

Biogas Generation Using Cattle Rumen Contents

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Received: January 18, 2019; **Published:** March 04, 2019

Abstract

This project work is focused on generation of biogas using cattle rumen contents as substrate, isolation microorganisms associated with the Generation of Biogas using the cattle rumen contents, evaluation the amount of biogas produced by the cattle rumen contents and to determine the variation in pH during biogas generation. Biogas digesters with capacity of 500g tins was designed and used. The substrate (cattle rumen contents) was in the ratio 2:1 with the water. The digesters were stirred thrice daily to avoid scum formation in the digesters and to allow for easy escape of the gas produced. The retention time used for this experiment was 56days during which the daily internal temperature reading was taken in order to determine temperature variation and also to determine the effect of sunlight on the production rate. Also microbial analysis, Biochemical Tests, Gram Staining and microscopy were carried out. Therefore, the average weekly production of biogas are; day1-7(40.25cm³), day8-14(178.5cm³), day 15-21(386cm³), day22-28(333.75cm³), day29-35(219.75cm³), day36-42(212.25cm³), day43-49(198.75), day50-56(31.50). The result obtained from this study indicates that *Bacillus* species were the most common bacteria isolated and identified during the research, suggesting that the species plays a vital role in the microbial activities for the production of biogas. The study also revealed that Cattle rumen content has great potential for the generation of Biogas and produced large quantity of Biogas during 15-21days retention time.

Keywords: Abattoir; Biogas; Cattle Rumen; Retention Time; Birnin Kebbi

Introduction

Biogas refers to a gas produced by the biological breakdown of organic matter in the absence of oxygen. Singh [1] also defined Biogas as a clean biofuel produced by micro-organisms or bacteria during anaerobic digestion of organic matter (cattle rumen content, cattle dung, poultry droppings, pig excreta, human excreta, kitchen waste). A biogas plant when successful is an appropriate and sustainable method to deal with anthropogenic waste [2].

The biogas production usually contains 50% and above methane (CH₄) and other gases in relatively low proportions namely, CO₂, H₂, N₂, O₂ and H₂S. Most of the organic acids and all of the H₂ are metabolized by methanogens, with the end result being production of a mixture of approximately 55% to 70% CH₄ and 30% to 45% CO₂, 1-10%hydrogen, 1-3% Nitrogen, 0.1% oxygen and

carbon monoxide and trace of hydrogen sulphide. The mixture of the gases is combustible if the methane content is more than 50%. The methanogens are slower growing and more environmentally sensitive to pH, air, and temperatures than the acidogenic bacteria. Usually, the methanogens require a narrow pH range (above 6), temperatures at or above 21°C, and adequate time (usually more than 15 days), to most effectively convert organic acids into biogas. The resultant energy in the anaerobic digestion (biogas) can be used directly for cooking by burning it in the presence of oxygen. It can also be converted into electricity by using it to convert water into steam, which turns a turbine connected to a generator [3].

However, organic matter can also decompose without any oxygen, by the process of anaerobic Fermentation. This happens due to the bacteria present in the matter which acts during the absence

of oxygen. Landfills see a lot of such decay, especially when the waste material becomes wet and receives little sunlight. As a result, a lot of methane and nitrous oxide is produced and released into the atmosphere [4].

Biogas is a form of energy produced when organic materials such as animal excrement or products that are left over from agriculture are fermented easily and at low cost. The advantage of biogas is that it replaces other energy sources for example charcoal, firewood, electricity, liquid petroleum gas and oil. After animal excrement had been fermented in the gas plant it becomes a good quality and odorless substrate, which is better than fresh manure in improving the soil for the agriculture. As an energy source, it prevents deforestation and animal excrement from causing pollution, smell, flies and water pollution in the community [5]. Also, the problem of Abattoir waste disposal is posing challenge to the general public as this waste constitutes a nuisance to the environment as well as an eyesore to the public. Therefore, if these wastes could be used to generate energy, it would be a welcomed solution to the problem of waste Pollution, disposal and control [6]. Nowadays the use of bio-gas has spread from small farms to big animal farms. It is expected that biogas will be a significant source of energy in the future to preserve the environment, solve the pollution problem and to promote better health to community [7]. The study covered the production of biogas from cattle rumen contents.

There is energy scarcity all over the world and fluctuation in prices of energy. Fortunately, Nigeria is an agricultural country that can use Abattoir waste and agricultural residues in biogas Generation. There is need to generate energy from other sources, especially from agricultural residues, which are generated in large quantities from farming activities. The large quantities of agricultural residues produced in Nigeria can play a significant role in meeting her energy demand. Cassava and yam are ones of the most important agricultural products in Nigeria, especially in southern and western parts of the country. Residues in form of peels are generated from processing of these crops. Initial digestion studies carried out on cassava peels showed that the peels are poor producers of biogas probably as a result of their content of toxic cyanogenic glycosides [8]. This work is therefore on one of the techniques involved in Generation of biogas from rumen contents.

The broad aim of this project was to generate biogas from cattle rumen contents and also to isolate and identify microorganisms associated with the production of biogas. To achieve this, the project had the following specific objectives:

1. To produce biogas from cattle rumen contents.
2. To isolate microorganisms associated with the Generation of Biogas using the cattle rumen contents.
3. To evaluate the amount of biogas produced by the cattle rumen contents
4. To determine the variation in pH during biogas generation.
5. To find the produce of biogas prior to temperature effects.

Materials and Methods

The Materials used for the practical are; Clean container, Cool box, Fresh rumen content of Cattle, Distilled water, Beaker, pH meter, Four(4) 500g capacity tins, Araldite (for sealing digesters), Delivery tubes, 1000cm³ measuring cylinder, Bowl, Test tubes, Syringes, Nutrient Agar plates, Gram Staining reagents (Crystal Violet, Iodine, Safranin, Decolorizer), Glass slides, Urea medium in universal bottle, Glucose phosphate medium in a test tube, Drops of H₂O₂,naphthol solution, lead Acetate paper, human plasma in normal saline, Inoculating loop/wire loop, Bunsen burner.

Methods

- **Sample collection:** Fresh rumen content of Cattle was collected from the Birnin Kebbi central abattoir in Birnin Kebbi metropolis. The sample was collected when the animals were being slaughtered.
- **Sample Preparation:** A clean container with cover was used for the Collection of the sample. The container was placed in a Cool box and transported immediately to the Energy research Centre laboratory at Usmanu Danfodiyo University, Sokoto.
- **Slurry Preparation:** Two hundred grams (200g) of the sample was weighed and mixed with 400cm³ of distilled water in a beaker to give a ratio of 1:2. The initial pH of the mixture was determined.
- **Experimental Set-up:** Four sets of 500g capacity tins each containing four tins was used as digesters. The digesters were labeled N1, N2, N3 and N4. Equal concentration of the slurry was poured in to the digesters. The digesters were sealed with araldite adhesive to cover leakages and connected with delivery tube which conveys the gas from the digester to a 1000cm³ measuring cylinder and inverted into a bowl containing water for gas collection using water displacement method. The digesters were set up and allowed to undergo anaerobic digestion for a retention period of Eight weeks. The amount of gas produced was recorded at 12noon on daily basis and the amount of gas as well as pH were recorded [9].

Microbial Analysis

Media preparation

All media are prepared according to the manufacture instructions through; the media used were nutrient Agar (NA).7g of NA was dissolved in 250 ml, of Distilled water in clean conical flask.

The media was then heated to obtain a homogenized suspension. It was then autoclaved at 121°C for 10 minutes and then allowed to cool to 45°C, the media was then poured into different sterile Petri-dishes and allowed to solidify [10].

Serial dilution

The fresh sample and the digested slurry sample were carried out up to 10⁶ tube. 0.5 ml was obtained using sterile syringe from the 10⁵ tube and inoculated onto already prepared nutrient agar plates by spread plate method of inoculation. The plates were replicated three times. Modified Mackintosh and Fildes pattern of anaerobic jar was used to incubate the plates. The residual oxygen (O₂) in the anaerobic jar was evacuated by placing a kindled match stick, which quenched immediately the left-over oxygen was exhausted. The jar was incubated for a period of 72 hours at 37°C [11].

Colony count

Colonies that emerge on the plates, were counted and recorded as colony forming units per milliliter (cfu/ml) of the sample. The colonies were also subculture repeatedly on fresh plates to obtain pure isolates

$$\text{Colony forming unit} = \frac{\text{Number of colonies}}{\text{Inoculants size} \times \text{dilution factor}}$$

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

Subculture

The colonies were sub-cultured repeatedly on fresh plates to obtain pure isolates.

Gram Staining

The pure bacterial isolates were gram-stained as follows:

- **Step 1:** The colony was picked and fixed on the glass slide and it was heat fixed.
- **Step 2:** The primary stain (crystal Violet) was added/ poured on the slide for 1minutes. The crystal Violet dyes the cell wall of the bacterial species present. It was then rinsed with water.
- **Step 3:** Gram iodine(mordant) was then poured on the

slide. It was then washed and allowed for 1minutes. The iodine helps to fix the primary dye to the cell wall.

- **Step 4:** Decolorizer (Ethanol) was used next allowed for 30seconds which removes the primary stain from Gram Negative bacteria present. It was then washed.
- **Step 5:** Finally, counter stain(safranin) was applied for 1minute, to stain those cells (Gram Negative) that have lost the primary stain as a result of decolorization. It was then washed [11].

Microscopy

The back of the glass slides was wiped clean and a drop of oil (glycerin) was applied on the smear which was examined microscopically with x100 objective lens for the observation of Gram reaction and morphological characteristics of the bacterial cells. Gram-positive bacteria appears purple in colour, while Gram-negative cells retained the counter staining colour of safranin and appeared pink in colour. After Gram Staining and Microscopy, the isolates subculture into universal bottle containing nutrient agar in a slant form for subsequent used in biochemical test [11].

Biochemical Test

Coagulase

The following steps were used in carrying out the coagulase Test:

- **Step 1:** Three test tubes were labeled as "Test", "Negative control", and "positive control".
- **Step 2:** Each of the test tubes was filled with 1 ml of 1:6 dilution factor of human plasma in normal saline.
- **Step 3:** 0.1 ml of the overnight broth Culture to the tube labeled test. Also 0.1 ml of overnight broth Culture of known *S. aureus* to the tube labeled positive control and 0.1 ml of sterile broth to the tube labeled Negative control.
- **Step 4:** All the tubes were incubated at 37°C and observed up to four hours [11].

Catalase

A drop of 3% (v/v) H₂O₂ was placed on a slide. Using a glass slide, a bacterial Culture was then added. Presence of catalase was then observed by the formation of gas bubbles [12].

Urease

Slant of urea medium in universal bottle was inoculated with a loopful of the isolates by streaking. The bottles were then incubated for 24hours at 37°C. Change in coloration from yellowish orange to pinkish indicated urease positive [11].

Methyl red

Using a prepared Glucose phosphate medium in test tube, loopful of the isolates was inoculated and incubated at 37°C for 2 days. To the two-day old culture, drops of methyl red solution was added. It was then shaken slightly and examined for the formation of red ring at the interface [11].

Voges-Proskauer test

In Voges-Proskauer test methyl red was added first to the two-day old culture and 0.6 ml 5% naphthol solution was added and shaken. The test tubes were then sloped and examined after 15 minutes. A red colour indicated a positive Voges-Proskauer reaction [11].

Indole production test

loopful of the isolates was inoculated in a sterile nutrient broth. The incubation was done at 37°C for 48 hours. After incubation, 0.5 ml of Kovac's reagent was added and shaken. It was then examined after one minute. A red colour in the reagent layer indicated indole production (i.e. indole positive), [13].

H₂S production test

A test tube of nutrient broth with the test organism. The lead acetate paper strip was inserted in the neck of the tubes of the medium and stopper red well. It was then incubated at 35-37°C and it was examined daily for blackening of the lower part of the strip [12].

Citrate utilization test:

A sterile Simon's citrate medium, a loopful of 24 hours old culture was inoculated aseptically. It was then incubated at 37°C for 24 hours after which was examined daily for turbidity for a period of 3 days [14].

Oxidative/fermentation (of) glucose test

To perform the OF-glucose test, two tubes of OF-glucose medium were inoculated with the test organism. A layer of mineral oil was added to the top of the deep in one of the tubes to create anaerobic conditions. Oil was not added to the other tube to allow for aerobic conditions. The tubes were then incubated for 24-48 hours. If the medium in the anaerobic tube turns yellow, then the bacteria are fermenting glucose [9]. If the tube with oil doesn't turn yellow, but the open tube does turn yellow, then the bacterium is oxidizing glucose. If the tube with mineral oil doesn't change, and the open tube turns blue, then the organism neither ferments, nor oxidizes glucose. Instead, it is oxidizing peptones which liberates ammonia, turning the indicator blue [11].

If only the aerobic tube has turned yellow then the organism is able to oxidase glucose aerobically ("O"). By-products: CO₂ and although organic acids may be present at low rates [9]. If both tubes are yellow then the organism is capable of fermentation ("F").

If there is, however, growth evident on the aerobic tube yet the medium has not turned yellow, either: (a) glucose has been respired and evolved CO₂ without significant production of acid, or (b) the organism is respiring the peptone [9].

Lactose test

The media used was phenol red lactose broth. The medium is a nutrient broth to which 0.5-1.0% lactose is added. An inoculum from a pure culture was transferred aseptically to a sterile tube of the phenol red lactose broth. The inoculated tube was incubated at 35-37°C for 24 hours and the results are determined. A positive test consists of a color change from red to yellow, indicating a pH change to acidic (indicating a positive test) or magenta or hot pink in the presence of bases/alkali (indicating a negative test), [11].

Motility test

The test isolate was inoculated into motility medium by making a stab with needle to a deep 1-2 and short of the bottom of the tube. Incubated at 35°C for 24 hours at the end of the period of incubation examines the tube [15]. The line of inoculation would not be sharply defined and the rest of the medium would be somewhat cloudy if the restricted to the line of inoculation which become sharply defined the rest of the medium remains clear [12].

Results

The Biogas Generated by the samples was recorded in table 1. The rumen content of the cattle started producing in the first week increasing throughout the period of three weeks. The biogas was produced within optimum temperature of 25°C-30°C.

Identification of isolates and frequency of occurrence

The table 2 shows the bacteria isolated based on morphological and biochemical characteristics. The percentage frequencies of occurrence of the isolates in relation to all samples are shown in the table 3. The isolated bacteria were *Yersinia entrocolitica*, *bacillus megatherium*, *bacillus licheniformis*, *Escherichia coli*, *Pseudomonas aeruginosa*, *bacillus firmus*, *staphylococcus aureus*, *Salmonella spp*, *Proteus vulgaris*, *Bacillus alvei*, and *bacillus latus*.

The result of the practical shows that *Bacillus* sp (49%) are the predominant organisms isolated in the sample (Cattle rumen content). Other organisms are (25%) *Yersinia entrocolitica*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*.

Retention time (in days)	N1(cm3) Volume of	N2(cm3) Biogas	N3 (cm3) Produced	N4 (cm3) [cm3]	Temperature (oC)
1-7	0.00	70.00	27.00	64.00	30.00
8-14	105.00	215.00	187.00	207.00	32.00
15-21	309.00	484.00	329.00	422.00	30.00
22-28	279.00	382.00	297.00	377.00	31.00
29-35	190.00	267.00	192.00	230.00	30.00
36-42	169.00	231.00	222.00	227.00	31.00
43-49	176.00	224.00	192.00	203.00	32.00
50-56	24.00	39.00	27.00	36.00	31.00
Total	1252	1912	1473	1473	

Table 1: The Daily Volume of Biogas Produced at Retention Time of Eight (8) Weeks.**Key:** N1 = Cattle Rumen Content Digester 1;

N2 = Cattle Rumen Content Digester2

N3 = Cattle Rumen Content Digester3;

N4 = Cattle Rumen Content Digester4

	N1	N2	N3	N4	N5	N6	N7	N8	N9	N10	N11
Gram reactn	+	-	-	+	-	-	-	+	+	+	+
Coagulase	-	+	+	+	+	+	+	+	+	+	+
Catalase	-	-	-	-	+	+	-	+	+	-	+
Urease	+	+	-	-	+	-	-	+	-	+	-
MR	+	-	-	+	-	-	+	-	+	+	-
VP	-	-	-	-	+	+	-	+	-	-	+
Indole	-	-	+	-	-	-	-	-	-	-	-
H ₂ S	-	+	-	-	-	+	+	-	-	-	+
Citrate	-	+	-	+	+	+	+	+	-	-	+
Lactose	-	-	-	-	-	-	-	-	-	-	-
Motility	+	+	+	+	-	+	+	+	+	+	+
Glucose	+	+	+	-	+	+	+	+	+	+	+

Table 2: Species of bacteria isolated during biogas generation
base on morphological and biochemical characteristics.

KEY: N1= *Yersinia entrocolitica*; N2= *Proteus vulgaris*; N3= *Escherichia coli*; N4= *Pseudomonas aeruginosa*; N5= *Staphylococcus aureus*; N6= *Bacillus megaterium*; N7= *Salmonella spp*; N9= *Bacillus luitus*; N10= *Bacillus firmus*; N11= *Bacillus alvei*; MR= Methyl red; VP= Voges-Proskauer

Bacteria	Frequency of occurrence	% Occurrence of isolate
<i>Bacillus spp</i>	16	48%
<i>Yersinia enterocolitica</i>	2	6%
<i>Proteus vulgaris</i>	5	15%
<i>Escherichia coli</i>	3	9%
<i>Pseudomonas aeruginosa</i>	2	6%
<i>Staphylococcus aureus</i>	4	13%
<i>Salmonella spp</i>	1	3%
Total	33	100%

Table 3: Number and Percentage of occurrence of bacteria isolated in the cattle rumen content.

Digesters	Initial pH	Final pH
N1	7.27	6.83
N2	7.22	6.38
N3	7.19	5.81
N4	7.11	5.67

Table 4: pH of Digesters Before and After Biogas Production.

Key: **N1**= Cattle Rumen Content Digester 1;
N2= Cattle Rumen Content Digester2;
N3= Cattle Rumen Content Digester3;
N4= Cattle Rumen Content Digester4

Retention Time (In days)	Bacterial load/count (cfu/ml)
1-7	2.5×10^7
8-14	4.2×10^7
15-21	2.3×10^7
22-28	2.08×10^7
29-35	1.83×10^7
36-42	1.03×10^7
43-49	0.07×10^7
50-56	0.02×10^7

Table 5: The colony count of organisms isolated.

Figure 1: A graph of average volume of Biogas produced against retention time (in days).

Figure 2: A graph of volume of Biogas produced by Digester N1 against retention time (in days).

Figure 3: A graph of volume of Biogas produced by digester N2 against retention time (in days).

Figure 4: A graph of volume of Biogas produced by digester N3 against retention time (in days).

Figure 5: A graph of volume of Biogas produced by digester N4 against retention time (in days).

Discussion

The results from this Research showed *Bacillus* species appear to overlap from one stage to another during biogas production, suggesting a succession in species of bacteria during the process of gas production. But some species such as *Bacillus* where found to be present throughout the process of gas production [16]. The result obtained from this study indicates that *Bacillus* specie were the most common bacteria isolated and identified during the research, suggesting that the species plays a vital role in the microbial activities for the production of biogas. It should be noted that *Bacillus megatarium*, *Bacillus licheniformis*, *Proteus vulgaris* and *Escherichia coli* were isolated during the second week were able to produce about 707cm³ of biogas, while *Bacillus firmus*, *Proteus vulgaris*, *Pseudomonas aeruginosa* and *Bacillus alvei* were isolated in the fifth week (35days) and produced, 1544cm³ of biogas gas.

Bacillus lentus, *Bacillus pumilus*, *Proteus vulgaris* and *Salmonella* sp occurred in the sixth week (42 days) and were able to produce 1335cm³ of biogas. However, *Staphylococcus aureus* and *Bacillus brovis* were isolated from the fourth week (28 days) and produced 1820 cm³ of biogas. The ability of *Bacillus* species to overlap during the production were probably due to the fact the organisms can produce spore which help them to withstand the harsh anaerobic condition or heat evolve during the biogas production [16]. These findings were in line with that of Oluyega., et al. [17] in which *Bacillus*, *Yersinia*, and *Pseudomonas* species were found to be responsible for biogas production from cow dung.

The pH of the slurry appeared to be decreasing in all the digesters. It is not surprising as the decrease in pH may be as a result of anaerobic fermentation taking place. pH is an important factor that affects biogas production. It was reported that anaerobic bacteria required a natural environment [18] and thus, pH ranging from 6.4-7.2 is required for optimum biogas production. Also, the decrease in pH may be due to the action of acetogenic methanogens as they break down sulphur containing organic and inorganic compounds as well as the formation of fatty acids. It was reported by Oyeleke., et al. [19] that biogas produced at pH of 5 is greater than that of pH 10. Some microorganisms also evolved later in the process while others died off midway through the process. This may be explained in terms of Shellford's law of tolerance that the occurrence of any organism in any environment is determined not only by availability of nutrients but also by various physicochemical factors. Therefore, as the medium tend to become acidic, non-acid tolerance organisms were replaced by acid tolerant organisms.

Results from this work showed that biogas was produced from the Cattle rumen content at different retention time. After the first week, there was a sharp increase in the volume of biogas produced in the second week. However, from the seventh to the eighth week the volume of biogas produced continued to decline. Therefore, it can be deduced that the increase in the second week indicated the acclimatization of the biogas producing microorganisms after the hydrolysis of the waste in the first week by the hydrolyzing organisms. The biogas production reached its peak in the second week and the action of biogas producing organisms decline and were replaced by organisms that tend to utilized some of the products of their actions. This probably explained the continued decline in the volume of biogas produced in the seventh and eighth week.

Also, the volume of biogas produced in the second week differed significantly to that of the third and fourth week. However,

no such significant difference was observed in the volume of biogas produced in the third and fourth weeks. This was in conformity to the findings of Bagudo., *et al.* [20] in which 8772.50 cm³ of biogas was produced from cow dung. Wahyudi., *et al.* [21] also reported the production of 2500cm³ of biogas from content of sheep colon at two weeks retention time [22].

Conclusion

The results of the research Signifies/indicated that Cattle rumen contents of abattoir can serve as a suitable substrate for the production Biogas. Results from this work showed that biogas was produced from the Cattle rumen content at different retention time. After the first week, there was a sharp increase in the volume of biogas produced in the second week. It should be noted that *Bacillus megatarium*, *Bacillus licheniformis*, *Proteus vulgaris* and *Escherichia coli* were isolated during the second week were able to produce about 707cm³ of biogas, while *Bacillus firmus*, *Proteus vulgaris*, *Pseudomonas aeruginosa* and *Bacillus alvei* were isolated in the fifth week (35days) and produced,1544cm³ of biogas gas. *Bacillus lenthus*, *Bacillus pumilus*, *Proteus vulgaris* and *Salmonella* spp occurred in the sixth week (42 days) and were able to produce 1335cm³ of biogas. However, *Staphylococcus aureus* and *Bacillus brovis* were isolated from the fourth week (28 days) and produced 1820cm³ of biogas. The utilization of this substrate for biogas production could eliminate its disposal problems and create another abundant source of sustainable energy.

Recommendations

Based on the results and findings of this study, the following recommendations were suggested for future experiment:

1. More research bodies and organizations should be created by the government or tertiary schools to translate this study into a high performing technology.
2. A means of sustaining mesophilic temperature should be developed, as productivity of biogas is higher at this temperature region.
3. To maintain the temperature of digester, it should be thermally insulated to prevent loss of heat and the material for the construction should be non-heat reflector.

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Volume 3 Issue 4 April 2019

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