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Anaerobic Digestion of Broiler Litter from Different Commercial Farm Flocks

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Abstract: Rearing broiler chickens generates large quantities of waste material in the form of bedding. Anaerobic digestion (AD) is a technology that can be applied to this waste. This study aimed to evaluate the AD of broiler litter, either screened (S) or unscreened (US), from different flocks, collected from each production batch, totaling nine, from a commercial farm. Anaerobic digestion was conducted in batch biodigesters, and fraction separation was performed through screening prior to loading. The S substrate from the second and fifth flocks did not produce biogas. Reductions in total (TS) and volatile solids were highest for S substrates from the third flock (50.5% and 58.3%, respectively). Only the third flock's S substrates showed greater reductions in solids than the US substrates. Potential biogas and methane production were also highest in the third flock's bedding for both the S substrate (336.8 and 218.2 L/kg of TS, respectively) and the US substrate (296.8 and 213.4 L/kg of TS, respectively). The methane concentration in the S substrate was highest in the third flock (64.8%), while in the US substrate, it was highest in the third and fourth flocks (70.3%). Screening the litter reduced the process efficiency. We conclude that fraction separation is inadvisable for broiler litter.

Keywords: chicken manure; screening; solid–liquid separation; waste management

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1. Introduction

Poultry farming is a significant source of animal protein, offering a more affordable option than other animal-derived proteins [1]. In Brazil, 14.524 million tons of poultry meat were produced in 2023, positioning the country as the second-largest producer of broiler chickens globally [2].

Poultry production generates substantial waste throughout the animals' life cycle from hatchery to slaughter [3]. Among these residues, chicken litter is the most prominent due to its large volume and high contents of organic material and nutrients; it has an estimated global production of 20.708 million tons annually [4].

Chicken litter consists of agricultural byproducts (such as rice husks, wheat straw, and wood shavings) placed on the floor of poultry houses to provide comfort for the chickens, reduce locomotor issues, and absorb excreta, feed remnants, and feathers [5]. One advantage of litter is that it can be reused across different production batches, which helps to reduce disposal costs and the expenses associated with acquiring new absorbent materials [6]. Reusing litter for multiple flocks is common, primarily due to the cost savings from not needing new bedding material. It can also enhance the incorporation of nutrients, as excreta and feed leftovers from different flocks contribute to its nutrient content. However, it requires careful management due to the need for sanitary handling of the bedding after the removal of each flock. The most common management practices for reusing poultry litter include applying lime and disinfectants, turning the bedding, controlling moisture, and removing the top layer of bedding between flocks. These practices aim to reduce ammonia emissions and the proliferation of bacteria and diseases among animals while ensuring that the environment remains hygienic. Thus, litter becomes a nitrogen-, phosphorus-, and calcium-rich substrate that can be used as a soil conditioner in agricultural areas [7]. However, the direct application of litter can harm plants due to unstable organic matter, which also emits gases into the environment [8]. Techniques such as composting and anaerobic digestion can serve as effective alternatives to treat waste and add value to poultry production.

Anaerobic digestion has been explored for treating chicken litter, particularly for its potential to produce biogas and methane (CH_4), reduce energy costs on farms, and support both economic and environmental sustainability [9,10]. Despite its advantages, the anaerobic digestion of chicken litter can be challenging due to the high concentration of solids in the waste, which is fibrous and nitrogen-rich. The total solid concentration in chicken litter can reach up to 70%, much of which consists of mineralized content, which is dense and prone to rapid sedimentation. When diluted in water, this can hinder material homogenization and lead to clogging in the biodigester [11]. Additionally, litter's high fiber content delays the digestion process and biogas production, requiring a longer hydraulic retention time for microbial action [12]. An elevated nitrogen concentration can inhibit methanogenic microorganisms responsible for methane production, as the carbon/nitrogen (C/N) ratio may become unbalanced, leading to a carbon deficit for CH_4 production [13].

During the poultry production cycle, antibiotics are commonly used as therapeutic or prophylactic agents. These substances may leave residues in the chicken litter, as they are not fully metabolized by poultry, and up to 70% of them can be excreted [14,15]. When such litter is directed to anaerobic digestion, these antibiotic residues may delay or even inhibit microbial activity, potentially fostering the proliferation of antibiotic-resistant bacteria [16]

Similarly, the use of disinfectants when handling litter can negatively affect the anaerobic digestion process. These disinfectants are designed to be active against pathogens, producing antimicrobial compounds and bacteriocin-like substances that help to prevent disease spread and allow for prolonged bedding usage [17]. Despite various studies being conducted on the anaerobic digestion of chicken litter, gaps remain in understanding factors such as the influence of different breeding batches and the separation of solid–liquid fractions on methane yields and the quality of the resulting biofertilizer.

Based on the above considerations, the following hypotheses are proposed: (1) the reuse of litter influences the concentration of organic constituents and methane production during anaerobic digestion, and (2) the separation of fractions through sieving can enhance methane production. This research aimed to evaluate the influence of poultry breeding batches and the separation of substrate fractions on the degradation of organic constituents, biogas and methane production, and the quality of the resulting biofertilizer.

2. Materials and Methods

This research was carried out at the Experimental Warehouse and Agricultural Waste Management Laboratory of the Faculty of Agricultural Sciences of the Federal University of Grande Dourados (UFGD). It is located in Dourados, MS, Brazil, at 22°13′18″ South and 54°48′23″ West.

The experiment followed a completely randomized design in a 9 × 2 factorial scheme represented by different broiler chicken flocks and substrate sieving conditions: unscreened (US) and screened (SC). The biodigesters served as repetitions, totaling three per treatment.

The broiler litter used as the substrate for the biodigesters was collected from a commercial Dark House model poultry house near the city of Dourados in the state of Mato Grosso do Sul. The poultry house covers an area of 1957.5 m^2, with a capacity to house 25,000 birds per flock. All flocks consisted solely of males, with slaughter ages ranging from 41 to 51 days. The poultry house raises six flocks per year, with an average mortality rate of 3% per flock. The sanitary downtime between flocks is 10 days, during which lime is applied to the poultry litter after removing the birds, and a small layer of new absorbent material is added.

The material used as broiler litter was rice husks, which was placed in the poultry house during the first production flock and reused for two subsequent flocks, totaling three flocks with the same broiler litter. Starting from the fourth flock, broiler litter from a newer flock of birds was added (approximately 50% from each flock for the samples). Thus, flocks one, two, and three used the same litter, while from the fourth to the ninth flock, the material came from a different aviary in the same poultry plant, which started rearing its first flock when the first poultry house was rearing its fourth, as illustrated in Figure 1. These were the raw materials used to compose the substrates for the biodigester loads. The experimental scheme used in this research is shown in Figure 2.

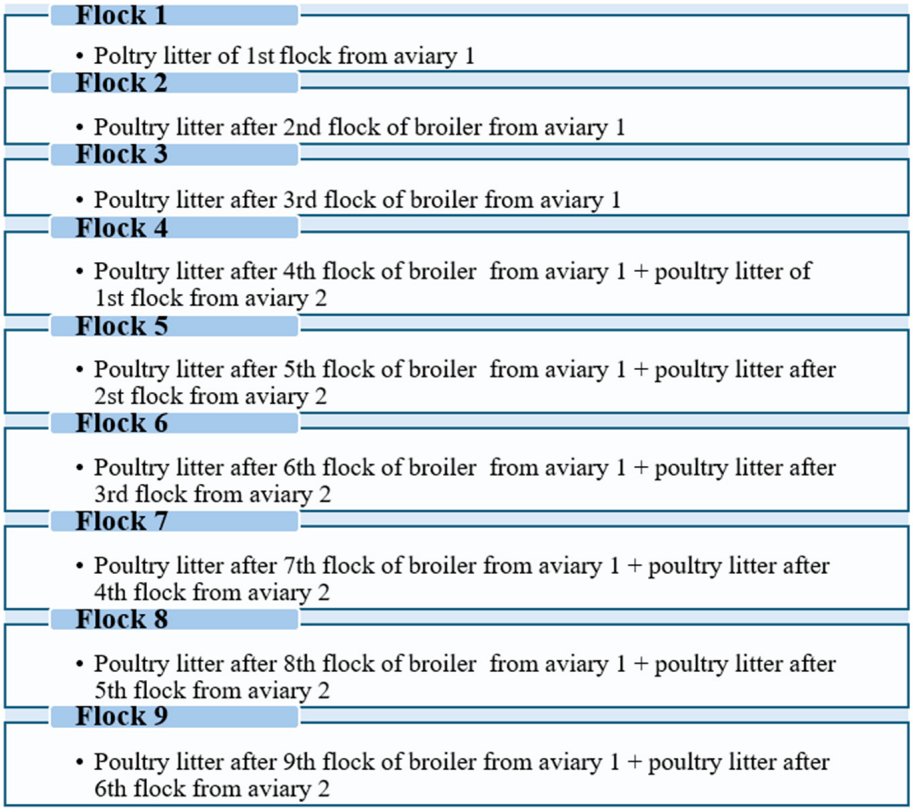


Figure 1. Representation of the poultry litter composition for each flock.



Figure 2. Schematic representation of the experiment.

Substrates for feeding digestors were formulated to create a TS (total solids) concentration equal to 4.0%. The inoculum represented 50% of the total volume (Table S1) and was previously prepared from the fermentation of sheep manure to accelerate the substrate's initial degradation. The VS inoculum to VS substrate ratio was 0.5:1. The inoculum was considered ready after an initial fermentation period of 90 days and upon reaching a methane content above 75%. The inoculum's digestion performance was evaluated in isolation (without the inclusion of other waste) so that the biogas production from the inoculum was measured and subtracted from the biogas production derived from the experimental substrates.

The original TS concentration was considered when adjusting the amount of each component (broiler litter and inoculum); tap water was used for dilution, and an industrial blender was employed to homogenize the influent. Afterward, the substrates were separated into two conditions (screened and unscreened). For the screened condition, the substrate passed through a 1 mm mesh sieve, resulting in an average retention of 50% of the solid fraction. After separation, only the liquid fraction was directed to the digestion process, with an average TS concentration of 2.6%. In the unscreened condition, the substrates, after dilution and homogenization, were directly sent to the biodigesters with an average TS of 4.6%. Table 1 presents the characteristics of the poultry litter used in the experiment, and Table 2 presents the input substrates for the biodigesters for each production flock.

The substrates were placed in 54 batch-type bench-scale biodigesters with 500 mL of fermentation content capacity. The biodigesters consisted of two PVC cylinders, one used as a water seal and the other as a gasometer for biogas storage, with a discharge valve for biogas release [18]. The substrates underwent digestion for different periods according to the production distribution (Supplementary Figures S1 and S2 and Table 2). The retention times were determined based on the daily biogas production, until it decreased to the equivalent of 1% of the total cumulative volume, following the recommendations of the [19].

The biodigesters were stored in the Agricultural Waste Management Laboratory in a covered area and under room temperature (average daily of 26.3 °C), protected from the

sun and rain. Biogas production was monitored daily, and methane levels were measured whenever production occurred during the experimental period. The vertical displacement of the gasometers was used to measure biogas production; the produced volume was calculated based on the area of the gasometer and the height of its displacement, and the value was corrected to standard temperature and pressure conditions [20]. A Gasboard-3200L infrared device (Cubic Sensor and Instrument Co. Ltd., Wuhan, China), equipped with sensors to determine the O_2, CO_2, and CH_4 levels, was used for the biogas composition analysis. The specific productions of biogas and methane were calculated considering the total volumes produced and the amounts of TS and VS added. The reductions in TS, VS, and fibrous constituents were calculated by considering the quantities of these components at the beginning (influent) and end (effluent) of the fermentation process. TS, VS, pH, COD, and BOD analyses were performed according to the methodology described by [21]. The N Kjeldahl, C, NDF, and ADF contents were determined as described by [22]. The samples were dried and subsequently ground for laboratory analyses.

Table 1. Characterization of fresh poultry litter.

Fresh Poultry Litter	pH	TS (%)	VS (%) *	C (%) *	N (%) *	C/N Ratio	NDF (%) *	ADF (%) *
Flock 1	9.70	80.23	78.32	19.12	2.01	9.51	58.48	39.15
Flock 2	9.55	77.88	74.74	17.71	2.11	8.39	52.76	31.69
Flock 3	8.89	86.38	83.45	17.48	2.27	7.70	41.76	20.53
Flock 4	8.35	85.13	87.31	20.17	2.82	7.15	38.60	15.89
Flock 5	8.93	78.25	78.44	22.65	2.61	8.68	48.77	22.12
Flock 6	8.80	77.44	80.72	22.30	2.70	8.26	41.20	15.22
Flock 7	8.86	77.74	80.53	19.20	2.70	7.11	44.54	15.68
Flock 8	8.74	78.27	82.12	21.28	2.70	7.88	43.11	16.06
Flock 9	8.87	80.65	83.25	18.71	2.57	7.28	39.62	16.80

TS, total solids; VS, volatile solids; NDF, neutral detergent fiber; ADF, acid detergent fiber; pH, hydrogen ion concentration. * % of TS content.

Table 2. Characterization of the initial substrates employed for anaerobic digestion.

Substrates	pH	TS (%)	VS (%) *	N (%) *	COD (mg/O_2/L)	BOD (mg/O_2/L)	HRT (Days)
Flock 1 SC	9.45	2.55	69.61	2.77	17,106.67	7700.00	98
Flock 2 SC	9.37	2.52	67.49	2.29	15,313.33	8940.00	167
Flock 3 SC	8.91	2.17	73.61	2.97	18,886.67	8560.00	133
Flock 4 SC	8.46	2.49	82.77	4.21	20,160.00	8377.00	167
Flock 5 SC	8.97	2.89	72.77	2.76	12,000.00	7240.00	167
Flock 6 SC	8.78	2.82	71.82	2.77	12,626.67	8480.00	121
Flock 7 SC	8.78	2.97	75.44	2.54	19,480.00	8200.00	121
Flock 8 SC	8.83	2.78	78.17	2.66	17,540.00	8680.00	98
Flock 9 SC	8.82	2.36	70.08	3.68	16,533.33	8560.00	161
Flock 1 US	9.43	4.53	79.26	1.96	20,250.00	15,550.00	70
Flock 2 US	9.28	4.78	77.28	1.48	25,133.33	21,850.00	121
Flock 3 US	8.98	4.12	79.94	2.17	28,433.33	18,750.00	133
Flock 4 US	8.53	4.36	83.27	3.02	29,833.33	20,400.00	98
Flock 5 US	9.01	4.32	78.19	2.42	22,333.33	16,800.00	121
Flock 6 US	8.86	4.09	81.87	2.42	26,900.00	22,250.00	121
Flock 7 US	8.85	4.72	82.64	2.44	28,700.00	18,100.00	167
Flock 8 US	8.73	4.60	81.96	3.24	24,383.33	17,010.00	121
Flock 9 US	8.80	4.46	81.41	2.77	27,116.66	13,370.00	77

COD, chemical oxygen demand; BOD, biochemical oxygen demand; US, unscreened; SC, screened; HRT, hydraulic retention time. * % of TS content.

The following formula was used to calculate TS and VS reduction (%):

$$Solid\ reduction\ (\%) = \frac{initial\ solid\ (\mathrm{g}) - final\ solid\ (\mathrm{g})}{initial\ solid\ (\mathrm{g})} \times 100$$

These formulas were used for the calculations of biogas and methane production by solids (L/kg of added TS or VS), and biogas by COD (L/g of added COD).

$$Biogas\ production = \frac{Biogas\ produced\ (\mathrm{L})}{Initial\ Solid\ (\mathrm{kg})\ or\ COD\ (\mathrm{g})}$$

$$Methane\ production = \frac{Biogas\ production \times CH4\ (\%)}{100}$$

The results were subjected to analysis of variance (ANOVA), considering the batches and sieving conditions as sources of variation, tested at a 5% significance level. The factors were analyzed independently using ANOVA to evaluate the influence of the production flock and sieving condition on the degradation of organic constituents and nitrogen concentration. In case of a non-significant interaction, the factors were analyzed separately. If there was a significant interaction between the factors, a decomposition was performed, and mean comparisons were conducted using Tukey's test ($p < 0.05$) for the qualitative factors (flocks and fraction separation).

3. Results and Discussion

We did not consider the results generated by the screened substrates produced from the second and fifth flocks' bedding for data analysis, as they did not produce biogas. This leads us to suspect that some antibiotic medication may have been used during the rearing of the birds, and it may have been present in the excreta that would serve as substrates for the biodigesters [23], considering that this study was conducted with poultry litter from commercial farms, where the sanitary challenge is higher compared with experimental conditions. Thus, it would cause a delay throughout the entire AD process [24,25]. A study by [26] reported that the highest concentration of antibiotics is found in the liquid fraction after fraction separation, which would justify these results only for the screened substrates.

The reductions in TS and VS are presented in Figure 3. The different rearing flocks significantly influenced TS reductions ($p < 0.05$) during the anaerobic digestion process. The broiler litter of the third-rearing flock had the greatest TS reductions in both the screened and unscreened substrates (50.5% and 47.9%, respectively).

TS reductions in unscreened substrates showed similar values across the other flocks, with a minimum of 42.3% for the ninth flock. Among the screened flocks, the minimum reduction was 22.9% for the sixth flock. Reusing poultry litter reduces costs and enhances nutrient concentrations for agriculture [27]. Safe reuse requires pathogen control through stacking and turning, which promotes heating, organic matter reduction, and nutrient mineralization. As a result, the material accumulated during rearing becomes less available to microorganisms in anaerobic digestion.

US substrates contain more insoluble solids due to the absorbent material commonly used as bedding, which leads to lower TS reductions, according to [28]. However, in our study, only the third flock's S substrates had a greater reduction in solids than US substrates (50.5 and 47.9%, respectively), while for the other flocks, the highest reductions occurred with intact substrates. As mentioned earlier, the use of antibiotics may hinder the reduction of solids, in addition to the potential for higher concentrations in the liquid fraction, which could be observed when fraction separation was performed. However, since the substrates were collected under commercial production conditions, the production unit did not provide information regarding the use of antibiotics during bedding collection.

Another possibility for treating the bedding occurs between flocks during the period known as sanitary downtime when lime or other disinfecting agents are often used to improve the bedding's sanitary condition. Between poultry batches, the bedding is typically turned over, and clumps are broken up for the application of chemical conditioners. The most commonly used conditioners include agricultural gypsum, aluminum sulfate, hydrated lime, and single super phosphate [29]. Bedding treatment is essential to improve its physical, chemical, and microbiological quality, thereby enhancing the performance of the poultry being raised [29].

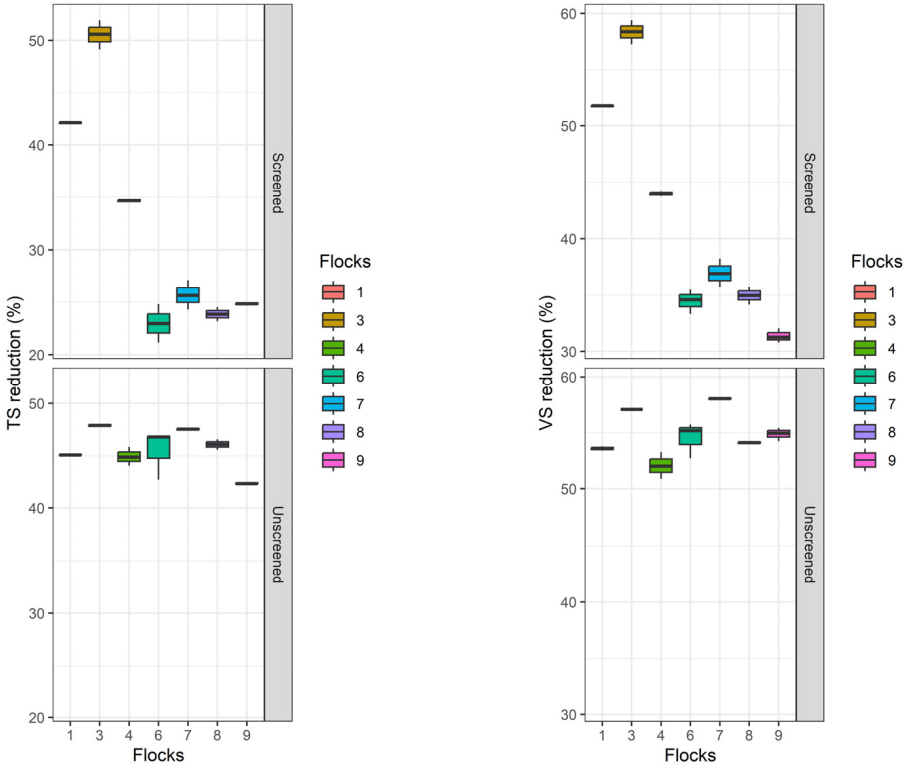


Figure 3. Reductions in total (TS) and volatile solids (VS) during the anaerobic digestion of screened and unscreened poultry litter substrates from different flocks.

Following a similar pattern to TS reductions, the VS reduction in US substrates during digestion also showed comparable values among flocks, with the highest reduction observed for the seventh flock's bedding, which did not differ from that of the third flock (average of 57.5 ± 0.5%). The US substrate with the lowest VS reduction was from the fourth flock under the NP condition (52.1%). These results align with those of [30], who conducted anaerobic digestion using rice husk poultry litter from the seventh flock, with or without fermentation as a pre-treatment. The litter that underwent prior fermentation had a 52% VS reduction, while the unfermented litter's VS reduced by 69%, likely due to the fresher material being more readily available to microorganisms. The management applied to the litter resembles the composting process, in which the more easily degradable material is reduced [31]. The same authors achieved average VS reductions of 44% with poultry litter digestion, which they considered a significant value, given that bedding material contains highly recalcitrant and lignified material.

The highest VS reductions in screened substrates were observed for batches from the third flock (58.3%), followed by the first (51.7%) and fourth (43.9%) flocks, with the lowest reduction for the ninth flock (31.4%). The authors of [30] attribute VS reductions as a factor that demonstrates the efficiency of the anaerobic digestion process. The higher the VS content, the greater the biodegradation of the organic material, which may result

in higher biogas production. In our study, the unscreened residues showed higher VS reduction values than the screened material, except for the third flock. Contrary to our results, the reduction values found by [28] were 46.7% and 40.3%, respectively, for screened and unscreened bedding, despite a longer hydraulic retention time for the unscreened bedding. However, the bedding was composed of peanut shells, which are difficult to degrade. The same occurred in the anaerobic digestion of bovine manure performed by [25], where screening led to the retention of about 40% of the fibrous fraction. In our study, the long retention period of the substrates (Table 2) may have favored the degradation of more resistant compounds, such as fibrous ones. Additionally, the reuse of the bedding across different flocks may have promoted a higher proportion of mineralized constituents in the substrates, which, when screened, remained in the liquid fraction, increasing the solid concentration but without creating the conditions for biogas production. The screening of the substrates may have also been responsible for the removal of microorganisms from the inoculum, which could have beneficially influenced biogas and methane production.

The results for biogas production are presented in Figure 4. The highest average biogas production occurred during the digestion of screened substrates originating from the third flock's bedding (336.8 L/kg of added TS), while the lowest production was observed in substrates made from the sixth and ninth flocks' bedding (an average of 52.1 $\pm$ 8.8 L/kg of added TS). These flocks were also responsible for the highest and lowest unscreened substrate values, 296.8 and 146.3 L/kg of added TS, respectively. This result may be directly related to the fact that, from the fourth flock onwards, the bedding was mixed with bedding from newer flocks, as according to [30], the higher the number of bedding reuses, the higher the biogas production. In substrates formed from bovine manure, [25] achieved maximum biogas production values of 150 and 83 L/kg of added TS with and without screening, respectively. The difference in production may be related to the different types of animal manure used, as the authors used only bovine manure, which had an NDF content above 30% due to the bovine diet.

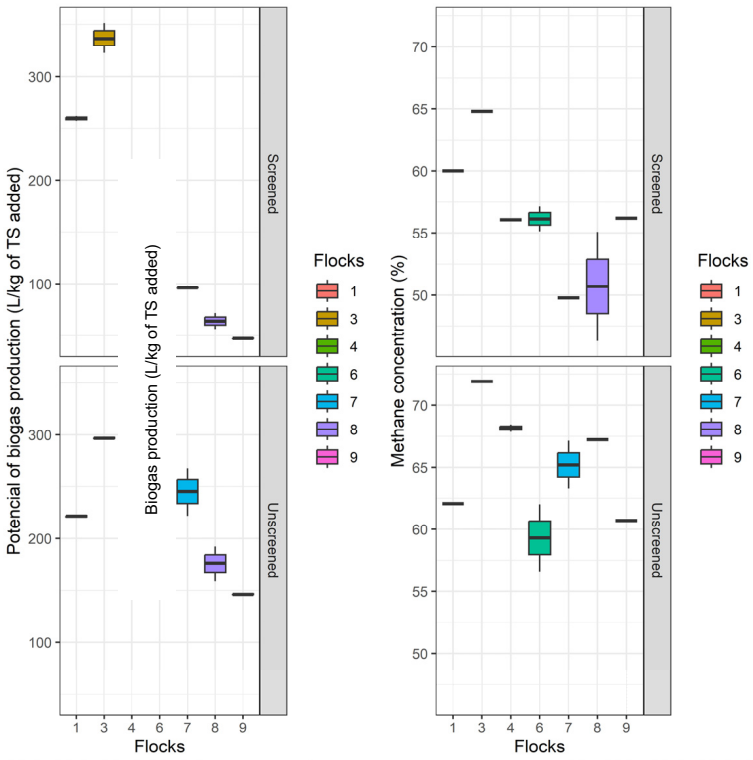


Figure 4. Biogas production (L/kg of added TS) and methane concentration (%) during the anaerobic digestion of screened and unscreened poultry litter substrates from different flocks.

The mono-digestion of substrates based on a single waste, such as animal manure, may not be as cost-effective as co-digestion due to nutrient imbalances, especially C/N, which can lead to process instability [32]. In our case, poultry bedding contains excreta from chickens, which provide bioavailable nutrients in addition to carbon-rich material and nitrogen (Table 1), which complement each other and can meet the microorganisms' demands during the digestion process. Other nutrients found in poultry litter are important for biofertilizer quality, such as potassium (2.6%), calcium (0.86%), magnesium (0.41%), and sodium (0.71%) [33]. Another important factor is the HRT adopted, as substrates like rice husks, which contain silica, are difficult to digest, thus requiring a longer HRT.

Among the two screening conditions, the order of the flocks influenced biogas yields, with the highest values observed for substrates derived from the bedding of the first and third flocks in screened substrates; from the fourth flock onwards, the highest yields were observed in unscreened substrates. Since the retention time of the substrates during digestion was long, as previously mentioned, this factor may have contributed to greater degradation of the unscreened substrates and, consequently, higher biogas yields. Lignocellulosic materials degrade slowly; however, when co-digested with animal manure, they can show satisfactory results for biogas production [34].

There was a significant effect ($p < 0.05$) of sieving on biogas production per added COD for substrates derived from batches 1, 4, 6, 7, 8, and 9, with higher productions observed in unsieved substrates (0.139, 0.103, 0.125, 0.124, 0.118, and 0.087 L/g COD, respectively). Since COD is an indicator of the organic matter concentration in the substrate, it serves as an energy source for microorganisms and is thus removed from the substrates for biogas formation. The key factors evaluated to determine the efficiency of substrate conversion into biogas are substrate composition, hydraulic retention time, and the microbial community present [35].

The substrates subjected to sieving may have had a lower concentration of fibrous material, which, despite being a degradation-resistant fraction, could have been converted into biogas due to the long retention time of the substrates. Additionally, as previously mentioned, sieving may have retained essential microorganisms for the digestion process, thereby hindering the degradation of the sieved substrates.

Ref. [36] evaluated the co-digestion process of poultry litter with swine wastewater using litter from eight production batches and also applying sieving. The authors observed that higher biogas production occurred when swine wastewater was added to the poultry litter, resulting in up to 0.08 L/g of added COD. Under ideal conditions, the expectation is that, for every kilogram of removed COD, between 0.35 and 0.45 m^3 of biogas is generated. However, this conversion depends on factors such as substrate composition, process efficiency, temperature, retention time, and the microbial community present.

The methane concentrations (Figure 4) of the biogas produced under mesophilic temperature conditions using substrates from different commercial poultry houses' bedding were close to those observed by [4], with an average of 61.5%. The methane concentration in unscreened substrates from the third flock was 71.9%, which was not substantially higher than that of substrates from the fourth flock, with a value of 68.6%. The lowest methane concentration in the biogas was 59.3% for the substrate from the sixth flock, which is similar to the minimum value found by [4]. In S substrates, the maximum methane concentration, which was found in the substrate from the third flock, was 64.8%. The minimum methane concentration occurred in the substrate from the seventh flock, with a value of 49.8% CH_4. When comparing the two screening conditions, only the substrates from the first flock had similar methane concentrations, while for the other flocks, US substrates had higher values. [25] did not find differences in methane concentrations in screened or unscreened

material with an HRT of 30 days, as with a longer HRT, bacteria can acclimate to the environment, potentially degrading the material's fibrous content.

Methane production expresses the relationship between biogas production and methane concentration. The highest methane yields were observed in substrates from the third flock for both screening conditions, and these values did not differ greatly from each other ($p > 0.05$), with an average of 218.8 ± 23.8 L/kg of added TS. The methane yields from the first flock's bedding were also not influenced by the screening condition ($p > 0.05$), with an average of 146.6 ± 10.0 L/kg of added TS. For the fourth to the ninth flocks' bedding, the US substrates showed higher yields than the S ones. However, in S substrates, reduced methane production was observed from the fourth flock onward, with no differences in production up to the ninth flock (average of 34.5 ± 3.7 L/kg of added TS). The ninth flock's bedding showed a methane production value of 88.8 L of methane/kg of added TS in the US condition, the lowest among the US substrates.

Methanogenesis is the final phase of anaerobic digestion, in which methanogenic bacteria produce methane through the use of hydrogen and CO_2 (hydrogenotrophic methanogenesis), acetate (acetoclastic methanogenesis), or methyl groups from methylated compounds (methylotrophic methanogenesis) [37]. This group of bacteria is more sensitive to changes in the internal environment of the bioreactor, and one of the benefits of co-digesting poultry manure with lignocellulosic material, such as poultry bedding, is reducing the risk of excess ammonia, which inhibits the anaerobic digestion process [34].

The biofertilizer from the first flock showed the lowest nitrogen concentration at the end of the process (Figure 5), both in screened and unscreened substrates: 2.2% and 1.3%, respectively. However, in material from later flocks, there was an increase in this concentration, followed by a decrease, which was also observed in the raw material (Table 1). Poultry manure has a high nitrogen concentration, which can be lost through ammonia volatilization. With the increased reuse of bedding, its carbon may become less available for synchronized use with the nitrogen available from the excreta, making it more prone to losses, and for this reason, the nitrogen content may decrease in older batches.

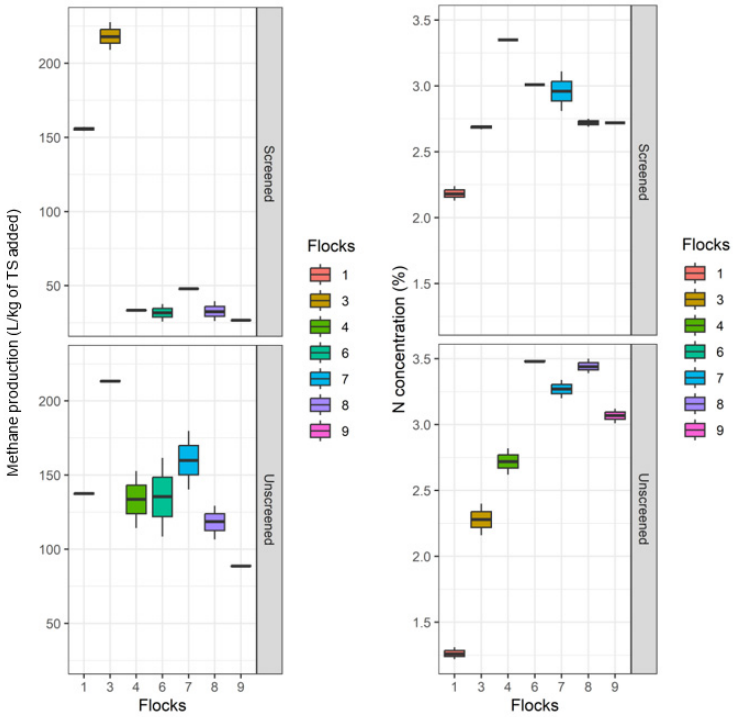


Figure 5. Methane production (L/kg of added TS) and N concentration (%) during the anaerobic digestion of screened and unscreened poultry litter substrates from different flocks.

As observed in this study, poultry bedding is a viable material for producing biogas and methane through anaerobic digestion. Concern for the environment and the disposal of waste generated from animal farming has gained prominence in recent decades, making anaerobic digestion a suitable technology for valorizing waste from poultry farming, generating clean energy, and producing an organic fertilizer rich in nutrients, contributing to the circular economy [38], which reduces costs associated with the purchase of chemical fertilizers. Ref. [39] conducted a case study of a plant producing biogas from poultry waste, which had been operational for 10 years, and reported satisfactory methane production and the acclimatization of methanogenic bacteria, increasing the efficiency of the process.

4. Conclusions

Screening of poultry bedding resulted in higher biogas production up to the third flock reared; however, no further increase in biogas and methane yields was observed from bedding from subsequent flocks. The screening of bedding was not beneficial for biogas production from the fourth flock onward; therefore, we do not recommend screening under the conditions evaluated in this study. Screening may be a viable pre-treatment for poultry bedding, but further studies on this management practice in repeatedly used bedding are needed for better clarification of the results. Research is still necessary on whether different bedding materials and the number of reuses influence the anaerobic digestion process. However, anaerobic digestion is strongly recommended for treating poultry waste, such as bedding, as it generates a high-quality biofertilizer that is safe for agricultural applications.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/poultry4020019/s1, Table S1: The amount of tap water, inoculum, and fresh poultry litter used for forming the substrate to load the biodigesters; Table S2: Averages of the results of anaerobic digestion of poultry litter from different commercial flocks, screened or unscreened; Figure S1: Distribution of biogas production over the biodegradation time in substrates prepared from poultry litter unscreened; Figure S2: Distribution of biogas production over the biodegradation time in substrates prepared from poultry litter screened.

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References

1. Bennett, C.E.; Thomas, R.; Williams, M.; Zalasiewicz, J.; Edgeworth, M.; Miller, H.; Coles, B.; Foster, A.; Burton, E.J.; Marume, U. The Broiler Chicken as a Signal of a Human Reconfigured Biosphere. *R. Soc. Open Sci.* **2018**, *5*, 180325. [CrossRef] [PubMed]
2. ABPA. Associação Brasileira de Proteína Animal—Relatório Anual; Relatório Anual-ABPA. 2024. Available online: https://abpa-br.org/abpa-relatorio-anual/ (accessed on 1 March 2025).
3. Orrico, A.C.A.; Schwingel, A.W.; de M. Costa, M.S.S.; Orrico Junior, M.A.P.; Borquis, R.R.A.; Alves, G.P.; de Oliveira, J.D.; Leite, B.K.V.; Garcia, R.G.; Vilela, R.N.S. Characterization and Valuing of Hatchery Waste from the Broiler Chicken Productive Chain. *Waste Manag.* **2020**, *105*, 520–530. [CrossRef]
4. Jurgutis, L.; Slepetiene, A.; Volungevicius, J.; Amaleviciute-Volunge, K. Biogas Production from Chicken Manure at Different Organic Loading Rates in a Mesophilic Full Scale Anaerobic Digestion Plant. *Biomass Bioenergy* **2020**, *141*, 105693. [CrossRef]
5. Do, A.D.T.; Lozano, A.; Van Laar, T.A.; Mero, R.; Lopez, C.; Hisasaga, C.; Lopez, R.; Franco, M.; Celeste, R.; Tarrant, K.J. Evaluating Microbiome Patterns, Microbial Species, and Leg Health Associated with Reused Litter in a Commercial Broiler Barn. *J. Appl. Poult. Res.* **2024**, *33*, 100490. [CrossRef]
6. Coufal, C.D.; Chavez, C.; Niemeyer, P.R.; Carey, J.B. Measurement of Broiler Litter Production Rates and Nutrient Content Using Recycled Litter. *Poult. Sci.* **2006**, *85*, 398–403. [CrossRef]
7. Alves, A.S.; Schultz, N.; Conforto, B.A.A.F.; Zonta, E.; de Carvalho, D.F. Soil, Water and Nutrient Loss under Simulated Rainfall Patterns in an Area Fertilised with Chicken Litter. *J. Hydrol.* **2023**, *620*, 129543. [CrossRef]
8. Kamp, J.N.; Feilberg, A. Covering Reduces Emissions of Ammonia, Methane, and Nitrous Oxide from Stockpiled Broiler Litter. *Biosyst. Eng.* **2024**, *248*, 73–81. [CrossRef]
9. Collins, B.A.; Birzer, C.H.; Kidd, S.P.; Hall, T.; Medwell, P.R. The Influence of Biochar Position in a Leach Bed System Anaerobically Digesting Chicken Litter. *J. Environ. Manag.* **2023**, *344*, 118404. [CrossRef]
10. Nacimento, R.A.; Rojas Moreno, D.A.; Luiz, V.T.; Avelar de Almeida, T.F.; Rezende, V.T.; Bazerla Andreta, J.M.; Ifuki Mendes, C.M.; Giannetti, B.F.; Gameiro, A.H. Sustainability Assessment of Commercial Brazilian Organic and Conventional Broiler Production Systems under an Emergy Analysis Perspective. *J. Clean. Prod.* **2022**, *359*, 132050. [CrossRef]
11. Li, Z.; You, Z.; Zhang, L.; Chen, H. Effect of Total Solids Content on Anaerobic Digestion of Waste Activated Sludge Enhanced by High-Temperature Thermal Hydrolysis. *J. Environ. Manag.* **2024**, *359*, 120980. [CrossRef]
12. Gyadi, T.; Bharti, A.; Basack, S.; Kumar, P.; Lucchi, E. Influential Factors in Anaerobic Digestion of Rice-Derived Food Waste and Animal Manure: A Comprehensive Review. *Bioresour. Technol.* **2024**, *413*, 131398. [CrossRef]
13. Kainthola, J.; Kalamdhad, A.S.; Goud, V.V. A Review on Enhanced Biogas Production from Anaerobic Digestion of Lignocellulosic Biomass by Different Enhancement Techniques. *Process Biochem.* **2019**, *84*, 81–90. [CrossRef]
14. Alonso, L.L.; Podio, N.S.; Marino, D.J.G.; Almada, N.S.; Gange, J.M.; Bernigaud, I.; Mórtola, N.; Wunderlin, D.A. Evaluating Antibiotic Occurrence, Degradation, and Environmental Risks in Poultry Litter within Argentina's Agricultural Hub. *Sci. Total Environ.* **2024**, *920*, 170993. [CrossRef] [PubMed]
15. Daghrir, R.; Drogui, P. Tetracycline Antibiotics in the Environment: A Review. *Environ. Chem. Lett.* **2013**, *11*, 209–227. [CrossRef]
16. Wang, Y.; Liu, Q.; Ran, H.; Peng, P.; Wang, Y.; Peng, G.; Wu, Y.; Wen, X. Residual Ciprofloxacin in Chicken Manure Inhibits Methane Production in an Anaerobic Digestion System. *Poult. Sci.* **2025**, *104*, 104539. [CrossRef] [PubMed]
17. De Cesare, A.; Caselli, E.; Lucchi, A.; Sala, C.; Parisi, A.; Manfreda, G.; Mazzacane, S. Impact of a Probiotic-Based Cleaning Product on the Microbiological Profile of Broiler Litters and Chicken Caeca Microbiota. *Poult. Sci.* **2019**, *98*, 3602–3610. [CrossRef]
18. de Oliveira, J.D.; Orrico, A.C.A.; Leite, B.K.V.; Schwingel, A.W.; Orrico Junior, M.A.P.; de Avila, M.R.; Machado, J.F.; Dias, A.M.D.F.; Macena, I.A.; dos Santos, W. Anaerobic Co-Digestion of Swine Manure and Forage at Two Harvesting Ages. *Ciênc. Rural* **2022**, *52*, e20200760. [CrossRef]
19. *VDI-4630*; Fermentation of Organic Materials: Characterisation of the Substrate, Sampling, Collection of Material Data, Fermentation Tests. Verlag des Vereins Deutscher Ingenieure: Düsseldorf, Germany, 2006.
20. Kunz, A.; Steinmetz, L.R.R.; do Amaral, A.C. Sociedade Brasileira Dos Especialistas Em Resíduos Das Produções Agropecuária e Agroindustrial-Sbera. In *Fundamentos da Digestão Anaeróbia, Purificação do Biogás, Uso e Tratamento do Digestato*; Food and Agriculture Organization: Rome, Italy, 2019.
21. APHA. *Standard Methods for the Examination of Water and Wastewater*, 22nd ed.; American Public Health Association: Washington, DC, USA, 2012.
22. Detmann, E.; Souza, M.A.; Valadares Filho, S.C.; Queiroz, A.C.; Berchielli, T.T.; Saliba, E.O.S.; Cabral, L.S.; Pina, D.S.; Ladeira, M.M.; Azevedo, J.A.G. *Métodos para Análise de Alimentos*; Suprema: Visconde do Rio Branco, Brazil, 2012.
23. Sarmah, A.K.; Meyer, M.T.; Boxall, A.B.A. A Global Perspective on the Use, Sales, Exposure Pathways, Occurrence, Fate and Effects of Veterinary Antibiotics (VAs) in the Environment. *Chemosphere* **2006**, *65*, 725–759. [CrossRef]
24. Kirby, M.E.; Mirza, M.W.; Leigh, T.; Oldershaw, L.; Reilly, M.; Jeffery, S. Destruction of Staphylococcus Aureus and the Impact of Chlortetracycline on Biomethane Production during Anaerobic Digestion of Chicken Manure. *Heliyon* **2019**, *5*, e02749. [CrossRef]

25. Leite, B.K.V.; Orrico, A.C.A.; Orrico Junior, M.A.P.; Aspilcueta Borquis, R.R.; da Costa, É.C.P.; da, S. Menezes, I.; de Oliveira, J.D.; Macena, I.A. Effect of Monensin Supplementation in the Bovine Diet on the Composition and Anaerobic Digestion of Manure with and without Screening. *Fermentation* **2024**, *10*, 474. [CrossRef]
26. Marti, E.; Gros, M.; Boy-Roura, M.; Ovejero, J.; Busquets, A.M.; Colón, J.; Petrovic, M.; Ponsá, S. Pharmaceuticals Removal in an On-Farm Pig Slurry Treatment Plant Based on Solid-Liquid Separation and Nitrification-Denitrification Systems. *Waste Manag.* **2020**, *102*, 412–419. [CrossRef] [PubMed]
27. Dornelas, K.C.; Mascarenhas, N.M.H.; dos Santos da Rocha, P.A.; Ton, A.P.S.; do Amaral, A.G.; Schneider, R.M.; dos Santos Lima de Brito, A.N.; Furtado, D.A.; do Nascimento, J.W.B. Chicken Bed Reuse. *Environ. Sci. Pollut. Res.* **2023**, *30*, 39537–39545.
28. Aires, A.M. *Biodigestão Anaeróbia da Cama de Frangos de Corte Com Ou Sem Separação das Frações Sólida e Líquida*. 134 f. Dissertação (Mestrado em Zootecnia)–Universidade Estadual Paulista, Faculdade de Ciências Agrárias e Veterinárias. 2009. Available online: http://repositorio.unesp.br/handle/11449/96593 (accessed on 1 March 2025).
29. Oliveira, M.C.; Ferreira, H.A.; Cancherini, L.C. Efeito de Condicionadores Químicos Sobre a Qualidade Da Cama de Frango [Effect of Chemical Conditioners on Poultry Litter Quality]. *Arq. Bras. Med. Vet. Zootec.* **2009**, *56*, 536–541.
30. Dornelas, K.C.; Schneider, R.M.; do Amaral, A.G. Biogas from Poultry Waste—Production and Energy Potential. *Environ. Monit. Assess.* **2017**, *189*, 407. [CrossRef]
31. Orrico Júnior, M.A.P.; Orrico, A.C.A.; de Lucas Júnior, J. Biodigestão Anaeróbia Dos Resíduos Da Produção Avícola: Cama de Frangos e Carcaças. *Eng. Agríc. Jaboticabal* **2010**, *30*, 546–554. [CrossRef]
32. Adnane, I.; Taoumi, H.; Elouahabi, K.; Lahrech, K.; Oulmekki, A. Valorization of Crop Residues and Animal Wastes: Anaerobic Co-Digestion Technology. *Heliyon* **2024**, *10*, e26440.
33. Orrico Júnior, M.A.P.; Orrico, A.C.A.; de Lucas Júnior, J. Compostagem Dos Resíduos Da Produção Avícola: Cama de Frangos e Carcaças de Aves. *Eng. Agríc. Jaboticabal* **2010**, *30*, 538–545. [CrossRef]
34. Neshat, S.A.; Mohammadi, M.; Najafpour, G.D.; Lahijani, P. Anaerobic Co-Digestion of Animal Manures and Lignocellulosic Residues as a Potent Approach for Sustainable Biogas Production. *Renew. Sustain. Energy Rev.* **2017**, *79*, 308–322.
35. Caetano, B.C.; Santos, N.D.S.A.; Hanriot, V.M.; Sandoval, O.R.; Huebner, R. Energy Conversion of Biogas from Livestock Manure to Electricity Energy Using a Stirling Engine. *Energy Convers. Manag. X* **2022**, *15*, 100224. [CrossRef]
36. Vicente Júnior, D.J. *Biodigestão e Co-Digestão Anaeróbias de Cama de Frangos Com Água Residuária de Suinocultura*. 68 f. Dissertação (Mestrado em Engenharia Agrícola)-Universidade Estadual do Oeste do Paraná. 2012. Available online: https://tede.unioeste.br/handle/tede/2876 (accessed on 1 March 2025).
37. Rani, J.; Pandey, K.P.; Kushwaha, J.; Priyadarsini, M.; Dhoble, A.S. Antibiotics in Anaerobic Digestion: Investigative Studies on Digester Performance and Microbial Diversity. *Bioresour. Technol.* **2022**, *361*, 127662. [CrossRef]
38. Tawfik, A.; Eraky, M.; Osman, A.I.; Ai, P.; Zhou, Z.; Meng, F.; Rooney, D.W. Bioenergy Production from Chicken Manure: A Review. *Environ. Chem. Lett.* **2023**, *21*, 2707–2727.
39. Bi, S.; Westerholm, M.; Hu, W.; Mahdy, A.; Dong, T.; Sun, Y.; Qiao, W.; Dong, R. The Metabolic Performance and Microbial Communities of Anaerobic Digestion of Chicken Manure under Stressed Ammonia Condition: A Case Study of a 10-Year Successful Biogas Plant. *Renew. Energy* **2021**, *167*, 644–651. [CrossRef]

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