

Four potentials of biogas yield from cow dung-CD

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ABSTRACT

The potentials of biogas yield from cow dung were analyzed using standard microbiological methods. The results revealed that optimum biogas yield from cow dung without starter culture was 345mls, 640mls and 720mls and in the treatment with starter culture was 490mls, 640mls and 830mls respectively in 1kg, 2kg and 3kg weights within 15 days. The 1kg, 2kg and 3kg weights gave a total biogas yield of 2339mls, 3302mls and 4436mls with starter culture and 1141mls, 2650.50mls and 3750mls without starter culture respectively. Percentage biogas yield of 15%, 33% and 49.70% was respectively recorded for the 1kg, 2kg and 3kg weight without starter culture while that with starter culture yielded 23.21%, 32.76% and 44.00% from the 1kg, 2kg and 3kg weights. Results showed that there was significant difference in the non-inclusion (without starter culture) [$F(2, 16) = 29.34, P < 0.001$] and inclusion (with starter culture) [$F(2, 16) = 25.87, P < 0.001$] of starter culture for the different weights of biogas generated from cow dung at the 1% level of significance. Positive correlation also existed between the digesters with or without starter culture.

Key words: Biogas, Energy, Water Hyacinth, Cassava Peels

INTRODUCTION

There may be no solution to the energy crisis in Nigeria and other developing countries except we develop an indigenous technology suitable and convenient to our peculiar circumstances especially, with respect to technological knowhow, raw material availability, human and economic resources and applicability by rural dwellers. This is because no developed country may be ready to transfer its already developed technology based on political power play, economic and capitalistic monopoly as well as security. Cross River State, Nigeria and indeed Africa is blessed with abundant, diverse and unexploited renewable energy resources that are yet to be used for providing clean fuel and help the energy crisis and poverty [13] [17] [12]. Guruswamy *et al.*, [10] and Alvarez and Gunnar, [2] identified two significant and important challenges of the millennium and the twenty first century to include; the development and use of renewable energy to decrease dependence on fossil fuel and management of the waste generated by human activities as a result of agricultural activities, industrial growth and population explosion which are associated with waste generation. Achieving the Millennium Development Goals (MDGs) in Africa also requires a significant expansion of access to modern and alternative renewable energy such as biogas which is of growing interest for the sustainable management of our waste and a major breakthrough in the search for a renewable energy for the reduction in over-dependence on non-renewable fossil fuel [19] [1]. Biogas is the product of organic matters decomposition under oxygen-free condition with microbial participation especially Methanogens. Biogas formation can occur naturally in swamps, marine sediments, and water logged soils, rice fields, deep bodies

of water, sanitary landfills and even in the digestive system of ruminants; and termites. It can also be recovered from lagoons used for waste treatment. Biogas is also called; swamp gas, sewer gas, marsh gas, gobar gas and digester gas 'will O the wisp gas, natural gas, landfill gas and sewage gas. Biogas, a mixture of gasses consist of 50 – 70%, methane 30 – 40%, carbon dioxide 5 – 10%, Hydrogen 1 – 2%, Nitrogen 0 – 3% , water vapour and traces of Hydrogen sulphide, carbon monoxide and oxygen. It is colourless, relatively odourless and flammable; it is also stable and non-toxic. It burns with a blue flame and has a calorific value of 4500 – 6000kcal/m³ when its methane content ranges from 60 – 70% [1] [12]. Generally, four different stages have been recognized in the production of biogas with several other intermediate products. These include; hydrolysis, acidogenesis, acetogenesis and methanogenesis. The efficiency, effectiveness and stability of anaerobic digestion and consequently biogas generation can vary significantly based on various operational factors such as; type of waste streams, digester design , temperature, moisture content, retention time, pH, agitation or mixing, bacterial species and organic loading rate. Presence of toxicants can also influence biogas production. Positive implications of biogas include; the reduction in environmental pollution, odour [15] [16], and in the destruction of most pathogenic organisms, worms, ova, etc. Biogas can also serve as a clean alternative to fuel energy source to oil, electricity and wood. The negative implications of biogas technology include; concentration of toxic compounds such as pesticides and heavy metals in plants and ground water contamination [20]. This research is aimed at determining the potentials of biogas energy generation from cow dung which is a nuisance in terrestrial environment and in our surroundings (foul odour due to uncontrolled fermentation) and various waste sources which abound in Cross River State and other parts of Nigeria.

MATERIALS AND METHODS

Cow dung

Ten kilogram (10kg) weight of fresh cow dung was collected from the Abattoirs in Nassarawa Village, Baccoco in Calabar Municipality, Nigeria and placed in sterile bags and transported immediately to the laboratory for analysis (See Plate 1).

Media

A wide spectrum of media was employed for the microbiological studies. These include; Nutrient agar, (NA), Potatoes Dextrose Agar (PDA) Sabouraud dextrose agar (SDA), Mineral salt medium (MSM), and MacConkey agar, (MA). The media were prepared as described by the manufacturer (OXOID and DIFCO 1984).

Microbiological analysis and sample preparation

1. Screening of bacteria and fungi from raw substrates and soil

Bacteria, fungi and Methanogens were screened from the substrates before and after digestion using analytical media and standard procedures.

Cow dung

Ten grams (10g) weight of well pulverized cow dung was mixed with 100mls of sterile distilled water in 250mls Erlenmeyer flask. The mixture was stirred and agitated thoroughly and allowed to stand for minutes (10mins).

Enumeration and identification of total viable bacteria (TVB) and fungi (TVF) from substrates and soil

One gram (1.0g) each of soil samples from study sites and control was suspended in 9 milliliters (ml) of sterile distilled water. One milliliter (1.0 ml) of the soil suspension was diluted serially (using physiological saline) in Ten-fold to the ranges 10¹ - 10⁵. Zero point one milliliter (0.1) aliquots of dilution 10² – 10⁵ each was seeded onto triplicate plates of nutrient agar using surface spreading technique. Agar plates for enumeration of total viable bacteria were supplemented with 50µg/ml of Nystatine (to inhibit fungal growth) and incubated at 30°C for 24 hours. Viable number of colonies per plates was multiplied by the reciprocal of the dilution factor and recorded as colony forming units (CFU) per gram of soil cfug⁻¹ [3]. For total heterotrophic fungi 1.0ml of 10⁻³ dilution were plated in triplicate on Potato Dextrose Agar (PDA) using surface spreading technique. Medium was supplemented with 100µg/ml of streptomycin and 15µg/ml of penicillin to inhibit bacteria growth. Cultural plates were incubated at 28°C for 72 hours. Colonies were enumerated as colony forming units (CFU) per gram of soil sample cfug⁻¹. Series of physico-chemical tests were carried out to identify isolates.

Screening of methanogens

Strict anaerobic cultivation was achieved using anaerobic jar and Anaerogen (An anaerobic system for the generation of anaerobic condition, OXOID 2.5L-AN0025A from Hardy diagnostic USA). The method described by [11] and the liquid medium cultivation described by [22] and [4] was used. Media for enumeration and isolation of hydrolytic and fermentation bacteria were prepared according to the method of [6].

Identification of hydrolytic and fermentative bacteria in pure culture was based on Virginia Polytechnic Institute manual (1975). Methanogenic bacteria were isolated on basal carbonated yeast tryptone media [17], supplemented with acetate, methanol, formate or hydrogen as energy sources. Methanogenic bacteria were identified based on [8]. Culture samples were taken during the last 10 days. After enumeration, the fermentative hydrolytic anaerobes were maintained in nutrient agar for further studies.



PLATE 1: Sampling of cow dung from slaughter house dumpsite at Baccoco Calabar Municipality, Calabar, Cross River State, Nigeria

Preparation of starter culture

The methods of [9] was employed, the support activated carbon (charcoal) was washed 5 times with acetate buffer pH (4-5) and finally re-suspended in the buffer overnight. Twenty kilogram weights were placed in storage containers and kept at 10°C in a refrigerator. Twenty kilogram weight of the slurry (residue w/v) of an old but active cow dung digester was mixed with 20kg weight of the pre - treated activated carbon and incubated at room temperature in anaerobic condition for 40 days. The adsorbed cells were used as crude starter culture for all digesting combinations.

The advantage of using the activated carbon as support for the immobilization was that it was relatively cheap and affordable, readily available, mild and poses no problem of cell and enzyme inactivation.

Innovation in digester design with gasometrical chamber

Biogas yield was measured daily using the gasometrical chamber which was an innovation, specially designed for this research. The chamber consisted of a gasometrical assembly which comprised of a graduated burette which was connected to the locally designed anaerobic digester through a rubber tube. The burette was also connected to a funnel with paraffin oil through a synthetic rubber tube (which could be transparent). The burette was linked to the tube from the anaerobic digester by a glass connector with two taps; the inlet and the outlet taps. The outlet tap was sealed with a flexible plastic tube with a strong clip (to avoid leakage). The total biogas yields were determined by opening the outlet tap of the anaerobic digester and the inlet tap to the graduated burette. The biogas generated was released through the tube which then displaced the paraffin oil in the graduated burette downward. The volume of gas yield was determined by the volume of paraffin oil displaced, i.e. gas yield was directly proportional to paraffin oil displaced (Figures 1, 2 and 3).

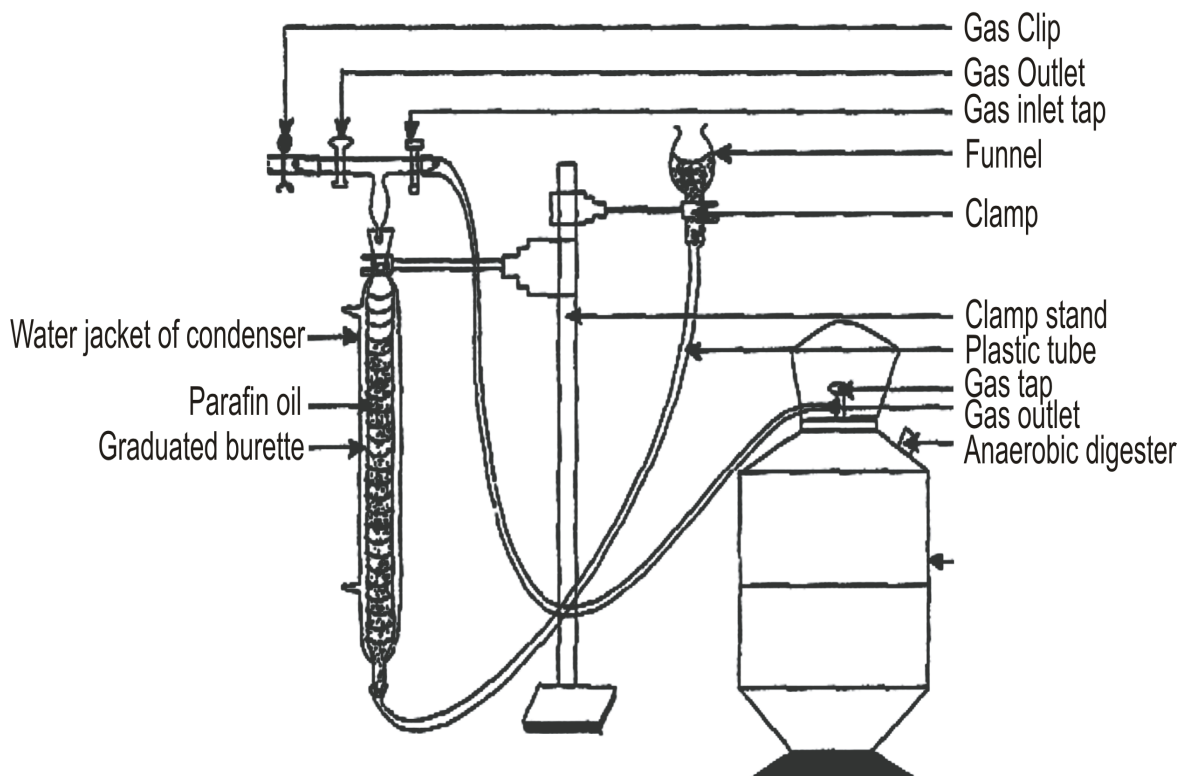


FIGURE 1: Anaerobic digester and gasometric chamber assembly

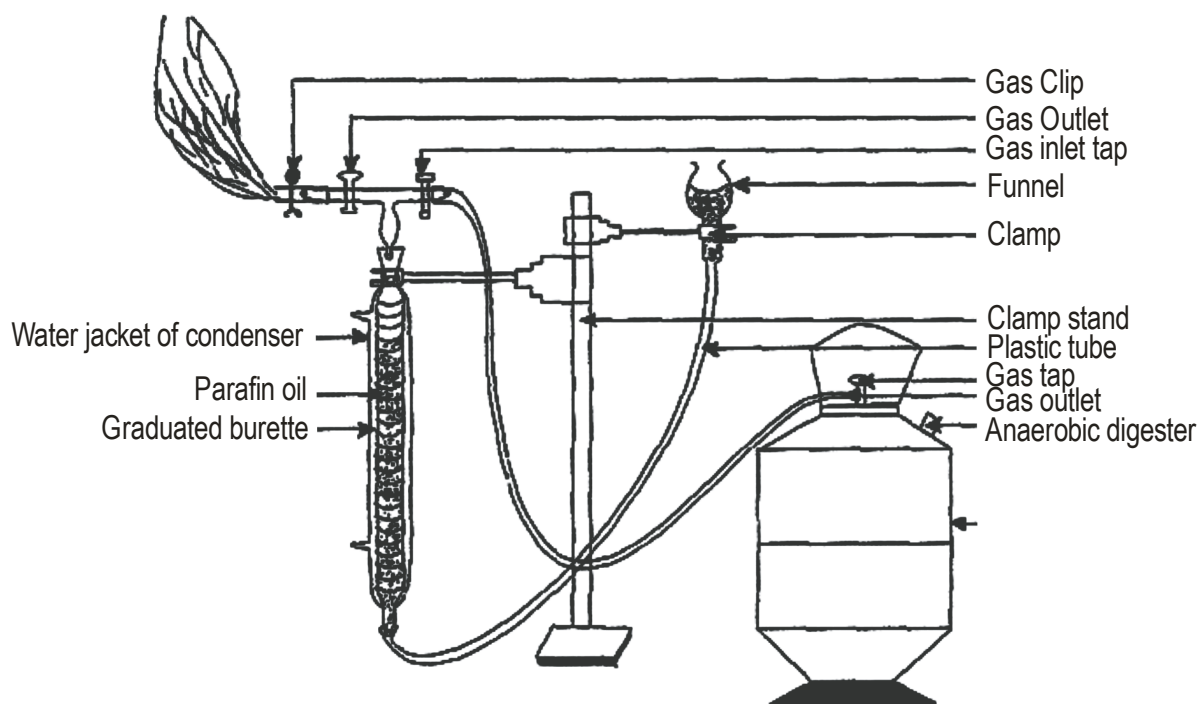


FIGURE 2: Anaerobic digester and gasometric chamber assembly showing flammable gas

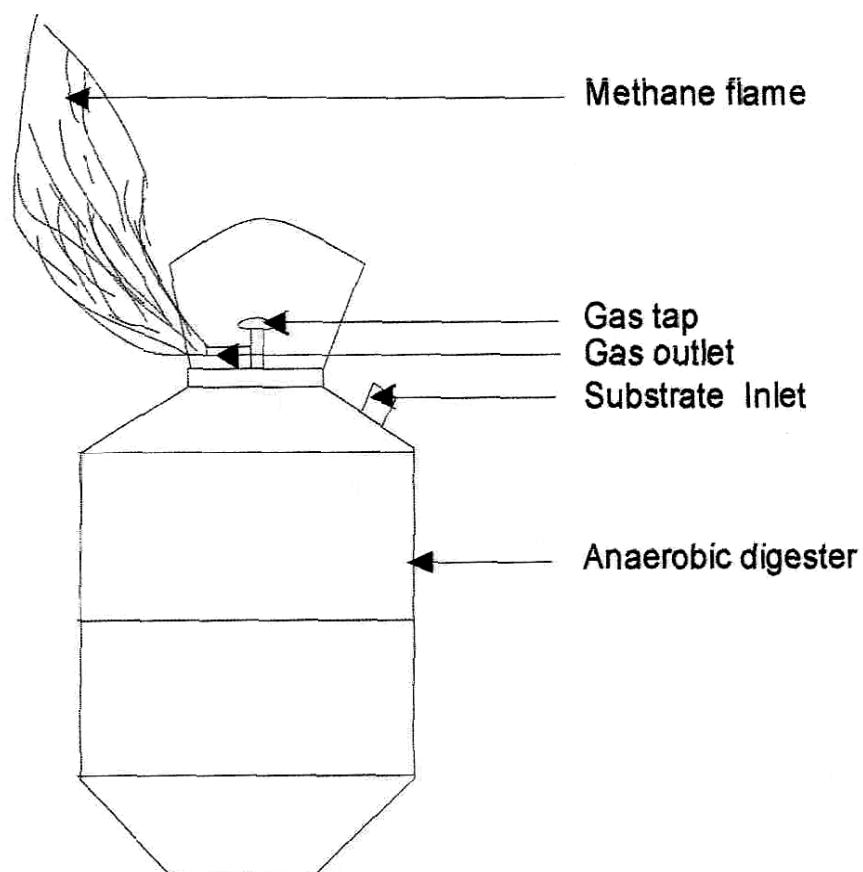


FIGURE 3: Burning methane gas Anaerobic Digester without gasometrical chamber



PLATE 2: Experimental Set - up



PLATE 3: Experimental Set-up showing the different weights of cow dung used for biogas generation

Measurement of methane content

The total amount of biogas was measured using the gasometrical chamber. Methane content was measured by subtracting the amount of flammable gas (methane from the total biogas obtained).

Flammable gas was detected using match stick flame to bum off the biogas evolved. Methane flame was dark blue and almost invisible in daylight (Figures 2& 3) [13]

$$\text{Percentage Methane (\%)} = \frac{\text{Total biogas volume}}{\text{Flammable gas evolved}} \times \frac{100}{1}$$

Note: This is a deviation from the gas analyzer (Shimadzu, class- GC14B, Japan) used by Anunputticul (2004) and the Saccharometer as suggested by Ellegard and Egneus (1984).

RESULTS

Evaluation of biogas yield from cow dung-CD

Optimum biogas yield from cow dung without starter culture was 345mls, 640mls and 720mls respectively in the 1kg and 2kg and 3kg weights within 15 days and in the treatments with starter culture, it produced optimum biogas yield of 490mls and 640mls and 830mls within 15 days from the 1kg, 2 kg and 3kg weights respectively (Fig. 4). The 1kg, 2kg and 3kg weights recorded total biogas of 1141mls, 2650.50mls and 3750mls respectively in treatment without starter culture, and 2339mls, 3302mls and 4436mls with starter culture (Table 1). Percentage biogas yield of 15%, 33% and 49.70% was respectively recorded for the 1kg, 2kg and 3kg weights without starter culture while with starter culture percentage yields was 23.21%, 32.76% and 44.00% from respective weights of 1kg, 2kg and 3kg (Table 1 and 2). The variations in the volume of biogas generated from cow-dung (CD) are summarized in ANOVA Table 2. There was correlation in the biogas generated in digester with and without starter culture (Fig. 5).

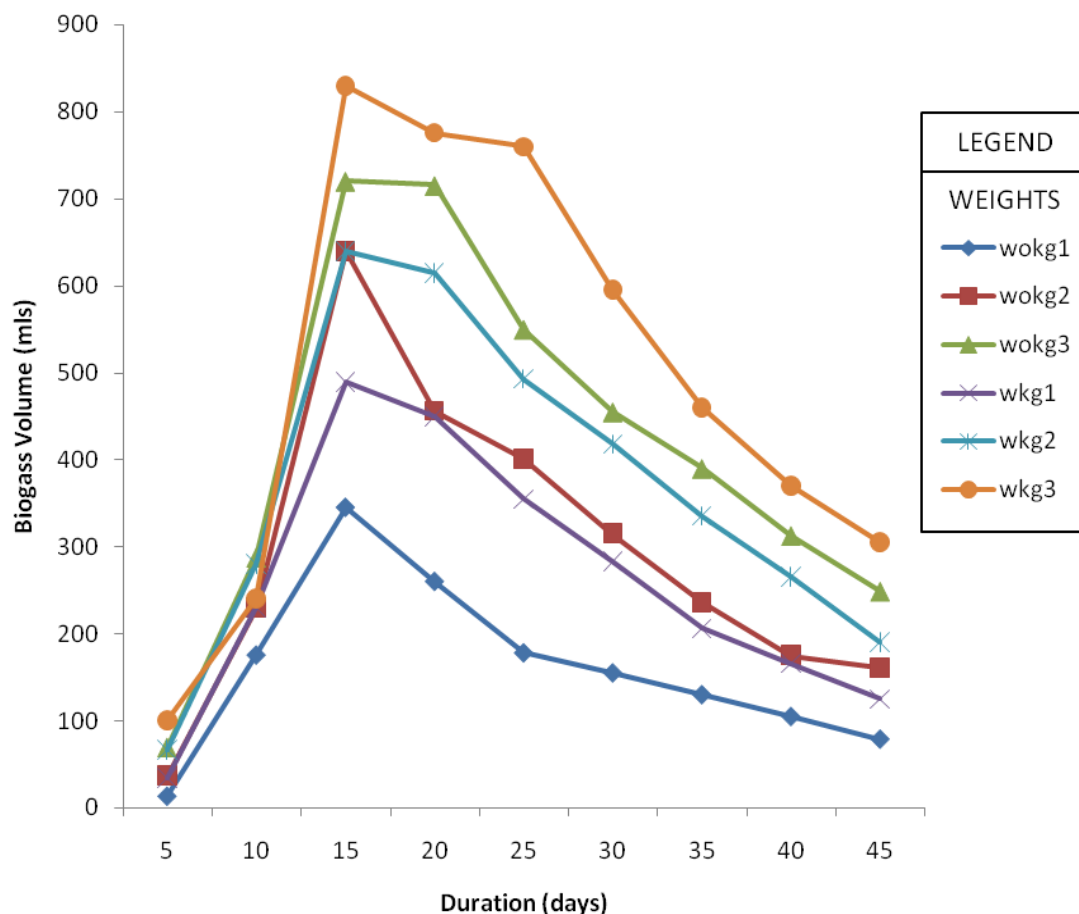


FIGURE 4: Optimum biogas yield from cow-dung with and without starter culture

TABLE 1 Total biogas yield from cow-dung–CD with and without starter culture (milliliters)

Digestion time (Days)	Volume of biogas (milliliters/5days)					
	substrate weight without starter culture			substrate weight with starter culture		
	1kg	2kg	3kg	1kg	2kg	3kg
5	13.5	36	70	34	66	100
10	175.5	230	288	230	280	240
15	345	640	720	490	640	830
20	260	456	715	450	615	776
25	178	401	550	355	493	760
30	155	315	455	283	418	595
35	130	236	390	206	335	460
40	105	175	313	166	265	370
45	79	161	249	125	190	305
Total	1141	2650	3750	2339	3302	4436

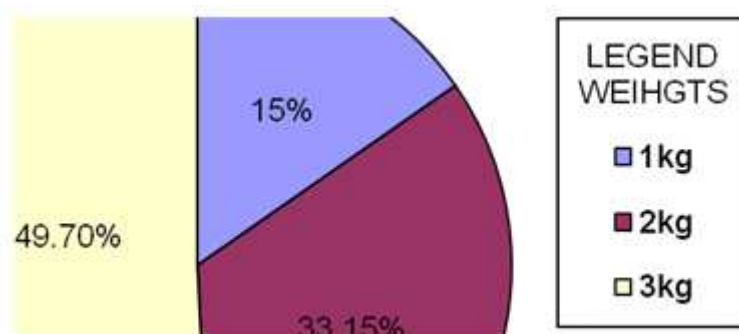


FIGURE 5: Percentage Biogas yield from cow-dung without starter culture



FIGURE 6: Percentage biogas production from cow-dung with starter culture

TABLE 2 Analysis of variance (ANOVA) summary results showing variations in volume of biogas produced from cow-dung with/without starter culture

SOURCES OF VARIATION	INOCULA	DF	SIGNIFICANCE	MSS	F-CAL	P-VALUE	F-CRITICAL
Weight	Without	2	296417.50	148208.70	29.34***	4.44E-06	3.63
	With	2	244842	122421	25.87***	9.7E-06	3.63
Periods(Days)	Without	8	635961.90	79495.23	15.74***	3.14E-06	2.59
	With	8	945587.30	118198.40	24.97***	1.23E-07	2.59
Error(Without)	Without	16	80828.54	5051.784			
	With	16	75727.33	4732.958			
TOTAL	Without	26	1013208				
	With	26	1266157				

*** = Significant at 1% level
 Source = Derived from experimental data (2008)

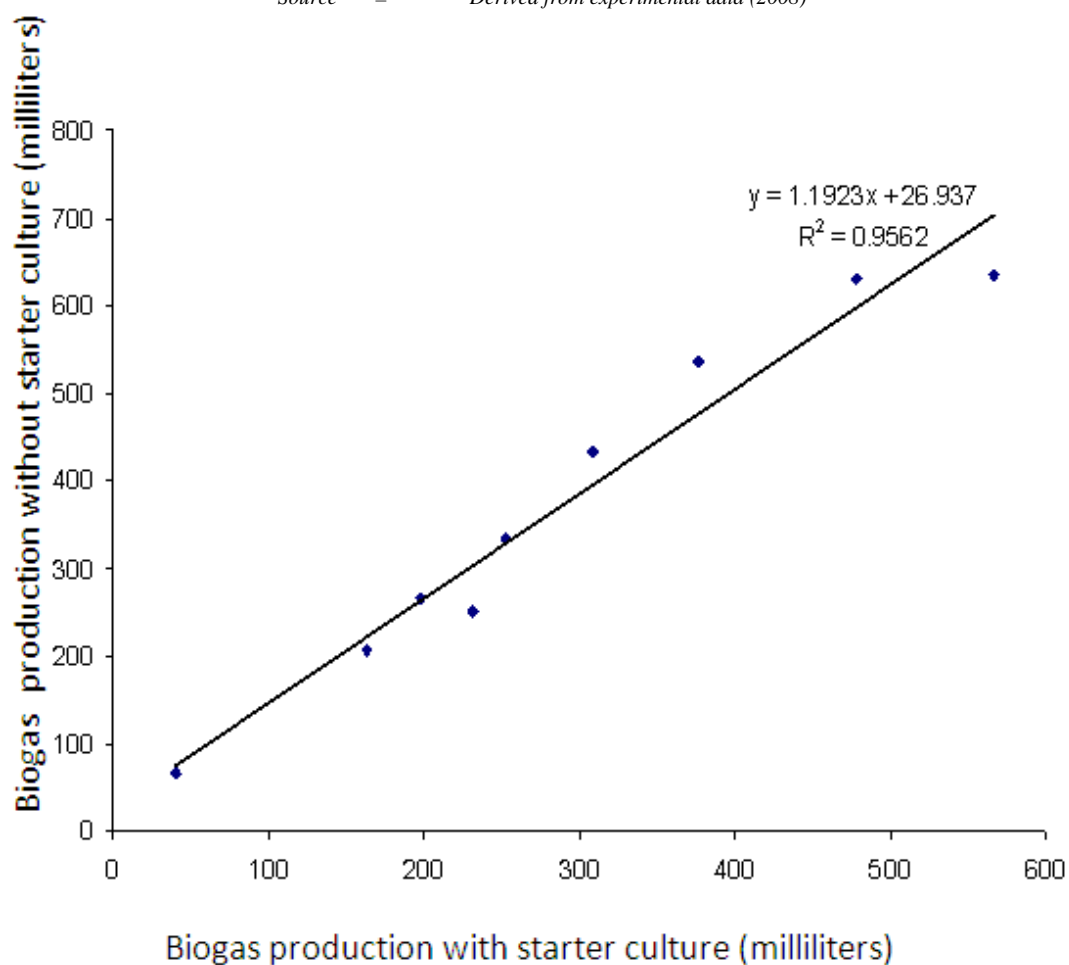


FIGURE 7: Relation between biogas production from cow-dung with and without starter culture

Cow dung

Optimum Biogas yield from cow-dung was obtained within a shorter retention time of 15 days in the three different weights of 1kg, 2kg and 3kg compared to water hyacinth, cassava peels and poultry droppings as single substrate, without or with starter culture. The biogas production trend was also more stable among the single substrate digesters.

Results show that there was significant difference in the non-inclusion (without starter culture) [$F(2, 16) = 29.34$, $P < 0.001$] and inclusion (with starter culture) [$F(2, 16) = 25.87$, $P < 0.001$] of starter culture for the different weights of biogas generated from cow-dung at the 1% level of significance (Table 2).

The results also indicated significant difference with [$F(2, 16) = 24.97$, $P < 0.001$] or without [$F(2, 16) = 15.74$, $P < 0.001$] inclusion of inoculums in the treatment (Table 2). Positive correlation also existed between the digester with or without starter culture (Fig. 5). This indicates that biogas production from cow-dung can be generated with or without starter culture in the treatment. This also shows that biogas yield can be increased by scaling up the substrate composition and addition of starter culture.

In their earlier study on energy from zoo animal waste [14] obtained biogas using cow dung as Starter culture from an active digestion. Animal wastes used were Elephant dung 25% and Rhinoceros dung. They reported that cow-dung shortened the lag time for new digesters. They obtained biogas and methane at the ratio of 1.1 liter gas from 1kg wet dung per day. Biogas and methane dropped sharply after 35 days of digestion time.

Muyiyya and Kasisir [18] obtained biogas yield of 13008ml from substrate combination of pig and cow-dung at a ratio of 2.1. Asikong *et al.*, [5] obtained biogas yield of 207 liters/kg-Vs at hydrostatic retention time of 17 days with ultimate methane yield of 184 L/kg vs. Chae *et al.*, [7] reported theoretical biogas and methane yields at standard temperature and pressure of 1.121 biogas /g/Vs.

Rabah *et al.*, [21] reported on the use of animal manure as one of the main biogas production resources in Sokoto, Nigeria. Also Rabah *et al.*, [21] reported that biogas from manure including cow dung represents a huge potential for reduction in global greenhouse gas emissions.

Yadvika *et al.*, [23] analyzed biogas production utilizing Agriculture and animal waste including cow dung. Itodo *et al.*, [13] designed a biogas stove for cooking in Nigeria using cattle dung as feedstock in the ratio of 1 part of dung to 2 parts of water at retention time of 30 days and daily loading rate of 100kg of slurry.

CONCLUSION

One of the major challenges of anaerobic digestion is the use of local technology to design a digester which will be sufficiently air tight to prevent leakage or introduction of air into it. This is because Methanogenic bacteria are highly sensitive to oxygen or air hence the entire system is destabilized and it takes a longer time to recover if ever it does. It is also obvious that higher temperature supports biogas generation at a shorter retention time than ambient temperature used in this study. There is the need to further research on a digestion model which will support biogas generation at ambient temperature since this conserves energy and can easily be applied by the rural dwellers. Methanogens naturally grow very slowly and this increases retention time, there is therefore the need for further study to screen novel bacteria and fungi which can grow faster with increased biogas generation. There is a further need to design a more effective way of storing the biogas generated for further use, especially by rural dwellers. Finally there is the challenge for sustainable research on biogas technology for it to create the expected impact as a source of renewable energy and a reliable alternative to the non renewable fossil fuel energy.

REFERENCES

- [1] Adeyanju, A. A. *Journal of Engineering and Applied Sciences* **2008**, 3 (3), 242 – 245.
- [2] Alvarez, R. and Gunnar L. *The effect of temperature variation on biomethanation Bioresource technology* **2008**, in press
- [3] American Public Health Association, *Standard methods for the examination of water and waste water* (16th Ed.). Washington D.C.: American Public Health Association, **1995**, 18-23.
- [4] Asikong, B. E. Biogas generation from Water hyacinth, Cassava peels, poultry droppings and Cow-dung in Cross River State, Nigeria, **2010**, The Ph.D. Thesis submitted to the Graduate School University of Calabar, Calabar, Nigeria.
- [5] Asikong, B. E., Epoke, J., Antai, E.E. and Eja M. E. *Journal of Nigerian Environmental Society- JNES* , **2012**, 7(2), 13-20.

- [6]Asikong B. E., Epoke, James, Matthew E. Eja, Effiom E. Henshaw and Antai, E.E. *Journal of Microbiology and Biotechnology Research*, **2013**, 3 (2), 19-25
- [7]Chae, K. J., Jang A. M. & Yim, S. K. *Bioresource Technology*, **2007**, 49 (5), 427-434.
- [8]Eja, M. E., Udo, S. M. and Etok, C. A. *Environmental Microbiology Practical Manual*, Saesprint Publishers, Calabar, **2005**, Pp. 56-69.
- [9]Geluk, M. A., Norde, W., Vankalsbeck H. K. I. and Van't Riet *Enzyme Micro. Technol* **1992**, 24, 748 – 754.
- [10]Guruswamy, T., Kannan, N. and Kumar, V., Design, *World Journal of Microbiology and biotechnology* **2003**, 84, 65
- [11]Hunter-Cenera, J. C., Fonda, M. E. and Belt, A. *American Annual Review of Industrial Microbiology and Biotechnology*, **1986**, 50, 1-23.
- [12]Igoni A. Hikiah, Ayotamuno M. J., Eze, C. L. Ogaji S. O. T. and Robert S. D. *Applied Energy*, **2008**, 8, 430 – 438
- [13]Itodo, I. N., Agyio, G. E. and Yusuf, P. *Journal of Energy in Southern Africa*, **2007**, 18 (3), 14 – 20.
- [14]Klasson, K. T., Nghiem, N. P. and Appolon, N. *Energy production from Zoo Animal Wastes*, Oak, Ridge National Laboratory, U. S. Department of Energy, U. S. A. **2003**,
- [15]Long, C. Review and Prospect of Biogas development, *China Biogas*, **1992**, 10, 104
- [16]Lung, M. S., Anderson, S. S. and Torry-Smith, M. Building of Flexible Biogas, iogas Digester in Tanzania, student Report, Technical University of Denmark, Copenhagen **1996**,
<http://www.fao.org/sp/egdirect/egre0022.htm>
- [17]Mashandete, A. M. and Parawira, W. *African Journal of Biotechnology*, **2009**, 8 (8), 116-125
- [18]Muyiyya, N. D. and Kasisira, L. L. *Agricultural Engineering International: the CIGR Ejournal*, **2009**, 11,1-7
- [19]Nagamiani, B. and Ramasamy, K. *International Journal of Physical Sciences* **2003**, 4 (7), 398 – 402.
- [20]Prescott L. M., Harley, J. P. and Klein, A. D. *Microorganism Interaction and Microbial Ecology in: Microbiology 6th Ed.* McGraw Hill Companies, Inc New York. **2005**, Pp 578
- [21]Rabah, A. B., Baki, A. S., Hassan, L. G., Musa, M. and Ibrahim, A. D. *Science World Journal*, **2010**,5(4), 23-26
- [22]Sakai, S., Imachi, H., Sekiguchi, Y., Ohashi, A., Harada, H. & Kamagata, V. *Applied and Environmental Microbiology*, **2007**, 73 (13) , 4326 - 4331.
- [23]Yadvika, S., Sreekrishnan, T. R., Sangeeta, S. and Vineet, R. *Bioresource Technology*, **2004**, 95, 1 – 10.