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The Influence of Catalyst on the Rate of Biogas Generation using Goat Dung

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Biogas is the mixture of gases produced by methanogenic bacteria while acting upon biodegradable materials in an anaerobic condition. It was observed that addition of char coal improved the quantity of biogas generated and also fastened the acid and methane forming stage during biometharization. In this study a comparative study of biogas production from goat dung was conducted under the same operating condition. 10 g of catalyst (char coal), 100 g goat dung was mixed with 700L of water. Biogas production was measured for a period of 5 weeks with an average temperature of 33.4^{0}C production started on the five days of first week and obtained maximum value on the fourth week. It shows that species of Escherichia coli, *Klebsiella* pneumonia, Pseudomonas *aeruginosa*, Citrobacterpolyxa, Bacillus subtilis, Staphylococcus aureus and Bacillus brevis have the ability to degrade harmful organic waste and convert it to useful product.

Keywords: Anaerobic digestion, biogas, goat, Nigeria, renewable energy

INTRODUCTION

Renewable energy technologies are currently competing favorably in the energy sector, all geared toward achieving a more sustainable and efficient energy use. Amongst the most renewable source; solar, hydro, geothermal, wind and biomass, biomass is amongst the most vastly explored in terms of research and application both in the developed and in under develops countries. It is a common knowledge that fossil fuel-based conventional grid extension constitutes the major centralized power systems from urban area to rural areas in most under develop countries. A scenario that is not only capital intensive but also economically unrealistic in most cases. It has been established that more than a quarter of the population of Nigeria experiences an energy crisis, especially those living in the rural areas of developing countries such as Nigeria (Nnaji *et al.,* 1986). In Africa, water pollution and access of energy resources present challenge to human health, environmental health and economic development. In twenty on sub-Saharan alternative renewable energy sources cannot be over emphasized. Anaerobic digestion consist of several interdependent, complex sequential and parallel biological reaction in the absent of oxygen, during which the products from one group of microorganism serve as the substrate for the next, resulting in transformation of organic matter mainly into a mixture of methane and carbon dioxide (Parawira, 2004). Anaerobic digestion of animal manure result in the production of a biogas composed mainly of methane and carbon dioxide. Uncontrolled decomposition of animal manure is undesirable as gases released are believed to have global warning effect. Methane is generated from the digestion of organic compounds in faces, primarily carbohydrate, proteins and lipid. Methane productivity can be measured in term of volatile solid destroyed, loaded, volume of gases produce, (Moller *et al.,* 2004). Organic and inorganic chemicals can be added to the slurry to improved gas production. Addictives can stimulate microbial activity under different operating condition. Some additives can greatly improved performance (Yadvika *et al.,* 2004). In Nigeria identified feedstock substrate for an economically feasible biogas

production include water lettuce, water hyacinth, dung cassava leaves and processing waste, urban refuse, solid waste, agricultural residues and sewage (Akimbani, 2009). Most developing countries relied on dung, straws and other biodegradable materials to meet most of their basic energy need. Considering the availability of raw materials, cost effectiveness and easy maintenance of the technology (Baki, 2004). There are several primary alternative energy sources that supplement fossil fuel; these include nuclear, geothermal, solar energy and biomass e.g. biogas technology (Garba *et al*., 1996) therefore source of energy such as biogas technology can be adapted to minimize or complement dependent on non renewable source. The global energy crisis has generated interest in the use of animal waste as a supplement for fossil fuel (Baki, 2004). Biogas can be obtained by anaerobic decomposable raw materials such as goat dung, poultry excreta, human waste municipal waste and agricultural residue etc. It is a colourless, odorless, mixture containing 60-70% of methane, 30-40% of carbon dioxide, 5-10% of hydrogen, 1-2% of nitrogen, 0-3% of hydrogen sulphide and water vapour as composition. Addictive can help to maintain conditions that are favorable in an anaerobic digester. These conditions include: pH, inhibition/promotion of acetogenesis and methanogenesis, e.t.c. Powered leaves of some plants have been found to stimulate biogas production between 18-40% (yadvika *et al*., 2004).

Biogas production is affected by factors like temperature, pH, and slurry concentration, nature of substance, stirring, seedlings and carbon-nitrogen ratio. The bio conversion in biogas generation is strictly anaerobic and acid sensitive methanogens, especially methane producing bacterium, *Methanobacillus, methanoccus and methanosarcina species* are the most metabolically active organisms known at present. The quantity of biogas produced depends on the amount of solid matter present in the slurry, their digestibility, carbon to nitrogen ratio of the substrate and moisture content. The anaerobiosis is usually affected by factors such as pH, temperature which could also be mesophilic or thermorphilic (Fernando and Dangoggo, 1986). Anaerobic digestion of organic materials is a four phase process in which cellulose materials are converted to methane. The four phase of biogas are the hydrolysis phase, acidogenesis phase, acetogenesis phase and methanogenesis phase. Two types of bacteria involve are the Actogens and Methanogens (Baki, 2004). Some microbes do take part in both hydrolysis and acidogenesis stage. There is a functional overlap (Maishanu, Seepkimbi, 1998). The aim of this research is to isolate the possible ways of improving the effect of raw materials for biogas generation.

(i) To determine the influence of catalyst charcoal on biogas generation using goat dung.
(ii) To compare the biogas produced using goat dung and goat dung + catalyst.
(iii) To isolate and identify the microbes associated with biogas generation using goat dung.

MATERIALS AND METHODS

Sample collection

Fresh sample of goat dung was collected from Adarawa village in Wammako Local government in Sokoto State. A clean container with cover was used for the collection of goat dung. The goat dung was collected immediately when the goat starts passing stool. The container was placed behind the goat and goat dung was collected safely without touching the ground to avoid contamination.

The container was covered and placed in a clean polythene bag to minimize the entrance of microbes while transporting it to the laboratory. The goat was feed on grass, corn husk and millet husk. The sample was taken to the chemical laboratory in the biomass unit for subsequently used in the slurry preparation (Lagrange, 1979). A set of two 100 ml capacity cylindrical tins were cleaned thoroughly and used as digester. The two digesters were labeled A1 and A2. 100 g of fresh goat dung was fed to each digester and 10 g of (slurry prepared), 10 g of catalyst (char coal), were also added and mix thoroughly with 700 ml of tap water respectively, to obtained a homogeneous mixture of west water, for digester a slurry of 1:7 west water (W/W) ratio was obtained with a pH of 6.08 (slightly alkaline was weighed and added.

Biogas experimental set up

Two 100 ml capacity cylindrical tins was cleaned thoroughly and use as the digester. A hole was bored on top of each digester and a horse-piper rubber tube (length = 50 cm, diameter = 0.7 cm) was inserted into the hole and glued using araldite adhesive. The slurry was fed into the digester through the normal inlet on the tin. The inlet of the digester was then scaled to ensure that it is alright (oxygen deficient environment). The unattached end of the horse-pipe for each digester was placed in plastic basin containing water and 100 cm^3 measuring cylinder, filled with water was inserted into the plastic basin as a water displacement method such as that the outlet of the horse-pipe tub was directed upward within the cylinder used as a measure of the volume of biogas produced.

The set up was made to be airtight since the process is strictly anaerobic. Reading was taken at 12:00 noon daily form the period of 5 week retention time, the digester was stirred manually on daily basis earlier before taking reading to prevent scum formation. The average temperature reading was 33.4ºC.

Sampling procedure

Sample of slurry for the microbiology and biochemical analysis was harvested before the digestion (fresh sample) and after five week, at the fifth week, sample was collected using sterile universal bottle and taken to the microbiology laboratory of Usmanu Danfodiyo University Sokoto for bacterial analysis.

Media preparation

The media used was nutrient agar. The required amount of these media was weighed according to the manufacturer's directives and mixed with required volume of distilled water. These was dissolved and heated on hot plate to obtain the homogeneous mixture and sterilized in an autoclave machine at 121ºC for 15 min. It was allowed to cool to about 45ºC and produce in to plate. The plate was left over night at room temperature to cheek for sterility before inoculation (Harrigan, Macance, 1976).

Bacterial colony count

Serial dilution of the sample was carried out pipetting 9 ml of distilled water into 5 test-tube (5 test tubes for each replicate of the samples). The test tubes was then plugged with cotton wool and covered with aluminum foil. These were autoclaved at 121ºC for 15 min and allowed to cool. Samples were serially diluted in the order of 1:10 (vol/vol). The diluents were inoculated on solidified nutrient ager in duplicate from 10^6. The plates were then incubated at 37ºC for 24 h. After 24 h, colonies were observed and counted. The number of bacteria per ml of the original sample of colonies on the plate was multiplied with dilution factor. One colony on the plate represents one cell of colony-forming unit (CFU) from the original sample. Different types of colonies were observed and then sub cultured to obtain pure cultures of the organisms.

INCUBATION

Aerobic incubation

Petri dishes was thoroughly cleaned and dried in dry heat oven. Samples from the influent were serially diluted in the order of 1:10 (vol/vol) and inoculated on the solidified nutrient ager plates in duplicate. All plates were then incubated at 37ºC for 24 h.

Anaerobic technique

The anaerobic jar was used for the incubation of facultative anaerobic bacteria (Maishanu, 1998). The nutrient ager plates were loaded into the jar to charge the jar with carbon dioxide. The jar was incubated at 37ºC for 24 h (Maishanu, 1998).

Characterization and identification of isolate

Gram staining

Sterilized wire loop was used to place a drop of water on the center of slide and the wire loop was sterilized again, growth was picked using the sterilized wire loop and transferred to the water drop. The growth was emulsified and speeded out over an area of 1cm radios to obtain thin smear. The slide was allowed to air-dried and then heat fixed by flame. The smear was stained with crystal violet solution for 1 min and then rinsed with water. The slide was then flooded with iodine solution (mordant) for 30 seconds and washed off with tap water. Acetone (a decolourizer) was dropped on the titled slide until all free color removed. The slide was rinsed again with water and finally safranin (acounter stain) as flooded on the slide for 60 seconds. The slide was washed off water blot and examined under the oil immersion objective (100) (Oyeleke and Manga 2008).

Biochemical tests

Morphology and staining characteristic are insufficient to identify organism. A battery of biochemical test is commonly used to identify the principle genera and specie (Arthur and Kathleen, 2002). The basic strategy for identifying bacteria based on biochemical test relies on the use of a dichotomous key, which is essentially a flow chart of test that gives either a positive or negative result. One series of reaction, the imvic (indole, methyl/red, vogesprsokauer and citrate) is a traditional panel that was used to differentiate several genera (Oyeleke and Magana, 2008).

Indole test

Twenty four hours old culture was inoculated into test tube containing sterile nutrient both and incubated at 37ºC for 48 h. 5 drops of kavac'sindole reagent were added to the culture and then shaken. A positive ($_+$) reaction was indicated by the appearance of a real ring coloration on top of the reagent above the broth medium within 1 min, while in negative (□) reaction, the indole reagents retains its yellow color (Barrow and Faltham, 1993).

Citrate utilization test

Twenty four hours old culture of the isolates was inoculated into slanted test tubs containing sterile

Table 1. Weekly biogas production and average daily temperature.

Retention time (DAYS)	Volume of biogas produce digester A (goat dung)	Average daily temperature ºC
1-7	97.15	35.1
8-14	1554	35.4
15-21	1693	33.4
22-28	1884	28.7
29-34	1492	34.2

R/T =Retention time
A= Goat dung

Table 2 Biochemical test of the isolates.

S/N	Lactose	Glucose	Sucrose	Citrate	Motility	H_2S	Indole	Urease	Gas	Gram r/n	Isolate
1	–	–	–	–	+	–	+	–	+	+ve rod	*Escherichia coli*
2	–	–	–	–	+–		-	–	+	+ve rod	*Escherichia coli*
3	+	–	+	+	+		–	–	–	+ve rod	*KlebsiellaPneumoniae*
4	–	+	–	+	+	+	–	+	–	+ve rod	*Pseudomonas aeriginosa*
5	+	+	–	+	+	+	–	+	+	+cluster	*Citrobacter polymyxo*
6	–	+	+	+	—		–	+	–	+rod	*Bacillus subtilis*
7	+	+	+	+	+	–	–	+	–	+cocci	*Staphylococcus aureus*
8	–	+	+	+	+	–	+	–	–	–rod	*Bacillus brevis*

Simmons citrate medium aseptically and incubated at 37ºC for 72 h, observing daily for the presence of growth and colour changes. Positive reaction was indicated by the development of deep blue colour (Oyekele and Manga, 2008).

Triple sugar iron (TSI) test

Triple sugar iron ager slants in test-tubes was inoculated with isolates (24 h old culture) using a sterile inoculating needle. The surface was steaked and the bottom was stabbed three times. The test-tubes was incubated at 37ºC for 24 h, after which they was examined for gas produced, Hydrogen sulphide production, glucose and lactose fermentation is indicated by the butt becoming yellow, while in lactose the media appeared yellow, gas production was determined by the appearance of bubbles. Motility was positive due to cloudy medium and the line of inoculate not sharply defined (Oyeleke and Manga, 2008).

Urease test

Urea agar slants in universal (steaking) with 24 h old culture and incubated at 37ºC for 48 h. Positive test was indicated by pink colour as a result of an enzyme urease which hydrolyzed the urea to carbon dioxide and ammonia, while negative test indicated by absence of pink colour (Oyeleke and Manga, 2008).

RESULTS AND DISCUSSION

Biogas production

The biogas produced by all samples and temperature were recorded. The goat dung started producing biogas after 5 day, of preparation in the first week and increases continuously throughout the period of production. The initial pH of the sample (goat dung) was 6.08 and the final pH is 5.90.The process of biogas generation has been under constant review as new organisms and substrates are being further identified with the process of methanogenesis. This study focused on the influence of catalyst (char coal) on the rate of biogas generation using goat dung. The result of this study shown in (Table 1) clearly shows differences in the amount of gas generated by simple goat dung under the same environmental condition. From the result reported in the (Table 1) it is evident that there is a profound effect of the catalyst on the total volume of biogas recorded. The results have show that there is an increase in the volumes of biogas produced from the first week. The result in (Table 1) indicates that the goat dung started its gas production on

Table 3. Frequency of occurrence of bacteria isolated from goat dung during biogas production.

Bacteria	Occurrence of isolate	% occurrence of isolate
Escherichia coli	2	0.25
Klebsiella pneumonia	1	0.125
Pseudomonas aeruginosa	1	0.125
Cirobacter polymyxo	1	0.125
Bacillus subtilis	1	0.125
Bacillus brevis	1	0.125
Staphylococcus aureus	1	0.125
Total	8	1.00

the first week. Digester A1 stops producing on the third week of the retention period, on the fourth week A2 production was high at the end of the production the result shows that digester A2 has the highest volume. However the result of the initial pH was 6.08 and final pH is 5.50.The result obtained from this study indicated that the catalyst used (char coal and slurry prepared) was very influential in average daily gas production, this has been reported in several place to have some effect on the performance of biogas digester (Baki, 2004). Table 2 shows that a total of eight bacterial species were isolated in which *Escherichia coli* were the predominant and most occurring species of bacteria and account for 0.25% isolates while *Pseudomonas aeruginosa* has 0.125% and *Citrobacter* polymyxo also counted 0.125% most of these species have been reported to be associated with one or two stages of anaerobic digestion (Maishanu and Seepkimbi, 1998). The frequency of occurrence of bacteria isolated from goat dung during biogas production as shown in (Table 3), the *Escherichia coli* occur in two isolates. *Klebsiella, Pneumoniae* occurs in one isolate, *citrobacter polymyxo* occurs in one isolate and *Psudomonas aeruginosa* occurs in one isolate. The Escherichia coli have the highest percentage of isolate because it occurs in duplicate. In this *study Escherichia coli* represent the most dominant isolate and counted for 0.125% and other bacteria have the same percentage, *Klebsiella Pneumoniae* 0.125% *Pseudomonas aeruginosa* 0.125% and *citrobacter polymyxo* also has 0.125%.

Conclusion

From the result of this research it can be seen that the addition of catalyst (char coal and slurry prepared) in the slurry influence the rate of biogas generation. The research shows that species of *Escherichia coli, Klebsiella pneumonia, Pseudomonas aeruginosa, citrobacter polyxa* have the ability to degrade harmful organic waste and convert it to useful product. Therefore the organism could be useful in industrial application based on the result obtain in this study. Goat dung can be used as a non- traditional energy source by mixing the goat dung. The anaerobic digestion solves sanitation problems by taking in human as well as animal mature, improving home and farm hygiene and the general environmental conditions. In this *study Escherichia coli* represent the most dominant isolate and counted for 0.125% and other bacteria have the same percentage, *Klebsiella Pneumoniae* 0.125% *Pseudomonas aeruginosa* 0.125% and *citrobacter polymyxo* also has 0.125%.

Recommendation

Base on the above result, the following recommendations were drawn:

(a) The utilities for operation of biogas technology are wastes which are free, cheap and in abundance. The investment is worthwhile and highly profitable. Nigeria and Africa have an alternative energy source that is ready to be displayed.
(b) There is also need to accentuate on disabusing the mind of the public on waste because most people are habitual in showing resistances or inertia to waste. Therefore there is need to coax on the idea that organic wastes is useless.
(c) To this end, there is need for government agencies and environmental managers to lay more emphasis on biogas production to minimize the growth of microorganisms of biogas as energy.

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