Effects of starter culture on biogas production from combination of poultry droppings and cow dung in Cross River State, Nigeria

*Asikong Bassey E.¹, Epoke James², Eja E. Matthew³ and Effiom E. Henshaw¹

¹Microbiology Department University of Calabar, Nigeria
²Medical Microbiology Department, Faculty of Health and Allied Science, University of Calabar, Nigeria
³Cross River University of Technology, Calabar, Nigeria

ABSTRACT

The Potentials of biogas production from combination of poultry droppings (PD) and cow dung (CD) to generate biogas were investigated using a locally-designed digester and a modified gasometrical chamber. Bacterial counts before digestion in the combination of poultry droppings and cow dung were 8.76 X 10⁷ and 5.45 X 10⁸ cfug⁻¹ with and without starter culture respectively. Bacterial counts after digestion were 4.25 x 10⁵ and 4.55 x 10⁵ with and without starter cultures. Fungal counts before digestion from PD + CD with and without starter cultures were 2.65 x 10⁴ and 4.20 X 10⁴ cfug⁻¹ respectively while after digestion, fungal counts were 2.35 X10² and 3.45 X10² cfug⁻¹ respectively. The mixture of poultry droppings and cow dung produced optimum biogas of 540ml, 655ml and 735mls in the 1kg, 2kg and 3kg weight respectively after 20 days of anaerobic digestion without starter culture and 600ml, 700ml and 796mls respectively for the respective weights of 1kg, 2kg and 3kg after 20 days of digestion with starter culture. The optimum biogas yield from the mixture showed increased biogas production in comparison to the respective substrates in single digestion with or without starter culture. Analyses of variance revealed significant difference in the generation of biogas from poultry droppings and cow dung with starter culture [F (2, 16) - 58.89, P < 0.001] and without starter culture [F (2, 16) -54.44, P < 0.001] as a result of the weights difference with positive correlation.

Key words: biogas production, poultry droppings, cow dung and starter culture.

INTRODUCTION

There may be no solution to the energy crisis in Nigeria and other developing countries except we develop an indigenous technology suitable and convenient to our peculiar circumstances especially, with respect to technological know-how, raw material availability, human and economic resources and applicability by rural dwellers. This is because no developed country may be ready to transfer its already developed technology based on political power play, economic and capitalistic monopoly as well as security. Cross River State, Nigeria and indeed Africa is blessed with abundant, diverse and unexploited renewable energy resources that are yet to be used for providing clean fuel and help the energy crisis and poverty [14, 18, and 13]. [11] and [3] identified two significant and important challenges of the millennium and the twenty first century to include; the development and use of renewable energy to decrease dependence on fossil fuel and management of the waste generated by human activities as a result of agricultural activities, industrial growth and population explosion which are associated with waste generation. Achieving the Millennium Development Goals (MDGs) in Africa also requires a significant expansion of access to modern and alternative renewable energy such as biogas which is of growing interest for the sustainable
management of our waste and a major breakthrough in the search for a renewable energy for the reduction in over-dependence on non-renewable fossil fuel [19 and 1]. Biogas is the product of organic matters decomposition under oxygen-free condition with microbial participation especially Methanogens. Biogas formation can occur naturally in swamps, marine sediments, and water logged soils, rice fields, deep bodies of water, sanitary landfills and even in the digestive system of ruminants; and termites. It can also be recovered from lagoons used for waste treatment. Biogas, a mixture of gasses consist of 50 – 70%, methane 30 – 40%, carbon dioxide 5 – 10%, Hydrogen 1 – 2%, Nitrogen 0 – 3%, water vapour and traces of Hydrogen sulphide, carbon monoxide and oxygen. Generally, four different stages have been recognized in the production of biogas with several other intermediate products. These include; hydrolysis, acidogenesis, acetogenesis and methanogenesis. Presence of toxicants can also influence biogas production. Positive implications of biogas include; the reduction in environmental pollution, odour [15 and 16], and in the destruction of most pathogenic organisms, worms, ova, etc. Biogas can also serve as a clean alternative to fuel energy source to oil, electricity and wood. The negative implications of biogas technology include; concentration of toxic compounds such as pesticides and heavy metals in plants and ground water contamination [22]. This research is aimed at determining the potentials of biogas energy generation from poultry droppings and cow dung which abound in Cross River State, Niger Delta area and other parts of Nigeria.

MATERIALS AND METHODS

Sample Collection
Poultry droppings and cow dung
Ten kilograms (10kg) weight of poultry droppings was obtained from the University of Calabar poultry farms and placed in sterile polythene bags, transported to the laboratory for analysis. Similarly Ten kilogram (10kg) weight of fresh cow dung was collected from the Abattoirs in Nassarawa Village, Baccoco in Calabar Municipality and placed in sterile bags, transported immediately to the laboratory for analysis within 24 hours of collection.

Processing of Samples and Isolation of Microorganism
Ten gram weight each of well pulverized poultry dropping and cow dung were mixed with 90mls of sterile distilled water in 250mls Erlenmeyer flask. The mixtures were vortex and agitated thoroughly and allowed to stand for ten minutes (l0mins). The supernatant were decanted and one milliliter volumes were prepared in ten-fold serial dilutions. Dilutions of 10^{-5} to 10^{-7} were plated in triplicate onto nutrient agar (supplemented with 50µgml^{-1} Nystatin to prevent fungal growth) using surface spreading plating. Plates were incubated for 24 – 48 hours at 35^0C. Colony forming units per gram (cfug^{-1}) of bacterial growth between 30 – 300 colonies were enumerated. Media and inoculation techniques were as described by [12].

For screening of fungi, dilutions of 10^{-3} to 10^{-4} of the supernatant were plated on Sabouraud’s dextrose agar supplemented with 100mgml^{-1} streptomycin and 15mgml^{-1} of penicillin (to inhibit bacterial growth). Triplicate plates were prepared and incubated for 72 to 96 hours. Plates with fungal colonies of between 20 to 100 were enumerated in cfug^{-1}. Methods of [21] and [12] were employed for isolation and characterization of fungi.

Preparation of Combination of two substrates; poultry dropping (PD) and cow dung for biogas production.
A modification of the methods of [10] and [12] where used. The poultry dropping was screened to exclude other extraneous materials and well pulverized. Similarly the cow dung was well mixed and homogenized. A consortium of the two substrates were mixed in the ratio of 0.5:0.5kg, 1:1kg, 1.5:1.5kg to yield total weights of 1kg, 2kg and 3kg respectively. Respective weights were mixed with water at the ratio of 1:3 and placed in the digesters. Duplicate of each weight was prepared, one without starter culture and the other with 1kg weight of starter culture from an old digester slurry mixed with charcoal. The digesters were tightly corked with rubber stopper to create anaerobic condition and connected to a gasometrical chamber. Biogas was monitored and measured daily over a period of 45 days using gasometrical chamber (Figure 1).

Preparation of starter culture
The methods of [10], were employed. The support activated carbon (charcoal) was washed 5 times with acetate buffer pH (4-5) and finally re-suspended in the buffer overnight. Twenty kilogram weights were placed in storage containers and kept at 10^0C in a refrigerator. Twenty kilogram weight of the slurry (residue w/s) of an old but active cow dung digester was mixed with 20kg weight of the pre -treated activated carbon and incubated at room temperature in anaerobic condition for 40 days. The adsorbed cells were used as crude starter culture for all
digesting combinations. The advantage of using the activated carbon as support for the immobilization was that it was relatively cheap and affordable, readily available, mild and poses no problem of cell and enzyme inactivation.

Innovation in digester design with gasometrical chamber
Biogas yield was measured daily using the gasometrical chamber which was an innovation, specially designed for the study. The chamber consisted of a gasometrical assembly which comprised of a graduated burette which was connected to the locally designed anaerobic digester through a rubber tube. The burette was also connected to a funnel with paraffin oil through a synthetic rubber tube (which could be transparent). The burette was linked to the tube from the anaerobic digester by a glass connector with two taps; the inlet and the outlet taps. The outlet tap was sealed with a flexible plastic tube with a strong clip (to avoid leakage). The total biogas yields were determined by opening the outlet tap of the anaerobic digester and the inlet tap to the graduated burette. The biogas generated was released through the tube which then displaced the paraffin oil in the graduated burette downward. The volume of gas yield was determined by the volume of paraffin oil displaced, i.e gas yield was directly proportional to paraffin oil displaced (Figure 1).

RESULTS AND DISCUSSION
Table 1 shows that bacterial counts before digestion-BCBD in the combination of poultry droppings and cow dung were 8.76 X 10^7 and 5.45 X 10^5 cfu g^-1 without and with starter culture respectively. Bacterial count after digestion-BCAD were 4.25 10^5 and 4.55 X10^5 cfu g^-1 with and without starter cultures. Fungal counts before digestion from PD+CD without and with starter cultures were 2.65 x 10^3 and 4.20 X 10^4 cfu g^-1 respectively while after digestion fungal counts were 2.35 X10^2 and 3.45 X10^2 cfu g^-1 respectively. This study revealed higher bacterial and fungal counts with starter culture in pre and post digestions. This shows that starter culture increased bacteria load and subsequently increased biogas than the substrates without starter culture. [18] and [26] reported similarly that the addition of starter culture of bacteria seed will enrich the bacteria of the digester and enhance the biogas generated. [8] reported that biogas formation is a microbial process. He obtained total viable bacterial and fungal counts of 18 x 10^6 cfu ml^-1 and 13 x 10^6 cfu ml^-1 from spent brewery grains. He further reported that starter culture age was influential in cumulative gas production Potentials of biogas from combination of cassava peels and poultry droppings-CP+PD. Also bacterial and fungal counts decreased after digestion. [19] observed similarly that while the digester slurry contained higher cellulolytic populations, the outlet recorded the least cellulolytic populations. [23] reported that anaerobic decomposition of waste is possible by a number of microorganisms especially bacteria, which work together to bring about the conversion of organic components of waste into suitable end product such as biogas.

TABLE 1 Total viable bacterial and fungal counts, from substrates slurry before and after anaerobic digestion in poultry droppings and cow dung.

<table>
<thead>
<tr>
<th>Culture mode</th>
<th>Raw substrates</th>
<th>Bacterial counts before digestion (BCBD), (cfug^-1)</th>
<th>bacterial counts after digestion (BCAD) (cfug^-1)</th>
<th>Fungal counts before digestion (FCBD) (cfug^-1)</th>
<th>Fungal counts after digestion (FCAD) (cfug^-1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Without starter culture</td>
<td>PD CD</td>
<td>8.63 x 10^7 8.65 x 10^7</td>
<td>5.54 x 10^7 6.45 x 10^7</td>
<td>3.42 x 10^7 3.55 x 10^7</td>
<td>2.65 x 10^7 2.55 x 10^7</td>
</tr>
<tr>
<td></td>
<td>6.54 X 10^7 2.55 x 10^7 3.75 x 10^7</td>
<td>8.76 X 10^7 6.54 X 10^7</td>
<td>4.25 X 10^7 2.55 x 10^7</td>
<td>2.65 x 10^7 3.75 x 10^7</td>
<td>2.35 x 10^7 2.40 x 10^7</td>
</tr>
<tr>
<td></td>
<td>PD CD</td>
<td>8.60 x 10^6 8.45 x 10^6</td>
<td>6.54 x 10^6 7.35 x 10^6</td>
<td>4.42 x 10^4 4.55 x 10^4</td>
<td>3.26 x 10^5 3.25 x 10^2 3.26 x 10^2</td>
</tr>
<tr>
<td></td>
<td>PD+CD</td>
<td>5.45 x10^6</td>
<td>4.55 X 10^6</td>
<td>4.20 x10^4</td>
<td>3.45 x 10^2</td>
</tr>
<tr>
<td></td>
<td>Control (Water Only)</td>
<td>5.50 X10^7</td>
<td>5.45 x10^7</td>
<td>3.10 x10^7</td>
<td>2.25 x10^2</td>
</tr>
</tbody>
</table>
TABLE 2 Analysis of variance (ANOVA) summary results showing variations in volume of biogas produced from poultry droppings and cow-dung with and without starter culture

<table>
<thead>
<tr>
<th>SOURCES OF VARIATION</th>
<th>Starter culture</th>
<th>DF</th>
<th>SIGNIFICANCE</th>
<th>MSS</th>
<th>F-CAL</th>
<th>P-VALUE</th>
<th>F-CRITICAL</th>
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<tr>
<td>Weight</td>
<td>Without</td>
<td>2</td>
<td>125189.40</td>
<td>62594.70</td>
<td>54.44***</td>
<td>7.26E-08</td>
<td>3.63</td>
</tr>
<tr>
<td></td>
<td>With</td>
<td>2</td>
<td>163438.90</td>
<td>81719.44</td>
<td>65.89***</td>
<td>1.89E-08</td>
<td>3.63</td>
</tr>
<tr>
<td>Periods</td>
<td>Without</td>
<td>8</td>
<td>859579.90</td>
<td>107447.50</td>
<td>93.46***</td>
<td>5.79E-12</td>
<td>2.59</td>
</tr>
<tr>
<td></td>
<td>With</td>
<td>8</td>
<td>956466.70</td>
<td>119558.30</td>
<td>96.39***</td>
<td>4.55E-12</td>
<td>2.59</td>
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<tr>
<td>Error</td>
<td>Without</td>
<td>16</td>
<td>18395.26</td>
<td>1149.704</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>With</td>
<td>16</td>
<td>19844.44</td>
<td>1240.278</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Error</td>
<td>Without</td>
<td>26</td>
<td>1003165</td>
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<td></td>
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<td></td>
</tr>
<tr>
<td></td>
<td>With</td>
<td>26</td>
<td>1139750</td>
<td></td>
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</tbody>
</table>

*** = Significant at 1% level

Source = Derived from Author’s experimental data (2008)
The mixture of poultry droppings and cow dung produced optimum biogas of 540ml, 655ml and 735mls respectively in the 1kg, 2kg and 3kg weight after 20 days of anaerobic digestion without starter culture and 600ml, 700ml and 796mls respectively for the respective weights of 1kg, 2kg and 3kg within 20 days with starter culture (Figure 2). The optimum biogas yield from the mixture of poultry droppings and cow dung showed increase in biogas production compared to the respective substrates in single digestion with or without starter culture. Analyses of variance revealed significant difference in the generation of biogas from poultry droppings and cow-dung with starter culture \( F \ (2, 16) - 58.89, P < 0.001 \) and without starter culture \( F \ (2, 16) -54.44, P < 0.001 \) as a result of the weights' difference (Table 2). The results also indicated that gas generated showed remarkable differences with starter culture. \( F \ (2, 16) = 96.39, P < 0.001 \) or without starter culture \( F \ (2, 16) = 93.46, P < 0.001 \) due to duration of the digestion. In their study with 39 combinations of different poultry droppings and cow dung with starter culture [19] obtained maximum methane production of 0.1470m³ per kg of total solid added and 0.3182m³ per kg of volatile solid from the combination of cow dung and poultry droppings in 1:1 ratio of 10% TS. They discovered that poultry litter is rich in Nitrogen and therefore must be mixed with cow dung to relieve the dependence of dung alone for gas production. In a related research by [20] they obtained peak biogas production on the 14th day from combination of poultry droppings and cow dung.

**FIGURE 2**: Optimum biogas yield from combination of poultry dropping and cow-dung- PD+CD with and without starter culture
**CONCLUSION**

This study has revealed that starter culture increases bacterial and fungal load and subsequently increased biogas production than the substrates without starter culture.

**REFERENCES**


