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Biogas production using co-digestion of Chicken droppings with *Ipomoea perfurea* grass

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Abstract

The depletion of fossil fuel reserves, energy crisis, industrialization, rapid growth in population and environmental issues across the globe have aroused interest and attention to be shifted to biofuels (biogas) production. This research focused on biogas production using co-digestion of Chicken droppings with Ipomoea perfurea grass through Simplex Centroid Design (S.C.D). The proximate compositions of the formulated substrates were determined on co-substrates A (Ipomoea perfurea), B (Chicken droppings), and A+B (equal mixture of Ipomoea perfurea and Chicken droppings). The volatility contents were 47.10, 52.60 and 55.80%, while moisture contents were 18.10, 16.01, and 14.20% and carbon contents were 29.26, 26.08, and 24.04% for formulations A, B, and A+B, respectively. The biogas production temperature (mesophilic), ranging between 26°C to 31°C, was observed after 42 days. The optimum yields for dried substrates were 334g/kg for mono substrates (B) and 357g/kg for co-substrates (A+B), while the optimum yield for fresh substrates was 410g/kg for mono-substrate A and 430.5g/kg for co-substrate A+B, respectively. The methane flammability test confirmed the biogas was combustible, and a pale bluish flame that burnt for 1minutes and 45seconds without soot was observed. The least and optimum C/N ratios for dried mono and fresh co-digested substrates were 14:1 and 21:1, respectively. The fresh co-digested substrates produced a better yield than the dried co-digested substrates in the production of biogas for cooking.

Keywords: Biogas, Mono-digestion, Co-digestion, Mesophilic temperature, Substrate.

INTRODUCTION

Energy is an essential factor and also an ingredient that stimulates, supports economic growth and development (Dar *et al.*, 2021). Global energy demand is fast growing with a rapid increase in population, industrialization, which accounts for about 88% of current fossil fuel demand (Marbaix *et al.*, 2021). Biomass residues available from agricultural products and other allied derivatives constitute a potential source for bioenergy production (Saleem, 2022).

Currently, there exist numerous forms of bioenergy such as bioethanol, biodiesel, bio-methanol, bio-hydrogen, di-methylfuran (D.M.F), and biogas (Sindhu *et al.*, 2019). Biogas remains a versatile mixture of gases, a sustainable source from anaerobic digestion of organic waste, such as animal waste (chicken droppings), plants/vegetation (grass), municipal waste and butchery remaining due to bioactivities of anaerobic microbes such as *Clostridium spp,* bacteroids and *Aspergillus spp* in the absence of oxygen (Godbole *et al.*, 2023). Anaerobic breakdown remains a natural process in which complex biomass resources are broken down to smaller monomers. This is widely employed as a replenish energy source that produces appreciable methane, carbon dioxide, and other constituents in traces (Hussain *et al.*, 2020). The breakdown process begins with biomass hydrolysis as the involved material breaks down biological polymer linkages to monomers such as sugars, which makes them available for microorganisms to utilize (Chukwuma *et al.*, 2021). The Acidogenic bacteria degrade and convert the multipart polymeric compounds like sugar and amino acids into carbon dioxide, hydrogen, ammonia and organic acids, where the acetogenesis bacteria convert the resultant organic acid into acetic acid, which is collected with additional ammonia, hydrogen and carbon dioxide. The methanogenic bacteria therefore, degrade and converted the product into methane and carbon dioxide (Enzmann *et al.*, 2018).

According to Liew *et al.* (2022), animal waste materials such as cow dung and Chicken droppings contain more readily digestible

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materials than other agricultural waste materials in biogas production. As such, studying various substrates before formulation for anaerobic digestion is commendable. Likewise, Wang *et al*. (2022) opined that co-digestion of poultry dung with added biological material recompenses abundant biogas yield, stocking of readily decomposable materials, better-quality and stability of nutrients in terms of C/N ratio, and decreases ammonia formation in anaerobic breakdown processes. This leads to weakening of toxic substances with superior quality gas and price decline due to the ability to process dissimilar substrates in a single set-up, owing to a higher concentration of lignin and lignocellulose bio-fibres of about 40 – 50 % total solids depending on substrates which increase a promising mixing atmosphere in anaerobic digesters (Elsayed *et al*., 2022).

METHODOLOGY

Sample Collection and Processing

Chicken droppings were acquired from NAPRI (A.B.U Zaria) in a clean polyethylene bag, sorted, and dried immediately to get rid of moisture. *Ipomoea perpurea* grass was obtained near a pond behind post graduate schools and the school of accounting at Ahmadu Bello University, Zaria. The grass identified at the Biological Science Department (Botany unit)herbarium A.B.U Zaria and a voucher number A.B.U V.N: 1635 was obtained and deposited. Size reduction was carried out to smaller particles of about 10mm using a cutter, part of the substrate was dried for 2weeks and crushed to powder using a mortar and pestle prior to proximate analysis, while part was used as a fresh substrate for the production. Mixing formulation was employed in designing the experiment as shown in Table 1, using Minitab software version 17 (Alfa *et al*., 2014).

Table 1: Design of Experiment

Substrate ID	Standard order	Run order	Grass	Chicken Droppings	Percentage (%)	Ratios
A	1	1	8,300	0	100	1:0
B	2	2	0	8300	100	0:1
A+B	3	3	4150	4150	50:50	1:1

Mixing formulation using (Mini tab software, Version 17) (Wall, 2015)
Key Note: A= Chicken droppings, B= *Ipomoea purpurea,* A+B= Chicken dropping+*Ipomoea purpurea*

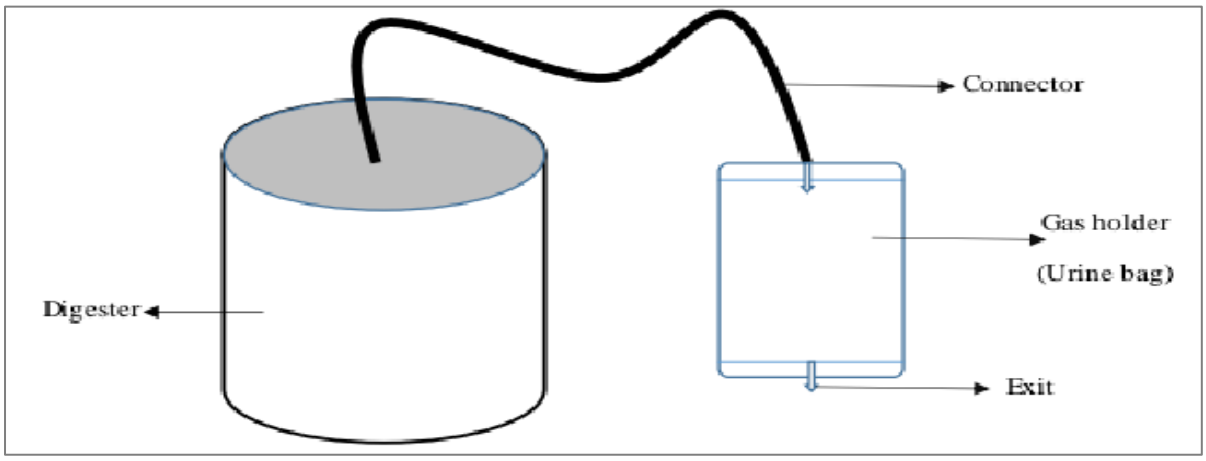


Fig 1. Schematic Experimental Design Set-up
Source: Oyewole *et al*. (2014)

Slurry Preparation and Digester Feeding

The percentage ratio of the chicken droppings and *Ipomoea perfurea* grass in grams. Eight thousand three hundred grams of chicken droppings were weighed using a digital weighing balance and dissolved in a water bowl to form a slurry in (1:2). The slurry was filled into an anaerobic digester (5L gallon), and the anaerobic digester was sealed using 4-minute sealant gum to maintain an air-tight condition for suitable metabolic activities. The same procedure was repeated for other respective formulations. The biogas was eventually collected using a transparent graduated urine bag container as a collector (Oyewole, 2010).

Culture Media Preparation

The culture media used include Nutrient broth, Nutrient Agar (NA), Sabouraud dextrose agar (SDA), Potato dextrose Agar (PDA), Eosin Methylene Blue Agar (EMB), Centrimide Agar & Mannitol Salt Agar were prepared according to the manufacturer's instructions.

Isolation of Bacteria and Fungi

One gram (1g) of the samples (A, B and A+B) were weighed and dissolved in 9mls of sterilized distilled water and allow for 10minutes and these were labeled as 10^{-1} followed by transfer of 1ml from10^{-1} to the next as 10^{-2}, 10^{-3}, up to 10^{-4} respectively (Aliyu, 2019). One milliliter (1 mL) from each of 10-4 was transferred into the petri plates, followed by NA and PDA for total bacterial and fungal counts. This was then followed by autoclaving at 30 ± 2 °C and at room temperature for 24 hours and 5 days (Aliyu, 2019).

The 10^{-4} dilution factor was spread using sterile bent glass onto nutrient agar and PDA plates for the enumeration and isolation of bacteria and fungi. The nutrient agar plates and PDA were incubated anaerobically at 37 °C for 24 hours and 5 days, respectively, inside an anaerobic candle jar (Adebayo, 2019). The bacteria and fungi that grew on different media were subcultured on respective nutrient agar and PDA plates. Bacterial and fungal counts were performed by placing the agar plate on a digital colony counter and counting (Oyewole, 2010; Islam *et al.*, 2019).

Gram Staining and Biochemical tests

Gram staining was done according to the standard method described by Osatogbe *et al.* (2024), while the biochemical profile includes;

Citrate Utilization Test

Using a straight wire loop, the tested colonies were emulsified in Kosser's citrate media and incubated at 37 °C for 24 hours. A blue colour with growth indicates a positive result; no change in colour indicates a negative result (Osatogbe *et al.*, 2024).

Indole Test

The tested organism was inoculated into peptone water and incubated at 37 °C for 24 hours. The indole reagent was added. Pink ring colour indicated a positive result. The absence of a pink ring on the surface indicated a negative result (Osatogbe *et al.*, 2024).

Motility Test

The isolate was inoculated into motility medium by a fine stab with a sterile needle to a depth of 2 cm length of the bottom of the tube. It was then incubated at 37 °C for 24 hours. The line of inoculation was defined, and the medium was cleared for non-motile organisms, while the line of inoculation that was not defined for motile organisms was somewhat cloudy (Osatogbe *et al.*, 2024).

Flame test for methane presence in biogas production

Methane, a principal constituent of biogas with flammable characteristics, was tested by igniting a flame on a Bunsen burner connected to the digester, where a bluish flame was obtained and lasted for 1 minute 45 seconds (Fatima et al., 2018).

pH and Temperature Measurements

This was carried out using a pH meter. The pH meter was inserted into the slurry, and the reading was recorded after stability. Likewise, the Temperature measurement was carried out using a digital thermometer. The thermometer was inserted into the slurry, and the reading was recorded after the slurry had reached stability (Akintayo *et al.*, 2020).

Determination of Moisture Content

This was carried out using the gravimetric method described by Demarchi (2013). A measured weight (2g) of samples of A, B, and A+B was weighed into a moisture can and labeled. The samples were dried in an oven at 105 °C for 3 hours. Cooled in a desiccator and reweighed, and returned to the oven for further drying, cooling, and weighing repeatedly was carried out at an hourly interval until when there were no further diminutions in the weight that is constant weight was obtained. The weight of moisture was calculated and expressed as a percentage of the weight of the sample analyzed, given by the expression:

$$\text{M. C} = \frac{\text{M3}-\text{M1}}{M2-M1} X\ 100 \quad \text{.....Eq. 1}$$

Where,

m1= weight of empty moisture Can

m2= weight of empty can + sample before drying

m3= weight of can + sample dried to constant weight

Determination of the Total Solids (T.S)

The total solids (T.S.) content was determined according to Vaishnavi (2023) using a standard method. A sample of 2g was taken into a pre-weighted silica crucible and dried at a temperature of 105 °C for one hour (1 hr). The silica crucible containing the sample was placed in an oven at a temperature of 105 °C for six hours (6 hours). Afterwards, the crucible was placed in the desiccator until its temperature reduced to room temperature, and then it was weighed. Finally, the % Total solid(T.S) was determined using the relation as the quantity of the sample remaining in the crucible:

Where,

M_2 = mass of crucible including sample and

M_4 = is the mass of crucible plus residue after heating @ temperature of 550 °C.

Determination of the Volatile Solid Content (V.S)

The percentage volatile content was determined by ignition of the dried samples in an inert atmosphere at 550 °C. After Volatile Solids (V.S) determination, the silica crucible was placed into a muffle furnace at a temperature of 550 °C for 2 hours, where the loss of mass on heating was the measure of Volatile substance (V.S), which was determined using the relation below:

$$V.S = \frac{M4-M1}{M3-M1} X\ 100 \text{..... Eq. 3}$$

Where,

M1 = mass of empty crucible

M_3 = mass of crucible plus residue after heating in an oven @ 105 °C,

M_4 = mass of crucible plus residue after heating @ temperature of 550 °C (Vaishnavi, 2023).

Determination of the Volatile Solid Content (V.S)

The percentage volatile content was determined by ignition of the dried samples in an inert atmosphere at 550 °C. After Volatile Solids (V.S) determination, the silica crucible was placed into a muffle furnace at a temperature of 550 °C for 2hrs, where the loss of mass on heating was the measure of Volatile substance (V.S), which was determined using the relation below:

$$A.C = \frac{M2-M3}{M2} X\ 100 \text{.....Eq. 4}$$

Where, M2 = Initial value, M3 = final value (Vaishnavi, 2023).

Determination of Carbon Content

This was determined using the method of James *et al.* (2017). Zero-point one gram (0.1g) of samples A, B, and A+B were weighed using an analytical balance. The samples were then introduced into their respective 250ml conical flasks. Next, 10ml of 0.167 mol/L K2Cr2O7 was measured using a measuring cylinder, followed by 20ml of concentrated H2SO4. H_2SO_4 was added to the respective samples (Through the surface of the sample container), and dark green coloration was seen after the addition. This was allowed to stand for 30minutes and cool, after cooling, 100 mL of distilled water was added to the respective dark green coloration mixture and additional 5 drops of Ferroin indicator was added to solution above and this was titrated against 0.5mols of iron 2 sulphate $FeSO_4$ until greenish colour was noticed, the % carbon content wasdetermined by the relation:

$$\%C = \frac{B-T \text{ x } M \text{ x } 1.33x0.003}{wt\ of\ sample} X\ 100 \text{..... Eq. 5}$$

Where

B = blank sample

T= titre value

M= molarity of Iron sulphate

Determination of Total Nitrogen Content

Zero-point one gram (0.1g) of the respective substrates A, B, and A+B was weighed and introduced into 100 mL of their respective conical flasks. Twenty milliliters (20ml) of Distilled water and 20ml of sulfuric acid were added to the respective substrates one at a time. A few copper catalyst particles were added to the substrates, then heated with the aid of a hot plate until it fumed, indicating the presence of Nitrogen. Filter paper was used to filter the mixture from the conical flask to the volumetric flask. A dark green to light yellow mixture was observed in the volumetric flask. Ten milliliters (10 mL) of the filtrate were introduced into a round-bottom flask, and 10 mL of 40% NaOH was added. An additional 10 mL of

2% boric acid was measured and introduced into a separate beaker, along with a few drops of mixed indicator. Then 0.01 Normal HCl was used to titrate against the distilled sample. The condenser was kept cool below 30 °C by allowing sufficient water to flow through, regulating heat, and minimizing frothing. Then, 50ml of the distillate was collected and distilled before stopping.

Then, % N content was determined by the relation:

$$\%N = \frac{0.14 \text{ x V.D x N x } 100\text{xT.V}}{wt\ of\ soil\ x\ Ad} \text{ ..... Eq. 6}$$

Where

V.D = volume of digest

N = Normality of Acid

TV = Titre value

Al = Aliquot of digest James *et al.* (2017).

Determination of composition of the biogas using Gas Analyser Non Diffusive Infra-red (NDIR)

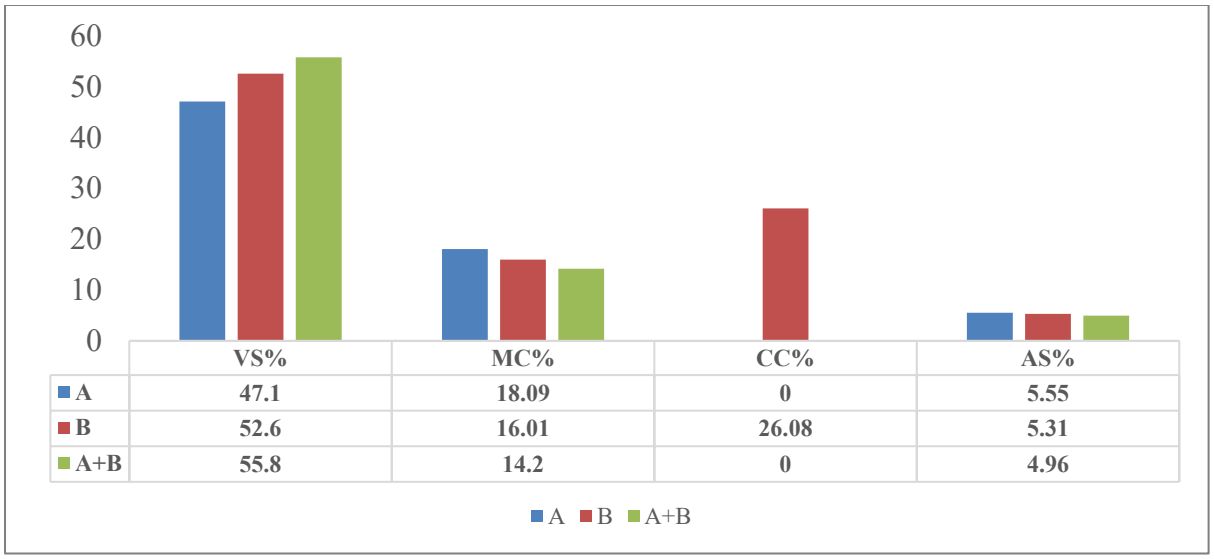
The gas analyser was calibrated using manufacturers instructions. All tubing was ensured to be very tight, and none was leaking. Direct sampling was employed, and the gas source was connected directly to the analyser. The gas system was flushed and purged to avoid contamination from previous samples. The instrument was turned "ON" and allowed to warm up. The samples were labelled and introduced into the analyser for analysis, and stability was allowed to be attained before recording the results of the respective samples. The values for all respective components analysed were recorded (Mezex *et al.*, 2017).

Data Analysis

The data obtained were analyzed using descriptive statistics, where mean values, experimental design, and mixing ratio formulations were determined with the application of Minitab software version 17 (Sachin *et al.*, 2023).

RESULTS

Figure 1 shows proximate composition results prior to the introduction of fresh/dried mono and co-digested substrates into the digester. The results revealed the potential of volatile organic matter for biogas production in relation to mono/ co-substrates, at 47.10%, 52.60%, and 56.80% for substrates A, B & A+B, respectively. The degree of volatility of the substrates was found to be higher with respect to the co-digested substrate A+B when mixed in an equal ratio than with mono substrates A and B.



	VS%	MC%	CC%	AS%
A	47.1	18.09	0	5.55
B	52.6	16.01	26.08	5.31
A+B	55.8	14.2	0	4.96

Figure 1:MeanProximate Analysis for Fresh *Ipomoea Perfurea* Grass and Chicken Dropping
Key: VS= Volatile solids, MC= Moisture content, CC= Carbon content, AS= Ash content

From Figure 1, the potentiality in terms of solubility content revealed 18.09, 16.01 and 14.20% for substrates A, B & A+B respectively, this shows that the magnitude of solubility was a bit higher in carbon-based substrates (A) than Nitrogen based substrates (B), and likewise when mixed on same proportion (A+B) the potentiality was found to be the least, but reverse might be the case on different proportion.

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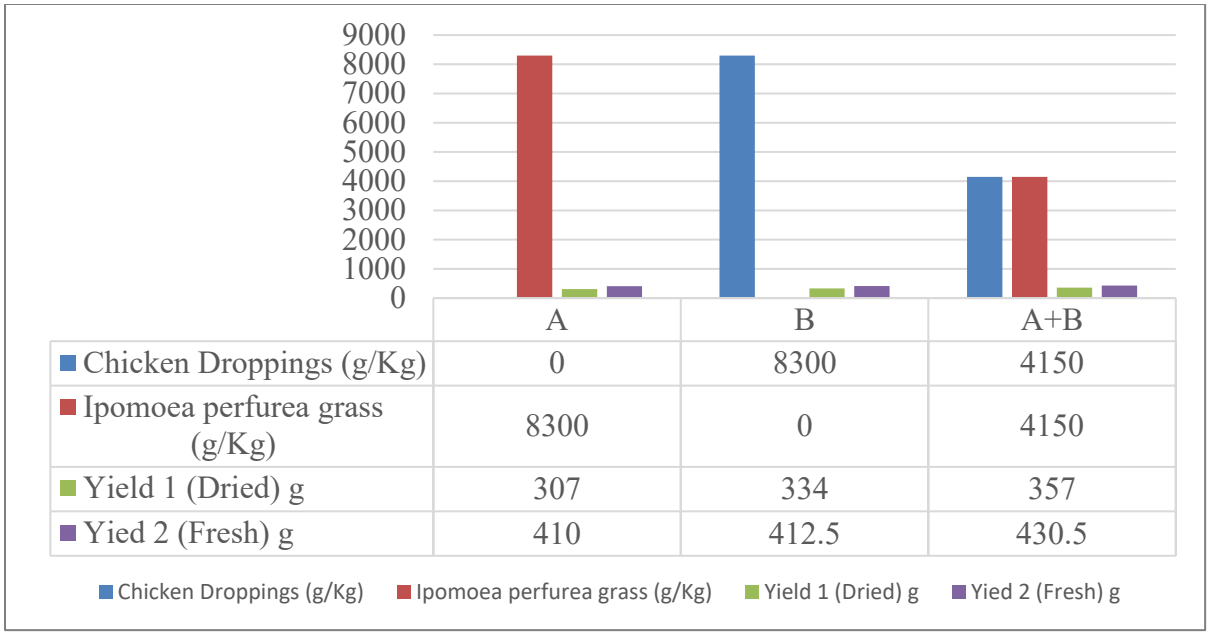
However, also from Figure 1, the potentiality in terms of carbon content was found to be 29.26 26.08 and 25.04% % for substrates A, B & A+B respectively, this shows that substrate A was found to be rich (high) in carbon as a source of energy to the microbes than B and likewise A+B was found least when mixed in equal proportion.

Although ash content determines the reusability of the ash for other purposes after digestion, such as biofertilizer production. The result revealed a high content of ash with respect to mono substrates A (5.55%), B (5.31%), but least in co-digested A+B (4.96) substrates,Figure 2 shows the biogas yield for dried and fresh *Ipomoea perfurea* grass as single substrates and with Chicken droppings as co-substrates. The results indicated that biogas production began from the first day across all substrates that consisted of substrate A (100%, 1:0) *Ipomoea perfurea* grass; B (100%, 0:1) Chicken dropping, A+B (50%:50%, 1:1) combination of the 2 substrates in equal proportion. The results also revealed that anaerobic microorganisms, such as Clostridium spp., bacteroids, and Aspergillus spp., were relatively active, and biodegradation began from the first day after introducing the substrates into the digester. This may be attributed to the nature of the substrate, the magnitude of the volatility of organic matter, and the synergetic effect that resulted in a carbon-nitrogen balance.



	A	B	A+B
Chicken Droppings (g/Kg)	0	8300	4150
Ipomoea perfurea grass (g/Kg)	8300	0	4150
Yield 1 (Dried) g	307	334	357
Yied 2 (Fresh) g	410	412.5	430.5

Figure 2: Mean Biogas yield from the Co-digestion of Chicken dropping and *Ipomoea perfurea* grass

Flammability Test

Confirmatory flame tests (Plate 3 and 4) show methane at the hydrolysis and methanogenesis stages. At the hydrolysis stage, immediately after opening the biogas collector and igniting the flame, the flame was extinguished, confirming a higher presence of CO2 at that stage than methane, as well as hydrogen, hydrogen sulfide, and traces of other gases. While at the methanogenesis stage (plate 4), immediately after opening the biogas collector and igniting the flame, a bluish flame was observed that lasted for 1 minute. 45 seconds confirming methane gas.

Table 3 presents the mean and standard deviation of bacterial and fungal populations (expressed as $\times 10^4$ colony-forming units, CFU) in three sample categories. Sample A, Sample B, and a combination of both (A + B). The results indicate notable differences in microbial load across the samples.

Table 4 shows the compositional profile of biogas production. The results revealed higher methane content with respect to fresh mono- and co-digested substrates than dried mono- and co-substrates, at 57, 56, and 60.5% for fresh mono/co-substrates A, B, and A+B, respectively, while 50, 49.5, and 51% for dried mono/co substrates, respectively. This indicates that the methane content was found to be higher in both fresh mono/co-formulated substrates and in dried substrates.

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However, the methane content determined the magnitude of flammability and extent of combustion from 41- 45% according to Ofoefule *et al*. (2010) who reported 'Biogas production from blends of Bambara nut Chaffs with some plants and animal waste'. This may be attributed to the nature of the substrates, their formulation, and the proportional volatility of the substrates, as well as the efficiency of microbial activity that led to effective digestion. Additionally, synergy between carbon and nitrogen contributed to a reasonable methane content in the biogas.

Plate 1: Biogas production in progress

Plate 2: Biogas in a urine bag collector


Plate3: Flammability test at Hydrolysis stage


Plate 4: Blue Flame at Methanogenesis stage

Table 3: Mean and Standard Deviation for Bacteria and Fungi Counts

Sample ID	Bacteria Count (10^4)	Fungi count (10^4)
A	107 ± 3.0	65.5 ±2.5
B	152 ± 4.0	44.5 ± 3.5
A + B	390 ± 2.0	49.5 ±3.5

Table 4: Compositional Analysis from the Co-digestion of Chicken Droppings and *Ipomoea perfurea* Grass

Parameters	**CO%**		**Methane (CH_4) %**		**CarbonDioxide (CO_2)%**		**Oxygen (O_2) %**		**Hydrogen (H_2) %**	
Subs. I.D	Dried	Fresh	Dried	Fresh	Dried	Fresh	Dried	Fresh	Dried	Fresh
A: *Ipomoea perfurea*										
	0	**0**	**51**	**56**	**22**	**21.5**	**15.5**	**9.5**	**10**	**11.5**
B: Chicken Droppings										
	0	**0**	**49.5**	**57**	**23**	**20.5**	**13.5**	**9.0**	**12.5**	**11.5**
A+B	**0**	**0**	**50**	**60.5**	**22.5**	**21**	**15**	**5.5**	**10.5**	**11.5**

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DISCUSSION

The volatile solids (VS) increased in the 1:1 mixture in this study is consistent with the report of Li et al. (2013), who observed better volatile solid degradation during the co-digestion of kitchen waste, maize stover, and chicken manure. According to Romero-Guiza *et al.* (2014), solid-state digestion benefits from lower moisture, which bolsters the advantages of the low moisture found in the 1:1 combination observed in this study. This study's carbon content results are similar to those of Samadi *et al.* (2024), who found that co-digestion optimized microbial digestion by balancing the high C/N ratio of lignocellulosic biomass with the low C/N ratio of poultry manure.The advantages of co-digestion were further supported by Okewale and Babayemi (2019) who similarly demonstrated that poultry droppings enhanced the anaerobic digestion performance of elephant grass. However, the low ash percentage observed in the 1:1 mixture corresponds with the report of Hasan et al. (2023), who highlight the positive correlation between decreased ash content and improved volatile matter and energy output. Likewise, the strong methane potential for co-digestion in this investigation was suggested by other studies, which found methane outputs ranging from 300 to 1200 mL/g volatile solids, depending on the substrate balance and synergy (Li *et al.*, 2015; Hassan *et al.*, 2023).According to studies, co-digestion techniques frequently shorten lag phases and increase microbial hydrolytic activity. The study's rapid commencement of biogas production across all treatments (A, B, and A+B) is consistent with this finding (Owamah et al., 2014; Zhang et al., 2020). For example, Zhang et al. (2020) reported effective lignocellulose breakdown in co-digestion systems, which they partially attributed to fungal activity. These findings are consistent with yours. With the A+B treatment, a synergistic effect is likely associated with improved C/N ratios. Co-digestion frequently improves nutrient balance and increases biogas yields above those of mono-digestion, a finding highly confirmed by various studies (Hassan *et al.*, 2020). The findings in this study with respect to yield shows an improved biodegradation and microbial activity from day one which are supported by other studies that revealed 20–24% higher bio-methane outputs and synergistic effects in the same C/N range when grass and manure are mixed (meadow grass + cattle dung) (Song *et al.*, 2023). It has been observed that Aspergillus and Penicillium species play important roles in the breakdown of lignocellulose during co-digestion, which helps produce biogas much earlier (Owamah et al., 2014). The rapid decline in volatile solids and the commencement of gas production in this investigation were likely due to their enzymatic activity.

The biogas ignition test reveals a visible flame that signifies successful combustion and verifies the presence of enough methane in the generated gas. To maintain ignition and a steady flame, methane combustion typically requires a minimum concentration of approximately 40% CH_4 in the gas mixture (Cellek *et al.*, 2024). Furthermore, stable combustion indicates that harmful amounts of inhibitory gases, like ammonia or hydrogen sulfide, were absent, which is consistent with other studies showing that well-balanced anaerobic digestion processes produce cleaner, flammable gas (Bastiaans, 2023). The observed flame likely results from a methane-rich mixture produced by the effective co-digestion of the substrates in this study. The flammability test observed in this study was in conformity with the work of Aliyu (2019), who reported 'Biogas production from cow dung for sustainable energy generation. The results were also in agreement with the work of Ajiboye et al. (2018), who reported the evaluation of the effect of sodium solution on biogas yield for the anaerobic digestion of poultry waste and digestate.

Sample A had a bacterial count of 107×10^4 CFU and a fungal count of 65.5×10^4 CFU, suggesting a relatively high level of fungal presence compared to the bacterial population. In contrast, Sample B exhibited a higher bacterial count of 152×10^4 CFU but a significantly lower fungal count of 44.5×10^4 CFU. This inverse pattern in microbial dominance suggests varying environmental or compositional conditions influencing microbial proliferation in the two sample types, as also reported in previous studies (Smith & Jones, 2020). Interestingly, the combined sample (A + B) showed a marked increase in bacterial count (390×10^4 CFU), which exceeds the sum of the individual means for Samples A and B. This may imply synergistic microbial interactions or changes in substrate conditions that promote bacterial growth when the two samples are combined (Kumar *et al.*, 2021). However, the fungal count in the combined sample (49.5×10^4 CFU) falls between the values observed in Samples A and B, indicating a possible stabilizing effect or competition between fungi and bacteria in the combined environment.

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The methane content in this study was higher than that reported by Ofoefule et al. (2010), who reported Biogas production from blends of Bambara nut Chaffs from plants and animals, which indicated the magnitude of flammability and extent of methane combustion at 41-45%. However, this may be attributed to the nature of the substrates, their formulation, and the proportional volatility of the substrates, as well as the efficiency of microbial activity that led to effective digestion, and, of course, synergy between carbon and nitrogen, which contributed to a reasonable methane content in the biogas. The profiles were in agreement with the work of Natthawud *et al*. (2017) who reported 'Biotechnological application of sustainable Biogas production through dry anaerobic digestion of Napier grass'. Likewise, dried mono- and co-substrates had a higher content in terms of carbon dioxide, oxygen, and hydrogen than fresh mono- and co-substrates.

The result obtained revealed a bit higher content of CO_2in dried *Ipomoea perfurea* grass both for mono and co-substrates ranging from 22 – 23%, but a bit lower in fresh co and mono-substrates ranging from 20.5 - 21.5 %, though excess CO_2 may serve as agent that hinders compression of biogas into the cylinder and the impurities inhibits methanogenic microorganism from conversion of materials for bioenergy production. However, the profile further revealed a lower oxygen content in fresh substrates, showing a more promising combustion range of 5.5–9.5% compared to dried substrates, which ranged from 13.5% to 15.5%.This was commendable because the oxygen content might be detrimental to the entire anaerobic digestion process. This indicates that methanogenic microorganisms are highly sensitive to oxygen and cannot thrive in oxygenated conditions, which consequently inhibits methane production. The results obtained were in agreement with the work of Pedizzi et al. (2016), who reported the Effect of oxygen on the microbial activities of thermophilic anaerobic biomass. Perhaps H_2 may serve as an important intermediate product in anaerobic digestion processes, aiding stability to ignition and combustion, and also acting as a reducing agent for the conversion of carbon dioxide into methane. The result revealed an H2 content of 11.5% for fresh substrates, but slightly higher in dried substrates, ranging from 10 to 12.5% accordingly. The result agreed with the work of Gawel et al. (2022), who reported hydrogenation from sour cabbage through dark fermentation.

CONCLUSION

Based on the study, the proximate analysis profile revealed the proximate compositions of substrates A, B, and A+B with respect to their volatility, moisture, ash, and carbon content. Co-substrates A+B had the best volatility content, carbon content, and the least ash content compared to the mono-substrate. In contrast, mono-substrates had higher solubility compared to co-substrates. However, varying the proportion of the substrates may alter the trend. The best biogas yields were A+B (357g/kg) and A+B (430.5g/kg) for dried and fresh substrates, respectively. The methane flammability test confirmed that the biogas was combustible and burned with a pale, bluish flame, producing no soot, for 1 minute and 45 seconds. The fresh / dried co and mono substrates revealed the same synergy with a Carbon-Nitrogen (C/N) ratio of 21:1, 15:1, and 14:1 for A+B, A, and B, respectively. The Optimum methane content for fresh substrates in the study was 60.5%, whereas dried substrates had an average of 51%.

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