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Original Article

Paper Waste Co-Digestion, Combined Milling, and Hydrothermal Pretreatment for Improved Biogas Production

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Abstract

This research, conducted in two experiments, focused on the effect of co-digestion and combined hydrothermal with milling pretreatment of paper waste (PW) and chicken manure (CM) on biogas production. In the first experiment, three reactors labeled B-D contained varying ratios of the feedstock (4:1, 3:2, 1:1) with control reactors A and E respectively containing milled paper waste (MPW) and chicken manure (CM) alone were prepared and assessed for biogas production. In the second experiment, the optimum co-digested mixture (reactor D) with a ratio of 1:1 and cumulative biogas volume (633.5mL/gVS) was selected from the first experiment and duplicated (reactor F) for the hydrothermal pretreatment. All digesters were operated at a temperature of 45oC for 32 days. The results revealed that reactor F, which contained the hydrothermally pretreated paper waste, showed a significant cumulative biogas yield of 1095.5 mL/gVS which was 1.7 times higher, surpassing control reactor D with a yield of 633.5 mL/gVS. Bacterial isolates present in the substrates and inoculum belonged to the following genera: Staphylococcus (50%), Lactobacillus (22.2%), Micrococcus (16.7%), and Bacillus (11.1%). The research outcome showed that co-digestion combined with hydrothermal pretreatment method led to enhanced biogas production. These results highlight how creative pretreatment methods can optimize waste-to-energy operations, supporting both renewable energy production and sustainable waste management.

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1 Introduction

Energy is a fundamental requirement for improving human comfort and meeting everyday basic needs. However, numerous countries, particularly developing ones, are facing energy crises due to heavy reliance on finite fossil fuels [1,2]. Globally, energy security, environmental protection, and economic growth are key drivers of national energy policies. Considering that fossil fuel sources like coal, gas, and oil are expected to be depleted in no distant time, there is a critical need to explore sustainable yet alternative energy sources [3]. Among the renewable energy options, biogas has emerged as a promising resource with both industrial and domestic applications, providing a proficient solution to the global energy crisis [4,5]. Biogas is a renewable energy source produced through the anaerobic digestion of organic matter in municipal waste, such as agricultural waste, manure, sewage or food scraps.

Anaerobic digestion is a biochemical process that involves the degradation of organic resources to simple substances through the activities of microorganisms in oxygen-free condition. Anaerobic digestion is currently an attractive and environmentally friendly biological process for the conversion and treatment of various complex biomass and toxic wastes [6]. Organic resources or waste are used as feedstocks in the production of biogas through anaerobic digestion. Jeswani et al. [7] reported that an estimate of 4.55 Mt of poultry litter is currently available in the UK, of which 2.73 Mt is suitable for conversion to energy. Currently, about 400 million tonnes of paper and paperboard is being produced, and which is estimated to reach up to 500 million tonnes by 2025 [8].

In the generation of biogas, the inclusion of a pretreatment step in anaerobic digestion processes increases the digestibility of lignocellulosic biomass and enhances biogas yields by promoting lignin removal and the destruction of complex biomass structures [9]. The different pretreatment techniques used for lignocellulosic biomasses are generally grouped into physical, chemical, physicochemical, and biological methods [9]. Hydrothermal pre-treatment of organic feedstocks at elevated temperatures/pressures (150–300 °C, initial pressure of 0–60 bar, 2–40 min) has garnered consideration for the production of biofuels from lignocellulosic substrate as it eliminates chemical addition and corrosion-resistant material requirements for hydrolysis reactors [10]. The recalcitrant structure of the lignocellulosic fraction gets easily broken, hemicellulose and lignin are degraded and the cellulose is hydrolysed effectively in the hydrothermal pretreatment, thus rendering them as soluble fraction in the anaerobic digestion process [10]. The paper waste's surface area available for microbial action during digestion is increased when its particle size is reduced through milling. This is important because anaerobic microbes can more easily break down smaller particles, which improves the production of biogas. Furthermore, when mixed with chicken manure, grinding makes the combination more uniform, which promotes better digestion and nutrient absorption. By using heat and pressure when water is present, hydrothermal pretreatment considerably improves the digestibility of paper waste. By dissolving intricate strands and upsetting the paper's structure, this procedure increases the accessibility of organic components to microorganisms [11]. The yield of biogas generated during anaerobic digestion can be greatly increased by hydrothermal pretreatment, which breaks down resistant components into simpler sugars. Anaerobic co-digestion enhances digestion and energy generation by increasing the availability of nutrients for microbes and organic load while reducing inhibitory chemical toxicity through co-substrate dilution [12]. Anaerobic co-digestion can result in synergistic interactions via balance of nutrients, supplementation of trace elements, dilution of toxic and inhibitory compounds, and promotion of microbial diversity [13].

Paper waste management is a complex issue that is mostly caused by the enormous amount that is produced annually. The massive volume of paper waste presents significant obstacles for recycling systems, especially as contaminated materials make it more difficult to turn waste paper back into products that can be used [14]. Materials that can seriously impede recycling operations and make it challenging to create clean, fresh paper include glue, ink, mud, and even food leftovers. Alternative disposal techniques including landfilling, incineration, or plain dumping are used when recycling is not practical. These choices do have some disadvantages, though; they emit a number of dangerous pollutants into the atmosphere, such as carbon monoxide and sulfur dioxide, which can seriously endanger community health and exacerbate larger environmental problems. Furthermore, more chicken dung is being produced as a result of the growing demand for chicken eggs and meat [15]. Even though compost is frequently made from this trash, the sheer volume poses its own difficulties. Ammonia, hydrogen sulfide, methane, and carbon dioxide are among the gases released during the breakdown of chicken dung; these chemicals can be harmful to human health and have a negative impact on air quality. To lessen their detrimental effects on the environment and public health, paper trash and chicken dung must be managed properly. Anaerobic co-digestion offers a sustainable solution to limit waste by turning it into a useful energy source like biogas, which helps to address these urgent challenges. The co-digestion of paper waste with other substrates, such as animal dung, foodwaste, and microalgae, has been studied by a number of researchers [16-18]

While a number of academics have worked on pretreating paper waste to improve the production of biogas, no research has been done on integrated co-digestion and hydrothermal pretreatment of paper waste. Furthermore, given its high generation rate in our area and the complex lignocellulosic structure of paper waste, combined hydrothermal pretreatment and anaerobic co-digestion of paper waste with livestock manure, such as chicken manure, is rare and deserving of investigation, according to our review of the literature that is currently accessible. Therefore, this research aims to examine the impact of combined co-digestion and hydrothermal pretreatment of paper waste for biogas generation.

2 Materials and methods

2.1 Sample collection

The materials utilized for this experiment consisted of waste paper as the primary substrate, chicken manure as the co-substrate, and cow dung as the inoculum. The waste paper was collected from receptacle bins around the University of Uyo, Permanent site, Uyo, Akwa Ibom State. The chicken manure was obtained from Vika Farms which operates a battery cage system, located at Mbak Etoi, in Uyo L.G.A, Akwa Ibom State and transported to the laboratory. The cow dung was collected in a Ziploc bag from the abattoir at Itam market in Itu LGA.

2.2 Pretreatment of paper waste

The paper waste used for this experiment was cut into small pieces using a pair of scissors and pretreated by milling to particle sizes of about 1-2mm diameter using a Corona Landers manual grinder (Landers and Cia, Colombia) with dimensions 11.4” L × 6.7” W × 16.1” H. Hydrothermal pretreatment was further carried out on the milled paper used for the combined pretreatment by subjecting it to a temperature of 121°C for 30 minutes in the autoclave. Fresh cow dung was digested anaerobically for 15 d at 45°C to dissipate background methane before use as inoculum [19].

2.3 Experimental design and bioreactors setup

This research was conducted in two experiments; in the first test, three (3) co-digestion reactors (Wheaton, USA) (labelled reactors B – D) contained varying proportions of the feedstock with control reactors A and E respectively containing milled paper waste (MPW) and chicken manure (CM) alone were prepared and assessed for biogas production. In the second experiment, the optimum co-digested mixture was selected from the first experiment and duplicated (reactor F) for the hydrothermal pretreatment.

A total of six batch bioreactors were utilized for the co-digestion of milled paper waste (PW) and chicken manure and labeled A-F as follows: A = 0:1 PW/CM (Control, chicken manure only), B = 4:1 PW/CM, C = 3:2 PW/CM, D= 1:1 PW/CM, E = 1:0 PW/CM (Control –milled Paper waste only), F =1:1 PW/CM (for the combined milling and hydrothermal pretreatment) as presented in Table 1. Each reactor was operated with a fixed amount of inoculum (5 g) at a thermophilic temperature of 45°C. The experiment was carried out in triplicates using batch mode, employing 100 ml amber borosilicate glass serum bottles as reactors with a working volume of 50 ml [20]. Soapy water was used to conduct a leak test on the digesters to detect any potential leaks [21]. The digesters were then sealed using a typical hand-operated crimper and a 20mm cap size (JG Finneran 9300-20, USA). The reactors were incubated in a water bath at static condition (45°C) for 32 days suitable for biogas producing bacteria. The biogas produced in the anaerobic reactors during digestion was measured through the butyl/PTFE septum using the liquid displacement apparatus containing lime water [Ca(OH)2]. Lime water was used because it allows for a detectable shift in the liquid levels when the carbon dioxide from the biogas bubbles through the lime water and combines with the calcium hydroxide to generate calcium carbonate. Lime water also reduces the possibility of gas escaping into the atmosphere, this makes possible for the precisely quantification of the gas volume produced [22]. By this approach, biogas is efficiently captured and measured. A gas transfer hose was connected to the butyl/PTFE septum valve installed on the lid of the biodigesters, and the end of this hose was placed inside a cylindrical glass column with a capacity of 0.5 L, which was placed upside down and filled with the solution (see Fig. 1). At 2-day intervals, the biogas produced was quantified volumetrically by connecting the reactor to the graduated cylinder. The amount of displaced solution was recorded as the volume of biogas in millilitre (mL).

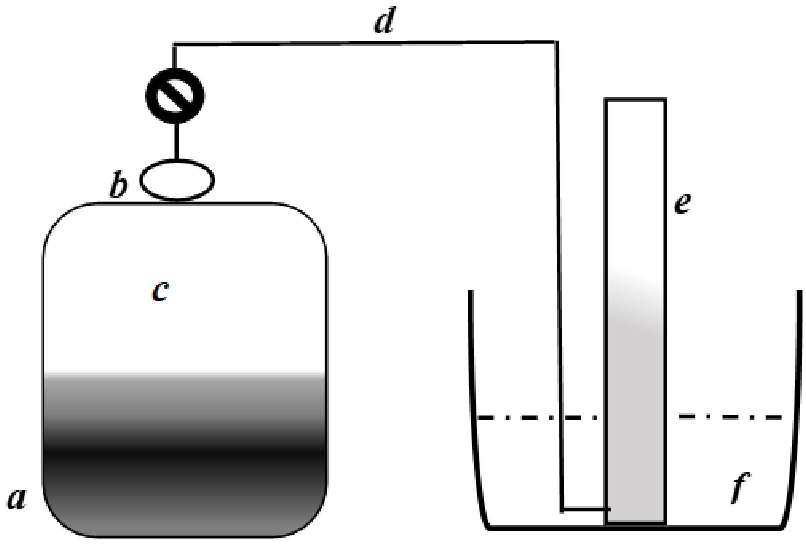

Fig. 1. Schematic illustration of experimental setup. *a* = biogas digesters in water bath at 45 °C treating waste mixtures; *b* = aluminum crimp seal with moulded septum; *c* = headspace for biogas collection; *d* = gas collection hose connected to reverse cylinder, *e* = graduated cylinder; *f* = beaker with lime water solution for liquid displacement.

Table 1. Mixing Ratio in Digesters for Co-digestion of Paper Waste (PW) and Chicken Manure (CM) at 45°C.

Milled Paper Waste				Inoculum (Cow Dung)	Milled + Hydrothermal Pretreated paper waste	
Reactor	**Mixing Ratio PW:CM**	**%PW added**	**S/I Ratio (based on % PW)**		**Reactor**	**Mixing Ratio PW:CM**
A (Control)	0:1	0	0:1	5g	F	1:1
B	4:1	80	8:1	5g		
C	3:2	60	6:1	5g		
D (Control)	1:1	50	5:1	5g		
E (Control)	1:0	100	10:1	5g		

2.4 Determination of physicochemical parameters

The pH of the mixture was measured during the experiment. The pH probe was immersed into the mixture, and the reading was taken using a pH meter. The total solid (TS) content and volatile solid (VS) content of the substrate was determined by gravimetry according to the Association of Official Analytical Chemist [23]. To determine the amount of extractives in the feedstock for lignin, hemicellulose and cellulose, the gravimetric methods reported by Mansor and others was adopted [24]. The volatile fatty acid content was determined in terms of acetic acid concentration as illustrated by [25]. For moisture content, the methods of [26] was adopted with modifications.

2.5 Bacteriological Analysis

Culturing of feedstock (paper waste and chicken manure) as well as inoculum (cow dung) for the detection and enumeration of heterotrophic indicator organisms was performed using the viable plate count method by pour-plating the desired aliquot in appropriate medium described earlier [27]. Nutrient agar was used to isolate total heterotrophic bacteria, MacConkey agar was used to detect total coliform.

Xylose Lysine Deoxycholate (XLD) agar was used in isolating Salmonella and *Shigella* species. The media were prepared according to the manufacturer's guidelines. All media were product of Oxoid, United Kingdom (UK), and were sterilized in the autoclave for 15 min at 121°C except XLD agar which was sterilized by heating to boiling at 100 °C. The samples were handled under aseptic conditions. Following a ten-fold serial dilution, 1ml from 10^5 dilution factor was cultured on all media and was incubated at 37°C for 24 hours. After incubation, discrete colonies appearing on culture media enumerated and reported as colony forming units per gram (CFU/g). The colonies were sub-cultured by repeated plating for further biochemical characterization including gram reaction, spore staining, catalase, coagulase, indole, methyl red, Voges Proskauer, citrate and sugar utilization tests as documented in the *District Laboratory practice for Tropical Countries; Part 2* [28]. All isolates were identified according to *Bergey's Manual of Determinative Bacteriology* [29].

2.6 Data Analysis

Experimental data were reported as values ± standard deviation for all experiments conducted in triplicates. Analysis of variance (ANOVA) for experimental data was performed using Excel 2016 to compare mean values of biogas production data from combined pretreatment reactors with those of single pretreatment, that is milling only with α fixed at 0.05. Calculated p values less than or equal to 0.05 ($p \leq 0.05$) where adduced to be statistically significant.

3 Results and discussion

3.1 Effect of combined milling and hydrothermal pretreatment on daily biogas yield

The purpose of this study was to determine the impact of combined co-digestion and hydrothermal pretreatment of paper waste on biogas yield. The combined use of co-digestion and hydrothermal pretreatment was shown to be an effective method for enhancing biogas production from paper waste co-digested with chicken manure. This has the potential to contribute to the development of more sustainable and environmentally friendly waste management practices.

The daily biogas production resulting from the co-digestion of milled paper waste (MPW) and chicken manure (CM) over a 32-day digestion period is illustrated in Fig. 2. Biogas production commenced on the second day following the loading of the substrate, co-substrate, and inoculum into the reactor. The biogas yield was notably higher in the co-digestion ratio of 1:1. The ranking of daily biogas yield in the co-digestion of only milled paper waste were as follows: reactor D (50% PW) > reactor C (60% PW) > reactor B (80% PW) > reactor A (0%PW) > reactor E (100%PW). Reactor A with no paper waste addition achieved its peak daily yield of 55 mL/gVS on the 6th day, while reactor B treating 80% paper reached its highest value of 60 mL/gVS on the 14th day. Reactor C with 60% paper waste demonstrated its highest peak value of 65 mL/gVS on the 5th, 7th, and 10th day. On the other hand, reactor D (digesting 50%PW) achieved its peak value of 87.5 mL/gVS on the 7th day, while reactor E reached its peak value of 15mL/gVS on the 12th day.

The daily biogas production resulting from the co-digestion of combined milled and hydrothermally pretreated paper waste and chicken manure (CM) is presented in Fig. 3. Biogas production commenced on the second day indicating a shorter lag phase. In reactor F, with a mixing ratio 1:1 involving combined pretreatment by milling and hydrothermal reaction, there was higher performance in terms of biogas yield compared to the control reactor D (Control), which utilized milled paper alone. Reactor F (combined pretreatment – CPT) achieved a maximum daily production of 120mL/gVS on the 11th day, while reactor D reached a maximum of 87.5mL/gVS. The daily biogas production commenced on the second day following substrate loading. Average daily biogas yield of the combined pretreatment was significantly higher ($p < 0.05$) than that of the milling pretreatment alone by 72.86%. This result agrees to an earlier report by [10], who observed a 132% average biogas yield in his study of improvement of anaerobic digestion of lingocellulosic biomass by hydrothermal pretreatment. In a previous study by [30], it was revealed that combination of hydrothermal and alkaline pretreatments reduced the recalcitrance of Miscanthus, and the results showed that the digestion performance was much better than single pretreatment.

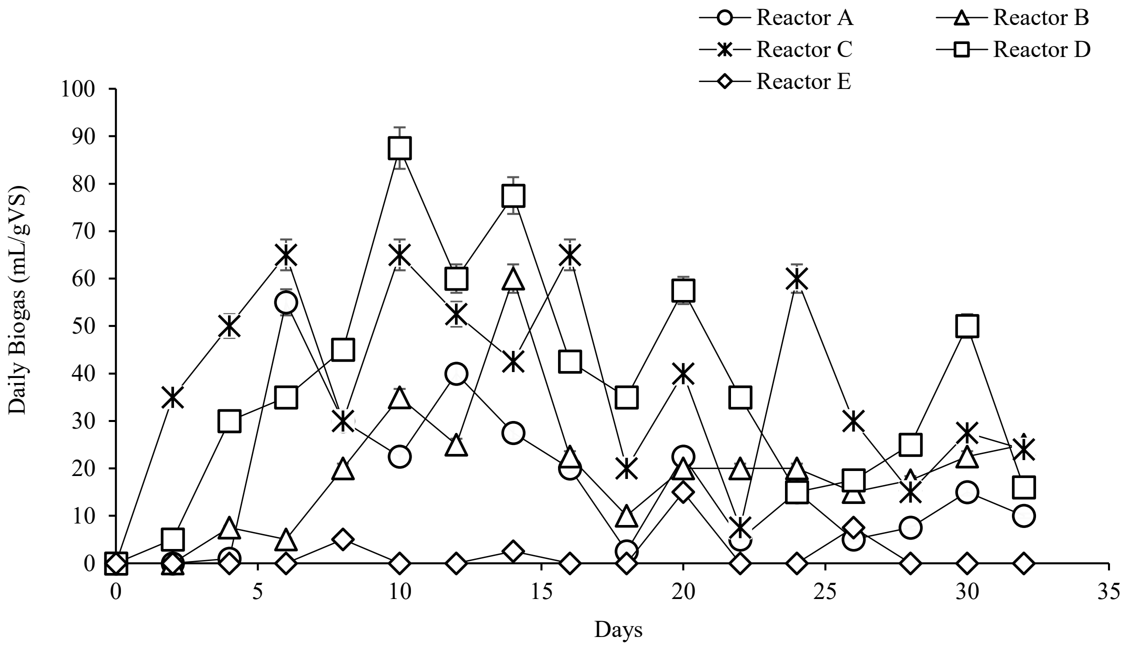

Fig. 2. Daily biogas yield from the anaerobic co-digestion of milled paper waste and chicken manure.

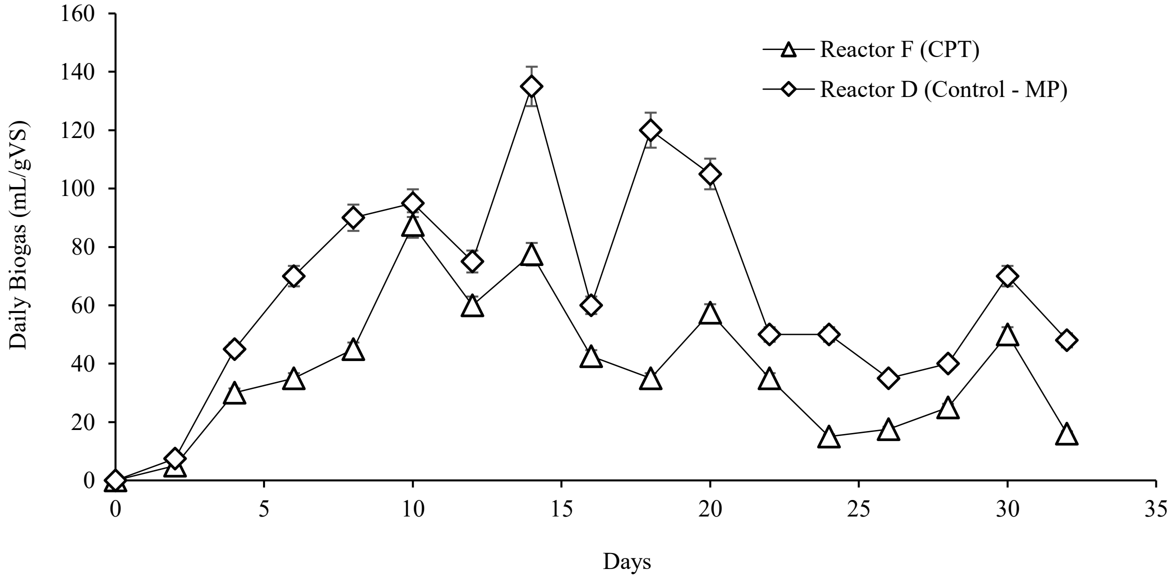

Fig. 3. Daily biogas yield from the anaerobic co-digestion of hydrothermally pretreated paper waste and milled paper with chicken manure.

3.2 Effect of combined milling and hydrothermal pretreatment on cumulative biogas yield

To assess the impact of different mixing ratios on the co-digestion of milled paper waste (MPW) and chicken manure (CM), the cumulative biogas yields were 278.5mL/gVS, 325mL/gVS, 629mL/gVS, 633.5mL/gVS, and 30mL/gVS for reactors A, B, C, D, and E, respectively, as shown in Fig. 4. To compare the effect of combined milling and hydrothermal pretreatment on paper waste for co-digestion with chicken manure against milling pretreatment alone, the result showed that the total biogas production from the combined pretreatment exceeded that from milled pretreatment alone as shown in

Fig. 5. Combined pretreatment reactor – CPT (F) exhibited the highest cumulative biogas yield of 1095.5mL/gVS, surpassing the control milled paper (Reactor D) which yielded 633.5mL/gVS. The cumulative biogas yields as shown in Fig. 3 were 278.5 mL/gVS (Reactor A), 325 mL/gVS (Reactor B), 629 mL/gVS (Reactor C), 633.5 mL/gVS (Reactor D), and 30 mL/gVS (Reactor E). Reactor F, which contained the combined pretreated paper waste (PW/CM 1:1) exhibited the highest cumulative biogas yield (1095.5 mL/gVS), greater than the control (milled paper) which yielded 633.5 mL/gVS. This cumulative biogas yield was 1.7 times higher in combined pretreatment than milling pretreatment alone. The results indicated that the hydrothermal pretreatment could degrade the organic particulates into soluble and even small-molecular substances to significantly improve the anaerobic digestibility and greatly facilitate biogas production [31]. According to Cybulska et al. [32], excluding chemicals from the process of pretreatment reduces its environmental impact and eliminates the costs in the pretreatment and product recovery, making the hydrothermal pretreatment a rather green process. The combined use of co-digestion and hydrothermal pretreatment on paper waste generated both increased biogas yield and a digestate that can be used as a fertilizer or soil amendment.

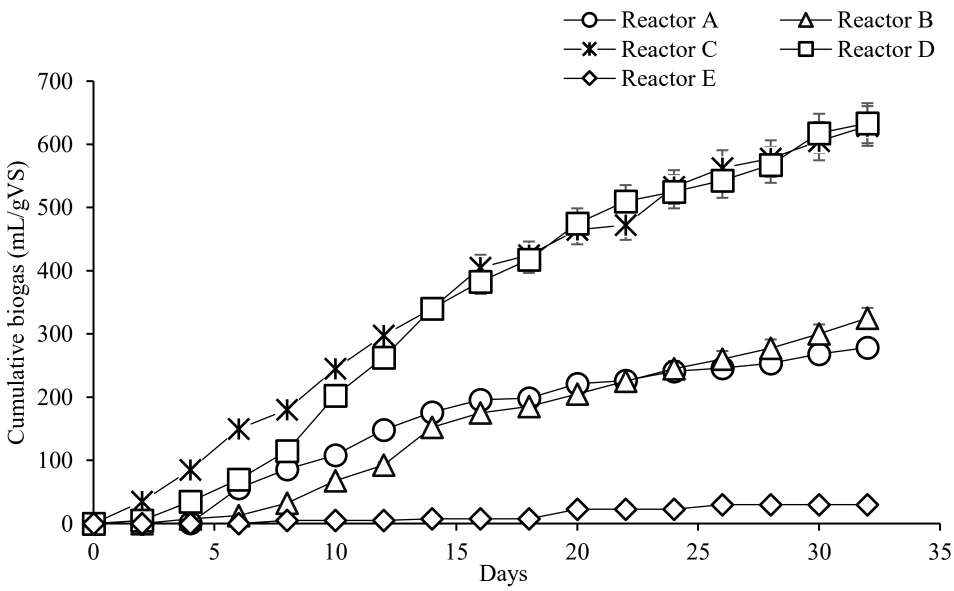

Fig. 4. Cumulative biogas yield from the anaerobic co-digestion of milled paper waste and chicken manure.

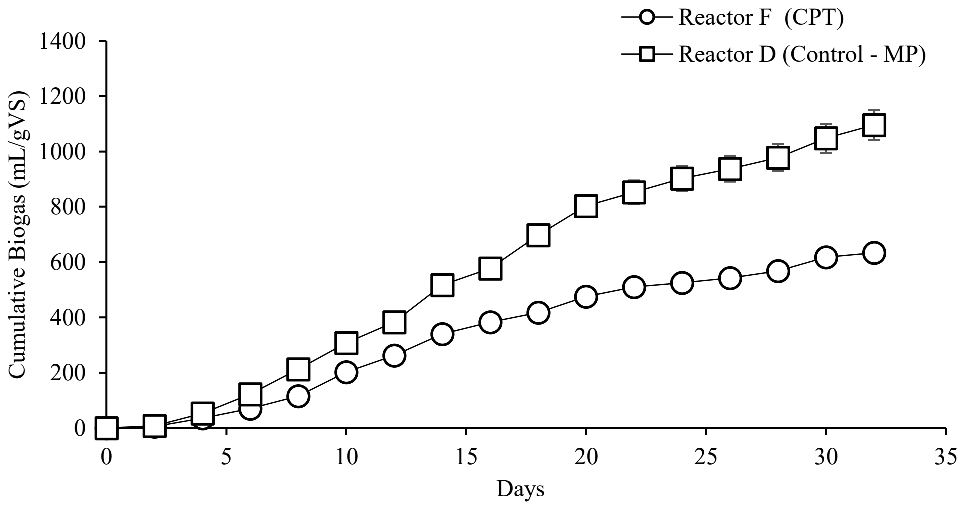

Fig. 5. Cumulative biogas yield from the anaerobic co-digestion of hydrothermally pretreated paper waste and milled paper with chicken manure.

3.3 Effect of pretreatment on the physicochemical properties of the substrate, co-substrate and inoculum

The physicochemical properties of the milled paper, hydrothermally treated paper, co-substrate and inoculum were evaluated and shown on Table 2. The pH values of the milled paper, hydrothermally treated paper, co-substrate and inoculum were 6.8, 6.1, 7.9 and 7.8 respectively. The pH values of the single substrates were within the optimum range for biogas production, that is 6.5-7.8 [33]. The C/N ratios of milled paper waste, hydrothermally treated paper, chicken manure and inoculum were 28.7%, 31.2%, 12.8% and 22.5%, respectively. The C/N ratio of hydrothermally treated paper waste was the highest which may have contributed to the increased biogas production. The total solids (TS) of milled paper waste, hydrothermally treated paper, chicken manure and inoculum were 305.2%, 286.5%, 98.6% and 30.0% respectively. Excessively high TS levels can have detrimental effect on biogas production, hindering the movement of microorganisms and the transfer of nutrients and metabolic products. The volatile solids (VS) of milled paper waste, hydrothermally treated paper, chicken manure and inoculum were 60.1%, 63.7%, 90.3% and 80.0% respectively. Generally, combined pretreatment method altered the lignocellulosic components of the substrates rendering it apposite for biogas generation by co-digestion. For instance, milled paper had a lignin content of 14.6 while hydrothermally pretreated paper had a lignin content of 5.2±0.1% indicating the effect of combined milling and hydrothermal pretreatment. The result corroborates with a previous study of [34], which showed that lower lignin content of the hydrothermally pretreated paper would make it easier for microorganisms to break down the organic matter in the paper, resulting in the production of more biogas. Milled paper had a cellulose content of 89.2±0.2% while hydrothermally pretreated paper had a cellulose content of 60.3±0.1%. Milled paper had a hemicellulose content of 66.1±0.5 while hydrothermally pretreated paper had a hemicellulose content of 10.4±0.3. The moisture content of hydrothermally treated paper, chicken manure and inoculum were 4.1±0.2, 23.8±0.1, and 10.1±0.5 respectively. The total volatile fatty acid (TVFA) of milled paper waste, hydrothermally treated paper, chicken manure and inoculum were 10.5 gL^{-1}, 11.62 gL^{-1}, 20.2 gL^{-1} and 40.9 gL^{-1} respectively which is consistent with the work of [35].

Table 2. Physicochemical Analysis of the Substrate, Co-substrate and Inoculum.

Parameter	Unit	Milled Paper	Milled+Hydrothermally treated paper	Chicken manure	Cow dung
pH	-	6.8±0.1	6.1±0.2	7.9±0.1	7.8±0.5
Total solids	%	305.2±0.3	286.5±0.5	98.6±0.2	30.0±0.1
Volatile solids	%	60.1±0.1	63.7±0.1	90.3±0.2	80.0±0.3
Volatile fatty	gL^{-1}	10.5±0.5	11.62±0.2	20.2±0.1	40.9±0.1
C/N ratio	%	28.7±0.1	31.2±0.1	12.8±0.3	22.5±0.1
Cellulose	%	89.2±0.2	60.3±0.1	-	-
Hemicellulose	%	66.1±0.5	10.4±0.3	-	-
Ligin	%	14.6±0.2	5.2±0.1	-	-
Moisture	%	-	4.1±0.2	23.8±0.1	10.1±0.5

3.4 Bacterial profile of paper waste (substrate), chicken manure (co-substrate) and cow dung (inoculum)

The total microbial count for the influent waste materials were enumerated before it was charged into the biogas reactors. A total of 18 bacterial isolates were obtained after morphological and biochemical analysis of the substrate, co-substrate and inoculum. The paper waste, chicken manure and inoculum had a total heterotrophic bacterial count (THBC) of 3.6 × 10^3 CFU/g ± 0.05, 1.93 × 10^5 CFU/g ± 0.03 and 1.12 × 10^5 CFU/g ± 0.01, respectively as shown in Table 3. The high load of bacterial count in inoculum is vital for anaerobic biodegradation of the substrateFor total coliform (TCC), no growth was observed for paper waste, chicken manure and cow dung, neither was there growth observed as well for the Salmonella-Shigella count (SSC). Diverse species of bacterial isolates were obtained from the samples. Four bacterial genera were isolated from the paper waste, chicken manure and cow dung (inoculum) and they included *Staphylococcus*, *Lactobacillus*, *Micrococcus* and *Bacillus*. The most occurring bacterial isolate was *Staphylococcus* sp with a percentage occurrence of 50%. The percentage of occurrence of total bacterial isolates obtained from the substrates and inoculum were *Staphylococcus* sp. (50%), *Bacillus* sp. (11.1%), *Micrococcus* sp. (16.7%) and *Lactobacillus* sp. (22.2%) as illustrated in Fig. 6. The isolation of *Staphylococcus* sp is in conformity with the study of [36] who obtained this isolate from analyzing bio-slurry from different biogas plants. In the study of [37], 24% of *Staphylococcus* sp was also isolated from cow dung. The isolation of *Bacillus* sp. agrees with the study of [38], who obtained the isolate from cow dung. *Bacillus* sp having the lowest frequency occurrence agrees with the study of [34] who obtained isolates from household waste used for biogas production. The isolation of *Lactobacillus* sp and *Micrococcus* sp agrees with the results of Adetunji et al. [39] who obtained these isolates in chicken manure. A microalgae pretreatment study by Ferdes et al. [40] showed that *Bacillus* sp have been used to break the thick cell wall of *Chlorella* sp to improve biogas yield. In the study of Co-digestion of cow dung and rice husk by [41], micrococcus helped to break down complex polymers into sugars. These organisms are fermentative bacteria similar to those previously reported in literature, and have been implicated in substrate hydrolysis, a rate limiting and essential stage during biogas production [42].

Table 3. Total Microbial Count of Substrate, Co-substrate and Inoculum.

Microbial Group	Paper Waste (CFU/g)	Chicken Manure (CFU/g)	Cow Dung (CFU/g)
THBC	3.6×10^3 CFU/g ± 0.05	1.93×10^5 CFU/g ± 0.03	1.12×10^5 CFU/g ± 0.01
TCC	Not detected	Not detected	Not detected
SSC	Not detected	Not detected	Not detected

Key: THBC= Total Heterotrophic Bacterial Count, TCC= Total Coliform Count, SSC= *Salmonella Shigella* Count

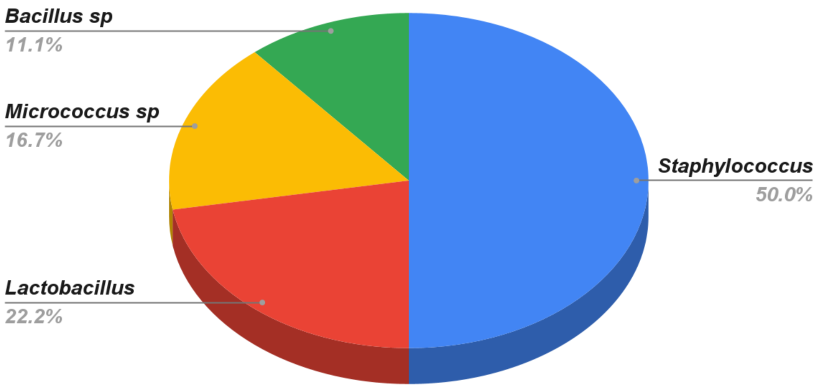

Fig. 6. Occurrence of bacterial isolates obtained from the substrates and inoculum.

4 Conclusion

This study demonstrated that paper waste which abound in large quantities can serve as suitable feedstock for biogas production, particularly when combined with a suitable co-substrate like chicken manure. The research findings also showed that the co-digestion of paper waste (PW) and chicken manure (CM) at different mixing ratios, particularly PW/CM 1:1 ratio, can result in higher daily and cumulative biogas yields. Additionally, the combined pretreatment by milling and hydrothermal treatment of substrate before co-digestion at equal paper waste to chicken manure ratio showed significant promise in enhancing biogas production compared to milling pretreatment alone, emphasizing the need for selection of appropriate mixing ratio and pretreatment techniques.

Declaration of Conflict of Interest

The authors declared that there is no conflict of interest with any other party on the publication of the current work.

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