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# Enhancing Biogas Production from Pumpkin (*Telfairia occidentalis*) Stalk Waste through Its Blending with Cow Dung and Alkaline Sodium Hydroxide Pretreatment

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***Authors' contributions***

*The first and corresponding author UE designed the study, wrote the protocol and first draft of the manuscript. Authors UE, IC and OG managed the analyses of the study. All authors read and approved the final manuscript.*

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## ABSTRACT

Improvement of biogas production from Pumpkin (*Telfairia occidentalis*) stalk waste through its blending with cow dung and alkaline sodium hydroxide pretreatment was investigated. Untreated pumpkin stalk waste (Un-PST) acted as the control. Initial verification carried out using this waste led to the present investigation. The batch digestion process anaerobically took place for 30 days under ambient atmospheric conditions. Bio-digesters of 41.00 $dm^3$ working volume were utilized. Cumulative volume of biogas yield from the cow dung blended with pumpkin stalk (PST-cd) was 3.57 $dm^3$/TS.kg and alkaline treated waste (PST-t) gave 4.48 $dm^3$/TS.kg, while untreated pumpkin stalk waste (Un-PST), yielded 1.50 $dm^3$/TS.kg. Each of these systems also produced flammable gas at different times: the Un-PST -15th day, PST-cd -5th day and PST-t-8th day, respectively. Paired sample T-Test carried out on volume of gas data indicated that t (t statistic) was 5.15 for paired sample Un-PST & PST-cd while 6.51 for Un-PST & PST-t with degree of freedom as 29; at 95% conf. interval. Hence, there was a significant ($p<0.05$) difference in volume of gas production

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between the untreated and treated waste systems. Overall, results indicate that the yield of biogas from the waste through the different treatments were significantly ($p<0.05$) enhanced for renewable energy production, sustainable environment and wealth recovery for urban and rural dwellers of developing countries such as Nigeria, when properly harnessed.

*Keywords: Biomass wastes; biogas yield; energy production; flammable biogas.*

## 1. INTRODUCTION

Human activities cannot be piloted without energy. Hence, as long as life exists, energy demands will continue to rise with economic and social development among nations. In the past decades, the demand was met globally through exploitation of conventional energy sources (crude oil, coal, bitumen, tar sand, etc) that are exhaustible. Besides, the world energy crisis in 1970 [1], had led to increase in prices of the conventional fuels due to socio-economic, technological, political and environmental factors thereby forcing most countries to embrace alternatives and renewable energy sources particularly from biomass. Biogas production is an anaerobic digestion process among other processes such as thermal pyrolysis, combustion and gasification. It has been viewed in recent times as a very good source of sustainable waste treatment/management especially in third world countries where waste disposal remained a major challenge [2]. An important by-product of this process is a residue rich in essential organic elements such as nitrogen, phosphorus and potassium, etc, needed for healthy growth of plants, known as bio-fertilizer. Application of this fertilizer to the soil enriches it without any detrimental effects on the environment [3]. The composition of biogas varies with the organic material to be biodegraded and the environmental conditions involved [4]. Generally, any organic waste material will contain adequate quantities of the nutrients essential for the growth of the microbes, but biological availability of the nutrients contained in the wastes vary with species, factors affecting growth and age of the animal or plant [5]. Various treatments such as soaking in water, size reduction, addition of organic solvents, alkali and acids, inorganic metals, etc., have been given to organic wastes to upgrade their biogas yield [6,7,8,9.10]. Also, one known treatment method for improving the biogas production of various feed stocks is co-digesting them with animal and/or plant wastes [11]. Blending of animal and plant wastes bring about sustained onset of flammable gas production with higher cumulative biogas yield during the chosen retention period [12]. This is because blending could enhance synergistic effect of the combined feed stocks. Previous studies have shown that dung from ruminants is known to contain native microbial flora that aids in faster biogas production [13]. Besides, acids and bases are known to de-lignify plant cell structures [14]. Hence, research findings of [15], on biogas production from pretreatment of bagasse and coconut fibers with HCl was shown to improve the yields of biogas from the materials by 31 and 74%, respectively. However, highest level of de-lignifications was shown in the treatment of agricultural residues with sodium hydroxide [16,17]. Results of investigation pointed out that alkali treatment in comparison with acid or oxidative reagents appeared to be most effective method of breaking the ester bonds between lignin, hemicelluloses and cellulose. Fortunately, every part of the world is endowed with various types of biomass that can be recycled [18]. Therefore, energy production from biomass wastes is non-exhaustible. *Telfairia* is a small genus of flowering plants in the squash family which is native to Africa while *Telfairia occidentalis* is a tropical vine grown in West Africa as a leafy vegetable and for its edible seeds. Common names for the plant include fluted guard, fluted pumpkin and "Ugu" [19]. Consequently, *Telfairia* stalk is a by-product from the leafy vegetable. This vegetable is commonly consumed by Ibos in the South Eastern part of Nigeria in West Africa, due to its health functional properties. The wastes have been used in composting and in recent times as animal feed. Preliminary investigation of biogas production from the stalk indicated that it produced flammable biogas but with low yield and longer onset of flammable gas production from its system. Hence, the present study was carried out to enhance pumpkin stalk waste renewable energy production by blending it with cow dung and alkaline sodium hydroxide treatment, under anaerobic digestion.

## 2. MATERIALS AND METHODS

Pumpkin stalk utilized for this investigation was procured from a restaurant in the University of Nigeria, Nsukka "campus community", Enugu state while fresh cow dung was obtained from an abattoir in Nsukka town's market. Chemicals such as sodium hydroxide pellets were purchased from a soap chemical dealer in a market of Nsukka town, without further purification. Acetic acid (99%) prepared by Sigma-Aldrich Laboratories, Germany, was also purchased from a scientific chemical shop in Nsukka. All other chemicals of analytical grade used for the physico-chemical and microbial analyses were provided in the Departments of Crop Science and Pharmacy-Microbiology Laboratories, University of Nigeria. Metallic prototype bio-digesters used had working volumes of 41.0 $dm^3$ each, constructed at the National Centre for Energy Research and Development engineering workshop of the same University. Materials further utilized were as follows: Weighing balance (50 kg "Five Goats," model no Z051099), gas collection accessories, liquid in glass thermometer (-10 to 110$^oC$), pocket-sized pH meter-RI02895 (Hanna Instruments Italy) and locally fabricated burner for checking flammable gas production.

## 2.1 Sample Preparation

Freshly collected pumpkin stalk wastes (Fig. 1) utilized for the study were left for eight weeks under atmospheric influence to dry so as to reduce acidic contents of the fresh wastes and undergo partial degradation. They were then cut to average of 125mm lengthwise and soaked in water (3 days) for further partial degradation by aerobic microbes which are known to be better decomposers of cellulose [20]. Concentrated sodium hydroxide solution (50% w/v) was prepared and left over-night to cool before the treatment.

## 2.2 Digestion Studies

The alkaline treated waste (PST-t) was charged when pH stabilized towards neutrality. The treatment was effected with 220 ml of sodium hydroxide solution and corrected with 100ml of acetic acid within 48 h of trials in a thermo-plastic trough. Blending of the fresh cow dung (cd) with pumpkin stalk waste (PST) was carried out in the ratio of 1:1. Untreated pumpkin stalk waste (Un-PST) was used as control. The waste variants and the control (PST-t, PST-cd and Un-PST) were prepared for charging by diluting 6kg of each variant and the control with 24kg of water from town's supply and the liquor from the soaked wastes, giving waste to water ratio of 1:4. They were then charged separately in different digesters of similar size (41.0 $dm^3$-Fig. 2) and the contents occasionally agitated to prevent formation of scum within the system. The ratio of the wastes to water was based on the moisture content of pumpkin waste after soaking. Experiment was batch operated for 30 days under daily variation of ambient temperature range 22.0-33.8$^0C$.

**Fig. 1. Fresh pumpkin stalk waste**

## 2.3 Analytical Methods

### 2.3.1 Chemical compositions of the undigested organic wastes

Moisture, ash and fiber contents of the undigested pumpkin waste; treated and blended with cow dung were determined using AOAC method [21]. Crude fat, protein and nitrogen contents were carried out using Soxhlet extraction and Micro-Kjedhal methods as described in Pearson [22]. Energy content analysis was done using bomb calorimeter according to AOAC [21]. Total solids (TS) and volatile solids (VS) were determined using the method described in Bhatia [23]. Carbon content was determined using modified method of Walkley and Black, described in Nelson and Sommers [24].

### 2.3.2 Microbial analysis

Total viable counts of biogas microbes were determined during the period of study at intervals of 4 days, using a modified method of Miles and Misra described by Okore [25].

**Fig. 2. Bio-digester**

#### 2.3.3 pH measurement

The pH of the systems was determined using the pocket sized pH meter at intervals of 2 days during the entire study period.

#### 2.3.4 Measurement and computation of cumulative and average daily biogas yields

Volume of biogas produced on daily basis was measured [26], within the active sunshine periods (9.00 am--4.00 pm). The digesters utilized being of 41.00 $dm^3$ working volume, was ideally expected to produce approximately 10.00 $dm^3$ per total mass of slurry (TS.kg dilution) of gas at optimum conditions, since ¾ volume (30.75 $dm^3$) of digester was charged with the diluted waste leaving the headspace for gas storage before collection [27]. Each reactor produced 6.50 $dm^3$ at full capacity with pressure recorded as 22 mmHg during study period. However, rate of biogas production was low due to diurnal temperature variations among other factors. Cumulative volume of gas yield for each digester system was calculated by summing up the daily average volume of biogas produced per TS.kg of slurry for 30 days retention period. Mean volume of biogas yield was also obtained by dividing the cumulative gas yield of each system by 30 days.

#### 2.3.5 Flammable gas composition analysis

This was determined using field gas analyzer, “crowcon Gasman” by the “Direct reading engineering method” (DREM).

#### 2.3.6 Data analysis

Data obtained from daily biogas yields were analyzed using paired sample t-test while total viable counts (TVC) and chemical compositions of undigested waste samples (wastes from sample preparation-dried and slightly degraded from atmospheric influence) were analyzed using one way analysis of variance in completely randomized design (CRD). The least significant difference (LSD) was used to determine level of differences in means while significance was accepted at $p<0.05$. The SPSS software package, 15.0 versions, was used.

## 3. RESULTS AND DISCUSSION

### 3.1 Daily biogas yields

Fig. 3 displays the daily biogas yields for the untreated pumpkin stalk waste (Un-PST) that acted as control, alkaline pretreated pumpkin stalk waste (PST-t) and cow dung blended with pumpkin stalk waste (PST-cd) systems. However, daily biogas yields for all the different waste systems were greatly affected by diurnal ambient temperature variations during digestion period and presented the pattern of the graphs. Each system started biogas production at different times as shown by the figure while the production of flammable gas after charging commenced on different days (Table 1). Effectiveness of biogas in cooking and lighting is based on the combustibility of the gas. If it burns, it means that the methane content is more than 45% [28]. Where a biogas system fails to produce flame, it may be useless to the end user for the purpose of energy utilization. Gas composition analysis indicated that all the systems produced flammable biogas with wet methane content of 60% and above (Table 1). However, PST-cd system had highest value of wet methane content and underscores the superiority of cow dung in quality and quantity flammable biogas production. This scenario obtained from PST-cd system agreed with the findings of [29,30]. Wastes from ruminants such as cows were found to be very good inoculums for biogas production process because they already contain native microbial flora in their feces [31,32]. The composition of flammable gas analyzed also showed 0% for carbon monoxide indicating environmentally friendliness of the process. Cumulative volume of gas yields are shown in Table 1. Un-PST system had the lowest value while PST-t system gave the highest. This relatively poor cumulative biogas yield obtained for the Un-PST system could be due to higher fiber and organic carbon contents of its undigested wastes. Most of the plant wastes are known for higher carbon contents and under biogas technology they yield low gas due to presence of lignin in their structures [33,34]. Nevertheless, acids and bases are known to de-lignify plant cell structures [14]. Research findings of Gas Par et al. [16] and Zhao et al. [17] indicated that highest level of de-lignifications was shown in the treatment of agricultural residues with alkaline sodium hydroxide. Besides, preliminary verification carried out with fresh untreated pumpkin stalk showed that the waste in the fresh state could not produce biogas which may be due to acidic nature of the waste which may have led to excess accumulated volatile fatty acids in the digester system during experimental period [10,35].

## 3.2 Daily Biogas Yield and pH Changes

Fig. 4 shows changes in the pH of untreated and treated pumpkin stalk systems utilized in the present study. For the production of sufficient amount of methane, optimum pH of digester should be maintained. This is because bio-digestion is an enzyme induced biochemical reaction. Hence, pH is an internal reactor parameter that can affect biogas microbes and consequently volume of gas produced in an anaerobic digestion. The slow growing methanogens are highly sensitive to pH changes and can only operate optimally within slightly acidic (6.5) to slightly alkaline (8.0) pH range [36]. Hence, improper range of pH values in a biogas reactor can lead to collapse of the system with subsequent reduction in the volume of gas production. Preliminary investigations carried out with fresh untreated pumpkin stalk showed that the waste in the fresh state could not produce biogas which may be due to its acidic nature (pH of 5.4 under soaking in water). There could have been excess accumulated volatile fatty acids in the digester system during experimental period [10,35]. Hence, partial degradation of the stalks in the present study was carried out before the gas production during digestion period. Fig. 4 also pointed out that if the untreated waste could be buffered and the pH maintained at the range favourable for the anaerobes the rural dwellers of developing countries can make use of this waste recycling process without further treatment in meeting up their daily energy needs. However, delay in flammable gas production and total gas yield from the untreated waste systems will remain a bottle neck in the process. Consequently, total volume of gas yield including daily gas yield may be increased when the system is buffered and at same time the waste de-lignified before digestion. This will help to fasten hydrolysis which is a rate determining step in anaerobic digestion of plant wastes [34].

## 3.3 Total Microbial Counts

Table 2 displays the total viable counts (TVC) during digestion period which represent the rate of growth of the microbes that converted the wastes to biogas. The PST-t had the highest improvement on the microbial load and this was fairly maintained towards end of study period. There are four stages under biogas production: hydrolysis, acidogenesis, acetogenesis and methanogenesis and each group of enzymes involved has its own optimum pH for a particular reaction to take place in the biogas system. This explains why chemical treatment of wastes is always carried out to achieve working pH ranges and increases the rate of biochemical reaction for feedstock digester system. Comparison of the viable counts from each system was done using one way analysis of variance (ANOVA) and least significant difference (LSD) was used to determine level of differences in means. There was a highly significant ($p<0.050$) difference in the total microbial load of PST-t system than the other systems at charging and flammable periods. This may be because plant wastes such as pumpkin stalk contain some recalcitrant and xenobiotics such as lignin that pose a lot of problems during anaerobic digestion process. Hence, chemical treatment using sodium hydroxide solution would have caused more nutrients to be available and improved pH to the required range giving rise to increase in the population of the anaerobic microbes in the chemically treated biogas system. However, there was no significant ($p>0.05$) difference in the viable counts of Un-PST and PST-cd at flammable gas production period even though PST-cd system was expected to have higher counts. This could be due to the two systems operating at almost equal levels of pH (Fig. 4) and the waste structures not modified before charging during study period. Data obtained from volume of biogas yields were also compared using Paired Sample T–test analysis. Results indicated that t (t statistic) was 5.15 for paired sample Un-PST & PST-cd while 6.51 for Un-PST & PST-t with degree of freedom as 29; at 95% conf. interval. Hence, there was a significant ($p<0.05$) difference in volume of gas production between the untreated and treated waste systems. However, the difference in total volume of biogas yield was higher for the alkaline treated waste system.

### 3.4 Volume of Biogas Yields and Chemical Compositions of Undigested Wastes

The chemical compositions of the undigested organic wastes (waste before dilution and charging commenced) are presented in Table 3. Significant improvements were observed in parameters such as crude fiber, organic carbon and protein contents of the treated wastes. Hydrolysis of most plant wastes is slow when compared to animal manures/wastes due to hard structure of the plants as a result of lignin and cellulose contents. Lignin is non-biodegradable while cellulose is difficult to microbiologically degrade except when treatment is applied [34]. Fiber is a combination of cellulose and lignin in various proportions. Hence, alkaline sodium hydroxide treatment enhanced significantly ($p<0.05$) de-lignifications of the Un-PST and would have contributed to the highest cumulative volume of biogas yield during experimental period. A remarkable reduction in organic carbon content of the Un-PST waste was also recorded due the treatments even though no significant ($p>0.05$) difference existed in the carbon contents after the treatment as shown in Table 3. Protein contents of the treated wastes were also significantly improved ($p<0.05$) but highest amount was obtained for PST-cd system. This might have contributed to the earliest onset of flammable gas production since adequate protein contents of the wastes would be required for the healthy growth of the anaerobes during digestion. Also, the population of the microbes might have been affected by nutrient reduction. Generally adequate chemical compositions such as crude protein, fat, carbohydrates, ash, carbon to nitrogen ratio (C/N ratio) are required for efficient biogas production [37]. Table 3 also shows the C/N ratio obtained for the variants and the control. The C/N ratio of a feedstock material under anaerobic digestion process is the balance of food a microbe requires in order to grow. Initially it was reported as 15 to 30:1 and 20 – 25: 1 [20,38]. However, current researches in this area maintains 20 to 30: 1 and above 20 to 35: 1, the system may become toxic [39,36].

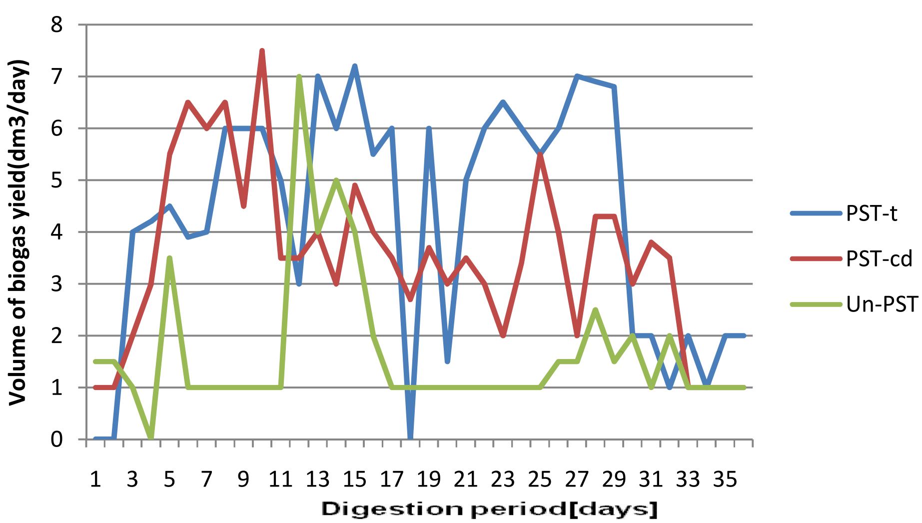


**Fig. 3. Daily biogas yield for all systems**

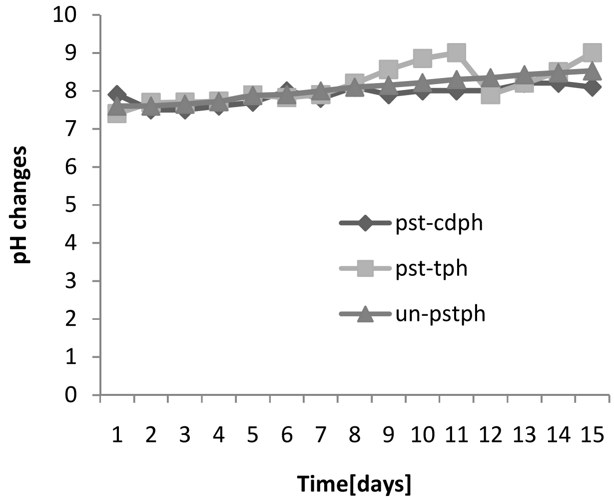


**Fig. 4. Changes in pH of all the systems at two days intervals during digestion period**

**Table 1. Bio-digester systems behaviour during study period**

| Parameters | Un-PST | PST-t | PST-cd |
|---|---|---|---|
| Lag days | 14 | 7 | 4 |
| Resumption of flammable gas production (days) | 15 | 8 | 5 |
| Cumulative gas yield ($dm^3$/TS. kg) | 1.50±.212$^{a}$ | 4.48±.0566$^{b}$ | 3.57±.0212$^{c}$ |
| Mean gas yield ($dm^3$/TS.kg /day) | 0.055±.007$^{a}$ | 0.149±.006$^{b}$ | 0.119±.006$^{b}$ |
| Moist methane content (%) | 60.0±.141$^{a}$ | 65.0±.424$^{b}$ | 80.0±.283$^{c}$ |

*Values are means ± standard deviations of triplicate determinations**,** Means within the same row that have common letters are significantly different (p >0.05),* ***Un-PST****...Untreated pumpkin stalk waste;* ***PST-t****…pumpkin stalk waste treated with sodium hydroxide solution; PST-cd…pumpkin stalk waste combined with cow dung*

**Table 2. Total viable counts (Cfu/ml) during study period**

| Periods | Un-PST | PST-t | PST-cd |
|---|---|---|---|
| Charging | 1.82 x $10^6$±7071.068$^{a}$ | 1.0 x $10^8$±1414213.562$^{b}$ | 3.75 x$10^7$±1767766.453$^{c}$ |
| Flammable | 8.23 x $10^6$±14142.136$^{a}$ | 8.2 x $10^7$±77071106.781$^{b}$ | 9.33 x $10^6$±77781.746$^{a}$ |
| Peak of production | 1.27 x $10^7$±70710.678$^{a}$ | 8.4 x $10^7$±1414213.562$^{b}$ | 9.0 x $10^6$±7071.068$^{c}$ |
| End of study | 4.92 x $10^5$±1414.214$^{a}$ | 3.6 x $10^7$±707106.781$^{b}$ | 2.03 x $10^7$±212132.034$^{c}$ |

*Values are means ± standard deviations of triplicate determinations, Means within the same row that have common letters are significantly different (p >0.05), Un-PST...Untreated pumpkin stalk waste; PST-t…pumpkin stalk waste treated with sodium hydroxide solution; PST-cd…pumpkin stalk waste combined with cow dung*

**Table 3. Chemical Compositions of Undigested Organic Wastes**

| Parameters | Un-PST | PST-t | PST-cd |
|---|---|---|---|
| Moisture (%) | 33.5±.212$^{a}$ | 43.0±.071$^{b}$ | 18.2±.141$^{c}$ |
| Ash (%) | 3.5±.566$^{a}$ | 3.0±.141$^{b}$ | 2.8±.141$^{b}$ |
| Crude fiber (%) | 49.15±.106$^{a}$ | 22.25±.106$^{b}$ | 25.8±.141$^{c}$ |
| Crude fat (%) | 1.2±.106$^{a}$ | 1.0±.014$^{a}$ | 1.25±.071$^{a}$ |
| Crude nitrogen (%) | 1.9±.113$^{a}$ | 1.54±.042$^{b}$ | 1.86±.057$^{a}$ |
| Crude protein (%) | 5.5±.071$^{a}$ | 9.63±.113$^{b}$ | 11.6±.141$^{c}$ |
| T. Carbohy (%) | 54.3±.212$^{a}$ | 43.37±.071$^{b}$ | 66.15±.106$^{c}$ |
| Total solids (%) | 66.5±.212$^{a}$ | 57.0±.141$^{b}$ | 81.8±.141$^{c}$ |
| Volatile solids (%) | 63.0±.071$^{a}$ | 54.0±.106$^{b}$ | 79.0±.212$^{c}$ |

| Parameters | Un-PST | PST-t | PST-cd |
|---|---|---|---|
| Org. carbon (%) | 42.0±.41[a] | 35.5±.707[b] | 34.4±.141[b] |
| Carbon/Nitrogen ratio | 22.11±.078[a] | 23.05±.071[b] | 18.53±.085[c] |
| pH at charging | 7.6±.141[a] | 7.4±.071[a] | 7.8±.071[b] |

*Values are means ± standard deviations of triplicate determinations, Means within the same row that have common letters are significantly different (p >0.05), Un-PST...Untreated pumpkin stalk waste; PST-t…pumpkin stalk waste treated with sodium hydroxide solution; PST-cd…pumpkin stalk waste combined with cow dung*

## 4. CONCLUSION

This experimental study has shown that pumpkin stalk is a potential organic waste for renewable energy production for developing countries such as Nigeria, where pumpkin vegetables are regularly consumed for their health benefits. The yield of biogas from the waste through the different treatments were significantly (p<0.05) improved for renewable energy production, sustainable environment and wealth recovery for urban and rural dwellers when properly harnessed. However, better optimization was obtained when chemically treated with sodium hydroxide solution. Consequently, waste recycling with sustainable environment, provision of energy needs and wealth creation for rural and urban dwellers of developing countries are promising benefits from this verification.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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