



## Research Article

# STUDIES ON THE POTENTIALS OF POULTRY DROPPINGS FOR BIOGAS GENERATION

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## ABSTRACT

Biogas is a type of biofuel that is naturally produced from the decomposition of organic waste. Anaerobic digestion has been considered as waste-to-energy technology, and is widely used in the treatment of different organic wastes. This study was carried out for biogas production from degradation or break down of poultry waste in producing biogas through anaerobic digestion. This was achieved by constructing four (4) local digesters and gas collection/passage pipe systems mounted in the laboratory of Department of Microbiology, University of Maiduguri. The digesters were used to digest the slurry (poultry waste and water) through microbial actions of the identified microorganisms. *E. coli*, *P. aeruginosa*, *B. subtilis* and *S. aureus* was isolated and identified. Proximate analysis of the substrate was also determined. The results revealed that nitrogen has  $523 \pm 0.17$  % (mg/ml), potassium has  $713 \pm 4.00$  % (mg/ml) and phosphorus has  $231 \pm 0.03$  % (mg/ml). Others such as Ash Content (%)  $27.27 \pm 0.18$ , Crude Protein Content (%)  $20.66 \pm 0.16$ , Crude Lipid Content (%)  $2.33 \pm 0.32$ , Carbohydrate Content (%)  $22.5 \pm 0.14$ , Crude Fiber Content (%)  $16.24 \pm 0.32$ , and Moisture Content (%)  $9.66 \pm 0.03$  were also determined. The total average yield of the gas produced was 575.71, 309.99, 114.29, and 558.58 for D1, D2, D3, and D4 respectively within six weeks of production. The elements value determined implies that the digest could be a good source of bio fertilizer. The pH values of the media in all the substrates digested were found in the optimal or at a neutral pH level of methanogenic bacteria of 6.12-7.20. This study revealed that poultry waste is a good substrate for biogas production and optimizing the slurries during the production stage is very essential.

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## INTRODUCTION

Biogas is a clean, environmentally friendly and renewable form of energy generated when micro-organisms degrade organic materials in an oxygen free environment (Fernandez *et al.*, 2005). The formation of biogas can occur either in natural environment or controlled conditions in constructed biogas plants, so called anaerobic digestion (AD). Swamps, marshes, river beds, rumen of herbivorous animal are some of the areas where biogas is formed naturally. The same microbial activities are achieved in both natural and controlled conditions (Lantz *et al.*, 2007).

Inadequate energy supply and environmental pollutions are serious problems confronting Nigeria with high population growth rate, there is need for alternative source of energy to replace the depleting fossil fuel (Dalhat 2015). Biogas generation from anaerobic digestion of readily available wastes could contribute to solving these problems. From the global perspective, the over-dependence on fossil fuels as primary source of energy has resulted in climate change, many environmental destruction and related human health problems (Budiyono *et al.*, 2010). The joint challenge of global pollution and depletion of fossil fuels is driving intense research into alternative renewable energy sources, among which is the biogas. Biogas is produced by the anaerobic digestion (AD) of organic waste through the synergistic metabolic activities of consortia of hydrolytic, acidinogenic, acetogenic and methanogenic bacteria on organic materials (Yebo *et al.*, 2011). The process of methanogenesis includes hydrolysis, acidogenesis or acetogenesis and methanogenesis (Ainswort *et al.*, 2001; Odeyemi, 2001; Charles *et al.*, 2003). Methane is the main component of biogas (50-70%). Other components include CO<sub>2</sub> (30-40%) and traces of H<sub>2</sub>S and H<sub>2</sub>O vapour (Odeyemi, 2001).

## MATERIALS AND METHODS

### Materials

The Materials used for this study are; Clean container, Cool box, Dry poultry waste, Distilled water, Beaker, pH meter, Four(4) 15-18 liter large plastic container, Delivery tubes, 1000cm<sup>3</sup> measuring cylinder, Bowl, Test tubes, araldite sealer, universal bottle, Glucose phosphate medium in a test tube, Drops of H<sub>2</sub>O<sub>2</sub>, naphthol solution, lead Acetate paper, human plasma in normal saline, conical flask, Syringes, Nutrient Agar plates, Gram Staining reagents (Crystal Violet, Iodine, Safranin, Decolorizer), Glass slides, Urea medium in universal bottle, Inoculating loop/wire loop and Bunsen burner.

### Collection of samples

Dried poultry waste was obtained from the Borno state poultry house, Bura-buri, Maiduguri, and transported to the Microbiology Department, University of Maiduguri for the research purpose.

### Proximate and Elemental Composition of the Substrate

The proximate and elemental composition of the sample will be carried out according to AOAC (2019) methods to determine the following compounds and elements. ash content, carbohydrate, crude fiber content, crude protein, lipids content, moisture content, nitrogen, phosphorus and potassium.

### Microbial Analysis

#### Media preparation

The culture media was prepared using standard laboratory methods as described by Cheesbrough (2003). These include: nutrient broth, nutrient agar (NA) and Sabouraud Dextrose Agar (SDA). All media are prepared according to the manufacture instructions.

### Isolation and Characterization of Microorganisms

#### Serial dilution

The slurry was serially diluted using a tenfold serial dilution and 0.1ml of 10<sup>-7</sup> dilution factor

was spread onto nutrient agar plates for the enumeration and isolation of bacteria and sabouraud dextrose agar for the enumeration and isolation of fungi. The nutrient agar plates were incubated aerobically and anaerobically at 37°C for 24 hours. The sabouraud dextrose agar plates were incubated at 28 °C for 3-5 days. Bacterial isolates were characterized using methods described by Cowan (1974) while fungi were characterized based on macroscopic and microscopic examination (Domsch and Gams, 1970).

#### **Colony count**

Colonies that emerge on the plates, were counted and recorded as colony forming units per milliliter (cfu/ml) of the sample. The colonies were also subcultured repeatedly on fresh plates to obtain pure isolates (Bello *et al.*, 2019).

Colony forming unit = Number of colony/Inoculant size x Dilution factor

Percentage frequency of occurrence= Number of isolate x 100/Total number of isolate

#### **Gram Staining**

Clean slides were obtained, a smear for all the isolates was made from each sub colony, air dry and heat fix. The smear was then flooded with crystal violet and allowed to stand for one minute, then wash with water and drain, then follow by flooding the smear with iodine solution and allow to stand for one minute, rinse and drain. It was then flushed with acetone alcohol (decolorizer) for three seconds and washed immediately to prevent excessive decolorization, then flood with safranin and left for one minute after it was washed and allowed to dry. The dried slides were viewed under oil immersion lens(x100) by applying a drop of oil immersion. Gram negative bacteria was seen

then counter stain and appear red pink (Cheesbrough, 2004).

#### **Microscopy**

The back of the glass slides was wiped clean and a drop of immersion oil was applied on the smear which was examined microscopically with x100 objective lens for the observation of Gram reaction and morphological characteristics of the bacterial cells. Gram-positive bacteria appear purple in color, while Gram-negative cells retained the counter staining color of safranin and appeared pink in color. After Gram Staining and Microscopy, the isolates subculture into universal bottle containing nutrient agar in a slant form for subsequent used in biochemical test (Oyeleke and Manga SB, 2008).

#### **Biochemical Test**

After identification, Gram-positive bacilli that produced spores were subjected to further biochemical tests for characterization of the isolated bacteria. The tests included; catalase, Coagulase, citrate, indole, motility, methyl red (MR) nitrate, Oxidase, Tripple sugar iron test Urease, voges-pro skaters and H<sub>2</sub>S sulfide tests (Oyeleke and Manga SB, 2008).

### **RESULTS**

Table 1 shows proximate and elemental composition of poultry waste. The results shows that Ash has the highest percentage mean concentration of 22.5 % followed by carbohydrate with 27.27 %, Crude protein 20.66, crude fiber 16.24 while moisture and lipids has 9.66% and 2.33% respectively. The results also shows the mean concentration of some elements with Potassium constituting major element with an average of 713.00 mg/ml and Phosphorus was found to have least concentration of 0.27 mg/ml.

**Table 1:** Proximate and Elemental Composition of Poultry Waste (all values are mean  $\pm$  standard deviation (S.D) of triplicate measurement)

PARAMETERS	COMPOSITION
Moisture Content (%)	9.66 $\pm$ 0.03
Ash Content (%)	27.27 $\pm$ 0.18
Crude Protein Content (%)	20.66 $\pm$ 0.16
Crude Lipid Content (%)	2.33 $\pm$ 0.32
Carbohydrate Content (%)	22.5 $\pm$ 0.14
Crude Fiber Content (%)	16.24 $\pm$ 0.32
Nitrogen (mg/ml)	523 $\pm$ 0.17
Potassium (mg/ml)	713 $\pm$ 4.00
Phosphorus (mg/ml)	231 $\pm$ 0.03

Table 2 shows percentage frequency of occurrence of the isolates in all the samples (poultry waste) with *E. coli* as the predominant organism isolated having (50%) and (30%) for other organisms respectively.

**Table 2:** Percentage of Occurrence of Bacteria Isolate in Poultry Waste

Bacteria	Frequency of Occurrence	% Occurrence of Isolate
<i>Escherichia coli</i>	10	50
<i>Bacillus subtilis</i>	6	30
<i>Staphylococcus aureus</i>	3	15
<i>Pseudomonas aeruginosa</i>	1	5
<b>Total</b>	20	100

Table 3 shows the initial and final pH of the four (4) digesters at a neutral pH level of 6.12 to 7.2 required for optimum biogas production.

**Table 3: Initial and Final pH of the Digesters**

Digester	Initial pH	Final pH
D1	7.00	6.53
D2	7.00	
D3	7.00	6.24
D4	7.20	6.12
		6.37

Table 4 shows the amount of gas produced by water displacement method using measuring cylinder of 1000cm<sup>3</sup>. The digesters were set up and allowed to undergo anaerobic digestion for a retention period of Six weeks. The amount of

gas produced was recorded at 12noon on daily basis and the amount of gas as well as pH was recorded. The experimental results obtained during the monitoring period in the study were presented in table 4

**Table 5:** The Daily Volume of Biogas Yield at a Retention Time of Six (6) Weeks

Retention Time In days	D1	D2	D3	D4
1-7	515.71cm <sup>3</sup>	35.71 cm <sup>3</sup>	5.71 cm <sup>3</sup>	455.71 cm <sup>3</sup>
8-14	37.14 cm <sup>3</sup>	128.57 cm <sup>3</sup>	2.86 cm <sup>3</sup>	71.43 cm <sup>3</sup>
15-21	1.43 cm <sup>3</sup>	40.00 cm <sup>3</sup>	32.86 cm <sup>3</sup>	14.29 cm <sup>3</sup>
22-28	11.43 cm <sup>3</sup>	68.57 cm <sup>3</sup>	40.00 cm <sup>3</sup>	14.29 cm <sup>3</sup>
29-35	7.14 cm <sup>3</sup>	27.14 cm <sup>3</sup>	22.86 cm <sup>3</sup>	2.86 cm <sup>3</sup>
36-42	2.86 cm <sup>3</sup>	27.14 cm <sup>3</sup>	10.00 cm <sup>3</sup>	0.00 cm <sup>3</sup>
<b>Total</b>	575.71 cm <sup>3</sup>	309.99 cm <sup>3</sup>	114.29 cm <sup>3</sup>	558.58 cm <sup>3</sup>

## DISCUSSION

The proximate and elemental composition of poultry waste revealed that Ash has the highest percentage followed by carbohydrate, then crude protein, crude fiber, moisture and lipids respectively. The results also show the mean concentration of some elements with Potassium constituting major elements and Phosphorus was found to have least concentration. The ash content, which is the amount of solid material remaining after the sample is incinerated and reflecting the mineral content. The results obtained in this study regarding the above-mentioned proximate analysis is similar to the finding of Odeyemi (2011) who conducted a study on poultry waste and its use as feed and supplementary feed for fish growth. Lawal *et al.* (2001) stated that poultry droppings contain a high level of urea which on decomposition produced a bulk of ammonia gas making it a good source of biofertilizer as well. The percentage frequency of occurrence of the isolates in all the samples (poultry waste) shows that *E. coli* is the predominant organism isolated than the other organisms. But some species were also present throughout the process of gas production (Tambuwal *et al.*, 2019). The result obtained from this study indicates that *E. coli* species were the most common bacteria isolated and identified during the research, suggesting that the species plays a vital role in the microbial activities for the production of

biogas. It should be noted that bacteria such as *Bacillus subtilis*, *pseudomonas aeruginosa* and *staphylococcus aureus* were also isolated during the production.

The initial and final pH of the digesters was also determined. The pH values of the media in all the substrates digested was at a neutral level, and were varied almost in the optimal limits of methanogenic bacteria of pH: 6.12 -7.2 which is in agreement with Hasen (2001) who reported that pH range from 6.8 through neutral to 7.4 is required for optimum biogas production. The pH of the slurry appeared to be decreasing in all the digesters. It is not surprising as the decrease in pH may be as a result of anaerobic fermentation taking place (Yadvika *et al.*, 2004). PH is an important factor that affects biogas production. It was reported that anaerobic bacteria required a natural environment (Gerardi, 2003) and thus, pH ranging from 6.4-7.2 is required for optimum biogas production. Also, the decrease in pH may be due to the action of acetogenic methanogens as they break down sulphur containing organic and inorganic compounds as well as the formation of fatty acids. It was reported by Oyeleke *et al.* (2017) that biogas produced at pH of 5 is greater than that of pH 10. Some microorganisms also appear later in the process of production during the log phase due to high alkaline of the slurry while others died off midway the process during the stationary and

the death phase due to depletion of nutrients in the slurry (Ahmadu, 2009).

Methanogenic bacteria are very sensitive to pH and do not thrive below a value of 6.0 (Ahmadu, 2009). Most microorganisms grow best under neutral pH conditions, since other pH values may adversely affect metabolism by altering the chemical equilibrium of enzymatic reactions, or by actually destroying the enzymes. The methanogenic group of organisms is the most pH sensitive. Low pH can cause the chain of biological reactions in digestion to cease (Oyeleke *et al.*, 2017).

The results from this research showed *Escherichia coli*, *Bacillus subtilis*, *Staphylococcus aureus* and *Pseudomonas aeruginosa* might be responsible for the anaerobic fermentation and degradation of the substrate (poultry waste) in the process of methane gas (cooking gas) production. *Escherichia coli* was isolated and found predominant in the batch process of gas production through the six weeks (42 days) of production, in relation to the other mentioned methanogenic microorganisms. This produce the

average mean of 575.71 cm<sup>3</sup>, 309.99 cm<sup>3</sup>, 114.29 cm<sup>3</sup>, and

The daily volume of biogas yield at a retention time of six (6) weeks (42 days) using measuring cylinder of 1000cm<sup>3</sup> was examined. The results shows a significant decrease in the total solids (gram) from 400g to 150g for all digesters (D1, D2, and D4) except for the third digester (D3) which decreased from 400g to 200g resulting in low gas production. This may be due to the utilization of the waste or slurry by microorganisms through anaerobic digestion. This agrees with the report of Oyeleke *et al.*, (2017), who stated that, the total solids and volatile solid reduce as methane yield increases.

### CONCLUSION

In conclusion, Biogas is produced by the biodegradation of poultry organic matter under anaerobic conditions. The organic matter is degraded in four steps: hydrolysis, acid genesis, acetogenesis/dehydrogenation and methanation by several microorganisms. The findings of this study further revealed that the bioproduct obtained during the production can be used to produced biofertilizer for boosting agricultural activities.

### REFERENCES

- Ahmadu T. O., (2009). Comparative Performance of Cow Dung and Chicken Droppings. *International Journal of Chemical and Biological Engineering*, **3** (1), 39-44.
- Ainswort, J. L., Atwood., and Rideont, J. (2001). Anaerobic Digester System, *Midland Texas, United*. *International Journal of Advanced Biotechnology and Research*, **8** (1), 143-147.
- Association of analytical chemistry (2019). Official methods of analysis, 21<sup>st</sup> Edition Washington DC, Association of Analytical Chemistry. 250-500.
- Budiyono., Widiassa I.N., Johari S., Sunrso., (2010). The Kinetic of Biogas Production Rate from Cattle Manure in Batch Mode. *International Journal of Chemical and Biological Engineering*, **2** (3), 39-44.
- Charles F., Dennis S., and James R. F., (2003). Generating methane Gas from manure. *Nigerian Journal of Renewable Energy*. **5**: 69-79.
- Cheesbrough M., "District Laboratory Practice in Tropical Countries Part 2". Second Edition, *Cambridge University Press* (2006). **32**.
- Dalhat M.A., (2015). Conversion of Cereal straw to Biogas and Compost using Cattle Manure as inoculums. Unpublished Msc Dissertation Presented to the Department of Water Resources and Environmental Engineering, *Ahmadu Bello University, Zaria Nigeria*. **3**.

- Domsch K. H., and Gams W., (1970). *The Pacific Journal of Science Technology.*, 7, 176-184.
- Fernandez A., Sanchez A., Font X., (2005). Anaerobic co-digestion of a simulated organic fraction of municipal solid wastes and fats of animals and vegetable origin. *Biochemical Engineering Journal.*, 26, 22-8. **3.**
- Gerardi, M. (2003). The Microbiology of Anaerobic Digesters. *Waste water Microbiology Series. John Wiley & Sons, Inc. United States of America.* **24.**
- Gomez, X., Cuetos, M., Cara, J., Moran, A., Garcia, A. (2006). Anaerobic co-digestion of primary sludge and the fruit and vegetable fraction of the municipal solid wastes conditions for mixing and evaluation of the organic loading rate. *Renewable Energy*, 31, 2017-2024. **3.**
- Hasen, W. R. (2001). Methane generating farm livestock waste. *Publication of farm management Colorado state university, Colorado.*, **5.**
- Igboro, S. B. (2011). Production of Biogas and Compost from Cow Dung in Zaria, Nigeria, unpublished PhD Dissertation Presented to the Department of Water Resources, Ahmadu Bello University Zaria.
- Lawal, A. K., Ajuebor, F. N. & Ojosu, J. O. (2001). Characteristics of piggery waste feed stock for determination of design parameters to biogas digester plant. *Nigerian journal of research and review in science.*, **2**:193-198.
- Lantz, M., Svensson, M., Björnsson, L., Börjesson, P. (2007). The prospects for an expansion of biogas systems in Sweden: Incentives, barriers and potential. *Energy Policy*, 35:1830-1843. **2.**
- Ocwieja, S. M., (2010). Life Cycle Thinking Assessment Applied to Three Biogas Projects in Central Uganda, being a Report Submitted in Partial Fulfillment of the Requirements for the Degree of Master of Science in Environmental Engineering, *Michigan Technological University*, available at <http://www.cee.mtu.edu/peacecorps/studentfiles/Ocwieja-Uganda.pdf> (17/5/2011). **11**
- Odeyemi, O. (2001). Biogas production. *Proceeding of the fifth Annual Conference of the Nigerian Society of Microbiology, Ado Ekiti*, 2<sup>nd</sup>-6<sup>th</sup> Dec., 2001, **5** pp. 17-24.
- Oyeleke and Manga SB. *Essential Laboratory Practical in microbiology* (2008). Papadopoulos, M.C., El-Boushy, A.R., Roodbeen, A.E., Ketelaars E.H. (1986). Effects of processing time and moisture content on amino acid composition and nitrogen characteristics of feather meal. *Anim. Feed Sci. Technol.*, 14, 279-290 **34**
- Oyeleke, S. B., Onigbajo, H. O. & Ibrahim, K. (2017). Proceeding of the 5th Annual Conference of Animal Science of Nigeria (SAN): **4** : 168-169.
- Tambuwal B. M., Bello A., Musa AR., Bello MR., and Baki AS., (2019). "Biogas Generation Using Cattle Rumen Contents". *Acta Scientifica Medical Sciences* **34.**, 22-30.
- Yadvika, Santosh, T. R. Sreekrishnan, S. Kohli, and V. Rana, (2004). "Enhancement of Biogas Production from Solid Substrates using Different Techniques. A Review," *Journal of Bioresource Technology*, vol. **95**, pp.1-10,.
- Yebo. L, S. Park and J. Zhu, (2011), Solid-state anaerobic digestion for methane production from organic waste. *Renewable Sustainable Energy Reviews* **2** (15) 821–826.