Quantitative and Qualitative Analysis of Biogas Produces From Three Organic Wastes

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Abstract
The use of biogas as a means of satisfying energy demands is a viable alternative to fuel wood which results in the indiscriminate felling of trees. Animal wastes as organic substrates in the production of biogas provide a cheap and eco-friendly method of managing wastes. In this study, three different animal wastes (Cow dung: pH$_1$=7.08, pH$_2$= 7.32; Goats’ droppings: pH$_1$=5.49, pH$_2$=5.26; and Chicken droppings: pH$_1$= 5.49, pH$_2$= 5.75) were used as substrates in the production of biogas, and the experiment was carried out at ambient temperature for a hydraulic retention time of three weeks. A set of three laboratory digesters was used in the experimental set up, and the performance of the animal wastes was assessed based on the volume and quality of the biogas produced. The amount of biogas produced by the animal wastes in decreasing order is as follows; chicken droppings (18.27 litres), cow dung (12.55 litres) and goats’ droppings (5.11 litres). The order of flammability of the biogas produced is as follows: cow dung > goats’ droppings > chicken droppings. The following methanogens were isolated; *Methanobacterium* sp., *Methanococcus* sp., *Methanospirillum* sp. and *Methanosarcina* sp. in the course of the experiment. In this study, cow dung produced the biogas of the highest quality and conclusively can be chosen as the best substrate for biogas production.

Keywords: Quantitative, Qualitative, Biogas, Organic Wastes, Methanogens

1. Introduction
In today’s energy demanding life style, the need for exploring and exploiting new sources of energy that are renewable and at the same time eco-friendly has become a mandate. The urban sectors in Nigeria have several alternative sources of energy to meet household needs [2]. On the other hand, the rural sectors still largely depend on fuel wood for cooking and other household purposes [13]. About 80% of the energy demands of rural households in northern Nigeria still come from fuel wood sources as reported by Sokoto Energy Research Centre. The remaining 20% is obtained from animal dung and other agricultural residues [3]. Over dependence on fuel wood has greatly resulted in rampant felling of trees in this already poorly vegetated zone, the result of which is the
consequences of desertification and soil erosion [17]. Many nations count on coal, oil and natural gas to supply most of their energy needs, but reliance on fossil fuels presents a big problem.

Fossil fuels are a finite resource. Eventually, the world will run out of fossil fuels, or it will become too expensive to retrieve those that remain. Fossil fuels also cause air, water and soil pollution, and produce greenhouse gases that contribute to global warming [19]. Biogas is a flammable gas produced when organic materials are fermented under anaerobic condition. It contains mainly methane and carbon (IV) oxide with traces of hydrogen sulphide and water vapour. It burns with a pale blue flame and it has a calorific value of between 25.9J/m– 30J/m, depending upon the proportions of methane and other constituent gases[21].

The gas is called by several other names such as dung gas, marsh gas, Gobar gas, sewage gas and swamp gas [12].

2. Materials and method

2.1. Sample Collection:

The animal dung (goat dropping, cow dung, and chicken droppings) used as the substrates in this research were obtained from Samaru village, Zaria, in the northern region of Nigeria. The substrates were sun-dried and ground to powder using wooden mortar and pestle as described by[17].

2.2. Experimental Set-up for Biogas Production

A set of three laboratory digesters of 6.5 litres capacity were loaded separately with 880 grams of each substrates and 3.6 litres of water was added to obtained slurries of 1:4, substrates: water ratio. This was then followed by occasional agitation as described by [5]. The pH of the slurries was determined using SUNTEX pH meter (SP-701) at the Department of Water Resources and Environmental Engineering, Ahmadu Bello University, Zaria, and the temperature was kept ambient (room temperature). A digestate (Slugde) about 10% of the total volume of each of the slurries [10] from a completed biogas plant was added to each digester to serve as the starter culture [17]. The digesters were then stoppered with rubber bands to prevent leakage and connected via rubber tubing each to a gas collecting jar (1L capacity measuring cylinder) inverted over a solution of 1% potassium hydroxide. The gas was collected by “upward delivery downward displacement” of the KOH solution, and the volume of the gas produced was recorded daily. The digesters were kept at ambient temperature (20-45°C) i.e. operated within the mesophilic range.

2.3. Quantitative and Qualitative Assessment of the Biogas Produced:

The parameters used for assessing the performance of the experimental substrates included; time of commencement of gas production, time to reach peak gas production and when production ceased, daily yield of gas and the total volume of gas produced over the period of 21 days called the hydraulic retention time (HRT) of the study [18]. The combustibility of the biogas produced was determined by lighting a match and passing the flame over the nozzle of the measuring cylinder in which the gas had been collected and the degree of flammability was recorded.

2.3.1. Removal of Carbon (IV) Oxide (CO₂):

Different researchers have used various methods for the absorption of CO₂. The absorption of CO₂ by the passage of biogas through concentrated alkaline solution such as KOH, NaOH, Ba(OH)₂ or Ca(OH)₂ is the most convenient method. Methane which neither reacts nor dissolves under this condition passes on.

\[
CO₂ (g) +2 \text{NaOH}_{(aq)} \rightarrow \text{Na₂CO₃} + \text{H₂O}_{(l)}
\]

2.3.2. Removal of hydrogen sulphide (H₂S):

Hydrogen sulphide may be eliminated from biogas by passing it through a concentrated solution of acidified lead (II) acetate or lead (II) nitrate. The acidified lead acetate solution absorbs the H₂S gas according the following equation.

\[
\text{H₂S} (g) +2\text{NaOH}_{(aq)} \rightarrow \text{Na₂S} + 2\text{H₂O}_{(l)}
\]

2.4. Microbial (Methanogenic bacterial) Analysis:

Sixty (60) millilitres of basal medium each in three (3) conical flasks labelled A, B and C was prepared as described by [9]. The basal media contained micronutrient and macronutrient solutions, phosphate solution (KH₂PO₄) and sodium formate, which served as the organic source of carbon. To the basal media, 1% agar-agar was added, brought to boil and then sterilized by autoclaving at 115°C for 20mins at 15lb/inch² as described by [9]. Following sterilization, the media were allowed to cool to about 48°C and rifampicin (about 600mg) was added to selectively isolate archaea and eliminate bacteria present [20]. To the conical flasks labelled A and B, 0.57g of sodium salt was added and mixed, and in addition, 2g of sodium nitrate and 1ml of 0.01% resazurin solution was added to the conical flask labelled B, and to the conical flask labelled C, methanol (2ml) was added and mixed.

The content of each flask was then dispensed into two sterile Petri dishes (duplicate plating) labelled appropriately, allowed to set and stored at 4°C until required. About 0.1ml of the digestate from a completed biogas production set up (starter culture) was serially diluted to 10⁻⁶ dilution and 0.1ml each from this dilution was plated aseptically onto the already prepared plates in duplicate. The inoculated plates were then incubated at 37°C for three to seven days in a candle jar.

3. Results

The performance of the three substrates tested for biogas production was based on different parameters as shown in the table of results below. Identification of the methanogens was
based on their resistance to rifampicin as well as their Gram reaction. They were all observed to be rifampicin-resistant; some were Gram positive, some Gram negative while others were Gram variable. Also, various shapes were observed among the isolates which include, cocci, rods and spirilli.

### Table 1: Performances of Different Substrates for Biogas Production

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Test Substrates</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cow dung</td>
</tr>
<tr>
<td>Initial pH</td>
<td>7.08</td>
</tr>
<tr>
<td>Day production started</td>
<td>2(^\text{nd})</td>
</tr>
<tr>
<td>Day flammability started</td>
<td>7(^\text{th})</td>
</tr>
<tr>
<td>Flammability test</td>
<td>+++</td>
</tr>
<tr>
<td>Day of peak production</td>
<td>19(^\text{th})</td>
</tr>
<tr>
<td>Day production ceased</td>
<td>-----</td>
</tr>
<tr>
<td>Total gas produced (cm(^3))</td>
<td>12,550</td>
</tr>
<tr>
<td>Final pH</td>
<td>7.32</td>
</tr>
</tbody>
</table>

Key: HRT = Hydraulic Retention Time; +++ = highly flammable; ++ = flammable; ± = flammability fluctuates; --- = No cessation in production for the entire HRT.

![Fig. 1: Volume of biogas produced against retention time](image_url)
4. Discussion, Conclusion and Recommendations

4.1. Discussion

Various factors influence the performance of different substrates in biogas production at varying degrees. Although, the temperature, the loading rate and the HRT were kept constant, therefore, the determinants of the biogas level produced by each substrate were the pH and the nature of the substrate. The initial pH of the goat droppings and chicken droppings slurries were the same but different from that of the cow dung slurries; while at the end of the pre-determined retention time, the pH of each of the digestate (biorest) differs from each other, these could not be unconnected with the nutrient composition of each of substrate that determines the type of metabolism and end product to be generated by the implicated microbial entities [18]. It has however been reported that about 40-60% of feed consumed by animals eventually ends as manure [1]. Hence, the major nutrients utilized by the “bottle neck” of methanogenesis (i.e. methanogens) are carbon and nitrogen usually derives from the manure.

The largest volume of biogas obtained was from the chicken droppings having a carbon-nitrogen (C-N) ratio of 5.8:1 which agrees with the work reported by [1]. The C:N ratio was however far from the ideal ratio of 20-30:1 thus, the performance could be attributed to the nutrients contents in this substrate which might include in addition to proteins and vitamins, other macronutrients such as calcium and phosphorus which are also required by the organisms involved in methanogenesis. Although, the chicken droppings had the highest performance regarding the volume of gas produced, but was found to be the least flammable with irregular fluctuations in flammability. This might be attributed to the presence of excess ammonia (incombustible gas) in the biogas produced from abundant uric acid in the droppings by the action of the microorganisms involved.

Cow dung closely followed chicken droppings in terms of the yield. The output was lower than that obtained from chicken droppings but higher than that of goat droppings. Even though, the pH of 7.32 was more close to neutrality, but the yield was not as high as that of the chicken droppings and this might not be unconnected with the relatively inadequate nutrients supply in the feed due to their monotonous feeding habit hence, this could only be attributed to the shortage of nutrients in the dung. This agrees with the finding of [18]. But interestingly, biogas produced from cow dung in this study was found to have the highest degree of flammability despite the time it took before production started and as well the lower yield, which might be due to high methane content and/or low levels of such incombustible gases as carbon dioxide, oxygen, nitrogen and ammonia as components of the biogas. This result corroborates with the finding of [17].

Goat droppings ranked the least in terms of yield but second most flammable, thus, the biogas produced from this substrate was not as high in volume as that obtained from the chicken droppings but more flammable than it, although, it was less flammable than the biogas obtained from cow dung as well as lower volume in comparison to the cow dung.

With regards to the microbiological analysis, the isolates were narrowed down to four genera of the family Methanobacteriaceae namely: Methanobacterium spp., Methanococcus spp., Methanospirillum spp. and Methanosarcina spp. since they are the only genera known to have been isolated in pure culture.

The plates labeled A, supplemented with sodium formate without nitrate and methanol supported the growth of all the four genera which were identified presumably by Gram staining. For the plate labeled B, which was fortified with sodium nitrate in addition to the sodium formate supported the growth of only Methanobacterium spp., while other members were even strictly inhibited. Being Methanosarcina spp., the only genus utilizing methanol as source of carbon, the plate labeled C, fortified with methanol revealed the growth of only Methanosarcina spp., identified by Gram-staining.

The Gram staining of the various colonies (non-distinct colonies but an oily extended film on the medium surface) revealed the Gram reaction and morphology of the isolated methanogens. The Methanospirillum spp. isolated were Gram variable, some were Gram positive while others were Gram negative, but all appeared as short and slightly curved rods. For the Methanobacterium spp., all the isolates were found to be Gram negative and rod shaped while the Methanococcus spp. isolated were all spherical in shape (some appearing singly, others in short chains), but Gram variable. Lastly, the Methanosarcina spp. was also Gram variable, spherical in shape and arranged regularly in clusters or in packets.
Plate I: Methanosarcina spp.

Plate II: Methanobacterium spp.
4.2. Conclusion

Organic wastes, particularly animal wastes constitute a nuisance to our environment and also a threat to public health, there is therefore a need to search for the beneficial ways of minimizing them without due harm to our environment. In order to alleviate the problems of rural and urban energy requirement, this research will provide reliable information on the exploration and exploitation of other energy sources for man’s economic benefits; this is connected to the rapid depletion of fossil fuels. Biogas is a suitable, standard, affordable and sustainable alternative source of renewable energy since the raw materials used are termed as wastes. Moreover, from this research, it could be deduced that a biogas generating plant could be easily constructed at a minimum cost affordable. Conclusively, on the basis of the biogas yield and quality of the gas produced, cow dung was found to be the best substrate for biogas production both domestically and industrially. Similarly, one of the limiting parameters in biogas production at industrial scale is the inoculum, but this study is considered important since it showed that the inoculum can be maximally obtained by culture on the compounded methanogenic medium which was found to strictly and selectively support the growth of methanogenic bacteria that principally mediate the indispensable biomethanation process.
Acknowledgement

The whole idea of this work was proposed by the co-author of this paper. I therefore dedicate this on-going effort to her ideology. Appreciation also goes to the entire staff of the Department of Microbiology, Ahmadu Bello University, Zaria for their contributions toward a successful completion of this research.

References