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ORIGINAL RESEARCH ARTICLE

Anaerobic Digestion of Maize Husk in Co-Digestion with Goat and Cow Dung for Enhanced Biogas Production

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ABSTRACT
In recent investigations, Anaerobic co-digestion has been superior to traditional anaerobic digestion (AD). The advantages of employing co-substrates for improved bioenergy generation and solids reduction have drawn researchers to investigate the co-digestion technology and understand the impact of multiple substrates on digester performance. This study aimed to generate biogas by co-digestion of maize husk with cow and goat dung as substrates, isolate the bacteria involved in the process, and assess the quantity and makeup of the biogas generated by the substrates. The substrates were fed to mini-digesters fabricated in the laboratory using 1L bottles for 49 days' retention time. It assessed the production potential of the substrates for biogas yield in mono-digestion and co-digestion. The average biogas yield (cm^3) and methane content (%) in the D1, D2, D3, D4, D5, and D6 were 9135 (58%), 8660 (71%), 9820 (69%), 6545 (65%), 5915 (48%) and 1965 (21%) respectively. The highest gas yield was observed in digesters with co-digestion of the substrates (D1 and D2) than the mono-digestion of the GD and CD by 35.2% and 24.4%, respectively, with an improvement in methane content. The process was carried out in a mesophilic condition and a pH range of 6.8-8.2. The study's findings showed that the most frequently isolated and identified bacteria were *Klebsiella pneumoniae* and *Bacillus species*, indicating that these species are essential to the microbial activities involved in biogas production. The investigation additionally showed that maize husk in co-digestion with cow dung and goat dung had great potential for generating and producing large quantities of biogas within 49 days' retention time.

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KEYWORDS
Biogas production, Anaerobic Co-digestion, Maize -husk, Cow dung and Goat dung, Retention time, and Methane content.

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INTRODUCTION
One of the most talked-about subjects of the twenty-first century is environmental degradation and its effects on people (Asiagwu *et al.,* 2012). Like the rest of the world, Nigeria's economy mostly relies on non-renewable fossil fuels like natural gas and petroleum. The reserves are being rapidly depleted as a result of this. Furthermore, using, refining, and burning these fossil fuels pose a serious risk to the environment, which is already fragile (Owamah and Izinyon, 2015). Utilizing organic resources (either as energy crops or waste streams) to create biogas, biomass is a renewable energy source. Compared to landfill gas emissions into the environment, biogas is a readily available energy source that greatly reduces greenhouse gas emissions (Nabuuna and Okure, 2005). Following the global energy crisis of the 1970s, it was recognized as one of the finest fossil fuel substitutes because it is a renewable natural gas supply. Anaerobic digestion (AD) of waste organic substances (biomass) to biogas seems to be the most widely used renewable energy source because it is one of the few biotechnological processes that can produce biofuel, lower pollution levels in the environment, and increase agricultural productivity by using the digestate as compost for organic farming (Owamah and Izinyon 2015).

However, the most effective combination of substrates and process variables must be used in the most economical way to ensure the sustainability and viability of industrial anaerobic digestion plants. Several writers have employed co-digestion, the anaerobic digestion of two or more biodegradable substrates in a digester, to maximize the substrates' capacity to produce biogas (Haider *et al.,* 2015). Because the co-digestion of various materials improves the carbon-to-nitrogen (C/N) balance and works well to support microbial growth and biogas production, it has been claimed to improve the anaerobic

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digestion process El-Mashad and Zhang, 2010). The area around the biogas plant should have abundant access to these co-substrates. There is energy scarcity all over the world and fluctuation in the prices of energy. Fortunately, Nigeria is an agricultural country that can use Abattoir waste and agricultural residues in biogas Generation. There is a need to generate energy from other sources, especially agricultural residues, which are generated in large quantities from farming activities. The large quantities of agricultural residues produced in Nigeria can significantly meet her energy demand. Maize and cassava are some of Nigeria's most important agricultural products, especially in the Northern and western parts of the country. Residues in husks and peels are generated from processing these crops. Initial digestion studies on cassava peels showed that the peels are poor producers of biogas probably due to their content of toxic cyanogenic glycosides (Okafor, 1998). Therefore, this work is one of the techniques involved in the Generation of biogas from agricultural and animal wastes. This study evaluated biogas production using co-digested maize husk and animal wastes, specifically goat and cow dung. The research was aimed at achieving the following specific objectives:

- Determine the physicochemical properties of the substrates (cow dung, goat manure, and maize husk).
- Determine the proximate composition of the substrate (maize husk).
- Determine the relative volume and composition of the biogas produced.
- Isolate and identify the most dominant bacteria within the digester using biochemical and molecular analysis.

MATERIAL AND METHODS

Sample Collection and Processing

The substrates used in this investigation are maize husk as agricultural waste, cow dung, and goat manure all of which were animal waste materials. The Maize husk was collected from nearby maize sellers along Kabuga and Janbulo streets, while the animal wastes were collected in a clean container at the fresh state from Hauren Shanu, along BUK road, Kano state, and stored anaerobically before use.

Pretreatment of Maize husk

The maize husk was sundried for three days to remove the moisture, crush and homogenize them, decrease their size, and increase the wastes' surface area to hasten degradation (El-Mashad and Zhang (2010). Before being used, the substrate was kept at room temperature in a closed container.

Proximate Composition of the Substrates

Proximate compositional analyses of the powdered samples were carried out to determine the lignocellulose compositions (lignin, hemicellulose, cellulose) and nutritional composition moisture, crude ash, crude fibre crude fat and crude protein content, following the methods described by the Association of Official Analytical Chemists (AOAC, 2010).

Fabrication of mini digesters

The digestion process was done using fabricated laboratory mini digesters with two setups. The first setup measured the biogas yield in terms of volume. Portable digesters were fabricated using 1L empty plastic gallons, a rubber strip, and a polyvinyl chloride (PVC) tube of 150cm in length and 0.8cm internal diameter. A hole was bored into the cover, and the tube was inserted into the hole bore on the plastic cover and tightened using a strip of rubber with one end of the PVC free (i.e., unattached), which conveyed the gas from the digester to 1000cm^3 capacity measuring cylinder which is filled with water and inverted into a bowl containing water for gas collection using water displacement method illustrated in (Figure 1). This was used as a digester under mesophilic conditions.

Figure 1 Fabricated Mini Laboratory Anaerobic Digester

The content of the digesters was mixed manually at a frequent interval, and the quantity of biogas produced was measured every 24 hours. A Set of digesters was run in duplicate. For each treatment, 250g of co-digested substrate and 500 ml water (1:2) was used for the biogas production under mesophilic conditions.

The second setup used a 1L plastic bottle and a 2L urine bag. The bottle cover was drilled and connected tightly to the tube of the urine bag, which served as the gas storage vessel (Figure 2). The urine bag collects and stores the

gas, which was later evaluated using a biogas analyzer to determine the methane content of the gas.

Figure 2 Mini Laboratory Digester for Biogas Collection Using a 2L Urine Bag

Preparation of Slurry for biogas production

For loading the substrate into the digester, different slurries were prepared for the biogas production. Each digester consists of a 250g dry mass of the feedstock homogenized in an equal volume of water (500ml) to obtain a working volume of 750ml through a retention time of seven weeks. The water was added to dilute the organic substance and enhance the growth of microorganisms [11].

D1 = 150g Goat dung + 100g Maize husk + 500ml water

D2 = 150g Cow dung + 100g Maize husk + 500ml water

D3 = 75g cow dung + 75g Goat dung + 100g Maize husk 500 ml water

D4 = 250g Cow dung + 500 ml water

D5 = 250g Goat dung + 500 ml water

D6 = 250g maize husk + 500ml.

Determination of pH

The pH was measured before and after by inserting the pH meter into the digestion bottle, and the reading was recorded.

Isolation and Identification of Anaerobic Bacteria

For the isolation, about 10ml of the slurry was poured into a conical flask containing 90ml of distilled water and then shaken to homogenize. This was further serially diluted until a dilution factor of 10 was obtained. One 1ml solution was cultured on a Nutrient agar using the spread plate method and incubated in an anaerobic jar at 35°C for 48 hours. Following incubation, distinct colonies obtained were further isolated as pure cultures and identified using culture. Gram's reaction, morphological and genotypic identification method.

Gram's staining

A thin smear of the 24h pure culture was prepared on a clean grease free glass slide, dried and fixed by passing over a gentle flame to identify the gram reaction and morphology of the organisms. The fixed smears were covered with 3 drops of crystal violet stain for 60 seconds and then washed with water. The smears were flooded with Lugol's iodine for 30 seconds and rinsed with water. The smears were then decolorized with 70% alcohol for 15 seconds and rinsed with water. Following decolorization, the smears were counter-stained with safranin for 60 seconds and then rinsed with water. Excess water was drained, and the back of the slide was wiped and allowed to air dry. After drying, the slides were examined under a microscope under an objective lens of ×100 (oil immersion). Gram-positive bacteria stained dark purple with crystal violet because they are not decolorized by alcohol, while gram-negative bacteria stained red/pink due to decolorization by alcohol and so take up the red counter stain (Cheesbrough, 2006).

Genotypic Identification

Primer

The following primers were used for amplifying 16s rRNA genes Fd2 (27F) {AGA GTT TGA TCC TGG CTC AG} Rd2 (1492R) {TACGGYTACCTTGTTACGACTT}.

DNA Extraction

The bacterial genomic DNA was extracted using the boiling method described by Dashi *et al.* (2009). Two colonies from overnight-grown cultures were used. The colonies were put in a test tube containing one ml of distilled water and boiled for 10 minutes in a water bath, and they were centrifuged for five minutes at 1000 rpm.

PCR reaction

The PCR reaction was done in a total volume of 12.5µL reaction containing 6.25µL master mix, 4.25µL sterile distilled water, 1 µL DNA template, 0.5µL forward primer, 0.5µL reverse primer used to amplify DNA fragment containing 16s rRNA like genes under the following conditions. Initial denaturation at 95°C for 5 minutes, followed by 30 cycles of denaturation at 95°C for 30 seconds, annealing temperature at 55°C for 30 seconds, and extension at 72°C for 30 seconds, followed by final extension at 72°C for 10 minutes.

Preparation of Agarose

The amplified PCR products were analyzed on 1.5% agarose gel electrophoresis. A 1000-1500bp DNA ladder (Cleaver, USA) was run with each gel, and the size of the amplified product determined the genotype. The gel was prepared by weighing 1.5g of agarose powder into 100m! Gilled water Tris/Borate/EDTA(TBE) buffer (Thermo Fisher Scientific, USA) was added admised properly. It was dissolved by heating in a microwave at 95-100°C for 3-5 minutes until it gave a clear solution. The conical flask was removed from the oven and allowed to cool at room temperature. About 0.5ul-1.0µl of an intercalating agent was added (ethidium bromide) staining agent to visualize the bands. The mixture was carefully poured into a casting material, and the appropriate comb was inserted to give the required well size. It was then allowed to solidify at room temperature for 20 minutes. The comb was gently removed in an upward upright direction after solidification of the gel, and the gel tray was placed in the electrophoresis tank

Sample Loading into Gel Wells

The casted gel was placed in an electrophoresis tank and filled with adequate TBE buffer to submerge the Gel was adequately added to the tank to submerged the gel. Exactly 0.5µl of the PCR products were carefully loaded using a 0.5-10ul micro pipette in an orderly manner. The DNA ladder and the control samples were loaded in separate wells. Depending on the tank size, the system was run at 100- 120 voltage for about 40-60 minutes. The system was turned off, and the gel was removed. It was subsequently visualized using the gel documentation system.

Viewing Gel Using the Gel Documentation System

The gel was carried in its holder from the gel tank to the gel documentation system (Omin Doc, clever scientific, UK) to be viewed by UV light and captured using inbuilt cameras. After removing the desired portion, the gel was discarded in a biohazard waste bin and stored at -20°C for further use.

Genotypic Identification

The following primers were used for amplifying 16s rRNA genes Fd2 (27F) {AGA GTT TGA TCC TGG CTC AG} Rd2 (1492R) {TACGGYTACCTTGTTACGACTT}.

Data Analysis

Descriptive statistics were used to assess the data, including mean, standard deviation, and frequency tables. The mean value ± SD (standard deviation) was used to show the values.

RESULT

Table 1 Physicochemical analysis of the waste. The volatile solid content representing the total amount of degradable organic matter in the substrate was high in both substrates, with cow dung representing a higher content at 88%, goat dung at 82%, and maize husk at 62%. Also, the C/N ratio was higher in maize husk with a value of 47.40, cow dung 14.18 and goat dung 12.81. Moisture content was higher in cow dung and goat than in maize husk, with a value of 59.40, 34.31, and 4.01, respectively.

Table 2 Proximate Composition of dried maize husk. The results show that Carbohydrates have the highest percentage mean of 53.36 %, followed by crude fiber at 19.40 % and crude protein at 9.66, while Fat content, Ash content, and Moisture content had 7.55%, 6.02%, and 4.01%, respectively.

The initial and final pH of the slurry in all six (6) digesters during the digestion process at a neutral pH level of 6.82 to 7.2 required for optimum biogas production is illustrated in Table 3.

Table 4 summarizes the relative volume of biogas produced at a retention time of seven (7) weeks under mesophilic conditions by water displacement method using a measuring cylinder of 1000cm^3. The digesters were set up and allowed to undergo anaerobic digestion for a retention period of seven weeks. A close observation shows that daily production started on the first week, reaching a peak in weeks two, three, and four with cumulative volumes of 9135cm^3, 8660cm^3, 9820cm^3, 6545cm^3, 5915cm^3 and 1965cm^3 of biogas for D1, D2, D3, D4, D5, and D6 respectively at the end of the 49 days' retention time. The largest volume of gas produced was 9820cm^3 in D3, while the lowest volume was recorded in D6 with a relative volume of 1965cm^3.

The result of weekly biogas production accumulation in all digesters is presented in Figure 3, which shows the increase in biogas production through the retention time.

Table 5 shows the result of the gas composition in all digesters. D2 was observed to have the highest methane content, with methane accounting for 71% of the total volume of the gas. The lowest methane content was recorded in D6, with a value of 21%. Figure 4 represents the cumulative volume of gas produced from each digester, together with the average content of methane present.

Table 6 Shows the Percentage frequency of occurrence of the isolates in all digesters, with *Klebsiella pneumonia* and *Bacillus subtilis* as the predominant organisms isolated, having (70%) and (30%) for other organisms. Figure 5 shows Agarose Gel Electrophoresis for 16s rRNA Genes of *Klebsiella pneumoniae*

Table 1 Physicochemical Analysis of the Wastes

Parameters	Maize husk	Cow dung	Goat dung
pH	5.9	6.81	6.31
Temperature (^{0}C)	29	30.7	22.0
Total solids (%)	77	93	87
Volatile solid (%)	62	88	82
Moisture content (%)	4.01	59.40	34.31
Ash content	3.1	3.37	5.5
Carbon content	45.98	31.90	41.0
Nitrogen content	0.97	2.25	3.20
C/N ratio	47.40	14.18	12.81

Table 2 Proximate Composition of the Maize Husk

Parameters	Percentage (%)
Moisture content	4.01
Ash content	6.02
Fat content	7.55
Crude protein	9.66
Crude fibre	19.40
Carbohydrates	53.36

Table 3 Initial and Final pH of the Digester Feedstock

Digesters	Initial pH	Final Ph
D1	7.18	6.02
D2	7.01	6.70
D3	7.10	6.10
D4	7.12	6.00
D5	6.82	5.98
D6	7.12	5.90

Key: D1= Cow dung + Maize husk; D2=Goat dung + Maize husk; D3=Cow dung + Goat dung + Maize husk; D4= Cow dung; D5=Goat dung; D6=Maize husk;

Table 4: The Relative Volume of Biogas Produced at a Retention Time of Seven (7) Weeks.

Retention time (in days)	D1 (cm^3) volume of	D2 (cm3) Biogas	D3 (cm3) Produced	D4 (cm3)	D5 (cm3)	D6 (cm3)
Week 1	900±1.41	760±0.42	995±2.21	335±0.84	200±0.56	0±0.00
Week 2	2100±1.56	1900±0.16	2925±0.84	1400±0.71	740±0.16	0±0.00
Week 3	4000±0.56	3650±1.41	3500±1.41	2530±0.00	1720±0.75	0±0.00
Week 4	1550±1.41	1540±0.42	1550±0.00	1500±2.82	2100±0.75	580±1.83
Week 5	570±1.83	700±2.82	600±0.71	650±.00	895±0.75	1230±1.56
Week 6	25±0.84	95±0.16	195±0.16	110±2.12	200±0.00	155±0.16
Week 7	10±0.00	15±0.00	55±1.41	20±2.82	60±1.41	0±0.00
Total	9135±1.83	8660±0.42	9820±0.56	6545±1.41	5915±0.71	1965±0.84

Key: D1= Goat dung + Maize husk; D2=Cow dung + Maize husk; D3=Cow dung + Goat dung + Maize husk; D4= Cow dung; D5=Goat dung; D6=Maize husk;

Table 5 Composition of biogas produced from all digesters after seven (7) weeks

Digesters	Methane CH4 (%)	Carbon dioxide CO2 (%)	Other gases (%)
D1	58	13	66
D2	71	17	19
D3	69	15	16
D4	65	28	9
D5	48	36	16
D6	21	13	66

Key: D1= Goat dung + Maize husk; D2= Cow dung + Maize husk; D3=Cow dung + Goat dung + Maize husk; D4= Cow dung; D5=Goat dung; D6 =Maize husk;

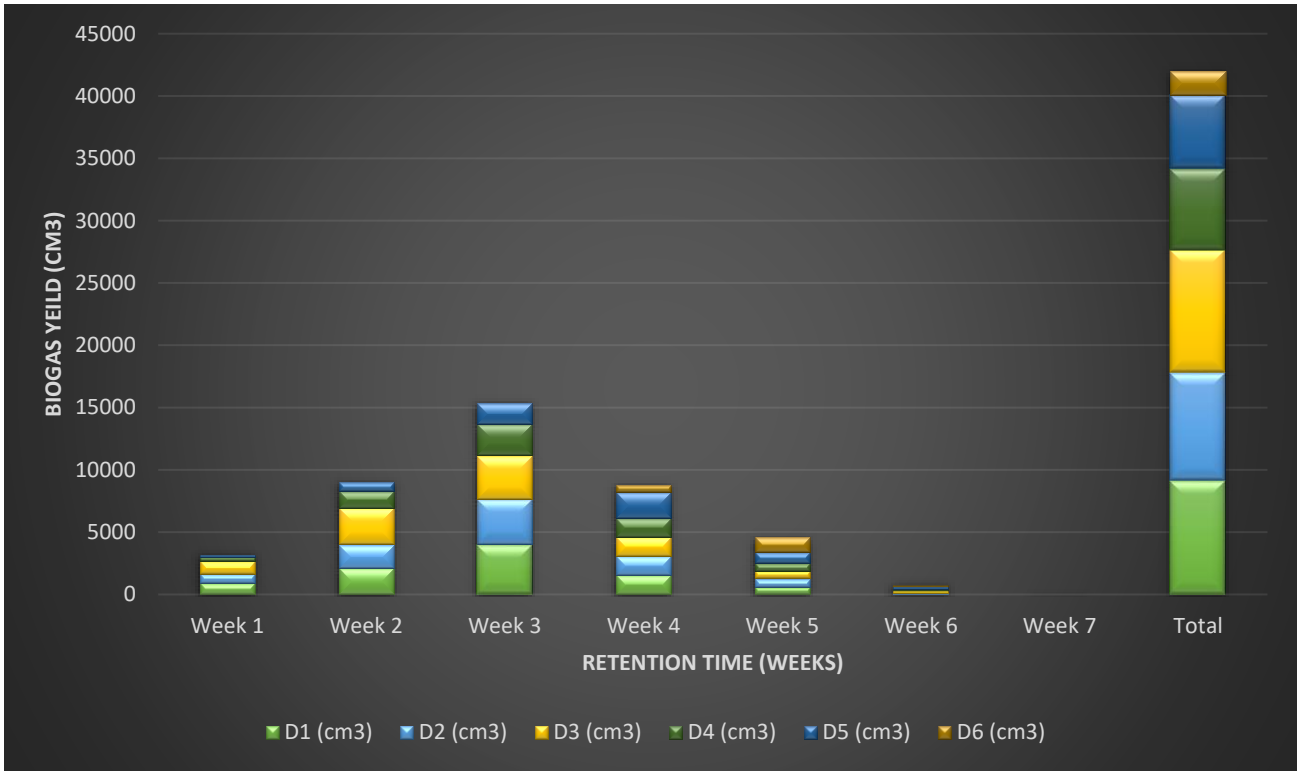

Figure 3: Weekly Increase in Biogas Production Through the Retention Time

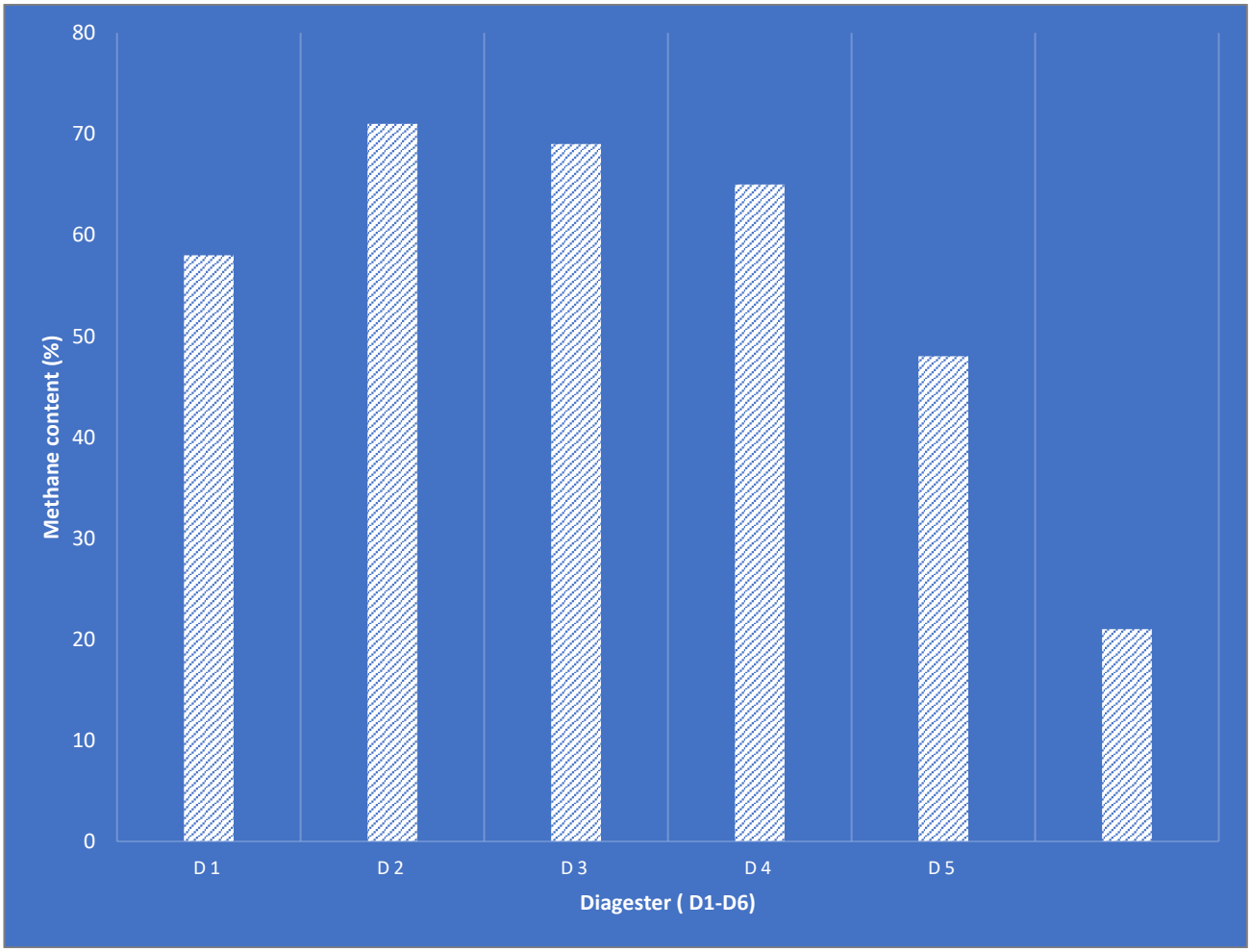

Figure 4 Content of Methane Gas from the Relative Volume Produced from each Digester

Table 6 Number and Percentage of Occurrence of Bacteria Isolated within the Digesters

Bacteria	Frequency of Occurrence	% of Occurrence
Klebsiella pneumonia	7	35
Bacillus subtilis	6	30
Escherichia coli	3	15
Staphylococcus aureus	2	10
Pseudomonas aeruginosa	2	10
Total	20	100

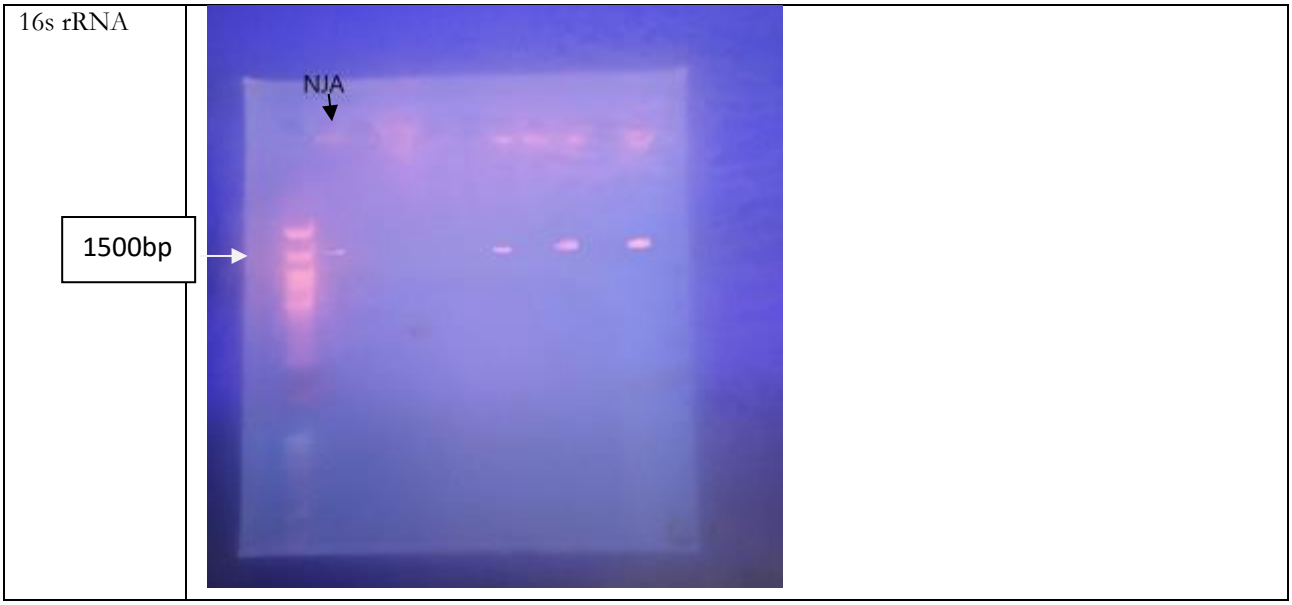

Figure 5: Agarose Gel Electrophoresis for 16s rRNA Genes of *Klebsiella pneumoniae*

DISCUSSION

The physicochemical parameters observed during the digestion processes. The parameters were temperature, pH, volatile solids, and C/N ratio. In this study, the digesters were operated under mesophilic conditions, similar to the finding of Ukapai and Nnabuchi (2012), with fluctuations ranging from 32.5 to 37.7^{0}C. Temperature changes mainly influenced these environmental fluctuations because the digestion processes were at room temperature. With respect to biogas yield, the highest yields were obtained at 37.7^{0}C in most of the digesters, while the lowest yields were obtained at 33.5^{0}C. From the results obtained, it was revealed that biogas production is optimal at mesophilic temperatures than at lower temperatures. Similar experiments were carried out by Onthong and Juntarachat (2017) within a temperature range of 30-38^{0}C. Alternatively, a thermophilic temperature (50-57^{0}C) ensures pathogen destruction and increases biogas production but can only be achieved by heating the reactors, which increases expenditure thus decreasing economic benefits. At temperatures below 15^{0}C, gas production is very slow and low. This is similar to the findings of (Voegeli *et al.*, 2014).

The proximate analysis of the substrates (Maize husk) in Table 2 was to determine the potentiality of the substrate for biogas production. The results show that dried maize husk has the highest Carbohydrate content, with a mean concentration of 53.36%, followed by crude fiber and Crude protein, with 19.40 % and 9.66%, respectively. The crude protein showed the potential of the maize husk as a nitrogen source required for growth and efficient enzyme expression, whereas the carbohydrate content indicates a high content of fermentable sugars required for growth and enzyme production by the organism. Ash and Moisture contents have 6.02% and 4.01%, respectively. However, the findings of this study agree with reports by Sara and Mahdi (2016), who reported a moisture content of 3.74% and an Ash content of 5.08%, respectively. Low moisture and ash content have been reported to result in high organic matter content and, hence, high biogas yield (Sambo *et al.*, 2015).

Table 3 In this study, the initial and final pH of the Six (6) digesters were at a neutral pH level of 6.88 to 7.0 required for optimum biogas production. It was reported that anaerobic bacteria require a natural environment; thus, pH ranging from 6-8 is required for

optimum biogas production (Zhang et al., 2015). The pH of the slurry appeared to be decreasing in the digesters. It is not surprising as the decrease in pH may be a result of anaerobic fermentation taking place. pH is an important factor that affects biogas production. It was reported by Oyeleke et al. (2017) that biogas produced at a pH of 5 is greater than that of pH 10. Some microorganisms also evolved later in the production process during the log phase, while others died off midway through the process during the stationary and the death phase due to the depletion of nutrients in the slurry (Ahmadu, 2009).

From the result of biogas production, it can be observed that digesters with co-digestion of the two substrates GD/MH in D1 and CD/MH in D2 have higher biogas production with relative volumes of 9135cm^3 and 8660cm^3 respectively than the mono-digestion of the GD and CD by 35.2%, and 24.4% respectively. Also, the co-digestion of the three substrates MH/CD/GD in D3 with a relative volume of 9820cm^3 was considerably higher than the co-digestion in D1 and D2 by 7% and 11%, respectively. With this, it can be inferred that the Anaerobic co-digestion enhances the performance of the digester and subsequently, more biogas production was attained. The lowest biogas yield was obtained in mono digestion of MH in D6 with 1965cm^3`, which showed a decrease yield of about 67-70% in the mono digestion of CD and GD in D4 and D5 with relative volume of 6545cm3 and 5915cm3 respectively, they produced appreciable amounts of biogas.

With respect to methane content in the gas, all the substrates in the digesters performed well in the production of flammable gas with the exception of D6 with an inflammable gas of 21% methane. Biogas is flammable at 45% above methane concentration. It was observed that digesters with co-digestion of GD/MH produced a high volume of gas, 9135cm^3/58%, while digesters with co-digestion of CD/MH produced more methane gas, 8660cm^3/71% and also higher methane content than digesters with mono-digestion of the substrates.

The percentage frequency occurrence of the isolates in all digesters in Table 6 shows that *Klebsiella pneumonia* and *Bacillus subtilis* are the predominant organisms isolated from the other organisms, and The query sequence was 94.8% similar to *Klebsiella pneumonia,* but some species were also present throughout the process of gas production (Baki, 2004). The ability of *Bacillus species* to overlap during the production was probably because the organisms can produce spores, which help them to withstand the harsh anaerobic conditions or heat that evolved during the biogas production (Baki, 2004).

CONCLUSION

Both substrates' biodegradable organic contents were very high, with cow dung having 88%, goat dung at 82%, and maize husk at 62%. The Carbon-Nitrogen ratio was higher in maize husk, at 47.10, and lower in cow dung, at 14.18, and goat dung, at 12.81. The production of biogas proceeded in a slightly alkaline pH range of 6.8-7.5 at a mesophilic temperature with minimal fluctuations ranging from 33.5-37.7^{0}C. Biogas production obtained from the substrates yielded a considerable amount of gas with improved methane content that is higher than the substrates' mono-digestion. These microorganisms, notably anaerobic bacteria isolated from this digestion process, include *Bacillus species, Pseudomonas aeruginosa*, *Klebsiella pneumonia*, *Escherichia coli*, and *Staphylococcus aureus*. Considering the findings of this investigation, maize husk in co-digestion was seen to be a promising feedstock in the generation of methane-rich biogas. It was observed that cow dung in co-digestion with maize husk at 3:2 performed best in the production of qualitative methane gas of about 71%, while goat dung with maize husk produced a higher volume of gas with 58% methane gas, which is more efficient than the mono digestion of the substrates. It also revealed that the implementation of co-digestion mode, compared to mono-digested systems, improved the overall efficiency of the treatment process and biogas yield, which would be associated with reduced operating costs. This, in turn, results in increased value-added products and reduced environmental footprint and supports local and national economies.

RECOMMENDATIONS

i. To ensure good conversion efficiencies and process stability in biogas production, it is necessary to accurately characterize the feedstock, especially the physicochemical properties. Based on such parameters, a decision can be made whether to use a single feedstock or a co-substrate in the anaerobic digestion.
ii. Since biogas is more productive in this temperature range, a way to maintain a mesophilic temperature should be devised.
iii. More research is needed to understand how different feedstocks are co-digested by microbial consortia.
iv. More research institutions and bodies should be established by the government or higher education institutions to turn this research into a high-performing technology.

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