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Biogas Generation using Cattle Rumen Contents

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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(40.25cm^3), day8-14(178.5cm^3), day 15-21(386cm^3), day22-28(333.75cm^3), day29-35(219.75cm^3), day36-42(212.25cm^3), 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 (2012) 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 (Ashok kumar, 1990).

The biogas production usually contains 50% and above methane (CH_4) and other gases in relatively low proportions namely, CO_2 , H_2 , N_2 , 0_2 and H_2 S. Most of the organic acids and all of the H_2 are metabolized by methanogens, with the end result being production of a mixture of approximately 55% to 70% CH_4 and 30% to 45% CO_2 , 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 above21°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 (Ioana and Cioabla, 2010).

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 receives little sunlight. As a result, a lot of methane and nitrous oxide is produced and released into atmosphere (Bejan and Rusu, 2007). 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 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 manor 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 community (Daxiong, 1990).

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 use generate energy; it would be a welcomed solution to the problem of waste Pollution, disposal and (Enweremadu *et al.*, 2004). Nowadays the use of bio-gas has spread from small farms to big animal farms. 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 community cilia and Kans war, 1998). 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 cacogenic glycosides (Orator, 1998). 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:

- To produce biogas from cattle rumen contents.
- To isolate microorganisms associated with the Generation of Biogas using the cattle contents.
- To evaluate the amount of biogas produced by the cattle rumen contents
- To determine the variation in pH during biogas generation.
- 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, Sarasin, Decolorize) , Glass slides, Urea medium in universal bottle, Glucose phosphate medium in a test tube, Drops of H_2O_2,napthol 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 Brining Debbi central abattoir in Brining Debbi 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 USANi Danfodiyo University, Soot.

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 (Abdulkadir, 2012).

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 250ml, 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 (Cowan and Steel 1983)

Serial Dilution

The fresh sample and the digested slurry sample were carried out up to 10^6 tubes. 0.5ml was obtained using sterile syringe from the 10^5 tube and inoculated onto already prepared nutrient agar plates by spread plate method of inoculation.

The plates were replicated three times. Modified Mackintosh and Files pattern of anaerobic jar was used to incubate the plates. The residual oxygen (O_2) 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 (Somekele and Mange, 2008).

Colony Count

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

Colony forming unit = $\frac{\text{Number of colonies.}}{\text{Inoculants size x dilution factor}}$ Percentage frequency of occurrence

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

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:

Step1: the colony was picked and fixed on the glass slide and it was heat fixed.

Step2: 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.

Step3: 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.

Step4: Decolorizer (Ethanol) was used next allowed for 30seconds which removes the primary stain from Gram Negative bacteria present. It was then washed.

Step5: Finally, counter stain (safari) was applied for 1minute, to stain those cells (Gram Negative) that have lost the primary stain as a result of decolonization. It was then washed. (Somekele and Manage, 2008).

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 color, while Gram-negative cells retained the counter Staining color of safari and appeared pink in color.

After Gram Staining and Microscopy, the isolates subculture into universal bottle containing nutrient agar in a slant form for subsequent used in biochemical test (Oyeleke and Mange, 2008).

Biochemical Test

Coagulase

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

Step1: Three test tubes were labeled as "Test", "Negative control", and "positive control".

Step2: Each of the test tubes was filled with 1ml of 1:6 dilution factor of human plasma in normal saline.

Step3: 0.1ml of the overnight broth Culture to the tube labeled test. Also 0.1ml of overnight broth Culture of known *S. aurous* to the tube labeled positive control and 0.1ml of sterile broth to the tube labeled Negative control.

Step4: All the tubes were incubated at 37°c and observed up to four hours. (Eyelike and Manage, 2008).

Catalase

A drop of 3% (v/v) H_2O_2 was placed on a slide. Using a glass slide, a bacterial Culture was then added. Presence of catalane was then observed by the formation of gas bubbles. (Cheese brought, 2006).

Urease

Slant of urea medium in universal bottle was inoculated with a lapful of the isolates by streaking. The bottles were then incubated for 24hours at 37°c. Change in coloration from yellowish orange to pinkish indicated unease positive (Somekele and Mange, 2008).

Methyl Red

Using a prepared Glucose phosphate medium in test tube, lapful of the isolates was inoculated and incubated at 37°c for 2days. To the two-day old culture, drops of methyl red solution were added. It was then shaken slightly and examined for the formation of red ring at the interface (Somekele and Mange, 2008).

Voges-Proskauer

Test: in Voges-Proskauer test methyl red was added first to the two-day old culture and 0.6ml 5% naphtha solution was added and shaken. The test tubes were then sloped and examined after 15minutes. A red color indicated a positive Voges-Proskauer reaction (Somekele and Mange, 2008)

Indole Production Test

loopful of the isolates was inoculated in a sterile nutrient broth. The incubation was done at 37°c for 48hours. After incubation, 0.5ml of Kodak's reagent was added and shaken. It was then examined after one minute. A red color in the reagent layer indicated insole production (i.e. insole positive), (Nwankwo and Joseph, 2014).

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 stopped red well. It was then incubated at 35-37°c and it was examined daily for blackening of the lower part of the strip (Cheese rough, 2006).

Citrate Utilization Test

A sterile Simon's citrate medium, a lapful of 24hours old culture was inoculated aseptically. It was then incubated at 37°c for 24hours after which was examined daily for turbidity for a period of 3days (Gebrezgabher *et al.*, 2010).

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 (Abdulkadir, 2012).

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 (Somekele and Mange, 2008).

If only the aerobic tube has turned yellow then the organism is able to oxidize glucose aerobically ("O"). By - products: CO_2 and although organic acids may be present at low rates (Abdulkadir, 2012). 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 CO_2 without significant production of acid, or (b) the organism is respiring the peptone (Abdulkadir, 2012).

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. Inoculums from a pure culture were 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), (Somekele and Mange, 2008).

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 (Abbasid, 1993). 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, (Cheese brought, 2006).

The Biogas Generated by the samples was recorded in table 4.1. 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 4.2 shows the bacteria isolated based on morphological and biochemical characteristic cs. The percentage frequencies of occurrence of the isolates in relation to all samples are shown in the table 4.3. The isolated bacteria were Yesinia entrocolitica, bacillus megatherium, bacillus lichen formis, Escherichia coli, Pseudo monas aeruginosa, bacillus firms, staphylococ cus aurous, Salmonella sop, Proteus vulgarism, Bacillus alive, and bacillus lint us.

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%) Yesinia entrocolitica, Pseudomonas aeruginosa, Staphylococcus aur ous.

RESULTS

Table1. *The daily volume of biogas produced at retention time of eight (8) weeks*

Retention time (in days)	N1 (cm^3) Volume of	N2 (cm^3) Biogas	N3 (cm^3) Produced	N4 (cm^3) [cm^3]	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	

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

Table2. *Species of bacteria isolated during biogas generation base on morphological and biochemical characteristics.*

	N1	N2	N3	N4	N5	N6	N7	N8	N9	N10	N11
Gram reaction	+	-	-	+	-	-	-	+	+	+	+
Coagulase	-	+	+	+	+	+	+	+	+	+	+
Catalase	-	-	-	-	+	+	-	+	+	-	+
Urease	+	+	-	-	+	-	-	+	-	+	-
MR	+	-	-	+	-	-	+	-	+	+	-
VP	-	-	-	-	+	+	-	+	-	-	+
Indole	-	-	+	-	-	-	-	-	-	-	-
H_2 S	-	+	-	-	-	+	+	-	-	-	+
Citrate	-	+	-	+	+	+	+	+	-	-	+
Lactose	-	-	-	-	-	-	-	-	-	-	-
Motility	+	+	+	+	-	+	+	+	+	+	+
Glucose	+	+	+	-	+	+	+	+	+	+	+

***Key: N1**= Yesinia entrocolitica; **N2**= Proteus vulgar is; **N3**= Escherichia coli; **N4**= Pseudomonas aeroginosa; **N5**= Staphylococcus aurous; **N6**= Bacillus elaterium; **N7**= Salmonella sop; **N9**= Bacillus lint us; **N10**= Bacillus firms; **N11**= Bacillus alive; **MR**= Methyl red; **VP**= Voges-Proskauer*

Table3. *Number and Percentage of occurrence of bacteria isolated in the cattle rumen content*

Bacteria	Frequency of Occurrence	% Occurrence of Isolate
Bacillus sop	16	48%
Yesinia enterocolitica	2	6%
Proteus vulgar is	5	15%
Escherichia coli	3	9%
Pseudomonas aeroginosa	2	6%
Staphylococcus aurous	4	13%
Salmonella sop	1	3%
Total	33	100%

Table4. *PH of Digesters before and after Biogas Production*

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

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

Table5. *The colony count of organisms isolated*

Retention Time(In Days)	Bacterial Load/Count (Cfu/Ml)
1-7	$2.5X10^7$
8-14	$4.2X10^7$
15-21	$2.3X10^7$
22-28	$2.08X10^7$
29-35	$1.83X10^7$
36-42	$1.03X10^7$
43-49	$0.07X10^7$
50-56	$0.02X10^7$

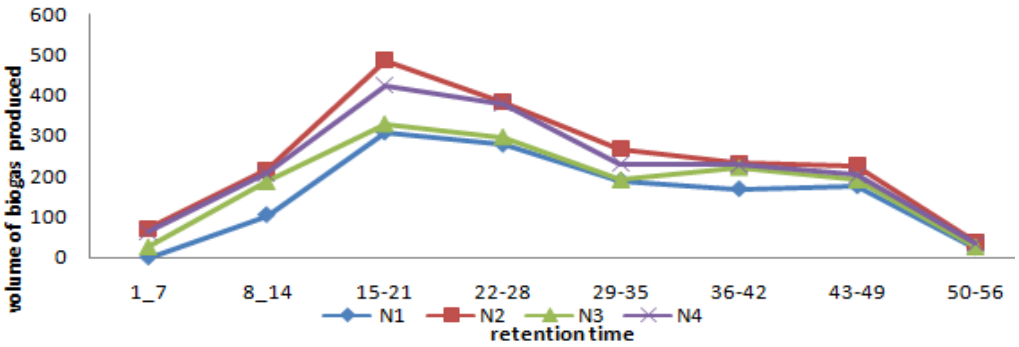

Fig1. *A graph of average volume of Biogas produced against retention time (in days)*

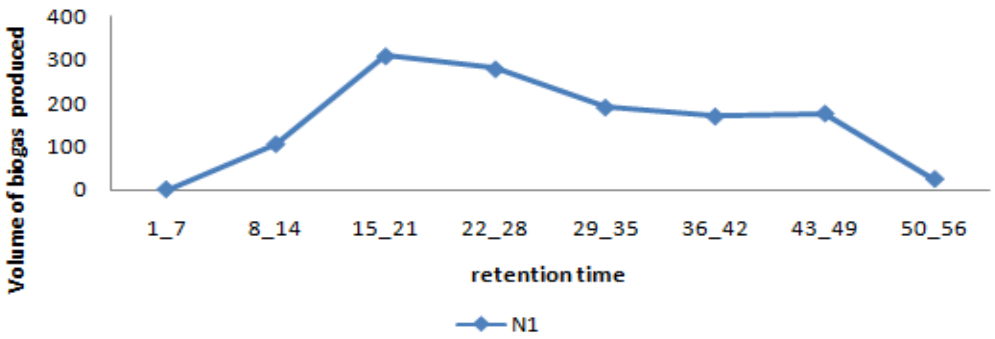

Fig2. *A graph of volume of Biogas produced by Digester N1 against retention time (in days)*

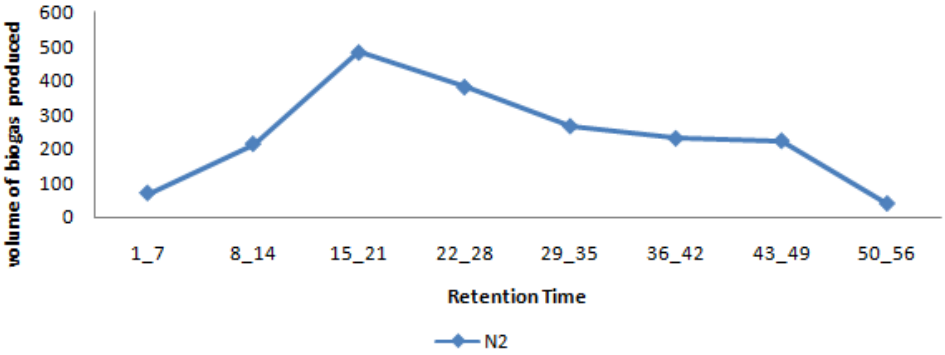

Fig3. *A graph of volume of Biogas produced by digester N2 against retention time (in days)*

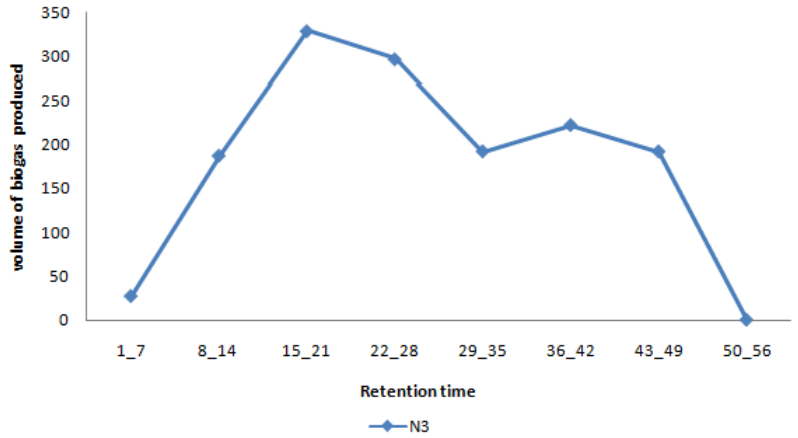

Fig4. *A graph of volume of Biogas produced by digester N3 against retention time (in days)*

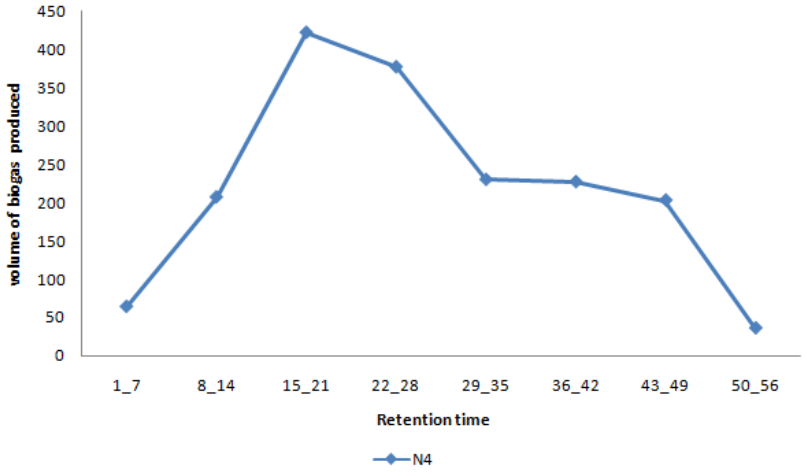

Fig5. *A graph of volume of Biogas produced by digester N4 against retention time (in days)*

DISCUSSION

The results from this Research showed *Bacillus* species appears 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 (Bake, 2004). 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 vagaries* and *Escherichia coli* were isolated during the second week were able to produce about 707cm^3 of biogas, while *Bacillus firms, Proteus vulgar is, Pseudomonas aerug inosa* and *Bacillus alive* were isolated in the fifth week (35days) and produced ,1544cm^3 of biogas gas. *Bacillus lentos, Bacillus pummels, Proteus vulgarism* and *Salmonella sp* occurred in the sixth week (42 days) and were able to produce 1335cm3 of biogas. However,

Staphylococcus aurous and *Bacillus braves* were isolated from the fourth week (28 days) and produced 1820 cm^3 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 (Bake, 2004). These findings were in line with that of Oluyega *et al*., (2006) in which *Bacillus*, *Yesinia*, 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 (Garb & Atoka, 1992) 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 sculpture containing organic and inorganic compounds as well as the formation of fatty acids. It was reported by Eyelike *et al.*, (2003) 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 Shell ford'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 eight 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 Baguio *et al*.,(2008) in which 8772.50 cm^3 of biogas was produced from cow dung. Wahid *et al*., (2010) also reported the production of 2500cm^3 of biogas from content of sheep colon at two weeks retention time.

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 moratorium, Bacillus licheniformis, Proteus vagaries and Escherichia coli were isolated during the second week were able to produce about 707cm3 of biogas, while Bacillus firms, Proteus vulgarism, Pseudomonas aeruginosa and Bacillus alive were isolated in the fifth week (35days) and produced ,1544cm3 of biogas gas. Bacillus lentos, Bacillus pummels, Proteus vulgarism and Salmonella sop occurred in the sixth week (42 days) and were able to produce 1335cm3 of biogas. However, Staphylococcus aurous and Bacillus braves 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:

- More research bodies and organizations should be created by the government or tertiary schools to translate this study into a high performing technology.
- A means of sustaining hemophilic tempera ture should be developed, as productivity of biogas is higher at this temperature region.
- 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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