










## CHARACTERIZATION, HORMETIC EFFECTS AND KINETIC MODELING OF CORN STALK-DERIVED BIOCHAR IN BIOGAS PRODUCTION

 Utibe A. Ofon<sup>1,2\*</sup>,  Uduak U. Ndubuisi-Nnaji<sup>1</sup>,  Anthony A. Adegoke<sup>1,3</sup>,  
 Godwin E. Udofia<sup>1,2</sup>,  Solomon E. Shaibu<sup>2,4</sup>,  Dele P. Fapojuwo<sup>5</sup>,  
 Opeyemi K. Fatunla<sup>1</sup>,  Idongesit S. Basse<sup>1</sup>, Olushola S. Ayanda<sup>6</sup>,  
 Mboutidem I. Ekaette<sup>1</sup>, Sulaiman O. Adewuyi<sup>6</sup>, Michael J. Klink<sup>7</sup>,  
Simphiwe M. Nelana<sup>7</sup>

<sup>1</sup>Department of Microbiology, University of Uyo, Uyo, Nigeria

<sup>2</sup>International Center for Energy and Environmental Sustainability Research (ICEESR), University of Uyo, Uyo, Nigeria

<sup>3</sup>Institute for Water and Wastewater Technology (IWWT), Durban University of Technology (DUT), Durban, South Africa

<sup>4</sup>Department of Chemistry, University of Uyo, Uyo, Nigeria

<sup>5</sup>Department of Chemistry, Nelson Mandela University, Port Elizabeth, South Africa

<sup>6</sup>Department of Chemistry, Federal University Oye-Ekiti, Oye-Ekiti, Nigeria

<sup>7</sup>Department of Chemistry, Vaal University of Technology, Vanderbiljpark, South Africa

**Abstract.** Anaerobic digestion remains a contemporary technology to manage organic matter with the recovery of valuable products including biogas which can be boosted with biochar supplementation. The properties of biochar in turn determines its effect on anaerobic digestion performance and biogas yield. In our study, corn stalk-derived biochar (CSB) was characterized using advanced techniques like CHN analysis, SEM imaging, EDX, FTIR and BET. The CSB was later applied to the anaerobic digestion of the paper waste codigested with chicken manure at four doses (0, 2.5, 5.0 and 7.5 gL<sup>-1</sup> on TS basis). CSB properties revealed significant characteristics [CEC (10 meq100g<sup>-1</sup>), surface area (15.6 m<sup>2</sup>g<sup>-1</sup>), pore size (20.1 nm) and pore volume (0.06 cm<sup>3</sup>g<sup>-1</sup>)] suitable for anaerobic digestion and sustained biogas production. Generally CSB mediated and significantly improved biogas production ( $p < 0.05$ ). Moderate CSB concentrations (2.5 g/L and 5 g/L) resulted in more stable and increased biogas yields by 2.34% and 25.76%, respectively than the control or the highest CSB concentration. However compared to control, high CSB concentration (7.5 g/L) boosted biogas production by 5.54 but produced toxic or inhibitory effect that led to lower biogas production relative to 2.5 and 5.0 gL<sup>-1</sup>. These outcomes signified a dose-dependent (hormetic) relationship between biogas generation and biochar dosage. The biogas data produced significant goodness of fit (0.995-0.998) when predicted with the kinetic model of the modified Gompertz equation. Overall, CSB as a sustainable alternative material has great potential in digestive engineering by anaerobic digestion, but attention must be paid to its dosage.

**Keywords:** Corn stalk biochar, biogas production, hormetic effect, anaerobic digestion, Gompertz equation.

**Corresponding Author:** Utibe A. Ofon, Department of Microbiology, University of Uyo, Uyo, Nigeria; International Center for Energy and Environmental Sustainability Research (ICEESR), University of Uyo, Uyo, Nigeria, e-mail: [utibeofon@uniuyo.edu.ng](mailto:utibeofon@uniuyo.edu.ng)

**Received:** 13 December 2024;

**Accepted:** 21 January 2025;

**Published:** 9 April 2025.

### How to cite (APA):

Ofon, U.A., Ndubuisi-Nnaji, U.U., Adegoke, A.A., Udofia, G.E., Shaibu, S.E., Fapojuwo, D.P., ... & Nelana, S.M. (2025). Characterization, hormetic effects and kinetic modeling of corn stalk-derived biochar in biogas production. *New Materials, Compounds and Applications*, 9(1), 65-83 <https://doi.org/10.62476/nmca.9165>

## 1. Introduction

In today's society, where environmental sustainability and renewable energy are burning issues of global concerns, discovering innovative solutions to organic waste management and energy production remains the need of the hour. Anaerobic digestion technology has emerged as a promising approach to address these challenges by converting organic waste materials into valuable biogas, a renewable energy source (Ndubuisi-Nnaji *et al.*, 2021; Zeng *et al.*, 2022). One can mention the benefit of converting an environmental liability (waste) into an environmental asset (bioenergy), adding value to residues that are usually erroneously wasted and depending on how they are used, inserting them into the context of the circular bioeconomy (Nadaleti, 2019). However, the efficiency and stability of anaerobic digestion processes can be influenced by various factors, including the characteristics of the feedstock and inhibitory substances (Ryue *et al.*, 2020).

One potential solution that has garnered significant attention in recent years is the use of biochar as accelerant to enhance biogas production during anaerobic digestion (Chen *et al.*, 2020). Biochar is pyrolysed biomass intended for anaerobic digestion improvement, through its use as a carbonaceous material for anaerobic digestion processes (Ghodake *et al.*, 2021; Karimi *et al.*, 2022; Li *et al.*, 2020). Biochar possesses unique properties such as high surface area, porosity and stability (Leng *et al.*, 2021). It has shown immense potential in improving soil fertility, carbon sequestration and pollutant remediation (Ma *et al.*, 2020). Moreover, many studies have indicated the numerous benefits of this addition in the acceleration of the biogas production process - anaerobic digestion (Liu *et al.*, 2021). Its positive impact on enhancing biogas production and mitigating process inhibition in anaerobic digestion systems are well documented in literature (Chiappero *et al.*, 2020; Zhao *et al.*, 2021; González *et al.*, 2022). In recent times, a number of studies have utilized different agrowaste for biochar preparation for use in the enhancement of biogas production. The agrowaste feedstock included sawdust (Kumar *et al.*, 2023); rice straw (Harun *et al.*, 2022); biogas residue (Ovi *et al.*, 2021); coconut shell (Singh *et al.*, 2024) and wood (Gupta *et al.*, 2022). Agricultural waste such as cornstalk - a potential substrate for biochar preparation, are known to be rich in biopolymers; cellulose, hemicellulose, lignin, among other organics (Liu *et al.*, 2019). Despite the potential benefits of using biochar to enhance biogas production through anaerobic digestion, these potential is influenced by the physical as well as the chemical characteristics of the biochar in use (Khalid *et al.*, 2021). There exists a critical gap in understanding the biochar characteristics not to mention the precise dose-response relationship (hormetic) effects of biochar on biogas generation, when applied to other waste substrates; in this case, the codigestion of paper waste and chicken manure. In particular, the synergistic effects of biochar on the anaerobic digestion of paper waste and chicken manure remain relatively unexplored to an extent in spite of that fact that these two waste streams hold significant energy recovery potential and are abundant in Nigeria and many regions of the world (Abdulfatah *et al.*, 2021; Ma *et al.*, 2021; Odejebi *et al.*, 2024; Ijaola *et al.*, 2024).

Studying the complex interaction between biochar characteristics and feedstock in the context of biogas production is vital for augmenting anaerobic digestion processes and developing sustainable waste management strategies. In this study, we carefully examined the properties of biochar and its dose-response relationship to gain valuable

insights for maximizing biogas yields, improve process stability and make way for efficient energy recovery from the paper waste and chicken manure feedstock.

## **2. Materials and Methods**

### ***2.1. Sample Collection***

The chicken manure (CM) sample was collected from Vika Farms Limited, a commercial farm in Mbak Etoi, Uyo. The farm adopts a battery cage system that generates copious quantity of chicken droppings. Paper waste (PW) was collected from offices within the Department of Microbiology, Faculty of Biological Sciences Building Complex, University of Uyo, for the anaerobic digestion process. Cow dung used as inoculum was collected from the Department of Animal Science Farm Yard, Faculty of Agriculture, University of Uyo, Nigeria. Corn stalk (CS) used for preparation of biochar was collected from the National Cereal and Research Institute, Aka Road, Uyo, Akwa Ibom State.

### ***2.2. Substrate Pretreatment***

The paper waste underwent a mechanical pretreatment process using milling machine to facilitate the degradation of cellulose and hemicellulose components during anaerobic digestion. The waste paper was shredded into small particles manually using scissors and milled using a manual Corona Lander grinder. This was followed by deinking by hydrothermal pretreatment which was carried out by boiling at 100 °C for 15 minutes to reduce its crystallinity and remove refractory compounds.

### ***2.3. Biochar Preparation***

Biochar was produced by the pyrolysis of feedstock (corn stalk) at 550 °C for 2 h. The feedstock were dried in an air oven at 80-100°C. Before oven drying and pyrolysis, corn stalk sample was left for 72 h under sun to dry so as to reduce the moisture content of the feedstock (El-Mashad *et al.*, 2022). The dried feedstock was pyrolyzed using a muffle furnace in oxygen-free conditions at 550°C for 120 min, with a heating rate of 15 K/min. After the pyrolysis, the corn stalk biochar (CSB) was ground and sieved through a 100-mesh sieve. The prepared biochar was stored at 4°C for subsequent use.

### ***2.4. Experimental Setup and Biogas Potential Assay***

Four (4) of 100 mL laboratory-scale reactors were used, as described by Ofon *et al.* (2022). The anaerobic digestion process was conducted in replicates of three with batch-type reactors where a mixture of feedstock, inoculum and biochar was added once to the reactors with a working volume of 70 mL (to allow headspace for biogas accumulation). The optimum paper waste chicken manure (PW: CM) mixing ratio (based on VS) was established at 65:35% according to a study by Zhao *et al.* (2020). To evaluate the effect of different doses of biochar on anaerobic digestion, four (4) dosages were utilized for this experiment: 0 g/L - control, 2.5 g/L, 5 g/L and 7.5 g/L of the biochar was added to the reactors. The biochar dose was selected according to the results obtained from a previous study by Chen *et al.* (2020).

After charging the reactors with the waste mixtures and biochar, the reactors were crimped using a 20 mm capsizer standard hand-held crimper (JG Finneran 9300-20, USA)

and incubated thermostatically at 45°C in a water bath for 44 days. The reactors were manually agitated twice daily to mix. Daily biogas yield was measured by the liquid displacement method in a graduated inverted cylinder by bubbling the gas in lime solution to absorb CO<sub>2</sub> (Ofon *et al.*, 2024). The experimental design for the anaerobic co-digestion of paper waste and chicken manure supplemented with biochar is represented in Table 1. Blank experiments digesting cow dung (inoculum) alone were used to correct and account for background methane in the inoculum as follows:

$$\text{Biogas production} = \frac{\gamma t(\text{total biogas volume from treatment}) - \gamma_0 (\text{biogas volume from control})}{\text{VS of substrate added}} \quad (1)$$

**Table 1.** Experimental design for the anaerobic co-digestion of paper waste and chicken manure supplemented with biochar

Digesters	Substrate composition	Biochar concentration (g/L)
1	PW : CM : I	CS 2.5
2	PW : CM : I	CS 5
3	PW : CM : I	CS 7.5
4	PW : CM : I	0 (control)

PW - paper waste, CM - chicken manure, I - Inoculum, CSB - corn stalk biochar.

### 2.5. Physical and chemical characterization of substrates and biochar

Biochar characterization was performed in Chemistry Laboratory, Nelson Mandela University, South Africa. The carbon, nitrogen and hydrogen contents were quantified using CHN Flash 2000 elemental analyzer (Thermo Scientific, UK) for paper waste, chicken manure and biochar samples. Elemental composition of biochar and paper samples were determined by energy dispersive X-ray spectroscopy (EDX) coupled with scanning electron microscopy (EDX-800). The Diamond Crystal ATR (Attenuated Total internal Reflectance) accessory was used to analyze surface functional groups using a Fourier transform Infrared (FTIR) spectrometer (Alpha II Bruker, United States). The biochar sample was placed directly on the diamond crystal plate and Opus 7.8 software was used to analyze the transmittance data. Paper waste, chicken manure and biochar were characterized for their pH, total and volatile solids contents, total volatile fatty acids, moisture and lignocellulose content. The pH was measured using well-calibrated pH meter (Hanna, USA), TS and VS were determined by gravimetric procedures following standard protocols of AOAC (2016), lignin, cellulose and hemicellulose fractions were determined according to the methods described by Mansor *et al.* (2019). Cation exchange capacity was determined by the methylene blue adsorption method described by Rihayat *et al.* (2018). Surface morphology and microscopic images of pretreated paper waste and biochar samples were generated by scanning electron microscopy (Nova Nano SEM 230, USA). The biochar was degassed at 80° C for 20 hours and then later was evaluated using nitrogen adsorption/desorption at -196°C using a Brunauer-Emmett-Teller (BET) analyzer (ASAP 2020 Micrometrics Inc.) to determine the surface area and pore size.

### 2.6. Microbiological Analysis

The detection, isolation and enumeration of total heterotrophic bacteria, coliform, anaerobic bacteria and Salmonella Shigella loads was analysed following using standard plate count procedures. Succinctly, serial dilution of paper waste (substrate), chicken manure (co-substrate) and cow dung (inoculum) was carried out in sterile phosphate

buffer saline to obtain  $10^6$  dilution and 1 mL of diluted samples were individually onto selected agar plates of nutrient agar (for heterotrophic count), MacConkey agar (selective for coliform), Schaedlar agar (for anaerobes) and Xylose Lysine Deoxycholate (XLD) selective for *Salmonella* and *Shigella*. All media were products of Oxoid UK and sterilized accordingly as instructed by the manufacturer. Except for Schaedlar plates, all cultures were incubated between 35-37 °C for 24-48 h. However, anaerobes were incubated in anaerobic jar with GasPak system for 48 h at 37 °C. Developing colonies were counted using the Quebec colony counter and reported as colony forming units per gramme (CFUg<sup>-1</sup>). All isolated organisms were characterized by Gram staining and a series of biochemical tests for their identification using Cheesbrough (2006) and Bergey's Manual of Determinative Bacteriology (Holt *et al.*, 1994).

### 2.7. Kinetics model and Data Analysis

To validate the biogas (CH<sub>4</sub>) production data during anaerobic digestion of paper waste, the modified Gompertz equation (Equation 2), a non-linear regression model was utilized. The single factor analysis of variance (ANOVA) was employed to compare means and test whether the varying biochar doses (0, 2.5, 5.0, 7.5 gL<sup>-1</sup>) affect biogas productivity (dependent variable). The One factor ANOVA was performed on all biogas data generated using the SPSS (Statistical Package for the Social Science, version 22) at  $\alpha = 0.05$ , representing 95% significant level to determine the effect of biochar dosages on biogas production. Predicted values were used to generate the fitness curve of the (measured) experimental data to the model (simulated data). Kinetic model simulation and validation was extrapolated from the coefficient of determination (R<sup>2</sup>) and standard error.

$$B(t) = B_o \times \exp \left\{ -\exp \left( \frac{R_{max} \times \exp(1)}{B_o} \right) (\alpha - t) + 1 \right\} \quad (2)$$

$B(t)$  = cumulative CH<sub>4</sub> yield at time  $t$  (ml g<sup>-1</sup>VS);  $B_o$  = bioCH<sub>4</sub> potential of feedstock (ml g<sup>-1</sup>VS);  $R_{max}$  = maximum CH<sub>4</sub> production rate (ml g<sup>-1</sup>VS.d<sup>-1</sup>);  $t$  = duration of digestion;  $\alpha$  = lag phase period;  $\exp(1) = 2.718282$ .

## 3. Result and Discussion

### 3.1. Physicochemical Characteristics of Substrate, Inoculum and Biochar

The physicochemical properties of the substrate (paper waste and chicken manure), inoculum and CSB are presented in Table 2. All the samples were analysed for pH, total solids (TS), volatile solid (VS), total volatile fatty acids (TVFA), C/N ratio, cellulose, hemicellulose, lignin and moisture content. The measured pH values in this study ranged from 6.8 to 7.9 for the substrates, inoculum as well as biochar and were within acceptable optimal range for stable and efficient anaerobic digestion. Variations in pH highly influence anaerobic digestion and low pH values may inhibit the microbial activity of methanogenic bacteria, which are very sensitive to operational parameters during anaerobic digestion. According to Ruiz *et al.* (2023) and Marchetti *et al.* (2020), the preferred pH value for anaerobic digestion processes aimed at biogas production is between 6.5 and 8.5. Pan *et al.* (2019), who studied the effect of biochar on anaerobic digestion of chicken manure, observed and reported that biochar produced at a higher temperature (550°C) were more alkaline than those produced at a lower temperature (350°C) supporting our relatively low pH of 7.5 tending towards alkalinity. The TS and

VS results showed that chicken manure reached the highest VS values (90.3%), thus indicating that most of the solids were volatile and available for degradation. CSB showed high VS percentages of 74.5. On the contrary, paper waste showed the lowest VS (60.1%), indicating that almost 39.9% of the TS in paper waste was inert matter that would be difficult to consume (break down) by bacteria in the anaerobic digesters, hence, the need for codigestion with chicken dropping. A high percentage of cellulose was detected from the paper waste (89.2%) and a relatively high (66.1%) hemicellulose, indicating a highly fibrous material which through pretreatment and hydrolysis can be converted to sugar, making paper waste ideal for biofuel production. However, compared to cellulose and hemicellulose, a relatively low (14.6%) lignin fraction was observed suggesting fewer inhibitors during hydrolysis and fermentation (Li *et al.*, 2018), all of which are favourable for biomass to bioenergy (biogas) production from the analysed paper waste.

Anaerobic digestion needs equilibrated concentrations of carbon (organic matter) and nitrogen (nutrients) to be efficiently carried out. Therefore, the C/N ratio is one of the most influential parameters in the anaerobic digestion process. In this study, paper waste and cow dung had the highest C/N ratios of 28.7 and 22.5, respectively, suggesting their suitability as good feedstock for anaerobic digestion. On the other hand, chicken manure had a low C/N ratio of 12.8. The elevated C/N ratio of paper waste compensated for the low C/N ratio obtained from chicken dung in order to achieve successful anaerobic digestion, hence, the rationale for co-digestion. The biochar's moderate CEC ( $10.0 \text{ meq}100\text{g}^{-1}$ ) helped in buffering the digester system and mitigated the effects of deleterious substances like ammonia. This contributed to a stable digestion process. A moderate surface area of  $15.6 \text{ m}^2/\text{g}$  was also observed, suggesting that biochar derived from corn stalk can serve as a support medium for anaerobic microbes (especially methanogens). Studies have shown that a moderate surface area can enhance the microbial population and increase the contact between microbes and substrates, leading to more efficient biogas production (Kundu *et al.*, 2023; Qui *et al.*, 2019).

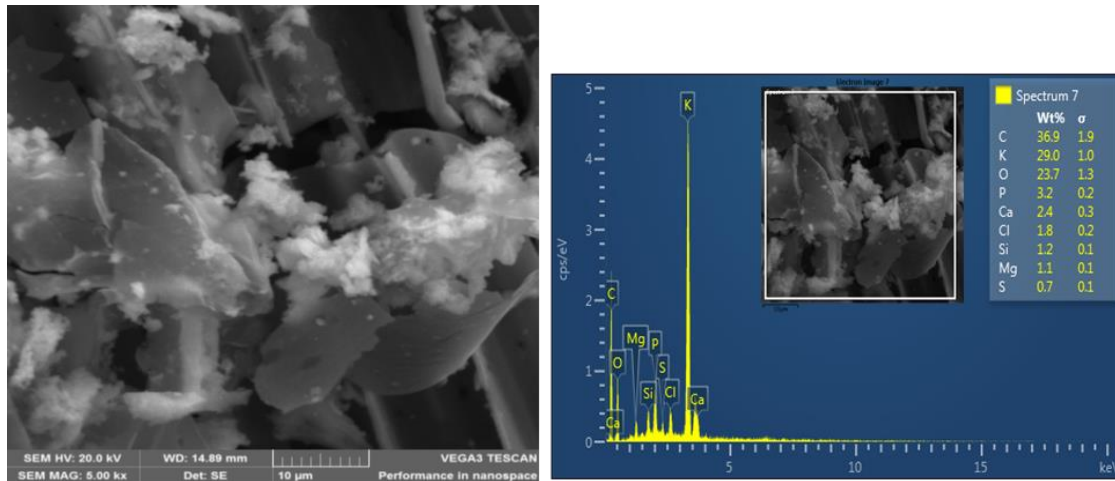
**Table 2.** Physicochemical characteristics of paper waste, chicken manure, cow dung and corn-stalk biochar

Properties	Paper waste (substrate)	Chicken manure (co-substrate)	Cow dung (inoculum)	Corn stalk (biochar)
pH	6.8	7.9	7.8	7.5
TS (%)	305.2	98.6	30.0	80.1
VS (TS%)	60.1	90.3	80.0	72.3
TVFA (g/L)	10.5	20.2	40.9	50.2
C (%)	80.4	24.5	65.1	41.6
N (%)	0.5	5.3	3.8	2.5
H (%)	6.5	5.2	6.3	5.8
C/N ratio	28.7	12.8	22.5	27.8
Cellulose (%)	89.2	NA	NA	6.3
Hemicellulose (%)	66.1	NA	NA	4.7
Lignin (%)	14.6	NA	NA	3.6
Moisture (%)	NA	23.8	10.1	NA
CEC ( $\text{meq}100\text{g}^{-1}$ )	NA	NA	NA	10.0
Surface area ( $\text{m}^2\text{g}^{-1}$ )	NA	NA	NA	15.6
Pore size (nm) mesopores	NA	NA	NA	20.1
Pore volume ( $\text{cm}^3\text{g}^{-1}$ )	NA	NA	NA	0.06

NA - Not Applicable

The surface area and mesoporous structure provided additional sites for microbial growth, which enhances the efficiency of substrate breakdown by anaerobic microorganisms, potentially increasing methane production. The pore size and pore volume of 20.1 nm and 0.06 cm<sup>3</sup>g<sup>-1</sup>, respectively allowed for moderate retention of organic matter, biogas and liquids within the biochar matrix for adsorption (Eltohamy *et al.*, 2023). This improved the contact between microbes and substrates, leading to higher degradation rates and biogas yields as observed in the study.

The SEM image at magnification ×5000 (Figure 1a) showed the detailed surface morphology of the CSB as porous and irregular structure with numerous visible pores, voids, surface texturing, high degree of roughness and layered structures providing more active sites and available surface area for anaerobic digestion microbial colonization and nutrient adsorption during anaerobic digestion. These surface features are a direct consequence of the controlled thermal degradation of lignocellulosic material during pyrolysis, leading to the formation of a porous carbon structure. There is the evidence of whitish particulate residues such as silicon, potassium, calcium or other inorganic compounds on the surface which is indicative of the precursor material and pyrolysis conditions as depicted in Figure 1b.



**Figure 1.** SEM image (a) and EDS spectrum (b) of corn stalk biochar

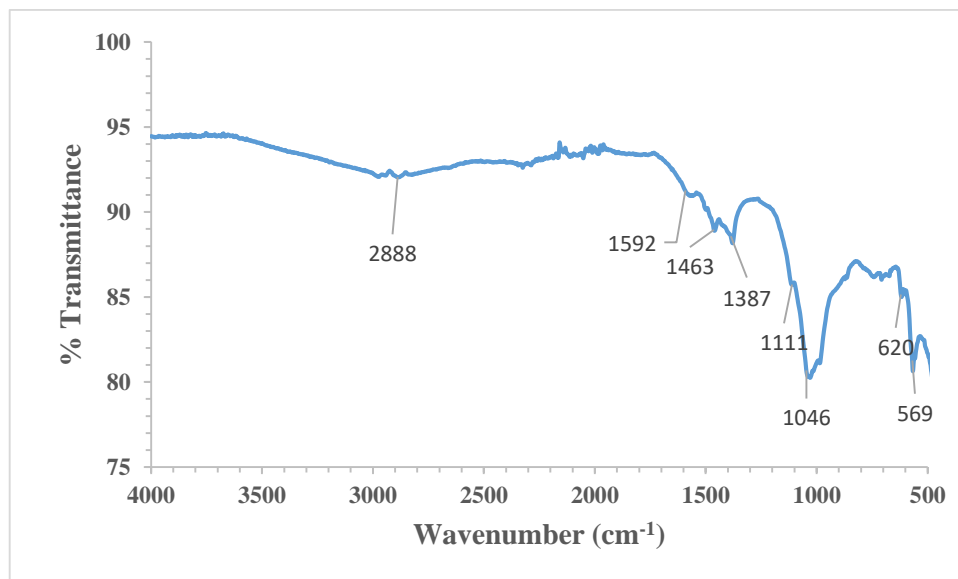
This significantly improved biogas production in biochar-amended digesters by promoting anaerobic digestion microbial attachment and mass transfer processes involving bioenergy generation. As a support medium, this surface structure of the biochar encourages biofilm formation that helps stabilize microbial communities to enhance the anaerobic digestion process efficiency through ammonia adsorption, reducing its toxicity that can lead to process failure (Zhang & Wang, 2021).

The EDS analysis identified that the biochar contains a high weight percentage (Wt%) of carbon (36.9%), potassium (29.0%) and oxygen (23.7%), along with other essential elements such as phosphorus (3.2%), calcium (2.4%) and magnesium (1.1%), among others (Figure 1b). Carbon is a major component of the CSB which is expected given the carbon-rich nature of biochar. This is consistent with the transformation of the corn stalk biomass into a carbonaceous material during the pyrolysis process. This provides the needed framework for microbial attachment and biofilm formation, enables adsorption and release of other organic and inorganic nutrients to maintain a balanced

microbial environment - a process called nutrient exchange (Wong & Ogbonnaya, 2021). Potassium as a required nutrient for microbial growth and activity serves as buffer and regulates osmotic pressure in the anaerobic digestion system (Zhang *et al.*, 2024). The presence of oxygen (O) is significant, likely associated with oxygen-containing functional groups such as hydroxyls, carbonyls and silicates, which are commonly found in biochar. These functional groups play a crucial role in determining the biochar's reactivity, surface chemistry and potential interactions with other substances. Oxygen which is part of the different oxides and organic compounds existent in the studied biochar can enhance the adsorption of nutrients and facilitate the binding of anaerobic digestion microbes, thus improving process efficiency (Wang *et al.*, 2020). Phosphorus though present in little amount enhances the breakdown of organic matter and methane production by promoting microbial metabolism in the form of ATP - the energy currency of a cell (Sun *et al.*, 2020). The detection of calcium (Ca) indicates the presence of calcium-based compounds, likely originating from the corn stalk or environmental sources. Calcium-containing minerals, such as calcium oxide (CaO) or calcium carbonate (CaCO<sub>3</sub>), may be present, contributing to the overall mineral content of the biochar. Magnesium (Mg) and potassium (K) further suggest the retention of alkaline earth metals and alkali metals, respectively, typically found in plant biomass. These elements may form alkaline salts or contribute to the biochar's basicity, which can influence its potential as a soil amendment or in other applications. Calcium and magnesium (micronutrient) act as buffer during anaerobic digestion process, averting acidification that can hinder biogas productivity by binding organic acids including volatile fatty acids (VFAs). Both elements also serve as co-factor for various microbial enzymes and enhances the overall anaerobic digestion process output (Paritosh *et al.*, 2020). The presence of silicon (Si) indicates the likely presence of silica (SiO<sub>2</sub>) or silicates, which are common in plant-based biomass. Silica is often retained during pyrolysis and can significantly impact the structural integrity and porosity of biochar. The detection of sulfur (S) points to the presence of sulfate compounds, potentially derived from sulfur-containing substances in the feedstock or from atmospheric sulfur during pyrolysis. Chlorine (Cl) suggests the incorporation of inorganic halide salts, which could originate from halogenated components in the feedstock. Phosphorus (P) is observed, likely as phosphates such as calcium phosphate (Ca<sub>3</sub>(PO<sub>4</sub>)<sub>2</sub>). These phosphate compounds could have been retained during pyrolysis and may have implications for the biochar's potential as a nutrient source in agricultural applications.

CSB contained various chemical bonds and functional groups that significantly affected its properties and use in the anaerobic digestion process (Figure 2 and Table 3). Peaks related to C-H bonds, including aldehydic C-H, CH<sub>2</sub> and CH<sub>3</sub> bending vibrations, showed the presence of alkyl, alkene groups and inorganic compounds (Coates, 2000). These groups may have contributed to the biochar's hydrophobic nature and its capacity to adsorb organic compounds, as well as its overall stability and structure, with alkene groups enhancing chemical reactivity. The FTIR spectrum of corn stalk-derived biochar (CSB) reveals both organic and inorganic functional groups, reflecting its biomass origin and the transformations it underwent during pyrolysis. A weak peak at 2888 cm<sup>-1</sup> suggests the presence of aldehyde groups, likely resulting from oxidation or functionalization, with a minimal concentration. Similarly, the peak at 1592 cm<sup>-1</sup> corresponds to weak C=O stretching in amide groups, indicating residual proteinaceous or nitrogen-containing compounds. In contrast, a strong peak at 1463 cm<sup>-1</sup> corresponds to CH- bending, highlighting the hydrocarbon-rich nature of the biochar. The peak at 1387 cm<sup>-1</sup> suggests

the presence of methyl or nitro-containing compounds, with a potential contribution from CH<sub>3</sub> bending or NO<sub>2</sub> stretching vibrations.



**Figure 2.** FTIR spectra of biochar used during codigestion of paper waste and chicken manure

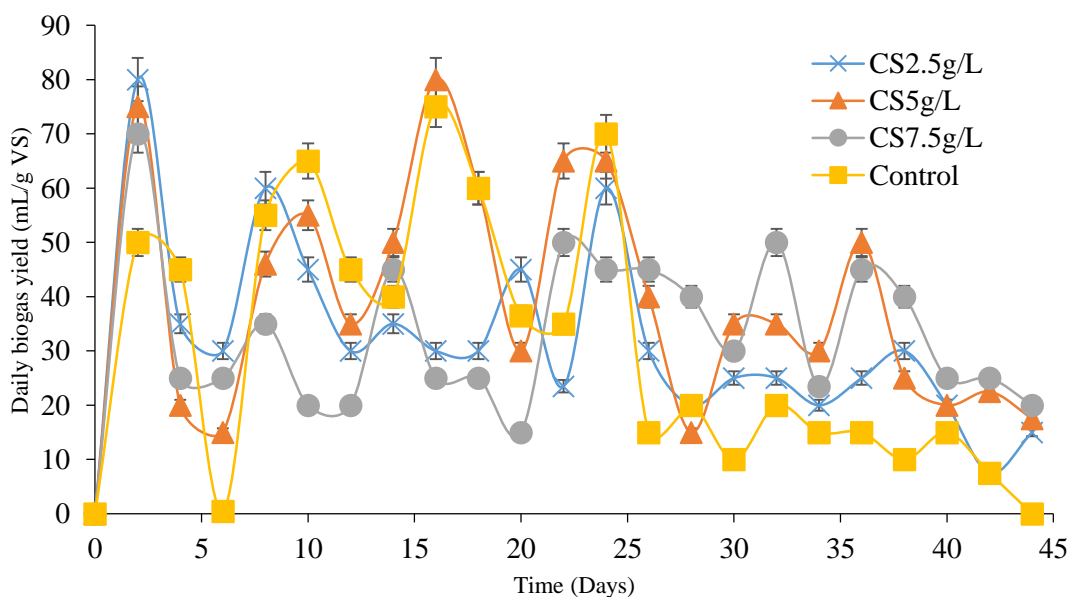
**Table 3.** Functional groups and chemical bonds of biochar obtained from corn stalk

Absorption peak (cm <sup>-1</sup> )	Chemical bond	Intensity	Functional group	Interpretation
2888	-C-H	Weak	Aldehyde	The absorption around 2888 cm <sup>-1</sup> corresponds to C-H stretching in aldehydic compounds. This suggests the presence of aldehyde groups in the material.
1592	C=O	Weak	Amide	This peak indicates weak C=O stretching in an amide group. This could suggest the presence of protein or amide-containing compounds, but the weak intensity might indicate a low concentration.
1463	CH <sub>2</sub> bend	Sharp, strong	Methylene	This strong, sharp peak corresponds to the bending vibrations of methylene (CH <sub>2</sub> ) groups, commonly found in hydrocarbons and polymer backbones.
1387	CH <sub>3</sub> bend, NO <sub>2</sub> stretch	Sharp	Methyl (CH <sub>3</sub> ), Nitro (NO <sub>2</sub> ) or C-F bonds	This peak represents a sharp bend of CH <sub>3</sub> , stretching in NO <sub>2</sub> or potentially C-F. It suggests the presence of either methyl, nitro groups or fluorine-containing compounds.
1111	Si-O	Weak	Si-O bond	This weak absorption likely corresponds to silicates in the CSB
1046	P-O	Sharp, strong	P-O bond	A strong, sharp P-O peak, confirming the presence of phosphates
620	Ca-Br, Ca-Cl	Sharp, weak	Bromo or Chloro compounds	The sharp, weak peak indicates the presence that inorganic halide salts are present in the CSB suggestive of halogenated compounds containing bromine or chlorine.
569	Ca-Br	Sharp, strong	Bromo compounds	This strong, sharp peak indicates the presence of C-Br bonds, strongly signifying that inorganic halide salts are present in the CSB

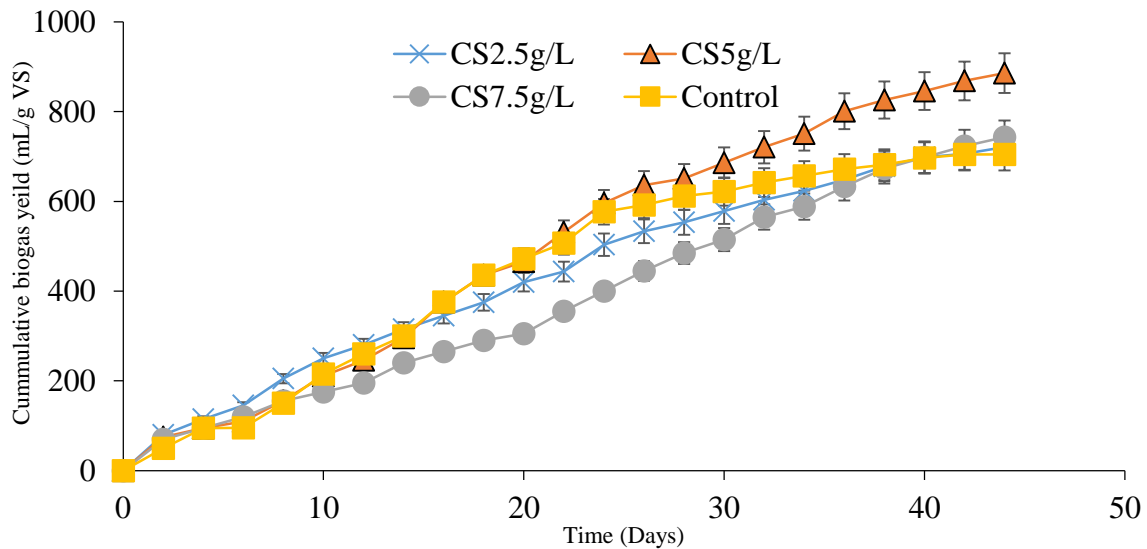
Additionally, the peak at  $1111\text{ cm}^{-1}$  represents Si-O stretching, suggesting the presence of silicates, likely silica, in the biochar. The strong peak at  $1046\text{ cm}^{-1}$  confirms the presence of phosphate groups, most likely calcium phosphate, derived from the corn stalk. Peaks at  $620\text{ cm}^{-1}$  and  $569\text{ cm}^{-1}$  indicate the presence of inorganic halide salts, with Ca-Br being more dominant, suggesting the incorporation of calcium bromide and chloride during pyrolysis. The presence of the silicate, phosphate groups and inorganic halides is corroborated by the EDS spectrum.

### 3.2. Effect of Biochar Dose on Daily and Cumulative Biogas Production

This study evaluated the performance of varying doses of CSB on biogas production during anaerobic co-digestion of paper waste and chicken manure over a 44-day incubation period. The effect of biochar dose on daily and cumulative biogas yield were investigated and the results are shown in Figure 3 and 4, respectively. In terms of daily production rate, all groups, including the control, showed sharp peaks in biogas yield early on, with 2.5 g/L and CS 5 g/L CS-biochar showing higher yields of 80 and 75 mL/gVS on the second day than the control. However, 0 g/L CSB produced a somewhat dampened peak (50 mL/gVS) with maximum value of 80 ml/gVS on day 16 compared to the other CSB concentrations, potentially indicating a slow initiation of the anaerobic digestion process under no biochar amendment condition. But between days 5 and 10 and days 20 and 25 the control group without corn stalk biochar (CSB) outperformed the experimental groups. During these periods, the experimental groups with added CSB may have experienced substrate inhibition due to the higher organic loading rate as excessive nutrients can disrupt microbial activity, especially in AD systems. The addition of CSB may have caused shifts in microbial population dynamics, with competition between different microbial species temporarily reducing the efficiency of the process. Due to its simplicity and reduced need for additional amendment, the observed increase in biogas generation from the control group (within days 5 to 10 and 20 to 25) may be advantageous and economical choice to minimize feedstock input costs while still achieving reasonable biogas yields.



**Figure 3.** Dose-dependent effect of corn stalk (CS) biochar on daily biogas production



**Figure 4.** Dose-dependent effect of corn stalk (CS) biochar on cumulative biogas production

The CS-derived biochar of 2.5 g/L and CS 5 g/L generally maintained higher maximum daily and more consistent peaks compared to the control, indicating that moderate levels of CSB enhanced biogas production. The control (no CSB added) had a noticeably lower biogas yield in the initial phase, followed by fluctuating and relatively lower peaks of daily biogas production throughout the experiment compared to the CS-treated groups. The reduced biogas yield in the control group confirmed the positive influence of CS-derived biochar on biogas production, as its absence resulted in lower microbial activity and/or substrate availability for biogas generation. Generally, low to moderate CSB concentrations (2.5 g/L and 5 g/L) significantly enhanced biogas yield ( $p < 0.05$ ) compared to the control. These concentrations potentially provided an optimal feedstock-to-microbe equilibrium and promoted higher microbial activity and biomethane production. Conversely, higher concentration of CSB (7.5 g/L) resulted in reduced biogas yield compared to the lower concentrations. This can be attributed to substrate inhibition, where excessive nutrients or chemicals limited microbial activity and performance. The high CSB dosage might also cause acidification and/or generate inhibitory by-products. Compared with the control ( $0 \text{ g/L}^{-1}$  corn stalk biochar), the cumulative biogas yield increased by 2.34%, 25.76% and 5.54 when the amount of CSB added was 2.5, 5 and 7.5 g/L, respectively. However, this data showed that further increase in the amount of biochar caused a decline in the cumulative biogas yield, which was 19.67 lower than digester with 5 g/L (Figure 4). While a slight improvement in cumulative biogas yield was observed in the digester supplemented with 7.5g/L biochar, the overlapping error bars indeed indicate that the increase may not be statistically significant. This means the apparent improvement might be due to random variability in the data rather than a true effect of the 7.5 g/L concentration. Biological systems like anaerobic digesters are intrinsically variable and influenced by multiple factors which could obscure trends. At higher concentrations like 7.5 g/L, substrate saturation or inhibition that might limit the activity of microbes can occur, potentially masking any significant differences in yield. A similar study was conducted by Ovi et al. (2020), who added rice husk biochar and palm tree-based biochar to an anaerobic digestion system, and the results suggested that biochar addition not only increased the cumulative methane yield but also improved the

reaction rate. Similarly, they also found that the optimal amount of biochar was 5.0 g/L and greater amounts lowered the biogas yield.

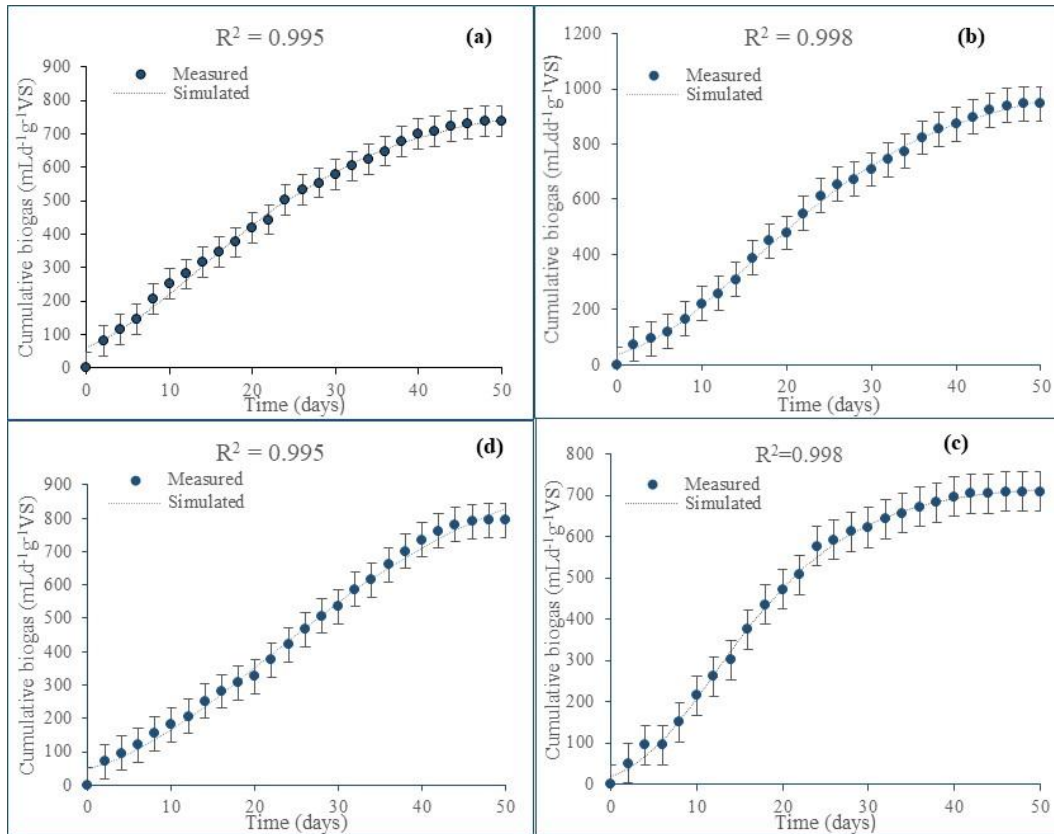
In a previous study by Abubakar et al. (2024), they confirmed that this was mainly because moderate biochar addition could effectively alleviate VFA accumulation, resulting in higher levels of biogas occasioned by methanogenic activity, while higher biochar concentration would lead to more propionic acid accumulation in the digester, thereby reducing the stability of anaerobic digestion process. A study conducted by Zeng et al. (2022) also showed 15.5-16.08% higher biogas production in biochar-added digesters in comparison to the control without biochar addition.

### 3.3. Modelling kinetics of biogas production by Modified Gompertz Model

The predicted parameters of the studied kinetic model are shown in Table 3. In the modified Gompertz used, the percentage difference between the measured (experimental) values and predicted data from the model indicated low percentage difference that ranged from 0.81-0.95%. Specifically, all biochar amended reactors showed lower percentage differences as follows; 2.5 g/L (0.95%), 5.0 g/L (0.88%), 7.5 g/L biochar (0.88%) while the control reactor without biochar amendment showed the highest difference between measured and simulated data. The little deviations observed in the theoretical (predicted) and experimental values (1%) suggest that all the modified Gompertz model could predict very accurately the performance of the reactors (Elsayed *et al.*, 2022). The highest maximum biogas production ( $B_0$ ) = 954.36 mLg<sup>-1</sup>VS was achieved with 5.0g/L biochar with biogas production rate ( $R_{max}$ ) of 28.22 mLg<sup>-1</sup>VS.d<sup>-1</sup>. This showed that 5.0 g/L concentration of biochar was most suitable for microbial fermentation for biogas production in the biogas digester. This was trailed by ( $B_0$ ) = 800.5 mLg<sup>-1</sup>VS from 7.5g/L biochar and  $R_{max}$  of 20.085mLg<sup>-1</sup>VS.d<sup>-1</sup>. Maximum biogas yield ( $B_0$ ) of 743.0 mLg<sup>-1</sup>VS and  $R_{max}$  20.774 mLg<sup>-1</sup>VS.d<sup>-1</sup> was obtained when biochar was added at 2.5 g/L concentration. Least predicted  $B_0$  and  $R_{max}$  = 709.5 mLg<sup>-1</sup>VS and 18.827 mLg<sup>-1</sup>VS.d<sup>-1</sup>, respectively was seen in the control reactors with no biochar addition. To estimate the reliability of the kinetic model results, the predicted biogas yields were plotted against the measured experimental values as depicted in Figure 5. The performance of the model and correlation coefficients ( $R^2$ ) ranged from 0.995 to 0.998 - an indication of a goodness of fit. This values were within the range reported by Liu and his co-workers (2020). The lag phase ( $\alpha$ ) of three (3) biochar amended experiments were within the range of 0.6-2.4 days, characteristically shorter than that of the reactor without biochar addition (2.8 days). A longer lag phase that ranged from 0.7-35.7 days was achieved by Wang et al. (2022) during the co-digestion of corn stalk with straw depolymerization wastewater. From the predictive model, biochar addition, shortened the lag phase by encouraging a speedy anaerobic digestion process start-up time, with correspondingly enhanced biogas yield.

**Table 3.** Kinetic study using Modified Gompertz Model

Biochar Treatment (g/L)	Simulated $B_0$ (ml g <sup>-1</sup> VS)	Measured (experimental) data (ml g <sup>-1</sup> VS)	Difference (%)	$R_{max}$ (ml g <sup>-1</sup> VS.d <sup>-1</sup> )	$\alpha$ (day)	$R^2$
0 (control)	709.5	725.3	2.17	18.827	2.804	0.995
2.5	743.0	736	0.95	20.774	-0.681	0.995
5.0	954.36	946	0.88	28.224	2.648	0.998
7.5	800.5	793.5	0.88	20.085	2.431	0.998

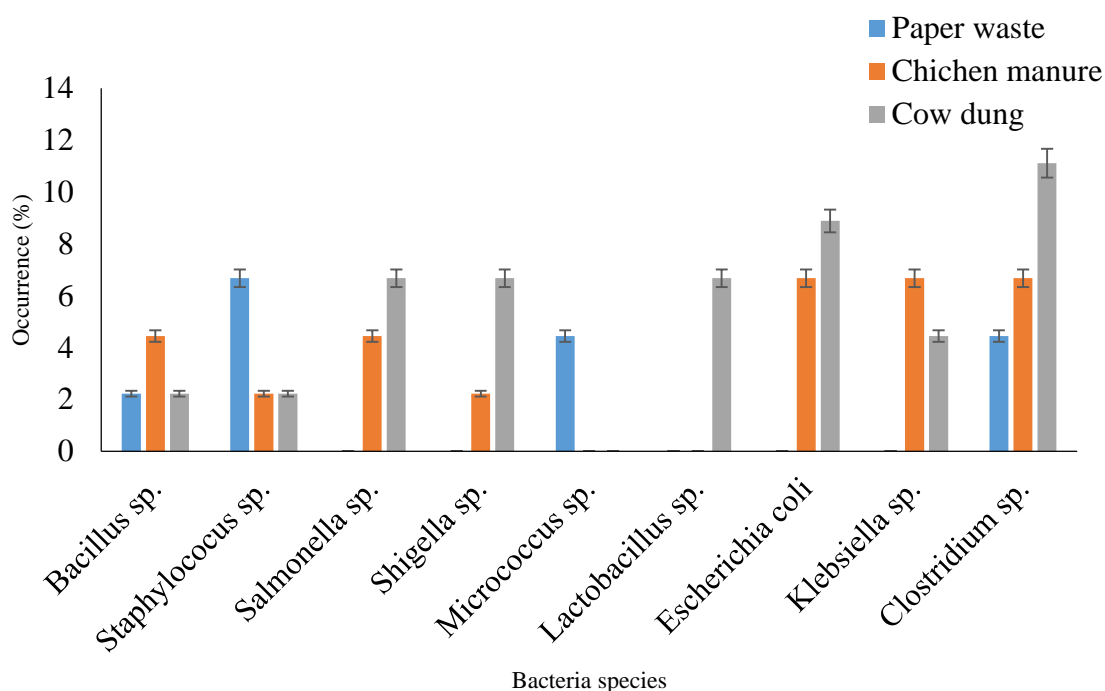


**Figure 5.** Simulation of dose response effect of biochar amendment (a) 2.5g/L; (b) 5 g/L; (c) 7.5 g/L; (d) 0 g/L (control) by Modified Gompertz Model

### 3.4. Microbial profile of pre-digestion substrate, co-substrate and inoculum

The microbial load in raw pre-digestion substrate and inoculum was observed for all bacterial groups and is shown in Table 4 and Figure 6. The microbial analysis of the substrates revealed significant differences in microbial counts across the various waste types. Chicken manure exhibited the highest total heterotrophic bacterial count ( $6.2 \times 10^5$  CFU/g), followed closely by cow dung ( $5.2 \times 10^5$  CFU/g), whereas paper waste recorded a considerably lower count ( $2.5 \times 10^3$  CFU/g). Similarly, the total coliform count was highest in chicken manure ( $3.2 \times 10^5$  CFU/g) and cow dung ( $2.8 \times 10^5$  CFU/g), with paper waste showing the lowest count ( $1.2 \times 10^3$  CFU/g). For anaerobic bacteria, cow dung demonstrated the highest count ( $1.6 \times 10^5$  CFU/g), followed by chicken manure ( $1.1 \times 10^5$  CFU/g), while paper waste again recorded the lowest count ( $1.3 \times 10^2$  CFU/g). Interestingly, no growth of *Salmonella* and *Shigella* was observed in paper waste, whereas chicken manure and cow dung had substantial counts of  $3.0 \times 10^5$  CFU/g and  $1.5 \times 10^5$  CFU/g, respectively. These results highlight the rich microbial composition of organic wastes like chicken manure and cow dung, compared to the relatively lower microbial load in paper waste. The result indicated that the highest total heterotrophic bacterial count, total coliform count and total *Salmonella-Shigella* count were observed in chicken manure (substrate), with values of  $6.2 \times 10^5$ ,  $3.2 \times 10^5$  and  $3.0 \times 10^5$  CFU/g, respectively. However, the highest total anaerobic bacterial count was observed in cow dung, with values of  $1.1 \times 10^5$  CFU/g. The bacterial isolates obtained from substrates and inoculum

in this study were *Staphylococcus* sp., *Lactobacillus* sp., *Escherichia coli*, *Bacillus* sp., *Clostridium* sp., *Salmonella* sp., *Shigella* sp. and *Klebsiella* sp (Figure 6).



**Figure 6.** Percentage occurrence of cultivable bacteria from substrates and inoculum before anaerobic digestion

**Table 4.** Total bacterial population present in pre-anaerobic digestion substrate and inoculum

Microbial Count	Paper waste	Chicken manure	Cow dung
Total Heterotrophic Bacteria Count (CFU/g)	$2.5 \times 10^3$	$6.2 \times 10^5$	$5.2 \times 10^5$
Total Coliform count (CFU/g)	$1.2 \times 10^3$	$3.2 \times 10^5$	$2.8 \times 10^5$
Total anaerobic bacterial count (CFU/g)	$1.3 \times 10^2$	$1.1 \times 10^5$	$1.6 \times 10^5$
Total Salmonella and Shigella count (CFU/g)	No Growth	$3.0 \times 10^5$	$1.5 \times 10^5$

These organisms are similar to those reported earlier (Ndubuisi-Nnaji *et al.*, 2020a; 2020b). The isolation of *Staphylococcus* sp. and *Escherichia coli* conforms to the study of Nwankwo (2014), who obtained these bacterial isolates from the digestion of paper waste and cow dung. The isolation of *Lactobacillus* sp. and *Clostridium* sp. corroborates with the study of Dudek *et al.* (2019), who obtained these bacterial isolates from the digestion of brewers' spent grain using biochar as an additive. *Staphylococcus* sp. and *Bacillus* sp. are often present in anaerobic digestion systems and contribute to the breakdown of organic matter. They help in the initial stages of feedstock decomposition by breaking down complex organic compounds into simpler forms. *Lactobacillus* sp. is known for its role in producing lactic acid during fermentation. In anaerobic digestion systems, it contributes to the acidification of the environment, creating conditions favourable for other syntrophic microorganisms involved in the digestion process. *Escherichia coli* (*E. coli*) is a versatile bacterium that can play a role in the breakdown of organic matter (Paulista *et al.*, 2020). However, certain strains of *E. coli* can also indicate contamination and may pose risks to human health, so their presence needs

to be monitored (Jiang *et al.*, 2020; Tian *et al.*, 2022). *Clostridium* sp. is crucial in the anaerobic digestion process as it performs the acetogenesis stage, an essential phase in anaerobic digestion before methanogenesis - producing acetate from simple organic compounds (Tukangan *et al.*, 2021). Nguyen *et al.* (2018) reported that *Salmonella* sp., *Shigella* sp. and *Klebsiella* sp. are potential pathogens that might find their way into anaerobic digestion systems, although they can be obtained as a result of possible contamination of feedstock or other sources, they have also been implicated with the degradation of polymers into monomers and other easily utilizable forms. The significance of these bacteria in anaerobic digestion systems lies in their diverse roles, from initial decomposition to specific stages of anaerobic digestion.

#### 4. Conclusion

The effects of biochar dose on the biogas production potential from anaerobic co-digestion of paper waste and chicken manure were elucidated. Biochar supplementation facilitated the anaerobic digestion process by creating a suitable environment for biogas production at a thermophilic temperature. Compared with the control (725.3 mL/g VS), biochar treatments (corn stalk) enhanced biogas yields. CSB at 5g/L demonstrated the highest increase at 886 mL/g VS, followed closely by 7.5g/L CSB at 793.5 mL/g VS, while 2.5 g/L CSB showed a slightly lower enhancement at 736.0 mL/g VS. Specifically, CSB supplemented at 5 g/L exhibited a significant ( $p > 0.05$ ) increase in the cumulative biogas yield, which was 25.76% higher than that of the control. Moderate biochar concentrations (2.5 g/L and 5 g/L) resulted in higher and more stable biogas yields than the control or the highest CS concentration. High CS-derived biochar concentrations (7.5 g/L) produced toxic or inhibitory effect on microbial activity, leading to lower biogas production. Overall, CSB as a sustainable alternative material has great potential in digestive engineering by anaerobic digestion due to its cost-effectiveness and excellent functions.

#### Acknowledgements

The authors appreciate the University of Uyo for the award of staff development grant bestowed on Utibe A. Ofon by the as well as the Institution-based Research (IBR) Grant provided by TETFUND Nigeria to Godwin E. Udofia. We are appreciative to the Laboratory staff at the Department of Chemistry, Nelson Mandela University, Port Elizabeth, South Africa for the assistance in characterization studies reported in this work.

#### References

- Abdulfatah, S., Dayyabu, I. & Adamu, I. (2021). Production of biogas from co-digestion of cow dung, chicken dropping and cow rumen wastes. *FUDMA Journal of Sciences*, 5(4), 413-417.
- Abubakar, A.M., Haruna, N.M., Ahmed, Z.D., Abdul, A.Z., Abubakar, M.A., Ali, A.M., ... & Thiagarajan, B. (2024). Unlocking biogas production potential: Evaluating the environmental impact and biodegradability of pharmaceutical and medical wastes. *Sigma Journal of Engineering and Natural Sciences*, 42(4), 1261-1291.
- Bhatnagar, N., Ryan, D., Murphy, R. & Enright, A.M. (2022). A comprehensive review of green policy, anaerobic digestion of animal manure and chicken litter feedstock potential-Global and Irish perspective. *Renewable and Sustainable Energy Reviews*, 154, 111884.

- Bhatt, A.H., Tao, L. (2020). Economic perspectives of biogas production via anaerobic digestion. *Bioengineering*, 7(3), 74.
- Cheesbrough, M. (2005). *District Laboratory Practice in Tropical Countries*, part 2. Cambridge University Press.
- Chiappero, M., Norouzi, O., Hu, M., Demichelis, F., Berruti, F., Di Maria, F., ... & Fiore, S. (2020). Review of biochar role as additive in anaerobic digestion processes. *Renewable and Sustainable Energy Reviews*, 131, 110037.
- Coates, J. (2000). Interpretation of infrared spectra, a practical approach. *Encyclopedia of Analytical Chemistry*, 12, 10815-10837.
- Dudek, M., Świechowski, K., Manczarski, P., Koziel, J.A. & Białowiec, A. (2019). The effect of biochar addition on the biogas production kinetics from the anaerobic digestion of brewers' spent grain. *Energies*, 12(8), 1518.
- Elsayed, M., Andres, Y. & Blel, W. (2022). Modeling of sludge and flax anaerobic co-digestion based on combination of first order and modified Gompertz models: Influence of C/N ratio and headspace gas volume. *Desalination and Water Treatment*, 250, 136-147.
- Eltohamy, K.M., Milham, P.J., Gouda, M., Menezes-Blackburn, D., Khan, S., Liu, B., ... & Liang, X. (2023). Size and composition of colloidal phosphorus across agricultural soils amended with biochar, manure and biogas slurry. *Carbon Research*, 2(1), 16.
- Ghodake, G.S., Shinde, S.K., Kadam, A.A., Saratale, R.G., Saratale, G.D., Kumar, M., ... & Kim, D.Y. (2021). Review on biomass feedstocks, pyrolysis mechanism and physicochemical properties of biochar: State-of-the-art framework to speed up vision of circular bioeconomy. *Journal of Cleaner Production*, 297, 126645.
- González, R., Peña, D.C. & Gómez, X. (2022). Anaerobic co-digestion of wastes: Reviewing current status and approaches for enhancing biogas production. *Applied Sciences*, 12(17), 8884.
- Gupta, J., Kumari, M., Mishra, A., Akram, M. & Thakur, I. S. (2022). Agro-forestry waste management-A review. *Chemosphere*, 287, 132321.
- Harun, S.N., Hanafiah, M.M. & Noor, N.M. (2022). Rice straw utilisation for bioenergy production: A brief overview. *Energies*, 15(15), 5542.
- Holt, R.D., Grover, J. & Tilman, D. (1994). Simple rules for interspecific dominance in systems with exploitative and apparent competition. *The American Naturalist*, 144(5), 741-771.
- Ihoeghian, N.A., Amenaghawon, A.N., Ogofure, A., Oshoma, C.E., Ajieh, M.U., Erhunmwunse, N.O., ... & Martin, A.D. (2023). Biochar-facilitated batch co-digestion of food waste and cattle rumen content: An assessment of process stability, kinetic studies and pathogen fate. *Green Technologies and Sustainability*, 1(3), 100035.
- Ijaola, A.O., Akamo, D., Bamidele, E.A., Jolaoso, L.A., Asmatulu, E., Farayibi, P.K., ... & Manovic, V.D. (2024). Biogas production, upgrading and utilization: A comprehensive review. *Journal of Engineering and Applied Sciences*, 11(1), 17-17.
- Jiang, Y., Xie, S.H., Dennehy, C., Lawlor, P.G., Hu, Z.H., Wu, G.X., ... & Gardiner, G.E. (2020). Inactivation of pathogens in anaerobic digestion systems for converting biowastes to bioenergy: A review. *Renewable and Sustainable Energy Reviews*, 120, 109654.
- Karimi, M., Shirzad, M., Silva, J.A. & Rodrigues, A.E. (2022). Biomass/Biochar carbon materials for CO<sub>2</sub> capture and sequestration by cyclic adsorption processes: A review and prospects for future directions. *Journal of CO<sub>2</sub> Utilization*, 57, 101890.
- Khalid, Z.B., Siddique, M.N.I., Nayeem, A., Adyel, T.M., Ismail, S.B. & Ibrahim, M.Z. (2021). Biochar application as sustainable precursors for enhanced anaerobic digestion: A systematic review. *Journal of Environmental Chemical Engineering*, 9(4), 105489.
- Kumar, J.A., Sathish, S., Prabu, D., Renita, A.A., Saravanan, A., Deivayanai, V.C., ... & Hosseini-Bandegharai, A. (2023). Agricultural waste biomass for sustainable bioenergy production: Feedstock, characterization and pre-treatment methodologies. *Chemosphere*, 331, 138680.
- Kundu, R., Kunnoth, B., Pilli, S., Polisetty, V.R. & Tyagi, R.D. (2023). Biochar symbiosis in anaerobic digestion to enhance biogas production: A comprehensive review. *Journal of Environmental Management*, 344, 118743.

- Leng, L., Xiong, Q., Yang, L., Li, H., Zhou, Y., Zhang, W., ... & Huang, H. (2021). An overview on engineering the surface area and porosity of biochar. *Science of the total Environment*, 763, 144204.
- Li, X., Li, M., Pu, Y., Ragauskas, A.J., Klett, A.S., Thies, M. & Zheng, Y. (2018). Inhibitory effects of lignin on enzymatic hydrolysis: The role of lignin chemistry and molecular weight. *Renewable Energy*, 123, 664-674.
- Li, Y., Xing, B., Ding, Y., Han, X. & Wang, S. (2020). A critical review of the production and advanced utilization of biochar via selective pyrolysis of lignocellulosic biomass. *Bioresource Technology*, 312, 123614.
- Liu, J., Zheng, J., Zhang, J., Yu, D. & Wei, Y. (2020). The performance evaluation and kinetics response of advanced anaerobic digestion for sewage sludge under different SRT during semi-continuous operation. *Bioresource Technology*, 308, 123239.
- Liu, M., Wei, Y. & Leng, X. (2021). Improving biogas production using additives in anaerobic digestion: A review. *Journal of Cleaner Production*, 297, 126666.
- Ma, H., Hu, Y., Kobayashi, T. & Xu, K.Q. (2020). The role of rice husk biochar addition in anaerobic digestion for sweet sorghum under high loading condition. *Biotechnology Reports*, 27, e00515.
- Mansora, A.M., Lima, J.S., Anib, F.N., Hashima, H. & Hoa, W.S. (2019). Characteristics of cellulose, hemicellulose and lignin of MD2 pineapple biomass. *Chemical Engineering Transactions*, 72(1), 79-84.
- Marchetti, R., Vasmarra, C., Bertin, L. & Fiume, F. (2020). Conversion of waste cooking oil into biogas: Perspectives and limits. *Applied Microbiology and Biotechnology*, 104, 2833-2856.
- Nadaleti, W.C. (2019). Utilization of residues from rice parboiling industries in southern Brazil for biogas and hydrogen-syngas generation: Heat, electricity and energy planning. *Renewable Energy*, 131, 55-72.
- Ndubuisi-Nnaji, U.U., Ofon, U.A. & Offiong, N.A.O. (2021). Anaerobic co-digestion of spent coconut copra with cow urine for enhanced biogas production. *Waste Management & Research*, 39(4), 594-600.
- Ndubuisi-Nnaji, U.U., Ofon, U.A., Asamudo, N.U. & Ekong, V.M. (2020a). Enhanced biogas and biofertilizer production from anaerobic codigestion of harvest residues and goat manure. *Journal of Scientific Research and Reports*, 26(3), 1-13.
- Ndubuisi-Nnaji, U.U., Ofon, U.A., Ekponne, N.I. & Offiong, N.A.O. (2020b). Improved biofertilizer properties of digestate from codigestion of brewer's spent grain and palm oil mill effluent by manure supplementation. *Sustainable Environment Research*, 30, 1-11.
- Nguyen, A.Q., Wickham, R., Nguyen, L.N., Phan, H.V., Galway, B., Bustamante, H. & Nghiem, L.D. (2018). Impact of anaerobic co-digestion between sewage sludge and carbon-rich organic waste on microbial community resilience. *Environmental Science: Water Research & Technology*, 4(12), 1956-1965.
- Nwankwo, J.I. (2014). Production of biogas from paper waste blended with cow dung. *Journal of Environmental Science, Toxicology and Food Technology*, 8, 58-68.
- Odejebi, O.J., Ajala, O.O. & Osuolale, F.N. (2024). Review on potential of using agricultural, municipal solid and industrial wastes as substrates for biogas production in Nigeria. *Biomass Conversion and Biorefinery*, 14(2), 1567-1579.
- Ofon, U.A., Ndubuisi-Nnaji, U.U., Shaibu, S.E., Fatunla, O.K. & Offiong, N.A.O. (2022). Recycling anaerobic digestate enhances the co-digestion potential of agro-industrial residues: Influence of different digestates as sources of microbial inoculum. *Environmental Technology*, 43(28), 4472-4483.
- Ofon, U.A., Ndubuisi-Nnaji, U.U., Udo, I.M., Udofia, E.S., Fatunla, O.K. & Shaibu, S.E. (2024). Biogas production potential from anaerobic co-digestion of food waste and animal manure. *Journal of Chemical Society of Nigeria*, 49(1), 79-93.
- Ovi, D., Chang, S.W., Wong, J.W., Johnravindar, D., Varjani, S., Jeung, J.H., ... & Ravindran, B. (2022). Effect of rice husk and palm tree-based biochar addition on the anaerobic digestion of food waste/sludge. *Fuel*, 315, 123188.

- Pan, J., Ma, J., Liu, X., Zhai, L., Ouyang, X. & Liu, H. (2019). Effects of different types of biochar on the anaerobic digestion of chicken manure. *Bioresource Technology*, 275, 258-265.
- Paritosh, K., Yadav, M., Chawade, A., Sahoo, D., Kesharwani, N., Pareek, N. & Vivekanand, V. (2020). Additives as a support structure for specific biochemical activity boosts in anaerobic digestion: A review. *Frontiers in Energy Research*, 8, 88.
- Paulista, L.O., Boaventura, R.A., Vilar, V.J., Pinheiro, A.L. & Martins, R.J. (2020). Enhancing methane yield from crude glycerol anaerobic digestion by coupling with ultrasound or *A. niger*/*E. coli* biodegradation. *Environmental Science and Pollution Research*, 27, 1461-1474.
- Qiu, L., Deng, Y.F., Wang, F., Davaritouchaee, M. & Yao, Y.Q. (2019). A review on biochar-mediated anaerobic digestion with enhanced methane recovery. *Renewable and Sustainable Energy Reviews*, 115, 109373.
- Rihayat, T., Salim, S., Arlina, A., Fona, Z., Jalal, R., Alam, P.N., ... & Juhan, N. (2018). Determination of CEC value (cation exchange capacity) of bentonites from North Aceh and Bener Meriah, Aceh Province, Indonesia using three methods. In *IOP Conference Series: Materials Science and Engineering*, 334(1), 012054. IOP Publishing.
- Ruiz, L.M., Fernández, M., Genaro, A., Martín-Pascual, J. & Zamorano, M. (2023). Multi-parametric analysis based on physico-chemical characterization and biochemical methane potential estimation for the selection of industrial wastes as co-substrates in anaerobic digestion. *Energies*, 16(14), 5444.
- Ryue, J., Lin, L., Kakar, F.L., Elbeshbishy, E., Al-Mamun, A. & Dhar, B.R. (2020). A critical review of conventional and emerging methods for improving process stability in thermophilic anaerobic digestion. *Energy for Sustainable development*, 54, 72-84.
- Singh, P., Dubey, P., Younis, K. & Yousuf, O. (2024). A review on the valorization of coconut shell waste. *Biomass Conversion and Biorefinery*, 14(7), 8115-8125.
- Sun, X., Atiyeh, H.K., Li, M. & Chen, Y. (2020). Biochar facilitated bioprocessing and biorefinery for productions of biofuel and chemicals: A review. *Bioresource Technology*, 295, 122252.
- Tian, T., Dai, S., Liu, D., Wang, Y., Qiao, W., Yang, M. & Zhang, Y. (2022). Occurrence and transfer characteristics of blaCTX-M genes among *Escherichia coli* in anaerobic digestion systems treating swine waste. *Science of the Total Environment*, 834, 155321.
- Tukanghan, W., Hupfauf, S., Gómez-Brandón, M., Insam, H., Salvenmoser, W., Prasertsan, P., ... & Sompong, O. (2021). Symbiotic bacteroides and clostridium-rich methanogenic consortium enhanced biogas production of high-solid anaerobic digestion systems. *Bioresource Technology Reports*, 14, 100685.
- Wang, G., Li, Y., Sheng, L., Xing, Y., Liu, G., Yao, G., ... & Chen, R. (2020). A review on facilitating bio-wastes degradation and energy recovery efficiencies in anaerobic digestion systems with biochar amendment. *Bioresource Technology*, 314, 123777.
- Wang, Z., Sun, Y., Qu, J., Yang, F., Li, J. & Yan, B. (2022). Effects on mesophilic anaerobic digestion performance of corn stalk with the addition/pretreatment of depolymerization wastewater. *Fuel*, 322, 124234.
- Wang, Z., Yun, S., Xu, H., Wang, C., Zhang, Y., Chen, J. & Jia, B. (2019). Mesophilic anaerobic co-digestion of acorn slag waste with dairy manure in a batch digester: Focusing on mixing ratios and bio-based carbon accelerants. *Bioresource Technology*, 286, 121394.
- Wong, J.W., Ogbonnaya, U.O. (2021). Biochar porosity: A nature-based dependent parameter to deliver microorganisms to soils for land restoration. *Environmental Science and Pollution Research*, 28, 46894-46909.
- Zeng, S., Harris, R. & Kan, E. (2022). Effect of alfalfa-derived biochar on anaerobic digestion of dairy manure. *Agronomy*, 12(4), 911.
- Zhang, C., Zhang, R. & Yuan, J. (2024). Potassium-mediated bacterial chemotactic response. *Elife*, 12, RP91452.
- Zhang, M., Li, J., Wang, Y. & Yang, C. (2019). Impacts of different biochar types on the anaerobic digestion of sewage sludge. *RSC Advances*, 9(72), 42375-42386.

- Zhang, M., Wang, Y. (2021). Impact of biochar supported nano zero-valent iron on anaerobic co-digestion of sewage sludge and food waste: Methane production, performance stability and microbial community structure. *Bioresource Technology*, 340, 125715.
- Zhao, S., Chen, W., Luo, W., Fang, H., Lv, H., Liu, R. & Niu, Q. (2021). Anaerobic co-digestion of chicken manure and cardboard waste: Focusing on methane production, microbial community analysis and energy evaluation. *Bioresource Technology*, 321, 124429.
- Zhao, W., Yang, H., He, S., Zhao, Q. & Wei, L. (2021). A review of biochar in anaerobic digestion to improve biogas production: performances, mechanisms and economic assessments. *Bioresource Technology*, 341, 125797.