

Full Length Research Paper

Studies on the microbial spectrum in anaerobic biomethannization of cow dung in 10 m³ fixed dome biogas digester

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Accepted 28 July, 2010

This study investigates the generation of biogas from cow dung using a constructed 10 m³ family-size digester at the National Center for Energy Research and Development, University of Nigeria, Nsukka. The cow dung was procured from Nsukka main market central abattoir. Production of biogas was monitored over a period of 20 days. Standard methods were used to determine pH, temperature, total viable counts, total solid, volatile solid, mould count, coliform count, ash content and moisture content of the digesting mixture. Results showed that cow dung commenced flammable gas production on the 2nd day after charging the digester. Optimum gas production was recorded on the 7th day. As digestion proceeded, pH increased to a maximum of 7.8 on the 7th and 8th day. Total viable counts increased by over 307%, while total solids and volatile solids decreased to 36.9 and 49.2% respectively. Result also showed that the waste produced maximum biogas within the optimum temperature range of 35 - 42°C but at different periods. The biogas generated sustained cooking of three different foods daily for two weeks without exhausting.

Key words: Biomethane, anaerobic digestion, innoculum, digestate.

INTRODUCTION

Biogas refers to a mixture of gases produced by anaerobic digestion of agricultural and animal wastes. It consists of varying percent of methane, carbon dioxide, nitrogen, hydrogen, ammonia and water vapor. Biogas is used for direct combustion in cooking or lighting applications, or to power combustion engines for motive power or electricity generation.

Like natural gas, biogas can be directly used for heating. A cubic meter of biogas with 60% methane content can substitute approximately 0.6 cubic meters of natural gas or 0.6 L of fuel oil during electricity generation in a combined heat and power (Kapdi, 2003). This type of energy generation is practically carbon dioxide-neutral as the green house gases released during the combustion have been previously consumed by the plants. Considerable quantities of biogas have been generated by anaerobic digestion of poultry droppings, pig and cow dungs. Fermented substrate (digestate) from the biogas

production can be used as high quantity fertilizer.

There are a number of bacteria that are involved in the process of anaerobic digestion including acetic acid forming bacteria (acetogens) and methane forming archaea (methanogens). These organisms feed upon the initial feedstock, which undergoes a number of different processes converting it to intermediate molecules including sugars, hydrogen and acetic acid before finally being converted to biogas. Different species of bacteria are able to survive at different temperature ranges. The ones living optimally between 35 - 40°C are called mesophiles of mesophilic bacteria. Some of the bacteria can survive at the hotter and more hostile conditions of 55 - 60°C, these are called thermophiles or thermophilic bacteria (Anon, 2007).

MATERIALS AND METHODS

Cow dung was procured from Nsukka central abattoir, weighed out and mixed with water in ratio of 2:1 (water : cow dung) and fed into a 10 m³ digester as shown in Figure 1. The content of the two-third capacity filled digester was properly mixed together with a wooden

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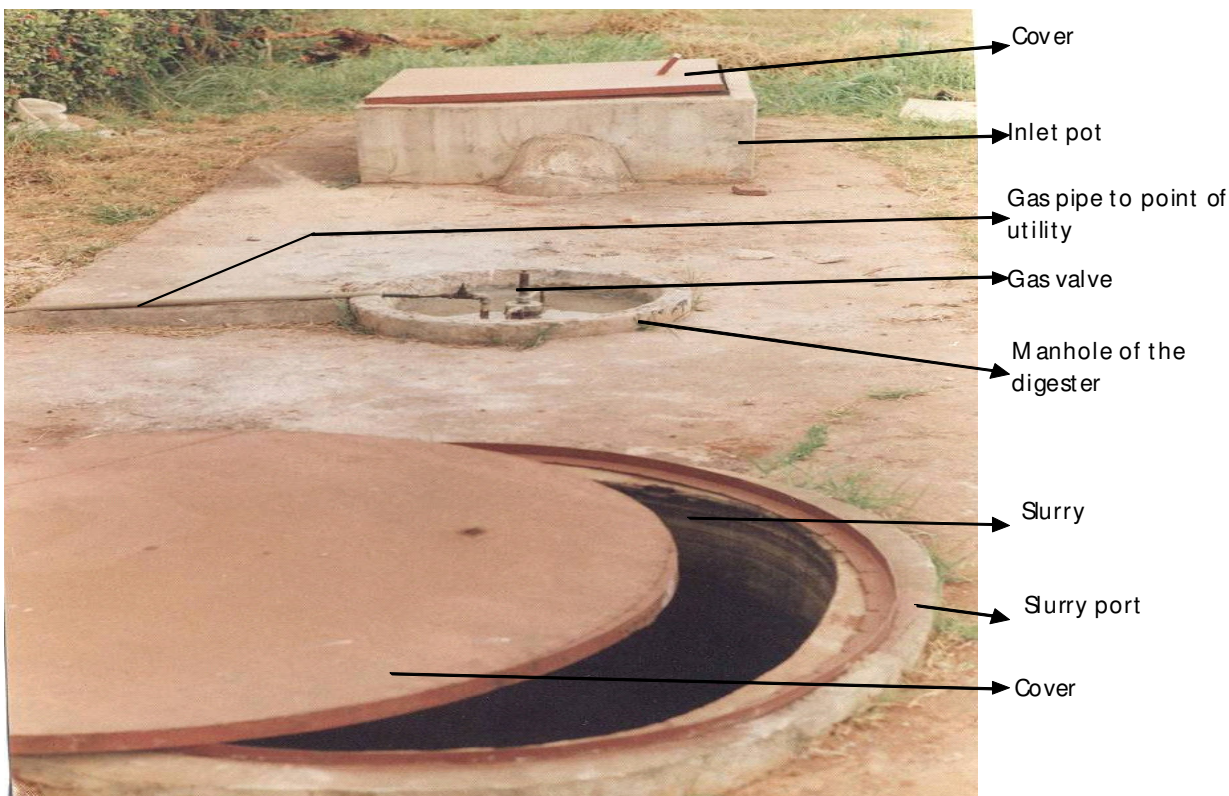


Figure 1. 10 m³ dome biogas digester.

pole and covered. The temperature of the biodigester was monitored daily with the aid of a thermometer.

Ash content determination

The method recommended by AOAC (1995) was used of which about 7 g of sample was weighed into a crucible of known weight and placed in temperature controlled furnace pre-heated to 900°C. After ashing, the crucible was transferred to a desiccator, cooled and weighed. The percentage ash was determined as follows:

$$\% \text{ Ash} = \frac{\text{Weight of dish with Ash} - \text{Weight of empty dish}}{\text{Weight of sample}} \times \frac{100}{1}$$

Moisture content determination

The moisture content was determined using AOAC (1995) method. About 15 g of sample was weighed in covered dish previously dried at 98-100°C, cooled in desiccator and weighed soon after reaching room temperature. Cover was loosened and heated at 98 -100°C to constant weight. At the end of drying, cover was immediately tightened on dish, transferred to desiccator and weighed soon after reaching room temperature.

$$\% \text{ moisture} = \frac{\Delta \text{SB} - \Delta \text{SA}}{\text{Wt of sample}} \times \frac{100}{1}$$

ΔSB = Weight of dish and sample before drying

ΔSA=Weight of dish and sample after drying

Total solids determination

This was determined using AOAC (1995) method. The moisture content was determined and the weight of the residue was reported as total solid (TS).

$$\text{TS} = \frac{\text{Weight of dry waste}}{\text{Weight of wet waste}} \times \frac{100}{1}$$

pH determination

This was determined using AOAC (1995) method. Electrodes were thoroughly wetted and prepared according to the manufacturer's instructions. Instruments were standardized with standard buffer solution of pH 4.0 and 9.0. Sample was analyzed as soon as possible. The reading was taken when equilibrium, as shown by the absence of drift, was established.

Total mould count

The mould count, using malt extract agar, was carried out using the method of Frazier and Westhoff (1995). 65 g of malt extract agar was dissolved in 1 liter of distilled water and sterilized in the autoclave together with required number of bijou bottles containing 9 ml of distilled water at 120°C for 15 min. 1 g of the sample was macerated in the 9 ml sterilized water. Dilution of up to 10⁻⁴ was

made by taking 1 ml from the first dilution bottle to the second bottle then 1 ml from the second dilution bottle to the third bottle and so on. The dilutions were shaken very well for proper mixing before they were transferred to the next dilution bottle. 1 ml of solution from the fourth and third dilution bottles were pipetted into sterile Petri dishes and 15 ml of the malt extract agar was added. The autoclave was then switched on to homogenize the mixture. Incubation was carried out at room temperature for 48 h. The mould colonies from the plate were counted and calculated as forming units per gram of sample.

Total viable count determination

This was determined using the method of Frazier and Westhoff (1995). Under aseptic condition, 1 g of the sample was macerated in 9 ml distilled water. 1 ml of the sample was pipette into a test-tube and then made into serial dilution of up to 10^{-4} . 1 ml of the appropriate dilution was pipette into a sterile Petri dish into which about 15 ml of sterile nutrient agar (culture medium) was poured. After inoculation, the plate was allowed to solidify. The dish was turned upside down to avoid condensation of water on the agar. The dish was incubated at 37°C for 24 h. After incubation, the number of colonies formed was counted.

Volatile solids determination

This was carried out according to the procedure in standard method of ALPHA (1976). 1 g of solid residue obtained from the total solid was transferred into a crucible charred over a flame and transferred to a cold muffle furnace and treated at 900°C for 2 h. The sample was removed from the furnace, cooled in a desiccator and weighed. The loss in weight represents the volatile solids (VS).

$$\text{VS} = \text{Total Solid} - \left\{ \frac{\text{Weight of dry solid (900}^{\circ}\text{C)}}{\text{Weight of wet waste}} \right\} \times \frac{100}{1}$$

Total coliform count determination

Total coliform count was determined using the method of Frazier and Westhoff (1995). The culture media, macConkey, was prepared and 9 ml of sterile distilled water was pipetted into each of the required number of bijou bottles. The medium and dilution bottles were sterilized in the autoclave for 15 min at 121°C . 1 g of sample was macerated into 9 ml sterilized water in a bijou bottle. From there, 10^{-4} dilution was made. 1 ml of the 10^{-4} dilution was pipetted into a sterile Petri dish and 15 ml of macConkey agar was poured into it and allowed to solidify. The plate was inverted and incubated at 37°C for 24 h. Colonies were then counted after incubation.

Test for combustibility of biogas

Methane which is a major component of the biogas has combustible characteristics. Combustibility test was carried out every day to know when methane gas was produced. This was tested by connecting a rubber hose to the biogas digester and burner and lighting the burner.

Collection of sample

The samples used for the isolation of macro-organism were

collected from the digester containing degrading cow dung.

Media preparation

Nutrient agar was used. Autoclave sterilization of all media was done at 121°C for 1 min at 15 lb temperature.

Processing of sample

About 10 g of each sample was weighed out and introduced into a flask containing 90 ml of sterile distilled water. The flask was then manually agitated to release attached vegetable cells and spores. It was then allowed to stand for some seconds so that the large particles could settle. This gave a dilution of 10^{-1} W/V. The resulting solution was further diluted serially to 10^{-5} W/V using a sterile pipette. These flasks were kept to be used in the plate inoculation.

Inoculation

Using sterile pipette, 1 ml of each of the 10^{-4} and 10^{-5} dilution was used to inoculate the nutrient agar plates. Plates were then inoculated at 37°C and observed daily. Subcultures were done on the previously prepared slants of the same medium, when growth was observed in the incubated plate. When pure cultures were obtained in the slants, they were then kept for identification.

Identification of bacterial isolates through Gram staining

The bacterial isolates involved in the degradation of the cow dung were identified through biochemical and physiological tests and Gram staining. Suspensions of the isolates were made using sterile saline. This was smeared on a slide and heat-fixed by passing over a flame 3 times. The slide was then flooded with crystal violet and drained off after 1 min. It was blotted and dried and oil was applied on the slide and viewed under the microscope using $\times 100$ magnification.

RESULTS AND DISCUSSION

Proximate composition of the cow dung

Table 1 shows the proximate composition of the cow dung and digestate. It is apparent that there was a decrease in the total solid, the volatile solid and ash content while total viable count increased. This is due to the actions of the micro-organism in the digester. During digestion, the anaerobes broke down the complex components of the waste, leading to a decrease in the total solids and volatile solids to 36.9 and 49.2% respectively. There was also a decrease in the ash content after digestion because the nutrients were depleted by the anaerobic during digestion.

Increase in the total viable count (TVC) after digestion could be due to the favorable condition of the digester during digestion. Anaerobes thrive in the absence of oxygen. Earlier works by McCarthy (1964) have shown that methane formation does not occur in environment where electron acceptors such as oxygen, sulphates or nitrites are readily available. The cow dung which was

Table 1. Proximate composition of cow dung.

Components	Amount before digestion (%)	Amount after digestion (%)	Percent change ($\Delta\%$)
Total solids	19.8	7.3	-63.1
Volatile solids	6.5	3.2	-50.8
Moisture content	80.2	-	-
Ash	50.7	18	-64.5
Total viable count	1.08×10^6	4.4×10^6	+307.4

Table 2. Biogas generation per day and changes in other physical parameters.

Time (days)	pH value	Ambient temperature ($^{\circ}\text{C}$)	Slurry temperature ($^{\circ}\text{C}$)	Biogas generated I liters (L)
1	6.21	28	26	5
2	6.25	34	32	10
3	6.70	34	30	15
4	7.0	36	33	20
5	7.50	32	27	25
6	7.60	34	32	30
7	7.80	36	25	33
8	7.80	34	33	40
9	7.11	35	25	45
10	7.20	25	24	50

initially at aerobic state before digestion had relatively smaller number of bacteria but after being degraded anaerobically, the number increased due to the anaerobic condition in the digester.

Changes in pH during fermentation

Immediately after charging the waste, the pH of the digester was 6.21 (day one) but as fermentation takes place the pH value of the waste started rising from 6.21 which is acidity to 7.11 which is alkaline, this was noticed from the 3rd day of fermentation when the pH value of the waste was 7.5. The reason for the low pH at the initial period of digestion of waste is attributed to the fact that initially, the acidogens were breaking down the organic matter and producing volatile fatty acids. As a result the acidity of the medium increased and the pH fell below neutral. Therefore, the acid formers were most probably displaced by the methane forming bacteria. This accounted for the breakdown of acids by the methadone to methane and the gradual rise of pH values from 7.5 - 7.11.

From Table 2, it was noted that pH of the waste was acidic on the first day of fermentation (6.21) but the pH value started rising from the 3rd (6.7) to 9th day (7.11). The highest gas production was noted on the 8th day of fermentation when the pH of the waste was 7.8 while the

temperature was 31.0 $^{\circ}\text{C}$. This shows that pH and temperature are important factors in biogas production. It was also noted that after the 9th day of fermentation, the pH started falling again from 7.11 to 6.10. The drop in pH value was evidence that the acidogens once more displaced the methanogens by the former acids thereby inhibiting methanogenesis and adversely affecting biogas generation from the waste. This was noticed when the volume of gas produced per day was checked, it was observed that the volume produced started declining after 12th day when the pH was 7.2. The result that the pH changes with time agrees with earlier work of McCarthy (1964), who reported that methane proceeds quite well as long as the pH is maintained between 6.1 - 7.8 with an optimum range between 7.0-7.2.

Methane is the major component of the biogas and it comprises about 75% of the gas produced. Biogas is a flammable gas and a fairly long-grade source of energy. It can provide intense localized heat compared to energy from solar panel which provides a generated warmth distribution (Meynell, 1982). One important use which requires high grade is cooking. It is also used for lighting and drying. The ability to utilize biogas in this operation is due to its ability to burn and its fuel value of 18-24 /m³ (Eze 2003). This was done on daily basis to determine when the gas started producing flammable gas and it was noted that the digester containing cow dung produced flammable gas after the 7th day of charging.

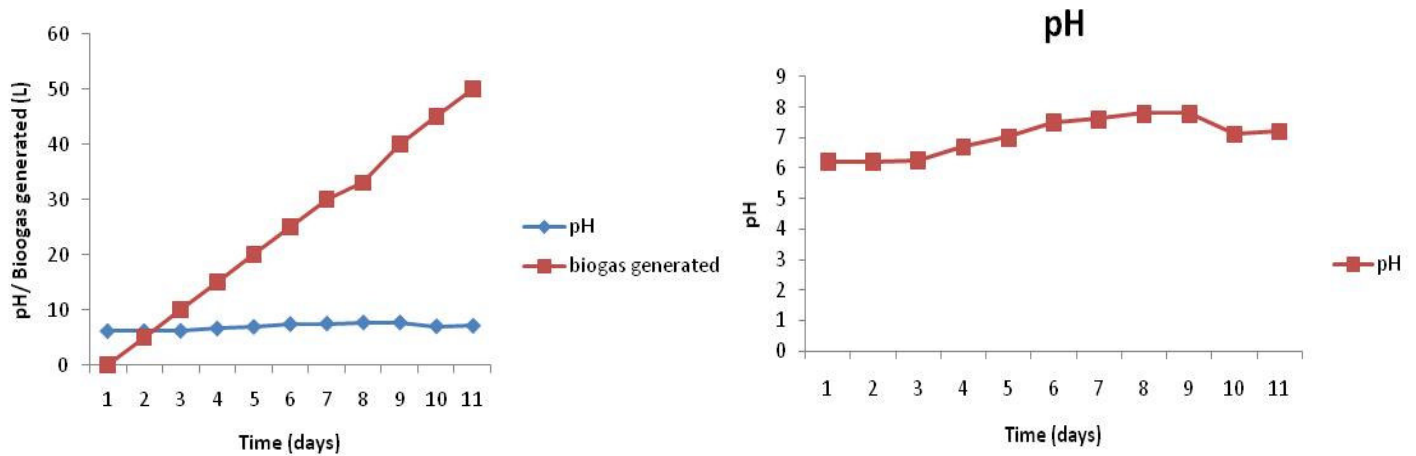


Figure 2a. Changes in slurry pH with time and biogas volume.

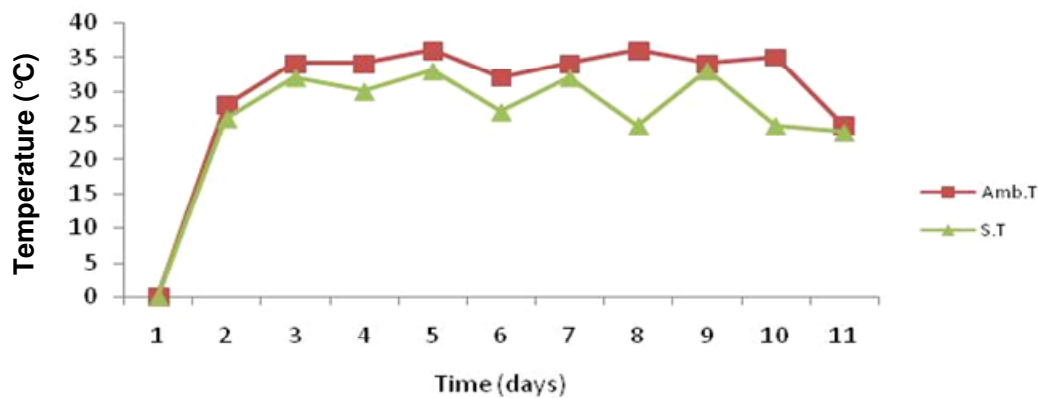


Figure 2b. Changes in ambient temp. over a time period. Amb.T. = Ambient temperature. S.T. = Slurry temperature.

Changes in total solids and volatile solids during the anaerobic digestion

Volatile solids are those made of organic matter as opposed to total solids which also contain the inorganic material such as carbohydrates, proteins, lipids, minerals and salts (Meynell, 1982).

Results observed are in accordance with the earlier work by Meynell (1982), who said that the reduction in total solids and volatile solids content through digestion of organic waste is a useful preliminary index of the efficiency of the digester at reducing the organic matter within the biodigester. The results obtained from the present study support Meynell's claim, who reported that high concentration of waste could be inhibitory to biogas production.

Changes in total viable count and total coliform count during the anaerobic digestion

The results obtained showed increase in the total viable

count (TVC) due to the fact that the organisms were predominately anaerobes and the environment was favorable for them, and there was decrease in the total coliform count. During the initial stage of fermentation, coliform predominated. This is due to the source of wastes as an agro-industrial and fecal contaminant respectively.

Combustibility of the biogas

Biogas was finally tested and it was confirmed that the biogas was combustible. This was tested by connecting a hose to the cylinder and to the biogas burner and lighting the burner. A bluish flame glowed and the glowing lasted for several seconds.

Table 2 shows the effects of slurry, the ambient temperature was slightly higher than that of the slurry temperature (Figure 2a). This results supports the earlier claim by Ferguson (2006). The graph in Figures 2b and c shows that temperature is one of the most important factors that affects the production of biogas, the ambient

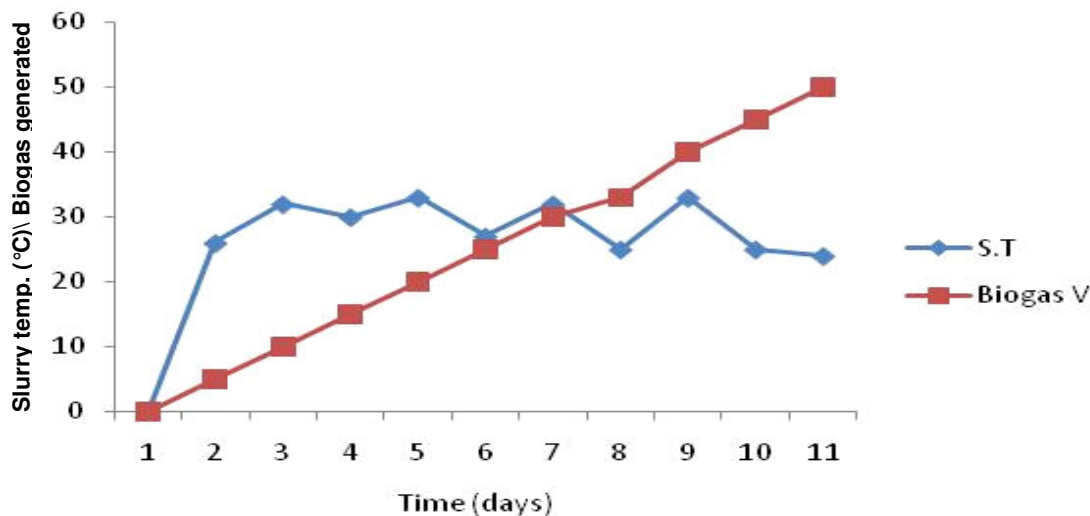


Figure 2c. Slurry temperature changes with biogas volume over time. S.T. = Slurry temperature. Biogas V = biogas generated.

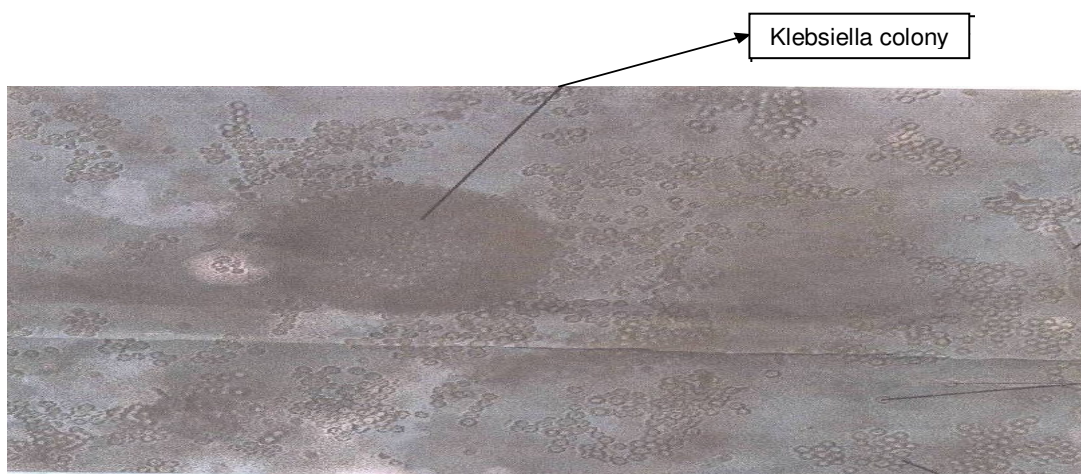


Figure 3. Microscopic structure of *Klebsiella*.

temperature was slightly higher than that of the slurry temperature.

Isolation and identification of bacteria

After isolation, two bacterial colonies were identified to be present. Pink pin-point colonies were observed when viewed under the microscope. They were suspected to be *Enterococci*, possibly *Klebsiella*. Figure 3 is the diagrammatic representation of this bacterium under microscope.

The second colony was identified to be bacillus which belongs to bacteroides, the main protein-hydrolyzing bacteria in the biogas digester. Under the microscope,

the organism was found to be large, pink and mucoid (Figure 4).

The organism found under the microscope was wrinkled white colonies that are about 5 – 10 mm in diameter. *P. restrictum* is also a spore forming micro-organism.

The presence of the mycellium was as a result of the cross combination of the hyphae which was formed by the spores after germination.

Conclusion

Cow dung was used to generate biogas at pre-determined ratio. The digestion was carried out for a

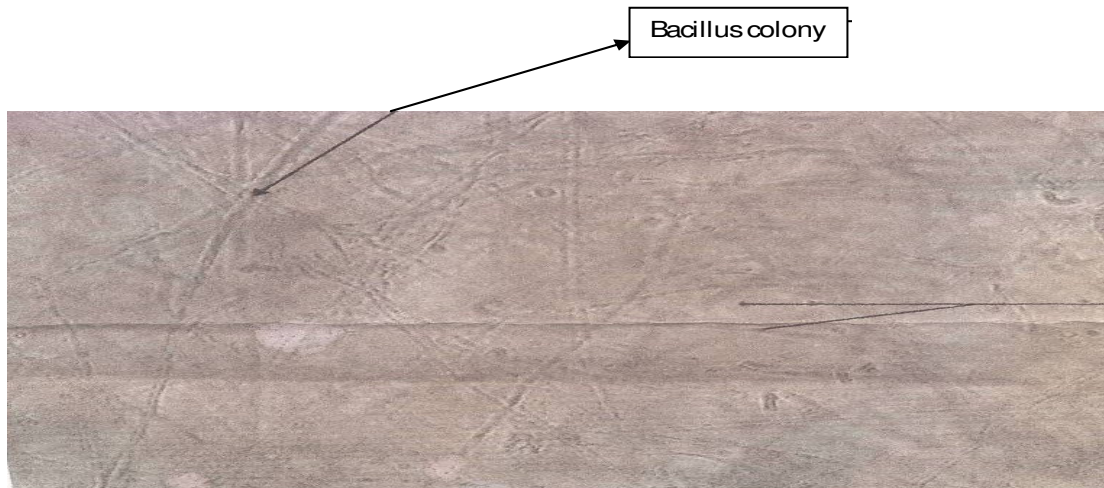


Figure 4. Microscope structure of *Bacillus*.

period of twenty days. Many factors were noted to have affected the production of biogas such as pH, temperature and microbial population. It was observed that the highest temperature recorded was 36 °C, which was good enough for efficient biogas production and effective operation. The ambient temperature was enough to operate the digester efficiently. Combustible biogas produced was found to be generated after 2 days of charging.

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