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COMPARATIVE EVALUATION ON THE POTENTIALS OF SHEEP RUMEN CONTENTS FOR BIOGAS GENERATION

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ABSTRACT

Biogas refers to a gas produced by the biological breakdown of organic matter in the absence of oxygen. It is as a clean biofuel produced by microorganism during anaerobic digestion of organic matter. The study was carried out to determine the potentials of sheep rumen contents for biogas production through anaerobic digestion. Proximate analysis was carried out on the substrate. The results shows that nitrogen, potassium and phosphorus have 0.58 ± 0.02 (mg/ml), 425 ± 5.00 (mg/ml), and 2.57 ± 0.03 (mg/ml) respectively. After proximate analysis, four (4) local digesters with capacity of 500g tins were designed and used for the collection of gas via passage pipe systems. The digesters were used to digest the slurry (mixture of Sheep rumen content and water) which were mixed in the ratio of 2:1 for a period of eight weeks (56 days) retention time until the biogas reduced significantly. The pH values of the slurries were adjusted to neutral. The digesters were stirred thrice daily to avoid scum formation in the digesters and allow for easy escape of gas produced. The total average yield of the gas produced were 820 cm3, 1070 cm3, 780 cm3, and 660 cm3 for D1, D2, D3, and D4 respectively. Isolation of Microorganisms were caried during the production where B. subtilis, E. coli, P. aeruginosa, and S. aureus were found. Biogas production from organic wastes which is an eco-friendly, which helps to solve most countries energy crisis.

Keywords: Biogas, Biofertilizer, Sheep Rumen Contents, Digester, Anaerobic Digestion and Slurry

INTRODUCTION

The country's economy mainly depends on the energy resources available and utilized. Energy has been exploited since the prehistoric times. With the advent of industrial revolution use of fossil fuels began growing and increasing till date. The dependence on fossil fuel as primary energy source has led to global climate change, environment degradation and human health problems (Ahmadu 2009). Biogas is a mixture of colorless, flammable gases obtained by the anaerobic digestion of plant and animal based organic waste materials. Biogas is typically made up of methane (50-70%) carbon dioxide (30-40%) and other trace gases. It is generally accepted that fuel consumption of a nation is an index of its development and standard of living (Abdulkadir 2012). There have been increases in the use of and demand for fuel in terms of transportation and power generation in many nations including Nigeria. These have so far been met in Nigeria largely from the nation's stock of fossil fuel such as crude oil, which is finite in nature. Fossil fuels are not environmentally friendly and are also expensive. The use of alternative and more environmentally-friendly energy sources such as biogas has been advocated (Abdulkadir 2012). A biogas plant when successful is an appropriate and sustainable method to deal with anthropogenic waste.

In Nigeria, the use of wastes from organic matter, though important, has been relegated to the background. There are abundant agricultural residues and municipal solid wastes, whose potentials are yet to be fully tapped for energy generation. The possibility of using such wastes for biogas production should be explored (Adaramola and oyewole, 2011). The raw materials used in commercial methane generation include plant residues, animal waste like cow dung and various urban wastes which are available in Nigeria. Biogas technology has advantages which include the following: generation of storable energy sources, production of a stabilized residue that can be used as a fertilizer, an energy-efficient means of manufacturing nitrogen containing fertilizer, a process having the potential for sterilization which can reduce public health hazards from fecal pathogens, and if applied to agricultural residues, a reduction in the transfer of fungal and plant pathogens from one year's crop to the next. (Akhila, 2010).

The two enormous problems that are increasingly threatening the good life of many nations include the task of waste management and inadequacy of energy supply. A nation's inability to dispose waste and to find enough energy greatly affects living conditions. The problem of fuel scarcity and sewage disposal in Nigeria and many developing countries is alarming. Energy generated from waste is therefore needful as it will serve the dual purpose of cleaning the environment and providing a cheaper source of energy (Alkan and Karthikayan, 2011).

Inadequate energy supply and environmental pollutions are serious problems confronting Nigeria with high population growth rate, access to adequate energy and healthy environmental demands for a diversification of sources of energy supply, if Nigeria is to achieve any meaningful growth and development, biogas generation from anaerobic digestion of readily available wastes could contribute to solving these problems (APHA 1995).

Thus, biogas production could be an effective means of recycling organic waste thereby achieving the goal one of the millennium development goals of eradicating extreme poverty (UN, 2005) via waste to wealth initiatives (Igboro, 2011). The yield and composition of biogas depends on the nature or type of substrate fed to the digester. The process of

producing biogas is by anaerobic fermentation of organic matter (substrate) which is gaining this development is due to its ability to provide relief to man from two of the problem encountered in the course of living from day to day. (Ansah and Emanuel 2019). Thus, biogas is a sustainable alternative to natural gas. Since anaerobic digestion only releases carbon to the gas phase, the other nutrients (nitrogen, phosphorus, and micronutrients) remain in the effluent, which makes it a high-quality organic fertilizer and soil amendment wastes (Anonymous 2012).

MATERIALS AND METHODS

Materials

The Materials that were used for the practical are; Clean container, Cool box, Dry poultry waste, Distilled water, Beaker, pH meter, Four(4) 15-18 litre large plastic container, Delivery tubes, 1000cm³measuring cylinder, Bowl, Test tubes, araldite sealer, universal bottle, Glucose phosphate medium in a test tube, Drops of naphthol solution, lead Acetate paper, human plasma in normal saline, conical flask, Syringes, Nutrient Agar plates, Gram Staining reagents (Crystal Violet, Iodine, Safranin, Decolourizer), Glass slides, Urea medium in universal bottle, Inoculating loop/wire loop, Bunsen burner.

Collection of Sample.

Fresh sheep rumen content was collected from Kasuwan Shanu abattoir, Maiduguri metropolis. The sample was collected in a clean container and transported to Microbiology laboratory, University of Maiduguri for analysis. The sample was then screened for fragments of unwanted materials (anything other than the sheep rumen contents).

Proximate and Elemental Composition of the Substrate

The proximate and elemental composition of the sample was carried out according to AOAC methods to determine the following compounds and elements such as ash content, carbohydrate content, crude fiber content, crude protein content, lipids content, moisture content, nitrogen content, phosphorus content and potassium content.

Microbial Analysis

Media preparation

The culture media was prepared using standard laboratory methods as described by Cheesbrough (2003). These include: nutrient broth, nutrient agar (NA) and of potato dextrose agar (PDA). All media are prepared according to the manufacture instructions Oyeleke and Manga 2008).

Nutrient Agar preparation

28 grams of dry powder nutrient agar was dissolve in 1 liter of distilled water. The suspension was allowed to stand for 10minutes. The mixture was then heat using magnetic stirrer for 10 minutes. The mixture was then sterilized using autoclave for 15minutes at 121ºC. The sterilized agar mixed and aseptically poured into petri dish. The plates were then dried using the hot air oven and stored in the refrigerator at 4ºC prior to use (Oyeleke and Manga 2003).

Potato dextrose agar preparation

19.5g powder of potato dextrose agar was dissolved in 500ml of distilled water and mix thoroughly until it dissolves totally. Foil paper was used to cover the conical flask. The dissolved potato agar was sterilize using autoclave for 15 minutes at 121ºC, the sterilized agar was allowed to cool before pouring into the petri dishes (Igboro *et al*., 2011).

Isolation and Characterization of Microorganisms

Serial dilution

The slurry was serially diluted using a tenfold serial dilution and 0.1ml of 10-7 dilution factor was spread onto nutrient agar plates for the enumeration and isolation of bacteria and sabauraud dextrose agar for the enumeration and isolation of fungi. The nutrient agar plates were incubated aerobically and anaerobically at 37 0C for 24 hours. The sabauraud dextrose agar plates were incubated at 28 0C for 3-5 days. Bacterial isolates were characterized using methods described by Cowan (1974) while fungi were characterized based on macroscopic and microscopic examination (Deublein and Steinhauser 2008).

Subculture

The colonies were sub-cultured repeatedly on fresh plates to obtain pure isolates (Tambuwal *et al.,* 2019).

Gram Staining Test

The pure bacteria were gram stained as follows.
The colony was picked and fixed on the glass slide, and it is then heat fixed. Then primary stain (crystal violet) was added/poured on the slide for 1 minute. The crystal violet dyes the cell wall of the bacteria species present. It was then rinsed with water. Gram iodine (mordant) was then poured on the slide. It was then washed and allowed for 1 minute. The iodine helps to fix the primary dye to the cell wall. Decolourizer (ethanol) was then used next and allowed to stay for 30 seconds which removes the primary stain from Gram negative bacteria present. It was then washed. Finally, counter stain (safranin) was then applied for 1 minutes, to stain those cells (gram negative) that have lost the primary stain because of the decolourizer. It was then washed as described by Baki (2009).

Biochemical Test

After identification, Gram-positive bacilli that produced spores were subjected to further biochemical test for characterization of the isolated bacteria. The tests include catalase, coagulase, citrate, indole, motility, methyl red (MR) nitrate, oxidase triple sugar iron test, urease, Voges-pro skaters and H2S sulfide tests (Tambuwal *et al.,* 2019).

Biogas Production

Slurry preparation

Ten (10) gram each of the sheep rumen contents were weighed and mix with 50cm^3 of distilled water in a 250ml conical flask to give ratio of 1:5 as recommended by Mattocks. The mixture was thoroughly stirred with a glass rod to achieve homogeneity (Tambuwal *et al.,* 2019).

Anaerobic digestion

Four sets of conical flasks each containing 250ml, was use as digesters. The flasks were labelled A1 and A2, B1 and B2, C1 and C2, D1 and D2. Each set was replicate three times. A total of 24 flasks was used. Each flask containing equal volumes of the slurry (10g sheep rumen contents: 50cm^3 water) was connect by a rubber delivery tube, which conveys the gas, to a burette and was fill with water and place in an inverted position in a glass trough containing water such that gas released from the digestion process was collected in the burette by water displacement method. The flask-end of each delivery tube was insert into the mouth of the conical flask and hold in place by cotton wool stuff at the mouth of the flask. The connecting point of the tube and flask was adhesive tape to prevent leakage of gas from the flask. Each of the four set of flasks were subjected by a different treatment. The

contents of the flasks were allowed to undergo digestion for a retention period of eight weeks with daily measurement of gas yields. (Baki, 2009).

RESULTS

Table 1 shows Proximate and Elemental Composition of Sheep Rumen Contents**.** The results shows that Crude fibre has the highest percentage mean of 48.3 % followed by Crude Protein Content with 21.65%, Carbohydrate Content 19.98%, Total Solids 6.73% and Moisture Content 5.83% respectively. The results also show the mean concentration of some elements with Potassium constituting major element with an average of 425.00 mg/ml. Sodium, phosphorus and calcium have a mean concentration of 32.66 mg/ml, 2.57 mg/ml and 0.80 mg/ml respectively. Magnesium was found to have least concentration of 0.27 mg/ml.

Table 1: Proximate and Elemental Composition of Sheep Rumen Contents (all values are mean ± standard deviation (S.D) of triplicate measurement)

PARAMETERS	COMPOSITION
Moisture Content (%)	5.83 ± 0.28
Ash Content (%)	11.5± 0.38
Total Solids	6.73 ± 1.89
Crude Protein Content (%)	21.65 ± 0.14
Crude Lipid Content (%)	6.1 ± 0.28
Carbohydrate Content (%)	19.98 ± 0.24
Crude Fibber Content (%)	48.3 ± 0.28
Nitrogen (mg/ml)	0.58 ± 0.02
Potassium (mg/ml)	425 ± 5.00
Calcium (mg/ml)	0.80 ± 0.01
Sodium (mg/ml)	32.6 ± 1.15
Phosphorus (mg/ml)	2.57 ± 0.03
Magnesium (mg/ml)	0.27 ± 0.02

Table 2 Shows the Percentage frequency of occurrence of the isolates in all the samples (fresh sheep rumen content) with *B. subtilis* as the predominant organism isolated having (70%) and (30%) for other organisms respectively.

Table 2: Number and Percentage of Occurrence of Bacteria Isolate in Fresh Sheep Rumen Content

Bacteria	Frequency of Occurrence	% Occurrence of Isolate
Escherichia coli	12	50
Bacillus subtilis	5	21
Staphylococcus aureus	4	17
Pseudomonas aeruginosa	3	12
Total	20	100

Table 3 Shows the initial and final pH of the four (4) digesters at a neutral pH level of 7.0 to 6.88 required for optimum biogas production.

Table 3: Initial and Final pH of the Digesters

DIGESTER	INITIAL PH	FINAL PH
D1	7.00	6.67
D2	7.00	6.15
D3	7.00	6.31
D4	7.00	6.08

Table 4. Shows Species of bacteria isolated during biogas generation base on morphological and biochemical characteristics. The organisms found were *Escherichia coli, Bacillus subtilis Staphylococcus aureus* and *Pseudomonas aeruginosa.*

Table 4: Species of Bacteria Isolated During Biogas Generation Base on Morphological and Biochemical Characteristics

Isolates	G/rxn	Ure	Glu	Lac	Suc	Mor	H_2S	MR	Citr.	VP	Cat	Coa	Ind	Org identified
1	+ve cocci	-	+	+	+	+	-	+	-	-	+	-	+	*E. coli*
2	+ve rod	+	+	+	+	+	+	-	+	+	+	-	-	*B. subtilis*
3	+ve rod	+	+	+	+	-	-	+	+	+	+	+	-	*S. aureus*
4	+ve rod	-	+	-	-	+	-	-	+	-	+	-	-	*P. aeruginosa*

Table 5 Shows the summary of the results of gas produced by water displacement method using measuring cylinder of $1000cm^3$. The digesters were set up and allowed to undergo anaerobic digestion for a retention period of Eight weeks. A

close observation shows that daily production started on the Fourth day, reaching peak on the first week and yielding 320 cm^3 of biogas. A cumulative of 3330 cm^3 of biogas was produced at the end of the 56 days retention period. Table 5 shows the results obtained during the retention period.

Table 5: The Daily Volume of Biogas Yield at a Retention Time of Eight (8) Weeks

Retention Time In days	D1	D2	D3	D4
1-7	90	320	100	70
8-14	310	180	170	180
15-21	0	30	70	70
22-28	90	140	80	100
29-35	10	20	100	0
36-42	60	170	140	40
43-49	130	90	100	110
50-56	130	120	20	90
Total	**820**	**1070**	**780**	**660**

DISCUSSION

This study was conducted to produce biogas through anaerobic fermentation of the substrate (sheep rumen content) by action of fermentative microorganisms. The bacterial isolates for this process are *Escherichia coli, Bacillus subtilis, Staphylococcus aureus, and Pseudomonas aeruginosa.* The organisms decompose the slurry to produce biogas. The average gas of 820cm^3, 1070cm^3, 780cm^3, and 660cm^3 was produced within the retention time of 8 weeks/56 days. This corresponds to that of Tambuwal *et al*., 2019) whom isolated *E. coli* during the second week of production and produced about 707cm^3 of biogas while bacillus species produced about 1544cm^3, *S. aureus* produced about 1820cm^3 of biogas, and *P. aeruginosa* produced about 1335cm^3 of biogas.

Table 1 Shows Proximate and Elemental Composition of fresh Sheep rumen content. The results shows that Crude fibre has the highest percentage mean of 48.3% followed by Crude Protein Content with 21.65%, Carbohydrate Content 19.98%, Total Solids 6.73% and Moisture Content 5.83% respectively. The results also show the mean concentration of some elements with Potassium constituting major element with an average of 425.00 mg/ml and Magnesium was found to have least concentration of 0.27 mg/ml. This agrees with (Ansah *et al*., 2019) findings who has conducted direct analysis studies of ruminant animals, including sheep, and his results indicates that Ash is the predominant composition found in the sheep rumen waste.

Table 2 shows the number and percentage of occurrence of bacteria isolate in fresh sheep rumen contents, *Escherichia coli* has frequency of occurrence 12, *Bacillus subtilis* has 5, *Staphylococcus aureus* has 4 and *Pseudomonas aeruginosa* 3 while the percentage occurrence of isolate bacteria, *E. coli* has 50, Bacillus *subtilis* has 21, *Staphylococcus aureus* has 17 while *Pseudomonas aeruginosa* has 12. The reason why *E. coli* has the highest percentage of occurrence is because they are found in the intestine and gut of animals and can be able to resist the extreme temperature during the biogas production (Bagudo, 2012).

Table 3 Shows the initial and final pH of the four (4) digesters at a neutral pH level of 7.00 to 6.88 required for optimum biogas production. The pH values of the substrates digested was at a neutral level and were varied almost in the optimal limits of methanogenic bacteria of pH: 7.2-6.12 which is in agreement with (Hasen, 2001) who reported optimal biogas production requires a pH range of 7.4 neutral to 6.88. Slurry pH appeared to drop in all digesters. This is not surprising as the decrease in pH could be due to the undergoing anaerobic fermentation. pH is a key factor affecting biogas production. Generally, the pH of all the slurries before and after the digestion are found to be slightly acidic. Since the acidity levels in the slurries were not too high the bioconversion of the substrates took place. The general decrease in the pH in the spent slurries may be attributed to formation of sulphide (S-2) in the slurries due to breakdown of biodegradable Sulphur containing organic and inorganic compounds and also due to the formation of fatty acids by acetogenic methanogens during the process of digestion.

Table 4 Shows Species of bacteria isolated during biogas generation base on morphological and biochemical characteristics. The organisms found were *Escherichia coli, Bacillus subtilis, S. aureus* and *P. aeruginosa.* The organisms show positive towards gram reaction, catalase, urease, glucose, fructose and sucrose while shows negative reaction towards indole except *Pseudomonas aeruginosa* shows indole positive. This is similar to (Tambuwal *et al*., 2019) discovery of isolating and identifying bacteria from cow dung to produce biogas.

Table 5 Shows the summary of the results of gas produced by water displacement method using a measuring cylinder of 1000cm^3. A digester was set up and subjected to anaerobic digestion with a residence time of Eight weeks. Close observations show that daily production shown on the fourth day peaks in the first week, producing 320cm^3 of biogas. A cumulative of 3330 cm^3 of biogas was produced at the end of the 56 day's retention period. Imam *et al*., (2013) investigated biogas production from fermentable materials were selected as cow dung, poultry waste and water hyacinth and sheep waste. Percentage of methane content (the main constituent) in biogas produced from different fermentable materials is almost the same. Castrillon *et al*., (2013) studied biogas production from sheep manure by adding food waste and was observed that methane content in biogas up to 78%. Owamah *et al.,* (2014) investigated the optimization of biogas production and quality from chicken droppings by anaerobic digestion with *Cymbopogon citratus*.

CONCLUSION

The result of this study clearly indicates that Sheep rumen content could be a suitable substrate for biogas production. Using this substrate for biogas production can eliminate its disposal problem and create another abundant source of sustainable energy, the results also demonstrate the applicability of the locally manufactured biodigesters as a biogas production mode. The remaining slurry in the biodigester after biogas production turned out to be concentrated compost that could be used to improve the

nutrients and productivity of agricultural soil. Animal and plant wastes are abundant especially in rural areas. The biogas generated from sheep rumen contents produces an energy resource that can be purified and stored in gas cylinder and used efficiently for direct heat conversion.

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