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Optimizing The Yield Of Methane In Biogas Production From Selected Organic Wastes

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Abstract: *The demand for clean energy and efficient waste disposal has prompted increased studies in renewable energy sources such as biogas. This study explored ways to increase methane production through the anaerobic digestion watermelon peels, plantain peels, and cow dung. The onset of methane production for these substrates started between day 12 and day 16 reflecting the different compositions. Co-digestion of watermelon and plantain peels with cow dung enhanced biogas yields significantly by optimizing the balance of nutrients and minimizing the inhibitory impact of lignin and ammonia. A cow dung-watermelon co-digested sample had the highest methane concentration (67.4%). The co-digestion of the three substrates also showed that the peak biogas production can be sustained over a longer time than for the individual substrates. A neutral initial pH was found to be oprimal for all systems but the ternary co-digested system is able to tolerate higher pH values up to 8 and produce biogas at an appreciable rate. .*

Keywords: *Biogas, methane, pH, co-digestion, plantain, watermelon, cow dung*

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I. INTRODUCTION

Recent industrial growth and rising populations have put a lot of stress on energy systems. This has led to a greater reliance on fossil fuels, which are limited and harmful to the environment (International Energy Agency (IEA), 2021). This reliance has greatly increased the emission of greenhouse gases (GHGs), global warming, and climate change. As a result, a worldwide growing focus is on finding alternative, renewable, and sustainable energy sources. These sources can help reduce these negative effects while also meeting the world's increasing energy needs. One promising option is biogas, which can turn organic waste into clean energy and help with waste management (Appels et al., 2008; Nasir et al., 2012). Biogas is a mixture of gases, mainly made up of methane (CH) and carbon dioxide (CO), with trace amounts of hydrogen sulfide (H S), hydrogen (H), nitrogen (N), and water vapor. It is produced through the biochemical process of anaerobic digestion (AD), in which microorganisms break down organic matter in the absence of oxygen (Khan et al., 2022). The primary energy carrier in biogas is methane, which constitutes about 50–70% of the total biogas volume. Its energy content and combustion properties are comparable to natural gas, making it suitable for electricity generation, heating, and also as a vehicle fuel when upgraded by purification. Thus, optimizing the methane yield in biogas production is a critical determinant of energy efficiency and process viability.

In anaerobic digestion, complex organic matter is initially hydrolyzed into simpler monomers, which are subsequently converted into volatile fatty acids (VFAs), hydrogen, and carbon dioxide. (Appels et al., 2008). These are then converted by methanogenic archaea to produce methane. (Wu et al., 2020; Wainaina et al., 2019). While the process of biogas production is well understood, its optimization is highly complex and influenced by a variety of biological, chemical, and physical parameters. One of the most critical factors is the substrate composition, which determines the biochemical pathways available for microbial metabolism. Substrates rich in carbohydrates (e.g., fruits, vegetables, and crop residues) are easily hydrolyzed and quickly fermented, often yielding higher methane in the short term. In contrast, lipid-rich substrates, such as oils and animal fats, have a higher theoretical methane potential but can cause long-chain fatty acid (LCFA) accumulation, leading to microbial inhibition (Kabir et al., 2020). Proteinaceous waste, while valuable, can result in ammonia accumulation due to the deamination of amino acids, potentially leading to ammonia toxicity in methanogenic populations (Elsayed et al., 2022). The carbon-to-nitrogen (C/N) ratio is another crucial aspect of substrate optimization. An optimal C/N ratio, mostly between 22:1 and 30:1, is essential to maintain microbial growth and activity (Othman et al., 2022; Yen & Brune, 2007). A high C/N ratio may cause nitrogen limitation, reducing microbial proliferation, while a low C/N ratio can result in ammonia buildup and subsequent process inhibition (Zahan et al., 2016). To overcome these limitations, co-digestion strategies are frequently employed, where different types of organic waste are combined to balance nutrients and improve digestion kinetics. Fruit wastes such as watermelon, which is high in sugars and moisture but low in nitrogen, can be co-digested with nitrogen-rich substrates like animal manure to enhance biogas output and system stability (Egbedike & Alaka, 2021). Other important parameters that affects microbial metabolism and biogas production include temperature, maintenance of anaerobic environment, pH, organic loading rate and the hydraulic retention time of the digester.

The optimum pH for methanogens is reported to range between 6.8 and 7.5. pH values below 6.2 or above 8.5 may inhibit enzymatic activity and microbial function, especially due to the accumulation of VFAs or ammonia (Wu et al., 2020). Also, various inhibitory compounds such as ammonia, sulfides, heavy metals, antibiotics, and even salt can adversely affect microbial performance. These inhibitors may originate from the substrate itself or external sources, and their management through pretreatment, buffering, or co-digestion is crucial for stable reactor operation (Zhang et al., 2017). Recent reports have presented newer optimization strategies, including bioaugmentation, where specific microbial cultures are added to enhance digestion; substrate pretreatment (mechanical, thermal, chemical); and monitoring technologies using sensors and modeling to maintain optimum reactor conditions in real time (Khan et al., 2022).

This research explored the effect of co-digestion of three different readily available organic substrates, cow dung, watermelon peels and plantain peels on biogas generation and the methane content of the gas. The effect of pH on the total methane produced was also evaluated. These fruit wastes were chosen for their high biodegradability, considerable carbohydrate content, and abundant availability in many parts of the country.

II. MATERIALS AND METHODOLOGY

2.1 Sample Collection and Identification

The waste samples of each fruit waste and cow dung were collected separately from food vendors within KNUST and the Ayeduase community area. Sample digestion bottles, silicone and glue were purchased from the local market. Sodiun citrate, sodium phosphate were obtained from Thermo Fisher Scientific, UK.
Several samples with varying compositions were prepared and identified as in Table 1 for this study.

Table 1: Sample Composition and Identification

Sample ID	Amount of Substrate (g)		
	Plantain peels	Watermelon peels	Cow Dung
CD	-	-	350
W	-	-	350
P	350	-	-
WCD1	-	200	150
WCD2	-	240	110
PCD1	200	-	150
PCD2	240	-	110
PWCD	120	120	110

2.2 Sample and Digester Bottle Preparation

All unwanted materials, including plastics, stones, and other non-biodegradable contaminants, were carefully removed from the fruit waste samples by hand-picking. The sorted samples were then thoroughly washed with distilled water to eliminate dirt, debris, and potential inhibitors such as oils, pesticides, or heavy metals. The samples were then sliced into smaller pieces and blended in distilled water to achieve a uniform slurry with a particle size of approximately 60 microns. The initial pH of the mixed samples were adjusted to between 6.8 and 7 with solutions of sodium hydroxide or citric acid. Each sample was then placed in 500 mL incubator bottles containing a working volume of 300 mL and a headspace of 200 mL. Cow dung was used as both a substrate and an inoculum.

2.3 Digester Bottle Preparation

500mL incubator bottles were used as the biogas digesters. The lids of each incubator bottle were modified by drilling holes to accommodate gas outlet tubes, which were fitted with secure joints and sealed using epoxy glue to prevent gas leakage. The bottle caps were sealed with silicone caulk, ensuring an airtight system. Before sealing, each digestion bottle was loaded with 350 mL of the prepared substrate slurry, consisting of blended fruit peel mixture and fresh cow dung inoculum in the different ratios shown in Table 1. This volume ensured adequate headspace for biogas collection and pressure buildup. The digesters were degassed, sealed and gently mixed before placing an incubator initially set at 37 °C. A schematic sketch of the digester bottle is shown in Fig. 1

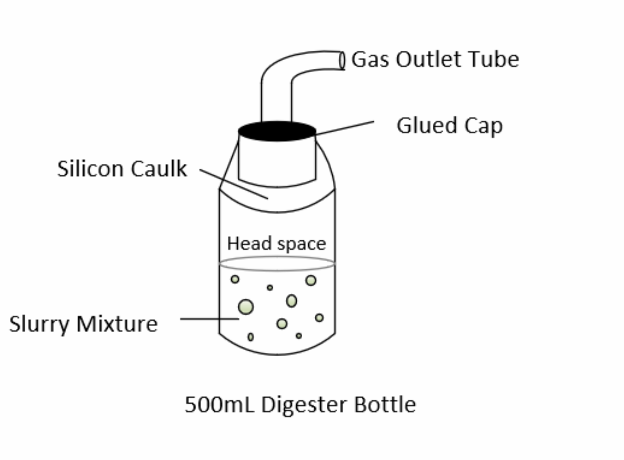

Figure 1. Schematic diagram of digester bottle

2.4 General Statistical Analysis

All experiments were conducted in at least triplicates and the reported data points are the means of these measurements.

2.5 Determination of Moisture Content

5 g of each sample was added to an already weighed crucible and placed in a preheated oven maintained at 105°C. The crucibles were subsequently cooled and weighed until a constant weight was obtained between successive measurements. The moisture content of each sample was determined based on the weight loss during the drying process, which reflects the amount of water originally present in the sample.

2.6 Determination of Total Solid

After the moisture content was determined, the final constant weights of the dried samples were used to evaluate the total solids content. The total solids represent the remaining dry matter in the sample after all moisture has been removed through oven drying.

2.7 Determination of Volatile Solid

The residue obtained from the total solid determination was transferred into a muffle furnace and ignited at 550 °C for 30 minutes to combust all the organic matter. The samples were then cooled in ambient air and in a desiccator and were weighed. The loss in weight during ignition, corresponding to the amount of organic material burned off, was used to determine the volatile solids content.

2.8 Gas Analysis

Biogas composition was analyzed using a Biogas 5000 Analyzer. Before use, the analyzer was purged with ambient air to eliminate residual gases and properly calibrated. The Biogas 5000 analyzer measured methane (CH), carbon dioxide (CO), hydrogen sulfide (H S), oxygen (O), and water vapor (H O). Gas volume was determined using the water displacement method.

III. RESULTS AND DISCUSSION

3.1 Characterization of Inoculum and Substrates

Substrate composition significantly influences the efficiency of anaerobic digestion (AD), particularly concerning methane generation. In this study, the total solids (TS), volatile solids (VS), ash content (AC), and moisture content (MC) of watermelon peels, plantain peels, and cow dung were determined using standard procedures (APHA, 2005). These parameters were selected because of their direct implications for microbial activity, digestibility, and methane yield, as supported by extensive prior research (Zhang et al., 2021; Li et al., 2023). The results are shown in Table 2.

Table 2: Physicochemical Properties of Substrates

Type of sample	**%TS**	**%VS**	**% MC**	**%AC**
Watermelon peels	19.7±0.82	1.1±0.04	80.3±1.61	1.1±0.08
Plantain peels	31.5±0.75	3.6±0.06	68.5±0.98	3.6±0.05
Cow dung	35.6±0.66	3.7±0.05	64.4±1.01	3.7±0.06

3.2 Total Solids (TS)

Total solids (TS) is a crucial parameter for evaluating substrate suitability for anaerobic digestion, which represents the total amount of solid matter (both organic and inorganic) in a given substrate. In this study, cow dung, plantain peels, and watermelon peels exhibited TS values of 35.6%, 31.5%, and 19.7%, respectively, as shown in Table 3.0. These findings are consistent with values reported in previous literature. For cow dung, Kumar et al. (2022) reported TS values ranging from 33% to 36%, influenced by the animal's diet and the age or handling of the manure. Plantain peels have been documented to have TS values between 30% and 34%. Gupta et al. (2021) recorded a TS value of 30.2% for fresh plantain peels, which are similar in state to the samples used in this study. In contrast, watermelon peels generally have lower TS values due to their high water content. Zhao et al. (2021) and Wang et al. (2023) reported TS values for watermelon peels ranging from 17% to 22%. The 19.7% found in this study is well within this range.

In biogas production, total solids content (TS) is an are important parameter in determining the amount of biodegradable material available for microorganisms. Substrates with higher TS values generally contain more degradable content per unit volume, which can boost biogas yield. According to Li et al. (2023), TS levels between 20% and 40% are ideal for wet anaerobic digestion systems. The TS values recorded in this study, particularly for cow dung and plantain peels, align with this favorable range, suggesting strong potential for effective anaerobic digestion.

3.3 Moisture Content (MC)

The moisture content for the samples were found to be 64.4% for cow dung, 80.3% for watermelon peels, and 68.5% for plantain peels as shown in Table 2. Moisture content (MC) is a vital factor in anaerobic digestion (AD) as it directly affects substrate solubility, microbial movement, enzymatic function, and nutrient diffusion. These figures are within the generally accepted optimal range of 60–85% for wet digestion processes (Li et al., 2023). For cow dung, moisture content usually varies between 60% and 70%, influenced by aspects such as the animal's diet, age of the manure, and conditions of storage. Kumar et al. (2022) noted MC levels of around 65% for freshly gathered cow manure, closely matching the 64.4% found in this study. Similarly, plantain peels are recognized for having moderate moisture levels, typically between 65% and 75%. Gupta et al. (2021) recorded an MC of roughly 69% for ripe plantain peels, which is in close agreement with the 68.5% found in this research. Additionally, watermelon peels are noted for their high water content. Earlier studies by Zhao et al. (2021) and Wang et al. (2023) reported MC values between 78% to 85%, depending on factors like ripeness and storage time. The MC of 80.3% measured in this study is within that range and accurately represents the natural composition of watermelon peels, primarily composed of water-retaining tissues.

Sufficient moisture promotes hydrolysis and eases microbial access to organic materials, thus enhancing digestion efficiency (Awasthi et al., 2020). Nonetheless, excessively high MC can dilute the concentration of volatile solids (VS), which reduces the availability of fermentable materials per unit volume. This phenomenon was evident in the watermelon peel samples, which, although showing a favorable MC of 80.3%, had the lowest VS content (1.1%), limiting their overall potential for digestion.

3.4 Volatile Solids (VS)

Volatile solids (VS) denote the biodegradable organic component of total solids (TS), primarily composed of carbohydrates, proteins, and lipids. These organic materials serve as the primary substrate for microbial activity during anaerobic digestion (AD), and the VS content is commonly used as an indicator of the degradable potential of feed stocks (Zhang et al., 2021; Awasthi et al., 2020). As shown in Table 2, the VS contents measured were 3.7% for cow dung, 3.6% for plantain peels, and 1.1% for watermelon peels. Kumar et al. (2022) found VS values between 3.8% and 4.2% in cow dung samples sourced from smallholder farms, which closely matches the 3.7% observed in this research. Plantain peels tend to be abundant in biodegradable carbohydrates and fiber, with prior studies reporting VS values spanning from 3.0% to 4.0%, which depend on

factors like ripeness and drying techniques. Gupta et al. (2021) in their work reported a VS content of 3.5% in ripe, fresh plantain peels, which aligns well with the 3.6% recorded in this study.
Watermelon peels are characterized by lower VS levels owing to their high moisture content and lower organic density. Wang et al. (2023) and Zhao et al. (2021) reported VS values ranging from 1.0% to 1.5% for fresh watermelon peel, primarily due to their high water content and minimal lignocellulosic composition.

3.5 Biogas and Methane Production

The cumulative and daily biogas production from the individual substrates is shown in Fig. 2 and Fig. 3 respectively.

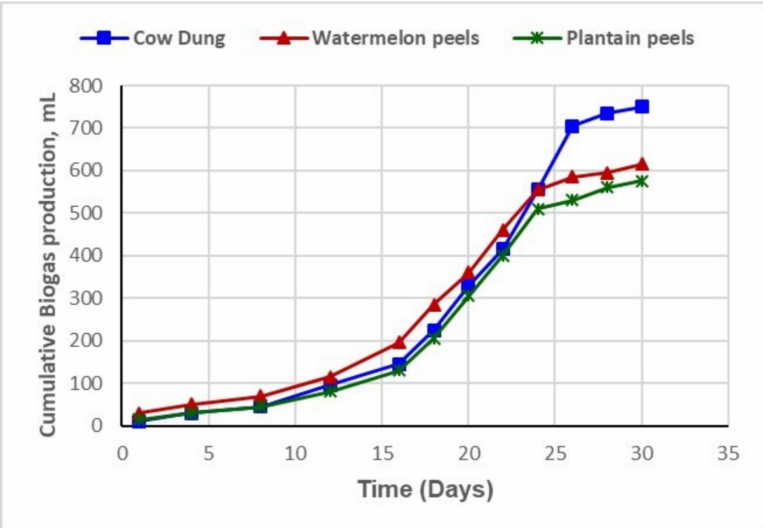

Figure 2: Cumulative Biogas production by the mono-digested substrates

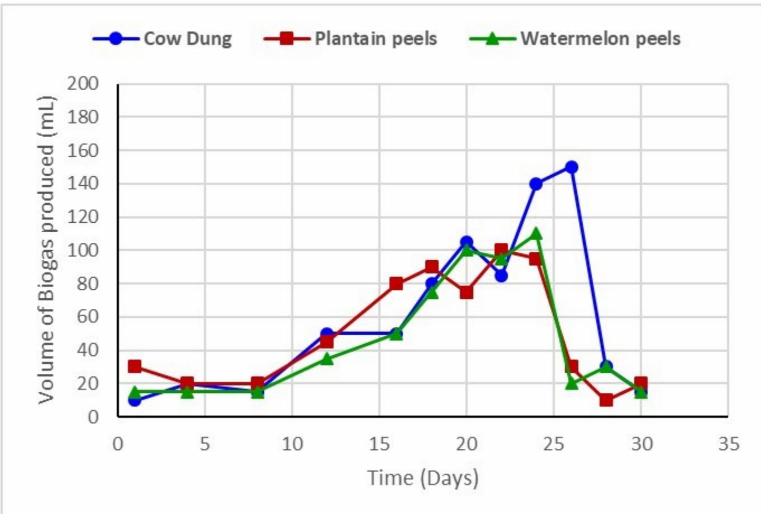

Figure 3: Daily Biogas production by mono-digested substrates

Cow dung, as a substrate produced the highest amount of biogas cumulatively as expected. This substrate contained the bacterial culture (methanogenic archaea) responsible for substrate degradation in an environment that did not alter significantly. This trend is consistent with the findings of Wang et al. (2023), who noted that animal manure, particularly cow dung, serves not only as an excellent inoculum but also as a substrate with rich buffering capacity and microbial density, supporting stable methanogenesis. The least cumulative biogas production was obtained from plantain peels (P). Plantain peels contain considerable levels of proteins and amino acids. These nitrogenous substances break down, during anaerobic digestion leading to the release of ammonium ions (NH) into the digester which may be converted to free ammonia (NH), a known is toxic to methanogens (Chen et al., 2008; Kumar et al., 2022). Although Hayes (2023) reports that some methanogens can adapt and still produce methane under such stressed conditions, their efficiency remains significantly reduced compared to uninhibited systems. Another form of inhibition encountered was the

observed foam formation. This was probably due the high solids content and the presence of natural surfactants such as saponins and glycosides, which decrease surface tension and support foam stability (Zhao et al., 2022). The low pH observed in setup P (4.2–5.5) may have also resulted from the buildup of volatile fatty acids, which would further aid in foam stability and instability within the reactor (Chen et al., 2008).
The production of methane from these substrates was not detected until day 12 for cow dung and watermelon and day 16 for plantain peels as shown in Table 3 and in Fig. 4

Table 3: Methane production by all samples

Sample ID	First day of methane production	Maximum methane fraction, %	Day of maximum methane production
CD	12	63.1	26
P	16	14.5	24
W	12	21.3	26
WCD1	12	67.4	24
WCD2	12	56.3	26
PCD1	16	25.7	26
PCD2	16	27.2	24
PWCD	16	52.8	28

These observations can be attributed to the specific nature and composition of these substrates. The lignin content of these plant materials tends to resist methanogenesis initially. (Wagner et al., 2013; Hendriks & Zeeman, 2009; Jin et al., 2021). The delay in methane production could also be attributed to the methanogens being in the lag phase of their growth cycle. During this phase, microorganisms adapt to their new environment by synthesizing transport proteins for substrate uptake, producing necessary enzymes for digestion, and initiating genetic replication (Angelidaki et al., 2011; Xu et al., 2022). Another contributing factor to this dormant period of methanogenesis may be the characteristics of the inoculum's original growth medium, which may differ significantly from the batch bioreactor conditions (Choudhary et al., 2022). Similar adaptation delays were reported by Zhang et al. (2023), where up to 10 days of lag phase were observed during the digestion of lignocellulosic residues in semi-continuous batch reactors. For cow dung, the peak methane yield of 63.1 % was obtained on day 26 which usually indicates the microorganisms are in the exponential phase of growth with increased metabolic rates. (Chen et al., 2008; Shen et al., 2021).

The average peak yield of methane from all substrates is shown in Fig 4.

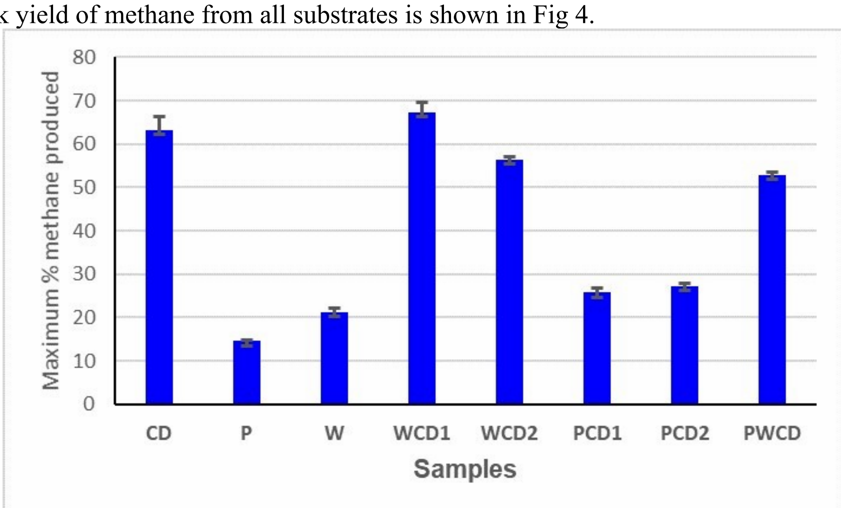

Figure 4: Average Peak yield of Methane

Watermelon peels presents a suitable moisture content, and ease of degradation by microorganisms and a balanced carbon-to-nitrogen (C/N) ratio. Methane generation from this substrate and its combinations was consistently higher compared production from those containing plantain peels. The poor performance of plantain peels in methane production can be attributed to its high lignin content, reported to be as high as 25.7% (Philomena Kanwulia & Christian N. Idogwa, 2016), in contrast to watermelon peels which has a lignin content of about 10% (Ibrahim, 2016). This finding aligns with Chiumenti et al. (2018), who reported lower biogas yields from the treatment of high-lignin substrates. Recent studies by Jin et al. (2021) and Mirmohamadsadeghi et al. (2023) further support this observation, emphasizing that lignin acts as a physical and chemical barrier to microbial attack, significantly limiting hydrolysis and methane yield unless advanced pretreatment techniques are applied.

For a two-substrate co-digestion, the cumulative and daily biogas production is shown in Fig. 5 and Fig. 6

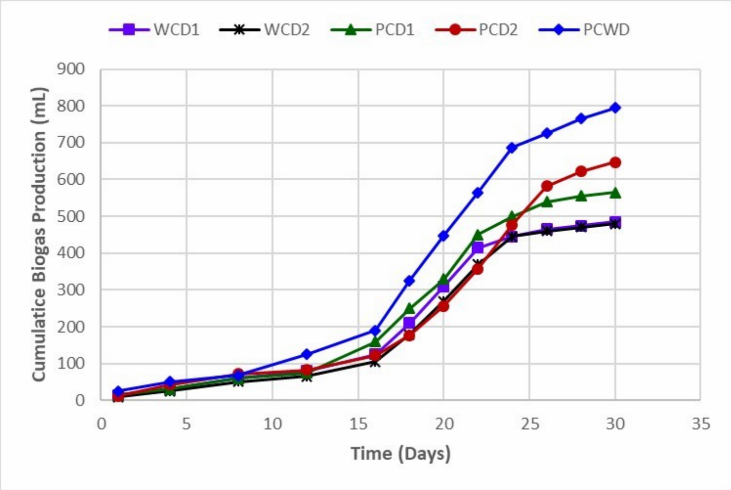

Figure 5: Cumulative Biogas production by co-digested binary substrates

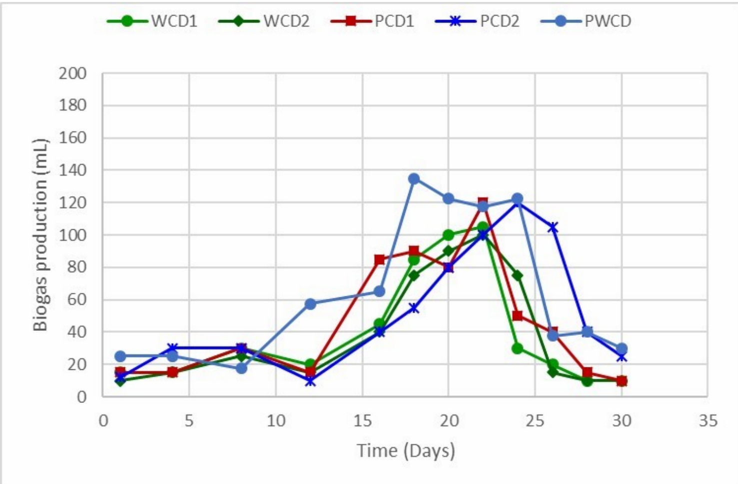

Figure 6: Daily Biogas production by co-digested binary substrates

The onset of methane production delayed longer in set-ups containing the plantain peels and these observations have been attributed to the degradability of the substrates. Faster-degrading substrates lead to an earlier onset of methanogenesis (Li et al., 2011; Al-Mashhadani et al., 2020). Recent work by Li et al. (2023) also confirms that co-digesting fruit peels with cattle manure significantly improves hydrolysis efficiency, reducing the lag phase and enhancing methane generation. The inclusion of watermelon peels tend to produce a higher percentage of methane as shown in Table 3. According to Bhajan and Sanjag (2013), substrates with high moisture content, optimal C/N ratio, ease of digestion, and low lignin content tend to have higher biogas potential, especially when paired with an active inoculum. Similar findings were reported by Singh et al. (2022), who observed up to 35% improvement in methane yield through the co-digestion of agricultural residues with manure.

These results suggest enhanced biogas yields when the substrates are co-digested. Co-digestion has been widely recognized to improve biogas yields due to better nutrient balance, especially the carbon-to-nitrogen (C/N) ratio, and dilution of inhibitors (Mata-Alvarez et al., 2014; Elalami et al., 2021).

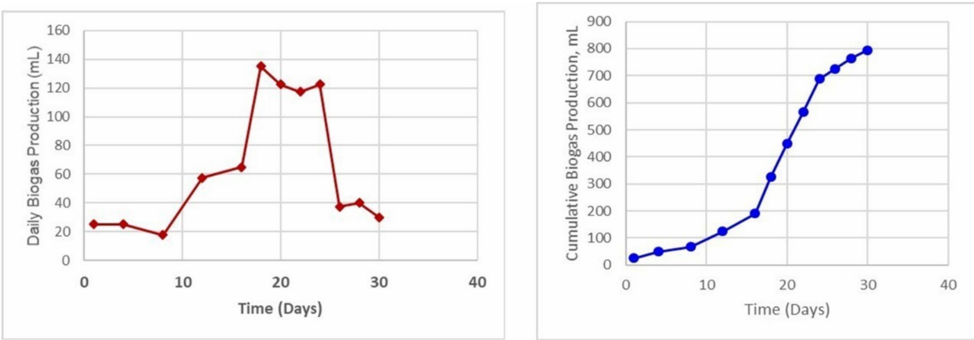

Figure 7: Daily and cumulative biogas production by ternary co-digested substrate

The daily and the cumulative biogas generation from the ternary co-digestion (CD, W and P) are shown in Fig. 7. It can be seen that the peak biogas production is sustained over a longer period than in the case of single or two-substrate systems. By balancing the C/N ratio between carbohydrate-rich and nitrogenous substrates, co-digestion improves overall degradation and gas yield (Zhang et al., 2014; Moreno et al., 2023). The maximum biogas yield of 880 mL was obtained using the ternary co-digestion set up over the 30 days. This method is not only efficient but also economically favorable, reducing the cost and complexity of using multiple mono-digestion setups.

3.6 Effect of pH on Methane production

The pH of the bioreactor is a critical parameter influencing the stability and efficiency of anaerobic digestion, as it affects both the microbial community structure and the subsequent metabolic pathways (Carotenuto et al., 2016; Chen et al., 2008). For each substrate combination, the pH of the mixture varies with composition at beginning and end of the 30-day period. Table 4 shows the initial and final pH values.

Table 4 Initial and final pH of samples

Samples	Initial pH	pH after 30 days
CD	7.46	7.32
W	6.28	6.07
P	5.01	4.20
WCD1	6.98	6.89
WCD2	6.47	6.48
PCD1	6.26	5.74
PCD2	6.08	5.90
PWCD	6.40	6.66

The initial pH values of samples ranged from 5.01 to 7.46 and some had to be adjusted to the 6.8 to 7.8 range which is optimal for anaerobic digestion. (Appels et al., 2008; Kumar et al., 2022). The drop in the final pH is indicative of the production of acidic intermediates as bacteria decompose organic matter and metabolic imbalances that affect methanogenic activity. (Anggarini et al., 2015; Chen et al., 2008). Methanogens are sensitive to pH changes and exhibit optimal activity within a narrow range. As the pH changes, their metabolic function declines, leading to reduced methane production and a further build-up of volatile fatty acids (Zhao et al., 2023). The pH is also thought to be affected by the concentration of ammonia produced by the deamination of nitrogenous compounds and these high concentrations of ammonia are known to be toxic to methanogens, especially under low-pH or high-temperature conditions (Rajagopal et al., 2013; Yenigün & Demirel, 2013; Li et al., 2023).

It can be observed from Table 3 that all substrate mixtures apart from pure cow dung had very low final pH values. Plantain peels, for example are rich in nitrogen and susceptible to protein degradation. These acidic conditions correlate strongly with poor methane yield highlighting the adverse impact of acidic product accumulation and possible ammonia inhibition.

This observation aligns with recent studies by Singh et al. (2022) and Shen et al. (2024), which show that substrates with low buffering capacity or high lignin/protein content often trigger pH instability and methanogen suppression. Importantly, the setups that included co-digestion with cow dung or watermelon

maintained better pH balance, likely due to improved C/N ratios and enhanced buffering, thus supporting higher methane yields. This further emphasizes the relevance of substrate synergy in sustaining microbial activity and optimizing biogas performance.
The initial pH variation on the ternary co-digested substrate (PWCD) was investigated within the known range for methanogens. The final pH of the PWCD system was 6.6, so we decided to investigate the effect of pH in the range 6 to 8 on the performance of the digester. The results are shown in Fig. 8.

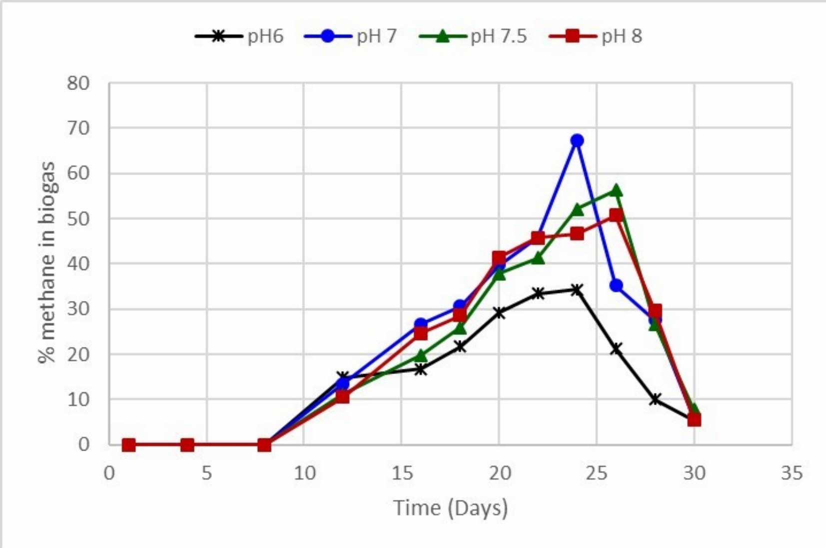

Figure 8: Effect of pH on peak methane content of biogas

The set up at pH 7 performed best yielding the highest methane peak of 67.4% on day 24. This indicates that neutral pH conditions are probably the optimal conditions for this collection of methanogenic bacteria. Methane generation at pH 7.5 and 8 show a slightly lower performance indicating that slightly basic conditions still support methanogenic activity, although not as effectively as the neutral pH conditions. Biogas yields and methane generation at pH 6 was observed to be significantly lower probably due to the suppression of methanogens, which are sensitive to lower pH environments. Acidic conditions disrupt the metabolic functions of methanogenic archaea, leading to the accumulation of volatile fatty acids (VFAs) and a reduction in methane output (Jayaraj et al., 2014).

These results align with the findings of (Jayaraj et al., 2014) and (Hussain et al., 2021) , who noted that biogas production from food waste gave the highest methane yield peak at pH 7, followed by pH 8 and 6, with significantly low yields at more acidic or alkaline levels. Also, Callaghan et al. (2002) found that extreme pH levels hinder enzymatic activity and microbial growth, resulting in reduced gas yields and volatile solids degradation. The current findings in this study emphasize that optimal methane production and optimization is closely tied to pH and the type of substrate. It appears substrate combinations with a high nitrogen content such as plantain peels may be digested at slightly higher pH without significant loss in biogas production and the methane content of the gas. Lower initial pH of the mixture should be avoided.

IV. CONCLUSION

This study shows that the addition of watermelon peels and plantain peels to the traditional cow dung substrate has profound effect on the biogas and methane yields. The combination of the three substrates produced the highest methane yield, showing the benefits of mixing different types of waste which can help balance the carbon-to-nitrogen (C/N) ratio and reduced the overly accumulation of harmful compounds like lignin and ammonia. It was shown that slightly higher pH values than neutral do not affect the performance of the digester very much when but lower pH values reduced the biogas and methane generation.

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