

# Study of the Microbial Composition and Comparative Biogas Production from Fresh, Dried and Cow-Dung Seeded Pumpkin (*Cucurbita maxima*) Residue

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## Abstract

The research study was aimed at identifying the microorganism(s) associated with biogas production as well as comparing the biogas production potential of the fresh, dried and cow dung seeded pumpkin wastes as substrate. The samples were procured from different fruit seller locations within Sokoto metropolis and the study conducted within a period of five weeks (35 days). Standard microbiological methods were used for isolating and identifying the microbes while fabricated anaerobic bio-digesters were used to ascertain the biogas production from the substrates. Analysis revealed that the pH varied between 6.30 to 7.20 and 5.0 to 6.2 before and during/after the anaerobic digestion respectively. Anaerobic bacteria isolated were identified as *Enterobacter spp* and *Escherichia coli*. The results showed 4850, 1430 and 1770 cm<sup>3</sup> biogas production from the fresh, dried and cow dung co-seeded substrates respectively during the retention periods with a calorific value of 4773 kcal/kg. The volume of biogas produced varied significantly ( $p < 0.05$ ) between the substrate treatments and the digestion intervals (days). GC-MS analysis confirmed the identity of the produced methane. This study confirmed the role of bacteria as well as the capability of the substrate to produce biogas production in an *in vitro* bio-digester system.

## Introduction

Developing countries like Nigeria not only faces energy scarcity which present challenges to human health and economic development but is also plagued with

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additional dilemma of environment pollutions due to the heavy dependency on fossil fuels and biomass [1]. Biogas is a flammable gas produced when organic materials are fermented under anaerobic condition [2, 3]. It holds promise as an alternative energy source capable of being used for generating electricity, car fueling, cooking among others [4]. The rate of biogas production depends on the nature of the substrate, temperature, pH, loading rate, toxicity, stirring, nutrients, slurry concentration, digester construction and size, carbon to nitrogen ratio, retention time, alkalinity, initial feeding, total volatile acids, chemical oxygen demand (COD), biological oxygen demand (BOD), total solids (TS), and volatile liquids among others [5].

Biomass such as agricultural wastes, municipal waste, and green waste (kitchen waste) present a promising renewable energy opportunity [6]. In most cities in Nigeria, waste is disposed off as landfills or discarded indiscriminately which causes health hazard and diseases like malaria, typhoid, and cholera [7], hence biological conversion of biomass to methane has received an increasing attention in recent years. Over the years, several techniques have been used for the conversion of organic materials to biogas but anaerobic digestion has gained prominence over the years. The anaerobic digestion process involves four stages namely: hydrolysis, acidogenesis, acetogenesis and methanogenesis [8-11] with each stage carried out by consortia of microorganisms which partly stand in syntrophic interrelation [12].

Agricultural wastes have become an integral source of biogas production especially in areas where agriculture forms an important part of the economy [13]. These wastes not only lead to soil loss and farmland damages if left unchecked but could pose danger to health of the populace especially if they litter the environment as they are known to be perfect habitat for pathogenic microorganism. This paper hence presents results of the production of biogas from fresh, dried and cow-seeded pumpkin residue as well as ascertaining the microbial consortia implicated in the biogas production.

## **Materials and Methods**

### ***Study location***

The experiment was conducted at the Sokoto Energy Research Centre (SERC) central laboratory, Usmanu Danfodiyo University (13.1246° N, 5.1994° E) Sokoto, Sokoto State Nigeria.

### ***Source of sample***

Pumpkin residues were collected from different fruit seller locations within Sokoto metropolis while cow (*Bos primigenius*) dung was collected from Dundaye village both in Sokoto metropolis.

### ***Media used***

Starch agar, carboxymethyl cellulose agar, egg yolk agar and nutrient-gelatin agar (Biotech Lab Ltd, UK) and triple sugar Iron agar (Hardy Diagnostic, USA) were the media used for the study.

### ***Preparation of media and reagents***

All the media and reagents used were prepared and preserved according to the manufacturer's specifications.

### ***Digester design***

Cylindrical cans were used as the digesters and plastic basins were used as water trough. To each can, a hole was bored on the lid of the can and a polyvinyl chloride tube was inserted into the hole and fixed with araldite adhesive to ensure that the digester was airtight. This served as the outlet for the generated biogas. The free end of the rubber tube was then inserted, through water contained in a partly filled water trough into an inverted measuring cylinder that has also been filled with water, to serve as the biogas collection system. The biogas was collected by downward displacement of water.

### ***Preparation of slurry and loading of digesters***

Both wastes were collected fresh, chopped by hand into small pieces (2-3 mm length) and sorted into three portions. The fresh portion was dried from two week before being reduced to fine powder with the aid of mortar and pestle. Its slurry was prepared by macerating the dried substrate with distilled water in the ratio of 1:4. While, the second portion was finely grounded with water in an electric milling machine in the ratio 1:4, the third portion was seeded with cow dung in the ratio 1:6 water to waste (ratio arrived at after preliminary trial). The slurries were quickly loaded into their respective biodigesters. Each sample category was performed in triplicate.

### ***Preparation of spent slurries for microbial isolation***

10 g of the spent slurries were weighed and aseptically crushed into homogenous mixture. It was transferred into 90 ml of sterile distilled water contained in a 100 ml volumetric flask. The mixture was agitated and allowed to settle.

### ***Isolation and assessment of bacterial populations***

The method described by Aderonke *et al.* [14] was adopted with slight modification. The prepared substrates correspond to 10<sup>-1</sup> dilution. 10 ml of aliquot from the 10<sup>-1</sup> dilution was measured into another volumetric flask containing 90 ml of sterile distilled water to obtain 10<sup>-2</sup> dilution. Further dilutions were carried out until a dilution level of 10<sup>-7</sup> was reached. Samples were taken once every three days for total heterotrophic counts. For bacterial screening, dilutions 10<sup>-5</sup> to 10<sup>-7</sup> of the samples (upon serial dilution) were plated on starch agar, carboxymethyl cellulose agar, egg yolk agar and nutrient-gelatin agar (hydrolytic bacteria media). Plates were incubated for 48 hours at 35°C. Colony forming units per gram (CFU g<sup>-1</sup>) of bacterial growth between 30-300 colonies were enumerated. The colonies formed were sub cultured and identified using cultural, morphological and biochemical methods.

### ***Characterization of the isolates***

Cultural and morphological characteristics of pure colonies were used to perform preliminary characterization [15]. The cell shape and arrangement characteristics were viewed under compound microscope after standard staining. 3% (w/v) KOH test [16] was used to determine the gram characteristics of the isolates. Among the biochemical tests conducted were catalase, coagulase, lactose, glucose, sucrose, citrate, indole, H<sub>2</sub>S production, urease, gas, methyl red (MR), Voges-proskauer (VP), spore formation, oxidase and motility tests

### ***Measurement of gas production***

Biogas production was measured daily on volume basis as evidenced by the displacement of water in the inverted measuring cylinder that has been filled with water to serve as the biogas collection system

### **Statistical Analysis**

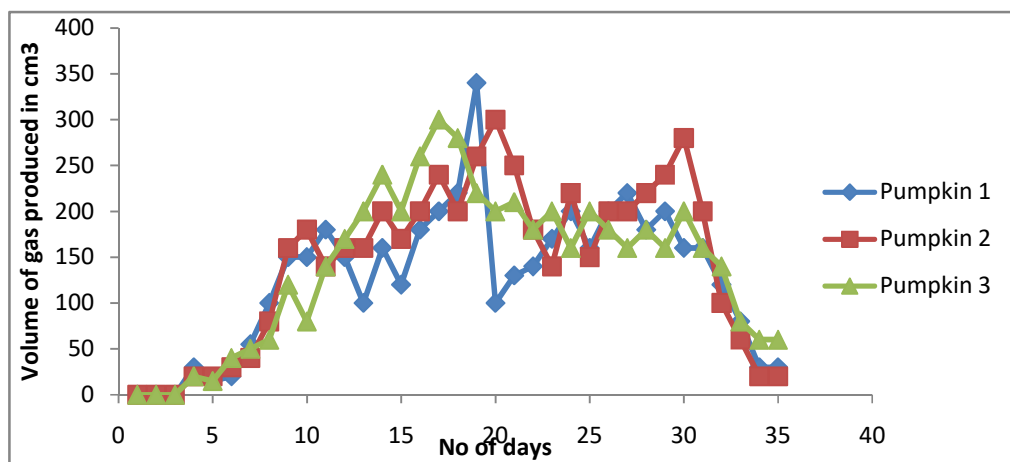
Statistical analysis of data obtained from the different treatments were carried out using student t-test and analysis of variance (ANOVA). All data were expressed as means  $\pm$  standard deviation of triplicate trials.

### **Results and Discussion**

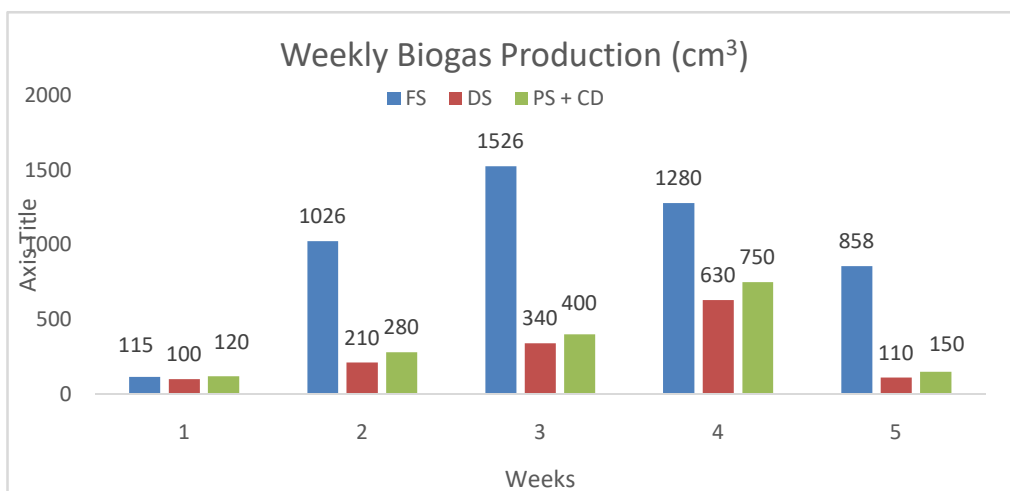
#### ***Daily biogas production***

This research work was done during the dry season with a temperature range of 30 –

34°C and during the 35 day retention time. Figures 1 and 2 show the daily biogas production of the fresh pumpkin substrate and weekly volume of biogas produced by the fresh, dried and cow dung seeded pumpkin substrate.



**Figure 1.** Daily biogas produced from pumpkin substrate throughout the retention period.



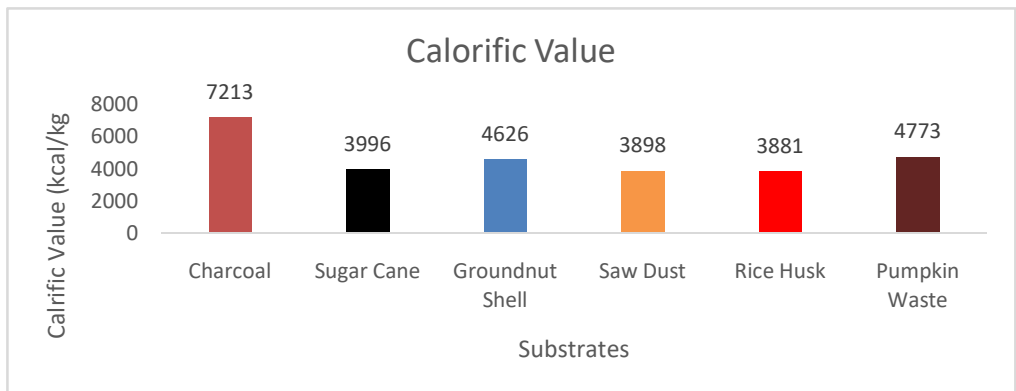
**Figure 2.** Weekly Biogas Production.

**Key:** FS = Fresh substrate; DS = Dried substrate; PS + CD = Pumpkin substrate seeded with cow dung.

The results presented in Figure 2 showed that the fresh substrate produced the highest volume of biogas produced when compared with the dried sample as well as that co-seeded with cow dung. This findings contradicts the acknowledged fact that cow dung is

the best biogas producer amongst animal wastes when blended with suitable substrates as a result of the rich flora of microorganism supplied by the animal waste which is capable of hydrolyzing a large amount of polysaccharide, which includes starch and biodegradable organic matter compared to the fresh and dried substrates when used independently [17]. The digester containing the fresh substrates produces more biogas than their dried counterpart. This may be because of a tougher lignin, cellulose and hemicelluloses in the dried sample as suggested by Adelekan and Bamgboye [18], who reported that lignin suppresses biodegradation and the higher the lignin content, the lower the biogas yield. The decline in the volume of biogas produced in weeks 4 and 5 when compared to week 3 when the volume of biogas peaked could be attributed to the decline in pH and increase in temperature of the medium, deposition of microbial metabolites (results not presented) as well as the gradual exhaustion of available nutrient from the substrates [19].

Figure 3 shows the calorific value of the produced biogas from the pumpkin substrate in comparison with established briquettes.



**Figure 3.** Calorific value of the produced biogas from pumpkin residue in comparison with conventional briquettes (Harban *et al.* [20]).

One of the most important characteristics of a fuel is its calorific value, that is the amount of energy per kg it gives off when burnt. The calorific value can thus be used to determine the competitiveness of a processed fuel in a given market situation in addition to other factors like the ease of handling, burning characteristics among others [20]. The reported calorific values of the sample if dried, blended and made as briquette will have good heating ability and compared reasonably with established briquettes as reported by Harbans *et al.* [20].

**Morphological and biochemical characterization of the isolated microbial isolates**

Tables 1 and 2 presents the morphological and biochemical characterization of the bacteria isolates.

**Table 1.** Morphological characteristics of bacteria isolates obtained from the substrates

Substrate	Isolate	Colony Morphology		Gram Reaction	Cell Morphology	
		Colour	Form		Arrangement	Motility
Pumpkin Residue	PU <sup>1</sup>	Grey	Rod	-	Rod	+
	PU <sup>2</sup>	Off-white	Rod	+	Rod	-

**Table 2.** Biochemical characterization of the bacteria isolates obtained from the substrates

Substrate/Test	Pumpkin Residue	
	PU <sup>1</sup>	PU <sup>2</sup>
Catalase	-	-
Coagulase	+	-
Lactose	+	-
Glucose	+	-
Fructose	+	-
Citrate	+	-
Indole	+	-
H <sub>2</sub> S	+	-
Urease	+	-
Gas	-	-
MR	-	-
VP	-	-
Spore	-	-
Oxidase	+	+
Bacteria Isolated	ES	EC

Key: (+) = Positive, (-) = Negative, ES = *Enterobacterspp*, EC = *Escherichia coli*, PU = Pumpkin.

Jaenicke *et al.* [21] reported that members of methanogens frequently dominate methanogenic sub-communities in different anaerobic digester systems and this research has shown that different methanogenic sub-communities within biogas producing consortia are crucial in the anaerobic degradation process for synthesis of methane. The bacteria isolated from the pumpkin residue is in agreement with the findings of Demirel and Scherer [22] who reported that the anaerobic transformation of organic wastes is a process which involves many different groups of bacteria such as hydrolyzing, acidifying, acetogenic and methanogenic bacteria which in the final stage produce CO<sub>2</sub> and methane, which are the main products of the digestion process.

## Conclusion

This research confirms that the organic waste materials from pumpkin fruit can be utilized by microorganisms for biogas production. The usage of this substrate for biogas production is possible due to the accessibility by cellulolytic, lipolytic, proteolytic and amylolectic microorganisms which play a vital role in biogas production from organic wastes.

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