

Upscaling Biogas Production using Fruit Waste (Pawpaw, Watermelon and Banana) Co Digested with Cow Dung, and Gutter's Sludge

Suleiman Abubakar Isa¹, Aisha Idris², Hafsa Musa Abubakar³, Ibrahim Yusuf⁴

^{1,2,3,4}Department of Microbiology, Faculty of Life Sciences, College of Natural and Pharmaceutical Sciences, PMB 3011 BUK Kano, Bayero University Kano State, Nigeria.

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Abstract

Nigeria faces a significant challenge of fruit waste due to improper handling and transportation practices, leading to damage and microbial contamination, resulting in environmental pollution. These wastes can be utilized to produce biogas which is environmentally safer and cleaner. This study explores the potential of using fruit waste (Pawpaw, Watermelon, and Banana) as substrates co-digested with cow dung and gutter sludge to upscale biogas production. Physicochemical analyses of the substrates and inoculums was conducted, followed by construction of pilot biogas digesters, and then isolation of methanogenic bacteria. A combination of single and mixed fruit ratio were added into the digesters with the cow dung and gutter sludge as co-digesters. For the up scaling, a 25L digester was constructed and the best mix ratio from the pilot digesters were fed into the digesters. Isolation of methanogens was carried out to access which substrate or inoculum had greater potential in the production of biogas individually or when co-digested by the presence of methanogenic colonies after incubation. The results revealed similar physicochemical parameters among the fruit waste substrates, while cow dung and gutter sludge showed variations, particularly in carbon-nitrogen ratios. The pilot study demonstrated that the mix ratio containing all three fruits, co-digested with cow dung and gutter sludge, produced the highest methane yield (11.2%). In upscaling, a 25L digester produced gas efficiently, albeit with some depletion over time, possibly due to methanotroph activity or exposure to sunlight. Methanogenic colonies were successfully isolated from lower dilutions of gutter sludge. This study highlights the potential of fruit waste as a valuable substrate, particularly when co-digested with cow dung and gutter sludge, which can serve as an effective inoculum due to the presence of methanogens.

Keywords: Fruit Waste, Cow dung, gutter's sludge, methanogens, methane, biogas

Introduction

Many countries, especially developing ones, face energy crises due to heavy reliance on fossil fuels, which are projected to deplete within the next century. Consequently, there is a growing need for sustainable and environmentally friendly energy

sources. Anaerobic digestion (AD) has emerged as an effective method for producing renewable energy by converting organic matter into biogas, primarily methane and carbon dioxide. AD involves a series of microbial processes, including hydrolysis, acidogenesis, acetogenesis, and methanogenesis⁽²⁸⁾ (2). Anaerobic digestion has gained increasing interest

Corresponding Author: Ibrahim Yusuf, Department of Microbiology, Faculty of Life Sciences, College of Natural and Pharmaceutical Sciences, PMB 3011 BUK Kano, Bayero University Kano State, Nigeria.

E-mail: iyusuf.bio@buk.edu.ng

both as an efficient way of treating wastes and as a source of renewable energy, during anaerobic digestion, bacterial communities' bio-transform the substrate to alcohols, CO₂, H₂, and volatile fatty acid (VFA) by hydrolysis and acidogenesis processes. Then, acetogenic bacteria groups oxidize the VFA producing acetate, CO₂ and H₂ gases. During the final stage, methanogenesis, acetoclastic, or hydrogenotrophic methanogens convert acetate or CO₂ to CH₄. These microbial steps are not consecutive but occur in parallel with different relaxation time, governed by the relative abundance of different species ⁽²⁸⁾.

Fruit waste, such as that from Pawpaw, Watermelon, and Banana, is an abundant and cost-effective resource, as fruit consumption has increased substantially. Unfortunately, these fruit wastes are often mismanaged, leading to various health and environmental issues ^(12; 26). Anaerobic co-digestion offers a way to get beyond the limitation of mono-digestion by simultaneously digesting two or more feedstock's ⁽²⁵⁾. The synergistic effect of encouraging a more diversified microbial population, which increases system stability and methane yield, are among the key advantages of co digestion, better nutritional balance, appropriate carbon-to-nitrogen (C/N) ratio ⁽²⁰⁾, enhanced buffering capacity, supplementing of trace elements ⁽³¹⁾ and safer and higher-quality digestate for agricultural uses ⁽¹⁾. In anaerobic digestion, different Sludge's can be used as inoculum because they are known to contain a wide consortium of microorganisms particularly methanogens, moisture and nutrients. In this study gutter's sludge was utilized as inoculum and this sludge is a mixture of water that is no longer suitable for its original purpose including domestic waste water from home and businesses as well as industrial waste water from factories, commercial establishment and storm water runoff. Gutter's sludge also contains organic matter, chemicals and microorganism and these components makes it suitable to be used as an inoculum ⁽²⁹⁾. Hence, this study aimed at up scaling biogas production using fruit wastes particularly those of watermelon, banana and pawpaw co-digested with cow dung and gutter's sludge.

Materials and Methods

Sample Collection and Processing

This research involved the use of three different fruit waste types: watermelon, pawpaw, and banana, all of which were sourced from the Naibawa Yan Lemo fruit market in clean polythene bags the day before the study commenced. Fresh cow dung was obtained from the local cattle market in Hauren Shaunu, Gwale Local Government Area (L.G.A), Kano, while gutter sludge was collected from the open gutters in the residential area of Dorayi Chiranchi, located in Kumbusto L.G.A, using a clean shovel. It was then transferred into a 20L jerry-can. The fruit wastes were weighed accordingly, homogenized in an electric blender, and subsequently introduced into the digesters.

Designing, Fabricating, Feeding, and Running of the Anaerobic Digesters

The pilot and up scaled digesters were set up as follows:

Pilot Digester

For the pilot digester, a small hole was drilled into the bottle cover of 1.5L clear plastic bottles. Urine plastic bags were attached to these bottles to function as gas collectors through the small hole, which was sealed with candle wax to maintain a completely anaerobic process ⁽³²⁾. These digesters were placed inside black polythene bags to prevent light penetration, and they were then positioned within a box on the floor. Eight digesters (in duplicate) were established and labeled as digester A to H, each containing different mix ratios as outlined below:

The fermentation process was allowed to proceed for four weeks, and the percentage composition of gases, including methane (CH₄), carbon dioxide (CO₂), and oxygen (O₂), was periodically measured during the fermentation process using a Geo Tech Gas Analyzer provided by the Department of Agricultural and Environmental Engineering, Bayero University, Kano.

Upscaled Anaerobic Digester

In this experiment, an anaerobic digester consisting of a 25L black drum (to prevent algae growth), a connecting tube, and a balloon tire used

as a gas collector chamber was constructed. The optimal mix ratio identified in the pilot experiment was chosen to feed the 25L digester. To avoid process disruption or balloon deflation, the anaerobic digester was positioned outdoors. The fermentation process continued for 7 days under sunlight. The gas produced was connected to a burner, and its burn duration was measured.

Mix ratio for the upscaled digester:

700g of watermelon + 700g of pawpaw + 700g of banana + 700g of cow dung + 18L of gutter sludge.

Microbial Analysis of the Substrates and Inoculums

Isolation of Methanogenic Bacteria

To isolate methanogenic bacteria, the method followed by ⁽²⁹⁾ was adopted. In the process, a thioglycolate medium was prepared, consisting of 2.0 grams of yeast extract, 1.0 gram of beef extract, 5.0 grams of peptone, 5.0 grams of glucose, 5.0 grams of sodium chloride, and 0.0002 grams of methylene blue, with the addition of 1.0 gram of agar per liter. The pH of the medium was adjusted to 7.2 ± 0.2 .

To initiate the isolation, a sterile syringe was used to inoculate one milliliter of each sample at the base of sterilized test tubes containing the prepared medium. After sterilization, the test tubes were incubated in an anaerobic jar at 37°C. Following observations of bacterial growth, serial dilutions of a 72-hour bacterial culture were performed in ratios of 10^{-1} , 10^{-2} , 10^{-3} , 10^{-4} , and 10^{-5} . These dilutions were then transferred to Petri dishes.

The Petri dishes were filled with a modified medium, and the samples were mixed with the medium by gently swirling the dishes both counterclockwise and clockwise. The medium was left undisturbed to solidify for approximately 20 minutes. Two plates were used for each series of inoculated plates, and they were subsequently placed in an anaerobic jar. To ensure the absence of any remaining oxygen, a lit candle was placed inside the jar.

The sealed jar was then incubated at 37°C for a period of 72 hours. The presence of methanogenic colonies on the surface of the Petri dishes was confirmed by the appearance of a distinctive blue-green fluorescence, characteristic of methanogenic bacteria. To further validate the identification of methanogenic colonies, the colonies were examined using a UV light chamber. Fluorescent light was used to assess the presence of growth, and long-wave UV light was employed to visualize the methanogenic colonies, following the procedure described by ⁽⁸⁾.

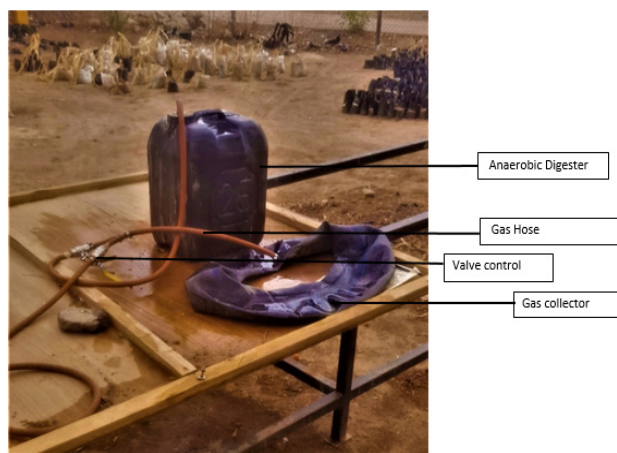


Plate 1: Upscale Digester Set up

Results

Physicochemical Analysis

The pH values of both substrates and inoculums fell within the range of 5.21, observed in pawpaw (the lowest), to 6.81 in cow dung (the highest). Within this range, both substrates and inoculums were found to be slightly acidic. Notably, cow dung exhibited the lowest percentage of volatile solids (V/solids) at 17.8% and a moisture content of 43.59%. On the other hand, pawpaw displayed the highest carbon-to-nitrogen (T/C) ratio, measuring at 610 mg/L, and the highest moisture content at 66.70%. Watermelon had the highest total carbon content at 4.4 mg/L, while cow dung contained the highest total solids percentage, which stood at 56.41%. (Refer to Table 1 for detailed information.)

Table 1: Physicochemical analysis of both substrates and inoculums

Parameters	Gutters sludge	Cow Dung	Banana	Pawpaw	Watermelon
pH	6.31	6.81	4.86	5.21	5.34
V/Solid (%)	17.80	17.20	31.20	35.30	32.10
M/C (%)	64.17	43.59	63.93	61.29	66.70
T/Solids (%)	35.83	56.41	36.01	38.63	33.30
T/N(mg/L)	228	2.6	410	610	321
T/C(mg/L)	3.1	1.4	2.3	3.8	4.4
C/N ratio	0.01	0.75	0.00	0.00	0.01
OM (%)	5.56	3.37	5.38	5.00	5.56

Keys:

V/solids: volatile solids, T/Solids: Total solids, T/N: Total Nitrogen, T/C: Total Carbon, OM: Organic matter, C/N: Carbon and nitrogen, M/C: Moisture content.

Methane Yield from the Pilot Study

The percentage of methane produced over a 25-day period for various mix ratios is illustrated in Graph 1. These mix ratios include BI, W, PI, CI, WMI, B, P, and C. On Day 5, the CI mix ratio yielded the highest methane production at 8.9%, followed by WMI at 8.15%, and BI at 4.95%. The alternative mix ratios did not yield any methane at this stage. By Day 10, all mix ratios experienced a decline in methane production.

On Day 15, only BI, CI, and PI mix ratios demonstrated methane generation, accounting for 3.3%, 11.2%, and 3.5%, respectively. The alternative mix ratios did not produce any methane. By Day 20, only three mix ratios exhibited methane production, with CI at 2.7%, B at 0.1%, C at 1%, and BI at 0.1%. The alternative mix ratios still did not produce methane.

On Day 25, only CI and BI displayed methane production, measuring 1.55% and 0.2%, respectively, while the other mix ratios showed no methane production throughout the entire experiment. Overall, BI, CI, and WMI demonstrated the highest methane production, while PI, B, P, and C consistently displayed very low methane production or none at all.

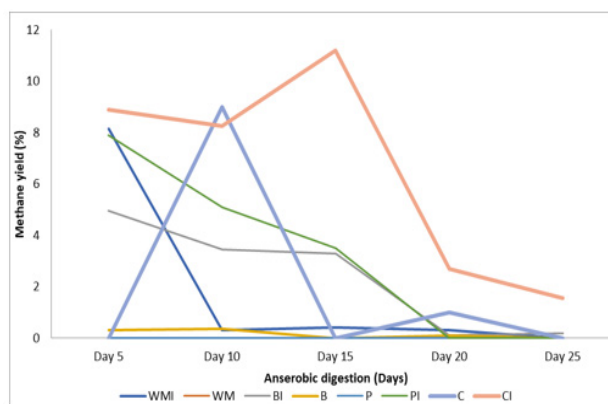


Figure 1: methane yielded (%) for pilot study

Keys: B: Banana, WM: Watermelon, P: Pawpaw, C: Cocktail, BI: Banana and Inoculum, WMI: Watermelon and inoculum, PI: Pawpaw and inoculum, CI: Cocktail and inoculum.

Methane Production (Up Scaling)

For upscaling the production of biogas, the most effective feeding ratio, which included the cocktail and inoculum, was employed. In Plate II, captured 24 hours after feeding, there was no visible gas present. However, by the time Plate III was taken, approximately 72 hours after feeding, there was a noticeable presence of gas as the gas collector became visibly swollen. In Plate IV, captured 96 hours after feeding, the gas started to deplete, and by the following day, there was little to no gas remaining in the gas collector.



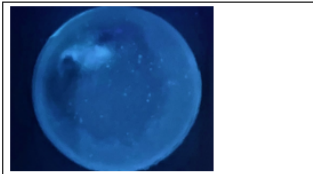
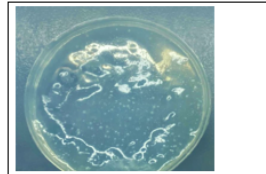
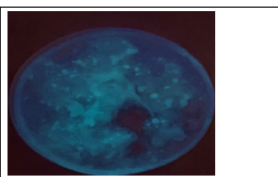
Plate II: Gas Collector 24 Hours After

Plate III: Gas collector after 72hrs

plate IV: Gas Collector 96 Hours

Isolation of Methanogens from Substrates and Inoculum

All samples, including both substrates and inoculums, were inoculated onto the surface of thioglycolate medium. After the appropriate incubation period at the specified temperature, colonies developed on all plates. However, upon examination under fluorescence and UV light, only the plates that were inoculated with gutter sludge dilutions of 10^{-4} (Plate V) and 10^{-5} (Plate VI) displayed colonies that were illuminated (glowing) under long-wave UV light. This glowing served as confirmation that these colonies were indeed methanogenic colonies.

Plate V: Gutters sludge 10^{-4} under long wave UV light showing a cluster of methanogenic colonies and other methanogenic colonies dispersed throughout the petri dishPlate VI: Gutters Sludge 10^{-4} under visible light showing all the Colonies Present on the Petri dishPlate VII: Gutters Sludge 10^{-5} under long wave UV light showing the Methanogenic Colonies Present on the Petri dishPlate VIII: Showing Gutters Sludge 10^{-5} under visible light Showing all Colonies Present on the Petri dish

Discussion

According to ⁽⁵⁾ and ⁽¹⁸⁾ the performance of a biogas digester is significantly influenced by the physicochemical parameters of the substrates and inoculum. Among these parameters, pH and temperature are known to strongly affect the process of anaerobic digestion.

The pH levels of all the fruits used in this study were found to be acidic, with banana having the lowest pH at 4.80, watermelon at 5.34, and pawpaw at 5.21. These values are comparable to the findings of ⁽¹⁴⁾, who reported pH values of 4.9 for banana,

3.6 for pawpaw, and 4.3 for watermelon. It's worth noting that this acidic pH may have contributed to the poor performance of digesters containing only fruit waste. ⁽²³⁾ Mentioned that the optimal pH for a digester using fruit substrates can vary depending on the type of fruit but is generally in the range of 6.0 to 7.5. Among the fruits in this study, watermelon came closest to this optimal range with a pH of 5.34.

The temperature of both the substrates and inoculum in this study ranged from 22°C to 30°C. Cow dung and pawpaw had the highest temperatures, at 30.7°C and 30.2°C, respectively, while gutter sludge had the lowest temperature at 22°C. This temperature range is consistent with the range of 25°C to 30°C observed on the 25th to 45th day of digestion in ⁽¹⁴⁾. It also aligns with the findings of ⁽³⁾ and the standard mesophilic range of 25°C to 45°C, which is considered optimal for biogas production. It's worth mentioning that ⁽¹⁰⁾ reported a decrease in metabolic activity of microorganisms, leading to reduced biogas yields at temperatures between 20°C and 25°C. However, increasing the temperature to the range of 30°C to 35°C resulted in about 40% more biogas production. This temperature effect might be one of the reasons why the digesters containing only fruit substrates, especially watermelon and banana, performed poorly.

The carbon-to-nitrogen (C/N) ratio of the substrates and inoculum varied significantly. Cow dung had a C/N ratio of 3:4, while ⁽¹⁹⁾ reported a C/N ratio of 5:1 for cow dung, which resulted in low methane yield due to an unstable carbon-nitrogen balance. In this study, cow dung had a more stable carbon-nitrogen ratio, which might have contributed to the improved performance of digesters with inoculum. However, the optimal C/N ratio can vary depending on the feedstock, with a C/N ratio between 20:1 and 30:1 considered optimal for biogas production ⁽¹⁵⁾. Gutter's sludge and watermelon had C/N ratios of 1:10, indicating a higher nitrogen concentration relative to carbon. Banana and pawpaw had C/N ratios of 1:200 and 3:500, respectively, which are considered low due to the excessive nitrogen concentration relative to carbon. A low C/N ratio can lead to excess ammonia production, which can inhibit the activity of methanogenic bacteria and reduce biogas yield ⁽¹⁶⁾. This could also explain the poor performance of digesters containing only substrates.

Among all the digesters, the one with the cocktail and inoculum (CI) performed the best, with methane production reaching as high as 11.2% on day 15. This finding aligns with the work of ⁽²¹⁾, who reported a percentage of 12.8% for mixed fruit and inoculum. In contrast, digesters containing only fruit wastes exhibited poor performance, with methane yields as low as 0.3% for banana and pawpaw and no methane production at all for watermelon. Compared to mono digestion, co-digestion offers better nutrient supply, digestion stability, and degradation rates ^(22, 9) reported increased biogas production through co-digestion with cow dung due to the improved stability of the C/N ratio, which aids in the better degradation of microorganisms.

The depletion of gas observed during the upscaling of biogas production can be attributed to several factors. In this study, gas accumulated in the gas chamber, which was a tire tube, and then disappeared from within the tube after 72 hours. This depletion could be caused by various reasons, with the action of methanotrophs and temperature being noteworthy. Methanotrophs are microorganisms capable of oxidizing methane to methanol or formaldehyde ⁽²⁷⁾. ⁽⁶⁾ Reported that methanotrophs can utilize oxygen for methane oxidation and, in its absence, can use other electron acceptors, including nitrates, iron, and sulfate. Since cow dung was used as an inoculum in this study, and ⁽¹³⁾ reported the presence of methanotrophs in the digestive tracts of some farm animals, including cows, the presence of methanotrophs may have contributed to the decrease in methane content and biogas production in this study, as reported by ⁽²⁴⁾.

Conclusion

1. This study found significant difference in physicochemical parameters, particularly carbon-to-nitrogen ratio, organic matter and total solids
2. Co-digesting fruit waste with cow dung and gutter sludge resulted in the highest methane yield.
3. Methanogens, essential for methane production, were isolated in the gutter sludge but not present in fruit waste and cow dung.

Conflict of Interest: None

Funding Source: Self

Ethical Clearance: There was no need to obtain ethical clearance for this research because there are no life subjects.

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