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ANAEROBIC CODDIGESTION OF RAW EFFLUENT AND FLOAT SLUDGE FROM POULTRY SLAUGHTERHOUSE: EVALUATION OF THE A/M RATIO AND THE INFLUENCE OF FLOAT SLUDGE CONTENT ON BIOGAS PRODUCTION

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ABSTRACT
Poultry slaughterhouses generate large volumes of effluents with a high content of organic matter and lipids. Among the alternatives available for treatment, anaerobic digestion provides the transformation of these effluents into biogas. The objective of this work was to evaluate the anaerobic codigestion of fresh sludge from the flotator to the raw effluent of a poultry slaughterhouse. The codigestion assays were carried out in reactors (500 mL of total volume and 300 mL of useful volume), operated in batch under mesophilic conditions (30 ± 1 °C), with an incubation time of 108 days. An experimental design of the Central Rotational Composite Design (RCCRD) was adopted, comprised of a 2^2 factorial with four trials at levels +1 and -1, four trials at the levels of axial points (-1.414 and +1.414) and one more triplicate at the central point (0), the response variables were: accumulated production of CH_4 (L) and yield CH4 (L CH4/g SV added), five levels of fresh sludge addition from the flotator (V/V) (15.9, 20, 30, 40 and 43.5%) and five levels of food/microorganism ratio (A/M) (0.3, 0.5, 1.0, 1.5 and 1.7) were tested. The results of the batch tests did not indicate a significant effect for the response variables: accumulated yield of CH_4 and accumulated production of CH4. However, the R1, R10, and R7 reactors demonstrated better efficiency in the conversion of organic matter into biogas, with yields of 0.83, 0.80, and 0.71 L CH4/g SV added respectively for each reactor.

Keywords: Agroindustry. Wastewater treatment. Methane. Anaerobic digestion.

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INTRODUCTION

In 2023, Brazil was the world's second-largest producer of chicken meat and the largest exporter, with Paraná accounting for 39.47% of the volume slaughtered in the country (ABPA, 2024). The growth and expansion of poultry activity in the State of Paraná are associated with the generation of effluents with a high organic load during the processing of animal protein.

The increase in chicken meat production in Brazil is one of the factors that contribute to the generation of liquid effluents, considering that the amount of water generated per slaughtered bird can vary from 15 to 30 liters. In addition, there is the contribution of water used in the cleaning of industrial facilities and equipment. In addition to the large volumes of effluents generated, they present heterogeneity, consisting of organic matter, nutrients, solids, and oils and greases. (KUSHWAHA et al., 2010; THEBALDI et al., 2011, DALLAGO et al., 2012). Usually, the treatment process employed is composed of physicochemical flotation, followed by a biological system, which can be composed of anaerobic ponds, biodigesters, aerated ponds, and/or activated sludge systems (HABCHI et al., 2024; FAGNANI, 2023; BUSTILLO-LECOMPTE; MEHRVAR, 2017).

Flotation sludge (LF) is one of the residues resulting from the physical-chemical treatment stage of the liquid effluent generated in the chicken slaughtering processes. Air is injected into the flotillator by diffusers, producing microbubbles that, through the upward flow, are responsible for carrying a fraction of the solids contained in the wastewater to the upper surface of the tank. Then, a mechanical scraper removes the flotated material, which is usually sent to a cooking unit at a temperature of 95ºC. After cooking, the sludge is sent to three-phase centrifuges (tridecanter) (FAGNANI, 2023; RESTREPO, 2019).

Anaerobic digestion has been pointed out in the literature as a sustainable technology for the stabilization of flototer sludge, but the monodigestion of this residue may be related to toxicity due to the presence of ammonia and excess fats (SALEHIYOUN et al., 2020; PAGÉS-DÍAZ et al., 2015).

Recently, studies have emerged that relate improvements in process performance with co-digestion with carbon-rich waste (DAMACENO, 2022; RESTREPO, 2019). Co-digestion is carried out from mixtures of two or more substrates: biomass or waste, which complement each other synergistically, in order to favor the production of biogas. Anaerobic codigestion has advantages over the digestion of individual wastes (monodigestion), such as the dilution of toxic components or even the addition of nutrients to the medium. (MATA-ALVAREZ et al., 2014).

In this way, the use of fresh sludge from the flotator as a substrate for anaerobic digestion enables cost reduction, such as the use of steam for cooking and equipment for the dehydration of this material, as well as transport to the composting yards. This study aims at the energy use through the anaerobic codigestion of the fresh sludge from the flotator added to the raw effluent of a poultry slaughterhouse.

OBJECTIVE

Evaluate the anaerobic codigestion of fresh sludge from the flotator with the raw effluent from a poultry slaughterhouse, identifying the best proportion of flotated sludge and the feed/microorganism ratio (A/M) to optimize biogas production (CH_4).

METHODOLOGY

The experiment was carried out at the Laboratory of Biological Reactors (LAREB). Monitoring and carrying out physicochemical analyses at the Environmental Sanitation Laboratory, both located in block H of the State University of Western Paraná, Cascavel campus.

Anaerobic co-digestion assays were performed in laboratory-scale reactors (500 mL of total volume and 300 mL of useful volume) with 200 mL of *headspace*. Operated in batch under mesophilic conditions (30 ± 1 °C). The substrates used came from the Effluent Treatment Station (ETE) of a poultry slaughterhouse located in the western region of Paraná. They are 1) fresh sludge from the flotator and 2) raw effluent. Each of the samples was diluted based on the COD of the substrate mixture and inoculated with anaerobic sludge standardized to a concentration of 2000 mg $^{L-1}$ of SSV. (NING et al.., 2018).

The reactors were manually stirred once a day to increase the contact between the microorganisms and the substrate. Before sealing the flasks, the oxygen was purged by bubbling with gaseous nitrogen (pressure of 5 psi for 5 minutes), ensuring anaerobic conditions inside the reactor. Thus, Table 6 shows the percentages of raw effluent and fresh sludge from the flotator and the A/M values for each of the DCCR treatments.

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Table 1 Percentages of fresh flotograph sludge and A/M values of DCCR treatments

Treatments	A/M Ratio	Fresh sludge content of the flotator (%, v/v)
R1	0,5	20
R2	1,0	30
R3	1,5	40
R4	1,5	40
R5	1,0	30
R6	1,0	30
R7	0,3	15,9
R8	1,7	43,5
R9	1,0	30
R10	1,0	30
R11	1,0	30

Legend: A: food; M: microorganism.

The COD of each of the treatments was calculated based on the COD of the substrate mixture (LF+EB), where each treatment represented a content of the fresh sludge of the flotator added. The reactors were inoculated with 10% inoculum (v/v) 30 ml. The alkalinity of the mixtures was adjusted to 0.5 gNaHCO/influent cod, and the assay lasted 108 days.

Biogas volume was measured daily using a digital pressure gauge (model HT-1890; maximum pressure of 7030.7 mmH2O; operating temperature of 0 to 50ºC) and plastic syringe. The digital manometer was used to measure the pressure inside the reactor, and the volume collected in the syringe was obtained by equalizing this pressure with the atmospheric pressure. Biogas production was monitored until the daily volume was less than 1% of the accumulated volume, based on the criteria established by the VDI 4630 (2006) standard. The parameters used for determination were obtained at the end of the experiment and the response variables: accumulated CH4 production (L) and CH4 yield (L CH4/g SV added)

DEVELOPMENT

EFFECTS OF INDEPENDENT VARIABLES ON CRSD

The analysis of the influence of the factors aimed to evaluate the best conditions for the anaerobic codigestion process of the fresh sludge from the flotator with the raw effluent of a poultry slaughterhouse in the investigated ranges.

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Table 2 - Planning matrix (DCCR) with the factors and results obtained for the response variables: accumulated production of CH4 (L) and CH4 yield (L CH4/g SV added)

	Factors (actual values)		Response variables	
Reactor	Fresh floton sludge (%, v/v)	Ratio (A/M) (cod/gSSV)	Cumulative production CH4 (L)	Yield CH4 (L CH4/g SV added)
R1	-1 (20)	-1 (0,5)	0,36	0,83
R2	+1 (40)	-1 (0,5)	0,15	0,13
R3	-1 (20)	+1 (1,5)	0,11	0,10
R4	+1 (40)	+1 (1,5)	0,57	0,53
R5	-1,414 (15,9)	0 (1)	0,15	0,22
R6	+1,414 (43,5)	0 (1)	0,22	0,26
R7	0 (30)	-1,414 (0,3)	0,31	0,71
R8	0 (30)	+1,414 (1,7)	0,09	0,07
R9	0 (30)	0 (1)	0,29	0,44
R10	0 (30)	0 (1)	0,52	0,80
R11	0 (30)	0 (1)	0,30	0,29

Table 2 presents the planning matrix, obtained by applying the Central Rotational Composite Design (DCCR), with the respective response variables. The food/microorganism ratios between 0.5 and 1 gCOD/gSSV provided the highest yields of CH_4 (L CH4/g SV added) in the final phase, being 0.83 CH4 (L CH4/g SV added) for the R1 reactor and 0.80 CH4 (L CH4/g SV added) for the R10 reactor.

In practice, the A/M ratio is not a parameter that is strictly monitored in the industry, This ratio may have affected the estimated effects of the DCCR since none was significant (beyond the average). Treatments with a higher W/M ratio resulted in higher CH_4 production (L) and higher CH_4 yield (L CH_4/g SV added). In particular, treatments R1, R4, R7, and R10 showed the highest values of accumulated CH_4 production.

Compared to the cumulative methane yield of lipid-rich pig slaughterhouse residues (SSW) for initial loads of 10–40 g SV/L, it increased rapidly to over 800 mL/g SV at A/M ratios of 0.7 and 1 during the first 25 days. However, it took more than 35 days for the cumulative methane yield to reach 800 mL/g SV at an A/M ratio of 1.5, and 50 days were required for A/M ratios of 2 and 2.5 with initial loads of 10-40 g SV/L. These results showed that higher A/M ratios prolonged the conversion of lipid-rich pig slaughterhouse residues to methane, while lower A/M ratios of 0.7 and 1 promoted the conversion of lipid-rich pig slaughterhouse waste to methane in less than 25 days (LATIFI et al., 2019).

The highest yields of biogas and methane in the experiments reported by Ning et al. (2018) were by 0.631 m3/kg-SV added to the batch reactors and 0.462 m3/kg-SV added to the digester on a larger scale, obtained with the removal of 66% of volatile solids (SV). The optimized parameters obtained in the first phase were also used for verification on a larger scale (20 liters), where the biogas and methane yields found were 0.574 and 0.402 m3 /kg-

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SV added, respectively. The results showed that optimal anaerobic digestion of slaughterhouse waste in a 20-liter digester can lead to 63% SV removal and 88% reduction in chemical oxygen demand (COD) over a retention period of 42 days. In addition, the substrates used in this experiment can be used on a larger scale in order to verify their behavior in terms of SV removal and accumulated CH4 production (NING et al., 2018).

The regression for the data showed R^2=0.38, representing 38% of the variation in production efficiency. The data related to the results of the analysis of variance (ANOVA) of the model are presented in Table 2.

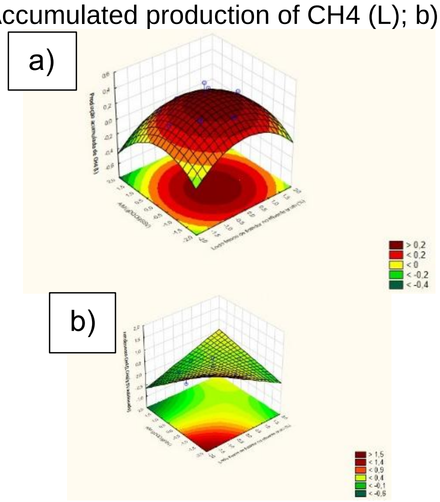
Table 2 - Analysis of Variance (ANOVA)

Source of variation	Quadratic Sum	Degrees of Freedom	Medium Square	Fcal	F tab
Regression	0,06	2	0,03	2,5	4,46
Residue	0,1	8	0,01		
Pure error	0,06	7	0,01		
Total	0,16	10			

ANOVA indicated that the model obtained cannot be considered predicted since, for a confidence level of 10%, the F test indicated a calculated F lower than the tabulated F. Even so, from these results, response surfaces were generated in order to verify the experimental regions that have the best responses, represented in Figure 1.

However, when the accumulated production and yield variables are related (Figure 1a), it is verified that the best production occurs close to the central points, as indicated in the graph. And that the income variable does not have a significant effect with a confidence interval of 90% (p<0.10).

Figure 1 - Response surfaces. a) Accumulated production of CH4 (L); b) Yield of CH4 (L CH4/g SV added)



MODELING METHANE PRODUCTION

The effect of the addition of fresh sludge from the flotator was also evaluated by calculating the reaction performance parameters for the response variables: maximum CH4 production (P), maximum specific velocity (Rm), and lag phase duration or lag time, (λ), using the modified Gompertz model (ZWIETERING et al., 1990).

According to Gompertz's model, a maximum final production of 462586.84 mL/g SSV was estimated for the R4 reactor and 72095.63 mL/g SSV for the R6 reactor. The reactors under study referring to DCCR that did not present lag phase time were R3, R5, and R8. Labatut et al. (2010) evaluated that in each phase of anaerobic digestion, it would be possible to observe different kinetics, considering the characteristics of the substrates and the possibility of converting substrates into different intermediate products.

Table 3 presents the kinetic parameters obtained from the aforementioned model in the planning matrix, constructed by the application of the DCCR.

Table 3 - Planning matrix (DCCR) with the real factors and the results of the kinetic parameters obtained from the modified Gompertz model

	Factors (actual values)		Kinetic parameters		
Reactor	Fresh Flotator Sludge (%)	Ratio (A/M) (cod/gSSV)	Cumulative production CH4 (L)	Rm (mL/h)	λ (h)
R1	-1 (20)	-1 (0,5)	0,36	1,38	262,40
R2	+1 (40)	-1 (0,5)	0,15	0,29	49,87
R3	-1 (20)	+1 (1,5)	0,11	0,23	0,00
R4	+1 (40)	+1 (1,5)	0,57	12,73	15796,94
R5	-1,414 (15,9)	0 (1)	0,15	0,21	0,00
R6	+1,414 (43,5)	0 (1)	0,22	2,82	9037,88
R7	0 (30)	-1,414 (0,3)	0,31	1,02	161,68
R8	0 (30)	+1,414 (1,7)	0,09	0,15	0,00
R9	0 (30)	0 (1)	0,29	0,45	252,74
R10	0 (30)	0 (1)	0,52	0,46	519,02
R11	0 (30)	0 (1)	0,30	0,72	105,14

Figures 2 and 3 show the estimates made for the adjustment of the modified Gompertz model, obtained in the production of methane from the co-digestion of fresh flotation sludge and raw effluent in the batch test (DCCR) for reactors R1 to R11.

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Figure 2 Estimates for adjustment of the modified Gompertz model, obtained in the production of methane from the co-digestion of fresh flototer sludge and raw effluent in the batch test (DCCR) for reactors R1 to R6. **Grades:** R1 (34% EB + 65% LF, A/M 0.5), R2 (75.9% EB + 24% LF, A/M 0.5), R3 (35% EB + 65% LF, A/M 1.5), R4 (75.9% EB + 24% LF, A/M 1.5), R5 (26.7% EB + 73.3% LF, A/M 1), R6 (83.2% EB + 16.8% LF, A/M 1).

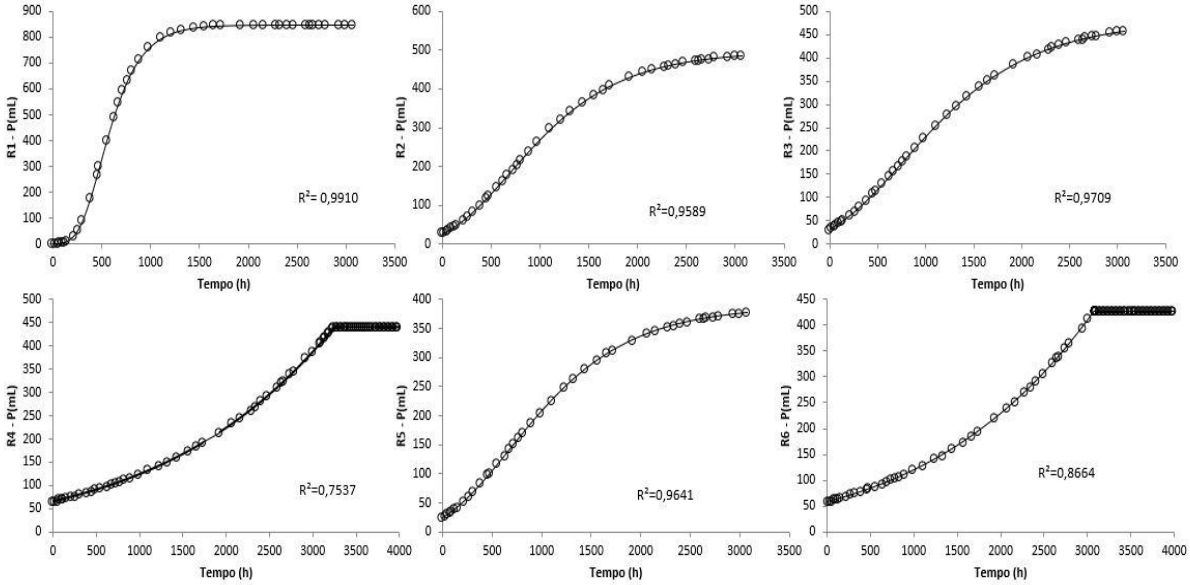
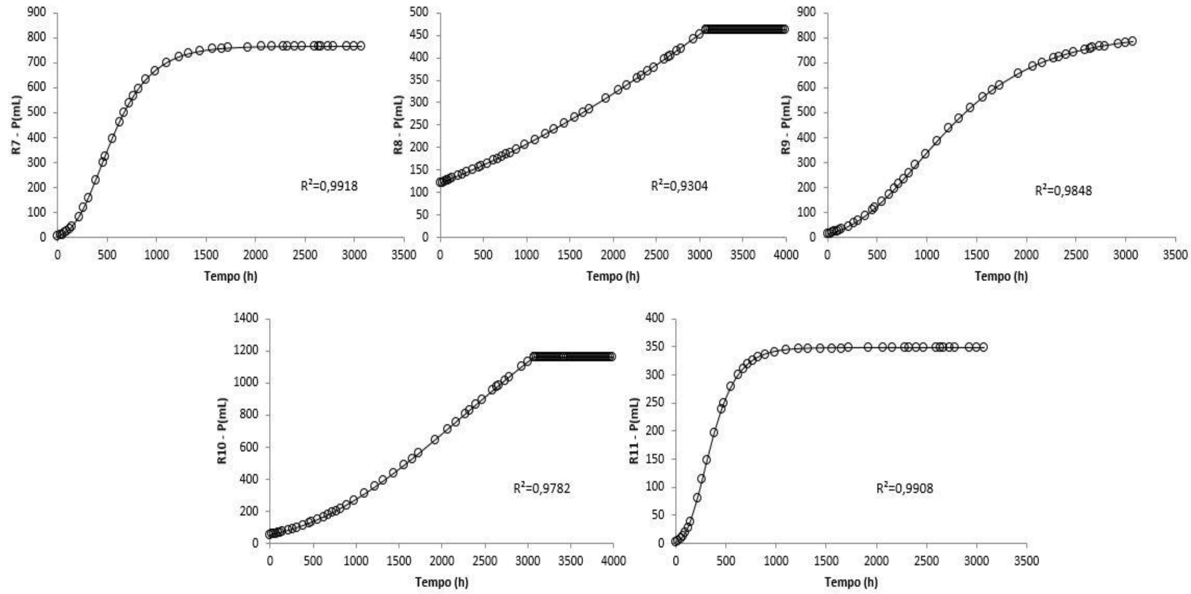


Figure 3 - Estimates for the adjustment of the modified Gompertz model, obtained in the production of methane from the co-digestion of fresh flototer sludge and raw effluent in the batch test (DCCR) for reactors R7 to R11. **Grades**: R7 (55.4% EB + 44.6% LF, A/M 0.3), R8 (55.4% EB + 44.6% LF, A/M 1.7), R9, R10 and R11 (55.4% EB + 44.6% LF, A/M 0.1).



However, it was observed that the modified Gompertz model was not adequate to describe the accumulated biogas production curves by presenting coefficients of determination (R^2). Except for the R1, R7, and R11 reactors, as they showed signs of stabilization of biogas production, presenting the highest linear correlation coefficients between the treatments.

Ware and Power (2016), in a study on poultry slaughterhouse waste, also demonstrated that cumulative methane curves can emerge in various forms, and it was possible to classify them into three groups: L-shaped, elongated S-shaped, and step curves. Another aspect mentioned by these authors was the importance of not attributing the validity of the models only to the values of R^2 in comparison with the logistic model and the R 2 model. R4 and R6 and in Figure 3 the reactor R10.

FINAL CONSIDERATIONS

Considering the results obtained about the potential of methane production in the codigestion of fresh flotation sludge and raw effluent from a poultry slaughterhouse in batch reactors, it can be concluded that the statistical analysis indicated that the model obtained cannot be considered predicted for a confidence level of 10%. However, the behavior of the response variables in the quadratic models of the accumulated CH4 production (L) and CH4 yield (L CH4/g SV added) based on the independent variables (fresh flotogen sludge, v/v and A/M ratio (gCOD/gSSV)) indicated higher values of accumulated $CH_{4\ production}$ among the mixtures that contained a higher percentage of fresh flototer sludge.

The results indicate that the best A/M ratio to optimize methane yield is between 0.3 and 1, with emphasis on 0.5 gCOD/gSSV. In addition, the R1, R10, and R7 reactors demonstrated better efficiency in converting organic matter into biogas, with yields of 0.83, 0.80, and 0.71 L CH4/g SV added respectively for each reactor.

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