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IMPROVING BIOENERGY PRODUCTION FROM ANAEROBIC CO-DIGESTION OF PAPER WASTE AND CHICKEN MANURE USING COCONUT SHELL BIOCHAR

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ABSTRACT

This study assessed the effect of biochar addition on anaerobic co-digestion of paper waste (PW) and chicken manure (CM) using standard experimental procedures. Coconut shell pyrolysed at 600˚C (CSB_{600}) supplemented at different doses (2%, 4%, 6%, and 10%w/v) were tested for its effect on biogas production potential in batch anaerobic digestion system. Five (5) digesters (100ml amber borosilicate glass serum bottles), each with homogenized PW:CM in the ratio of 2:1 was used in this study. All digesters were maintained under thermophilic temperature (45˚C) for 44 days. The result showed that 2% CSB_{600} led to an increase in biogas yield of 1135.5mL/gVS compared to control yield of 775mL/gVS. Consequently, cumulative biogas generation was improved by 46.51% for CSB_{550}. Generally, the addition of adequate dose (2%, 4% and 6%) of biochar enhanced cumulative yield while excess amount exhibited deleterious effect on biogas production. Physicochemical parameters of the substrate, inoculum and biochar were determined. Microbial population analysis of substrates and inoculum revealed the presence of *Micrococcus* sp, *Staphylococcus* sp, *E. coli, Bacillus* sp, *Lactobacillus* sp, *Salmonella* sp, *and Shigella* sp. The utilization of biochar in anaerobic digestion system demonstrated a useful strategy for enhancing biogas production.

Keywords: Biochar, Coconut shell, Biogas, Paper waste, Chicken manure

INTRODUCTION

The growing global demand for energy, rising greenhouse gas (GHG) emissions, and climate change have sparked interest in utilizing renewable energy sources, which can help reduce the consumption of fossil fuels and mitigate related environmental issues. (Ma *et al*., 2021). From 1990 to 2020, global energy consumption increased from around 8,800 million tonnes of oil equivalents (Mtoe), including coal, gas, oil, electricity, heat, and biomass, to 16,400 Mtoe. In 2019, renewable energy sources accounted for about 14% of the primary energy supply, while fossil fuels were responsible for 81% of greenhouse gas (GHG) emissions (Chiappero *et al*., 2020). In addition, a significant amount of organic waste generated by industry, agriculture, and daily human activities remains improperly managed. This growing accumulation of organic waste requires global attention, and making for a pressing need for more effective and sustainable disposal methods. Without proper management, this waste not only leads to substantial resource loss but also poses serious risks to the ecology, environment and human health. (Wang *et al*., 2021). These wastes can be converted to useful renewable energy via anaerobic digestion. In recent years, anaerobic digestion has gained significant attention. Furthermore, utilizing AD to process organic waste helps in reducing greenhouse gas emissions (Ngo *et al*., 2022).

Anaerobic digestion (AD) is a process that transforms organic waste into bioenergy (biogas) through a microbial sequence involving four stages: hydrolysis, acidogenesis (primary fermentation), acetogenesis (secondary fermentation), and methanogenesis. (Ofon *et al*., 2022). Biogas, a key byproduct of anaerobic digestion, holds significant value for utilization. It serves as a clean and eco-friendly alternative to fossil fuels. (Wang *et al*., 2021). According to Ngo *et al*. (2024), anaerobic digestion (AD) is a biological process that utilizes microbial activity to convert organic waste into valuable biogas. Typical feedstock for anaerobic digestion (AD) include livestock manure and lignocellulosic agricultural residues such as bagasse and straw, municipal organic waste, sheep rumen, food waste, garden waste, tree waste, wastes from pulp and paper industry and winery waste (Kaltum et al., 2022; Salim et al., 2023; Abubakar et al., 2023). These organic wastes can be utilised to generate electricity and produce other useful digestate products. Lignocellulosic biomass, a viable source of natural sugar polymers, is widely used as a substrate in the anaerobic digestion (AD) process due to its abundance and easy availability. (Wang *et al*., 2021). Lignocellulosic materials hold significant energy potential, with their cell walls comprising up to 55% cellulose and 35% hemicellulose. These components can be hydrolyzed by microorganisms to generate energy-rich products like biogas and biofuels. (Ma *et al*., 2021). In 2018, the China Paper Association reported that paper consumption reached 10.35 million tons (Li *et al*., 2018). Alternative methods for waste paper disposal, such as dumping, incineration, and landfilling, have significant environmental drawbacks and offer limited economic advantages compared to reutilization. As a result, developing more sustainable approaches for managing waste paper is essential (Jansson *et al*., 2020). The high C/N ratio and the unique structure of lignocellulose make it susceptible to volatile fatty acid (VFA) accumulation during the initial stages of the typically prolonged anaerobic digestion (AD) process, hence the need for codigestion. So, the co-digestion of waste paper with other nitrogen-containing substrates like food wastes, animal dung, and algal sludge have been comprehensively investigated by researchers to essentially improve paper waste AD performance (Ajeej *et al*., 2015, Li *et al*., 2018, Begum *et al*., 2021, Zhao *et al*., 2021). Using chicken manure as a co-feedstock for anaerobic digestion (AD) is particularly significant for many countries, as livestock manure frequently constitutes an incredible sources of municipal organic waste (Ngo *et al*., 2022). Compared to chicken manure, corrugated board (CB) and waste office paper (OP) are rich in cellulose, but missing in nitrogen, thereby making the c co-digestion of waste paper (CB and OP) with chicken manure necessary.

Biochar, a low-cost additive with its numerous beneficial properties has shown operational efficiency at even low concentrations during AD (Ngo *et al*., 2022). Biochar, known for its solid carbonaceous properties, serves as a precursor for activated carbon. It is produced by eliminating oxygen during the thermal-chemical conversion of biomass. Various waste materials thast includes agricultural residues, animal manure, wood, and waste sludge, can be utilized as raw materials for its production (Khalid *et al*., 2021). The accumulation of volatile fatty acids (VFA) can be reduced by adding biochar, which enhances syntrophic interactions within an anaerobic reactor. (Html *et al*., 2020). The high specific surface area, cation exchange capacity and porosity of biochar offers it several advantages including contaminant removal during AD. Its incorporation into the anaerobic digestion (AD) process has shown enhanced microorganism immobilization, and improved buffering capacity, increasing resultant methane (CH_4) yields, by promoting direct interspecies electron transfer (DIET) between acid-forming bacteria and methanogens (Khalid *et al*., 2021, Manga *et al*., 2023). Several factors may explain the advantages of biochar, including its ability to alleviate acid inhibition caused by VFA accumulation during the early stages of digestion and to improve the efficiency of organic acid utilization by microorganisms. (Devi and Eskicioglu, 2024). Coconut shell, a waste biomass is generated in quantum by the coconut industry in several countries of the world including Nigeria (Ajien *et al*., 2023). This waste biomass is ordinarily mismanaged through direct disposal by open burning that results in significant waste of green energy (Ighalo *et al*., 2023), environmental pollution, and its attendant health and ecological issues (Kalidasan *et al*., 2023). As a result, there is a strong need for alternative methods of managing coconut waste. One innovative approach involves converting coconut biomass into biochar—a black, carbon-rich material described by its high porosity and strong resistance to decomposition together with a high degree of aromatization,. (Ajien *et al*., 2023). For this study, coconut shell biochar is produced through pyrolysis, a process that utilizes thermal energy to decompose biomass at high temperatures in an atmosphere deficient of oxygen (Wang *et al*., 2021). This study focused on the use coconut shell biochar to improve the biogas yield from the anaerobic co-digestion of paper waste and chicken manure through the promotion of microbial metabolism of organic waste.

MATERIALS AND METHODS

Sample Collection

Paper waste as a substrate was obtained from staff offices at the University of Uyo main Campus, Nwaniba, Uyo. Fresh chicken manure as a co-substrate was gotten from Vika Farms Limited, located at Mbak Etoi, Uyo, Akwa Ibom State. The cow dung (inoculum) was gotten from Akwa Ibom state central abattoir and livestock depot, Nasarawa Itam, Akwa Ibom state. The samples were collected using a clean aluminium foil and stored in sterile plastic bags before being transported to the Microbiology Laboratory, University of Uyo, Uyo for analysis.

Preparation of Biochar

The feedstock used in this study for biochar production was coconut shell and by pyrolyzing the feedstock at the optimum temperature and duration, biochar was produced. Prior to pyrolysis, this feedstock was dried at 80–100˚C in an air oven. Using a muffle furnace operating in an oxygen-free environment, the dried feedstocks were pyrolyzed for 120 minutes at 600˚C at a heating rate of 15 K/min. The biochar was crushed and sieved using a 2mm mesh sieve following pyrolysis. For later use, the produced biochar was kept in storage at 4˚C.

Experimental Design

In order to investigate the role of coconut shell biochar on biogas production potential of paper waste and chicken manure, a batch anaerobic digestion test was carried out using 100mL amber borosilicate glass serum bottles (Wheaton 223766, USA) and 20mm aluminum crimp seal with PTFE\Butyl septa for headspace vial (Wheaton W22422A USA) as reactors with a working volume of 80mL (Ndubuisi-Nnaji *et al.,* 2020). Five (5) reactors were used including control reactor, each reactor was charged with paper waste and chicken manure in the ratio of 2:1 (60:40) (Zhao *et al.,* 2021), 5g of cow dung(inoculum) and varying biochar dosages (2%, 4%, 6% and 10%) as additive in all the reactors. The content of the reactors were denoted as follows: paper waste as PW, chicken manure as CM, cow dung as CD and coconut shell biochar (CSB) and they were designated as follows: 2%CSB reactor contained 65%PW + 35%CM + 5gCD + 1.5gCSB(2%), 4%CSB reactor contained 65%PW + 35CM + 5gCD + 3.0gCSB(4%), 6%CSB contained 65%PW + 35%CM + 5gCD + 4.5gCSB(6%), 10%CSB reactor contained 65%PW + 35%CM + 5gCD + 7.5gCSB(10%). Biochar dosages were added based on total solids (TS) contents. The content of the bioreactors is further explained in Table 1. After charging the reactors with the mixtures, all the reactors were capped using a 20mm cap size standard hand operated crimper (JB Finneran 9300-20, USA) to ensure anaerobiosis. The reactors were incubated thermostatically at 45˚C in the water bath with five (5) minutes of daily agitation. The experiment was conducted for 44 days (until there was no significant biogas production). Biogas generated was quantified volumetrically through downward displacement of liquid by connecting the reactor to an inverted graduated cylinder, the volume of liquid displaced was taken and recorded as the volume of biogas (Ndubuisi-Nnaji *et al.,* 2020).

Table 1: Experimental design and Reactor composition

	2% Biochar	4% Biochar	6% Biochar	8% Biochar	10% Biochar	No Biochar (Control)
PW (%)	60	60	60	60	60	60
CM (%)	40	40	40	40	40	40
CD (%)	5	5	5	5	5	5

Determination of Physicochemical Parameters

The pH of the feedstocks, inoculum and biochar was determined using a pH meter (H198107 pHep). The pH meter values were obtained when the pH probe was submerged in the sample solution. The amount of the three components of lignocellulosic materials in the samples was determined by the method cited by Mansor *et al.,* (2019). The total solids, volatile solids and was determine using methods of Zobeashia *et al*., 2021. For volatile fatty acid, the methods as illustrated by Siedlecka *et al*. (2008) was adopted with slight modifications. The organic carbon and nitrogen in the samples were determined using the Walkley and Black method and Kjelhdal method with slight modifications.

Microbiological Analysis

The microbial population of all the samples were enumerated by standard plate count technique. Nutrient agar was used to isolate heterotrophic bacteria, MacConkey agar was used to isolate total coliforms, and Xylose Lysine Deoxycholate (XLD) agar was used for the isolation of *Salmonella* and *Shigella* species. After performing a 10-fold serial dilution, the bacterial species were cultured using the pour plating method and incubated for 24-48 hours at 37°C. After incubation, colonies were enumerated as Colony Forming Units per gram (CFU/g). Following the count, several colonies were subcultured to produce pure cultures. Biochemical assays were carried out to determine the microbial isolates according to Cheesbrough, 2005. All the isolates were identified according to Bergey's Manual of Determinative Bacteriology.

RESULTS AND DISCUSSION

Effect of Coconut Shell Biochar on Daily and Cumulative Biogas Yield

The impact of CSB on daily biogas yield is illustrated in Figure 1. Across all treatment groups, biogas production commenced on the first day of digestion. Among the treatments, 2% CSB achieved the highest biogas yield of 95 mL/gVS on the fourth day. In comparison, 4% and 6% CSB reached their peak biogas volumes of 80 mL/gVS and 75 mL/gVS on the fourth and eleventh days, respectively. The initial peak in daily biogas production, observed on the fourth day, may be attributed to the methanogenic conversion of easily degradable materials present in the anaerobic digestion (AD) medium (Li et al., 2020). A decline in biogas production after the 25th day could be explained by the partial degradation of readily degradable substrates into volatile fatty acids through hydrolytic acidification, leading to methanogen inhibition (Xu and He, 2021). A similar pattern was reported by Zhao et al. (2021), who linked the reduction in biogas yield to the accumulation of inhibitory intermediates.

The effect of CSB on cumulative biogas production is shown in Figure 2.The 2%, 4%, and 6% of CSB generated the highest cumulative biogas yield of 1135.5mL/gVS, 1066mL/gVS and 972.5mL/gVS compared to control yield, which generated 775mL/gVS volume of biogas. 10% CSB also showed a lower yield of 740mL/gVS. As a result, for 2% and 4% CSB, respectively, the cumulative biogas yield increased by 46.51% and 37.54%. According to the data obtained, using coconut shell biochar at a 2% concentration first increases the production of biogas, which peaks at 1135.5 mL/gVS. This implies that biochar increases the conditions for microbial activity or improves the reactor environment at lower concentrations, which raises the yields of biogas. However, as the concentration of biochar grows to 4%, 6%, and ultimately 10%, there is a notable drop in biogas output. This drop can be due to numerous factors: increased biochar concentrations could upset nutritional balance, impair microbial activity, or produce issues such as over-saturation in the reactor, which impairs mixing and gas exchange, as reported by Shi *et al*. (2022). Reactors with minimal dosages of biochar produced more biogas than those with greater concentrations, although the reactor without biochar produced more than the reactor with 10% biochar. This pattern suggests that at lower concentrations, biochar can greatly increase biogas generation; however, at greater concentrations, its efficiency decreases and it may even become harmful (Ambaye *et al*., 2021). Dudek and his colleagues conducted a research on the effect of biochar addition on biogas production kinetics from the anaerobic digestion of brewers' spent grain in 2019. They reported that minimal biochar dosages (≤ 8%) increased biogas production while biochar overdose (≥10%) inhibits biogas production (Dudek et al., 2019). Similarly, Ihoeghian *et al*. (2023) reported that 5% biochar dosage effectively enhanced biogas production when he and his colleagues conducted a research on biochar-facilitated batch co-digestion of food waste and cattle rumen content.

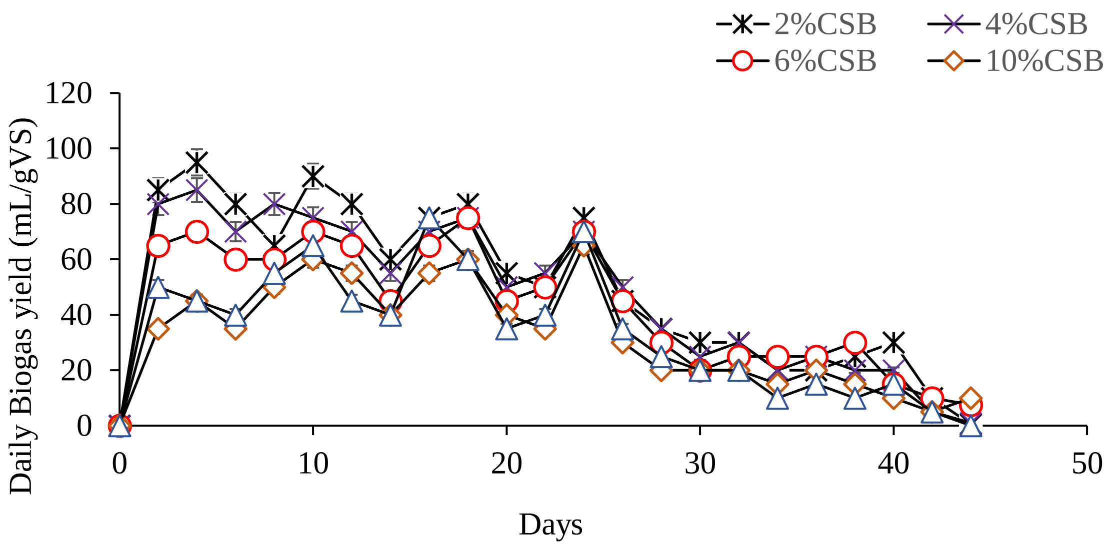

Figure 1: Effect of Coconut Shell Biochar on Daily Biogas Production

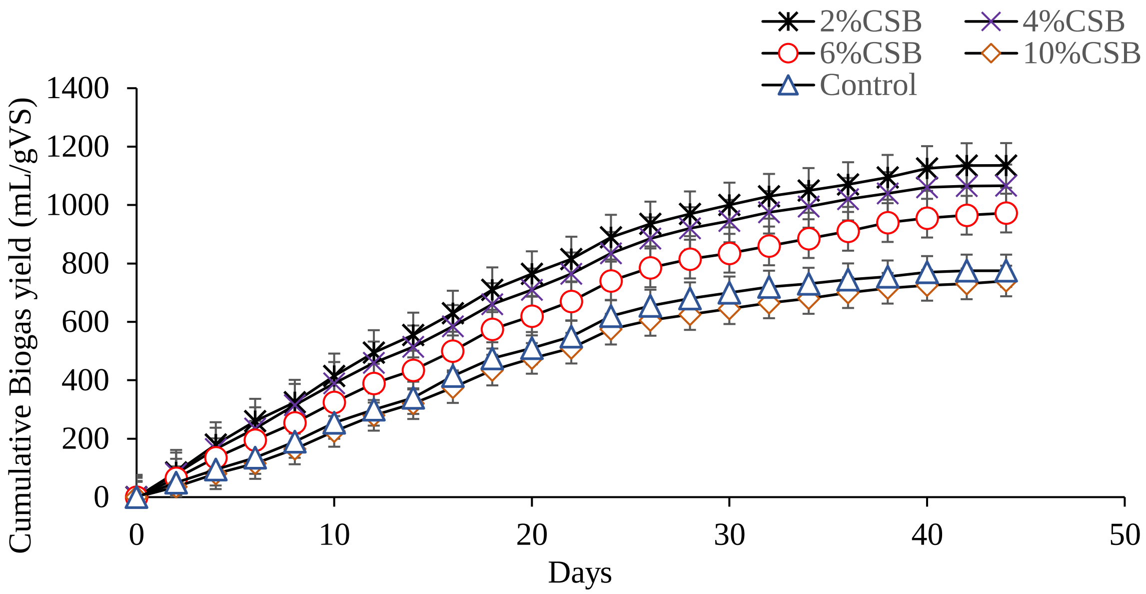

Figure 2: Effect of Coconut Shell Biochar on Cumulative Biogas Production

Physicochemical properties of the Substrates, Inoculum and Biochar

The Table 2 presents the Physicochemical characteristics of substrates (paper waste and chicken manure), inoculum (cow dung) and biochar (coconut shell). Properties such as pH, Carbon-nitrogen ratio, total solids, volatile solids, volatile fatty acid, cellulose, hemicellulose, lignin and moisture content were properly analysed. The pH levels are particularly important since they influence microbial activity. PW has a pH of 6.8, which is suitable for anaerobic digestion, while CM and CSB have higher pH values, suggesting that they could provide a more alkaline environment. This might enhance the activity of methanogenic bacteria, which thrive in neutral to slightly alkaline conditions. However, an excessively high pH, like that of CSB (9.5), could inhibit some microbial populations if not managed properly. Total solids (TS) content reveals the concentration of organic matter available for digestion. PW shows a very high TS, which might suggest it's a solid waste or that the measurement requires clarification. In contrast, CM and CSB have lower TS values of 98.6 and 86.7 respectively, indicating they could contribute more effectively to the digestibility of the mixture, allowing for better flow and mixing in digesters (El Ibrahimi *et al.*, 2021).

The volatile solids (VS) percentage is another critical factor, as it reflects the amount of organic material that can be converted into biogas (Induchoodan *et al*., 2022). Here, CM has a higher VS (80.0%), suggesting it's rich in readily digestible organic matter. PW, with a lower VS, might not contribute as effectively, but the combination of these materials could lead to increased biogas production. Total volatile fatty acids (TVFA) levels are indicative of the degradation process. A high TVFA concentration in CSB (181.3 g/L) suggests an abundance of fermentable material. This corresponds with Chinwe *et al*. (2024) who studied the decomposition process of household waste. The author suggested that while a high TVFA is beneficial, if the co-digestion is not well-balanced, it could lead to acid accumulation that might inhibit methane production. The carbon to nitrogen (C/N) ratio is also vital for microbial growth. An ideal ratio supports effective biogas production, and here, PW, CM, and CSB have ratios that suggest a generally favorable balance for co-digestion. However, CM's lower ratio may require careful management to prevent nitrogen overload, which could hinder the digestion process. In terms of structural composition, the high cellulose and hemicellulose content in PW indicates a strong potential for methane production. CSB does contribute some cellulose and hemicellulose, which could provide beneficial structural properties despite being lower in quantity. Moisture content is essential for supporting microbial activity (Li *et al*., 2019). CM and CD have appropriate moisture levels, this aspect is crucial for facilitating the anaerobic digestion process.

Table 2: Physicochemical analysis of substrate, inoculum and Biochar

Properties	PW	CD	CM	CSB
pH	6.8	7.8	7.9	9.5
TS (%)	305.2	30.0	98.6	86.7
VS (%TS)	60.1	80.0	90.3	64.1
TVFA (g/L)	10.5	40.9	20.2	181.3
C\N ratio	28.7	22.5	12.8	30.1
Cellulose (%)	89.2	NA	NA	15.9
Hemicellulose (%)	66.1	NA	NA	11.3
Lignin (%)	14.6	NA	NA	7.7
Moisture	NA	10.1	23.8	NA

Microbial Load of Paper waste (substrate), Chicken Manure (co-substrate) and Cow dung (inoculum)

Table 5 displays the microbial load in the inoculum and substrate. According to the results, chicken dung (substrate) had the highest total heterotrophic bacteria count (THBC), measuring 3.0 × 10^5 CFU/g. While no growth of coliform, *Salmonella*, or *Shigella* was found in paper waste, the highest total coliform and total *Salmonella*-*Shigella* counts were also found in chicken dung, with values of 2.8 × 10^5 CFU/g and 2.2 × 10^5 CFU/g, respectively. *Micrococcus* sp, *Staphylococcus* sp, *E. coli*, *Bacillus* sp, *Klebsiella* sp, *Lactobacillus* sp, *Salmonella* sp, and *Shigella* sp were the bacterial isolates that were acquired from this investigation following biochemical examination. Figure 7 displays the proportion occurrence of various microorganisms. The presence of *Micrococcus* sp, *Staphylococcus* sp, and *Bacillus* sp in paper waste can be explained by their capacity to use cellulose, a significant component of paper. Certain species of *Bacillus*, *Staphylococcus*, and *Micrococcus* have been shown to create enzymes known as cellulases that convert cellulose to glucose, which they can then use as fuel (Chukwuma *et al.*, 2020). *Shigella* sp, *E. coli*, and *Lactobacillus* sp were all discovered in cow dung, whereas *Salmonella* sp was found in both cow dung and chicken manure, while *Klebsiella* sp. was only found in chicken manure. The isolation of *E. coli* and *Staphylococcus* sp was consistent with that of Nwankwo (2014), who recovered these organisms through the digestion of cow dung and paper waste. *Shigella* sp, *Salmonella* sp, *Lactobacillus* sp, and *Bacillus* sp isolations are consistent with the research of Ndubuisi-Nnaji *et al*. (2020). By using goat manure to codigest harvest waste, the authors encountered these bacterial isolates.

E. coli, *Salmonella enterica*, *Shegella flexineri*, and *Bacillus* were the most common bacteria found in poultry manure, according to a similar study by Zhao *et al*. (2021). Since the microbes derived from this study are often facultative anaerobes, they participate in the hydrolysis and acidogenesis stages of anaerobic digestion, which create metabolic intermediates prior to methanogenesis. Therefore, using cow dung as an inoculum to co-digest paper waste and chicken manure may have produced a wide range of microbial consortiums for the microbial synergism required for a robust AD process.

Table 3: Bacterial count from the microbial analysis of paper waste, chicken manure and cow dung

Microbial Count	Paper waste	Chicken manure	Cow dung
Total Heterotrophic Bacteria Count (CFU/g)	1.9 × 10^3 ± 0.08	3.0 × 10^5 ± 0.05	2.9 × 10^5 ± 1.00
Total Coliform count (CFU/g)	Not detected	2.8 × 10^5 ± 0.08	2.5 × 10^5 ± 0.02
Total Salmonella and Shigella count (CFU/g)	Not detected	2.2 × 10^5 ± 0.03	1.2 × 10^5 ± 0.07

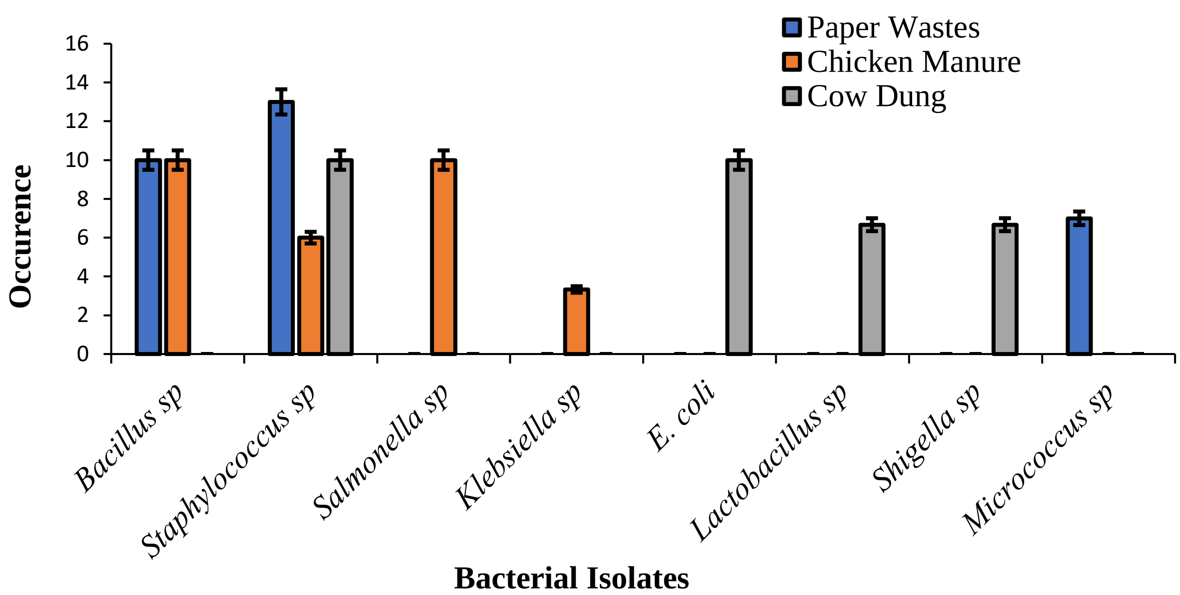

Figure 3: Percentage occurrence of bacteria species from substrates and inoculum

CONCLUSION

The strategy of increasing the efficiency of the anaerobic co-digestion of paper waste and chicken manure with the incorporation of coconut shell biochar showed significant promise as indicated in our results. Addition of biochar promoted improved organic material breakdown and conversion by enhancing the microbial habitat and maximizing nutrient availability, which eventually resulted in higher biogas generation. In addition to addressing waste management issues, this creative approach supports the production of renewable energy. The results highlight the potential of using agricultural by-products like coconut shell biochar to provide more efficient and ecologically friendly waste treatment solutions as research in this field develops. Adopting such approaches can lead to waste-to-energy systems that are more resilient, which will be advantageous for the environment and the economy.

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