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Valorisation Potential of Kolanut Pod for Biogas Production: A Physicochemical and Biochemical Characterization

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ABSTRACT

Kolanut (Cola nitida) pods are an abundant agricultural residue that are often indiscriminately discarded after harvesting, resulting in offensive odour, environmental pollution, and the proliferation of disease-causing organisms and harmful animals. This study evaluated the potential of kolanut pods as lignocellulosic biomass for biogas production via anaerobic digestion. Freshly harvested kolanut pods were mechanically pre-treated by washing, sun-drying, pulverising, and sieving with an ASTM standard laboratory sieve No. 35 to obtain a uniform particle size before characterisation. Proximate and ultimate analyses were conducted to determine the Dry Matter (DM), Organic Dry Matter (ODM), Organic Carbon (OC), Organic Nitrogen (ON), Carbon-to-Nitrogen (C/N) ratio, and Higher Heating Value (HHV), while Scanning Electron Microscopy coupled with Energy-Dispersive X-ray spectroscopy (SEM/EDX), Fourier Transform Infrared spectroscopy (FTIR), and microbiological analyses were used to evaluate the morphological, elemental, functional, and microbial characteristics of the biomass respectively. The results showed DM and ODM values of 91% and 83%, respectively, with OC, ON, C/N ratio, and HHV of 14.25%, 0.48%, 30:1, and 17.9 MJ/kg, respectively. SEM revealed a heterogeneous, porous surface favourable for microbial colonisation, while EDX identified essential mineral elements, including Si, Ca, K, Mg, Al, and Na. FTIR confirmed the presence of cellulose, lignin, and phenolic functional groups, and microbiological analysis identified Bacillus cereus, Escherichia coli, and Proteus mirabilis. The favourable physicochemical properties, porous morphology, essential mineral composition, and presence of anaerobic bacteria indicate that kolanut pod is a promising feedstock for anaerobic digestion. Its valorisation could provide a sustainable solution for agricultural waste management.

INTRODUCTION

Energy is a fundamental resource that underpins domestic and industrial activities and plays a vital role in socioeconomic development. However, the continued dependence on fossil fuels for energy generation has contributed significantly to environmental degradation, ecological imbalance, and climate change through the emission of greenhouse gases and other harmful pollutants. These emissions adversely affect human health and accelerate the depletion of natural resources. Consequently, there is an increasing need for environmentally sustainable and renewable energy sources that can mitigate the negative impacts of conventional energy systems. Among the promising alternatives, biogas has emerged as a clean and renewable energy source capable of simultaneously addressing energy demand, organic waste management, and greenhouse gas mitigation (Akinbami *et al.*, 2001; Aworanti *et al.*, 2023; Subbarao *et al.*, 2023; Alengebawy *et al.*, 2024).

Biogas is a gaseous fuel produced by the fermentation of organic matter or biomass. When this organic matter is fermented, methane (CH4) and carbon dioxide (CO2) are produced, which may be present alongside small quantities of hydrogen Sulphide (H2S), moisture and siloxanes, with methane being the principal constituent (Iweka *et al*., 2021). Since organic materials are inherently biodegradable, the gases produced during their decomposition can be captured and utilized for clean and renewable energy generation, thereby promoting sustainable waste management and reducing environmental contamination by harmful substances (Kabeyi and Olanrewaju, 2022).

Biogas is the methane-rich gas that is produced from the anaerobic digestion of organic residues. Anaerobic digestion is a method that is being popularly employed to transform biodegradable material into valuable sources of energy and, in parallel, reduce waste volumes (Samani *et al*., 2017).

The technology of catalytic co-digestion of agricultural residues with cattle dung helps improve the retention days, enhance the amount of production and improve the composition of the methane gas (Verma, 2002). Several agricultural residues have been used as substrates for biogas production, ranging from animal waste/manure and crop residues to dedicated energy crops, as these have attracted the attention of researchers, but little work has been done on the biodegradability of kolanut pod through anaerobic digestion for biogas generation. Kola tree belongs to the *Sterculiaceae* family and is indigenous to West Africa, especially the nations of Nigeria, Sierra Leone, Liberia and Ivory Coast (Dewole *et al*., 2013; Nyadanu *et al*., 2020).

Ebana et *al.*(2015) in "Chemical composition and antimicrobial analysis of the pods and seeds of *cola nitida* and *colanitida*" established that the average moisture content of both species (*cola nitida* and *colarostada* pods) was 81.75% while their seeds had an average of 54.60%. Crude protein ranged from 9.60 - 11.91% for the pods and 10.50 - 11-91% for the seeds. Lipid for both was less than 2%. The fibre of the pod was 15% while that of the seed ranged from 2% to 3.6%. The toxicant levels were generally low. Fabunmi (2018) on the conversion of kolanut waste to beneficial products for environmental protection, submitted that enzymes such as *xylanase, pectinase, protease, cellulase and amylase* were produced from kolanut husk. Many works have not been done on evaluating the lignocellulose content and anaerobic bacteria inherent in African-based kolanut pods to establish their usefulness in anaerobic technology for biogas production.

MATERIALS AND METHODS

Material Procurement and Processing

Kolanut pods were freshly harvested from local farms in Lagbedu Orile, Ogo-Oluwa Local Government Area of Ogbomoso, located at latitude 8°08′00″ N and longitude 4°16′00″ E. Upon collection, the pods were thoroughly cleaned with water to remove dirt particles and grit. The cleaned samples were sun-dried for a period of seven days to achieve significant moisture reduction. The dried kolanut pod residues were milled using a Janke and Kunkel hammer mill and subsequently sieved using a standard laboratory sieve with an aperture size of 0.50 mm (500 μm). The biomass fraction passing through the 1.0 mm sieve (ASTM Standard Sieve No. 18) and retained on the 0.5 mm sieve (ASTM Standard Sieve No. 35) was collected and used for all subsequent analyses. The particle size was selected following the recommendations of ASTM E1757 (2019) for biomass sample preparation, as it increases substrate surface area, facilitates microbial attachment and enzymatic activity, and promotes efficient biodegradation of organic matter. The Figures of both the fresh and processed kolanut pods are presented in Plate 1. The processed samples were then subjected to physicochemical characterisation using a Hitachi SU 3500 Scanning Electron Microscope coupled with Energy Dispersive X-ray Spectroscopy (SEM/EDX) and a Shimadzu FTIR-8400S Spectrometer for Fourier Transform Infrared (FTIR) analysis.

(a) Fresh kolanut pod

(b) Processed kolanut pod

Plate 1: Kolanut pod

Methods of Characterisation

Proximate Analysis of the Kolanut Pod

Proximate characterisation of the kolanut pod was performed at the Department of Animal Production and Health Laboratory, Ladoke Akintola University of Technology, Ogbomoso, Nigeria, using standardised APHA (2017) procedures, using the average values of three replicates. The analysis encompassed the determination of moisture content, ash content, volatile matter, organic matter, and fixed carbon fractions. Accurate measurements of sample and water masses were obtained using a top-loading balance. In addition, the nutritional constituents of the agricultural residue, including crude protein, crude fat, moisture, ash, and carbohydrates, were quantified through a combination of solvent extraction techniques and the Kjeldahl method for nitrogen determination.

The Dry Matter (DM) and Organic Dry Matter (ODM) contents in the kolanut pod were measured according to Standard Methods APHA (2017). The total solids of substrates in a digester are a combination of organic and inorganic matter in the feedstock (Adams and Happiness 2010). To measure total Dry Matter (DM), an empty crucible or moisture dish is weighed, W_1(g), then a fresh amount of the feedstock was added to the empty crucible and weighed as W_2(g). This was dried in an oven, maintained at 105 °Cuntil a constant weight was obtained. It was then cooled in a desiccator and the dried sample was weighed W_3(g). Percentage total Dry Matter (DM), which is the ratio of the weight of the dried sample to the wet sample, was calculated using Equation 1 (APHA 2017; VDI 4630 2016).

$$\%DM = \frac{W_3 - W_1}{W_2 - W_1} x\ 100 \qquad (1)$$

Where: W_1= weight of empty crucible (g), W_2= weight of crucible + wet sample (g), W_3= weight of crucible + oven-dried sample (g). Moisture content was estimated as given in equation 2:

$$\text{Moisture Content (\%)} = 100 - \text{DM (\%)} \qquad (2)$$

Organic Dry Matter is the combustible organic fraction remaining after subtracting the ash content from the dry matter. The process of measuring the Organic dry matter of an organic substrate involves using the oven-dried sample obtained from DM determination. The oven-dried sample was placed in a muffle furnace at 550°C for 2–4 hours to form ash, and then cooled in a desiccator. The residue (ash) was weighed W_4(g). The ash content was calculated according to equation 3.

$$Ash\ (\%DM) = \frac{W_4 - W_1}{W_3 - W_1} x\ 100 \quad (3)$$

Where W_4 = weight of crucible + ash after ignition (g)

The Organic Dry Matter (ODM) was therefore calculated directly according to equation 4(APHA 2017; VDI 4630 2016)

$$ODM\ (\%DM) = \frac{W_3 - W_4}{W_3 - W_1} x\ 100 \quad (4)$$

The Ultimate Analysis of the Kolanut Pod

The ultimate composition of the kolanut pod was determined in the laboratory using standard APHA analytical procedures (APHA 2017) using the average value of three replicates. The analysis focused on quantifying the major elemental constituents, namely carbon, hydrogen, oxygen, nitrogen, sulphur, and other associated elements. Atomic Absorption Spectrophotometry (AAS) was utilised for elemental determination in the liquid sample extracts. The technique operates on the principle that free atoms absorb light at element-specific wavelengths, typically between 190 and 900 mm, thereby enabling accurate quantification of the elemental concentrations present in the kolanut pod samples. Carbon (C), Hydrogen (H), and Nitrogen (N) were determined experimentally using a CHNS (Carbon-Hydrogen-Nitrogen-Sulphur) elemental analyser. The oxygen (O) was determined by equation 5 when Sulphur is negligible:

$$\%O = 100 - (\%C + \%H + \%N + \%Ash) \quad (5)$$

The gross calorific value (Higher Heating Value) of the biomass (kolanut pod) was estimated according toParikh's equation 6 (Parikh *et. al.*, 2005). This calorific value obtained indicates the energy potential of the biomass and its suitability as a feedstock for bioenergy production.

$$\text{HHV} = 0.3536(\text{FC}) + 0.1559(\text{ODM}) - 0.0078(\text{A}) \quad (6)$$

Where, FC = Fixed Carbon, ODM = Organic Dry Matter and A =Ash content.

Scanning Electron Microscopy (SEM)/Energy Dispersive X-ray Spectroscopy (EDX) Analysis of the Kolanut Pod

The SEM/EDX analysis of the kolanut pod was done using a Hitachi SU 3500 scanning electron microscope to determine its external morphology image, crystalline structure, orientation and elemental composition. The sample was prepared by cleaning its surface, rinsing it, dehydrating it, mounting it and coating it with a thin layer of approximately 20nm of gold. The major purpose of coating the sample was to increase its conductivity and to prevent the build-up of high voltage charges on the specimen. 0.3 g of each sample was sprinkled on a two-sided carbon tape and the carbon tape was fixed to the SEM stub. A stream of light was applied to the machine and this generated signals that produced a two-dimensional image. This showed microstructural morphology information in the SEM compartment. The EDX component was also attached to the SEM analysis and this determined the elemental composition of the samples.

Fourier Transform Infrared Spectroscopy (FTIR) of the Kolanut Pod

The FTIR spectroscopy analysis of the kolanut pod was carried out to identify the functional groups in the kolanut pod. The analysis was conducted using a Shimadzu FTIR-8400S spectrometer. The instrument operates with a mid-infrared range, typically between 4000 cm^{-1} and 400 cm^{-1}, providing a comprehensive spectrum of the material's vibrational modes. The FTIR spectra collected from the sample showed the presence of various functional groups, which correspond to the characteristic peaks of specific molecular bonds, offering insight into the composition and structure of the kolanut pod.

Biochemical and Morphological Characterisation of the Kolanut Pod

Morphological and biochemical methods were used for the characterization and identification of isolates of the kolanut pod according to Bergey's Manual of Bacteriology (Whitman *et al*., 2015). Four replicate isolates of the kolanut pod were characterised to determine the anaerobic bacteria present in the kolanut pod and their growth medium. Gram staining was done to classify the two main groups of bacteria (Gram-positive and gram negative bacterial) because Gram-positive bacteria retain the crystal violet stain and appear purple, while Gram-negative bacteria do not retain the primary stain but take up the counterstain and appear pink or red. The Simmons citrate test was carried out to determine whether a microorganism can utilize citrate as its sole source of carbon and energy and its growth in citrate solution. The catalase test was done on the kolanut pod to identify the presence of the enzyme catalase, which helps in speeding up the breakdown of hydrogen peroxide during the process of metabolism.

The oxidase test was performed to determine the ability of the isolated microorganisms to produce cytochrome c oxidase, an enzyme involved in the electron transport chain during aerobic respiration. The test aided in the identification of bacterial species based on their respiratory enzyme systems. Sugar fermentation tests were also conducted to evaluate the ability of the microorganisms to ferment specific carbohydrates, including glucose, lactose, sucrose, fructose, and mannitol, as well as to determine the metabolic end-products generated during fermentation. In addition, starch hydrolysis tests were carried out to assess the production of amylase, an enzyme responsible for the degradation of starch into simpler sugars. Casein hydrolysis tests were performed to identify microorganisms capable of secreting extracellular proteolytic enzymes that hydrolyze casein into peptides and amino acids. Furthermore, gelatin hydrolysis tests were conducted to determine the production of gelatinase, an enzyme that breaks down gelatin into soluble peptides and amino acids. All tests were performed in accordance with the procedures outlined in Bergey’s Manual of Determinative Bacteriology (Whitman *et al*., 2015).

RESULTS AND DISCUSSION

Proximate and Ultimate Characteristics of Kolanut Pod

The findings of the proximate and ultimate analyses are presented in Table 1. The kolanut pod recorded Dry Matter and Organic Dry Matter values of 91% and 83%, respectively. These values are higher than the minimum requirement of 75% for both Dry Matter and Organic Dry Matter, reported by Ebana *et al*., (2015) and Fabunmi (2018) for biomass materials suitable for biogas production. The high dry matter content indicates a low moisture level, while the substantial Organic Dry Matter content suggests the presence of adequate biodegradable organic constituents required for microbial metabolism during anaerobic digestion. Consequently, the kolanut pod can be considered a suitable feedstock for efficient biogas generation.

The high levels of dry matter and organic dry matter recorded for the kolanut pod agree with the observations of Maghanaki *et al*., (2013) and Veerabadhran *et al*. (2021), who stated that biomass substrates suitable for anaerobic digestion should possess a considerable proportion of organic matter to sustain microbial growth and methane-producing activities. Although the kolanut pod exhibited favorable DM and ODM values, its lignin

Table 1: Proximate and ultimate analysis of kolanut pod

Proximate		Ultimate	
Parameter	%	Element	%
DM	91.00	Oxygen	19.00
ODM	83.00	Hydrogen	2.38
Ash content	8.00	Nitrogen	0.48
Crude fibre	20.00	Carbon	14.25
Lignin	21.10	C/N ratio	30:1
Moisture content	9.00	Calorific value (MJ/kg)	17.9
Fat content	2.00		
Crude protein	25.38		
Fixed Carbon	80.00		

content of 21.10% may adversely affect the biodegradation process. Lignin forms a protective matrix around cellulose and hemicellulose, thereby reducing their accessibility to anaerobic microorganisms. Consequently, the use of catalysts or pretreatment strategies may be necessary to enhance the degradation of the organic fraction. This observation is consistent with the findings of Nguyen *et al.*, (2007) and Asgari et *al*., (2011), that lignin-rich biomass substrates are more resistant to microbial attack and often require longer retention times to achieve satisfactory biogas yields.

The ultimate analysis results indicate that the kolanut pod possessed a carbon-to-nitrogen (C/N) ratio of 30:1, which is considered suitable for efficient methane production during anaerobic digestion. An appropriate C/N ratio is essential because methanogenic microorganisms consume carbon more rapidly than nitrogen and require a balanced nutrient environment for optimal growth and metabolic activity. This observation is in agreement with the findings of Khalid *et al*., (2011); Panpong *et al*., (2014); Hagos *et al*., (2017) and Alqaralleh *et al.,* (2017), that biomass substrates intended for biogas production should maintain a C/N ratio of at least 20:1, while Ibro *et al.,* 2024; Obileke *et al.,* 2025 and Alengebawy *et al* 2024 reported that the optimal C/N ratio for organic residues suitable for biogas production should range from 20:1 to 30:1. In addition, the calorific value of 17.9MJ/kg recorded for the kolanut pod indicates its potential as a viable renewable energy resource. The combination of a favorable C/N ratio and appreciable energy content further highlights the suitability of kolanut pod as a feedstock for sustainable biogas production.

Scanning Electron Micrograph (SEM) and Elemental Composition of Kolanut Pod

The SEM image and EDX micrograph image of the kolanut pod are presented in Plate 2 and Figure 1, respectively. It illustrates the surface morphology of the kolanut pod. The micrograph revealed a heterogeneous and irregular surface structure with a network of well-defined pores and cavities distributed across the biomass matrix. These porous features are advantageous for biogas production because they facilitate microbial adhesion, improve mass transfer, and enhance the accessibility of degradable organic components. The kolanut pod was characterised by a particle size of 20.36 µm, a height of 2.41 mm, and a bulk density of 2.12 g/cm³. The combination of a rough surface texture and high porosity observed in the kolanut pod significantly increases the effective surface area

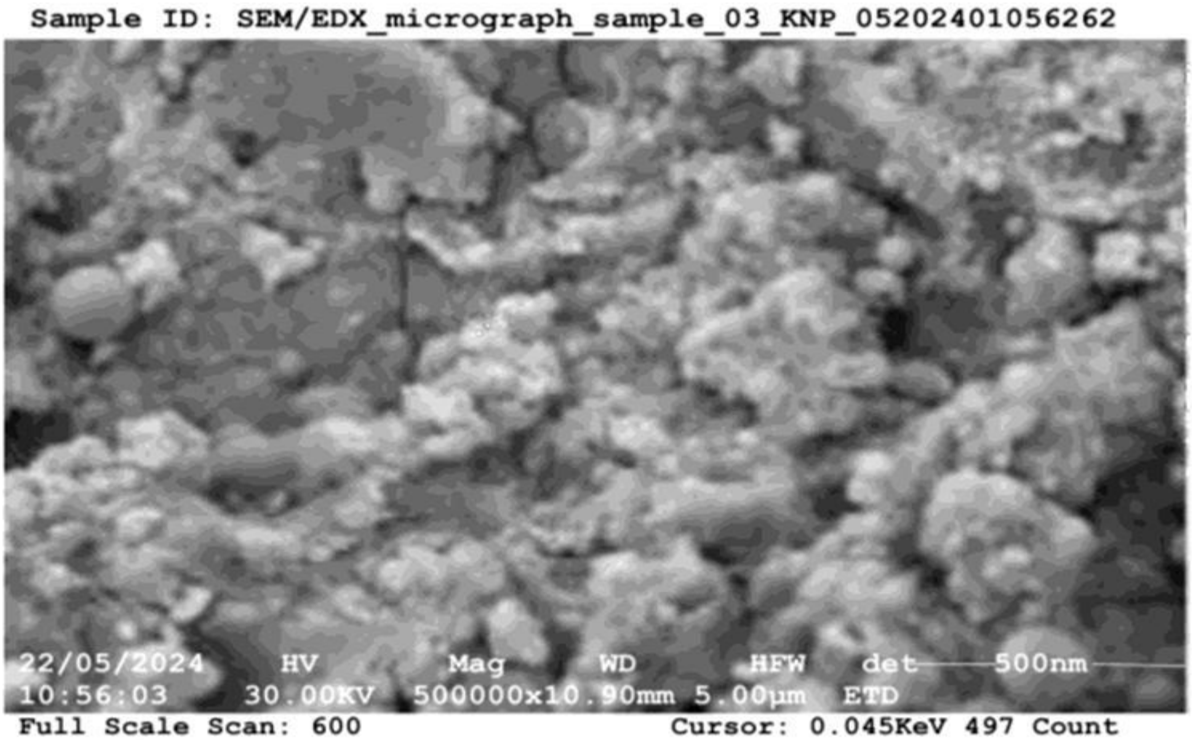

Plate 2: SEM image of kolanut pod.

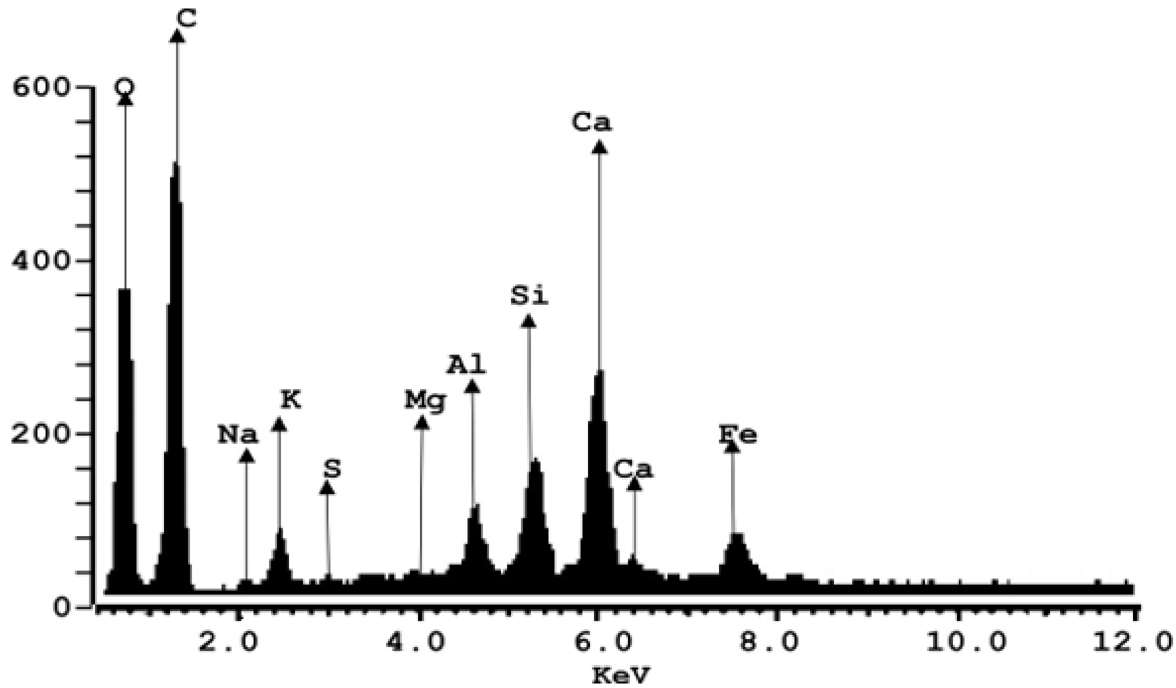

Figure 1: EDX micrographs of kolanut pod.

available for microbial colonisation and catalytic reactions, thereby potentially improving the efficiency of the anaerobic digestion process. This is in agreement with the findings of Adaramaja *et al*., (2024) that dispersed pores in organic substrate increase the surface area for microbial activities and catalytic action during anaerobic fermentation. Table 2 shows the elemental composition of the kolanut pod from EDX analysis. This further confirmed the suitability of kolanut pod as biogas

Table 2: Elemental composition of the kolanut pod from EDX analysis

Element	Composition in % wt
Oxygen (O_2)	51.36
Silicon (Si)	22.15
Aluminum (Al)	1.98
Magnesium (Mg)	2.35
Potassium (K)	3.29
Calcium (Ca)	13.96
Sodium (Na)	0.28
Sulphur (S)	1.16
Iron (Fe)	1.4
Phosphorus (P)	2.85
Chlorine (Cl)	0.21

substrates. The oxygen content of 51.36% indicates the potential for aerobic pre- digestion. The oxygen entrapped within the digester during substrate loading may support a brief aerobic or microaerobic phase before anaerobic conditions are fully established. During this period, aerobic and facultative microorganisms consume the residual oxygen and partially solubilize biodegradable organic matter, thereby enhancing hydrolysis and facilitating the subsequent anaerobic digestion process. Similar observations were reported by Botheju *et al*. (2009, 2010) and Botheju and Bakke (2011), who noted that limited oxygen availability can increase organic matter solubilisation and improve the initial stages of anaerobic digestion.
Likewise, the presence of essential macro-elements such as Silicon (Si), Calcium (Ca), and Magnesium (Mg) suggests kolanut pod supports microbial metabolism and buffers the digestion process against acidification. The calcium content of 13.96% in the kolanut pod aids in stabilising pH levels, a critical factor for optimal anaerobic digestion. This is in agreement with the submission of Yu *et al.* (2006), Appels *et al*. (2008) and Water Research X Review (2025), that moderate calcium content buffers the pH medium of slurry and aids the digestion process. Moreover, the presence of trace elements like Iron (Fe) and Phosphorus (P) plays a crucial role in enzyme activation and microbial growth, thus enhancing biogas production. This supports the findings of Joo *et al.,* (2018) that biogas production from biomass can be enhanced with the presence of Fe, Ca, Mg, Zn and other metals in kitchen waste.

Fourier Transform Infrared Spectra (FTIR) of the Kolanut Pod

The FTIR spectrum of the kolanut pod (KNP), shown in Figure 2, revealed the presence of several functional groups associated with lignocellulosic biomass. The identified absorption peaks in the KNP corresponded primarily to cellulose, lignin, and phenolic compounds, which constitute the major structural components of plant-derived materials. The occurrence of these compounds indicates that the kolanut pod possesses significant potential for utilisation in the production of biochar, bioethanol, biopolymers, and bioplastics. In addition, the lignocellulosic composition revealed by the FTIR analysis suggests the availability of organic constituents that can be biologically converted into methane during anaerobic digestion. Consequently, the FTIR results further demonstrate the suitability of kolanut pod as a sustainable feedstock for biogas generation and other renewable energy applications.

There is a large band centered at 3400-1650cm^{-1} due to hydrogen vibrations of the OH groups of alcohols, phenols, or carboxylic acids. The peak at 2500-1750cm^{-1} is due to CH vibrations of aliphatic groups. The band centered between 2360-2339 cm^{-1} is due to C=C of terminal alkynes. Bands between 1735 and 1721cm^{-1} are due to C=0 of aldehydes, ketones, carboxylic acids and esters. The bands around 1655 and 1639 cm^{-1} are characteristic of aromatic amides and ketones (C-C, C=O).

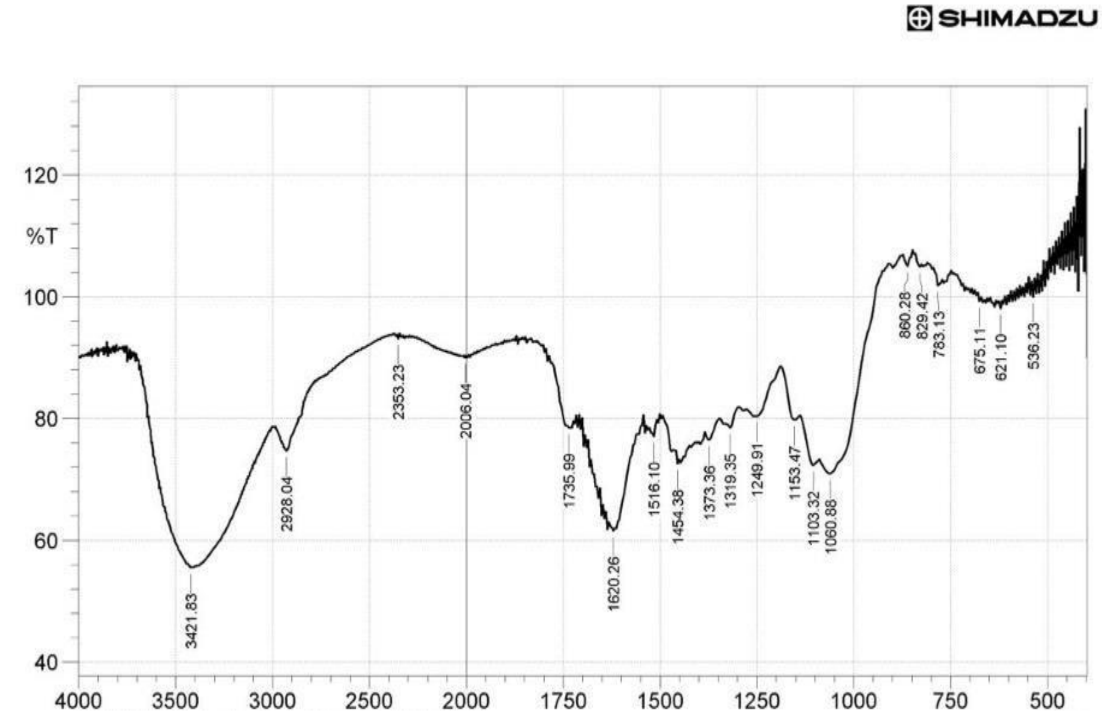

Figure 2: FTIR spectrum of kolanut pod

The bands between 1561 and 1544 cm-1 are caused by the absorbance of N-H amides. The bands centered around 1462 to 1425cm^{-1} are due to COO^- aliphatic groups, asymmetric CH bending of CH_2 and CH_3 groups.

Microbial Characterization of the Kolanut Pod Substrates

The results of the microbial characterisation of the kolanut pod, used for this research, are shown in Table 3. The notation "R" observed in the cell morphology column indicates rod-shaped bacterial cells (*bacilli*). The presence of these bacterial isolates suggests that the bioactive compounds naturally occurring in the kolanut pod do not significantly inhibit microbial growth, thereby supporting its suitability as a substrate for anaerobic digestion. The isolates demonstrated effective growth under mesophilic temperature conditions and in a moderately alkaline medium with a pH of 9.2. Results of the starch hydrolysis test further indicated that the substrate contained only an insignificant level of starch. The microbial population associated with the kolanut pod was composed primarily of bacterial species, and these are: *Bacillus cereus*, *Escherichia coli*, and *Proteus mirabilis*. Their occurrence may contribute to the initial degradation of organic matter and facilitate subsequent methane production during anaerobic digestion.

These bacteria detected in the kolanut pod are important contributors to the anaerobic degradation of organic matter and the subsequent production of methane. Their presence supports the suitability of the substrate for biogas generation. Similar findings were reported by Aworanti *et al*., (2017) in an investigation of the biomethanisation potential of the mixture of cattle dung with pineapple peels. Moreover, the microbial characterisation revealed positive motility among the isolates, indicating their capacity for active movement within the growth medium. This characteristic allows the microorganisms to escape unfavourable

Table 3: Morphological and microbial characterization of the substrates

Code	Gram Reaction	Cell Morphology	Catalase	Oxidase	Casein Hydrolysis	Gelatin Hydrolysis	Methyl red	Voges Proskaer	Nitrate Redn	Growth 60^0	Growth 30^0	Coagulation	Urease	Growth at pH 3.9	Growth at pH 9.2	Starch Hydrolyses	Growth NaCl
KNP1	+	R	+	+	+	+	+	+	+	+	-	-	-	+	+	-	+
KNP2	+	R	+	+	+	+	-	-	+	-	-	-	-	-	+	+	+
KNP3	+	R	+	+	+	+	-	-	+	-	-	-	-	-	+	+	+
KNP4	-	R	+	-	-	-	+	+	-	-	-	+	-	-	+	-	-

Table3: (continued). Morphological and microbial characterization of the substrates

Code	Citrate Utilization	Motility	Indole test	Glucose	Fructose	Maltose	Lactose	Sucrose	Galaitose	Xylose	Arabinose	Raffinose	Rhamnose	Dulaitol	Mannitol	Identity
KNP1	D	+	-	+	+	+	+	-	+	-	-	-	+	D	-	*Bacilluscereus*
KNP2	+	+	-	+G	+	-	-	+	+	+	-	-	-	-	-	*Proteusmirabilis*
KNP3	+	+	-	+G	+	-	-	+	+	+	-	-	-	-	-	*Proteusmirabilis*
KNP4	-	+	+	+	+	+	+	D	+	D	+		D	D	+	*Escherichiacoli*

Key:	R	Rod-like shape. (It can be cylindrical or hot dog)
	+ sign	Present/significant/active/functional trait
	- sign	Absent/not significant/non-active
	+ G	Gas present. (As the microbes ferment carbohydrates, gases are released).
	D	Delay. (This indicates the organisms produced the enzymes slowly).
	KNP	Kolanut Pod

environmental conditions and migrate toward regions with higher nutrient concentrations. The ability to detect and respond to chemical gradients through chemotaxis enhances nutrient uptake, microbial colonisation, and substrate degradation, thereby promoting more effective anaerobic digestion and methane production.

CONCLUSION

This research only characterised kolanut pod as a potential feedstock in anaerobic technology for biogas production. The findings showed that the KNP biomass contains suitably appreciable levels of Dry Matter (DM), Organic Dry Matter (ODM), and an appropriate Carbon-to-Nitrogen (C/N) ratio, all of which are essential parameters for efficient biogas generation. Furthermore, the highly porous and dispersed surface morphology of the kolanut pod provides an extensive surface area that supports microbial attachment and activity, which can enhance the digestion of organic material in anaerobic technology. Microbiological analysis revealed the presence of *Bacillus cereus*, *Escherichia coli*, and *Proteus mirabilis* in the kolanut pod, which are microorganisms that can thrive within mesophilic temperature ranges of 4–44 °C, 7–40 °C, and 10–43 °C, respectively, suggesting their potential involvement in the biodegradation of organic matter.

RECOMMENDATION

The outcome of this study hereby provides useful baseline information and data on the composition and biodegradability potential of kolanut pod. It is hereby recommended that this viable agricultural residue be employed for an anaerobic digestion experiment to investigate its biomethane generation potential.

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