Biogas production using guinea corn and rice husk

Abdulrahim Umar1*, Rabah Abdullahi Bala1, Baki Aliyu Sarkin2, Orjiude Josiah Ejike3 and Idris Abdullahi Dabban3

1Department of Microbiology, Usmanu Danfodiyo University Sokoto, Nigeria
2Energy Research Centre, Usmanu Danfodiyo University Sokoto, Nigeria
3Department of Microbiology, Ahmadu Bello University Zaria, Kaduna, Nigeria

ABSTRACT

Preliminary experiment on the possibility of biogas production from guinea corn and rice husks. The two samples (guinea corn and rice husk) were collected from a farm land in Rabah Local Government Area Sokoto, Nigeria. The guinea corn husks were dried and grounded using wooden pestle and mortar. The experimental set up was carried out by measuring 75g each of their pure form and mixture with label A, B, C and D before diluting with water in a ratio of 6:1, 3:1, 1:8 and 1:1 respectively and 10kg of freshly collected rumen content was added to each sample. The total volume of biogas was determined, moisture content, ash content, fiber content and N, P, K values were determined for digested and undigested slurries, as well as bacteria from anaerobic and aerobic culture were isolated from the digested slurries. Results indicated that both guinea corn and rice husk produced 1000cm$^3$ and 100cm$^3$ of biogas respectively for the period of 28 days with guinea corn having the highest biogas production, the digested slurries were rich in both mineral and nutrient contents and also some methanogenic bacteria and bacteria species identified are P. aeroginosa (11%), Bacillus spp (34%), Staphylococcus spp (16%), Enterococcus spp (9%), Vibrio cholerae (11%) Enterobacter spp (5%), Salmonella spp (5%), Morganella morganii (5%), Lactobacillus lactis (2%) and Serratia marcescens (2%). Guinea corn and rice husk can be used to produce significant amount of biogas, and digested substrates (slurries)can also be used as biofertilizers.

Key words: Biogas Production, Bacteriology, Microbial Load, Proximate Analysis, Guinea Corn Husk, Rice Husk, Methanogenic Bacteria.

INTRODUCTION

Over the past centuries, the concept of energy, source and method of producing it has always been a scientific challenge to man. The world energy problems usually emerge from the dwindling energy supply, energy distribution, utilization and waste management, environmental effect base on methods of energy production, and the problem of worldwide population growth [12].

Various sources of energy have been used by man in order to meet his basic life essentials such as food, clothing and shelter. Starting with one’s energy and sunlight, then advances to wood and wind power, then developed engine power, fuelled by wood, coal, petroleum, nuclear energy and microbial products. Man has utilized energy by modifying and manipulating land, water, plants and animals to obtain food, clothing and shelter. In order to achieve greater level of outputs, other energy inputs (mostly derived from fossil fuel reserves) have been used by man to enhance solar energy transformation [1].

It is generally accepted that fuel consumption represent a measure of a nation’s index of development, and improved standard of living. As nations develop industrially and in status, most individual own cars, generating plants and other forms of mechanical devices, which operates from one form of fuel to the other. As a result of this, there has been an increase in the uses and demand for fuel in terms of transportation and power generation. These have been
extracted from the stock of fossil fuels such as crude oil and to lesser extent coal and natural gas. This energy requires great deal of finance to extract them and finite in nature because it takes millions of years to accumulate by the activities of micro-organisms, temperature and pressure [18]. Worldwide there is a growing concern in the generation and utilization of energy derived from fossil fuels as they are not environmentally friendly causing global warming especially depletion of the ozone (O₃) layer [18]. In Nigeria due to the present economic reform and environmental effect, the nation is trying to divert attention from the fossil fuels, that has been the country’s primary and sole energy source for decades, to more economically and environmentally acceptable alternative sources of energy such as biogas [18].

Biogas technology is a modern technology based on the decomposition of organic materials by bacteria in anaerobic condition and appropriate temperature. When biogas is produced it leaves digested slurry in the digester, which has been a better measure of biofertilizer than its original form. This is an environmentally acceptable process because it releases no pollutant into the environment. In most developing countries, animal dung, agricultural residues and fire woods are used as fuel. As a result, the air is polluted with smoke by burning these fuels. Thus causing serious health problems and should be avoided [26].

Biogas production is accomplished by methanogenic bacteria, which reduce the molecules into methane (CH₄), carbon dioxide (CO₂), etc., which brings about the final step in the breakdown [10]. Hansen [15] states that acetate utilizing methanogens are responsible for 70% of methane produced in a biogas reactor.

In most developing countries, there is a vast biogas resource untapped, but instead they depend on wood, coal or crude oil for the fuel. These possess a great danger in the way of deserts encroachment as well as soil erosion particularly for locations close to deserts. These can be meaningfully checked by adapting the use in cooking, lighting as well as serving as fuel for running thermal engines and the slurries for a better fertilizer [18].

Studies conducted by Zuru [27] showed that, there is difference in biogas production between cow dung, the stem, leaves and the mixture of both the stem and the leaves of the plant. It also shows that the total gas produced by each after the retention period of 6 weeks was 19.31, 8.59, 3.70 and 3.48 cm³, for cow dung, stem, leaves and mixture of both stem and leaves. They concluded that the order of total biogas yield of the substrate is cow > stem > leave > mixture.

Bagudo [5], Wahyudi [25] and Rabah [22] produced biogas using cow dung, content of sheep colon, and abattoir waste respectively. All in their findings agreed that there is significant difference (p < 0.05) in the volume of the in the first and second week similarly in the second and third week.

Rabah [22] carried out a research on the production of biogas using abattoir waste at different retention time. They discovered that Bacillus species play very important role in biogas production, and despite the role they play are able to overlap from one stage to another during the process of gas production. The research showed that during anaerobic fermentation the pH decreases continuously and deduce that it could be due to the action of acetogenic methanogens breaking down sulphur containing organic and inorganic compounds, as well as the formation of fatty acids.

Similarly, Oluyega [20] produced biogas from cow dung where they found that certain bacteria such as Bacillus, Yersinia and Pseudomonas species were responsible for biogas production. The area of renewable energy research has undergone tremendous developments.

Biogas production is also a temperature dependant process, this can take place either in psychrophilic (less than 25°C) mesophilic (25-40°C) or thermophilic (45-60°C) temperature [17].

Also Garba and Sambo [13] looked into the effect of concentration, temperature, pH, nutrient addition and retention time. In the case of concentration they noted that the 4:1 and 5:1 ratio are the right proportion to obtain optimal result i.e. waste to water ratio. The experiment showed that the digesters with this ratio yield the highest gas. As for the temperature, some certain range of temperature (30°C to 40°C and 40°C to 60°C), there is higher level of gas production and at any temperature below and above the range there is lower or no gas production, the set-up also showed several experimental conditions of varying pH levels ranging from 4.0 to 9.0 and observed that the digester that produce the highest gas has the pH of 7.0 (neutral).

Baki [6] discovered that groundnut shell was an excellent source of energy and the retention period taken by the substrate was encouraging. The gas collected from the large scale digester to the biogas cylinder was used for domestic cooking and that gas produced was colourless flammable gas, clean and cause no pollution to the
environment. It was also observed that temperature plays vital role since the geographical location of Sokoto was on savannah and the higher temperature, the more the activities in the digester take place. They also found out that the digested slurry when analyzed has the composition of \( K = 35.3 \text{g/kg} \), \( P = 1.77 \text{mg/kg} \), \%N = 0.08, \%Ca = 0.09 and \%Mg = 0.05.

The digested slurry (biofertilizer) was spread on the farm and immediate decomposition took place implying that the farm became more fertile and there was an increase in crop production as well as healthier growth.

Garba et al [14] based on their findings, noted that ornamental plants especially those containing latex are potential sources of biogas and as such could also form enhanced fertile manure farm application.

Tambuwal [24] worked on the physio-chemical studies on biogas production using plant, pigeon droppings and animal manure, and they discovered that biogas production was greater at higher temperature and concluded that Sokoto weather was favourable for biogas production.

The urgent need to divert from conventional sources of energy and adopt different types of bioenergy cannot be over emphasized. Therefore this study was aimed at finding out the possibilities of transforming guinea corn husk and rice husk to produce biogas. At the same time determine the volume of biogas produced, the amount of nutrient content present in both undigested and digested samples as well as isolate and identify bacteria responsible in facilitating the biogas production.

**MATERIALS AND METHODS**

**2.1 Preparation of Slurry**

The substrate used for the production of biogas were designated and prepared according to [6] as follows:

Eight cylindrical tins (500g) were collected, cleaned and labelled A, B, C and D respectively.

Sample A contains a mixture of 50g of guinea corn husk and 25g of rice husk, 450cm\(^3\) of tap water was added to each of the sample to obtain slurry with water to mixture ratio of 6:1.

Sample B contains a mixture of 50g of rice husk and 25g of guinea corn husk, 225cm\(^3\) of tap water was also added to each of the samples to obtain slurry with water to mixture ratio of 3:1.

Sample C contain 75g of guinea corn husk only, 600cm\(^3\) of tap water was added to obtain slurry with water to guinea corn husk ratio of 8:1.

Sample D contain 75g of rice husk only, 75cm\(^3\) of tap water was added to obtain slurry with water to rice husk ratio of 1:1. Each slurry contains 10kg of the rumen content.

The experimental set-up for the biogas production was designed and biogas collected by downward displacement of water. The experiment was monitored daily and readings were taken every 24 hours at 12 noon every day for the period of 28 days.

**2.2 BACTERIOLOGICAL ANALYSIS**

**2.2.1 Media Preparation**

Tryptone Soy Agar was used for culturing, the media was prepared according to manufactures’ instructions and sterilized in an autoclave at 121°C for 15 minutes. The surfaces of the agar plates were dried in an oven before inoculation of the samples [8]. Buffered Peptone Water was prepared by weighing 0.5g of BPW using analytically weighing balance and dissolving it in 500ml distilled water. It was then autoclaved at 121°C for 15 minutes.

**2.2.2 Sample Collection and Processing**

The samples consisted of guinea corn husk, rice husk and rumen content. The husks were collected from a farm land in Rabah Local Government Area Sokoto, Nigeria. While the rumen content of a slaughtered cow was collected. This was done by tearing open the stomach of the animal, and the contents collected using a polythene bag, the collection was made in an abattoir in Sokoto State metropolis. The guinea corn and rice husks were separated from their seeds manually by beating the entire logs with sticks and the husks were further grinded to fine pieces using mortar and pestle to ensure homogeneity. After digestion slurries were diluted in a tenfold serial dilution up to the dilution of \( 10^{-5} \) using buffered peptone water as the diluent [2].
2.2.3 Inoculation and Incubation
For the tryptone soy agar, the spread plate method was used and 0.1 ml of the dilution was transferred onto a dried agar surface of the plates and a sterile glass rod spreader was used to spread the sample suspension on the surface of the agar plates. The spreader was sterilized by dipping in absolute ethanol and flamed by passing it through a Bunsen flame and allowed to cool for 20 seconds. All cultures were prepared in duplicates and incubated in both aerobic and anaerobic conditions (using the gas jar) at 37°C for 48 hours [8]. All the colonies appearing at the surface of the duplicate media plates after the incubation period were counted using the digital illuminated colony counter and the count expressed as colony forming unit per gram (cfu/g) of the samples. The actual number of colonies was determined using the formula,

\[
\text{Actual number of colonies} = \frac{\Sigma C}{(N1 + 0.1N2)D}
\]

Where C is the sum of the colonies counted on all the dishes retained, N1 is the no. of dishes retained in the first dilution, N2 is the no. of dishes retained in the second dilution and D is the dilution factor corresponding to the first dilution plated [3].

2.2.4 Characterization and Identification of Isolates
The colonies of the bacteria developed on the surface of the plates were purified by sub-culturing until pure isolates are obtained. Representative surface colonies were identified using colonial morphology, microscopical examination and biochemical characteristics based on standard procedures [7], [8], [15].

2.3 PROXIMATE ANALYSIS
The proximate analysis was carried out before and after digestion of the slurries, which is according to the standard official method of analysis of the Association of Official Analysis of Chemist [4] and those of Onyeike and Osuji [21].

2.3.1 Determination of Moisture Content
A clean crucible was dried in a constant weight in a hot air oven at 105°C, cooled in a desiccator and weighed (W1). Two grams of each sample (slurry) was accurately weighed into the previously labelled crucible and reweighed (W2). The crucible containing the sample was dried in the oven to a constant weight (W3). The percentage moisture content was calculated thus:

\[
\% \text{ moisture content} = \frac{W_2 - W_3}{W_2 - W_1} \times 100
\]

2.3.2 Determination of Ash Content
The porcelain crucible was fried in an oven at 100°C for 10 minutes, cooled in a desiccator and weighed (W1). Two grams of each of the sample was placed into the previously weighed porcelain crucible and reweighed (W2). It was first ignited and transferred into a furnace which was then set at 55°C. The sample was then left in the furnace for eight hours to ensure proper ashing. The crucible containing the ash was then removed and cooled in the desiccator after which it was then weighed (W3). The percentage ash content was calculated as:

\[
\% \text{ Ash Content} = \frac{W_2 - W_3}{W_2 - W_1} \times 100
\]

2.3.3 Determination of Crude Lipid Content
A clean, dried 500ml round bottom flask containing few anti-bumping granules was weighed (W1) and 200ml of petroleum ether (40-60°C) for the extraction was poured into the flask fitted with soxhlet extraction unit. The extractor thimble containing 2.0g of the sample was fixed into the soxhlet extraction unit. The round bottom flask and a condenser were connected to the soxhlet extractors and cold water circulation was put on. The heating mantle was switched on and the heating rate adjusted until the solvent was refluxing at a steady rate. Extraction was carried out for 6 hours, the solvent recovered and the oil was dried in oven at 70°C for one hour. The round bottom flask and oil was cooled and then weighed (W2). The lipid content was calculated thus:

\[
\% \text{ Crude Lipid Content} = \frac{W_2 - W_1}{\text{Weight of Sample}} \times 100;
\]
2.3.4 Determination of Nitrogen And Crude Protein
Two grams of each powdered sample in an ash less filter paper was dropped into 500cm$^3$ kjeldahl flask. Three grams of digesting catalyst (selenium) and 10ml conc. H$_2$SO$_4$ was also dropped into the kjeldahl flask. The sample was digested until a clear green colour was obtained. The digestion cooled and diluted to 100ml with the distilled water. 20ml of diluted digest were measured into 500ml kjeldahl flask containing ant-bumping chips and 40ml of 40% NaOH was slowly added by the side of the flask. A 250ml conical flask containing a mixture of 50ml 20% boric acid and 4 drops of mixed indicators was used to trap the ammonia being liberated. The conical flask and the kjeldahl flask were then placed on the kjeldahl distillation apparatus with the tubes inserted into the conical flask and kjeldahl flask. The flask was heated to distil out the NH$_3$ evolved. The distillate was collected into the boric acid solution, when the boric acid turned to green, it was allowed for 10 minutes to compete distillation of the ammonia present in the digest. The distillate was then titrated with 0.1M HCl.

Calculation:

\[
\% \text{ Nitrogen (N}_2\text{)} = \frac{14\times MX\times TV \times 100}{\text{Weight of Sample (mg)} \times XVa}
\]

Therefore,

\[
\% \text{ Crude Protein} = \% \text{ Nitrogen (N}_2\text{)} \times 6.25;
\]

Where

- $M$ = Actual Molarity of Acid
- $TV$ = Titre Volume of HCl used
- $VT$ = Total Volume of Diluted Digest
- $Va$ = Aliquot Volume Distilled

2.3.5 Determination of Crude Fibre
Two (2.0) grams of the sample powder was weighed out into a round bottom flask containing 20ml of 10% H$_2$SO$_4$ were added and boiled gently for 30 minutes. The hot solution was quickly filtered under suction, the insoluble matter was washed several times with hot water until it was acid free. It was quantitatively transferred into flask and 20ml of 10% NaOH was added and the mixture boiled again under the reflux for 30 minutes and quickly filtered under suction. The soluble residue was washed with boiling water until it was base free and was dried to constant weight in the oven at 100°C and recorded as $C_1$. The weight sample ($C_1$) was then incinerated in a muffled furnace at 550°C for 2 hours, cooled in the desiccator and reweighed ($C_2$)

Calculation:

\[
\% \text{ Crude Fibre} = \frac{C_1 - C_2}{\text{Weight of Sample}}
\]

2.3.6 Determination of Carbohydrate (By Difference)
The total carbohydrate content was determined by difference. The sum of percentage moisture, ash, crude lipid, crude fibre and crude protein was subtracted from 100.

Calculation: \(\% \text{ Total Carbohydrate} = 100-(\%\text{moisture + }\%\text{ash + }\%\text{fat + }\%\text{protein + }\%\text{fibre})\)

2.4 MINERAL ANALYSIS
2.4.1 Determination of Potassium and Sodium Content
The photometer was set – up and calibration read out using potassium while aspirating distilled water as blank solution. The meter reading was also set at 100% transmittance, while aspirating the top concentration of the standard, all other intermediate standard solutions were then run and % transmittance were recorded. A standard curve of transmittance versus concentration was plotted. A portion of aliquot digest sample solutions was aspirated and % transmittance reading was recorded. The concentration of K$^+$ content of the sample solution was read from the standard curve.

2.4.2 Determination of Phosphorus
Five (5) millilitre of 20% HCl is added to dissolve the ash residue, and mixed properly. A 50ml of the distilled water was used to dilute; the solution was then filtered to remove the particle. 2ml of the prepared sample was dispensed into 50ml volumetric flask, 2ml of phosphorus extraction solution was added, 2ml of ammonium – molybdate reagent was also added and distilled water. 1ml of dilute stannous chloride was added into the sample and blue
colouration was observed; again distilled water was added to make up to 50ml of the volume. For standard curve, 0.2, 0.4, 0.6, 0.8 and 1.0 concentrations were prepared. The colour absorbance of the sample and that of the standard were determined using spectrophotometer at a wavelength of 660nm. The conc. of phosphorus in samples corresponding to the absorbance was obtained from the standard curve.

\[
\% \text{ Phosphorus (Mg/Kg)} = \frac{\text{Absorbance} \times 0.61 \times \text{DF} \times \text{DF}}{30.97}
\]

2.4.3 Determination of Calcium (Ca) and Magnesium (Mg)

Five (5)millilitre of 20% HCl was added to dissolve the ash residue, and mixed properly. A 50ml of the distilled water was used to dilute; the solution was then filtered to remove the particle. 1ml of the prepared solution and 19ml of distilled water were placed in a conical flask to dilute up to 20ml. 1ml of 10% NaOH was also added to the solution. A tip of Murexid indicator (in powdered form) was also added to give pink colour. Titration was performed against 0.01 molar EDTA and the end point was signified by a purple colour change in colour for calcium (Ca), while a blue colour change in colour signifies for magnesium (Mg).

RESULTS

The plates inoculated with the dilution of \(10^{-5}\) were retained for the aerobic and anaerobic viable plate count. The results are shown in table 1. It indicated a highest count in sample Danaerobic digester and lowest in sample B anaerobic digester. Each slurry was found to have significant amount of bacteria, expected to have facilitated the biogas production.

<table>
<thead>
<tr>
<th>Plate code</th>
<th>Bacteria Load (cfu/g)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Aerobic</td>
<td>Anaerobic</td>
</tr>
<tr>
<td>A</td>
<td>(3.1 \times 10^5)</td>
<td>(5.5 \times 10^5)</td>
</tr>
<tr>
<td>B</td>
<td>(4.6 \times 10^5)</td>
<td>(1.9 \times 10^5)</td>
</tr>
<tr>
<td>C</td>
<td>(4.8 \times 10^5)</td>
<td>(5.0 \times 10^5)</td>
</tr>
<tr>
<td>D</td>
<td>(4.2 \times 10^5)</td>
<td>(7.9 \times 10^5)</td>
</tr>
</tbody>
</table>

Key: cfu/g = Coliform Forming Unit / Gram

The result from this study shows that Bacillus spp are dominant in facilitating biogas production as shown in figure 1 followed by staphylococcus spp. Lactococcus lactis and Serratia marcescens were the least.

The result of this study shows that biogas production was at its pick in week 1 with sample C (75g of guinea corn husk) and lowest in week 4 with sample D (75g of rice husk) having the lowest.
This study have revealed in Table 2. That values of proximate analysis obtained from the slurries after biogas production is rich in carbohydrate, fibre, crude protein, ash small amount of nitrogen.

<table>
<thead>
<tr>
<th>DETERMINATION (%)</th>
<th>SAMPLE A</th>
<th>SAMPLE B</th>
<th>SAMPLE C</th>
<th>SAMPLE D</th>
</tr>
</thead>
<tbody>
<tr>
<td>MOISTURE</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>UNDIGESTED</td>
<td>1.0</td>
<td>2.0</td>
<td>2.5</td>
<td>0.5</td>
</tr>
<tr>
<td>DIGESTED</td>
<td>81.5</td>
<td>88.5</td>
<td>83</td>
<td>90.5</td>
</tr>
<tr>
<td>ASH</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>UNDIGESTED</td>
<td>11.5</td>
<td>16.0</td>
<td>18.5</td>
<td>10.0</td>
</tr>
<tr>
<td>DIGESTED</td>
<td>1</td>
<td>1</td>
<td>1.5</td>
<td>0.5</td>
</tr>
<tr>
<td>FIBRE</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>UNDIGESTED</td>
<td>29.5</td>
<td>35.5</td>
<td>28.0</td>
<td>43.0</td>
</tr>
<tr>
<td>DIGESTED</td>
<td>15.5</td>
<td>17.0</td>
<td>13.0</td>
<td>20.0</td>
</tr>
<tr>
<td>LIPID</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>UNDIGESTED</td>
<td>1.5</td>
<td>2.0</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>DIGESTED</td>
<td>0.5</td>
<td>1.0</td>
<td>1.0</td>
<td>0.5</td>
</tr>
<tr>
<td>NITROGEN</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>UNDIGESTED</td>
<td>0.83</td>
<td>1.01</td>
<td>0.83</td>
<td>1.55</td>
</tr>
<tr>
<td>DIGESTED</td>
<td>0.22</td>
<td>0.41</td>
<td>0.18</td>
<td>0.65</td>
</tr>
<tr>
<td>CARBOHYDRATE</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>UNDIGESTED</td>
<td>52.32</td>
<td>40.19</td>
<td>47.32</td>
<td>36.32</td>
</tr>
<tr>
<td>DIGESTED</td>
<td>81.62</td>
<td>78.44</td>
<td>83.37</td>
<td>74.94</td>
</tr>
<tr>
<td>CRUDE PROTEIN</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>UNDIGESTED</td>
<td>5.1</td>
<td>6.31</td>
<td>5.18</td>
<td>9.68</td>
</tr>
<tr>
<td>DIGESTED</td>
<td>1.38</td>
<td>2.56</td>
<td>1.13</td>
<td>4.06</td>
</tr>
</tbody>
</table>

This study have shown that only phosphorus (P) appreciated in values after the mineral analysis while others decreases.
This study have revealed that sample A, B and C have potential to become acidic while only sample D have potential of becoming alkaline in nature.

**Table 4. RESULT FOR pH OF DIGESTED AND UNDIGESTED SLURRY**

<table>
<thead>
<tr>
<th>SAMPLES</th>
<th>UNDIGESTED</th>
<th>DIGESTED</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>6.7</td>
<td>5.37</td>
</tr>
<tr>
<td>B</td>
<td>6.4</td>
<td>5.64</td>
</tr>
<tr>
<td>C</td>
<td>6.8</td>
<td>5.21</td>
</tr>
<tr>
<td>D</td>
<td>5.8</td>
<td>7.38</td>
</tr>
</tbody>
</table>

**DISCUSSION**

Microorganisms present in the digested slurries were isolated and identified base on their gram reaction and biochemical characteristics. Some of the species are *Enterococcus* spp, *Stapylococcus* spp, *Enterobacter* spp, *Bacillus* spp, *Pseudomonas* spp, *Lactobacillus* spp e.t.c.

Most of the isolate identified are those associated with the substrate used for biogas production, for example the enteric organisms are those present in the rumen content of cow the source of rice husk while species such as *Bacillus cereus* are those associated with grains. The organisms mostly have the ability to produce gas, hence why they are responsible for biogas production.

Organisms isolated such as *bacillus* spp and *pseudomonas* spp correspond to those isolated by Oluyega [20] and Rabah [22], then deduced that they are responsible and play very important role in biogas production.

The weekly production of biogas from guinea corn husk, rice husk and their mixture is presented in table 4.1. The highest values of 436.6, 143.3, 940 and 80cm$^3$ for sample A, B, C, and D respectively were obtained within the first week, and the least was 6.6, 3.3, 20, and 20 obtained on the third and fourth week as shown in the table.

However, the pattern of biogas production using these substrates is unique i.e rapid production was experienced within the first week and rapid decline in the second week, then fluctuation is recorded in the third and fourth week for sample B, C, and D. The rapid biogas production could be as a result of microorganisms utilizing the substrates to carry out metabolic activities.

The result obtained from this study is similar to that of Bagudo [5] and Rabah [22] stating that there is significant difference (p <0.05) in the volume of gas produced within the first and second week, and also the second and third week with no difference within the third and fourth week of production [11] while this experiment shows that biogas production was optimum within the first week.

But studies conducted by Wahyudi [25] and Bagudo [5] using cow dung and content of sheep colon reported biogas production within the first week similar to this but optimum between the third and fourth week, although this could be as a result of seeding effect of the substrates.

The difference in the volume of biogas produce corresponds to the mixture ratio of water to substrate, the highest to the lowest production are 1000, 516.5, 159.8 and 100cm$^3$ which is similar to their mixture ratio of water to substrate.
given as 8:1, 6:1, 3:1 and 1:1. This result can be related to the findings of Garba and Sambo, [13] and deduce that ratios of 4:1 and 5:1 are the right proportion to obtain optimal result but this research shows that even higher ratios can also produce more biogas.

The different compositions of the substrates also affects the biogas production, as digesters with higher grams of guinea corn husk produce more biogas compared to that of rice husk.

As shown in the table 4.1, the temperatures in the first week to fourth week were 34.0°C, 31.47°C, 32.6°C, and 33.1°C respectively. The highest temperature 34.0°C was recorded in the first week, hence the reason for highest biogas production. While the lowest temperature 31.47°C was recorded in the second week, hence the massive fall in the volume of gas produced.

This was in conformity to the findings of Garba and Sambo, [13] which shows that some certain range of temperature (30°C to 40°C and 40°C to 60°C) there is higher level of gas production, and at any temperature below and above the range there is lower or no gas production.

pH is an important factor of biogas production. As such the pH of undigested and digested slurry were recorded as shown in table 4.2. The pH decreases from the undigested to digested slurry becoming more acidic in sample A, B and C while sample D initially acidic increases in pH. The decrease in pH may be due to the action of acetogenic methanogens as they break down sulphur containing organic acid and inorganic compounds as well as the formation of fatty acids [22]. The increase in pH in sample D could be as a result of low fatty acid formation by the organisms as shown in the analysis of lipid content.

However, samples with initial pH close to neutrality 6.8, 6.7, and 6.4 have the highest biogas production. While sample D having initial pH of 5.8 recorded the lowest volume biogas produced. This is in conformity with the work of Garba and Sambo, [13] who observed digesters with highest biogas production at the pH of 7.0.

Generally plant material consists of moisture, ash and volatile solids (organic matter). The values for moisture content for guinea corn husk is 2.5% before digestion and 83% after digestion while rice husk is 0.5% before digestion and 90.5 after digestion, also on the other hand ash content for guinea corn husk is 18.5% before digestion and 1.5% after digestion while rice husk is 10% before digestion and 0.5% after digestion as shown in table 4.4. The ash content is often a good determining factor for the mineral content of plant based samples; the higher the ash content, the higher the mineral element content. It can be seen that undigested husks of the four samples all give higher ash content than the digested husks. This showed that undigested has more mineral element such as sodium, potassium, and nitrogen than the digested husks with phosphorus as an exception.

During anaerobic digestion, microorganisms action indicated that the relative moisture, ash and volatile solid in a given plant material influences the amount of biogas produced.

The phosphorus content determined in this study is higher in digested than in undigested slurries. Therefore, it can be used to enrich the soil after biogas production.

The role played by potassium in the synthesis of carbohydrates cannot be over emphasized, it assist in the synthesis of protoplasmic protein and is believed to increase vigour in plants [23]. This research showed that the potassium level for guinea corn husk, rice husk and their mixture is higher in undigested slurries than in digested. The result for analysis carried out also showed required value for potassium (K), phosphorus (P), nitrogen (N₂), calcium (Ca), and magnesium (Mg) for use on farm land in order to become more fertile and increase crop production as well as healthier growth as stated byBaki [6].

Even though, the ash content in this study shows the reduction in nutrient of the digested slurries. Taking in to consideration of their analyzed compositions they still satisfy the requirement for use as biofertilizer.

**CONCLUSION**

The production of biogas from guinea corn husk, rice husk and their mixture has been successful, with sample C having the highest biogas production of 1000cm³ while sample D has the lowest biogas production of 100cm³. At the temperature of 34°C and pH of 6.8 all the sample produced the highest amount of biogas. Microorganisms (aerobic and anaerobic) that are mostly gas producers were isolated and identified, and despite utilizing the nutrient available the digested slurries were still rich in mineral nutrient.
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REFERENCES


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