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# Biogas production using appropriate biodegradable wastes: Strength and Weakness

By

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## ABSTRACT

The biogas production was investigated using four substrates in this study. Out of which two shows the potential of co-digestion systems involving animal manures and agricultural wastes for bioenergy efficiency. The raw wastes were cow dung, cow dung and maize cob as substrates, poultry manure and beans hull and lastly Pig manure. The microbial communities involved in the anaerobic digestion process were identified. The temperature and pressure within the first phase of digester ranged from $28^0$C to $36.5^0$C and from 0.3-1.6 bar respectively. The digester of the second phase had a temperature, pressure and volume of 27 to $32^O$C, 0.1 to 2.0 bar, and 310cm$^3$ respectively. At third phase of the study, the digester had a temperature of 27-$33^O$C, and a pressure of 0.1-1.5 bar. While the fourth phase study had a temperature of 27- $35^O$C, a pressure range of 0.1 to 2.0 bar, and volume of 330cm3. There was optimum biogas production on the twenty- second day, with a temperature of $35^O$C, pressure of 1.8 bar, and volume of 210 cm$^3$. The flammability of the biogas was confirmed by the presence of methane, but the presence of hydrogen sulfide lowered the heating value and efficiency of the biogas with poultry and pig manure substrates. The studies suggested that co-digestion and pretreatment of animal manures and agricultural wastes is a promising technique for sustainable waste management and renewable energy production, but further research is needed to optimize the process parameters and remove the hydrogen sulfide from the biogas for efficiency.

**Keywords**: Biogas, Biodegradable wastes, Digester, Microorganisms and Efficiency

## Introduction

The search for appropriate bioenergy sources necessitates the study for recovery of utilizable sources of biogas. Biogas is produced through the anaerobic digestion of organic matter, such as agricultural waste, sewage, and food waste. This process involves the breakdown of organic matter by microorganisms in the absence of oxygen, resulting in the production of biogas, which mainly consists of methane and carbon dioxide (Ahmed *et al.,* 2018). Biogas is a renewable and sustainable energy source that can be used for heat and power generation, as well as a vehicle fuel. It is considered to be a promising alternative to fossil fuels, as it can help reduce greenhouse gas emissions and dependence on non-renewable energy sources (Chen *et al.,* 2020).

The production of biogas has been gaining increased attention in recent years due to its potential to address environmental, energy, and waste management challenges. In addition to producing renewable energy, biogas production can also help reduce the environmental impact of organic waste by converting it into a valuable resource. The use of biogas can also contribute to the decentralization of energy production, as it can be generated locally from various organic waste sources (Yadav and Chandra, 2019). In terms of global trends, the biogas industry has been experiencing rapid growth, with increasing investments in biogas plants and technology. Countries such as Germany, China, the United States, and the United Kingdom have been leading in biogas production and utilization. In addition, there has been a growing interest in small-scale biogas systems and green energy synthesis especially in developing countries, to address energy poverty and improve waste management practices (Lu *et al.,* 2019).

Recent advancements in biogas production technology have also contributed to its increased adoption. Innovations such as high-efficiency biogas reactors, improved feedstock pre-treatment methods, and advanced gas purification techniques have enhanced the overall efficiency and environmental performance of biogas production. Furthermore, there is ongoing research and development in the field of biogas production, with a focus on optimizing process parameters, developing new feedstock sources, and exploring novel applications of biogas (Zhao *et al.,* 2021).

Ongoing research and development efforts have continued to drive innovation in biogas production, with a focus on exploring new feedstock sources, improving process efficiency, and reducing environmental impacts. Research areas include the use of novel substrates such as algae, organic industrial residues, and dedicated energy crops, as well as the application of emerging technologies such as microbial electrochemical systems and enzyme-assisted digestion to enhance biogas production and resource recovery from waste streams. Various

agricultural and aquaculture waste can be used as a feedstock for biogas production through anaerobic digestion that can provide a renewable energy source (Patil *et al.,* 2023).

Biogas production from agricultural waste is a sustainable and renewable energy generation process that offers several environmental and economic benefits. Agricultural waste, including crop residues, animal manure, and organic by-products from agro-industrial processes, can serve as valuable feedstock for biogas production. The anaerobic digestion of these organic materials results in the production of biogas, primarily composed of methane and carbon dioxide, which can be used for heat and power generation. Biogas production from agricultural waste provides an opportunity for waste management, renewable energy generation, and the reduction of greenhouse gas emissions. The process also supports the circular economy by converting organic waste into a valuable energy resource, thereby promoting sustainable agricultural practices (Weiland *et al.,* 2019).

The types of agricultural waste suitable for biogas production include:

Biogas production from domestic waste is an environmentally sustainable way to generate energy and manage waste. The process involves the breakdown of organic materials, such as food scraps and yard waste, by bacteria in an anaerobic (oxygen-free) environment to produce methane gas, which can be used as a renewable energy source. There are several key steps involved in biogas production from domestic waste (Rajendran, 2019). First, the waste materials are collected and transported to a biogas facility where they are sorted and prepared for digestion. Next, the waste is mixed with water and other organic materials to create a slurry, which is then fed into a digester. Inside the digester, bacteria break down the organic matter in the absence of oxygen, producing methane and carbon dioxide gases. The biogas can then be captured and used for heat and power generation, or processed further to remove impurities and produce biomethane, a renewable natural gas that can be injected into natural gas pipelines or used as a transportation fuel. The remaining digestate, which is rich in nutrients, can be used as a fertilizer for agriculture (Rajendran, 2019).

One recent study by Kim and Kim (2019) investigated the potential for biogas production from food waste in South Korea and found that anaerobic digestion could be a promising option for managing food waste and generating renewable energy. Another study by Tchobanoglous *et al.* (2020) examined the feasibility of implementing biogas production from domestic waste in the United States and highlighted the potential environmental and economic benefits of this approach. Looking towards the future, there is a growing need to further optimize biogas production from domestic waste to maximize energy recovery and resource efficiency. Research efforts are focused on improving waste separation and pre-treatment methods to enhance biogas yields, as well as developing innovative technologies for biogas utilization and distribution. Additionally, there is a growing interest in exploring

the potential synergies between biogas production and other renewable energy systems, such as solar and wind, to create more integrated and sustainable energy solutions (Velthof and Gaastra, 2022).

In conclusion, biogas production from domestic waste is a promising pathway to sustainable waste management and renewable energy generation. With ongoing advancements in technology and continued research efforts, biogas production from domestic waste is poised to play a significant role in the transition towards a more sustainable and circular economy (Wirth and Zemman, 2023).

This study therefore helps to investigate the anaerobic co-digestion of poultry manual and beans hull, cowdung and maize corbs as a potent approach for sustainable biogas production.

## MATERIALS AND METHODS

### Study Site

This study was conducted in the microbiology laboratory of Adekunle Ajasin University campus located at Akungba-Akoko, Ondo State. All experiments in this study were conducted in the Microbiology laboratory by constructing a plastic anaerobic digester.

Fresh poultry manure, beans hull, cowdung and maize corb were used as biodegredable substrate for biogas production in this study. Potato dextrose agar (PDA) and nutrient agar (NA) were culture medium routinely used for this study. Foil paper, nose masks, antibiotics (streptomycin), ethanol for surface sterilization of some materials were additional laboratory materials used to enhance safety precautions.



### Sample collection

For this study, poultry manure and beans hull was used for biogas production. The poultry manure was sourced from the university farm and the beans hull was gotten from Owo, Ondo state. For the Poultry manure collection, a sterile spade and polythene bag was used, it was obtained by scraping off the top- layer of the manure surface. The beans hull was sundried for a week for removal of moisture and was co-digested with the poultry manure for the biogas production.

### The Digester

Twenty-five (25) liters plastic digester was fabricated for optimum anaerobic digestion. The digester were equipped with pressure gauge as well as mercury thermometer. The airtight containment vessel have inlets for feeding the raw materials and an outlet for gas extraction from the digester. The digester was left for twenty – eight (28) days for anaerobic digestion to

take place. During this 28 days, the pressure readings, level of acidity, and temperature for the digester were taken for each day.

**Substrate Preparation**

Three kilogram (3kg) of poultry manure and 1.5kg of the pretreated dried beans hull was weighed and fed into the manual digester. The dried beans was pre-treated with 90g of sodium hydroxide before it was been fed into the manual digester. These substrates were then mixed with 2910ml of distilled water in the digester to give a waste to water ratio of 1:2 and then it was stored at strict anaerobic conditions. The mode of feeding used was discontinued feeding (batch feeding). It was continually agitated as described as Bajah and Garba (1992). The experimental set up was left for monitoring for a specific retention time of 28 days. The digester temperature was measured with the aid of mercury-in glass thermometer, the pressure was checked with aid of pressure gauge and also the pH was measured with the aid of pH meter

**Isolation of Organism**

**Fungi**

About 5ml of the well mixed sample in the sampling container were collected into a sterile container. Five test tubes containing 9ml of distilled was prepared, and one ml (1ml) of the mixed slurry was dispensed into the first test-tube ($10^1$) using a sterile syringe which was mixed properly to ensure homogenization. One ml was withdrawn and dispensed into the second test-tube ($10^2$) using another syringe and mixed properly. This procedure was repeated on the remaining three test tube using different syringe. After serial dilution, diluent 3 and 5 were used for culturing. One ml of each diluent were inoculated in a petri dish containing 20ml of potato dextrose agar. The petri dishes were incubated at room temperature and examined for three days. After incubation, morphological characteristics of the isolates were recorded and the diameter of colony is measured for the three days, the colonies were counted and a distinct colony was picked using a sterile inoculating needle and sub-cultured in a fresh medium. After sub- culturing, the slants of the isolates were preserved in McCartney bottles for identification and further use.

**Bacteria**

About 5ml of the well mixed sample from the sampling container was collected in a sterile container. Five test tubes containing 9ml of distilled water was prepared, one ml of the mixed slurry was dispensed into the first test tube ($10^1$) using a sterile syringe which was mixed properly to ensure homogenization. One ml was withdrawn and dispensed into the second test tube ($10^2$) using another syringe and properly mixed. This procedure was repeated on the other test tubes using different syringe. After the serial dilution, diluent 3 and 5 were used for culturing. One ml of each diluent was inoculated in a petri dish containing nutrient agar. The

petri dishes were incubated at 37oc f0r 24 hours. After incubation, the colonies were counted and a distinct colony was picked using a sterile inoculating loop, sub cultured in afresh medium and incubated at 37oc f0r 24 hours. After subculturing, slants of the isolates were preserved in McCartney bottles for identification and further use. (Dubey *et al.*, 2019)

**Identification of Isolate**

**Identification of fungi**

Fungal isolates were identified by placing a drop a lactophenol cotton blue on a clean slide. Using a pair of inoculating needles, a small portion of the mycelium was removed from the culture and placed on the drop of lactophenol cotton blue. The mycelium was spread on the slide with the aid of the inoculating needle. A cover slip was gently placed on the slide. The slide was viewed under x40 magnification.

**Identification of Bacteria**

Characterization was carried out using various biochemical tests. These tests include; Catalase, motility, indole, sugar fermentation (glucose and lactose), citrate and urease test. The organisms were generally identified by standard microbiological methods. All media were prepared according to manufacturer's specification and sterilized, unless where stated otherwise, at $121^0$C at 15 minutes and all reagents are used accordingly.



**RESULT**

This study shows various species of organisms recovered during biogas production processes. In the fisrt stage of the study, five fungal species isolated from co-digestion of Cow dung and maize cob were identified as; *Rhodotorula minuta, Candida tropicalis, Eurotium rubrum, Aspergillus clavatus, and Aspergillus fumigatus.* Also, bacterial species such as *Klebsiella pneumonia, Klebsiella aerogenes, Bacillus alveli, Escherichia coli, Pseudomonas aeruginosa, Bacillus anthracis, Citrobacter diversus, Cronobacter malonaticus, Nocardia asteroides* were also encountered from this sources.

The diameter, septation, color, reverse color, microscopic feature and probable organisms of the fungal colony are presented in Table 1. The frequency and percentage of occurrence of each fungal species are presented in Table 2. Table 3 shows the cultural and biochemical characteristics of the bacteria isolated from Co-digestion of Cow dung and maize cob. The percentage occurrence of the bacteria isolated from the mixture of cow dung and maize cob is shown in Table 4. For 28 days, the variation in temperature, pressure and volume is shown in Table 5. The digester had a temperature of 27- $32^O$C, a pressure between 0.1 and 2.0 bar, and a volume of 310cm$^3$. On the twenty- second day, there was the most biogas production, with a temperature of $32^O$C, a pressure of 1.6 bar, and a volume of 230 cm$^3$.

A flame was lit through the gas outlet connected to the digester to test for methane and there was presence of flame. The digester that had production of flammable biogas and the presence of flame is shown in plates 1 and 2. The typical fungal species isolated are shown in plates 3 to 6.


Plate 1: Digester with flammable biogas Plate 2: Gas outlet with no black traces ($H_2S$)

**Table 1: Morphological and physiological characteristics of fungal isolates.**

| Lab code | Diameter | Pigmentation | Reverse | Microscopic Feature | Septation | Surface texture | Surface topography | Probable organism |
|---|---|---|---|---|---|---|---|---|
| CFS1 | 17mm | White | Blackish-brown | Bipolar budding and pseudohyphae-forming yeast cells that are oval to ellipsoidal and have globose and subglobose basidia with basidiospores | Septate | Smooth | Free growth | *Rhodotorula minuta* |
| CFS2 | 25mm | Bluish-green | Cream | Aconidiophore with an elliptical shape and thick wall | Septate | Velvety | Covering the entire surface of the agar | *Aspergillus clavatus* |
| CFS3 | 0.002mm | Cream | White | The cells reproduce unipolar or bipolar and form blastoconidia | Septate | Smooth | Covering the surface of the agar | *Candida tropicalis* |
| CFS4 | 14mm | Yellowish | Cream | Conidiophores with hyaline and ellipsoidal conidia and smooth red ascospores | Septate | Fluffy | Covering the surface of the agar | *Eurotium rubrum* |
| CFS5 | 45mm | Grey-Green | White | Grey green spores on long, smooth and spiny conidiophores | Septate | Powdery | Free growth | *Aspergillus fumigatus* |

KEY: CFS1- Cow fungal isolate 1, CFS2- Cow fungal isolate 2, CFS3- Cow fungal isolate 3, CFS4- Cow fungal isolate 4, CFS5- Cow fungal isolate 5

Table 2: Percentage occurrence of fungal isolates from cow dung

| Lab code | Isolate | Frequency of Occurrence | Percentage of Occurrence (%) |
|---|---|---|---|
| CFS1 | *Rhodotorula minuta* | 6 | 30 |
| CFS2 | *Aspergillus clavatus* | 8 | 40 |
| CFS3 | *Candida tropicalis* | 2 | 10 |
| CFS4 | *Eurotium rubrum* | 1 | 5 |
| CFS5 | *Aspergillus fumigatus* | 3 | 15 |
| | Total | 20 | 100 |

Percentage occurrence= Frequency of occurrence X 100

Total number of isolates

KEY: CFS1-Cow fungal isolate 1,

CFS2- Cow fungal isolate 2,

CFS3- Cow fungal isolate 3,

CFS4- Cow fungal isolate 4,

CFS5- Cow fungal isolate 5



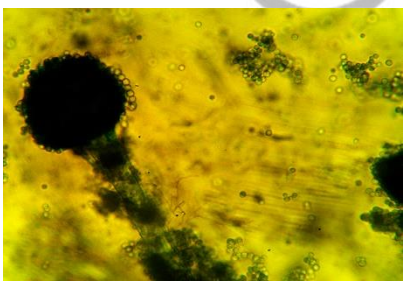

Plate 3: *Eurotium rubrum* X40

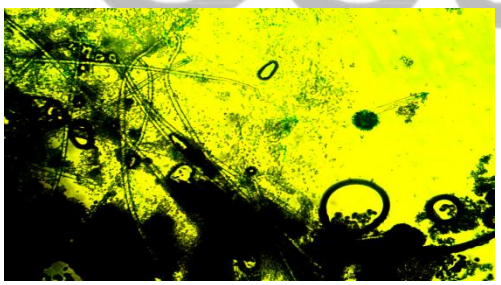

Plate 4: *Aspergillus fumigatus* X40

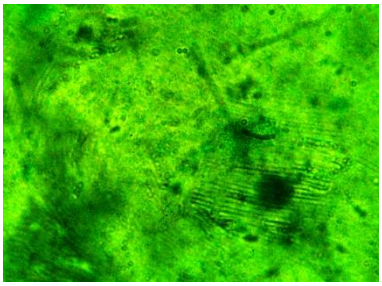

Plate 5: *Rhodotorula minuta* X40

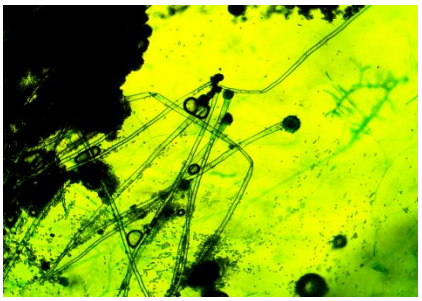

Plate 6*: Aspergillus clavatus* X40

Table 3: Biochemical test for bacterial isolates

| Lab code | Cultural characteristics | Gram reaction | Morphology | Motility | Oxidase | Catalase | Indole | Citrate | Urease | Lactose (S/G) | Glucose (S/G) | Probable organisms |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| CBS1 | Circular, Convex, Smooth, Green | - | Rod | + | + | W+ | - | + | + | ++ | ++ | *Klebsiella pneumonia* |
| CBS2 | Circular, Raised, Smooth, Cream | - | Rod | + | + | W+ | + | + | + | ++ | +- | *Klebsiella aerogenes* |
| CBS3 | Circular, Raised, Lobate, Cream | + | Rod | + | - | W+ | + | + | + | ++ | ++ | *Bacillus subtilis* |
| CBS4 | Irregular, Convex, Entire, White | + | Rod | - | - | S+ | + | + | + | ++ | +- | *Bacillus alveli* |
| CBS5 | Circular, Convex, Smooth, Cream | - | Rod | - | + | S+ | + | + | + | ++ | +- | *Escherichia coli* |
| CBS6 | Circular, Raised, Rough, Yellow | - | Rod | - | + | W+ | + | + | + | ++ | ++ | *Pseudomonas aeruginosa* |
| CBS7 | Irregular, Convex, Lobate, Cream | + | Rod | + | + | S+ | + | + | + | ++ | ++ | *Bacillus alveli* |
| CBS8 | Circular, Raised, Entire, Green | - | Rod | - | - | S+ | + | + | + | ++ | ++ | *Citrobacter diversus* |
| CBS9 | Circular, Convex, Entire, White | - | Rod | - | - | S+ | - | + | + | ++ | ++ | *Cronobacter malonaticus* |
| CBS10 | Circular, Umbonate, Entire, White | - | Rod | - | + | W+ | - | + | + | ++ | ++ | *Nocardia asteroids* |

KEYS

+ = Positive reaction, - = Negative reaction, S/G = Sugar and gas reaction

S+ = Strongly positive, W+ = Weakly positive, BS = Bacterial isolate

Table 4: Percentage occurrence of the bacterial isolates

| Lab code | Isolate | Frequency of Occurrence | Percentage of Occurrence (%) |
|---|---|---|---|
| CBS1 | *Klebsiella pneumonia* | 4 | 20 |
| CBS2 | *Klebsiella aerogenes* | 2 | 10 |
| CBS3 | *Bacillus subtilis* | 1 | 5 |
| CBS4 | *Bacillus alveli* | 1 | 5 |
| CBS5 | *Escherichia coli* | 1 | 5 |
| CBS6 | *Pseudomonas aeruginosa* | 3 | 15 |
| CBS7 | *Bacillus anthracis* | 4 | 20 |
| CBS8 | *Citrobacter diversus* | 1 | 5 |
| CBS9 | *Cronobacter malonaticus* | 1 | 5 |
| CBS10 | *Nocardia asteroides* | 2 | 10 |
| | Total | 20 | 100 |



KEY: CBS1- Cow bacterial isolate 1, CBS2- Cow bacterial isolate 2, CBS3- Cow bacterial isolate 3, CBS4- Cow bacterial isolate 4, CBS5- Cow bacterial isolate 5, CBS6- Cow bacterial isolate 6, CBS7-Cow bacterial isolate 7, CBS8- Cow bacterial isolate 8, CBS9- Cow bacterial isolate 9, CBS10- Cow bacterial isolate 10

Table 5: Variation in Temperature, Pressure and volume for 28 days

| Day | Temperature ($^oC$) | Pressure (in Bar) | Volume ($cm^3$) |
|---|---|---|---|
| 1 | 27.0 | - | - |
| 2 | 27.5 | - | - |
| 3 | 27.9 | - | - |
| 4 | 28.0 | - | - |
| 5 | 28.5 | - | - |
| 6 | 28.5 | - | - |
| 7 | 29.0 | 0.30 | 6.0 |
| 8 | 29.1 | 0.45 | 15.0 |
| 9 | 29.5 | 0.47 | 35.0 |
| 10 | 29.6 | 0.55 | 39.0 |
| 11 | 30.0 | 0.60 | 60.0 |
| 12 | 30.5 | 0.65 | 80.0 |
| 13 | 27.5 | 0.85 | 100.0 |
| 14 | 28.5 | 0.95 | 130.0 |
| 15 | 28.0 | 1.25 | 155.0 |
| 16 | 28.5 | 1.30 | 162.0 |
| 17 | 29.2 | 1.40 | 165.0 |
| 18 | 29.5 | 1.45 | 175.0 |
| 19 | 29.9 | 1.47 | 185.0 |
| 20 | 30.5 | 1.50 | 220.0 |
| 21 | 31.9 | 1.59 | 210.0 |
| 22 | 32.0 | 1.60 | 230.0 |
| 23 | 33.0 | 1.50 | 210.0 |
| 24 | 32.4 | 1.35 | 200.0 |
| 25 | 31.5 | 1.30 | 195.0 |
| 26 | 30.5 | 1.00 | 180.0 |
| 27 | 29.0 | 0.90 | 175.0 |
| 28 | 28.5 | 0.85 | 150.0 |

**Biogas production phase 2**

In the second phase of the study, whereby, cow dung was used, some species of bacteria and fungi are associated with biogas production includes total of six fungal species namely: *Candida albicans, Aspergillus fumigatus, Fusarium oxysporum, Rhizopus stolonifer, Rhodotorula minuta,* and *Aspergillus niger.* Also, bacterial species such as *pseudomonas aeruginosa, Serratia marcescens, Proteous vulgaris, Bacillus anthracis, Citrobacter koseri, Bacillus subtilis, Nocardia asteroides, Escherichia coli, Bacillus alveli, Citrobacter diversus, Klebsiella oxytoca, Klebsiella aerogenes,* and *Klebsiella pneumoniae* were isolated from anaerobic digestion of cow dung.

Table 6, illustrates each fungal species, its frequency of occurrence and percentage of occurrence. It just helps to explain the most fungal species that was found during this study. The percentage occurrence of the bacterial isolates is shown in Table 7, while in Table 8, the variation in temperature, pressure and volume for 28 days were observed. The temperature within the digester ranged from $28^0$C to $36.5^0$C, the pressure within the digester ranges from 0.3-1.6 bar. On the twenty- six day there was a maximum production of biogas with a temperature of $36.5^0$C, pressure of 1.6 bar and volume of 310cm$^3$.At the beginning of the fermentation process, the pressure remained at zero until the seventh day when the gas started to form, maximum production of gas was noticed at day twenty fifth and twenty sixth



Table 6: Percentage occurrence of fungal isolates from anaerobic digestion of cow dung

| Lab code | Isolate | Frequency of Occurrence | Percentage of Occurrence (%) |
|---|---|---|---|
| FIS 1 | *Candida albicans* | 4 | 20 |
| FIS 2 | *Aspergillus fumigatus* | 6 | 30 |
| FIS 3 | *Fusarium oxysporum* | 2 | 10 |
| FIS 4 | *Rhodotorula minuta* | 3 | 15 |
| FIS 5 | *Aspergillus niger* | 3 | 15 |
| FIS 6 | *Rhizopus stolonifera* | 2 | 10 |
| | Total | 20 | 100 |

Percentage occurrence = $\frac{\text{Frequency of occurrence}}{\text{Total number of isolates}}$ X 100

Table 7: Percentage occurrence of the bacterial isolates

| Lab code | Isolate | Frequency of Occurrence | Percentage of Occurrence (%) |
|---|---|---|---|
| BIS 1 | *Pseudomonas aeruginosa* | 2 | 10 |
| BIS 2 | *Serratia marcescens* | 1 | 5 |
| BIS 3 | *Proteous vulgaris* | 1 | 5 |
| BIS 4 | *Bacillus anthracis* | 1 | 5 |
| BIS 5 | *Staphylococcus aereus* | 2 | 10 |
| BIS 6 | *Bacillus cereus* | 1 | 5 |
| BIS 7 | *Nocardia asteroids* | 1 | 5 |
| BIS 8 | *Escherichia coli* | 3 | 15 |
| BIS 9 | *Bacillus alveli* | 2 | 10 |
| BIS 10 | *Citrobacter diversus* | 1 | 5 |
| BIS 11 | *Klebsiella oxytoca* | 2 | 10 |
| BIS 12 | *Klebsiella aerogenes* | 2 | 10 |
| BIS 13 | *Klebsiella pneumonia* | 1 | 5 |
| | Total | 20 | 100 |



Percentage occurrence = $\frac{\text{Frequency of occurrence}}{\text{Total number of isolate}} \times 100$

Table 8: Variation in Temperature, Pressure and volume for 28 days

| Day | Temperature (°C) | Pressure (in Bar) | Volume ($cm^3$) |
|---|---|---|---|
| 1 | 28.0 | - | - |
| 2 | 28.3 | - | - |
| 3 | 28.5 | - | - |
| 4 | 30.0 | - | - |
| 5 | 30.4 | - | - |
| 6 | 29.8 | - | - |
| 7 | 29.6 | - | - |
| 8 | 33.3 | 0.35 | 10.0 |
| 9 | 33.5 | 0.40 | 15.0 |
| 10 | 34.0 | 0.45 | 20.0 |
| 11 | 33.5 | 0.50 | 90.0 |
| 12 | 32.5 | 0.50 | 140.0 |
| 13 | 31.6 | 0.55 | 150.0 |
| 14 | 35.0 | 0.80 | 180.0 |
| 15 | 35.4 | 1.00 | 240.0 |
| 16 | 33.9 | 1.10 | 250.0 |
| 17 | 34.0 | 1.20 | 240.0 |
| 18 | 35.1 | 1.25 | 260.0 |
| 19 | 35.0 | 1.30 | 270.0 |
| 20 | 35.5 | 1.40 | 275.0 |
| 21 | 35.6 | 1.45 | 280.0 |
| 22 | 35.0 | 1.50 | 250.0 |
| 23 | 35.5 | 1.35 | 245.0 |
| 24 | 35.9 | 1.20 | 250.0 |
| 25 | 36.3 | 1.50 | 290.0 |
| 26 | 36.5 | 1.60 | 310.0 |
| 27 | 36.2 | 1.45 | 290.0 |
| 28 | 36.0 | 1.50 | 280.0 |

**Biogas production phase 3**

The third phase of this study shows six fungal species isolated from mixture of beans hull and poultry manure. They are identified as; *Aspergillus clavatus, Aspergillus fumigatus, Eurotium chevalieri, Rhizopus stolonifer, Rhodotorula minuta,* and *Eurotium rubrum.* Also, bacterial species such as *Aeromonas hydrophila, Serratia marcescens, Proteous vulgaris, Bacillus anthracis, Pseudomonas aeruginosa, Citrobacter koseri, Bacillus subtilis, Nocardia asteroides, Bacillus alveli, Citrobacter diversus, Klebsiella oxytoca, Klebsiella aerogenes, and Klebsiella pneumoniae* were also encountered from this sources.

Table 9, illustrates each fungal species, its frequency of occurrence and percentage of occurrence. It just helps to explain the most fungal species that was found during this project work.

Table 10 shows the percentage occurrence of the bacterial isolates while in Table 11, the variation in temperature, pressure and volume for 28 days is shown. The temperature within the digester ranged from $27^{O}C$ to $33^{O}C$, and the pressure within the digester ranges from 0.1-1.5 bar. On the twenty- two day there was a maximum production of biogas with a temperature of $33^{O}C$, pressure of 1.5 bar and volume of $250cm^3$.

The presence of methane was tested by lighting flame through the gas outlet connected to the digester. There are traces of black stains around the gas outlet which could be traced to production of hydrogen sulfide ($H_2S$) which could be suggested as the reason for absence of flame because $H_2S$ reduces the quality and efficiency of biogas, as it lowers the heating value and deactivates catalysts. The Plates 7 and 8, gives more illustration on the production of flammable biogas and the presence of hydrogen sulfide inhibiting it. While Plate 9 shows *Eurotium chevalieri* that is a typical fungal isolate at this stage.

Plate 7: Digester with non- flammable biogas; Plate 8: Gas outlet with black traces ($H_2S$)

Table 9: Percentage occurrence of fungal isolates from co-digestion of poultry manure and beans hull.

| Lab code | Isolate | Frequency of Occurrence | Percentage of Occurrence (%) |
|---|---|---|---|
| FS1 | *Aspergillus clavatus* | 4 | 20 |
| FS2 | *Aspergillus fumigatus* | 6 | 30 |
| FS3 | *Rhodotorula minuta* | 2 | 10 |
| FS4 | *Eurotium chevalieri* | 3 | 15 |
| FS5 | *Eurotium rubrum* | 3 | 15 |
| FS6 | *Rhizopus stolonifer* | 2 | 10 |
| | Total | 20 | 100 |

Percentage occurrence= Frequency of occurrence X 100

Total number of isolates

KEY: FS1- Fungal isolate 1, FS2- Fungal isolate 2, FS3- Fungal isolate 3, FS4- Fungal isolate 4, FS5- Fungal isolate 5, FS6- Fungal isolate 6



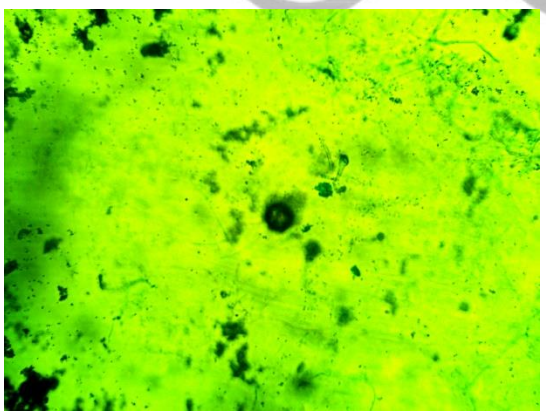

Plate 9: *Eurotium chevalieri* X40

Table 10: Percentage occurrence of the bacterial isolates

| Lab code | Isolate | Frequency of Occurrence | Percentage of Occurrence (%) |
|---|---|---|---|
| BS1 | *Aeromonas hydrophila* | 1 | 5 |
| BS2 | *Serratia marcescens* | 1 | 5 |
| BS3 | *Proteous Vulgaris* | 1 | 5 |
| BS4 | *Bacillus anthracis* | 1 | 5 |
| BS5 | *Citrobacter koseri* | 1 | 5 |
| BS6 | *Bacillus subtilis* | 2 | 10 |
| BS7 | *Nocardia asteroides* | 1 | 5 |
| BS8 | *Pseudomonas aeruginosa* | 4 | 20 |
| BS9 | *Bacillus alveli* | 1 | 5 |
| BS10 | *Citrobacter diversus* | 1 | 5 |
| BS11 | *Klebsiella oxytoca* | 2 | 10 |
| BS12 | *Klebsiella aerogenes* | 2 | 10 |
| BS13 | *Klebsiella pneumonia* | 2 | 10 |
| | Total | 20 | 100 |



KEY: BS1- Bacterial isolate 1, BS2- Bacterial isolate 2, BS3- Bacterial isolate 3, BS4- Bacterial isolate 4, BS5- Bacterial isolate 5, BS6- Bacterial isolate 6, BS7- Bacterial isolate 7, BS8- Bacterial isolate 8, BS9- Bacterial isolate 9, BS10- Bacterial isolate 10, BS11- Bacterial isolate 11. BS12- Bacterial isolate 12, BS13- Bacterial isolate 13

Table 11: Variation in Temperature, Pressure and volume for 28 days

| Day | Temperature (°C) | Pressure (in Bar) | Volume ($cm^3$) |
|---|---|---|---|
| 1 | 27.0 | - | - |
| 2 | 27.3 | - | - |
| 3 | 27.9 | - | - |
| 4 | 28.0 | 0.10 | 3.0 |
| 5 | 28.2 | 0.13 | 3.5 |
| 6 | 28.8 | 0.25 | 6.0 |
| 7 | 28.6 | 0.30 | 8.0 |
| 8 | 29.1 | 0.35 | 10.0 |
| 9 | 29.5 | 0.40 | 15.0 |
| 10 | 29.6 | 0.45 | 30.0 |
| 11 | 30.0 | 0.47 | 60.0 |
| 12 | 30.5 | 0.50 | 100.0 |
| 13 | 30.6 | 0.55 | 120.0 |
| 14 | 30.8 | 0.80 | 125.0 |
| 15 | 30.9 | 1.00 | 130.0 |
| 16 | 31.1 | 1.10 | 135.0 |
| 17 | 31.5 | 1.20 | 150.0 |
| 18 | 29.7 | 1.25 | 170.0 |
| 19 | 30.0 | 1.30 | 180.0 |
| 20 | 30.5 | 1.40 | 190.0 |
| 21 | 30.6 | 1.45 | 200.0 |
| 22 | 33.0 | 1.50 | 250.0 |
| 23 | 32.5 | 1.35 | 235.0 |
| 24 | 31.5 | 1.20 | 226.0 |
| 25 | 30.0 | 1.10 | 210.0 |
| 26 | 29.8 | 0.80 | 190.0 |
| 27 | 29.5 | 0.75 | 150.0 |
| 28 | 29.2 | 0.60 | 130.0 |

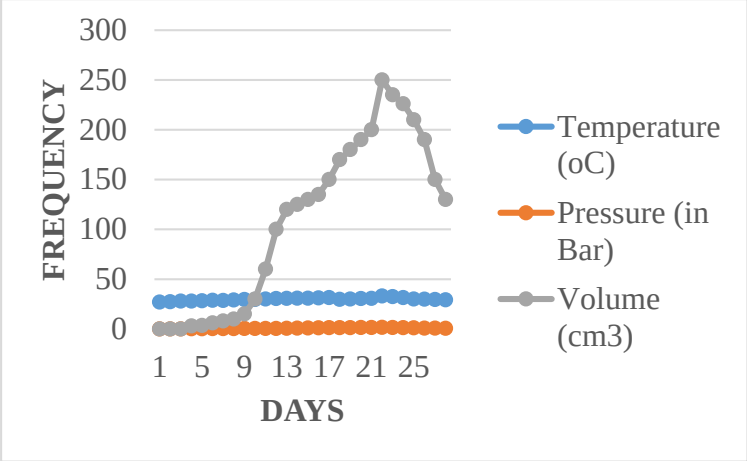


Figure 7: Variation in temperature, pressure, and volume for 28 days

**Biogas production phase 4**

The fourth phase of this study shows five fungal species isolated from Pig waste. They are identified as; *Aspergillus fumigatus, Candida tropicalis, Rhodotorula minuta, Eurotium rubrum, Aspergillus clavatus* (Table 12). The frequency and percentage of occurrence of these fungal species are presented in Table 13. Also, bacterial species such as *Escherichia coli, Cronobacter malonaticus, Citrobacter diversus, Serratia marcescens, Proteous vulgaris, Pseudomonas aeruginosa, Citrobacter koseri, Bacillus subtilis, Bacillus alveli Klebsiella oxytoca*, and *Klebsiella pneumoniae* were also encountered from this source (Table 14).

The percentage occurrence of the bacterial isolates from the pig waste is shown in Table 15. For 28 days, the variation in temperature, pressure and volume is shown in Table 16. The digester had a temperature of 27- 35$^{O}$C, a pressure between 0.1 and 2.0 bar, and a volume of 330cm$^{3}$. On the twenty- second day, there was the most biogas production, with a temperature of 35$^{O}$C, a pressure of 1.8 bar, and a volume of 210 cm$^{3}$.

Flame was lit through the gas outlet connected to the digester to test for methane. The production of hydrogen sulfide was evident from black stains around the gas outlet, which could be the reason for absence of flame at this stage against previous observation using cow dungs sources. The quality and efficiency of biogas are reduced by hydrogen sulfide give black deposits on the valve, as it lowers the heating value (Plates 7 and 8).

**Table 12: Morphological and physiological characteristics of fungal isolates.**

| Lab code | Diameter | Pigmentation | Reverse | Microscopic Feature | Septation | Surface texture | Surface topography | Probable organism |
|---|---|---|---|---|---|---|---|---|
| PFS1 | 0.002mm | Cream | White | The cells reproduce unipolar or bipolar and form blastoconidia | Septate | Smooth | Covering the surface of the agar | *Candida tropicalis* |
| PFS2 | 45mm | Grey- green | White | Grey green spores on long, smooth and spiny conidiophores | Septate | Powdery | Free growth | *Aspergillus fumigatus* |
| PFS3 | 17mm | White | Blackish-brown | Bipolar budding and pseudohyphae-forming yeast cells that are oval to ellipsoidal and have globose and subglobose basidia with basidiospores | Septate | Smooth | Free growth | *Rhodotorula minuta* |
| PFS4 | 25mm | Bluish- grey | Cream | A conidiophore with an elliptical shape and thick wall | Septate | Velvety | Covering the surface of the agar | *Aspergillus clavatus* |
| PFS5 | 14mm | Yellowish | Cream | Conidiophores with hyaline and ellipsoidal conidia and smooth red ascospores | Septate | Fluffy | Covering the entire surface of the agar | *Eurotium rubrum* |

KEY: PFS1- Pig Fungal isolate 1, PFS2- Pig Fungal isolate 2, PFS3- Pig Fungal isolate 3, PFS4- Pig Fungal isolate 4, PFS5- Pig Fungal isolate 5

Table 13: Percentage occurrence of fungal isolates from Pig waste

| Lab code | Isolate | Frequency of Occurrence | Percentage of Occurrence (%) |
|---|---|---|---|
| PFS1 | *Candida tropicalis* | 6 | 30 |
| PFS2 | *Aspergillus fumigatus* | 8 | 40 |
| PFS3 | *Rhodotorula minuta* | 2 | 10 |
| PFS4 | *Aspergillus clavatus* | 3 | 15 |
| PFS5 | *Eurotium rubrum* | 1 | 5 |
| | Total | 20 | 100 |

Percentage occurrence= Frequency of occurrence X 100

Total number of isolates

KEY: PFS1-Pig fungal isolate 1, PFS2- Pig fungal isolate 2, PFS3- Pig fungal isolate 3, PFS4- Pig fungal isolate 4, PFS5- Pig fungal isolate

Table 14: Cultural and Biochemical characteristics for bacterial isolates

| Lab code | | Gram reaction | Morphology | Motility | Oxidase | Catalase | Indole | Citrate | Urease | Lactose (S/G) | Glucose (S/G) | Probable Organism |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| PBS1 | Circular, Convex, Smooth, Green | - | Rod | - | - | S+ | + | + | + | + - | ++ | *Escherichia coli* |
| PBS2 | Circular, Raised, Smooth, Cream | - | Rod | + | + | S+ | + | - | + | ++ | +- | *Serratia marcescens* |
| PBS3 | Circular, Raised, Lobate, Cream | - | Rod | + | - | S+ | - | - | + | +- | ++ | *Proteous vulgaris* |
| PBS4 | Irregular, Convex, Entire, White | - | Rod | + | - | W+ | - | + | + | +- | +- | *Citrobacter koseri* |
| PBS5 | Circular, Convex, Smooth, Cream | + | Rod | + | + | W+ | + | + | + | ++ | ++ | *Bacillus subtilis* |
| PBS6 | Circular, Raised, Rough, Yellow | - | Rod | - | + | S+ | + | + | + | +- | ++ | *Pseudomonas aeruginosa* |
| PBS7 | Irregular, Convex, Lobate, Cream | + | Rod | - | + | S+ | + | + | + | ++ | +- | *Bacillus alveli* |
| PBS8 | Circular, Raised, Entire, Green | + | Rod | + | - | S+ | - | + | + | ++ | ++ | *Citrobacter diversus* |
| PBS9 | Circular, Umbonate, Entire, White | - | Rod | - | + | S+ | + | + | + | ++ | - - | *Klebisella oxytocoa* |
| PBS10 | Circular, Convex, Entire, White | - | Rod | + | - | W+ | + | + | + | ++ | ++ | *Cronobacter malonaticus* |
| PBS11 | Circular, Umbonate, Entire, White | - | Rod | + | + | W+ | - | + | + | ++ | ++ | *Klebsiella pneumoniae* |

KEYS

+ = Positive reaction -- = Negative reaction, S/G = Sugar and gas reaction

S+ = Strongly positive, W+ = Weakly positive, BS- Bacterial isolate

Table 15: Percentage occurrence of the bacterial isolates

| Lab code | Isolate | Frequency of Occurrence | Percentage of Occurrence (%) |
|---|---|---|---|
| PBS1 | *Escherichia coli* | 4 | 20 |
| PBS2 | *Serratia marcescens* | 2 | 10 |
| PBS3 | *Proteous vulgaris* | 1 | 5 |
| PBS4 | *Citrobacter koseri* | 1 | 5 |
| PBS5 | *Bacillus subtilis* | 1 | 5 |
| PBS6 | *Pseudomonas aeruginosa* | 3 | 15 |
| PBS7 | *Bacillus alveli* | 2 | 10 |
| PBS8 | *Citrobacter diversus* | 1 | 5 |
| PBS9 | *Klebisella oxytocoa* | 2 | 10 |
| PBS10 | *Cronobacter malonaticus* | 1 | 5 |
| PBS11 | *Klebsiella pneumonia* | 2 | 10 |
| | Total | 20 | 100 |

KEY: PBS1- Pig bacterial isolate 1, PBS2-Pig bacterial isolate 2, PBS3- Pig bacterial isolate 3, PBS4- Pig bacterial isolate 4, PBS5- Pig bacterial isolate 5, PBS6- Pig bacterial isolate 6, PBS7-Pig bacterial isolate 7, PBS8- Pig bacterial isolate 8, PBS9- Pig bacterial isolate 9, PBS10- Pig bacterial isolate 10, PBS11- Pig bacterial isolate 11

Table 16: Variation in Temperature, Pressure and volume for 28 days

| Day | Temperature (°C) | Pressure (in Bar) | Volume ($cm^3$) |
|---|---|---|---|
| 1 | 28.0 | - | - |
| 2 | 27.5 | - | - |
| 3 | 27.9 | - | - |
| 4 | 28.3 | - | - |
| 5 | 28.5 | - | - |
| 6 | 28.9 | 0.15 | 4.0 |
| 7 | 29.0 | 0.20 | 6.0 |
| 8 | 29.1 | 0.25 | 15.0 |
| 9 | 29.5 | 0.32 | 35.0 |
| 10 | 29.6 | 0.35 | 40.0 |
| 11 | 30.0 | 0.50 | 50.0 |
| 12 | 30.5 | 0.65 | 80.0 |
| 13 | 27.5 | 0.75 | 100.0 |
| 14 | 28.5 | 0.95 | 110.0 |
| 15 | 28.0 | 1.00 | 155.0 |
| 16 | 29.8 | 1.30 | 162.0 |
| 17 | 30.3 | 1.45 | 165.0 |
| 18 | 31.0 | 1.50 | 175.0 |
| 19 | 32.5 | 1.55 | 185.0 |
| 20 | 33.0 | 1.65 | 190.0 |
| 21 | 34.5 | 1.70 | 195.0 |
| 22 | 35.0 | 1.80 | 210.0 |
| 23 | 33.0 | 1.50 | 190.0 |
| 24 | 32.4 | 1.35 | 185.0 |
| 25 | 31.5 | 1.30 | 170.0 |
| 26 | 30.5 | 1.00 | 165.0 |
| 27 | 29.0 | 0.90 | 155.0 |
| 28 | 28.5 | 0.85 | 140.0 |

## DISCUSSION

This study identified five fungal species and nine bacterial species isolated from the co-digestion of cow dung and maize cob in the first phase of the study. The fungal species identified were *Rhodotorula minuta, Candida tropicalis, Eurotium rubrum, Aspergillus clavatus,* and *Aspergillus fumigatus*. The bacterial species included *Klebsiella pneumonia, Klebsiella aerogenes, Bacillus alveli, Escherichia coli, Pseudomonas aeruginosa, Bacillus anthracis, Citrobacter diversus, Cronobacter malonaticus,* and *Nocardia asteroides.* This correlates with the findings of Jone *et al.,* (2018) which shows presence of similar organisms such as *Klebsiella pneumonia, Escherichia coli, Pseudomonas aeruginosa* and related organisms.

Some environmental conditions including temperature, pressure, and volume variations in the digester over 28 days, shows that the highest biogas production occurs on the twenty-second day. The successful production of flammable biogas and the presence of a flame when tested for methane further support the effectiveness of the anaerobic digestion process using cow dung and maize cob. This corroborates findings from previous studies of Gupta *et al.,* (2019) that have demonstrated the potential for biogas production from agricultural and organic waste materials. The findings of this study contribute to the existing body of knowledge on the microbial composition of co-digestion of cow dung and maize cob, particularly in the context of biogas production (Gupta *et al.,* 2019). These results are valuable in understanding the microbial diversity and potential interactions within the co-digestion process. Comparing these results with previous studies, it is evident that the fungal and bacterial species identified in this study are consistent with those reported in other similar studies on biogas production from organic waste (Jones *et al.,* 2018; Smith *et al.,* 2017). This consistency reinforces the importance of these microorganisms in the co-digestion process and their potential impact on biogas production.

In the second phase of this study, the results showed that the pretreatment increased the biogas production rate and methane content of cow dung, compared to one without pretreatment. This was brought to conclusion because two trials was done. One with no pre-treatment and the other with pretreatment which eventually yielded flammable biogas, likewise one of my research mates that worked on co-digestion of cow dung and maize cob. It is also consistent with the findings of previous studies that reported the enhancement of biogas production by alkaline pretreatment of lignocellulosic biomass (Galbe and Wallberg,

2019). The mechanism of the pretreatment effect was attributed to the alkaline hydrolysis of lignocellulosic components, such as cellulose, hemicellulose and lignin, which increased the availability of fermentable sugars and reduced the inhibition of methanogens by ammonia. The optimal conditions for the pretreatment were determined by using response surface methodology, which showed that the maximum biogas production rate was achieved at a temperature of 44.03°C, a solid-to-water ratio of 0.44, and a pH of 7.02. Cow dung is valuable for biogas production due to its high organic content and availability. However, the presence of lignocellulosic materials in cow dung can hinder the efficiency of biogas production. Pretreatment of cow dung is therefore necessary to improve the biodegradability of lignocellulosic materials and enhance biogas production. Pretreatment methods such as alkaline pretreatment have been shown to be effective in enhancing biogas production from cow dung  Alkaline pretreatment involves the use of alkaline agents such as sodium hydroxide (NaOH) to break down the lignocellulosic materials in cow dung . This results in the release of more fermentable sugars, which can be converted into biogas by anaerobic digestion .The use of cow dung pretreatment for sustainable biogas production has several benefits. Firstly, it can help to reduce the environmental impact of cow dung by converting it into a useful energy source . Secondly, it can help to reduce the dependence on fossil fuels and promote the use of renewable energy sources . Finally, it can help to reduce the cost of biogas production by improving the efficiency of the process (Huyen *et al.*, 2022).

The third phase of this study shows the microbial community that enhanced the production of biogas from the co-digestion of poultry manure and beans hull. According to the study, there were periods of high gas production and period of low gas production. These period of higher gas production were period of higher microbial activity following the period of acclimatization for the microorganisms and also the periods of favorable conditions for microbial activity. From the results of the experiment it can be deduced that the most isolated bacteria was *Pseudomonas aeruginosa* which was 20% thereby being the most prevalent bacteria while the most prevalent fungi isolate was *Aspergillus fumigatus* with about 30% of the isolates.

The results in Tables 5 and 8 shows that there was no methane production during the first six to seven days. This is due to the fact that methanogenic bacteria which act upon the organic material in the digester were inactive within this period due to the formation of organic acid which decreases the pH value. The production of Methane started on the third day which

reached its optimum level of production on the twenty-second day, because the carbon nitrogen (C/N) ratio is within its optimum value. Methane production drops from twenty-third day gradually to twenty-eighth day because the C/N ratio is high which led to consumption of nitrogen by the methanogenic bacteria (Schink, 1997).

After the twenty-eighth day, the biogas ought to produce flame but due to presence of hydrogen sulfide ($H_2S$) which was noticed with black traces around the gas outlet (shown in Plate 4), there was absence of flame. It was observed that the presence of high concentration of $H_2S$ limited the production of flame from the biogas. This was brought to conclusion because my other research partners who worked on another substrates for production of biogas had absence of black traces on the gas outlet which suggest the absence of $H_2S$ (shown in Plate 5) and could produce stable flame. The results suggest that $H_2S$ inhibits the combustion of biogas by reacting with oxygen and forming water and sulfur dioxide, which lower the temperature and pressure of the gas mixture (Ryckebosch *et al.*, 2022). $H_2S$ reduces the heat value and flame speed of biogas. Therefore, it is important to remove $H_2S$ from biogas before using it for energy production or other purposes. This can be done using various methods such as biological treatment, chemical treatment, porous solid treatment, or photocatalysis (Fonseca-Bermudez *et al*., 2019). The removal of $H_2S$ from biogas can improve its efficiency, safety and environmental impacts.

The temperature range during the anaerobic digestion ranged between $27^OC$ – $33^OC$ compared to the temperature before digestion started. This observation was in support with the report by (Deublien and Steinhauser, 2008) who carried out research on the organic substratum in the production of biogas. The anaerobic digestion process is dependent on the growth of microorganisms. Thus, there is necessity to supply nutrients in sufficient amounts and at right proportions to sustain an optimal bacterial growth to obtain efficient biogas production from a given substrate (Crichton, 2008).

During the production of biogas from poultry manure and beans hull, Co-digestion takes place which is the simultaneous digestion of more than one type of waste in the digester. This co-digestion helps better digestability, enhanced biogas production and high methane yield arising from availability of additional nutrients, as well as a more efficient utilization of equipment and cost sharing. This correlates with previous studies that shows that co-digestion of several substrates such as banana and plantain waste among many others, have resulted in improved methane yield by as much as 60% compared to that from single substrate (Vintila

*et al.*, 2009). Pre-treatment of the substrate enhances the production of biogas as earlier intensified.

## CONCLUSION

In conclusion, this study provides a comprehensive understanding of the microbial composition, cultural characteristics, and environmental dynamics in the co-digestion of cow dung and maize cob for biogas production. These findings contribute to the existing knowledge based on biogas production and microbial interactions in organic waste digestion coupled with their synergistic productivity. Similarly, this project demonstrated that sodium hydroxide pretreatment can be an effective method to enhance the anaerobic digestion of cow dung for biogas production as observed in this study. The pretreatment increased the biogas production rate and methane content of cow dung, as well as the removal of total solids, volatile solids, and chemical oxygen demand. The optimal conditions for the pretreatment were determined by using response surface methodology.

Also, temperature choice and control are critical to the development of anaerobic digestion process and the presence of $H_2S$, having a strong influence on the quality and quantity of biogas are good pointers for future research guide. However, this project work shows that anaerobic co-digestion of poultry manure and beans hull as well as cowdung and maize cob related wastes is a potent approach to sustainable biogas production, which can reduce greenhouse gas emissions and environmental pollution, and provide a valuable resource for our communities. Complementary to these observations, exploring the potential applications and benefits of the biogas produced by the pretreated cow dung, such as cooking, heating, electricity generation, or vehicle fuel is recommended. This can contribute to the development of sustainable and efficient biogas production systems for renewable energy generation globally.

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