International Journal of Agriculture and Environmental Research

ISSN: 2455-6939

Volume: 11, Issue: 02 "March-April 2025"

GREENHOUSE GASES PRODUCTION POTENTIAL DURING ANAEROBIC BIODIGESTION OF MANURE FROM CATTLE FED WITH DIFFERENT FEED ADDITIVES AND THEIR COMBINATIONS

[1]Guilherme Acácio de Sene, [2*]Fábio Luís Henrique, [3]Flavio Perna Junior, [4]Ramos Jorge Tseu, [3]Paulo Henrique Mazza Rodrigues

[1]DSM-Firmenich, Brazil.

[2]Lipid Marker Omics Sciences, Brazil.

[3]Department of Animal Production and Nutrition, College of Veterinary Medicine and Animal Science, University of São Paulo, Brazil.

[4]Department of Animal Production, College of Veterinary Medicine, Eduardo Mondlane University, Mozambique.

*Corresponding author

DOI: https://doi.org/10.51193/IJAER.2025.11205

Received: 17 Feb. 2025 / Accepted: 28 Feb. 2025 / Published: 15 Mar. 2025

ABSTRACT

Manure management, particularly anaerobic digestion, is an alternative for reducing the environmental impacts of cattle raising and energy generation. The aim of this study was to produce biogas and biofertilizer from waste from Nellore cows fed sodium monensin, essential oils, exogenous enzymes, and their combinations. The experimental batch-type biodigesters were placed inside a climatic chamber (30–35 °C). They were organized in a completely randomized design in a 2 × 2 × 2 factorial arrangement, with waste tested from Nellore cows fed the presence or absence of essential oil, exogenous enzyme, and monensin, with four repetitions totaling 32 experimental units (represented by the manure of the animals that received the different additives and their associations). The use of monensin reduced the amount of N inserted into the biodigesters, reduced the nutrient removal efficiency, and reduced biogas production by 39.26%, in addition to altering the composition of the biofertilizer produced. The other tested additives together with the combination of additives did not influence the biodigestion process. Therefore, the addition of monensin reduces nutrient removal efficiency, compromises biogas production, and reduces the concentration of nutrients in the biofertilizer, while essential oils and enzymes do not

ISSN: 2455-6939

Volume: 11, Issue: 02 "March-April 2025"

affect the biodigestion process. In addition, no associative effect was observed among the tested additives.

Keywords: Biogas, Enzyme, Essencial oil, Methane, Monensin

1. INTRODUCTION

Agriculture is a significant source of greenhouse gas (GHG) emissions worldwide, accounting for 10%–12% of global anthropogenic GHG emissions. When analyzing non-carbon dioxide (CO_2) emissions, enteric fermentation of ruminants is the main contributor to approximately 30%–40% of agricultural emissions, followed by the emission of waste deposited in pastures, responsible for approximately 15% of agricultural emissions (1), which can represent up to 27% of the total emissions of methane gas (CH_4) by ruminants (2; 3) in intensive production systems, where these wastes accumulate in small areas.

Due to this, the correct management of animal waste becomes an important tool to reduce environmental impacts, with anaerobic biodigestion being a promising alternative, as in addition to reducing GHG emissions, it is capable of recycling waste in the form of biofertilizers, in addition to producing biogas, which is considered an alternative source of energy.

Anaerobic biodigestion can be defined as a complex interaction of microorganisms that degrade organic components present in waste, mainly in the form of CH4 and CO2 (4). In this interaction, the nutrients contained in the waste ensure the survival and reproduction of microorganisms, allowing the degradation of the organic fraction into the form of biofertilizers as well as the production of biogas (5).

Bovine waste is a suitable substrate for anaerobic biodigestion (6). However, several factors can change the characteristics of waste and influence its potential for biogas production, including animal feed (7; 8). Among all these factors, the composition of the material directly influences its degradation potential. Therefore, the extent of biogas production is dependent on animal feed. To increase the feed efficiency of ruminants and reduce the CH_4 emissions, several studies have reported a wide variety of nutritional techniques aimed at manipulating the ruminal environment to increase ruminant feed additives. In addition to acting in the manipulation of the ruminal environment, it can affect the use of nutrients and, consequently, the characteristics of waste excreted by animals.

Ionophores can increase the apparent energy digestibility by 2% and the apparent nitrogen digestibility by 3.5% (9). Some studies have also demonstrated that the action of essential oils makes the digestive process of food more efficient, increasing the digestibility of dry matter (DM), organic matter (OM), and neutral detergent fiber (NDF) (10). Enzyme supplementation has the main goals of removing or destroying antinutritional factors and increasing the total digestibility

ISSN: 2455-6939

Volume: 11, Issue: 02 "March-April 2025"

of the diet (11; 12). Furthermore, improvement in nutrient digestibility with the addition of enzyme preparations in ruminant feed has been demonstrated by several studies (13; 14; 15).

Therefore, it is expected that an increase in the digestibility of nutrients in the diet will result in changes in the composition of waste excreted by animals and, therefore, influence anaerobic digestion and, consequently, the production of biogas. Therefore, this study aimed to evaluate the potential for biogas production and the characteristics of biofertilizer from waste from Nellore cows fed with sodium monensin, essential oils, exogenous enzymes, and their combinations, as an alternative for the management of waste from cattle.

2. MATERIAL AND METHODS

2.1 Study location and ethical issues

This research project was conducted at the College of Veterinary Medicine and Animal Science of the University of São Paulo (USP), Campus Fernando Costa, and Pirassunga/SP, Brazil. The project was approved and authorized in accordance with the norms of the National Council for the Control of Animal Experimentation (CONCEA) by the Ethics Committee in the Use of Animals, Faculty of Animal Science and Food Engineering, University of São Paulo – FZEA/USP (CEUA/FZEA), filed under CEUA No. 4788111017.

2.1.1 Treatments and experimental design

The experiment was carried out in two phases: the feeding phase and the anaerobic digestion phase, as follows:

2.1.1.1 Feeding phase

Eight non-pregnant and non-lactating Nellore bovine females, with an average live weight of 480 ± 55 kg, were housed individually and covered with sand beds, cement troughs, and automatic drinking fountains.

The animals were divided into two contemporary 4 × 4 Latin squares in a 2 × 2 × 2 factorial arrangement and fed daily (8:00 and 16:00 h) using isoenergetic and isoprotein diets, which differed according to the presence or absence of the tested additives. Being: diet without the addition of essential oil (OE-A); diet added with 31.7 mg/kg DM of an essential oil blend composed of 43% cinnamaldehyde and 7% garlic oil (OE-P); diet without added enzyme (EN-A); diet with the addition of 1027 mg/kg DM product containing an "enzyme blend" composed of cellulase, xylanase, amylase, protease, phytase, beta-glucanase and pectinase (EN-P); diet without added monensin (MA); diet with the addition of 30.6 mg of sodium monensin/ kg DM (MP). This phase was divided into four periods of 22 days, with 16 days for adaptation to experimental diets and 5 days for fecal collection. Urine collection was carried out on the 22nd day of each period.

ISSN: 2455-6939

Volume: 11, Issue: 02 "March-April 2025"

Feces were collected manually through the rectum at 8:00 am and 4:00 pm, frozen at -20°C, and pooled to form a single composite sample for each animal in each period. Urine samples were obtained every 6 h during stimulation by vulvar massage and then stored at -20ºC in a single vial, which formed a single composite sample within 24 h. Next, enhance (43% cinnamaldehyde + 7% garlic oil) (Novus International Inc., Indaiatuba, Brazil) was used as the source of essential oil, Allzyme® SSF (Alltech Inc., Nicholasville, USA) was used as the source of the enzymatic blend, and Rumenpac® (Grupo MCassab, São Paulo, Brazil) was used as the source of sodium monensin.

2.1.1.2 Anaerobic digestion phase

2.1.1.2.1 Substrate preparation, experimental design, and treatments

Fecal and urine samples, collected and frozen in the feeding phase, were thawed and diluted in water. A mixture of feces and urine (waste) was prepared using a theoretical ratio of 83%:17%, respectively. Then, this mixture was diluted with water and, finally, the inoculum was added.

Batch-type benchtop biodigesters were used, and 3 kg of substrate were prepared, of which 2 kg were used to fill the biodigesters and 1 kg to carry out the substrate characterization analyses (Table 1).

The substrate composition was done with the following proportions: 40% manure, 3.3% inoculum and 56.7% water. The sludge from the bovine manure treatment pond was used as an inoculum, which presented 0.164% of total solids (TS). Thus, the substrates were prepared in order to guarantee an estimate of 6% of TS.

The biodigesters were arranged in a completely randomized design in a 2 x 2 x 2 factorial scheme with 4 replications, totaling 32 experimental units (represented by the manure of the animals that received the different additives and their associations).

2.1.1.2.2 Biogas Production

Anaerobic biodigestion was performed under mesophilic conditions (30 to 35ºC), ideal for digestion kinetics (16). The biodigesters were placed inside a climatic chamber with an electrical resistance heating system and a digital temperature controller.

The batch-type biodigesters consisted of three straight cylinders with diameters of 15, 10, and 7.5 cm, with an average capacity to ferment 2 liters of substrate each, according to (17).

The reading of biogas production was performed according to the accumulation in the gasometer. It consisted of measuring the height with a ruler fixed to the gasometer, according to its vertical displacement. The reading value was multiplied by the internal cross-sectional area of the gasometer. After each reading, the gasometers were emptied using the biogas discharge log. The

ISSN: 2455-6939

Volume: 11, Issue: 02 "March-April 2025"

correction of the biogas volume for conditions of 1 atm at 20ºC was performed according to the methodology described by (18). To correct the volume of biogas, the expression resulting from the combination of the Boyle and Gay-Lussac laws was used:

$$(V0P0) /T0 = (V1P1) /T1$$

Where: V0 = corrected biogas volume, m^3 ou L; P0 = corrected biogas pressure, 10322.27 mm H_2O; T0 = corrected biogas temperature, 293.15 K; V1 = gas volume in the gas meter; P1 = biogás pressure at the time of reading, 10344.11 mm H_2O; T1 = biogas temperature, in K, at the time of reading.

Considering the mean atmospheric pressure of Pirassununga equal to 10273.11 mm H_2O and the pressure given by the gasometers of 71 mm H_2O, the following expression was obtained to correct the volume of biogas:

$$V0 = (V1/T1) \times 293.7703$$

Biogas samples were taken together with the measurement of the biogas volume. Samples were collected using a 60 mL syringe connected to the gas register at the top of the gasometer. Before the sampling itself, the biogas was collected and used to flush the bottle (twice), after which 50 mL of biogas were injected to analyze its composition. After collecting the biogas, the gasometers were emptied; this allowed a new accumulation of gases. The test was terminated when biogas production ceased, which occurred 164 days after filling the biodigesters.

The concentration of CH_4, CO_2, and N_2O was determined by gas chromatography (Trace 1300, Thermo Fisher Scientific®, Rodano, Milan, Italy) in a temperature-controlled environment (25°C), according to (19). The biogas samples were diluted in glass flasks, with known volume, 16.78 times in atmospheric air. Then, 6 mL was injected into the chromatograph injector (split/splitless), of which 4 mL was used for flushing the injection system, and 2 mL was used for analysis. The system with flame ionization detector (FID) is responsible for measuring CO_2 and CH_4 and the system with electron capture detector (ECD) is responsible for the quantification of N_2O.

The chromatograph was calibrated with 3.1% CH_4, 3.1% CO_2 and 0.49% N_2O diluted in atmospheric air. Two gas mixtures were used as reference, one with 50% CH_4 and 50% CO_2 and the other with 10% N_2O in equilibrium with He (mol/mol).

The volumes of CH_4, CO_2 and N_2O produced (m^3or L) were calculated using the biogas production and composition data from each digester according to the equation:

$$Vol = (VolBIOGAS \times \%Gas)/100$$

ISSN: 2455-6939

Volume: 11, Issue: 02 "March-April 2025"

Where: Vol = volume (m^3 ou L); VolBIOGAS = volume of biogas produced (m^3 ou L);% Gas = contente gas of interest in biogas (%)

The production of CH_4, CO_2 or N_2O was calculated by dividing the total production of each gas by the amount of VS added or removed (difference between SV added in the filling time of the biodigesters and VS eliminated during fermentation).

The Gompertz model was used to study the kinetics of biogas and its components production. (20), according to the equation:

$$Y_t = A \exp [-B \exp (-kt)]$$

Where: Y_t: gas production (L/g VS added) at time t (day); A: model asymptote, indicates the production stabilization value (L/g VS added) in relation to time t;B: integration constant, no biological meaning.kt: maximum growth rate, logarithmic function of production growth (L/gVS added) per unit of t.

The time (t) at the inflection point was determined as follows:

$$t1 = \ln B/k$$

Where: t1: time (days) at the inflection point; ln: natural logarithm; k: production constant.

Gas production at the inflection point was determined as:

$$y1 = A/\exp$$

Where: y1: gas production at the inflection point; exp: base of the natural logarithm (2.7183)

2.1.1.3 Removal of nutrients

The substrates added and recovered in each biodigester were weighed and multiplied by the DM percentage to calculate the DM content in grams. The added and recovered nutrients, expressed in grams, were calculated by multiplying those added or recovered and expressed in DM grams, which were expressed as a percentage and divided by 100 according to the following equation:

Nutrient (g) = Nutrient added or recovered (%) x DM (g)/100

Nutrient removal, in percentage, was calculated from the content of added and recovered nutrients and expressed in g/kg of DM according to the following equation:

Nutrient removed (%) = [Nutrient added (g) – Nutrient removed (g)] x 100/Added nutrient (g)

2.1.1.4 Laboratory Analysis

ISSN: 2455-6939

Volume: 11, Issue: 02 "March-April 2025"

The substrate samples before and after anaerobic digestion were collected, dried in an oven with ventilation and constant air renewal at 65ºC for 72 hours, according to (20). Then, they were ground (1 mm) and stored in properly sealed bottles. DM was determined at 105ºC for 4 hours in an oven (method 930.15; 21). Mineral matter (MM) was obtained by calcination in a muffle at 550ºC for 5 hours (22). The contents of ST (ST = 100 - moisture) and SV (SV = ST - MM) of the substrates were determined with adaptations to the methodology described in (23). The total N content was determined by the micro-Kjeldahl technique (method 920.87; 22). Neutral detergent fiber (NDF) was determined by the method described by (24). The hydrogen ion potential (pH) was measured by a portable pH meter (Hanna Instruments®, HI 8424, Italy).

2.2 Statistical Analysis

Data were analyzed using the Statistical Analysis System (25). Before data analysis, they were evaluated for the presence of discrepant information (outliers) and normality of the residuals using the Shapiro-Wilk test. When the normality premise was not met, the data were transformed. Data were subjected to analysis of variance, which separated as causes of variation the effect of factors and their interactions, period effect, animal effect inside squared, along with squared effect. The effect of factors was analyzed using analysis of variance using 0.05 significance.

3. RESULTS

3.1 Biodigestion and nutrients removal

The waste added to the biodigesters from animals fed with sodium monensin presented a 20.03% lower amount of N ($P<0.05$) than the waste from animals that did not receive such additive, with no significant difference being observed ($P> 0.05$) for other added nutrients.

The amounts of TS and NDF remaining after the biodigestion process were 25.02% and 66.24% higher for manure from animals that received sodium monensin ($P<0.05$), when compared to manure from animals that did not receive sodium monensin.

The removal efficiency of TS, NDF and N were lower ($P<0.05$) for biodigesters supplied with animal manure fed with sodium monensin, when compared to those supplied with animal manure that did not consume such additive (Table 1).

ISSN: 2455-6939

Volume: 11, Issue: 02 "March-April 2025"

Table 1: Biodigestion and removal efficiency of nutrients from anaerobic batch type biodigesters supplied with waste of Nellore cows fed with essential oil, an enzyme blend, monensin and their interactions.

Variable	Factors						Average	SEM	P value						
	Essential Oil		Enzyme		Monensin				EO	E	M	EO*E	EO*M	E*M	EO*E*M
	Negative	Positive	Negative	Positive	Negative	Positive									
Added nutrients															
TS, g	117.3	117.1	117.3	117.2	117.3	117.2	117.2	0.084	NS	NS	NS	NS	NS	NS	NS
VS, g	101.1	101.6	101.4	101.4	102.0	101.8	101.4	0.448	NS	NS	NS	NS	NS	NS	NS
FDN, g	50.82	52.14	50.47	52.48	51.16	51.79	51.53	0.913	NS	NS	NS	NS	NS	NS	NS
N, g	5.72	5.70	5.77	5.65	6.34	5.07	5.74	0.150	NS	NS	<0.001	NS	NS	NS	0.08
Recovered nutrients															
TS, g	68.68	72.86	67.81	73.73	62.9	78.64	70.38	2.158	NS	0.08	<0.001	NS	NS	NS	NS
VS, g	55.41	61.47	55.37	61.51	55.37	61.51	58.41	2.312	NS	NS	NS	NS	NS	NS	NS
NDF, g	30.15	33.43	30.27	33.32	23.88	39.7	31.49	1.747	NS	NS	<0.001	NS	NS	NS	NS
N, g	1.92	1.99	1.87	2.04	1.99	1.92	1.95	0.055	NS	NS	NS	NS	NS	NS	NS
Removal efficiency															
TS, %	41.80	38.25	42.53	37.51	46.69	33.63	40.36	1.83	NS	0.08	<0.001	NS	NS	NS	NS
VS, %	45.11	39.48	45.37	39.21	45.72	38.87	42.36	2.293	NS	NS	NS	NS	NS	NS	NS
NDF, %	40.19	35	39.39	35.8	53.01	22.18	38.24	3.655	NS	NS	<0.001	NS	NS	NS	NS
N, %	65.50	54.09	67.01	62.58	68.3	61.3	65.12	1.545	NS	NS	0.02	NS	NS	NS	0.08

SEM: standard error of mean; EO: Essential Oil; E: Enzyme Blend; M: Monensin; EO*E: Interaction between essential oil and enzyme; EO*M: Interaction between essential oil and monensin; E*M: Interaction between enzyme and monensin; EO*E*M: Interaction between essential oil, enzyme and monensin; TS: total solids; VS: volatile solids; N: nitrogen; NDF: neutral detergent fiber.

ISSN: 2455-6939

Volume: 11, Issue: 02 "March-April 2025"

3.2 Biogas Production

Biodigesters supplied with animal waste fed with sodium monensin showed a reduction of 39.26% in biogas production (L). Consequently, lower production of CH_4 and CO_2 in absolute values (liters), 36.17% and 45.08% respectively, and relative values (L/g of feces and L/g of VS) ($P<0.05$) when compared to biodigesters supplied with manure from animals that did not consume this additive. However, there was no change in the composition of the biogas produced ($P>0.05$), which was composed of 71.92% CH_4, 27.55% CO_2, and 0.06% N_2O, using the mean values between the factors analyzed (Table 2).

Manure from animals that consumed sodium monensin had lower ($P<0.05$) production rate (A) and lower ($P<0.05$) production at the inflection point (y) for both CH_4 (Figure 1) and CO_2 variable.

The use of sodium monensin in the diet increased the production rate (A) and production at the inflection point (y) for the N_2O variable ($P<0.05$), however, the total production of this gas was not changed ($P >0.05$) (Table 2).

ISSN: 2455-6939

Volume: 11, Issue: 02 "March-April 2025"

Table 2: Gas production (total biogas, CH_4, CO_2 and N_2O) in batch type biodigesters with waste of Nellore cows fed with essential oil, an enzyme blend, monensin and their interactions

Variable	Factors						Average	SEM	P value						
	Essential Oil		Enzyme		Monensin				OE	E	M	OE*E	OE*M	E*M	OE*E*M
	Negative	Positive	Negative	Positive	Negative	Positive									
Biogas, L	40.46	40.29	39.86	40.89	50.24	30.51	40.58	2.40	NS	NS	<0.001	NS	NS	NS	NS
CH_4															
CH_4, L	29.22	28.79	29.11	28.91	35.41	22.6	29	1.65	NS	NS	<0.001	NS	NS	NS	NS
CH_4, %	71.45	72.28	72.73	71.01	70.32	73.41	71.92	0.82	NS	NS	0.07	NS	NS	NS	NS
CH_4/feces, L/g	0.029	0.029	0.029	0.029	0.036	0.023	0.029	0.0016	NS	NS	<0.001	NS	NS	NS	NS
CH_4/added VS	0.28	0.28	0.28	0.28	0.34	0.22	0.28	0.016	NS	NS	<0.001	NS	NS	NS	NS
A, L/g	0.32	0.3	0.3	0.31	0.35	0.26	0.31	0.014	NS	NS	0.001	NS	NS	NS	NS
k, L/g.day	0.04	0.036	0.037	0.034	0.041	0.03	0.036	0.0029	NS	NS	NS	NS	NS	NS	NS
t, day	52.32	47.6	43.56	56.35	44.22	55.69	48.86	3.62	NS	0.09	NS	NS	NS	NS	NS
y, L/g	0.12	0.111	0.113	0.115	0.131	0.097	0.115	0.0052	NS	NS	0.001	NS	NS	NS	NS
CH_4/removed VS, L/g	0.7	0.82	0.7	0.83	0.9	0.62	0.76	0.068	NS	NS	0.05	NS	NS	NS	NS
CO_2															
CO_2, L	11.22	11.01	10.73	11.5	14.35	7.88	11.05	0.83	NS	NS	<0.001	NS	NS	NS	NS
CO_2, %	27.6	27.65	26.31	28.94	28.76	26.49	27.55	0.75	NS	0.09	NS	NS	NS	NS	NS
CO_2/feces, L/g	0.011	0.011	0.01	0.011	0.014	0.08	0.011	0.0008	NS	NS	<0.001	NS	NS	NS	NS

ISSN: 2455-6939

Volume: 11, Issue: 02 "March-April 2025"

CO_2/added VS	0.11	0.108	0.105	0.113	0.14	0.078	0.108	0.0082	NS	NS	<0.001	NS	NS	NS	NS
A, L/g	0.12	0.116	0.112	0.123	0.143	0.092	0.121	0.0087	NS	NS	0.006	NS	NS	NS	NS
k, L/g.day	0.036	0.034	0.037	0.033	0.042	0.028	0.037	0.0034	NS	NS	0.09	NS	NS	NS	NS
t, day	51	47.58	42.24	56.33	44.14	54.43	47.41	3.92	NS	NS	NS	NS	NS	NS	NS
y, L/g	0.044	0.0426	0.041	0.0455	0.052	0.034	0.044	0.0032	NS	NS	0.006	NS	NS	NS	NS
CO_2/removed VS, L/g	0.27	0.318	0.257	0.331	0.368	0.221	0.294	0.029	NS	NS	0.01	NS	NS	NS	NS
N_2O															
N_2O, mL	18.58	20.99	26.33	13.25	10.08	29.49	19.94	6.21	NS	NS	NS	NS	NS	NS	NS
N_2O, %	0.056	0.061	0.071	0.047	0.017	0.1	0.059	0.02	NS	NS	0.058	NS	NS	NS	NS
N_2O/feces, mL/g	0.018	0.021	0.026	0.013	0.01	0.03	0.02	0.0063	NS	NS	NS	NS	NS	NS	NS
N_2O/added VS	0.184	0.205	0.259	0.13	0.097	0.291	0.196	0.061	NS	NS	NS	NS	NS	NS	NS
A, mL/g	0.318	0.135	0.383	0.071	0.099	0.354	0.189	0.061	0.07	0.005	0.01	NS	NS	NS	NS
k, mL/g.day	0.034	0.035	0.036	0.032	0.04	0.029	0.035	0.0027	NS	NS	0.07	NS	NS	NS	NS
t, day	55.17	47.55	46.22	56.5	44.24	58.48	49.61	3.69	NS	NS	0.06	NS	NS	NS	NS
y, mL/g	0.117	0.049	0.141	0.026	0.036	0.13	0.069	0.022	0.07	0.005	0.01	NS	NS	NS	NS
N_2O/removed VS, mL/g	0.433	0.464	0.57	0.327	0.194	0.702	0.451	0.142	NS	NS	0.1	NS	NS	NS	NS

SEM: stanadard error of mean; EO: Essential Oil; E: Enzyme Blend; M: Monensin; EO*E: Interaction between essential oil and enzyme; EO*M: Interaction between essential oil and monensin; E*M: Interaction between enzyme and monensin; EO*E*M: Interaction between essential oil, enzyme and monensin; VS: volatile solids; A: asymptotic production (L/g added VS); k: production constant (L/g added VS per day); t: time at inflection point (day); y: production at inflection point (L/g added VS).

ISSN: 2455-6939

Volume: 11, Issue: 02 "March-April 2025"

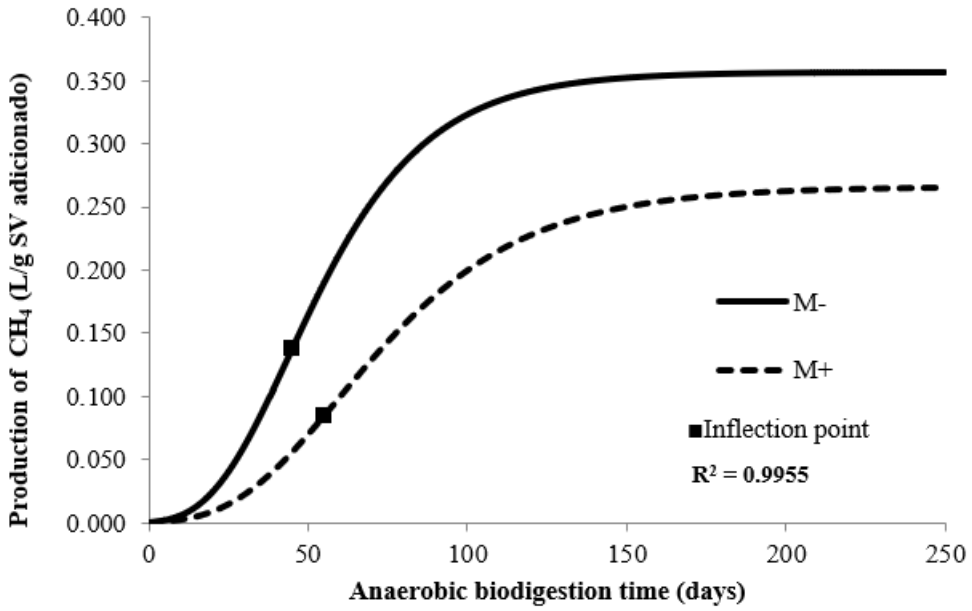

Figure 1: CH_4 production, adjusted by the Gompertz model, in batch-type biodigesters supplied with waste from Nellore cows, fed with or without sodium monensin.

3.3 Biofertilizer Composition

Waste from animals fed with sodium monensin resulted in biofertilizers with higher total carbon content ($P<0.05$) and lower levels of N, P_2O_5, and K_2O ($P<0.05$), resulting in a higher C: N ratio without changing the amount of organic carbon (Table 22). Additionally, the biofertilizer obtained from animal waste that received sodium monensin had a lower pH value ($P<0.05$) (Table 3).

ISSN: 2455-6939

Volume: 11, Issue: 02 "March-April 2025"

Table 3: Composition of biofertilizers obtained in batch type biodigesters supplied with waste from Nellore cows fed with essential oils, a blend of exogenous enzymes, sodium monensin and their associations.

Variable	Factors						Average	SEM	P-value						
	Essential Oil		Enzyme		Monensin				EO	E	M	EO*E	EO*M	E*M	EO*E*M
	Negative	Positive	Negative	Positive	Negative	Positive									
Total C (g/kgMS)	429.3	437.2	429.6	436.9	413.6	452.9	432.4	4.35	NS	NS	<0.001	NS	NS	NS	NS
Organic C (g/kgMS)	163.6	162.6	162.5	163.7	163.8	162.4	163.1	0.84	NS	NS	NS	NS	NS	NS	NS
Total N (g/kgMS)	27.82	27.75	28.02	27.55	31.74	23.83	27.78	0.95	NS	NS	<0.001	NS	NS	NS	NS
P_2O_5 (g/kgMS)	18.21	18.18	18.78	17.6	20.75	15.64	18.31	0.55	NS	0.07	<0.001	NS	NS	NS	NS
K_2O (g/kgMS)	27.00	25.50	26.73	25.77	30.27	22.22	26.40	0.94	NS	NS	<0.001	NS	NS	NS	NS
C/N	15.62	16.32	15.92	16.02	13.22	18.71	15.9	0.60	NS	NS	<0.001	NS	NS	NS	NS
pH	7.70	7.73	7.72	7.71	7.79	7.64	7.72	0.030	NS	NS	0.02	NS	NS	NS	NS

SEM: stanadard error of mean; EO: Essential Oil; E: Enzyme Blend; M: Monensin; EO*E: Interaction between essential oil and enzyme; EO*M: Interaction between essential oil and monensin; E*M: Interaction between enzyme and monensin; EO*E*M: Interaction between essential oil, enzyme and monensin; C/N: Carbon: Nitrogen ratio.

ISSN: 2455-6939

Volume: 11, Issue: 02 "March-April 2025"

4. DISCUSSION

The use of cattle waste as a substrate for anaerobic digestion is a good alternative to carbohydrate, protein, and fat (6). However, certain factors can alter the potential for biogas production. According to (26), the composition of the material directly influences the potential for degradation of the substrate. Thus, the extent of biogas production from manure is dependent on animal feed.

It is important to emphasize that different additives used as modifiers of ruminal metabolism do not decrease CH_4 production through a single mechanism of action. Sodium monensin is a classic manipulator of the rumen environment, directing H_2 that would be used for the production of CH_4 for the production of propionic acid, thus causing a change in ruminal patterns with increased energy efficiency (27). Essential oils such as cinnamaldehyde and garlic oil have antimicrobial properties and the potential to modulate rumen fermentation, being mostly investigated in in-vitro experiments (28; 29; 30; 31; 32; 33). Among its main advantages, the low risk of microbial resistance stands out because these compounds present, in most cases, several active principles that give different modes of action (34). Exogenous enzymes appear as alternatives that can promote improvements in digestibility and use of the offered diet. The associated use of different exogenous enzymes showed an improvement in rumen fermentation when compared to the use of isolated enzymes. Even in conditions where the rumen has a high rate of fermentation in the diet, it is still possible to observe the elimination of degradable fibers and starch in feces (35; 36). Because the feed additives mentioned above do not have a unique and exclusive mechanism of action, nothing prevents their effects from being additive - the result of the combination is equal to the sum of the parts - or even synergistic - the combined result is greater than the sum of the parts.

The use of essential oils and exogenous enzymes did not affect the nutrients added to the biodigesters or biodigestion process. Similarly, another study evaluating the effects of exogenous enzymes (37) found no significant differences in the production of gases from the feces of dairy cows. For example, cattle fed cottonseed and vitamin E added to their diets did not show significant differences in the fecal emissions of biogas, CH_4, and N_2O, with the addition of cottonseed increasing the concentration of CH_4 in the feces and reducing that of CO_2 but did not affect the total production of CH_4, CO_2, and N_2O in the biodigesters. (38).

In the present study, sodium monensin did not affect the pH of the material introduced into the biodigesters or the amounts of TS, VS, and NDF added. However, this reduced the amount of N added. This reduction is justified by the ability of sodium monensin to increase the use of N in the diet (9), thereby influencing the biodigestion process. For the biodigestion process to occur under satisfactory conditions, nutrients must be present in sufficient quantities (39), with N being the main one, since during the anaerobic decomposition process, microorganisms use NH_3 and organic

ISSN: 2455-6939

Volume: 11, Issue: 02 "March-April 2025"

forms of N for their growth (40). Additionally, (41) indicates the importance of N concentration, because if the C:N ratio is not adequate, bacteria cannot consume all the carbon present, and the process performance will be low.

Despite the lower availability of N with the use of sodium monensin, the efficiency of VS removal did not change and had an average value of 42.36%, which can be considered within the appropriate range (30–45%). The composition of the biogas produced was not altered and was within the parameters presented in (42). The mixture of gases was composed of 50-80% CH_4 and 20-40% CO_2. In the present study, the biogas produced was composed of 71.92% CH_4, 27.55% CO_2, and 0.06% N_2O, using the mean of all the analyzed factors. However, the use of sodium monensin reduced the removal efficiency of TS and NDF, indicating lower fermentation activity, resulting in a 39.26% drop in biogas production due to the lower production of CH_4 and CO_2. However, (43), evaluating the supplementation of monensin and Acacia mearnsii tannins in the diet of Nellore cows, observed antagonistic interactions when the additives were associated with the aim of reducing the emission of gases in the feces of these animals. According to (44), part of the monensin consumed by animals can be recovered in feces. This effect was not observed in the urine or tissues of animals. (45), when determining the pattern of excretion and tissue distribution of monensin in cattle, reported a 95% recovery of the active metabolites of monensin in the feces of animals.

The effect of cattle diet on the environmental impact caused by manure concluded that the use of ionophores in the diet delayed the start of biogas production and altered the total production (46). These results are similar to those found in the present study. In addition to lower biogas production, a lower rate of CH_4 and CO_2 production was observed with sodium monensin in the diet. In addition, according to (46), such a change in the biodigestion process is due to monensin being responsible for preventing the growth of acetate-producing bacteria, reducing the availability of C and H for methanogenic archaea.

The lower efficiency of TS and NDF removal observed in the biodigesters that received manure from animals fed monensin resulted in a higher concentration of TS and NDF in the remaining material (Table1). Consequently, this resulted in a higher concentration of C total in the biofertilizer obtained at the end of the process. It dilutes the amounts of N, P_2O_5, and K_2O (Table 3) because the transformation of C into CH_4 is responsible for concentrating the nutrients in the biofertilizer (47). Thus, the lower production of CH_4 and CO_2 in these biodigesters (Figure 1, Table 2) justifies the higher C total concentration and lower concentrations of N, P_2O_5, and K_2O in the obtained biofertilizer, consequently changing the C: N ratio and moving away from the optimal ratio of 10:13:1 in the stabilized residue (48).

ISSN: 2455-6939

Volume: 11, Issue: 02 "March-April 2025"

When analyzing the effluent pH, we observed that sodium monensin provided a slight drop (Table 3) but did not remove it from the ideal range between 7 and 8.5 pointed out by (49) and (50), characterizing it in terms of its basic pH as a soil pH corrector.

5. CONCLUSION

The addition of sodium monensin reduces nutrient removal efficiency, compromises biogas production, and reduces nutrient concentrations in biofertilizers. Both EO and E did not affect the anaerobic biodigestion process. In addition, no associative effect was observed between the tested additives during the anaerobic biodigestion process.

REFERENCES

[1]. IPCC, 2014: Climate Change 2014: Mitigation of Climate Change. Contribution of Working Group III to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change [Edenhofer, O., R. Pichs-Madruga, Y. Sokona, E. Farahani, S. Kadner, K. Seyboth, A. Adler, I. Baum, S. Brunner, P. Eickemeier, B. Kriemann, J. Savolainen, S. Schlömer, C. von Stechow, T. Zwickel and J.C. Minx (eds.)]. Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA.

[2]. Külling, D. R.; Dohme, F.; Menzi, H.; Sutter, F.; Lischer, P.; Kreuzer, M. (2002). Methane emissions of differently fed dairy cows and corresponding methane and nitrogen emissions from their manure during storage. Envinomental Monitoring and Assessment, v. 79, p. 129 - 150, https://doi.org/10.1023/a:1020248700255.

[3]. Hindrinchsen, I. K.; Wettstein, H. R.; Machmuller, A.; Kreuser, M. (2006). Methane emission, nutrient degradation and nitrogen turnover in dairy cows and their slurry at different milk production scenarios with and without concentrate supplementation. Agriculture, Ecosystems & Environment, v. 113, n. 1/4, p. 150-161, https://doi.org/10.1016/j.agee.2005.09.004.

[4]. Côte, C.; Masse, D. I.; Quessy, S. (2006). Reduction of indicator and pathogenic microorganisms by psychrophilic anaerobic digestion in swine slurries. Bioresource Technology, v. 97, n. 1, p. 686-691, https://doi.org/10.1016/j.biortech.2005.03.024.

[5]. Alvarez, R.; Lidén, G. (2008). Semi-continuous co-digestion of solid slaughterhouse waste, manure, and fruit and vegetable waste. Renewable Energy, v.33, n.2, p.726-734, https://doi.org/10.1016/j.renene.2007.05.001.

[6]. Ahring, B. K.; Ibrahim, A. A.; Mladenovska, Z. (2001). Effect of temperature increase from 55 to 65°C on performance and microbial population dynamics of an anaerobic reactor treating cattle manure. Water Research, v. 35, n. 10, p. 2246-2452, https://doi.org/10.1016/S0043-1354(00)00526-1.

[7]. Amon, T.; Amon, B., Kryvoruchko, V.; Zollitsch, W.; Mayer, K.; Gruber, L. (2007). Biogas production from maize and dairy cattle manure – Influence of biomass composition on the methane yield. Agriculture, Ecosystems and Environment, v. 118, p. 173–182, https://doi.org/10.1016/j.agee.2006.05.007.

[8]. Jarret, G.; Cerisuelo, A. L.; Peu, P.; Martinez, J.; Dourmad, J. (2011). Impact of pigs diets with different fibre contents on the composition of excreta and their gaseous emissions and anaerobic digestion. Agriculture, Ecosystems & Environment, v. 160, p. 51-58, https://doi.org/10.1016/j.agee.2011.05.029.

[9]. Spears, J. W. (1990). Ionophores and nutrient digestion and absorption in ruminants. The Journal of Nutrition, v. 120, p. 632-638, https://doi.org/10.1093/jn/120.6.632.

[10]. Yang, W. Z.; Benchaar, C.; Ametaj, B. N.; Chaves, A. V.; He, M. L. Mcallister, T. A. (2007). Effects of garlic and juniper berry essential oils on ruminal fermentation and on the site and extent of digestion in lactating cows. Journal Dairy Science, Champaign, v. 90, p. 5671 – 5681, https://doi.org/10.3168/jds.2007-0369

[11]. Gemeda, B. S., Hassen, A. (2015). Methane production of two roughage and total mixed ration as influenced by cellulase and xylanase enzyme addition. Scientia Agricola. v.72, (1):11-19 https://doi.org/10.6084/m9.figshare.14305456.

[12]. Hassen, A. et al. (2023). Role of Exogenous Enzymes in Feed Digestibility and Reducing the Emission Intensity of Enteric Methane Production in Ruminants. In: Salem, A.Z.M., Hassen, A., Anele, U.Y. (eds) Exogenous Enzymes as Feed Additives in Ruminants. Springer, Cham. 77-102, https://doi.org/10.1007/978-3-031-27993-5_5.

[13]. Judkins, M. B.; Stobart, R. H. (1988). Influence of two levels of enzyme preparation on ruminal fermentation, particulate and fluid passage and cell wall digestion in whether lambs consuming either a 10% or 25% grain diet. Journal of Animal Science, v. 66, p. 1010-1015, https://doi.org/10.2527/jas1988.6641010x.

[14]. Beauchemin, K. A.; Rode, L. M.; Maekawa, M.; Morgavi, D. P.; Kampen, R. (2000) Evaluation of a Nonstarch Polysaccharidase Feed Enzyme in Dairy Cow Diets. Journal of Dairy Science, v. 83, n. 3, p. 543–553, https://doi.org/10.3168/jds.S0022-0302(00)74914-9.

[15]. Cruywagen, C.W.; Goosen, L. Effect of an exogenous fibrolytic enzyme on growth rate, feed intake and feed conversion ratio in growing lambs. (2004). South African Journal of Animal Science, v. 34 (6) (supplement 2), 71-73.

[16]. Metcalf, L.; Eddy, H. Wastewater engineering: Treatment and resource recovery. 5.ed. New York: McGraw-Hill, 2014, 2048 p.

[17]. Perna Junior F., Nogueira R.G.S., Carvalho R.F., Tseu R.J., Rodrigues P.H.M. 2024. Effects of Acacia mearnsii Tannins on greenhouse gas emissions from cattle manure: a anaerobic

ISSN: 2455-6939

Volume: 11, Issue: 02 "March-April 2025"

digestion study. London Journal of Research In Science: Natural and Formal, 24(5), 63–73, S631-S631.5.

[18]. Lucas Junior, J. (1994). Algumas Considerações sobre o uso do estrume de suínos como substrato para três sistemas de biodigestores anaeróbios. 1994. 137f. Tese (Livre Docência Construções Rurais) - Faculdade de Ciências Agrárias e Veterinárias, Universidade Estadual Paulista, Jaboticabal.

[19]. Kaminski, M.; Kartanowics, R.; Jastrzebski, D.; Kaminski, M. M. (2003). Determination of carbon monoxide, methane and carbon dioxide in refinery hydrogen gases and air by gas chromatography. Journal of Chromatography, v. 989, p. 277-283, https://doi.org/10.1016/S0021-9673(03)00032-3.

[20]. Kafle, G.K., Chen, L., 2016. Comparison on batch anaerobic digestion of five different livestock manures and prediction of biochemical methane potential (BMP) using different statistical models. Waste Management 48, 492-502. https://doi.org/10.1016/j.wasman.2015.10.021.

[21]. AOAC. Association Of Official Analytical Chemists. Official methods of analysis. 15 ed., Washington, DC, USA, 1990.

[22]. AOAC. Association Of Official Analytical Chemists. Official methods of analysis. 16 ed.,Virginia, USA, 1995.

[23]. APHA – American Public Health Association. Standard Methods For the Examination of Water and Wastewater, 22ª ed. Washington, DC, USA, 2012.

[24]. Van Soest, P. J.; Robertson, J. B.; Lewis, B. A. (1991). Methods for dietary fiber, neutral detergent fiber, and nonstarch polysscharides in relation to animal nutrition. Journal of Dairy Science, v. 74, p. 3583, https://doi.org/10.3168/jds.S0022-0302(91)78551-2.

[25]. SAS Institute Inc.. Statistical Analysis System – SAS [cd-rom]. Cary: SAS Institute; 2013.

[26]. Orrico Junior, M. A. P.; Orrico, A. C. A.; Lucas Junior, J.; Sampaio, A. A. M.; Fernandes, A. R. M.; Oliveira, E. A. (2012). Biodigestão anaeróbia dos dejetos da bovinocultura de corte: influência do período, do genótipo e da dieta. Revista Brasileira de Zootecinia, v. 41, p. 1533-1538, https://doi.org/10.1590/S1516-35982012000600030.

[27]. McGuffey, R. K.; Richardson, L. F.; Wilkinson, J. I. D. (2001). Ionophores for dairy cattle: current status and future outlook. Journal of Dairy Science, v. 84, suppl. E, p. 194-203, https://doi.org/10.3168/jds.S0022-0302(01)70218-4.

[28]. Busquet, M.; Calsamiglia, S.; Ferret, A.; Cardozo, P.W.; Kamel, C. (2005). Effects of cinnamaldehyde and garlic oil on rumen micro- bial fermentation in a dual flow continuous culture. Journal of Dairy Science, v. 88, p. 2508–2516, https://doi.org/10.3168/jds.S0022-0302(05)72928-3.

[29]. Busquet, M.; Calsamiglia, S.; Ferret, A.; Carro, M.D.; Kamel, C. (2005). Effect of garlic oil and four of its compounds on rumen microbial fermentation. Journal of Dairy Science, v. 88, p. 4393–4404, https://doi.org/10.3168/jds.S0022-0302(05)73126-X.

[30]. Busquet, M.; Calsamiglia, S.; Ferret, A.; Kamel, C. (2005). Screening for the effects of natural plant extracts and secondary plant metabolites on rumen microbial fermentation in continuous culture. Animal Feed Science and Technology, v. 123/124, p. 597–613, https://doi.org/10.2527/2005.83112572x.

[31]. Calsamiglia, S.; Busquet, M.; Cardozo, P.W.; Castillejos, L.; Ferret, A. (2007). Invited Review: Essential oils as modifiers of rumen microbial fermentation. Journal of Dairy Science, v. 90, n. 6, p. 2580-2595, https://doi.org/10.3168/jds.2006-644.

[32]. Mateos, I.; Ranilla, M.J.; Tejido, M.L.; Saro, C.; Kamel, C.; Carro, M.D. (2013). The influence of diet type (dairy versus intensive fattening) on the effectiveness of garlic oil and cinnamaldehyde to manipulate in vitro ruminal fermentation and methane production. Animal Production Science, v. 53, p. 299–307, https://doi.org/10.1071/AN12167.

[33]. Blanch, M.; Carro, M.D.; Ranilla, M.J.; Viso, A.; Vazquez-Añón, M.; Bach, A. (2016). Influence of a mixture of cinnamaldehyde and garlic oil on rumen fermentation, feeding behavior and performance of lactating dairy cows. Animal Feed Science and Technology, v. 219, p. 313-323, https://doi.org/10.1016/j.anifeedsci.2016.07.002.

[34]. Brooker, J.D.; Acamovic, T. (2005). Phytochemicals in livestock production systems. Animal Feed Science and Technology, v. 121, n.1/2, p. 1-4.

[35]. Gallardo, I., Bárcena, R., Pinos-Rodríguez, J. M., Cobos, M., Carreón, L., & Ortega, M. E. (2010). Influence of exogenous fibrolytic enzymes on in vitro and in sacco degradation of forages for ruminants. Italian Journal of Animal Science, 9(1). https://doi.org/10.4081/ijas.2010.e8

[36]. Tricarico, J.M.; Abney, M.D.; Galyean, M.L.; Rivera, J.D.; Hanson, K.C.; Mcleod, K.R.; Harmon, D.L. (2007). Effects of a dietary Aspergillus oryzae extract containing alpha-amylase activity on performance and carcass characteristics of finishing beef cattle. Journal of Animal Science, v. 145, p. 802–811. https://doi.org/10.2527/jas.2006-427.

[37]. Tseu R.J., Paucar L.L.C., Perna Junior F., Carvalho R.F., Nogueira R.G.S., Cassiano E.C.O., Vasquez D.C.Z., Solórzano L.A.R., Rodrigues P.H.M. 2022. Effect of exogenous enzymes on feed digestion and anaerobic digestion of Holstein cow feces. Iranian Journal of Applied Animal Science (2022) 12(2), 223-236.

[38]. Nogueira, R. G. S.; Perna Júnior, F.; Tseu, R. J.; Rodrigues, P. H. M. (2023). Dietary effects of cottonseed and vitamin E on greenhouse gas emissions from cattle feces analyzed in biodigesters. Pesq. agropec. bras. 58, https://doi.org/10.1590/S1678-3921.pab2023.v58.03037

ISSN: 2455-6939

Volume: 11, Issue: 02 "March-April 2025"

[39]. Kinyua, M. N. Rowse, L. E. Ergas, S. J. 2016. Review of small-scale tubular anaerobic digesters treating livestock waste in the developing world, Renewable and Sustainable Energy Reviews, 58, p.896-910, https://doi.org/10.1016/j.rser.2015.12.324.

[40]. Chernicharo, C. A. DE L. Princípios do tratamento biológico de águas residuárias: Reatores anaeróbios. 2ª edição. Editora UFMG. Belo Horizonte, MG. 2007, p. 379, https://doi.org/10.1590/S1413-41522007000300001.

[41]. Sgorlon, J. G.; Rizk, M. C.; Bergamasco, R.; Tavares, C. R. G. (2011). Avaliação da DQO e da relação C/N obtidas no tratamento anaeróbio de resíduos fruti-hortículas. Acta Scientiarum. Technology, v. 33, p. 421-424, https://doi.org/10.4025/actascitechnol.v33i4.8259.

[42]. Coldebella, A.; Souza, S. N. M.; Souza, J.; Koheler. A.C. Viabilidade da cogeração de energia elétrica com biogás da bovinocultura de leite. Congresso Internacional sobre Geração Distribuída e Energia no Meio Rural – AGRENER GD 2006. Campinas, 2006.

[43]. Tseu R. J., Perna Junior F., Carvalho R. F., Sene G. A., Peres A. H., Tropaldi C. B., Dos Anjos F., Rodrigues P. H. M. (2021). Gas emission from waste of cows fed monensin and Acacia mearnsii Tannins. Iranian Journal of Applied Animal Science, 11(3), 443-455.

[44]. Davison, K. L. (1984). Monensin absorption and metabolism in calves and chickens. Journal of Agriculture and Food Chemistry, v. 32, p. 1273-1277, https://doi.org/10.1021/jf00126a016.

[45]. Herberg, R.; Manthey, J.; Richardson, L.; Cooley, C.; Donoho, A. (1978) Excretion and tissue distribution of [14C] monensin in cattle. Journal of Agriculture and Food Chemistry, v. 26, n. 5, p. 1087-1090, https://doi.org/10.1021/jf60219a004.

[46]. Ribeiro, G. M.; Sampaio, A. A. M.; Fernandes, A. R. M.; Henrique, W.; SUGOHARA, A.; Amorin, A. C. (2007). Effect of the protein source and physical processing of the concentrate on the finishing of feedlot young bulls and environmental impact of dejections. Revista Brasileira de Zootecnia, Viçosa, MG, v.36, n.6, p.2082-2091, https://doi.org/10.1590/S1516-35982007000900019.

[47]. Molinuevo-Salces, B.; Gómez X.; Morán, A.; García-González, M. C. (2013). Anaerobic co-digestion of livestock and vegetable processing wastes: Fibre degradation and digestate stability. Waste Management, v. 33, p. 1332-1338, https://doi.org/10.1016/j.wasman.2013.02.021.

[48]. Mbuligwe, S. E.; Kassenga, G. R. (2004). Feasibility and strategies for anaerobic digestion of solid waste for energy production in Dar es Salaam city, Tanzania. Resources, Conservation and Recycling, v. 42, n. 2, p. 183-203, https://doi.org/10.1016/j.resconrec.2004.02.010.

ISSN: 2455-6939

Volume: 11, Issue: 02 "March-April 2025"

[49]. Mshandete, A.; Bjornosson, L.; Kivaisi, A. K.; Rubindamayugi, M. S. T.; Mattiasson, B. (2006). Effect of particle size on biogas yield from sisal fibre waste. Renewable Energy, v. 31, p. 2385-2392, https://doi.org/10.1016/j.renene.2005.10.015.

[50]. Rabiu, A.; Yaakub, H.; Liang, J. B.; Samsudin, A. A. (2014). Increasing biogas production of rumen fluid using cattle manure collected at different time as a substrate. Journal of Agriculture and Veterinary Science, v. 7, p. 44-47, e-ISSN: 2319-2380, p-ISSN: 2319-2372.

