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Comparative Performance of Biogas Production using Water melon, Orange and Banana Fruit Wastes

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Abstract

The study was designed to evaluate the performance of three alkali-treated fruit wastes in the production of biogas. Fruit wastes (Banana wastes, orange wastes and watermelon wastes) were collected from fruit vendors within the Ahmadu Bello University main campus Samaru and transported to the laboratory, Department of Microbiology, ABU, Zaria. The fruit wastes were washed, pretreated with 0.5% NaOH solution, washed to neutrality and then allowed to dry. The fruit wastes (200grams each)were separately blended and transferred into a 2L digesters containing 400mL to prepare a slurry. Freshly collected rumen content was added into the digester and the biogas produced was collected and recorded daily. The quality of the biogas was determined by flammability testing and the methanogens involved in the production of biogas were isolated by inoculating the digestate onto enriched Bold Basal Medium. The highest biogas produced (total volume of 1,254cm³ and a mean of 94.5cm³) was observed using treated watermelon wastes followed by treated banana wastes(total volume of 805cm³ and mean of 57.5cm³) and then treated orange wastes (total volume of 291cm³ and mean of 22.39cm³). The biogas with the highest flammability was produced using treated watermelon wastes and treated banana wastes. Four genera of methanogens were identified namely Methanobacterium, Methanospirillum, Methanosarcina and Methanococcus. Treated water melon waste had a better quantity of biogas production and better flammability, hence performed better.

Keywords: Biogas, Fruit, Wastes, Methanogens, Flammability.

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Introduction

One of the major problems in the world today is depletion of reserved fossil fuel which is a cause of the increase in energy price. Energy demand increases with increase in population leading to generation of high amount of wastes, deforestation and global warming. With the ongoing challenges of uncontrolled waste generation and decrease in fossil fuel, the best possible alternative to land fillings and incineration is anaerobic digestion of waste (Gargi, 2016; Hovarth *et al*., 2016).

Anaerobic digestion is a technology of recovering energy from organic wastes(Budiyono *et al*., 2018) through the production of biogas. Biogas is a mixture of different gases such as methane, carbon-dioxide and some traces of hydrogen sulphide and ammonia. The proportion of methane in the biogas usually determines the quality of the biogas which is measured in terms of its flammability when ignited. Methane is the main component of biogas which can be used in place of fossil fuel in both heat and power generation and in vehicles (Matsakas *et al.,* 2016). Therefore, anaerobic digestion of fruit wastes can be used in the production of biogas for cooking and generation of electricity while the Biological treatment through anaerobic digestion is globally accepted as the best method of waste disposal. This is because, it reduces the amount of biodegradable wastes producing biogas and nutrient-rich effluent which is used either as bio-fertilizer or soil conditioner (Ismail *et al.,* 2012). Interestingly, anaerobic digestion also reduces the public health risk of untreated waste disposal since aerobic pathogens are killed under low oxygen tension. In an attempt to optimize biogas yield, a process known as co-digestion where multiple substrates are digested in a single reactor is now in use (Manyi-Loh *et al*., 2013).

Biogas production also depends on the surface area of the substrate and therefore grinding the substrate into smaller size increases the surface area which provides larger contact surfaces for bacterial colonization. Anaerobic digestionof wastes into biogas generally involves four stages namely; hydrolysis (breakdown of carbohydrates, proteins and lipids into monosaccharides, amino acid and fatty acids respectively), acidogenesis (fermentation of the monomers into simple organic acids), acetogenesis (conversion of the organic acids into acetic acid) and methanogenesis (decarboxylation of acetic acid into methane and CO_2) (Sagagi, 2009; Javkhlan *et al*., 2014).

In developing countries such as Nigeria, there are difficulties associated with hydroelectric power and biogas generating capacity (Rouf *et al*., 2018). Increase in population with advances in urbanization and lack of proper disposal options from the household waste has led to loss of aesthetic nature of environments and health problems (Ferronato and Torretta, 2019).
This study was aimed at comparing the quantity and quality of biogas produced from three different fruit wastes generated within Ahmadu Bello University main campus Zaria, Nigeria. The results of this study will serve as a guide in the use of fruit waste as a substrate for biogas production.

Materials and Methods

Sampling Area

All the fruit wastes and the inoculum used in this study were collected from within Samaru, Zaria located on the coordinates 11°04'N 7°42'E (https://en.wikipedia.org/wiki/Zaria).Fruit wastes (peels) of orange, watermelon and banana were collected from fruit vendors at

Suleiman hall and Social Center within Ahmadu Bello University main campus. Fresh rumen content was also collected in clean sample bottles from Zango abattoir, Zaria.

Collection of Samples

Waste samples of each fruitwere separately collected in a cleanpolythene bag and transported to the Laboratory, Department of Microbiology, Ahmadu Bello University, Zaria for analyses., The fresh rumen content sample (2kg) on the other hand was immediately cap-screwed (to avoid exposure to air which might kill the anaerobic microorganisms needed), transported to the Laboratory and used as inoculum during the biogas production.

Fabrication of Anaerobic digesters and the Experimental Setup

A total of three digesters were locally fabricated by drilling holes on the upper side of 2L plastic containers and a flexible rubber pipe was inserted into the hole and glued together so as to prevent leakage of biogas and to create anaerobic condition. The other end of the rubber tubing was inserted into an inverted measuring cylinder in an opened plastic bowl containing 1% NaOH. The measuring cylinders were fixed to a steel clamp connected to retort stand to ensure stability. The set up was kept at room temperature throughout the retention period (see Plate I). Daily observations and recordings of the biogas produced which is indicated by the displacement of NaOH solution were taken as produced (Olatunde *et al*., 2018).

Preparation and Pretreatment of the Fruit Waste Samples

The fruit waste samples were sorted out, washed gently with distilled water to remove dirt and then dried under shade for 48hours. The fruit wastes were pretreated with 0.5%w/v sodium hydroxide (NaOH) as described by Arumugam and Manikan (2011). The fruit wastes were separately soaked in 0.5%w/v NaOH in an opened plastic container and allowed to stand for five days. The alkali-treated fruit wastes were then rinsed thoroughly to a neutral pH (7.0 ± 0.2).

Production of Biogas

Preparation of Substrate Mixture

The pretreated fruit wastes were processed by separately blending about200g of each of the fruit to increase the surface area and ultimately the rate of bacterial digestion. A total volume of 400mL of distilled water was added separately into each of the well labelled vessels containing the blended pretreated fruit wastes to obtain slurries.

Loading of the Reactors and Purification Set-up

Afterward, the slurries were separately transferred into the appropriately labelled 2L fabricated anaerobic digester (see Plate I), then 400g of the rumen content each was added to the slurries as inoculum. (Gargi *et al*., 2016). Each experiment lasted for a retention period of two (2) weeks. Gas production was measured at interval of 24 hours by volume displacement method. The biogas production was carried out at ambient temperature. The gas obtained was also burned to test its combustibility. The gas produced was collected by 'upward delivery and downward displacement' of NaOH solution (Musa and Raji, 2016) which purifies the biogas produced by removing carbon dioxide (incombustible) and traces of other gases such as hydrogen sulphide as shown in the equations below;

$$CO_{2(g)} + 2NaOH_{(aq)} \longrightarrow Na_2CO_3 + H_2O_{(l)} \quad \text{.....(1)}$$

$$H_2S_{(g)} + 2NaOH_{(aq)} \longrightarrow Na_2S + 2H_2O_{(l)} \quad \text{.....(2)}$$

Quantitative and Qualitative Assessment of the Biogas Produced

The quantitative performance of the experimental substrates was assessed base on the following parameters; commencement of gas production, daily gas yield, time to reach peak gas production, time when production ceases and the total volume of gas produced over the period of retention time of 14 days.Flammability testing was used to assess the quality of the biogas produced. The flammability of the biogas produced was carried out by loosening the clamps fixed to the measuring cylinder first, followed by lighting a match flame and passing it over the nozzle of measuring cylinder in which the gas was collected. Results were recorded based on appearance of blue or bright colored flame indicating the presence of methane i.e. combustible biogas or not (Musa and Raji, 2016; Olatunde *et al*., 2018). The degree of flammability was recorded as high, moderate or low.

Plate I: Experimental set up for biogas production

Isolation of Methanogens

Preparation of isolation medium

A total of 300mL of bold basal medium (BBM) was prepared using appropriate amounts of mineral salts and 2% nutrient agar as the base. Four different stock solutions were prepared, the first stock contained 5g NH_4Cl, 0.5g of NaCl, 0.5g of $MgCl_2$ and 0.25g of $CaCl_2$ in 50 mL of distilled water. The second stock solution contained 10g of K_2HPO_4 in 50mL of distilled water, followed by the third stock solution which contained 0.1g of $MnCl_2$, 0.0025g of $AlCl_3$, 0.0025g of $CoCl_2$, 0.0046g of $NiCl_2$, 0.025g of EDTA, 0.05mL of HCl and 0.005g of $NaSeO_3$ dissolved in 50mL of distilled water as described by Khalifah *et al*.(2013).

Vitamin solution was also prepared in 500mL conical flask by adding 0.5g of vitamin A and 0.05g of vitamin C in 50 mL of the water to 2.8g of nutrient agarin 100mL of distilled water. The solution was boiled and allowed to cool until it reaches 50 ^oC. The stocks were added to the prepared agar and swelled evenly, it was autoclaved for 15 minutes at 121 ^oC and was allowed to cool in a water bath. Acetate was added and, the prepared medium were divided into two, the first portion was poured into a sterile conical flask to which 2ml of methanol were added and labeled A. To the second portion 2g of $NaNO_3$ was added and labeled B. The media were poured into the sterile Petri dishes and allowed to solidify. The digestate was serially diluted, then 1mL of dilution 10^{-5} was inoculated by pour plating technique aseptically. Incubation was carried out anaerobically at 37° C for seven days. The isolates were Gram stained and viewed microscopically using oil immersion objective (Musa and Raji, 2016; Long *et al*., 2017).

Results

Figure 1 showed the volume of biogas produced in cm^3 using each of the treated fruits throughout the hydraulic retention period. The peak of biogas production was observed on the 9^{th} day for treated watermelon waste ($210cm^3$) and treated banana waste ($152cm^3$) while

the peak of biogas production using treated orange waste (170cm^3) was observed on the 10th day.

The performance of the different fruit wastes to produce biogas throughout the retention period is presented on Table 1. The highest volume of biogas produced (total = 1254cm^3; mean = 94.5cm^3) was observed using treated watermelon waste followed by treated banana waste (total = 805cm^3; mean = 57.5cm^3) and treated orange waste (total = 291cm^3; mean = 22.39cm^3). Treated banana waste had the earliest onset of gas production (3rd day), followed by treated watermelon waste (4th day) and treated orange waste (6th day).

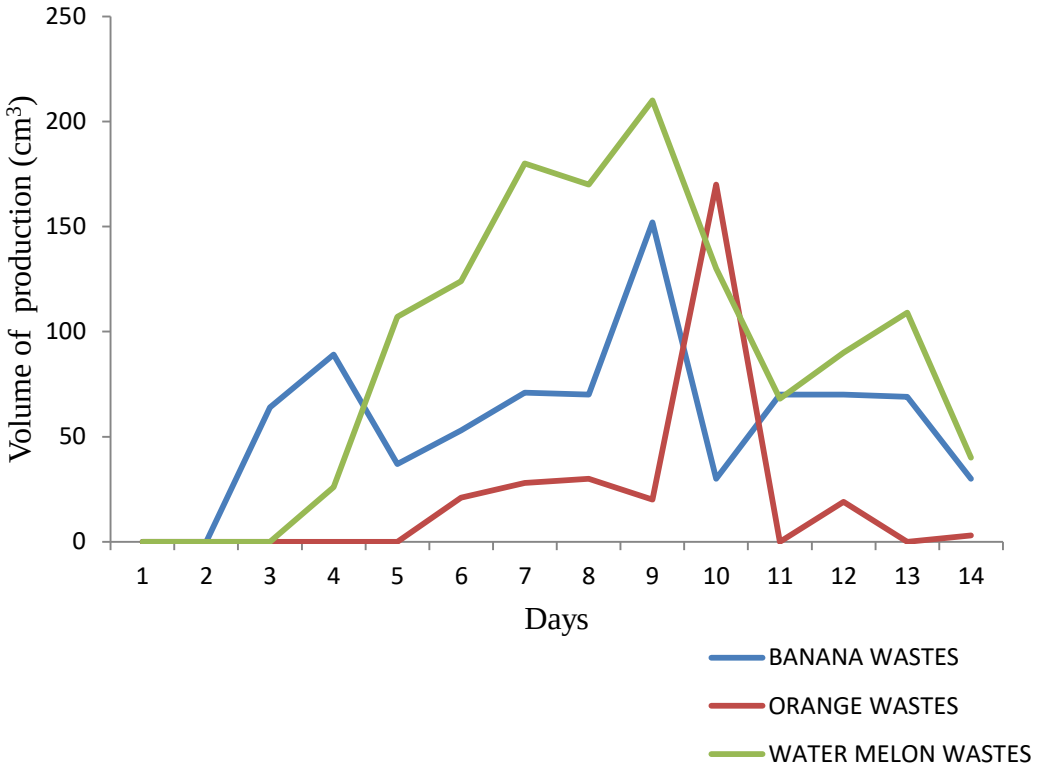

Figure 1: Volume of biogas produced during retention period of the treated fruit waste

Table 2 and Plate II show the level flammability biogas produced using each of the substrate throughout the retention period. Flammable biogas was produced using treated banana waste from the 6th day upward, while flammable biogas was produced from treated watermelon wastes from 7th day upward. However flammable biogas was produced using treated orange waste after 10 days of setup.

Table 1: Performance of the different Samples for Biogas Production

Parameters	**Treated watermelon waste**	**Treated banana waste**	**Treated orange waste**
Total volume of biogas produced (cm^3)	1,254	805	291
Mean of biogas produced (cm^3)	94.5	57.5	22.39
Range of biogas produced (cm^3)	0-105	0-152	0-170
Onset of biogas production (days)	4th	3rd	6th
Onset of flammable biogas {methane} production (days)	7th	6th	10th
Peak biogas production (days)	9th	9th	10th
Temperature of the digestion	Room temperature	Room temperature	Room temperature

Table 3 showed the Gram's reaction of methanogens isolated from the digestate of treated fruit wastes on bold basal medium with different treatments. The genera of methanogens isolated were: *Methanobacterium, Methanosarcina, Methanococcus* and *Methanospiral*.

Table 2: Flammability Test for the biogas produced from the treated fruit wastes

Days	**TWW**	**TBW**	**TOW**
6th	-	+	-
7th	+	+	-
8th	+	+	-
9th	+++	+++	-
10th	+	+	++
11th	+	+	-
12th	+	+	-
13th	++	+	-
14th	+	+	-

Key: Highly flammable =+ ++, Moderately flammable =+ +, Flammable = +Not flammable = -, TWW= treated watermelon wastes, TOW= treated orange wastes, TBW= treated banana wastes.

Table 3: Genera of Methanogens isolated from the digestate of treated fruit wastes

Isolates code	Growth on Medium A	Growth on Medium B	Gram reaction and morphology	Inference
TWW 1	+	-	Gram positive rod	*Methanobacterium* sp.
TWW2	_	+	Gram positive cocci	*Methanosarcina* sp.
TWW 3	_	+	Gram variablecocci	*Methanococcus* sp.
TBW 1	+	_	Gram variable slender rod	*Methanospirillum* sp.
TOW 1	+	_	Gram positive rod	*Methanobacterium* sp.
TOW 2	+	_	Gram variable slender rod	*Methanospirillum* sp.
TOW 3	_	+	Gram variable cocci	*Methanococcus* sp.

Key: Medium A = Growth on BBM + Acetate +Methanol; Medium B = Growth on BBM +Acetate +$NaNO_3$

(a) (b) (c)

Plate II: Pictorial representation of flammability of biogas produced from the treated fruit waste

Discussion

The production of biogas is a step by step process indicating that minimal time is required before the activities of methanogens start which is the conversion of some intermediate's substrates into biogas (Budiyono *et al*., 2018). In this study, biogas production was not observed during the first few days, this correspond to the hydrolysis and acidogenesis stages in anaerobic digestion process (Javkhlan *et al*., 2014). So also, the biogas produced immediate after the hydrolysis and acidogenesis stages are not flammable and it correspond to the acetogenesis stage where acetate and CO_2 are produced mainly. Onset of production of flammable biogas (methane) on the 6th, 7th and 10thday using TBW, TWW and TOW respectively signifies the commencement of methanogenesis which is the fourth stage in anaerobic digestion process (Javkhlan *et al*., 2014).

The highest volume of biogas observed using TWW might be due to ease of degradation of TWW orvarying proximate composition of the substrates. Though TWW had the highest volume of biogas produced, biogas from TBW was better in terms of flammability. The total volume of biogas production from TOW was low and the flammability of biogas from TOW was also the least. These could be attributed to the presence of D-limonene which is an antimicrobial substance that is highly toxic to many organisms (Wikandari *et al*., 2015) and presence of excess ammonia which is incombustible (Budiyono *et al*., 2018) in orange waste. The treatment carried out was equally proportioned for all the wastes, possibly the need for additional treatment for the TOW would have decrease the fluctuation by in-activating the

anti-microbial substance. Another possible reason could be due complexity of the orange wastes resulting in the requirement of much longer time for the production of combustible biogas (Loannis *et al*., 2017).

The genus of methanogens isolated from the digestate were *Methanospirillum* sp., *Methanobacterium* sp., *Methanosarcina* sp. and *Methanococcus* sp. These methanogens are known to produce methane from the acetate produced by the previous microbial communities during the acetogenesis stage. This result agrees with the findings of Musa and Raji (2016) and Feng *et al*., (2010).

Conclusion

Biogas was produced from all the treated fruit wastes with TWW having the highest volume of biogas production and TBW producing the most flammable biogas. The methanogens involved in the methane production were *Methanospirillum* sp., *Methanobacterium* sp., *Methanosarcina* sp. and *Methanococcus* sp.

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