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## ANAEROBIC CO-DIGESTION OF SWINE AND LAYING HEN WASTE FOR BIOGAS GENERATION AND DIGESTATE QUALITY

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### KEYWORDS

anaerobic digestion,  
resource recovery,  
mesophilic,  
psychrophilic.

### ABSTRACT

Anaerobic digestion (AD) stands out as a degradation pathway for the treatment of agro-industrial waste. This study aimed to evaluate the biogas production ( $P_b$ ) from the co-digestion of swine (SW) and laying hen waste (LHW) under different temperature conditions (psychrophilic x mesophilic). The studies were carried out on a laboratory scale in anaerobic reactors (1.25 L) in a batch system for 60 days. Three mixtures in volumetric proportions (%) of 25/75 (P1), 50/50 (P2), and 75/25 (P3) (SW/LHW) were studied. The mixtures were characterized before and after AD in terms of TS, VS, and COD under temperatures of 18 and 36 °C, with  $P_b$  measured daily.  $P_b$  was higher at 36 °C for all mixtures compared to the psychrophilic condition (18 °C). Among the mixtures, the highest  $P_b$  value was observed for P3, reaching 0.34 and 0.60 m<sup>3</sup> biogas kg<sup>-1</sup> COD<sub>removed</sub> for 18 and 36 °C, respectively. The digestate showed an increase in the contents of micro-and macronutrients for P1, P2, and P3 after AD, which indicates its use for agricultural purposes. The co-digestion of swine and laying hen waste is a promising proposal in terms of management and energy recovery of biogas, especially for mesophilic conditions in mixtures with a predominance of laying hen waste.

### INTRODUCTION

Poultry meat and eggs stand out among the most consumed foods of animal origin in the world (Martinelli et al., 2020). Laying hen waste is considered a source of active pollution due to emissions of hydrogen sulfide, methane, ammonia, and ammonium hydroxide (Han et al., 2018). In this scenario, the confinement of these animals in large poultry farms leads to the accumulation of feces, urine, and leftover feed, which may be a problem due to their high polluting potential when released without treatment into the environment (Mahmud et al., 2021; McAuliffe et al., 2017).

According to the portal Embrapa Suínos e Aves (2022), Brazil is the world's fourth-largest producer and exporter of pork, with 4,325 million tons produced and 1,322 thousand tons exported in 2021. Methane (CH<sub>4</sub>),

carbon dioxide (CO<sub>2</sub>), ammonia (NH<sub>3</sub>), nitrous oxide (N<sub>2</sub>O), and hydrogen sulfide (H<sub>2</sub>S) stand out among gaseous compounds emitted under natural conditions by the disposal of swine waste in the environment without treatment (Cardoso et al., 2015). However, poor management of manure from animals raised in confinement causes numerous environmental problems, as they are most often disposed of in water courses or even in the soil, promoting contamination via fecal coliforms and water eutrophication (Lôpes-Pacheco et al., 2021).

Many alternatives for treating agricultural waste have been addressed by several authors (Cruz et al., 2019; Mendonça et al., 2017; Mendonça et al., 2018; Pecchi & Baratieri, 2019; Santos, 2017; Scarlat et al., 2018) to take advantage of the fertilizer and energy generation potential

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of these residues. Studies have indicated that anaerobic digestion is an efficient alternative that combines biofuel production with sustainable waste management (Cardoso et al., 2020; Neshat et al., 2017; Tambone et al., 2019; Verdi et al., 2019), being used and recommended even in regions with low temperatures (Castro et al., 2017; Martí-Herrero et al., 2014; Telles, 2019).

The optimization of biogas production in anaerobic digestion, as well as higher efficiency in the degradation process, can occur by controlling some factors involved in the process, especially temperature (Cao et al., 2019; Jaimes-Estévez et al., 2021; Lian et al., 2020). Waste treatment via anaerobic digestion is a viable alternative in places where the mean annual temperature is below 20 °C (Rusín et al., 2021, TiwarI et al., 2021). In this case, biogas production can be equated to degradation in the mesophilic range, as observed by Jaimes-Estévez et al. (2021), who reported a biogas production (0.60 Nm<sup>3</sup> biogas kg<sup>-1</sup> VS) higher than expected at the psychrophilic temperature (18 °C), which can be explained by the activity of the consortium of anaerobic microorganisms. Wei & Guo (2018) studied the co-digestion of swine waste, barley straw, and bovine manure at temperatures of 15 and 35 °C and obtained a production of 0.225 and 0.246 m<sup>3</sup> biogas kg<sup>-1</sup> VS, indicating a similar biogas production for the two temperatures under study. Lendormi et al. (2022) observed that the biogas production after acclimatization of swine manure in a reactor operated at 13 °C was 0.222 m<sup>3</sup> biogas kg<sup>-1</sup> applied COD, which corresponded to 68% of the biogas production for the temperature of 37 °C.

Furthermore, the use of anaerobic co-digestion has been reported as a strategy to increase biogas production and waste management, proving to be advantageous compared to mono-digestion (Ma et al., 2020), which could be identified in studies with mesophilic (Li et al., 2020; Magbanua et al., 2001) and psychrophilic (Telles, 2019) degradation conditions. Regarding the studies that carry out the co-digestion of swine and laying hen waste with other substrates, Li et al. (2018) evaluated the co-digestion of corn straw and tomato pulp with swine and laying hen waste separately in different proportions and found that the 4:1 ratio of tomato pulp residue with swine and laying hen waste produced 0.365 L g<sup>-1</sup> applied VS and 0.322 L g<sup>-1</sup>

applied VS of methane, respectively. Shen et al. (2019) pointed out that anaerobic co-digestion of durian with laying hen waste produced higher values of biogas compared to the mono-digestion of durian.

In this context, there are still few studies on the co-digestion of swine and laying hen waste and the comparison of the influence of psychrophilic and mesophilic temperatures on the degradation process of these substrates. Magbanua et al. (2001) promoted the co-digestion of these residues under mesophilic conditions (35 °C) and observed the potentiation of biogas production from co-digestion but the final quality of digestates and other temperature ranges were not evaluated. The present research aimed to evaluate the co-digestion of swine and laying hen waste in terms of biogas production and digestate quality for temperatures of 18 and 36 °C and study the impact of the progressive addition of laying hen waste to swine waste (mono-digestion condition).

## MATERIAL AND METHODS

Anaerobic degradation studies were carried out using the substrates swine waste (SW) and laying hen waste (LHW). The waste used in the experiment came from Lohmann Brown laying hens, collected after one day of accumulation. The swine waste (Naima lineage) was collected after 3 days of storage in an equalization tank and the inoculum was collected inside the digester in a farm located in the municipality of Lagoa Dourada (MG). Animal production followed the complete cycle, with waste treatment carried out in a covered lagoon digester with a capacity of 1350 m<sup>3</sup> operated at ambient temperature (±22 °C) (INMET, 2019).

The study of swine waste degradation on the co-digestion strategy with laying hen waste was conducted based on the following methodological steps: (i) collection and characterization of SW and LHW substrates; (ii) preparation of the different studied proportions between substrates; (iii) feeding of reactors under different operating conditions for temperature (psychrophilic and mesophilic); and (iv) degradation test and final characterization of digestates after the end of the study. Figure 1 shows more details of the methodological stages of the experiment.

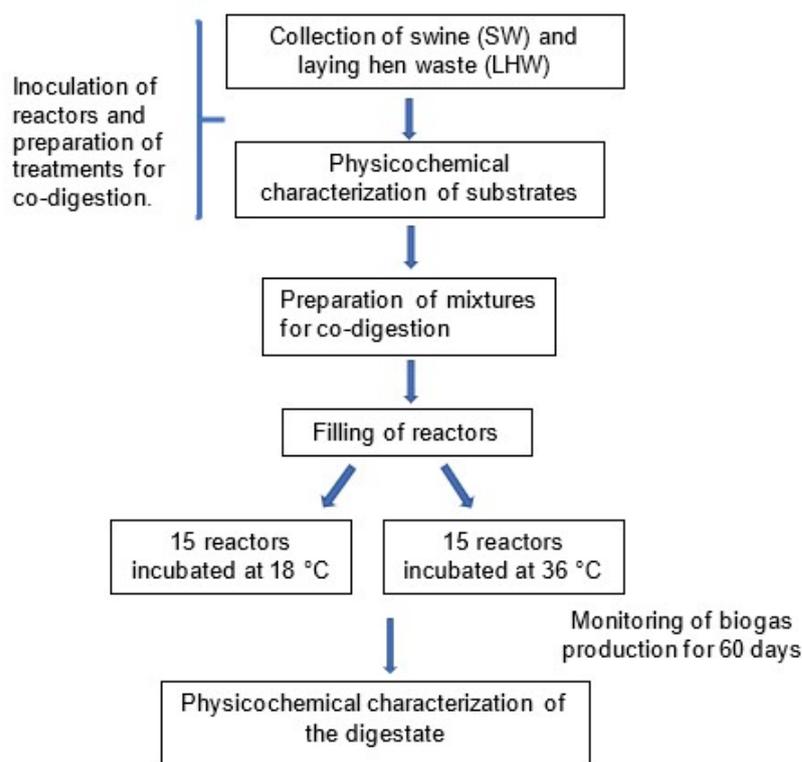


FIGURE 1. Flowchart with the experimental steps.

The co-digestion of the substrates swine (SW) and laying hen waste (LHW) was evaluated from three proportions, namely: P1 (25% LHW / 75% SW), P2 (50% LHW / 50% SW), and P3 (75% LHW / 25% SW). Also, the treatments were analyzed in terms of the influence of different temperature conditions on the degradation process ( $18\pm 0.5$  and  $36\pm 0.5$  °C). The volumetric capacity of the digesters used in the study was 2 L. The produced biogas was collected and directed through transparent crystal hoses ( $\frac{1}{2}' \times 2$  mm thick) to a bulkhead with gasometers made with 100 mm pipes 700 mm in height and one of the openings sealed with 100 mm caps. The base of the gasometers was made with 200 mm pipes 20 cm in height and one of its ends sealed with 200 mm caps. The tubular reactors were installed inside two chambers with temperature control, aiming to evaluate the efficiency of biogas production in anaerobic benchtop reactors. For this purpose, the treatments with four replications + one control were evaluated, resulting in 15 reactors for each of the evaluated temperatures, mesophilic (36 °C) and psychrophilic (18 °C) conditions. Chamber temperatures were controlled by Digital Stc-1000 thermostats (Electrofrio, Brazil). For the mesophilic condition chamber, two tubular armored resistors for 110-volt incubators were installed, which were activated via a thermostat to maintain a temperature of  $36 \pm 0.5$  °C inside the chamber. The chamber of the psychrophilic condition was connected to the thermostat that activated the cooling of the freezer, keeping it at a temperature of  $18\pm 0.5$  °C.

Initially, the inoculation of reactors in the three proportions was performed by mixing 675 mL SW with 675

mL of inoculum (pH=7.95; IA:PA=0.15; 0.30 VS%). The co-digestion proportions (P1, P2, and P3) were conditioned from sequential feedings of the reactors, in which 50% of the useful volume was discarded, followed by the replacement of the volume taken by additional mixtures (laying hen waste and swine waste) until the desired concentration was reached. Two, three, and four replenishments were performed for the proportions P1, P2, and P3, respectively. In all conditions, the reactors were filled with about 2/3 of their total capacity (1,350 mL), with a headspace of 1/3 of the total volume. After establishing the proportions P1, P2, and P3, the experiment began (operating day 0) in a batch, with biogas production being evaluated for 60 days.

#### Characterization of substrates and biogas production

The physicochemical characterization of in natura substrates (SW and LHW) was carried out in terms of pH, alkalinity, conductivity, COD, TS, VS,  $P_{total}$ , K, Na, Ca, Mg, Fe, Zn, Cu, Mn, TKN, and  $N_{NH3}$ . In addition, the contents of reactors on operational days 0 and 60 (digestate) were characterized in the co-digestion process. Initially, 100 mL aliquots of each replication of the proportions were collected for characterization. The same physicochemical parameters analyzed in the in natura substrates were determined in the co-digestion studies, adding the microbiological analysis (total coliforms *E. Coli*). Table 1 shows the methods used to characterize the evaluated parameters, as shown in the Standard Methods (APHA et al., 2017).

TABLE 1. Parameters characterized in the different evaluated mixtures.

Parameter	Reference	Analysis method	Unit
pH	4500-H <sup>+</sup> B	Potentiometry	--
Conductivity	2510 A	Conductimetry	$\mu\text{S cm}^{-1}$
alkalinity	2320 B	Titrimetry	$\text{mg L}^{-1} \text{CaCO}_3$
COD	5220 D	Colorimetry	$\text{mg L}^{-1}$
TS	2540-B	Gravimetry	%
VS	2540-B	Gravimetry	%
P total	4500-P F	Colorimetry	$\text{g kg}^{-1}$
K	3500- K B	Flame photometry	$\text{g kg}^{-1}$
Na	3500- Na B	Flame photometry	$\text{g kg}^{-1}$
Ca, Mg, Fe, Zn, Cu, Mn	3500 – parameter (A)	Atomic absorption	$\text{g kg}^{-1}$
TKN	4500-Norg B	Titrimetry	$\text{mg L}^{-1}$
N <sub>NH3</sub>	4500-NH <sub>3</sub> C	Titrimetry	$\text{mg L}^{-1}$

The volumetric biogas production in each of the reactors was measured in a gasometer composed of two concentric PVC tube cylinders, where its displacement was measured daily using a graduated ruler (Figure 2). The biogas volume produced under normalized conditions ( $\text{Nm}^3$ ) was determined according to [eq. (1)], resulting from the combination of Boyle's and Gay-Lussac's laws. After reading the displacement, the biogas was burned in a Bunsen burner attached to the gas valve. The biogas yield was calculated using daily biogas production data and expressed in mL of biogas per g of removed and applied COD.

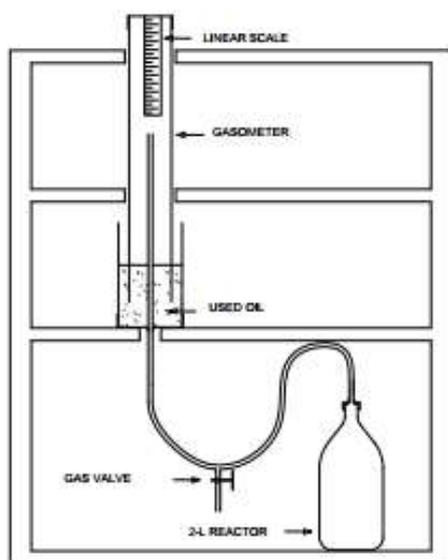


FIGURE 2. Representation of the anaerobic reactor and gasometer used to measure the volumetric biogas production.

$$V_0 = \frac{V_1 \times P_1 \times T_0}{T_1 \times P_0} \quad (1)$$

Where:

$V_0$  is the corrected biogas volume ( $\text{Nm}^3$ );

$P_0$  is the corrected biogas pressure (10332.2745 mm  $\text{H}_2\text{O}$ );

$T_0$  is the corrected biogas temperature (293.15 K);

$V_1$  is the biogas volume in the gasometer (product between the displacement and its cross-section) ( $\text{m}^3$ );

$P_1$  is the biogas pressure at reading (mm  $\text{H}_2\text{O}$ ), and

$T_1$  is the biogas temperature at reading (ambient temperature) ( $^{\circ}\text{C}$ ).

The indication of the best proportion for co-digestion was carried out from the observation of the condition associated with the biogas production at the two studied temperatures. A completely randomized design was adopted, consisting of three treatments and four replications in each temperature condition. The data were analyzed by ANOVA using the software SAEG 9.1 (Anon, 2007). The mean values of production and biogas generated in the treatments were statistically compared by Tukey's test with a 5% significance level. The biogas produced by each experimental unit was measured through the vertical displacement of the gasometer, as shown in Figure 2.

## RESULTS AND DISCUSSION

### Characterization of substrates

Table 2 shows the physicochemical characterization of the substrates (swine and laying hen waste). The pH of the substrates was within the range understood as ideal for the anaerobic digestion process (6.0–8.0) (Chernicharo, 2019). In addition, the values of ammoniacal nitrogen for the manure were below the value of  $3 \text{ g L}^{-1}$  pointed out by Chernicharo (2019) as toxic to microorganisms in the development of the anaerobic digestion process. Higher TS and COD values were also observed in the laying hen waste, while the swine waste showed a higher presence of biodegradable organic matter (VS/TS), as also reported by Bułkowska et al. (2015) and Provenzano et al. (2014).

TABLE 2. Characterization of swine waste (SW) and laying hen waste (LHW).

Variable	Unit	SW	LHW
pH	--	7.00 (0.06) <sup>a</sup>	6.82 (0.02)
Conductivity	μS cm <sup>-1</sup>	5.98 (0.06) <sup>a</sup>	11.37 (0.36)
COD	g L <sup>-1</sup>	28.51 (1.10)	64.57 (1.34)
TS	%	2.57 (0.08) <sup>a</sup>	27.00 (0.58)
VS/TS	%	83.86 (0.19) <sup>a</sup>	58.39 (0.70)
P total	g kg <sup>-1</sup>	27.11 (1.06)	23.00 (0.76)
K	g kg <sup>-1</sup>	65.25 (0.07)	10.38 (0.34)
Na	g kg <sup>-1</sup>	22.45 (0.02)	3.15 (0.04)
Ca	g kg <sup>-1</sup>	44.30 (2.20)	148.31 (0.92)
Mg	g kg <sup>-1</sup>	26.80 (0.81)	5.24 (0.07)
Fe	g kg <sup>-1</sup>	187.13 (1.75)	48.07 (0.58)
Zn	g kg <sup>-1</sup>	772.39 (0.91)	21.72 (0.42)
Cu	g kg <sup>-1</sup>	142.54 (0.43)	16.17 (0.53)
Mn	g kg <sup>-1</sup>	40.11 (0.40)	16.65 (0.32)
TKN	g L <sup>-1</sup>	23.32 (0.07)	32.93 (0.04)
N <sub>NH3</sub>	g L <sup>-1</sup>	2.02 (0.03)	1.51 (0.02)

COD = chemical oxygen demand; TS = total solids; VS = volatile solids; TKN= total Kjeldahl nitrogen; N<sub>NH3</sub> = total ammoniacal nitrogen. <sup>a</sup> sample number n=5, and the others represent the sample number n=3.

Swine waste showed higher contents of heavy metals (Zn and Cu). According to Barros et al. (2019) and Yan et al. (2022), the purpose of inserting these elements in the feed is to prevent diseases (diarrhea) and stimulate animal growth. The anaerobic digestion process can be impaired due to the high mobility and low biodegradability of heavy metals in the waste, and the content of these metals in the digestate can be high (El Rasafi et al., 2021).

**Influence of co-digestion and temperature on the degradation of swine and laying hen substrates**

The co-digestion study was carried out from the biogas production considering a mono-digestion scenario related to the anaerobic degradation of swine waste and the use of the strategy of periodic addition of laying hen waste to prepare different co-digestion mixtures (P1, P2, and P3).

In preliminary analyses, the methane content in the biogas was evaluated using the kit developed by Embrapa Swine & Poultry in partnership with the company Alfakit, following the analytical methodology recommended by the

product developers (Kunz et al., 2007). The preliminary characterization showed a mean value of 55% CH<sub>4</sub> in the biogas although it was not the focus of the study. Regarding the accuracy of results, Oliveira et al. (2021) reported no statistical difference (5% significance) between the levels of CH<sub>4</sub> measured in the portable gas analyzer (Alfakit) and the gas chromatography when analyzing the biogas produced in a covered lagoon digester to treat swine effluents.

Figure 3 shows the accumulated biogas production under conditions of mono-and co-digestion of substrates (SW and LHW) over the degradation period under mesophilic and psychrophilic conditions. Biogas production in the co-digestion was superior to the production in the mono-digestion in all conditions, which suggests that the substrate mixture of laying hens presented a positive synergistic effect in the degradation process. Thus, the simultaneous processing of two or more substrates through anaerobic digestion is a promising strategy for biogas production (Awosusi et al., 2021; Magbanua et al., 2001).

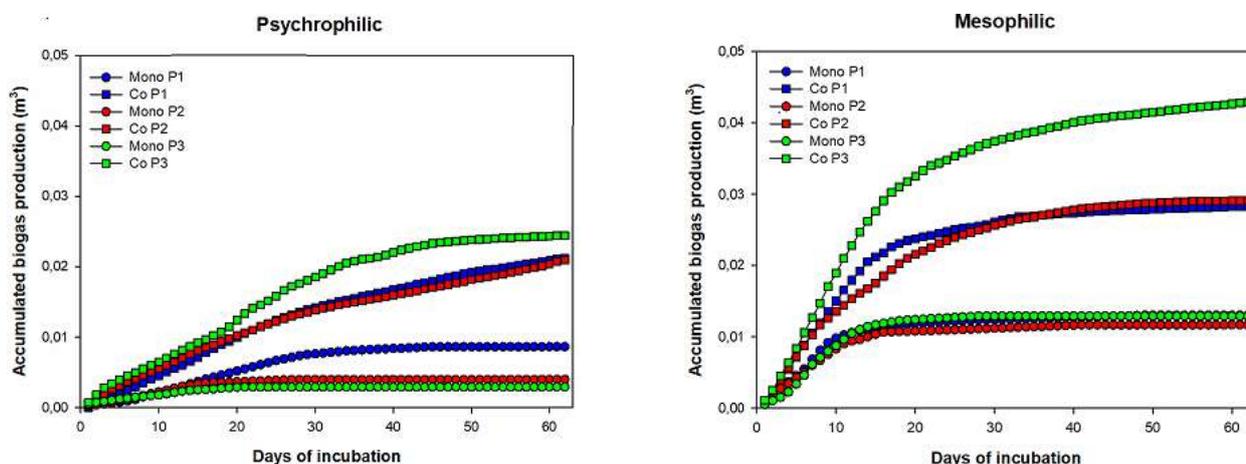


FIGURE 3. Accumulated biogas production in mono-and co-digestion of swine (SW) and laying hen waste (LHW).

The evaluation of the influence of temperature for the same proportion showed an increase in production, as also reported by Telles (2019), who mixed SW and LHW compared to SW alone. In addition, Deng et al. (2016) and Fleck et al. (2017) suggested that the degradation rate is favored at temperatures equal to or higher than 30 °C.

TABLE 3. Accumulated biogas volume produced in the co-digestion.

Treatment	Biogas production (L)	
	18 °C	36 °C
P1	21.1 Ab	28.3 Ba
P2	20.9 Ab	29.1 Ba
P3	24.4 Ab	42.9 Aa

P1: 25% LHW + 75% SW; P2: 50% SW +50% LHW; P3: 75% LHW + 25% SW. Means followed by at least one uppercase letter in the column and the same lowercase letter in the row for each variable do not differ from each other by Tukey's test at the 5% probability level.

Table 3 shows the statistical study that compares the total biogas production between the treatments for co-digestion and the influence of temperature on the degradation process. All treatments showed that biogas production in the mesophilic condition was statistically higher than that produced in the psychrophilic condition. Zhang et al. (2014) evaluated the effect of adding sewage sludge to swine waste in the co-digestion process and observed similar results. In addition, other studies have also reported that temperatures above 30 °C favored the performance of anaerobic digestion (Deng et al., 2016; Fleck et al., 2017; Magbanua et al., 2001). P3 (75% LHW / 25% SW) at the temperature of 36 °C showed a higher production with statistical significance when evaluating the influence of the contribution of the laying hen waste on co-

digestion. Magbanua et al. (2001) also evaluated a higher biogas production in treatments with high levels of LHW relative to SW, although this trend was observed up to 40 days of degradation. Moreover, Arias et al. (2021) evaluated the degradation of swine waste and corn straw at a temperature of 35 °C and obtained a biogas yield close to that found in this study for the best reactor supply condition, with 674 L kg<sup>-1</sup> applied VS. Table 4 shows some unit relationships related to the P3 treatment. Contrary to what has been reported by Wei & Guo (2018) and Lendormi et al. (2022), the biogas production for this study in the mesophilic range was considered superior to the psychrophilic range. However, the high production of biogas at the temperature of 18 °C stands out; in practical terms, the recovery of biogas as an energy source is already advantageous. Further studies should consider an energy balance to evaluate possible advantages associated with heating the effluent (mesophilic phase) to enhance biogas production.

TABLE 4. Biogas unit relationships in P3 (75% LHW/25% SW) under the co-digestion condition.

		Unit	18 °C	36 °C
Biogas production rate		L kg <sup>-1</sup> COD <sub>applied</sub>	490 <sub>(28)</sub>	860 <sub>(72)</sub>
		L kg <sup>-1</sup> COD <sub>applied</sub>	340 <sub>(12)</sub>	600 <sub>(32)</sub>
		L kg <sup>-1</sup> VS <sub>applied</sub>	450 <sub>(16)</sub>	790 <sub>(80)</sub>
		L kg <sup>-1</sup> VS <sub>applied</sub>	290 <sub>(12)</sub>	520 <sub>(48)</sub>

Sample number (n=4).

#### Effect of incorporating laying hen waste to swine waste and digestate quality

Table 5 shows the characterization of the mixtures prepared to supply the anaerobic reactors.

TABLE 5. Physicochemical characterization of the mixtures before the digestion process under the influence of the temperatures evaluated in P1, P2, and P3.

Variable	Unit	P1	P2	P3
pH	--	8.02 <sub>(0.01)</sub>	7.68 <sub>(0.03)</sub>	7.67 <sub>(0.19)</sub>
COD	g L <sup>-1</sup>	47.57 <sub>(2.49)</sub>	72.37 <sub>(4.14)</sub>	86.02 <sub>(9.29)</sub>
TS	%	4.33 <sub>(0.11)</sub>	4.92 <sub>(0.18)</sub>	6.65 <sub>(0.11)</sub>
VS	%	2.71 <sub>(0.15)</sub>	3.13 <sub>(0.12)</sub>	4.35 <sub>(0.12)</sub>
P <sub>total</sub>	g kg <sup>-1</sup>	20.62 <sub>(0.94)</sub>	21.63 <sub>(0.35)</sub>	22.04 <sub>(0.64)</sub>
K	g kg <sup>-1</sup>	37.41 <sub>(5.03)</sub>	60.50 <sub>(1.31)</sub>	47.32 <sub>(0.20)</sub>
Na	g kg <sup>-1</sup>	11.31 <sub>(0.60)</sub>	11.28 <sub>(0.37)</sub>	8.55 <sub>(0.17)</sub>
Ca	g kg <sup>-1</sup>	72.85 <sub>(3.36)</sub>	80.20 <sub>(4.01)</sub>	65.45 <sub>(3.13)</sub>
Mg	g kg <sup>-1</sup>	18.53 <sub>(1.01)</sub>	18.28 <sub>(0.36)</sub>	16.92 <sub>(0.18)</sub>
Fe	g kg <sup>-1</sup>	186.25 <sub>(5.14)</sub>	216.37 <sub>(2.79)</sub>	210.55 <sub>(5.67)</sub>
Zn	g kg <sup>-1</sup>	365.30 <sub>(6.51)</sub>	277.47 <sub>(4.26)</sub>	234.50 <sub>(1.08)</sub>
Cu	g kg <sup>-1</sup>	63.57 <sub>(1.52)</sub>	42.16 <sub>(1.00)</sub>	34.34 <sub>(0.81)</sub>
Mn	g kg <sup>-1</sup>	53.73 <sub>(1.06)</sub>	54.41 <sub>(1.96)</sub>	56.43 <sub>(2.41)</sub>
TKN	g L <sup>-1</sup>	25.73 <sub>(0.46)</sub>	33.27 <sub>(0.05)</sub>	41.84 <sub>(0.40)</sub>
N <sub>NH3</sub>	g L <sup>-1</sup>	2.60 <sub>(0.03)</sub>	3.47 <sub>(0.04)</sub>	3.87 <sub>(0.04)</sub>

COD = chemical oxygen demand. TKN = total Kjeldahl nitrogen; N<sub>NH3</sub> = ammoniacal nitrogen. Sample number (n= 4). MPN = most probable number. P1 (25% LHW and 75% SW), P2 (50% LHW and 50% SW), and P3 (75% LHW and 25% SW).

A small increase in pH values was observed in all proportions after the anaerobic digestion process, and the digestates had pH values slightly above the range understood as ideal for the anaerobic digestion process (6.0–8.0) (Chernicharo, 2019). Table 6 shows the physicochemical characterization of P1, P2 and P3 after anaerobic digestion. The increase in pH at the end of anaerobic digestion can be attributed to an increase in ammoniacal nitrogen in the digestate, a fact also observed by Li et al. (2018) in a study of co-digestion of dairy cattle waste and corn straw. In addition, the high buffering capacity of swine waste stands out, as reported by Wang et al. (2020), which probably favored the growth of microorganisms. The IA:PA ratio for the digestates under the temperature condition of 36 °C was closer to the ideal, demonstrating higher stability in mesophilic temperatures. According to Ripley et al. (1986), the ratio of intermediate alkalinity and partial alkalinity above 0.3 in an anaerobic process indicates some alteration in the system balance.

There was consumption of P during the anaerobic digestion in the mixtures P1 and P2 under both temperature conditions. P3 showed availability of P after the degradation process, with values higher than those found in digestates studied by Dinnebier et al. (2021) and Monlau et al. (2016), probably due to the chemical precipitation of the inorganic fraction with some metallic cations such as Ca<sup>2+</sup>, Mg<sup>2+</sup>, and Fe<sup>2+</sup> inside the reactor or even the use of P by microorganisms to form microbial cells and organic compounds (nucleic acid and humic acid) (Li et al., 2019).

All evaluated conditions showed Zn and Cu availability in the anaerobic digestion process. The availability of metals for the same mixture of substrates (SW/LHW) was more evident under the mesophilic condition, following a trend of reduced availability of these metals at the temperature of 18 °C as there was a higher

contribution of laying hen waste (LHW) in the mixtures. The highest availability rates of metals Zn (73.9%) and Cu (71.4%) were identified in P3 (T= 36 °C). Jin & Chang (2011) also reported an increase in Zn (62.0%) and Cu (116%) concentrations under degradation at ambient temperature. The increased concentrations of these metals may be related to the animal diet (Yan et al., 2022) and the storage conditions of the waste before anaerobic digestion (Gopalan et al., 2012). In general, all mixtures for the co-digestion of substrates under the psychrophilic and mesophilic conditions showed an increase in the concentration of nutrients in the digestate, possibly due to the organic matter decomposition, in which the mineralized nutrients became available in the solution, except for TKN and P in P1 and P2. The temperature of 36 °C promoted higher availability of K, Na, Zn, and Cu, whereas the higher availability of Mn and Ca was observed at the temperature of 18 °C.

In general, all studied proportions had an increase in the concentration of ammoniacal nitrogen in the digestate, with no evidence that the degradation temperature had a significant influence on the conversion process of organic nitrogen to ammonia despite high N<sub>NH3</sub> concentrations being a common feature in laying hen waste (Alba Reyes et al., 2021).

The best results of the efficiency of TS, VS, and COD removal were associated with the P3 treatment (75% LHW + 25% SW), which can be related to the higher biogas production, as shown in Table 7, resulting from the conversion pathway of organic matter in the original substrates of biogas reactors. In general terms, the solids removal efficiencies (TS and VS) under the mesophilic condition were higher than that found under the psychrophilic condition.

TABLE 6. Physicochemical characterization of the mixtures after the digestion process under the influence of different temperatures for P1, P2, and P3.

Variable	Unit	P1		P2		P3	
		(18 °C)	(36 °C)	(18 °C)	(36 °C)	(18 °C)	(36 °C)
pH	--	8.09 (0.03)	8.11 (0.04)	8.08 (0.05)	8.18 (0.03)	8.18 (0.02)	7.91 (0.03)
IA:PA	--	0.17 (0.05)	0.15(0.01)	0.38 (0.04)	0.26 (0.02)	0.27 (0.02)	0.22 (0.02)
COD	g L <sup>-1</sup>	32.88 (4.00)	24.09 (4.70)	29.39 (1.12)	25.80 (0.72)	20.97 (7.12)	28.82 (0.82)
TS	%	3.25 (0.20)	2.9 (0.05)	3.75 (0.07)	3.90 (0.13)	4.40 (0.03)	4.06 (0.18)
VS	%	1.60 (0.09)	1.26 (0.02)	1.86 (0.06)	1.61 (0.06)	2.28 (0.04)	1.93 (0.08)
P <sub>total</sub>	g kg <sup>-1</sup>	13.90 (0.77)	14.19 (0.36)	13.61 (0.50)	14.18 (0.75)	36.49 (0.85)	33.06 (0.66)
K	g kg <sup>-1</sup>	63.10 (3.37)	74.11 (2.16)	73.83 (1.77)	80.79 (2.26)	34.16 (2.11)	66.46 (3.10)
Na	g kg <sup>-1</sup>	15.30 (0.58)	17.13 (0.33)	14.93 (0.60)	16.03 (0.81)	12.75 (1.20)	14.76 (0.19)
Ca	g kg <sup>-1</sup>	168.6 (20.05)	149.37 (8.91)	197.50 (14.03)	168.40 (14.54)	110.62 (3.90)	98.92 (8.64)
Mg	g kg <sup>-1</sup>	30.05 (1.36)	30.90 (1.02)	26.4 (1.16)	27.28 (0.51)	28.4 (0.68)	23.68 (1.16)
Fe	g kg <sup>-1</sup>	266.87 (18.77)	264.49 (1.65)	288.18 (10.31)	250.26 (25.42)	302.37 (4.73)	269.10 (4.50)
Zn	g kg <sup>-1</sup>	550.20 (7.75)	592.36 (20.7)	401.14 (14.89)	422.22 (24.84)	315.59 (13.77)	407.78 (9.90)
Cu	g kg <sup>-1</sup>	98.60 (1.59)	94.04 (4.89)	58.67 (1.28)	67.60 (3.85)	45.84 (1.74)	58.75 (1.63)
Mn	g kg <sup>-1</sup>	76.15 (5.30)	69.17 (2.17)	74.74 (8.29)	73.11 (7.37)	90.01 (0.81)	74.67 (3.11)
TKN	g L <sup>-1</sup>	4.57 (0.05)	4.55 (0.12)	5.95 (0.09)	5.98 (0.05)	6.64 (0.20)	6.71 (0.05)
N <sub>NH3</sub>	g L <sup>-1</sup>	3.59 (0.04)	3.75 (0.06)	4.54 (0.01)	4.43 (0.05)	4.87 (0.02)	5.17 (0.11)

IA:PA = intermediate-to-partial alkalinity ratio; COD = chemical oxygen demand; TKN = total Kjeldahl nitrogen; N<sub>NH3</sub> = ammoniacal nitrogen. P1: 25% LHW + 75% SW; P2: 50% SW + 50% LHW; P3: 75% LHW + 25% SD. Sample number (n= 4). MPN = most probable number; NQ = not quantified.

TABLE 7. Removal efficiency (%) of TS, VS, and COD for SW and LHW co-digestion.

	Removal efficiency (%)		
	Total solids		
Temperature	P1	P2	P3
18 °C	24.88 (4.07)	20.56 (0.53)	33.72 (1.62)
36 °C	32.89 (3.26)	23.46 (1.83)	38.80 (2.57)
	Volatile solids		
	P1	P2	P3
18 °C	39.73 (6.20)	40.46 (1.12)	47.41 (0.88)
36 °C	52.78 (2.18)	48.39 (2.08)	55.37 (2.19)
	COD		
	P1	P2	P3
18 °C	31.56 (5.91)	59.07 (3.11)	66.40 (0.01)
36 °C	49.84 (8.43)	64.16 (2.08)	66.50 (0.03)

Sample number (n=4). P1: 25% LHW + 75% SW; P2: 50% SW + 50% LHW; P3: 75% LHW + 25% SW.

## CONCLUSIONS

The best results in terms of volumetric biogas production were observed in the co-digestion condition when comparing the mono-digestion (swine waste), regardless of the evaluated temperature. A positive synergistic effect was evaluated from the addition of laying hen waste to swine waste.

The mesophilic temperature showed more favorable results in terms of volumetric biogas production compared to the psychrophilic temperature condition.

The increased addition of laying hen waste for the evaluated treatments suggests more favorable pathways for the conversion of organic matter to biogas, especially for the mesophilic condition.

In short, the co-digestion of swine and laying hen waste is an interesting strategy in terms of waste management and biogas energy recovery, especially for mesophilic conditions in mixtures with a predominance of laying hen waste.

The digestate produced in the mixtures with co-digestion under the psychrophilic and mesophilic conditions showed an increase in the concentration of nutrients, which favors the use of the digestate for agricultural purposes.

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