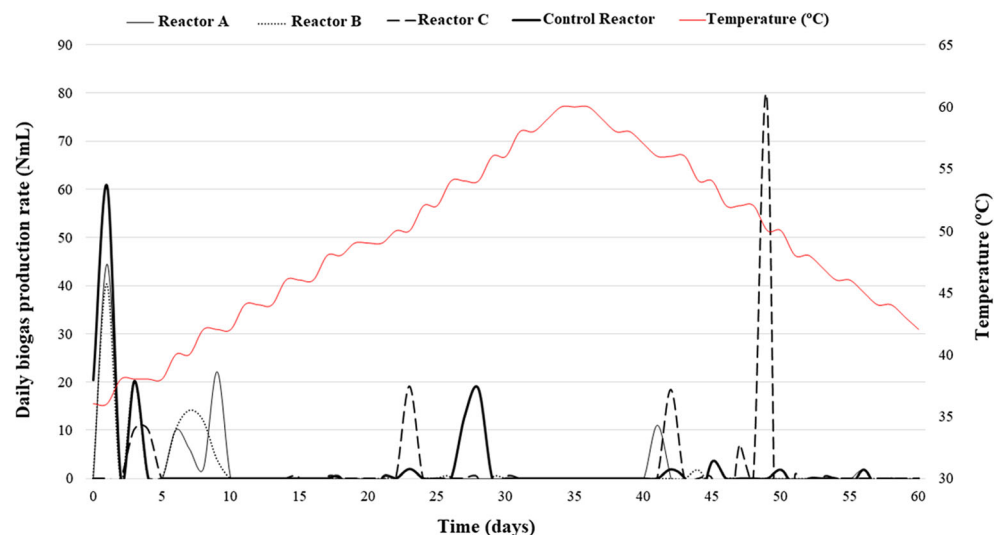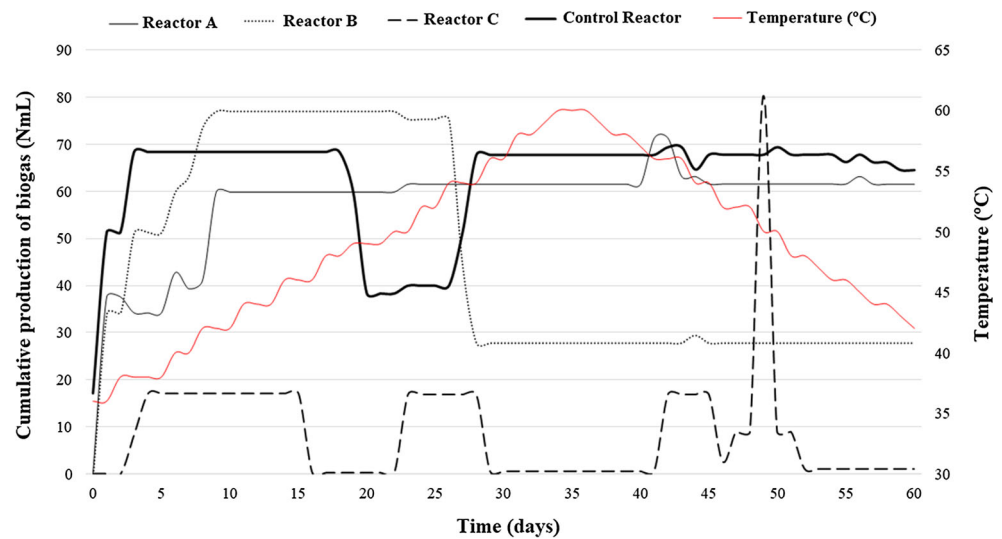**Fig. 5.** Daily biogas production rate with temperature influence

Fig. 6. Cumulative production of biogas in reactors with temperature influence

It is also observed in Fig. 6 that Reactor B obtained a cumulative production of 76.95 mL and its stabilization occurred around the 10th day of the test. In this proportion, rice straw contributed 7.55 mL of total production, corresponding to approximately 10% of cumulative biogas production. In this case, the proportion tested accelerated biogas production, which is evidenced by the shortening of the hydrolysis-acidification step, as mentioned in a study by Han et al. [74] when the residue digested with bovine support means consists of steel slag in different granulometries.

content in the mixture [64–71]. Therefore, as the proportion contained high concentration of agricultural waste, the test confirms the need for pretreatment of waste with lignocellulosic characteristics, such as rice straw [72]. The application of pretreatment enables the reduction of possible negative effects on biogas production [73].

Finally, Reactor C presented the highest cumulative biogas production (80.36 NmL) and the one attributed to the rice straw was 10.96 NmL. Eventhough Reactor A showed a large cumulative production, it was also considered inefficient because this process presented biogas production lower than Control Reactor.

In this study, temperature variation was carried out following the strategy reported by [75]. For the microorganisms to adapt to changing environmental conditions, the temperature was gradually increased by 2 to 2 ° C every three or 5 days. If this temperature change was carried out in a brutal way, without any control, it could cause the death of some populations of microorganisms, resulting in an increase of some species and reduction of others, a fact that would decrease biogas production [50]. Figure 7 presents the effects of temperature variation in each reactor.

Reactor A (Fig. 7a) presented good adaptation to the mesophilic and thermophilic conditions; however, continuous production occurred significantly in the thermophilic range,

supporting the findings by Li et al. [2] who concluded that higher temperatures improve biodegradation of rice straw.

However, in the mesophilic range, biogas production remained constant, corroborating the results found by Liu et al. [76] when they concluded that the mesophilic temperature (more precisely 44 °C) showed greater efficiency in biogas production from codigesting animal waste with agricultural waste.

It was also observed in Fig. 7 that Reactor B (Fig. 7b) obtained the highest cumulative biogas production at the upper limit temperature of the mesophile (40 °C) between the interval of 1 and 17 days and according to Cabbai et al. [77], the mesophilic upper limit is ideal for optimal biogas production. Thus, it was observed that as temperatures increased, approaching thermophilic temperatures, biogas production in Reactor B decreased and presented a different behavior from that found in Reactor B (higher proportion of rice straw in the mixture with bovine waste). The temperature requirement identified in the tests has a significant economic value, especially in large-scale commercial processes, as the higher temperature implies a higher investment in terms of system heating requirements [2]. Lianhua et al. [78] studied the rice straw codigestion for biogas production under both conditions (mesophilic and thermophilic) and the authors concluded that the process in the mesophilic condition resulted in higher methane production (62%) when compared with the thermophilic condition. As temperatures increased, approaching thermophilic temperatures, biogas production in Reactor B decreased, remaining at 27.84 NmL at the end of the experiment. The biogas production remained stable until the end of the test and a stable system usually occurred around the 30th day, with the cumulative biogas production reaching 75.28 mL.

However, according to Fig. 7c, Reactor C showed that the adaptability of the microorganisms to both conditions was not significant for biogas production, since biogas production was
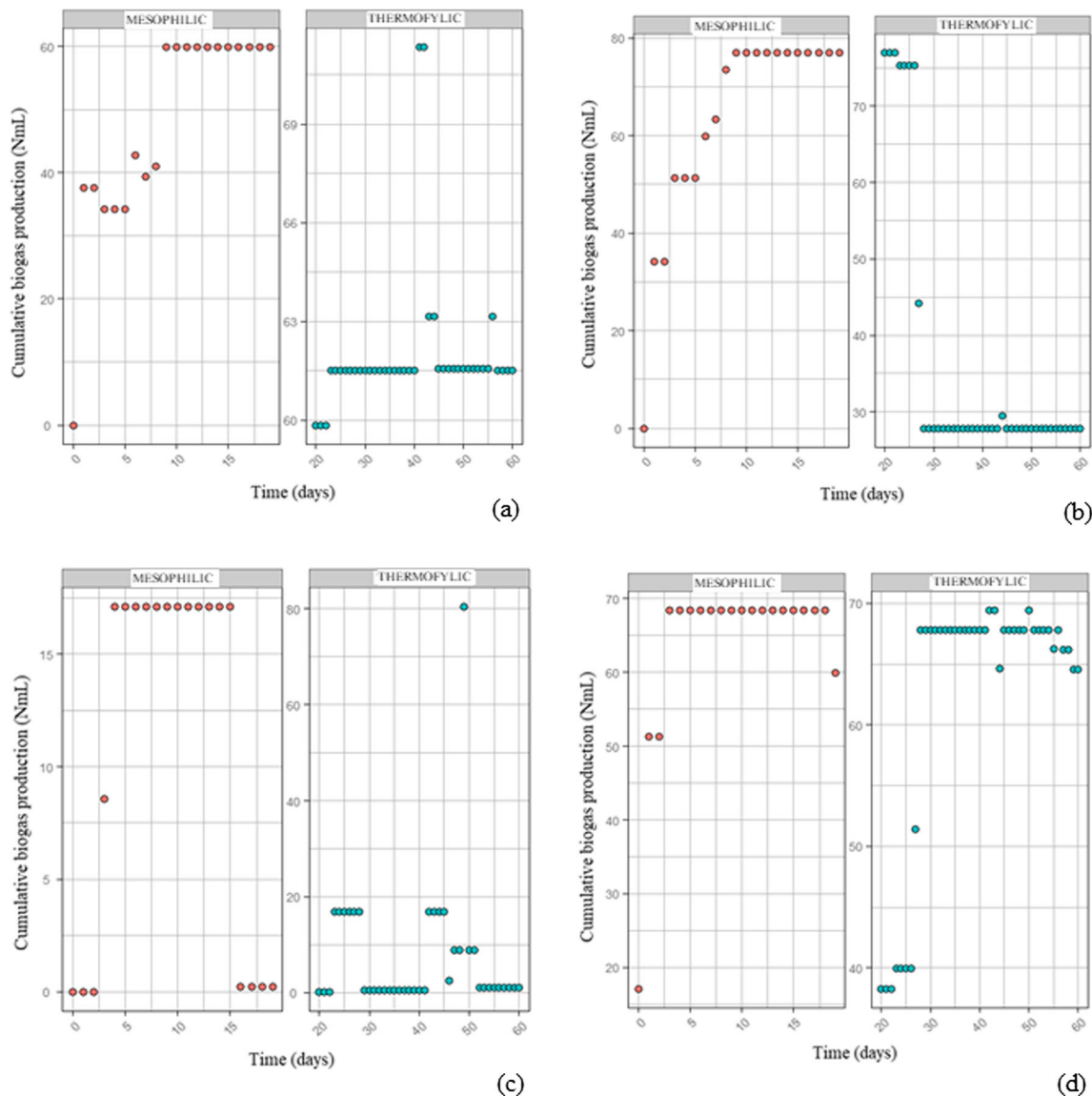
Fig. 7. Influence of temperature range on biogas production: Reactor A (a); Reactor B (b); Reactor C (c); Control Reactor (d)

way below the minimum produced in the Control reactor. The average biogas production in the codigestion process in Reactor C was 20 NmL. It may be inferred that the low biogas production was caused by high solids concentration which resulted in an acid environment with the high hydrolysis rate [79, 80].

The control reactor (Fig. 7d) was considered the biogas production standard for comparative purposes of efficiency of the codigestion process. The reactor showed adaptability to mesophilic and thermophilic conditions; however, the mesophilic condition was highlighted due to a higher production of biogas and subsequent stabilization of the production plateau.

In general, it is observed that each ratio of bovine waste with rice straw behaves differently when exposed to different temperatures, confirming what [81] reported on their study,

that temperature is the most significant influence in the anaerobic digestion process. Thus, the increased production of biogas in mesophilic conditions was also cited by [82], where it was found that the methanogenic microorganisms are very sensitive to temperature variations. The authors [82] reinforce that the thermophilic condition presents higher conversion rates of solids to biogas compared with the mesophilic condition; however, despite having better stability and higher microbial diversity, it can have low methane levels due to increased demand for nutrients caused by the wide variety of microorganisms present.

Hydrogen Ionic Potential—Digestate

According to [35, 48–58, 60–62, 64–82], the ideal pH value to induce microorganism growth is between 6.5 and 7.5. The

initial pH values of the reactors were entirely adequate for biogas production, except in Reactor B, which had an initial pH of 5.6 (Fig. 8), considered low for the anaerobic digestion process, as it inhibited methanogenic activity caused by the accumulation of volatile fatty acids (VFAs). However, all final pH values were in the range for the growth of microorganisms.

The pH values may be related to the anaerobic digestion steps, since according to [83] in step IV, the pH tends to increase, ranging from 6.8 to 8.0, the range in which the reactors are at the end of 60 days. Increasing pH at the end of the digestion process may be associated with Henry's Law, which regulates the solubility of gases in liquids, along with Dalton's Law, since CO_2 remains in equilibrium varying between gaseous and aqueous forms [83]. According to Schirmer et al. [84], with the production of CO_2 during the digestion process, produced CO_2 reacts with water and forms bicarbonate, resulting in a pH increase [85].

Increasing pH and reducing VS, as seen above, are indicative of the conversion of organic matter into biogas [48]. Daiem et al. [86] explain that in the methanogenic phase of the anaerobic digestion process, acids and hydrogen gas are converted to methane and carbon dioxide, resulting in a natural pH increase near neutral (6.8 to 8.0), corroborating with the results found in the literature.

Statistical Analysis

Table 3 presents the summary of the ANOVA test for the biogas production in the reactors.

Interpreting the results of the ANOVA test, it was found that all anaerobic codigestion reactors (rice straw with bovine waste) were statistically significant, since the F values were higher than the $F_{critical}$ ($F > F_{critical}$). Moreover, all anaerobic codigestion reactors showed to be statistically significant, $p < 0.05$.

The Tukey test was applied at a significance level of 5% to verify the statistical differences between the reactors. From the Tukey test results, it was found that all proportions presented statistical differences for the parameter time (days); however,
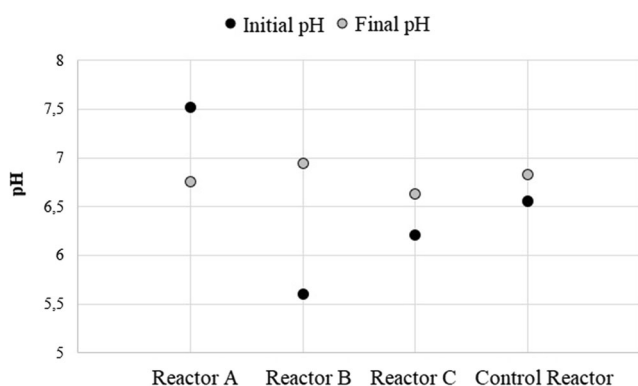
Table 3. ANOVA test summary

Reactors	F	$F_{critical}$	p value
A	67.69	3.05	1.19×10^{-22}
B	17.57	3.04	1.07×10^{-7}
C	126.88	3.05	4.18×10^{-35}
Control	94.82	3.04	7.49×10^{-29}

only B and Control reactors differed statistically for biogas production with emphasis on temperature effect, therefore validating the results found in biogas production under temperature effect (higher biogas production for Reactor B).

For the calculation of principal component analysis (PCA) (Fig. 9), cumulative biogas production over 60 days was used, associated with the parameters: time and temperature. The parameters considered to be significant for biogas productions were the ones that showed the higher value in relation to the main components. The sum of such values can explain most of the total data variation [87].

Component 1 supplies the greater explanation for the results of biogas production in the 1:1 proportion, 84%, so it can be considered the most important. Among the variables, time showed to be the most important and therefore most influential on the biogas production. As for the 1:3 proportion, component 1 supplies the greater explanation for the results of biogas production, 82%, so it can be considered the most important. Among the variables, in this case, both time and temperature showed to be the most important and therefore most influential on the biogas production. As for 1:10 proportion, component 1 supplies the greater explanation for the results of biogas production, 70%, so it can be considered the most important. Among the variables, time showed to be the most important and therefore most influential on the biogas production. Component 1 supplies the greater explanation for the results of biogas production in the 0:1 proportion, 75%, so it can be considered the most important. Among the variables, time showed to be the most important and therefore most influential on the biogas production.

Final Considerations

The BMP tests for the evaluated treatments proved to be a viable, easy-to-use, and low-cost operational alternative for laboratory tests to monitor anaerobic codigestion and to determine the biogas production potential for the wastes used. It is worth noting that the association of bovine waste with rice straw provided an equilibrium of nutrients (nitrogen, phosphorus) responsible for anaerobic codigestion.

When compared with each other, the reactors that used rice straw residue presented remarkably distinct biogas
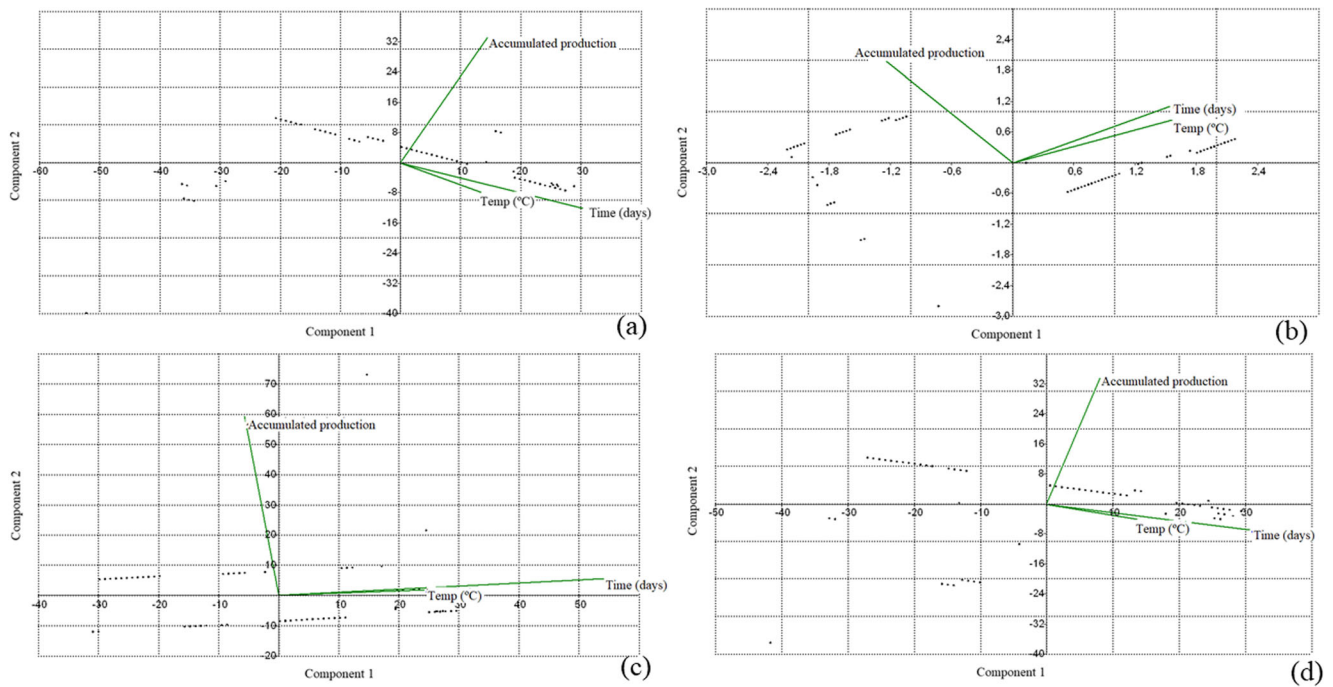

Fig. 8. pH values in reactors with different proportions of residues

Fig. 9. Influence of temperature range on biogas production: Reactor A (a); Reactor B (b); Reactor C (c); Control reactor (d)

production. Reactors A and C did not reach the proposed objective and presented biogas production below the one in the Control reactor; this can be explained by the temperature difference proposed by the test. However, Reactor B presented the highest cumulative biogas production in the 60 days of the test, with 76.95 NmL, and the contribution of rice straw was 7.55 NmL. In this test, the ratio tested 3 (bovine waste) to 1 (rice straw) was able to keep the system balanced and, as a result, achieved the highest biogas production among all reactors tested.

As for temperature, all reactors showed good adaptation for both conditions: mesophilic and thermophilic. This occurrence is a very significant factor as it shows synergism between the tested residues. Despite the verified adaptability, the mesophilic condition was defined as the most favorable for biogas production due to greater stability and lower energy requirement, what is ideal for biogas production.

In general, comparing biogas production curves in BMP tests can be quite complicated, given the different test conditions, such as residue type and temperature for each experiment. It can be concluded that the success of the application of anaerobic codigestion in biogas production depends on the quality and quantity of waste and applied waste/straw ratio, directly influencing the anaerobic digestion process, since it was possible to observe differences in biogas production at proportions used. The implementation of biogas production on an industrial scale, using rice straw with bovine waste, offers a sustainable alternative for managing the disposal of agricultural waste, that is, turning an environmental liability into a great potential for energy production.

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