



EVALUATING THE EFFICIENCY OF TERMITE-MEDIATED DEGRADATION OF SUGARCANE BAGASSE, MAIZE COBS, AND COCONUT HUSKS FOR BIOGAS PRODUCTION

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Bassey, I. E., Babatola, J. O., Ojo, O. M., Onagbola, E. O., Ekom, R. I. (2026): Evaluating the Efficiency of Termite-Mediated Degradation of Sugarcane Bagasse, Maize Cobs, and Coconut Husks for Biogas Production. FUTA Journal of Engineering and Engineering Technology 20(2), 50-67

Received Date: 23.02.2026

Accepted Date: 03.07.2026

Abstract

The escalating global energy demand and environmental concerns associated with fossil fuels have intensified the search for sustainable alternatives. Agro-solid waste offers a large volume of lignocellulosic material, a sustainable, underutilised resource material to develop a wide portfolio of renewable energy products. However, their complex lignocellulosic structure often limits microbial accessibility with the existing standard anaerobic digestion processes, necessitating the search for sustainable alternatives to circumvent this bottleneck. This study aims to evaluate the efficiency of termite-mediated degradation of selected lignocellulosic agro-wastes for biogas production using in-situ termireactors. Twelve in-situ termireactors, fabricated with polycarbonate material, were inserted into active termite colonies to mimic their natural habitat. Sugarcane bagasse, maize cobs, and coconut husks were the selected cellulose sources due to their abundance, complementary chemical profiles, and the waste management challenges they present in tropical and subtropical regions. The termites' termitaries were fed selected feedstocks, and the emitted gas was collected at mesophilic temperatures. Feed consumption increased steadily for all substrates, with maize cobs showing the highest consumption, at 0.63 kg on day 13 and 0.84kg on day 30, due to its favourable lignocellulosic structure. Daily biogas production increased with retention time, highlighting the impact of feedstock composition. Analysis of produced gas by Gas Chromatograph Mass Spectrometry shows maize cobs as the most efficient substrate, yielding 66.3% methane against 61.81 and 57.81% from sugarcane bagasse and coconut husks, respectively.

Keywords: Substrates, Lignin, Biogas, Termigradation, Agro Solid Waste.

Introduction

Agro Solid Wastes (ASW) and their management are critical issues, the world over, especially in Nigeria and other developing economies. Annually, huge amount of solid waste is generated, which increasingly pose severe challenges to environmental safety, public health and human welfare.

The increasing environmental and health concerns created by this condition make the recycling of wastes and residues from agricultural and industrial sectors very relevant.

Growing concerns over environmental health have made the recycling of agricultural and industrial waste more urgent than ever. Most of these residues

are natural building blocks that many believe are the future of sustainable, non-food resources. These building blocks are found in the form of tough microfibrils, highly prized for their remarkable biodegradability, biocompatibility, and ability to be constantly renewed in nature (Nunes *et al.*, 2020). Because of these versatile traits, researchers have successfully extracted cellulose from various waste streams to create everything from paper and textiles to automotive components and sustainable packaging (Husin *et al.*, 2019).

The need to recycle agricultural and industrial residues is driven by a global push for better environmental and health standards. These wastes largely represent a vast, untapped source of non-

food materials with potential to unlock a significant energy mix needed for sustainable and environmentally-friendly development (Nunes *et al.*, 2020). Today, cellulose extraction is a cornerstone of the circular economy, powering diverse industries ranging from building and textiles to food science and high-tech packaging (Husin *et al.*, 2019).

Cellulose extraction methods including chemical (Chopra *et al.*, 2022), biological (Sharma *et al.*, 2019), hydrothermal (Trisanti *et al.*, 2021), and microwave (MW)-assisted methods (Zhu *et al.*, 2023; Sun *et al.*, 2006), all target the degradation of hemicelluloses and lignin from biomass feedstocks. To truly address environmental concerns, the methods used to extract cellulose must be as sustainable as the agricultural materials themselves. Aligning our extraction processes with green standards ensures that the entire lifecycle of these residues remains eco-friendly.

It's been severally reported (Husin *et al.*, 2019, Chopra *et al.*, 2022, Sharma *et al.*, 2019, Trisanti *et al.*, 2021, Zhu *et al.*, 2023, Sun *et al.*, 2006) that lignocellulose comprises a significant portion of agro-solid waste at about 40-70%, including sugarcane bagasse, wood, maize shaft/cobs, cotton fibres as well as coconut husks. Cellulose, the main biodegradable plant polymer, is often shielded from optimal degradation by lignin, as well as hemicellulose (Nunes *et al.*, 2020, Rahimi, 2019 and Akinmusere *et al.*, 2019). While microbes can easily turn cellulose and hemicellulose into methane and carbon dioxide, lignin is far more stubborn. Its tough structure resists breaking down in the oxygen-free environment of a landfill, which also shields other organic materials from being processed. Overcoming this 'lignin barrier' is a major hurdle for landfill operators aiming to boost renewable energy and free up valuable space (Rahimi, 2019), which often requires expensive chemical or thermal pretreatments. There is a critical research gap in developing biologically-inspired reactor designs that can achieve high biomethane yields without energy-intensive external processing.

Following this, therefore, the main aim of this study is to evaluate the efficiency of termite-mediated degradation of selected lignocellulosic agro-wastes for biomethane production using in-situ termireactors.

As Rahimi (2019) notes, removing this barrier allows microbes to convert cellulose and hemicellulose into biogas, potentially doubling or even tripling a landfill's methane output. This biological efficiency is mirrored in nature by termites and as highlighted by Blissbern *et al.* (2023) termites naturally generate methane as they process

cellulose, offering a low-cost pathway for energy production.

Utilizing these biological processes could be a game-changer for energy access in Africa, where "energy poverty" remains a significant hurdle (Akinmusere *et al.*, 2019). Many in developing nations still rely on wood for basic needs due to a lack of awareness regarding renewable alternatives (Babatola, 2008). Transitioning to low-carbon technologies is essential, but there is a growing global consensus that a diverse energy mix is the most realistic way to hit climate targets (Zhu *et al.*, 2023).

One of the most promising areas of research involves the termites themselves. These insects, specifically the wood and plant-feeding varieties like Isoptera, have spent millions of years perfecting the breakdown of tough woody biomass (Dar *et al.*, 2025). Their guts host a complex "micro-factory" of symbionts capable of degrading lignocellulose with ease. By bioprospecting for the robust enzymes found in these tiny ecosystems, we may find the key to making biofuel production truly cost-effective (Sero *et al.*, 2025). Research into termite-aided waste digestion by Rahimi (2019), Abassi and Gajalakshmi (2015), Akinmusere, (2019), Ojo and Babatola, (2019) primarily focused on the in-vitro application of termite gut microorganisms to enhance anaerobic digestion (AD) rather than in-situ reactor systems within active mounds. These studies highlight the fabrication of in-situ chamber inserted into the termite mound to mimic the environmental conditions of the termite's natural habitat and hence evoke optimal degradation activities.

This study is poised to evaluate the efficiency of termite-mediated degradation of sugarcane bagasse, maize cobs, and coconut husks for biogas production using in-situ termireactors and the prospects of harnessing the associated methane gas for global warming control and clean energy generation. Exploring methane-trapping potential in the ASW management system could make methane elimination from the atmosphere a particularly effective method of combating global warming. Therefore, it is contended that recovery projects that generate methane for clean energy fuel have reasonable prospects for combating the effects of global climate change. The clear hypothesis here is that the introduction of termite-mediated digestion will significantly enhance the degradation of lignocellulosic agricultural wastes (sugarcane bagasse, maize cob, and coconut husk), thereby increasing the overall biogas and methane yield compared to the traditional AD process. This study aims to evaluate the efficiency of termite-mediated degradation of selected lignocellulosic agro-wastes for biogas production using *in-situ* termireactors.

Materials and Methods

The materials used for this research study included some selected agro-solid waste, identified termite species, constructed Plexiglas cages, gas bags with appropriate conduit connections as well as measuring devices – gas rotameter flow meters and thermo-hygrometer probes.

Experimental Location: The experiment was carried out in the Federal University of Technology, Akure (FUTA) located in southwestern Nigeria, at latitudes 7° 17' - 7° 19' and longitudes 5° 7' – 5° 9'

Choice and selection of relevant substrate

Three constituents of agro-solid waste (ASW) were collected – sugarcane bagasse, pieces of coconut husk, and maize cob from the piles of agro-solid waste generated at residential and agricultural areas of the Federal University of Technology, Akure (FUTA). These components of ASW have been reported to emit significant volumes of CH₄ from the process of termigradation (Abbasi and Gajalakshmi, 2015; Xie *et al.*, 2024; Fathima *et al.*, 2025; Saberi *et al.*, 2024).

Termite collection and identification

Several termite mounds were identified by field survey at FUTA, located in the rainforest region of Nigeria where the subterranean mound-building termite species are very abundant and their destructive activities are widely documented in the literature (Sandoval-Cobo *et al.*, 2020; Karthikeyan *et al.*, 2014). The focus of collection was on the soldier and the worker castes from the identified nests, using the direct collection method. At the identified nests, a direct hit with a shovel scooper was executed, and a sizable portion of the nest opened, and then allowed to close up. The soldier termites were attracted out by the opening to protect the nest from the intrusion. In about 30 – 40 minutes, the worker termites grouped out to rebuild and seal the opened portion of the nest; then they were picked up by aspirators and forceps into 70% ethanol in Petri dishes, labelled with dates and the collector's information, and then transported to the laboratory for identification. At the Department of Biology, the Federal University of Technology, Akure, the sample specimens from the four termitaria were identified as subterranean *Macrotermes subhyalinus* Rambur.

The active population of the termite's colony, consuming an average of 30,850 mg of substrate per day, was estimated using the Biomass-to-Consumption Proxy (Nanda, 2022).

This method validates the physical counts by linking termite presence to the actual work they are doing. The procedure for this estimate involves to

- Measure the dry weight loss of the substrate during the sample period.

- Subtract the predicted microbial degradation (based on the control reactor).

- Apply the Standard Foraging Constant (e.g., an average *Macrotermes* worker consumes approx. 0.22 mg of lignocellulose per day). Using the Equation (1):

Estimated Population

$$= \frac{\text{Daily weight loss (mg) attributed to termite digestion}}{\text{Average consumption per termite (0.8 mg/day)}}$$

$$\text{Estimated Population} = \frac{31,850 \text{ mg}}{0.22} \quad (1)$$

$$\text{Estimated Population} = 140,227 \text{ individuals}$$

Physical and Chemical Characterisation of the Substrate.

2.4. 1. Pre-Treatment of the Substrates

Maize cob, sugarcane bagasse, and coconut husks were sun-dried for three days to remove moisture from them and thereafter oven-dried at 110°C for 8 hours. They were then pulverised and sifted into small particles of size < 2 mm.

The characterisation of the biomass feedstocks is essential for the suitability and assessment of the quality of methane gas that is potentially produced by the termites on consumption of each feedstock.

To determine if a waste material is a good candidate for chemical processing, we first need to understand its core properties. Key indicators such as water content, thermal behaviour (proximate analysis), and elemental makeup (ultimate analysis) tell us how volatile a material is and how much energy it holds. Among these, elemental analysis is particularly vital, as it directly determines the feedstock's heating value. To ensure accuracy, we analysed our samples following the standard instrumental procedures typically used for coal and coke.

Physical Characterisation of the Substrate -

Proximate Analysis

The proximate composition analysis of the studied waste materials was carried out to classify the feedstocks in terms of their moisture content (MC), volatile matter (solids) (VS), fixed carbon (FC), and ash content. This is necessary to evaluate the suitability of the feedstock for the purposes of the research.

Determination of Moisture Content of Feedstocks

Moisture content is often defined as the quantity of water per unit mass of the dry solid. The wet basis water content was used in this work, which is the

ratio of the total mass of moisture in the original sample to the original wet mass of the sample.

10 g of the pre-treated samples were weighed in clean petri dishes and then placed in an oven at 110°C. The different weights of the samples were taken and recorded at every 10 minutes until respective constant weights were recorded as final weights, and the moisture content was then determined by using Equation (2):

$$\% \text{ Moisture content} = \frac{(\text{initial weight} - \text{final weight})}{(\text{initial weight})} \times 100 \quad (2)$$

Determination of Volatile Solid

To determine the volatile solids components of the selected samples, 5 g each was weighed initially in clean evaporating petri dishes and placed in a muffle furnace at 550°C for 1 hour. They were then allowed to cool down in a desiccator and reweighed again. The loss in weight is now the volatile matter present in the samples using Equation (3).

$$\% \text{ Volatile Matter} = \frac{\text{Initial weight of wet sample} + \text{weight after heating} - \text{Final weight after heating} + \text{crucible} - \text{initial weight}}{\text{Initial weight of wet sample} + \text{weight after heating} - \text{Final weight after heating} + \text{crucible} - \text{initial weight}} \times 100 \quad (3)$$

Ash Content

The ash contents of the selected samples were determined by combustion of the fixed carbon. The resulting ash fraction is not representative of the original ash due to the oxidation process employed in its determination.

So, moisture + vapors + fixed carbon + ash = 100 m%.

Sample Analysis

Each of the three selected samples was pulverised coarsely on a clean surface by hand, using rubber gloves. 25 to 50 g of the sample was placed in a prepared evaporating dish and weighed. The weighed sample was placed in an oven at 103 to 105°C overnight. Cooling was done to balance the temperature in a desiccator and was then weighed. Drying was repeated for one hour. Cooling, weighing, and desiccating steps were repeated until the weight change was less than 4%. At least 10% of all samples were analysed in duplicate, and determinations agreed within 5% of their average weight as shown in equations (4) and (5).

Calculations

$$\text{Volatile solid (\%)} = \frac{(A-D)}{A-B} \times 100 \quad (4)$$

$$\text{Fixed Solid (\%)} = \frac{(D-B)}{A-B} \times 100 \quad (5)$$

where:

A is weight of dried residue + dish, (mg)

B is weight of dish, (mg)

C is weight of wet sample + dish, (mg)

D is weight of residue + dish after ignition, (mg).

Ultimate Analysis

The ultimate analysis generally reports the elemental carbon (C), hydrogen (H), nitrogen (N), sulphur (S) composition, and oxygen (O), very often by difference in the feedstock. Identification of the termites was done to ascertain that they are actually soil-feeding termites.

Proximate and ultimate analysis of the three feedstocks, which are sugarcane bagasse, maize cob, and coconut husk, was done for the quantitative analysis.

Chemical Composition of the Substrate

The compositions of the selected substrates – sugarcane bagasse, coconut husk, and maize cob were analysed using the procedures described by Mensah *et al.* (2022). 1g each of the selected samples was boiled in 60 mL of acetone at 90 °C for 2 h. Afterwards, the fibre samples were separated, washed with deionised water, and dried at 110 °C. The obtained samples were weighed, and then the percentage (by weight) of contaminants inside the samples was calculated using the following Equation (6):

$$\% \text{ Contaminant} = \frac{W_A - W_B}{100} \quad (6)$$

The compositions of the selected samples, i.e., hemicelluloses, lignin, and cellulose, were then determined after removing the contaminants.

Determination of hemicelluloses

To determine the hemicellulose content, 1 g of each contaminant-free substrate was boiled in 150 mL of a 0.5 M NaOH solution at 80°C for three hours and 30 minutes. After boiling, the sample was separated and rinsed thoroughly with deionised water until it attained a neutral pH. The samples were then dried to a constant weight (WC), which was used to calculate the hemicellulose percentage by Equation (7).

$$\% \text{ Hemicelluloses} = \frac{W_B - W_C}{100} \quad (7)$$

Determination of lignin:

To isolate the lignin, we began by soaking 1 g (WA) of raw bagasse in 30 mL of sulfuric acid for four hours, followed by boiling at 100°C for one hour. After the heat treatment, we separated the solids and rinsed them thoroughly with deionised water until the pH reached neutral. To ensure the system was completely free of sulfate ions, we titrated the filtrate with an acid. Finally, the residue was dried at 110°C and weighed. We recorded this final mass as (the lignin weight) and calculated the total percentage using Equation (8)

$$\text{Lignin} = \frac{W_D}{100} \quad (8)$$

Determination of cellulose:

Determination of cellulose content of the substrate was achieved by subtracting the weight of the substrate from the total contaminants, hemicelluloses, and lignin. This difference was then converted into a percentage as shown in Equation (9).

$$\% \text{ Cellulose} = \frac{1-(W_A+W_C+W_D)}{100} \quad (9)$$

Design and construction of the Termireactor

Twelve termireactors were designed, constructed, and inserted into identified/existing active termite mounds to mimic the environmental conditions of the termite's natural habitat. Made of optically transparent polycarbonate material (Plexiglas), the termireactors were designed to enable optimal activities of termites in their mimic natural habitat, which are much more conspicuous above the ground surface to ensure convenience in monitoring and measuring relevant metrics of temperature, humidity, gas volume, and rate of flow. These reactors were designed to simulate the size and structural outlook of a typical termite mound, as in Plate 1.



Plate 1: Photograph of One of the Termite Mounds Used for the Study.

Experimental design for lignin degradation using Termireactors

The degradation of lignin was assessed by inserting the constructed Termireactors into an active termite's mound, as in Plate 2b. To ensure statistical reliability and capture the average behaviour of the wastes, three replicates (n=3) were established for every substrate group. This resulted in a total of 12 Termireactors operating simultaneously, including a parallel set of three reactors each containing the different substrates, placed in a temperature-

controlled environment without termite access to serve as the experimental control.

Measurement of biogas produced from termites was achieved by safely connecting the gas collector from the cages to gas bags placed on boxes provided outside the cages (Plate 2).



Plate 2: The Experimental Setup Showing the Termireactor with Gas Collection Bag, B.

Mechanical Design of the In-situ Reactor

The reactor is designed as a pyramidal cage that mimics the natural nesting structures of termites to ensure high colonisation rates. The reactor core cage allows worker termites (*Macrotermes*) to enter and consume the maize cobs, bagasse, and coconut husks while retaining the bulk substrate. Gas Collection Dome, a sealed, conical head at the top of the cuboid base, made of non-reactive plexiglass, was designed to capture the rising biogas. A perimeter seal was made as an airtight flange at the mound surface level to prevent external oxygen ingress (maintaining anaerobic conditions) and to prevent biogas leakage into the atmosphere. Monitoring Ports of two insulated tubes extending to the surface were the gas line, connected to a Tedlar gas sampling bag for effective gas flow and storage, while the sensor probe was designed to house a thermocouple and humidity sensors for monitoring the internal mound microclimate.

2.5.2 The Technical Lignin Degradation in Bioreactor Experiments

Termite samples were collected from six (6) different termite colonies within the Federal University of Technology, Akure, for identification and classification at the laboratory of the Department of Biology, Federal University of Technology, Akure. The samples were identified as Subterranean, *Macrotermes subhyalinus*.

Activities of feedstock degradation were observed in the termitaries on day 2 of the experimental run and the leftovers were weighed every 1 day again to determine the weight of substrate digested: Feed

Consumed = Final weight of feed – Initial weight of feed/1

Feeding the Termites

In a bid to create an eco-friendly environment, the predominant *Macrotermes subhyalinus* were fed within the created in-situ termitaries with three different cellulose substrates: maize shaft/cob, sugarcane bagasse, and coconut husks, which constitute a significant component of discarded agro-solid wastes.

In each of the termireactors, 500 g (fresh weight) of the substrate was placed. The equivalent dry weight of each substrate was concurrently determined by oven-drying a known mass to a constant weight at 105°C. The substrates were moistened with tap water to the extent that the contents became damp but not soggy. At the end of each day, the substrate remaining unconsumed by the termites was carefully removed from the termireactors and air-dried. Care was taken to brush off any soil particles adhering to the surface of the residue. Then, the residue was oven dried at 105°C to constant weight. It's been established that very little of what termites ingest is excreted, and the excreta are made up of organomineral aggregates, which are carried away by the worker termites to line the termite nests. (Nauer *et al.*, 2018; Fathima and Abraham, 2020). So, in this research work, the extent of substrate consumption was quantified on the basis of the remaining fraction of the substrate, as in Abbasi *et al.* (2015).

The associated biogas production volume and flow rate were monitored and recorded over thirty days in the created termireactors and the termites were fed simultaneously with these substrates for a period of 30 days in the in-situ termireactors respectively. The termites' behaviour was monitored, and the environmental conditions of temperature and humidity were adjusted by opening and closing the vents provided at the lower portions of the cages to ensure a regulated environment akin to the termites' habitat and obtain gas flow readings using the glass-tube rotameter gas flow meters affixed to the termireactors.

Determination of the optimal micro-climatic conditions for the most effective degradation of lignin-containing waste by termites in the termitaries.

The metabolic performance of these termites was increased through feeding and maintaining optimum temperature and relative humidity of the termitaries to enhance termite activities.

Temperature and humidity Readings

Temperature and humidity within the termites' laboratory colony were monitored with the use of a thermo-hygrometer probe affixed to the polycarbonate cage. The internal temperature of the cage was controlled by the provision of vents on each face of the pyramid, which were blocked to raise the temperature and unblocked to lower the temperature. The vents were opened in twos for 2 days, until all the vents were opened. During gas collection, all 8 apertures were blocked to ensure the gas released by the termites in the termitaries does not mix with exterior air from the environment

Temperature and humidity assessments

The temperature and humidity conditions of the termireactors were monitored inside the cage with the use of digital thermo-hygrometers HTC - 2 (Plate 3), products of Nanchang Huibing Electronics, China, to maintain appropriate conditions for optimal termite activities.

This device possesses digital displays of the readings and is able to record and store both the maximum and minimum temperature as well as relative humidity values over time. The digital devices were fixed permanently to the polycarbonate cages for recording the temperature and humidity conditions of the termite's environment daily throughout the research period for each of the four feed substrates.



Plate 3: Photograph of a Digital Thermo-hygrograph for Measuring Temperature and Relative Humidity

Gas collection

The gas generated by the termites' activities on the feed substrates is collected through the conduit hose attached to the cages (see Plate 2) to conduct emitted gas through the glass-tube rotameter gas flow meter to Tedlar gas bags housed in constructed boxes provided outside the termite reactors. This procedure was to facilitate measuring and recording the flow rate and volume of biogas emitted by the termites. This procedure was continued for 30 days, simultaneously for each feed substrate and the replicates.

Gas flow rate and volume measurement

The volume of gas produced from the simulated termite environment was measured with the use of a low flow glass tube rotameter flow meter model series LZM-3WB (Plate5), a product of Shangai Chikong Automation Industry Ltd. With a flow rate capacity of 10ml to 100ml/minute, this device is a low-flow glass tube fitted with a control valve which allows for the measurement of the minimum amount of gas released from the stimulated environment.

The float, a spherical indicator device, is located inside the flow tube and is engineered so its diameter is readily identical to the flow tube's inlet diameter. The technique for measuring the gas flow was accomplished by this freely moving float, finding equilibrium in a tapered glass tube. As gas flow increases, the float inside the tapered glass tube is lifted by the gas pressure, and its position on a calibrated scale indicates the flow rate (see plate 4a).

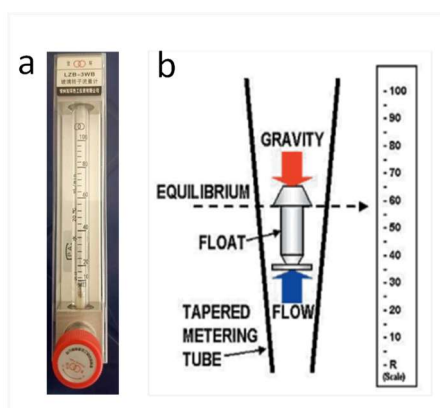


Plate 4: Photographs of (a) Glass-tube Gas Rotameter, and (b) Gas Flow meter.

The higher the flow rate, the higher the float rises, creating a larger flow area within the tube. The calibrated scale on the glass tube allows for direct reading of the flow rate of the emitted gas.

The tapered tube's gradually increasing diameter provides a related increase in the annular area around the float, and is designed in accordance with the basic Equation (10) for volumetric flow rate:

$$Q = kA\sqrt{gh} \quad (10)$$

where:

Q is the volumetric flow rate, in ml per minute

k is a constant

A is the annular area between the float and the tube wall

g is the force of gravity, h is the pressure drop (head) across the float

With h being constant in a VA meter, we have A as a direct function of flow rate Q. Thus, the rotameter is

designed to determine the tube taper so that the height of the float in the tube is a measure of flow rate and volume

Gas Analysis

The gas collected was analysed for gas composition and volumetric percentage using gas chromatography (GC). The gas sample in the headspace vial was absorbed into the carbon disulphate and placed in the jacketed headspace connected to the GC-MS. HP powered by HP ChemStation Rev. A 09 01 (1206) with FID and a capillary column, was used in this work.

The column type, length of column, internal dimension and film thickness are given, respectively, as CP-Sil SCB, 25.0m. 0.25 mm, 0.12 µm, respectively.

The temperature of the injector, the temperature of the detector, the initial oven temperature, and the rate of the final temperature are 150, 300, 35, 5, and 100°C./min. respectively. BIP plus grade helium, which is the ultimate zero-defect gas, was used as the carrier gas, which was the mobile phase that moved the solvent to the separating column. The flow rate for the carrier was 0.25ml/min, and the mode of injecting the sample into the inlet was split. As a result of its inertness, good purity, excellent performance, and well-established methodologies, helium is preferred for use as a carrier gas.

RESULTS AND DISCUSSION

The results indicated slow commencements and a gradual increase in biogas production with retention time, until attaining their peak, after which decreasing biogas production was observed across all three feedstocks. This can be attributed to microbial response to temperature and moisture requirements. This result is in agreement with the findings of Blissbern *et al.* (2023), who reported an increasing trend of biogas production from commencement and a gradual drop after day 30. Akinmusere (2019) also reported a high rate of biogas production from termigration of some food wastes with a drop towards the end of the experiment.

Physical and Chemical Properties of Feedstocks

The physical and chemical characteristics of the selected feedstocks are summarised in Table 1.

The proximate composition of the feedstocks, as shown in Table 1, reveals that the SB had a value of 2.34% moisture content, 2.41% ash content (AC), and 75.05 and 20.20% were determined for Volatile Matter (VM) and Fixed Carbon (FC), respectively. For MC, moisture content was 5.67%, AC – 1.43, VM – 75.05, and FC of 22.8%. The lignin

composition, CH, was highest at 31.22%, while MC exhibited the lowest at 21.78%.

Table 1: Proximate Analysis of the feedstocks

S/No.	Feedstocks	Moisture	Ash	Volatile	Fixed	Lignocellulosic Structure		
		Content (%)	Content (%)	Matter (%)	Carbon (%)	Cellulose (%)	Hemicellulose (%)	Lignin (%)
1	MC	5.67	1.43	75.05	22.8	39.21	17.53	21.78
2	SB	2.34	2.41	70.01	20.20	43.32	21.21	25.26
3	CH	8.13	7.86	41.68	22.33	35.22	15.21	31.22

SB is sugarcane bagasse; CH is coconut husk; and MC is maize cobs

Note: Moisture content values reflect sun-dried samples typically used in research, rather than fresh field-moist residues

The proximate composition of the feedstocks revealed that the SB had a value of 2.34% moisture content (MC), 2.41% ash content (VM), and 75.05 and 20.20% were determined for Volatile Matter (VM) and Fixed Carbon (FC), respectively. The lignocellulosic structure of sugarcane bagasse substrate was 43.32, 21.21, and 25.56 % for cellulose, hemicellulose, and lignin, respectively.

The results summarised in Table 1 indicate that moisture, ash, and volatile solids in a given ASW directly influence microbial activity and, consequently, biogas yield (Fathima *et al.*, 2025). While Fathima *et al.* (2025) reported slightly higher values for sugarcane bagasse (5.44% moisture and 1.75% ash), our findings align closely with Mensah *et al.* (2022), who recorded 5.66% moisture and 1.38% ash. However, lignin is noted to be the main recalcitrant structure of this biomass, limiting the biomethane production potential of these substrates, especially in the conventional anaerobic digestion (AD) conditions.

Physical Characterization of the Feedstock

The variation in biogas production often stems from the specific quality, quantity, and composition of the agricultural materials used in the digester. As Blissbern *et al.* (2023) observed, different agricultural wastes produce varying methane yields based on how easily they break down. For instance, garden waste typically yields less biogas than kitchen waste because it contains higher levels of lignocellulosic fibres, which are harder for microbes to digest (Blissbern *et al.*, 2023). The dip in biogas production toward the end of our experiment likely reflects the depletion of available nutrients, which slowed the organic breakdown process. To counteract such issues, Khong *et al.* (2012) suggest that "acclimatisation" - gradually adding biomass to adjust the mixture can improve microbial tolerance,

shorten the lag phase, and prevent toxic buildup. However, care must be taken with nutrient levels, as an excess of light ions can inhibit the process. Ultimately, biogas yield is a delicate balance of material quality, inoculum seeding, pH levels, and retention time.

This shows that the selected feedstocks, which are readily available agricultural solid waste, hold considerable potential as substrates for biogas production through termite-based digestion.

Impact of key operational parameters and retention time on biogas generation

Generation of biogas from the three substrates was observed from day 2 of the experimental runs for the three experimental set-ups. Temperature and relative humidity (RH) readings, as well as the emitted gas flow rate and gas volume values, were recorded on a daily basis for the 30 days.

Temperature and retention time for the different feedstocks within the bioreactor

The temperature data for sugarcane bagasse, maize cob, and coconut husk within the bioreactor over the 30-day retention period display zigzag patterns, characterised by alternating rises and falls rather than steady trends from day 2 to 12 of the experimental runs, as presented in Figure 1. This fluctuation is typical in anaerobic digestion systems, reflecting microbial metabolic activity, environmental interactions, and feedstock characteristics. For sugarcane bagasse, temperatures oscillated between a low of 27.24°C on day 6 and a high of 31.92°C on day 16. This variability suggests intermittent bursts of microbial activity, particularly during the acidogenesis and methanogenesis phases, which generate heat and are sensitive to substrate breakdown, as earlier observed by Olatunji *et al.* (2021).

The temperature pattern, though, shows fluctuation from day 2 to 11; however, it maintains a steady upward trend to day 13, from which the fluctuating pattern generally depicts a relatively flat trend ranging from 31.63 to 31.99°C. From the onset of

the process, the temperature rose slightly to 28.5°C, dropped after day 3, and then peaked again by day 5 and day 7, indicating the cyclical nature of biochemical reactions and microbial population dynamics (Mohammed *et al.*, 2024).

Maize cob also shows a zigzag pattern, ranging from 27.22°C (day 4) to 29.00°C (day 2). The irregular temperature swings could be attributed to its higher lignin content, which may slow degradation and cause less consistent microbial activity, leading to unstable heat generation. Fluctuations also reflect delayed microbial adaptation and substrate

resistance to enzymatic attack (Enaboifo and Adadu, 2020).

For coconut husk, temperature variation follows a similar zigzag trend, with values fluctuating between 29.00 (day 1) and 27.17°C (day 6). The initial high temperature likely results from rapid microbial colonisation of surface materials, while subsequent drops and rebounds reflect the slower breakdown of fibrous content and sustained microbial adjustments (Jaturapiree *et al.*, 2025). This pattern shows a general rise from 28.47 (day 9) to 31.62°C (day 15), from which a flattening pattern is recorded up to day 30.

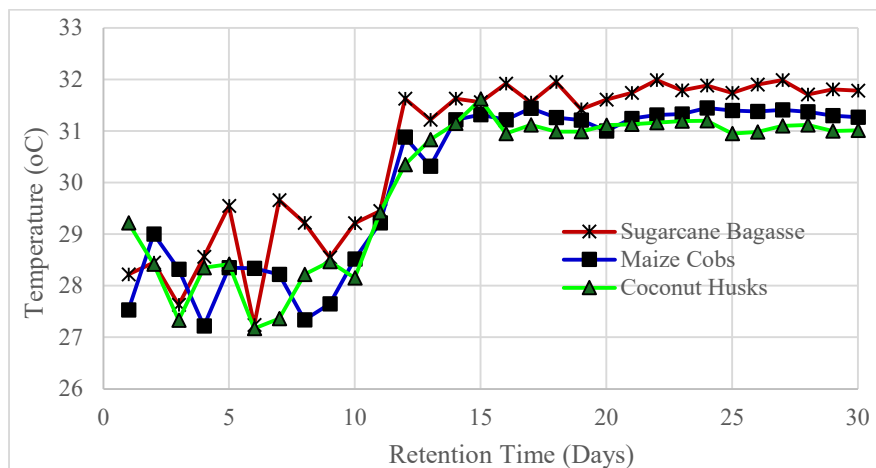


Figure 1: Temperature and retention time for the feedstocks

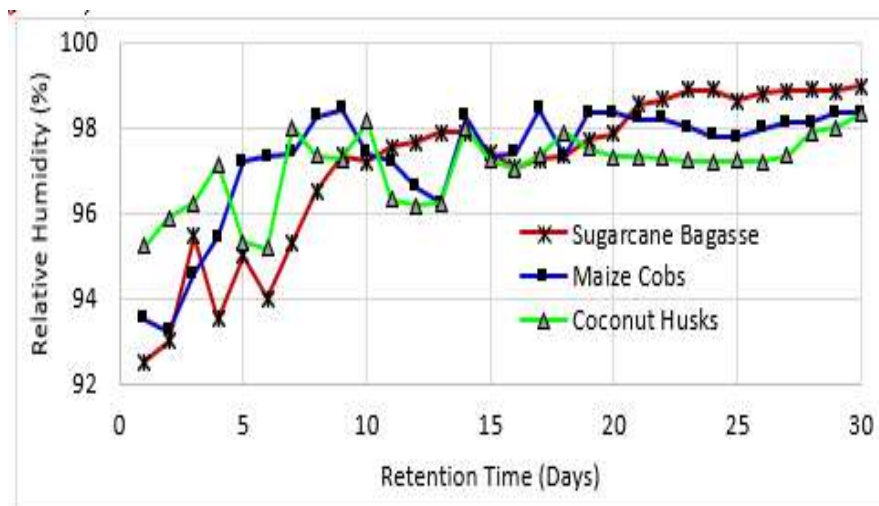


Figure 2: Relative humidity and retention time

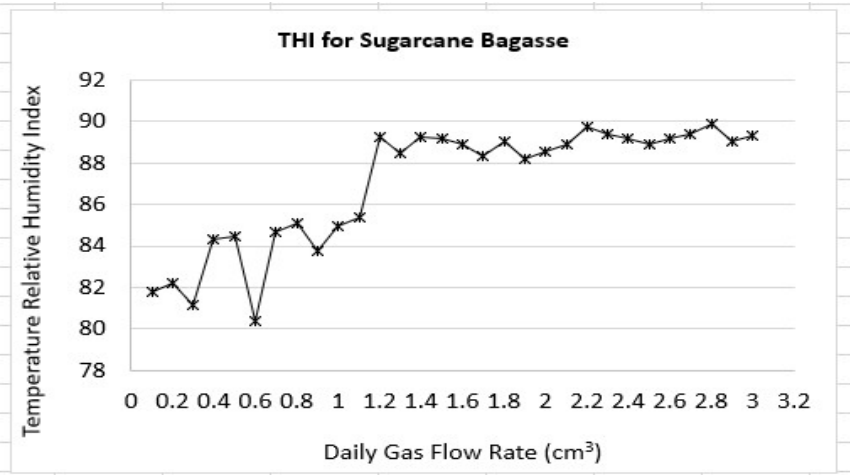


Figure. 3: THI against Gas flow rate for Sugarcane Bagasse

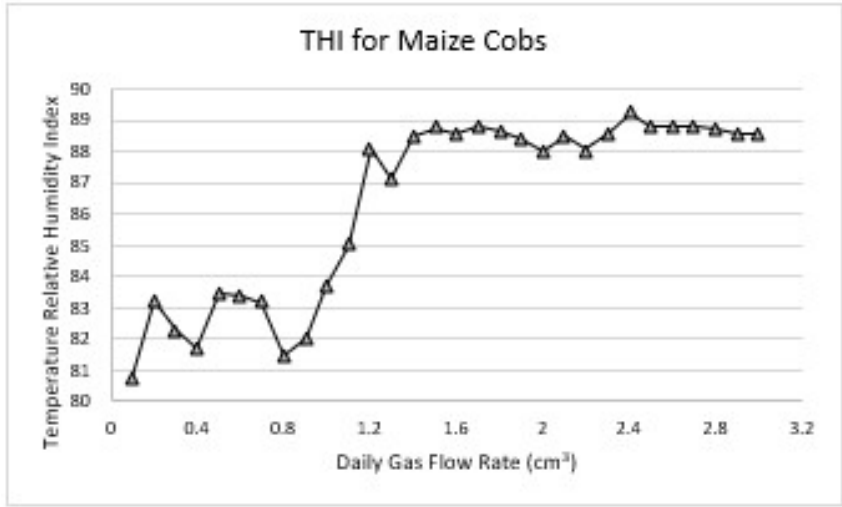


Figure 4: THI against Gas flow rate for Maize cobs

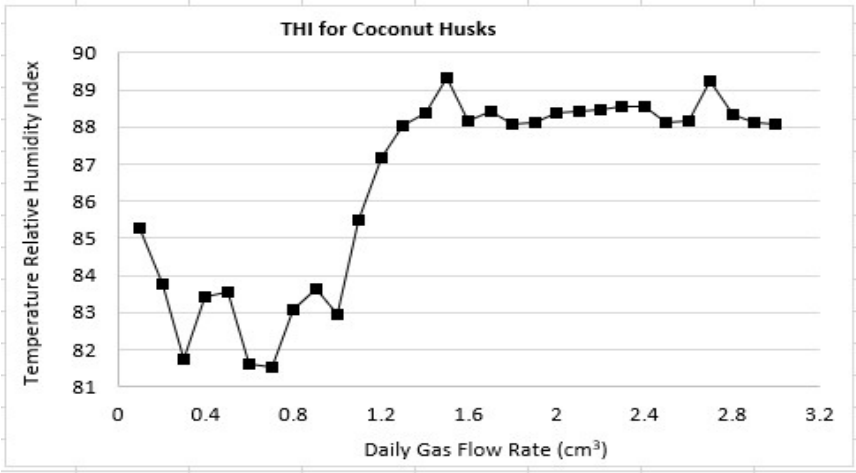


Figure 5: THI against Gas flow rate for Coconut Husks

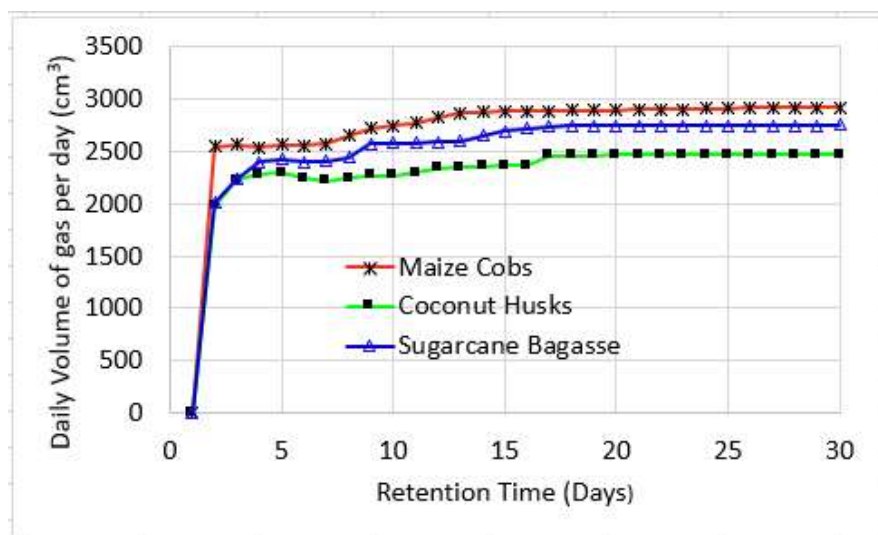


Figure 6: Feed consumption and retention time

Relative humidity and retention time

The relationship between relative humidity and retention time for the different feedstocks within the bioreactor exhibits a zig-zag pattern, where increases are generally observed over time but with intermittent fluctuations across days and feedstocks, as presented in Figure 2.

Relative humidity rises from day 1 to day 10 for all substrates, reflecting increased termite activity and moisture release during decomposition. Relative humidity for Sugarcane bagasse recorded 92.5% on day 1, fluctuating slightly between days and exhibiting an upward trend before reaching 97.2% on day 10, while maize cob and coconut husks show minor dips and rebounds along their paths to 97.41% and 98.15%, respectively. These undulating trends indicate variations in termite metabolism and substrate breakdown rates. The zig-zag behaviour was observed to correspond with biogas production trends, where gas output surges initially due to rapid hydrolysis and acidogenesis (Olatunji et al., 2021), then stabilizes differently depending on the feedstock. Sugarcane bagasse, with its high cellulose and low lignin content (Jaturapiree et al., 2025), sustains the highest biogas yield despite moderate RH, while coconut husk, with higher RH, maintains moderate gas output due to gradual fiber decomposition. Maize cob, although showing increasing RH, yields the least gas, likely due to high lignin content that limits microbial access (Enaboifo and Adadu, 2020).

Thus, the zig-zag relationship between RH and retention time underscores the fact that while RH generally supports microbial processes, the substrate's biodegradability plays a more decisive role in determining the consistency and volume of biogas produced.

Metabolic performance of termites with biogas production.

Termites have been reported to thrive best in warm, humid conditions, preferring temperatures around 25-32 °C and high humidity levels, typically 70-90%. To evoke optimal digestion behaviour from the study, therefore, the temperature-humidity index (THI) of the termite reactors was monitored, and the results are shown for the three feedstocks in Figures 3 - 5. For Sugarcane bagasse, THI values generally increased with an increase in daily gas production, ranging between 80 and 90%, as shown in Figure 3. This is consistent with the observation of Nanda et al. (2022) in their study to identify the characteristics of acoustic and temperature signals of subterranean termite infestation.

For maize cob, a similar pattern as for sugarcane bagasse was also recorded, as shown in Figure 4, confirming the favourable micro-climatic conditions of the termireactors for the termite's optimal digestion activities.

For coconut husks, the THI at the beginning of the experimental run was recorded at about 85 THI and then fluctuated between 81.5 and 83.5; however, it was within the range that allows optimal behaviour of the termites. This steadily rises and levels off between 88 and 93 at the close of the experimental observations as shown in Figure 5.

These show that the temperature-humidity index (THI) significantly impacts termite digestion and activity. Termites have been reported (Olatunji et al., 2021) to thrive in warm, humid environments, which are optimal for their gut microbial activities, enhancing cellulosic digestion of the feedstocks. High THI, indicating heat stress, can negatively affect termite activity, including feeding and digestion, potentially leading to decreased feedstock consumption and even increased mortality at

extreme temperatures. This is consistent with findings of Mensah *et al.*, (2022) that termites rely on symbiotic gut microbes to digest cellulose, the primary component of ASW. These microbes are sensitive to temperature and humidity fluctuations, and their activity can be impaired by extreme THI conditions. Studies have shown that termite wood consumption decreases under heat stress conditions, indicating a negative impact on their digestive processes (Sero *et al.*, 2025; Dar *et al.*, 2024, Xie *et al.*, 2024).

Weight of feed consumption and retention time for the different feedstocks

The relationship between weight of feed consumed and retention time for the different feedstocks within the bioreactor demonstrated a progressive increase, indicating enhanced microbial degradation with time as shown in Figure 6.

Maize cobs showed the highest average cumulative feed consumption for all the replicates, rising from 0.00 on day 1 to 0.835 kg by day 30, which aligns with its known high biodegradability due to its favourable lignocellulosic composition rich in cellulose and hemicellulose with relatively low lignin content (Jaturapiree *et al.*, 2025).

This structural composition allows for easier microbial access and enzymatic hydrolysis, leading to faster breakdown and higher conversion rates. Coconut husks, which recorded the lowest weight consumption (0.67 kg), contain more lignin, a recalcitrant compound that hinders microbial activity and slows digestion (Enaboifo & Adadu, 2020), thus explaining the slower increase over the retention period. Maize cobs recorded a moderate consumption of 0.74 kg by day 30, which is consistent with their fibrous matrix that gradually decomposes over time and sustains microbial action. The increasing weight of feed consumed across all feedstocks is consistent with findings from Olatunji *et al.* (2021), who observed that longer retention times enhance microbial colonisation and substrate breakdown in anaerobic digesters.

These results are in tune with the conclusion by Sero *et al.* (2025) that retention time is a key operational parameter influencing organic matter degradation and biogas yield. Therefore, while all feedstocks benefit from extended retention time, the rate and extent of feed consumption are largely governed by their chemical and structural composition, with sugarcane bagasse emerging as the most efficient substrate for sustained microbial digestion.

Daily biogas production and retention time for the different feedstocks

Daily biogas production increased rapidly for all three feedstocks between days 2 and 3, indicating an initial surge in termite activity (Figure 7).

This early rise is consistent with the hydrolysis and acidogenesis phases in biogas production, during which easily degradable compounds are rapidly converted by microbes (Olatunji *et al.*, 2021). Maize cobs consistently produced the highest volume of gas throughout the 30-day retention period for all the replicates, reaching an average of 2919 cm³/day by day 30.

Its high cellulose and hemicellulose content, coupled with relatively lower lignin, enhances its biodegradability and supports higher methane yields (Jaturapiree *et al.*, 2025).

Sugarcane bagasse showed relatively moderate gas production, higher than coconut husk, with a relatively stable output between days 2 and 30 for the three replicates, likely due to its fibrous composition that decomposes more gradually. Coconut husk produced the least gas overall at an average of 2,471 cm³ from the three replicates by day 30, and exhibited slight fluctuations after day 5, suggesting lower biodegradability or microbial efficiency, possibly due to higher lignin content, which resists microbial breakdown (Enaboifo and Adadu, 2020).

Overall assessment shows that biogas yield increases with retention time, but the production varies based on the feedstock type, with sugarcane bagasse demonstrating the most favorable characteristics for sustained gas production.

Cumulative biogas production and retention time for the different feedstocks

Figure 8 illustrates a steady linear increase in cumulative biogas production over the 30-day retention period, showing a progressive degradation of organic matter under the influence of the termite activities for all the feedstocks. These increases were sustained until reaching a maximum point at about 25 -27 days before decreasing linearly to zero with increasing retention time.

Maize cobs consistently yielded the highest gas volume (see Figure 8), which can be attributed to their high cellulose and hemicellulose content and lower lignin composition, making them more accessible to digestion (Jaturapiree *et al.*, 2025; Olatunji *et al.*, 2021).

Sugarcane bagasse followed closely, showing moderate biodegradability likely enhanced by its fibrous composition and potential microbial adaptation (Mohamed *et al.*, 2024), while coconut husks produced the least cumulative biogas, possibly due to its higher lignin content and structural recalcitrance (Enaboifo & Adadu, 2020).

The uniform upward trend in average cumulative biogas production across all feedstocks confirms the importance of retention time as a key factor in maximizing biogas yield, particularly for

lignocellulosic materials, which often require prolonged digestion for complete hydrolysis (Bassak *et al.*, 2025).

Differences in cumulative gas output also underscore the influence of feedstock composition and the necessity of appropriate pretreatment methods to enhance enzyme access and improve digestion efficiency (Olatunji *et al.*, 2021).

From the results, maize cobs demonstrated the most favorable characteristics for sustained biogas production, making it a promising feedstock for renewable energy generation.

It can be seen in Figure 9 that biogas production increases with retention time. The coefficient of correlation (R^2) for sugarcane bagasse, maize cob, and coconut husk shows good correlation values of 0.9958, 0.9959, and 0.9915, respectively, with the plots displaying ascending limbs since the values of

b are positive. The sugarcane bagasse has constant a and b with numerical values of 3041.6 ml/gm/day and 2719.3 ml/gm/day, respectively.

It was established from the plot that biogas production rate increases linearly with an increase in time until reaching a maximum point at about 27-30 days before decreasing linearly to zero with increasing retention time.

Assessment of Gas Quality

The quality of the obtained biogas was assessed at the gas laboratory of the Covenant University Central Instrumentation Facility (CUCIRF), Ota, Ogun State, using the Gas Chromatograph Mass Spectrometry (GCMS) which analysed the collected gas samples to obtain the specific percentage compositions of the gas generated from each of the termitaries during the experimental runs.

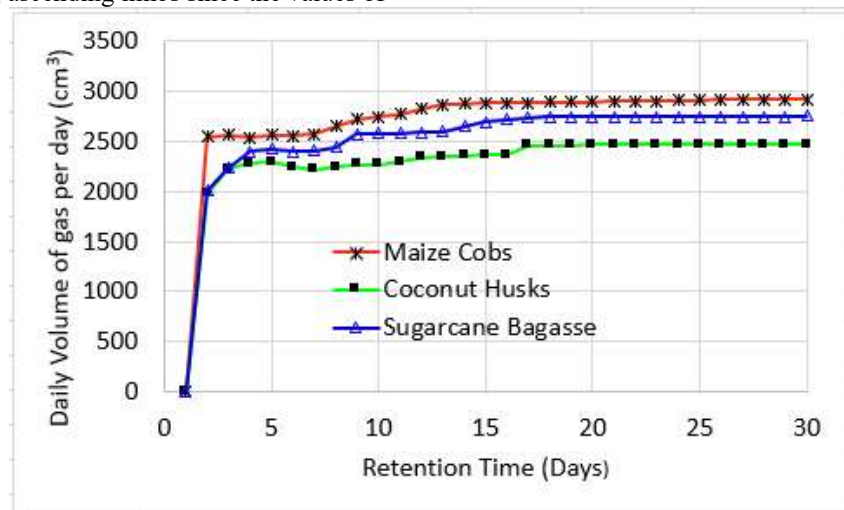


Figure 7: Daily biogas production and retention time

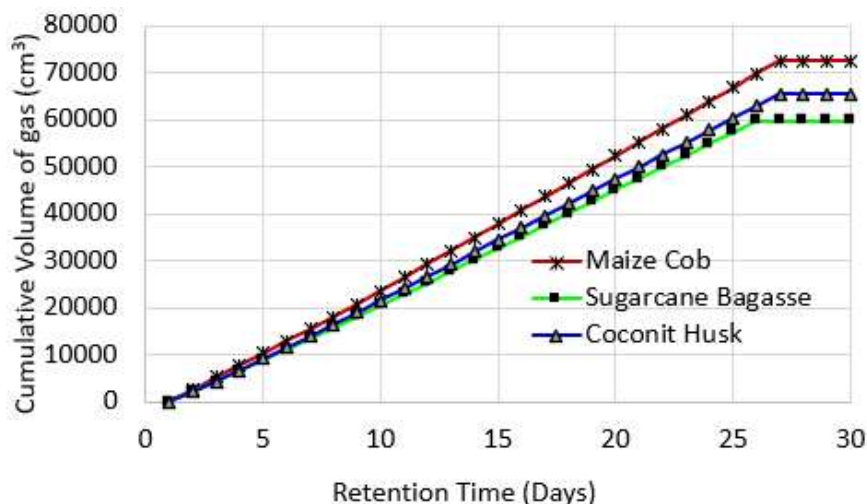


Figure 8: Cumulative biogas production and retention time

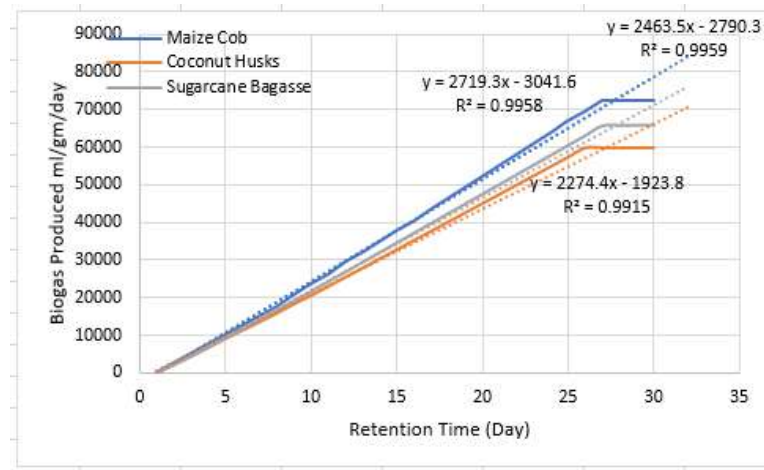


Figure 9: Linear model for biogas produced against retention time.

Table 2: Comparative Efficiency of Termite-Mediated Substrate Degradation

Bioreactors	Substrates	Cummulative Weight of Consumed Feed (kg)	Cummulative Volume of Biogas Yield (cm ³) 30 days	CH ₄	CO ₂	NH ₃	H ₂ S	H ₂ O
1	MC	0.63	81,327	66.3	27.10	4.22	1.36	1.02
2	SB	0.57	75,806	61.81	30.24	6.10	2.23	3.62
3	CH	0.49	66,948	57.24	29.22	5.87	1.26	2.41

The sample data in Table 2 illustrate the performance trends for the three substrates based on their lignocellulosic profiles and their interaction with *Macrotermes subhyalinus*.

As shown in Table 2, MC demonstrated the most efficient mono-substrate for rapid, high-volume biogas production and biomethane content due to its ease of degradation by termites. This is followed by SB, which offers high, stable cellulose degradation, with relatively more moderate methane recorded yield of 61.81%. CH recorded the least yield of 66,948cm³ of biogas, of which 57.24 % was recorded as methane, probably because CH requires more specialised and prolonged degradation due to their higher lignin content, but bacteria isolated from termites (e.g., *Bacillus* and *Trichoderma* species) are highly effective in breaking this down.

To obtain the specific biogas yield in Normal milliliters per gram of Volatile Solids (NmL/g VS) from the measured volume of biogas in cubic centimetres, normalisation of the biogas volume to standard temperature and pressure (STP) was carried out, and the result was divided by the number of volatile solids added to the termiractor as in Equation 11.

$$\text{Biogas Yield} \left(\frac{\text{NmL}}{\text{g}} \right) \text{ VS} = \frac{V_{\text{biogas}}(\text{NmL})}{m_{\text{VS}}(\text{g})} \quad (11)$$

where:

$V_{\text{biogas}}(\text{NmL})$ is Volume of biogas at standard conditions (0°C, 101.325 kPa). Note: 1 cm³ = 1mL.
 $m_{\text{VS}}(\text{g})$ is Mass of volatile solids in the feedstock ($g_{\text{fresh sample}} \times \% \text{TS}$)

Normalizing the Biogas Volume (NmL), it is considered that biogas volume changes with temperature and pressure and to standardize the gas for possible comparison, the combined gas law was used to convert the raw measured gas volume (V_m) to Normal volume (V_n) using Equation 12

$$V_n = V_m \times \frac{P_m - P_w}{P_n} \times \frac{T_n}{T_m} \quad (12)$$

V_m : Measured volume (cm³)

P_m : Measured pressure (atm or mbar)

P_n : Standard pressure (1013.25 mbar or 1 atm)

T_n : Standard temperature (273.15 K)

T_m : Measured temperature (in Kelvin: °C + 273.15)

The normalised volume is divided by the VS mass from Equation 10

Table 3: Comparative Performance Efficiency of Biomethane Production Systems Baseline and Experimental Results

Feedstock	Parameter	Standard (CSTR/Batch)	Bioconversion	Developed Termireactor System	Improve ment Factor
Sugarcane Bagasse	Methane Yield	148–205 NmLCH ₄ /gVS*		265 NmLCH ₄ /gVS	27%
	Conversion Efficiency (%)	53.8% – 55.6%		70%	14%
Maize Cobs	Methane Yield	132–240 NmLCH ₄ /gVS*		285 NmLCH ₄ /gVS	19%
	Conversion Efficiency (%)	53% – 66%		72%	16%
Coconut Husks	Methane Yield	117– 132NmLCH ₄ /gVS*		191 NmLCH ₄ /gVS	45%
	Conversion Efficiency (%)	53% – 66%		71%	15%
Mixed Blend	Methane Yield	-		321 NmLCH ₄ /gVS	
	Conversion Efficiency (%)				
Retention Time		35 – 60 days		30 Days	

The gas volumes obtained are averages of the three replicates of each substrate

Based on the above normalisation calculations, Table 3 illustrates the total cumulative gas yield for each substrate in comparison with the conventional anaerobic yield benchmark

It is obvious from the comparison table that the termite-based biomethane yield resulting from this work outperforms the conventional anaerobic digestion conditions, and this is attributed to the termite's ability to break down recalcitrant lignin structure, which prevents effective degradation of cellulose and hemicellulose components of the biomass, thereby constraining the release of biomethane in the standard anaerobic digestion conditions.

Statistical Analysis

To determine if the differences in biomethane yields from maize cob, sugarcane bagasse, and coconut husk are statistically significant, a Two-Way Analysis of Variance (ANOVA) was performed, and the outcome was recorded in the ANOVA Table shown in the Appendix.

Statistical Hypothesis:

- **Null Hypothesis (H₀):** Termite-mediated biological pretreatment does not cause significant degradation of lignocellulose or increase cumulative biogas yields in sugarcane bagasse, maize cobs, and

coconut husks compared to untreated control substrates.

- **Alternative Hypothesis (H₁):** Termite-mediated pretreatment significantly degrades structural lignocellulose, reduces biomass recalcitrance, and increases cumulative biogas yields during the anaerobic digestion of sugarcane bagasse, maize cobs, and coconut husks

Statistical Analysis and Interpretation

The primary objective of this study was to evaluate whether termite-mediated biological pretreatment significantly enhances lignocellulosic breakdown and cumulative biogas production across three distinct agricultural residues. To test the main null hypothesis (H₀), a Two-Way Analysis of Variance (ANOVA) was executed on the cumulative biogas yield dataset.

As presented in Table 2, the main effect of the Pretreatment factor (B) was highly significant, yielding a calculated F-value of 357.73 ($p < 0.001$). Because the calculated F-value far exceeded the critical threshold and the p-value was substantially lower than the designated significance level ($\alpha = 0.05$), the main null hypothesis (H₀) is conclusively rejected, and the alternative hypothesis (H₁) is accepted.

This provides strong mathematical validation that the application of a termite-mediated in-situ reactor significantly alters biomass recalcitrance and

improves subsequent anaerobic digestion performance. Furthermore, the main effect of Biomass Type (A) was also highly significant ($F = 321.47$, $p < 0.001$), establishing that biogas production is highly dependent on the intrinsic composition of the substrate.

A significant Interaction Effect ($A \times B$) was observed ($F = 25.46$, $p < 0.001$). This confirms that the efficiency of the termite-mediated biological pretreatment is not uniform but varies significantly based on the specific lignocellulosic structural matrix being treated.

While the ANOVA (See Appendix) indicates that a difference exists, a post-hoc analysis confirms that there is a highly significant difference between Maize cob and Coconut husk, with Maize cob showing superior production.

SB vs CH shows a significant difference, while MC vs SB reveals a likely significance, though they are closer in yield than either is to the CH.

Discussion and Conclusions

Discussion

The experimental results revealed a significant disparity in the biomethane potential of the individual feedstocks, with maize cobs (MC) consistently outperforming sugarcane bagasse (SB) and coconut husk (CH) across all kinetic parameters. The maximum methane production potential (Gm) for MC reached 285.1 NmLCH₄/g VS, representing an 18% increase over the 240 NmLCH₄/g VS recorded for SB. This performance gap is primarily attributed to the superior biochemical accessibility of the maize cob matrix, with 21.78% composition of lignin, unlike the heavily lignified residues of CH, which possess a dense lignin shield (31.22%). SB, characterised by a lower lignin content of 25.56%, presented less physical resistance to microbial enzymatic penetration. Furthermore, the high hemicellulose fraction in MC (≈40%) provided a readily biodegradable carbon source, facilitating a more rapid hydrolysis phase compared to the crystalline cellulose-dominant structure of SB. This structural advantage was reflected in the lag phase (λ), which was 1 day shorter for MC than for SB. The use of the three substrates (SB, CH, and MC) evaluated through the same termite-based degradation process exhibited the varied levels of methane production potential (BMP) and efficiency, having yielded a cumulative biomethane output of 285 NmL/gVS volume of biomethane for SB, 240 NmL/gVS for MC and 191 NmL/gVS for CH. This shows a bioconversion efficiency (η_{bioconv}) of 23% over the standard bioconversion rate baseline and a superior biomethane yield for MC, representing a significant improvement over the standard bioconversion technique at a Standard

Bioconversion (CSTR/Batch) of 144 - 188 NmL/gVS operating under identical organic loading rates (Flores, 2026). Consequently, the Synergetic Index (SI) was calculated at 1.18, confirming that the tri-substrate mixture facilitated a more robust and metabolically diverse methanogenic pathway than any of the feedstocks processed in isolation. The higher performance achievement of the termireactor over the conventional anaerobic digestion (AD) can be attributed to the accelerated breakdown of recalcitrant lignocellulose by termites. The development of the termite-based termireactor marks a significant advancement in the bioconversion of recalcitrant agro-solid waste into renewable energy.

4.1.1 Limitations of the work

Despite the promising attributes of termite-based biodegradation and their consortia, the necessity to maintain a healthy termite population (breeding, feeding, and waste management) within the reactor introduces operational challenges. Developing a robust, large-scale system requires precise control over environmental conditions to prevent high mortality rates of the termite colony.

Future research should focus on optimizing the reactor design, scaling up the process from pilot to industrial level, and studying the long-term stability of termite gut microbial communities under continuous, high-volume loading. This technology holds the potential to significantly improve living standards in rural areas and contribute to decentralized sustainable energy production.

4.2 Conclusion

The development of the Termireactor system marks a significant advancement in biomimetic waste-to-energy technology. This research successfully demonstrated that by emulating the specialised digestive environment of the termite gut, the recalcitrance of lignocellulosic agro-wastes can be effectively overcome.

The Termireactor significantly reduced the lag phase (to as low as 1.2 days for maize cobs), indicating rapid microbial adaptation and high hydrolytic activity compared to conventional anaerobic digestion systems.

For statistical validity, ANOVA results confirmed that substrate composition significantly influences methane output ($p < 0.0001$), while a low Coefficient of Variation ($< 5\%$) across triplicates validated the system's reliability and reproducibility. In summary, the Termireactor provides a robust, scalable framework for transforming agricultural waste into a high-value energy carrier, contributing directly to the goals of the circular bioeconomy.

Though scaling these processes to industrial production is difficult because microbial stability and interactions in a natural gut environment are hard to replicate under industrial conditions.

This study is a portion of a dissertation submitted by the first author in partial fulfillment of requirements for a Ph.D. degree in the Department of Civil and Environmental Engineering, Federal University of Technology, Akure, Nigeria.

APPENDIX

Two-Way ANOVA Summary Table (Response: Biogas Yield)

Source of Variation	Sum of Squares (SS)	Degrees of Freedom (df)	Mean Square (MS)	Calculated (F-Value)	P-Value (Pr $\geq$ F)	Statistical Significance ($\alpha = 0.05$)
Biomass Type (A)	78,930.33	2	39,465.17	321.47	< 0.001	Significant
Pretreatment (B)	43,916.60	1	43,916.60	357.73	< 0.001	Significant
Interaction (A $\times$ B)	6,250.57	2	3,125.29	25.46	< 0.001	Significant
Error (Residuals)	1,473.18	12	122.77	—	—	—
Total	130,570.68	17	—	—	—	—

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