

**BIOGAS PRODUCTION FROM COW DUNG FOR SUSTAINABLE ENERGY GENERATION*****Fatima Mukhtar¹, Aliyu Abubakar¹, Ummasalma Aliyu Saulawa¹ and Hadiza Garba²**¹Department of Microbiology, ²Ibrahim Shehu Shema Centre For Renewable Energy Research, Umaru Musa Yar'adua University, Katsina

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Abstract

Microbial degradation of organic material under aerobic and anaerobic conditions is a key process within the natural metabolism of ecosystem. This term is often used in relation to energy, waste management and is now commonly associated with the anaerobic generation of methane. Microbial degradation and Biogas production from cow dung was investigated. Fresh cow dung was collected from Abattoir, Katsina State, Nigeria. The sample was collected in a large clean plastic container. Batch type anaerobic digesters were designed using Karki's Biogas model. The produced biogas was collected by water displacement method. The production started on the sixth day of fermentation and followed an increasing trend. It reached its peak on the seventeenth day before a gradual fall in production rate. The Physico-chemical and microbiological analysis of the animal wastes were carried out before, during and after the digestion process. The pH range was 6.51 - 7.89. The temperature remained at mesophilic range (25-45°C) throughout the study. Data on % total solids, and ash content were also determined. The total aerobic and anaerobic bacterial counts ranged from 8.5×10^6 - 2.3×10^7 and 1.5×10^6 - 1.2×10^7 cfu/ml, respectively. The bacteria isolated were *Klebsiella sp*, *Escherichia coli*, *Bacillus sp*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Clostridium sp*. Biogas production from cow dung is a good and cheap alternative source of energy. The use of biogas will not only serve as a source of fuel but will also help in the management of waste. Large scale production of biogas from wastes should be undertaken as the wastes around you can be converted to wealth.

Key: Biogas, Fermentation, Physicochemical and Microbiological Analysis**INTRODUCTION**

Microbial degradation of organic material under aerobic and anaerobic conditions is a key process within the natural metabolism of ecosystem (Ojo, 2007). This term is often used in relation to energy, waste management and is now commonly associated with the anaerobic generation of methane.

Anaerobic digestion provides exciting solutions to alternative energy production, handling human, animal, municipal and industrial wastes safely, controlling environmental pollution, and expanding food supplies (Ofoefule and Uzodinma, 2006).

Biogas is a colorless, flammable gas produced via anaerobic digestion (fermentation) of animal, plant, human, industrial and municipal waste to produce methane (50-70%), Carbon dioxide (20-40%) and traces of other gases such as nitrogen, hydrogen, ammonia, hydrogen sulphide and water vapour (Ofoefule and Ibeto, 2010). However, the composition of the mixture depends on the source of biological waste and management of digestion process. Biogas production comprises of three biochemical process, which includes;

hydrolysis, acidogenesis/acetogenesis, and methanogenesis (Nagamani and Ramasamy, 1999). Complex molecules (carbohydrate, protein, fats) are broken down into smaller products (acetic acid, H₂/CO₂, monocarbon compounds and organic fatty acids larger than acetic) by fermentative bacterial action. Fatty acids longer than acetate are metabolized to acetate by obligate hydrogen-producing acetogenic bacteria. Hydrogen and carbon dioxide can be converted to acetate by hydrogenoxidizing acetogen or methane by acetoclastic methanogens (methanogenesis) (Nagamani and Ramasamy, 1999) at pH between 6.0-8.0 and ambient temperature between 25°C - 40°C in a bioreactor (digester) under anaerobic condition.

Cow dung can be defined as the undigested residue of consumed food material being excreted by herbivorous bovine animal species. Being a mixture of faeces and urine in the ratio of 3:1, it mainly consists of lignin, cellulose and hemicelluloses. It also contains 24 different minerals like nitrogen, potassium, along with trace amount of sulphur, iron, magnesium, copper, cobalt and manganese (Muhammed, 2015).

The aim of this research is to generate biogas from cow dung and the Specific objectives include: determination of the pH and temperature at which biogas is produced at optimum, determination of total aerobic and anaerobic count before, during and after the anaerobic digestion, to isolate and identify some possible bacteria involved in the biogas generation

MATERIALS AND METHODS

Sample Collection

Fresh cow dung was collected from Katsina Modern Abattoir, Katsina State, Nigeria. The sample was collected in a large clean plastic container and transported within 24 hours to the laboratory for analysis. All media used during this study were prepared according to manufacturer's instructions.

Digester Design

Batch type anaerobic digesters were designed in this research using a combination of the Karki's Biogas model. The laboratory scale digesters were constructed using five-litre empty plastic gallons with three openings one for slurry inlet, the second as gas outlet while the third for slurry collection. The openings of the digesters were drilled and fixed with valves. Each valve was further fitted with rubber ring to ensure no air is entering.

Slurry preparation

Preparation of slurry for biogas generation was carried out according to the methods described by Asikonget *al.* (2013). 500g of the wastes (cow dung) was weighed and mixed thoroughly with 1000ml of water in the ratio of 1:2.

Feeding of Digesters

The mode of feeding used was a discontinued feeding (batch feeding). This simply means loading the digester at once and maintaining a closed environment throughout the retention period. The mixture of the waste was poured into the digester and was allowed to undergo anaerobic digestion for a retention period 22 days.

Biogas Collection

The biogas was collected by downward displacement of water. The water displacement method of biogas collection is a method in which gas is allowed to replace water at equal volume of water displaced and this will be used to determine the volume of gas produced daily. The biogas produced from the digester was connected to a separate inverted 1000ml measuring cylinder. The volume of displaced water was recorded as the volume of gas produced.

Test for the Presence of Methane in the Biogas Produced

Methane which is a major component of the biogas has combustible characteristic. The presence of the methane was tested by lighting flame on a Bunsen burner connected to the digester.

Analysis of Physicochemical Parameters

The physicochemical composition of the feedstock were evaluated before and after digestion using standard procedures Parameters analyzed includes Temperature, pH, Moisture content, total solids, Ash content and Biochemical oxygen demand (BOD).

Bacteriological Analysis

Total Bacterial Count

Total viable plate count was conducted on undigested and withdrawn samples during digestion. Each sample was aseptically collected, introduced into peptone water broth medium in sterile tubes. 1ml from each sample was serially diluted in 9ml sterile physiological saline. Dilution 10^{-5} was spread on Nutrient agar. All inoculated plates in triplicates were incubated at 37°C for 24hrs under aerobic and anaerobic conditions to obtain total aerobic and anaerobic bacterial counts. After incubation, the number of emergent colonies on each plate were counted, recorded and each value represented the mean of triplicate plating.

Isolation and Identification of Bacterial Isolates

The spent slurry in the digesters was subjected to microbial analyses at the end of the anaerobic digestion. A small portion of digested slurry (sludge) was serially-diluted using the plating method of Harrigan and McCance. The 10^{-5} dilution was cultured on Nutrient agar, MacConkey agar, Mannitol salt agar (MSA), Cetrimide agar and Eosin Methylene Blue, after incubation the isolates were purified, gram stained and subjected to microscopy.

Spore Staining Test and Biochemical Characterization of the Isolates

Spore staining test and series of biochemical tests were carried out on the isolates in order to characterize and identify the organisms. These includes; indole, Methylred, VogesProskauer, Catalase, Citrate utilization, Coagulase, Hydrogen sulphide, Urease and Oxidase tests.

RESULTS

Anaerobic digestion and Biogas production

The anaerobic digestion of cow dung obtained from Katsina Modern Abattoir generated 400 ml of biogas over the three-week digestion period.

Figure 1 shows the graph of the daily gas production; the production started on the sixth day of fermentation with 110ml and followed an increasing trend. It reached its peak on the

seventeenth day before a gradual fall in production rate was recorded for the rest of the study period and 400 ml was obtained on the final day of the experiment.

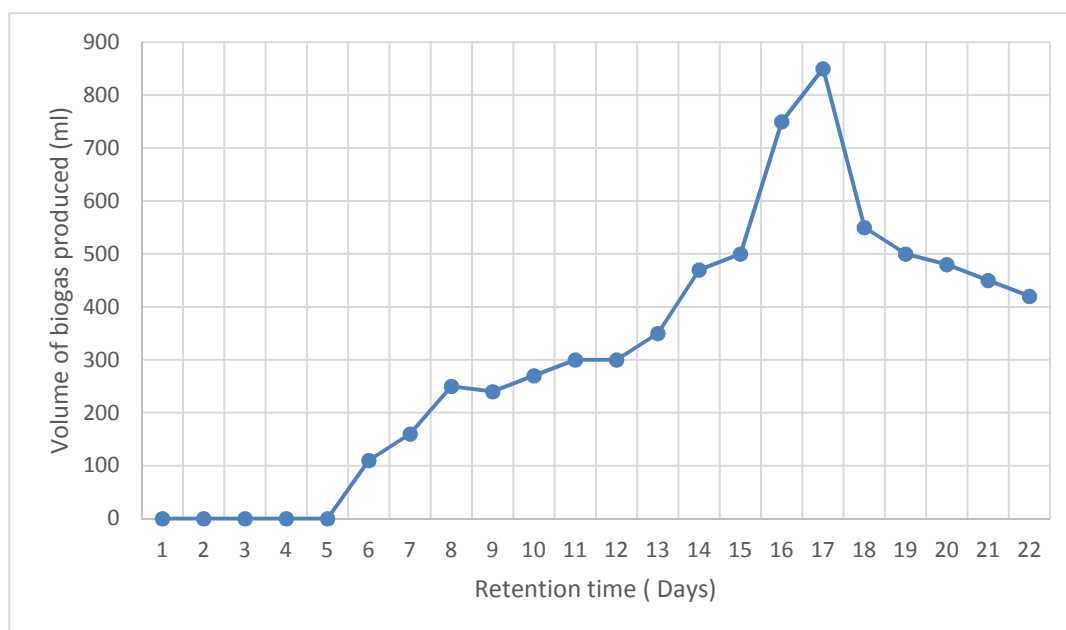


Figure.1 shows the daily biogas production.

Figure 2 shows the mean daily records of slurry temperature during the digestion process. The temperature remained at mesophilic range (25-33°C) throughout the study. The lowest temperature reading of 24.25°C was obtained

on the third and eleventh day while the highest of 30.5°C, 32.25 and 31.0°C were recorded on the sixteenth, seventeenth and eighteenth day of the digestion process respectively.

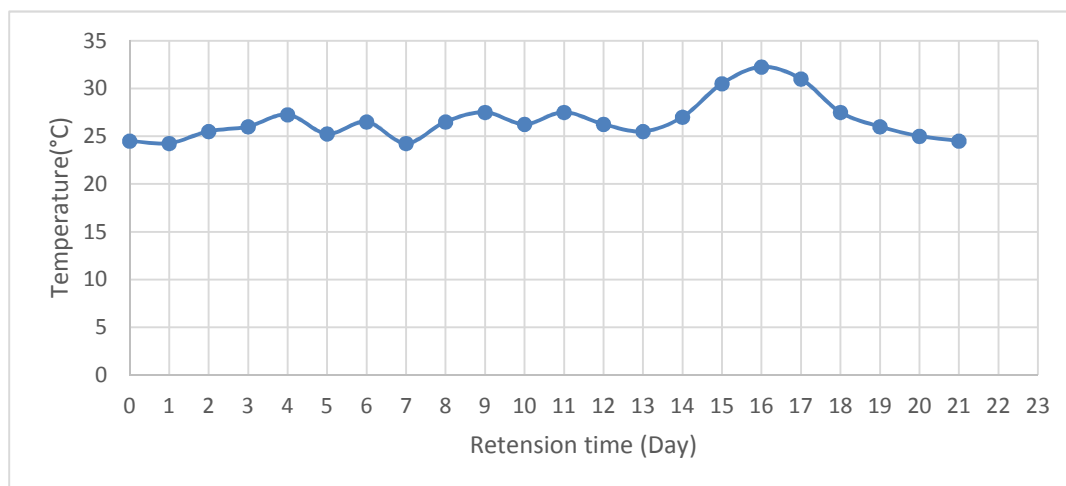


Figure 2: Daily Temperature of Digester Feedstock

Figure 3 is the graph showing pH changes of the digester feedstock on a weekly basis. The initial pH was 7.89, a sequential decrease in pH was

observed after a slight drop in the first week of fermentation. A final pH of 6.21 was recorded at the end of the experiment.

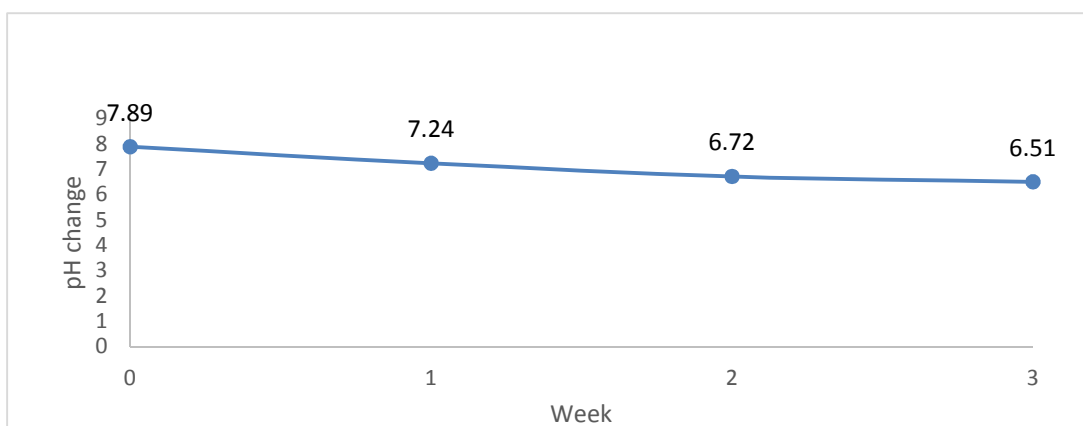


Figure 3: pH Changes during Digestion

Table 1 shows the changes in the physico-chemical parameters of the digester feedstock before and after the anaerobic digestion. The value obtained for BOD and ash content showed

a decrease after the anaerobic digestion also total solids also decreased in values after the digestion process.

Table 1: The physicochemical parameters of the digester feedstock before and after the anaerobic digestion.

Parameters	Before digestion	After digestion	Reduction
Moisture-Content (%)	85.6	78.67	7.93
Ash content (%)	2.70	2.42	0.28
Total solid (%)	7.83	6.75	1.08
BOD (mg/L)	2730.00	2634.00	96.0
pH	7.89	6.11	1.78

Test for the Presence of Methane in the Biogas Produced

Biogas was finally tested and it was confirmed that the biogas was combustible. A bluish flame glowed and the glowing lasted for several seconds.

Bacteriological Analysis

Table 2 reveals the mean bacterial count before and after the digestion process. The

count of aerobic organisms showed a decrease trend from 2.3×10^7 Cfu/ml in the first week of digestion to 8.5×10^6 Cfu/ml in the second week. A further steady decrease was observed on the third week of digestion. Anaerobic count was found to have an increasing trend from 1.5×10^6 Cfu/ml in the first week of digestion to 1.2×10^7 Cfu/ml in the last week.

Table 2: Total Bacterial Counts Before and During the Anaerobic Digestion Process

Days of digestion	TAC(cfu/ml)	TANC(cfu/ml)
0	2.3×10^7	1.5×10^6
7	1.9×10^7	2.0×10^7
14	9.4×10^6	2.2×10^7
22	8.5×10^6	1.2×10^7

Key: TAC- Total aerobic bacterial counts; TANC-Total anaerobic bacterial count;CFU- Colony forming unit

DISCUSSION

Energy is inevitable when we come to determine a nation’s economy; it is one of the indicators for sustainable growth and development. The depleting nature of

petroleum and coal threatens supply of fuel throughout the world, also, the problem of their combustion leads to research in different corners to get access to the new sources of energy.

But biogas is distinct from other renewable energies because of its characteristics of using, controlling and collecting organic wastes (Suyog, 2011). Biogas produces clean gas which mainly consists of methane and carbon dioxide, it is usually convenient for cooking and lightening (Mohammed, 2015)

This study revealed that biogas production was delayed till the sixth day. This can be traced to the fact that most cows feed on fibrous materials and microorganisms require a longer time to degrade fibrous materials. This finding is in conformity to that, from the works of Babatola (2012) and Ozoret *al.* (2014). The findings of this study showed that, biogas production was less and gradual in the first week of the study. This suggests that the biogas producing microorganisms are in the lag phase of growth where acclimatization or adaptations of the cells take place. This report is in consonance to that of Abubakar and Ismail (2010). It can also be deduced from this that, biogas production rate is equivalent or dependent on the growth of methanogenes. From the second week of the study, results indicated a progressive increase in biogas production, this continued to the third week of the study. This indicated that the methanogenes are in their exponential stage of growth. However this differs from the findings of Rabahet *al.* (2008) and that of Abubakar and Ismail (2010) where biogas production experienced a decline in the third week. These differences observed may be due to the different breeds of cows found in the different locations and nature of substrate.

The slurry temperature varies from 24.25 - 32.25°C (Fig. 4.2), these temperature ranges signifies that biogas production is achieved within mesophilic temperature range (25 - 45°C). The maximum biogas produced from each digester was attained at day 17th, in which the temperature of these days was 32.25°C, which is in agreement with the work of (Ukapai and Nnabuchi, 2012).

The pH of the cow waste feed-in was 7.89. The effluent-out at day 7 and day 14 showed the decreased pH (7.24 and 6.72 respectively). (Fig.3), this can be due to acidogenesis reaction that took place in the digester. At this phase, the organic matter was utilized by microorganisms on hydrolysis to produce

volatile fatty acids (VFAs). Thus, increase in VFAs decrease the pH during the initial stage, the effluent-out at day 22 showed slightly a decrease in pH (6.51) (Fig. 3).

Bacterial isolates from the digesters are suspected to include *Klesiella oxytoca*, *Escherichia coli*, *Bacillus sp.*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Clostridium spp.* (Table 4). These were probably responsible for the breaking down of complex organic substances to intermediates such as volatile fatty acids which were ultimately converted to biogas. Rabah *et al.* (2010) and Baki (2004) reported the isolation of *B. licheniformis* and *E. coli* from biogas digesters. Oluyega *et al.* (2006) reported that *Bacillus*, *Yersinia* and *Pseudomonas* species were responsible for biogas production in cow dung,

Conclusion

Biogas generation from cow dung was achieved; the production of the gas started on the sixth (6th) day and reaches its peak on the seventeenth day (17th). Decrease in pH and an increase of the temperature of the slurry digester were observed in the second week of production. Also all the physicochemical parameters analysed decrease after digestion. Microbial isolates are suspected to include; *Klesiella oxytoca*, *Escherichia coli*, *Bacillus spp.*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Clostridium spp.*

The findings of this study showed that cow dung could be used as a suitable substrate for biogas production. Biogas generation, if carried out at commercial scale, would not only provide an alternative source of energy but would also be a means of waste disposal in Nigeria.

Recommendations

1. Digestion of organic compound should be undertaken whenever possible because of its advantages which include the following: generation of storable energy sources and production of a stabilized residue that can be used as a fertilizer.
2. Nigeria with its warm climates is well-suited for the biogas digester technology, The government should think well in building huge plants to take advantage of the various factors that help in the success of the process

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