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Biogas production from cow dung using laboratory scale digester as potential tool for abattoir waste management

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Abstract

The main challenge of the present world is to harness the energy source which is environment friendly and ecologically balanced because the use of fossil fuels has led to global climate change, environmental degradation and human health problems. This has forced the world to search for another alternate energy source such as biogas. Biogas typically refers to a gas produced by the breakdown of organic matter in the absence of oxygen. Cow dung as a renewable source of energy supply has been proven to be very efficient. This work is focused on production of biogas using cow dung as a means of abattoir waste management. A Laboratory scale digester was constructed using three 750ml capacity plastic water bottles with slurry concentration of 1500g cow dung per 3000cm^3 distilled water over a retention time of three weeks. The biogas production started on the fourth day of fermentation and followed an increasing trend. Reaching its peak on the seventeenth day before a gradual fall in production rate. The average weekly production of biogas are; day1-7 (17.33cm^3), day 8-14 (99.00cm^3), day 15-21(172.33cm^3). The result obtained from this study also indicates that *Bacillus* species were the most common bacteria isolated.

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1.0 Introduction

The use of fossil fuels as primary energy source has led to global climate change, environmental degradation and human health problems (Gomez *et al.*, 2008). The main challenge of the present world is to harness the energy source which is environment friendly and ecologically balanced (Adeyaniu, 2008). This need has forced the world to search for another alternate energy source. Unfortunately, the new alternative energy sources like solar, hydro, wind etc., require huge economic investment and technical power to operate, which seem to be very difficult for developing countries like Nigeria (Akinbami *et al.*, 2001).

Biogas typically refers to a gas produced by the breakdown of organic matter in the absence of oxygen (Baki, 2004). 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 (Kossman *et al.*, 2001). It is a renewable energy source, like solar and wind energy. An increase in industrial, commercial, agricultural and environmental activities has resulted in the generation of large quantities of waste (Bello, 2019).

Biogas can be produced from regionally available raw materials and recycled waste and it is carbon (IV) oxide (CO_2) neutral. It is produced by the anaerobic digestion or fermentation of biodegradable materials such as manure, sewage, municipal waste, green

waste, plant material, and crops (Bagudo, 2012). Biogas production comprises three biochemical processes: hydrolysis, acidogenesis/acetogenesis, and methanogenesis (Labatut, 2010). By fermentative bacterial action, complex molecules (carbohydrate, protein, fats) are broken down into smaller products (acetic acid, H2/CO2, monocarbon compounds and organic fatty acids larger than acetic). Fatty acids longer than acetate are metabolized to acetate by obligate hydrogen-producing acetogenic bacteria (Fariku and Kidah, 2008). Hydrogen and carbon dioxide can be converted to acetate by hydrogen oxidizing acetogen or methane by aceticlastic methanogens (methanogenesis) (Li *et al*., 2002) at pH between 6.0-8.0 and ambient temperature between 25ºC - 40ºC in a bioreactor (digester) under anaerobic condition (Rabah, 2010; Carucci, 20015)

Cow dung is the undigested residue of consumed food material 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 amounts of sulphur, iron, magnesium, copper, cobalt and manganese. Cow dung constitutes one of the wastes generated from abattoirs, which poses a serious challenge to the general public as this waste constitutes a nuisance to the environment as well as an eyesore to the public (Rabah, 2010). Thus, 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. The biomass generated after digestion can be used both as animal feed and to improve soil fertility. This research aimed to produce biogas from cow dung using a laboratory- scale digester.

2.0 Materials and Methods

2.1 Sample collection

Fresh cow dung was collected from Azare Abattoir, Bauchi State, Nigeria, in a clean container. The container was placed in a Cool box and transported immediately to Bauchi State University's Microbiology laboratory for slurry preparation.

2.2 Slurry Preparation

Preparation of slurry for biogas generation was carried out according to the methods described by Asikong *et al*. (2013)**.** One thousand five hundred grams **(**1500g) of the cow dung was mixed thoroughly with 3000cm^3 of distilled water in the ratio of 1:2 (w/v). The initial pH of the mixture was determined.

2.3 Experimental Set-up

Batch type anaerobic digester used in this research was designed according to the method of Ahamed *et al.* (2016) with slight modification. The laboratory-scale digester was constructed using three sets of 750ml capacity plastic water bottles. The bottles were labelled as N1, N2, and N3. An equal concentration (1500g/3000cm^3) of the slurry was poured into the bottles. Each of the plastic water bottles was thoroughly washed, then rinsed with deionized water and conditioned for the growth of microorganisms. The bottles were perforated at the top of the lid and made airtight using Araldite and rubber seals. Araldite and rubber seals were used to secure an airtight link between the plastic bottle water and 320ml calibrated plastic container shown in Figure 1. The 320ml calibrated bottle for the gas collection was made airtight and inverted. A glucose drip (tube) fitted into the inverted calibrated bottle was made to discharge water into a measuring container.

At the start of each experiment, the calibrated plastic bottle was filled with water and inverted (Figure 1). As biogas was discharged from the digester, it entered the inverted 320ml calibrated bottle filled with water made free from undesirable gases. The biogas was first measured by the calibration on the inverted 320ml calibrated bottle and was then confirmed by the volume of displaced water collected in the measuring container. The digester was set up and allowed to undergo anaerobic digestion for a retention period of three (3) weeks. The amount of gas produced was recorded at 12 pm at an interval of one day (daily), as well as the initial and final pH of slurry were determined before and after the experiment (Bello, 2019).

2.4 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 an equal volume of water displaced and this was used to determine the volume of gas produced daily. The biogas produced from the digester was connected to a separate inverted 320ml calibrated bottle. The volume of displaced water was recorded as the volume of gas produced.

2.5 Tests for the Presence of Methane in the Biogas Produced

Methane which is the major component of the biogas has a combustible characteristic. The presence of the methane was tested by lighting a flame on a Bunsen burner connected to the digester. The gas that came out from the digester was checked to see whether it burns; the colour of the flame and the odour were also checked (Carucci, 2005).

2.6 Serial dilution

An aliquot of 0.5 ml was obtained using a sterile syringe from the 10^{-5} dilution of digested slurry samples and inoculated onto already prepared nutrient agar plates in triplicate by the spread plate method. An anaerobic jar was modified Mackintosh and Fildes pattern was used to incubate the plates for 72 hours at 37°C.

The residual oxygen (O_2) in the anaerobic jar was evacuated using a kindled match stick, which immediately quenched the left-over oxygen exhausted. The jar was incubated for a period of 72 hours at 37ºC as described by Babatola, (2008).

2.7 Colony count

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

2.8 Identification of Bacterial Isolates

The bacterial isolates were identified based on cultural characteristics, Gram staining reaction and biochemical tests as described by Bello *et al.* (2019).

3.0 Results

The biogas generated by the samples were recorded in Table 1. The Cow Dung started producing in the fourth day and increased throughout the period of three weeks. The biogas was produced within the optimum temperature of 25 -35°C. The biogas produced was finally tested and confirmed to be combustible through a bluish flame that glowed for several seconds.

3.1 Identification of isolates and frequency of occurrence

Table 2 shows the bacteria isolated based on morphological and biochemical characteristics. The percentage frequencies of occurrence of the isolates in relation to all samples are shown in Table 3. The bacteria isolated were *Yersenia entrocolitica, Bacillus megatherium, Bacillus licheniformis, Escherichia coli, Pseudomonas aeruginosa, Bacillus firmus, Staphylococcus aureus, Salmonella spp, Proteus vulgaris, Bacillus alvei, and Bacillus lintus.* The result indicate that *Bacillus* sp (49%) are the predominant organisms isolated in the sample (Cow dung).

4.0 Discussion

This study reveals that biogas production was delayed till the fourth day, which could be related to the fact that most cows feed on fibrous materials and microorganisms require a longer time to degrade fibrous materials. This finding corroborates well with previous reports by Babatola, 2008 in Akure, and Ukpai and Nnabuchi *et al.* (2012) in Abakaliki, both in Nigeria.

The absence of biogas production in the first three days could result from multiple carbon sources in the cow dung (substrate). As one carbon source is exhausted due to an anaerobic condition, the microbial cells divert their energy source for growth to a new carbon supply (Tyagi *et al.,* 1981). A close examination of the findings of this study shows that biogas production was less and gradual in the first week of the investigation. This suggests that the biogas producing microorganisms are in the lag phase of growth, where acclimatization or adaptations of the cells take place. It can also be deduced from this that biogas production rate is equivalent or dependent on the growth of methanogens. From the second week of the study, results indicated a progressive increase in biogas production, which continued to the third week of the study. This indicates that the methanogens are in their exponential stage of growth. However, this differs from the findings of Rabah *et al. (*2010) in Sokoto and that of Abubakar *et al.* (2012), 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. Also, climatic factors, the nature or quality of feed or pasture that the cows were exposed to, are factors that could contribute to the differences in the rate of biogas production (Abubakar *et al.,* 2012).

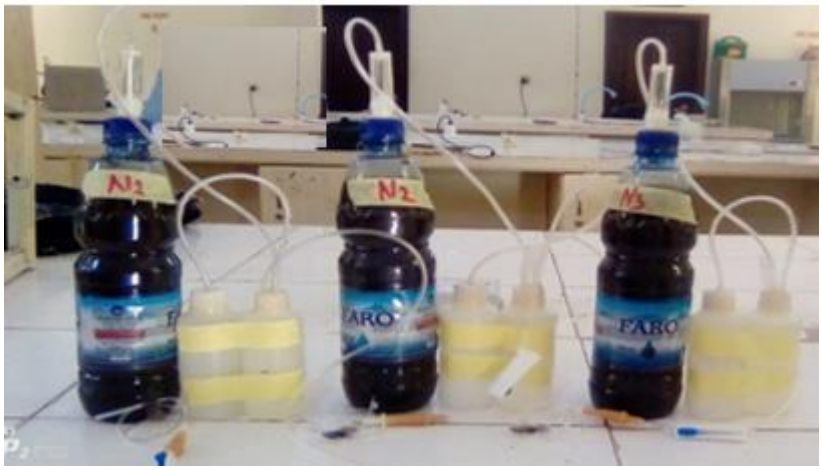

Figure 1. Experimental set-up of the digester for biogas production using cow dung. The three bottles labeled N1; N2 and N3 contains equal concentration of cow dung slurry.

Table 1: The daily volume of biogas produced at retention time of three (3) weeks

Retention time (in days)	Volume of Biogas Produced			Temperature (^{o} C)	Average Gas Produced
	N1(cm^3)	N2(cm^3)	N3 (cm^3)		
1	0.00	0.00	0.00	32	0.00
2	0.00	0.00	0.00	30	0.00
3	0.00	0.00	0.00	30	0.00
4	3.00	2.00	1.00	33	2.00
5	5.00	3.00	3.00	32	3.67
6	4.00	5.00	4.00	31	4.33
7	7.00	9.00	6.00	33	7.33
8	6.00	0.00	1.00	30	2.33
9	4.00	10.00	8.00	32	7.33
10	9.00	10.00	11.00	33	10.00
11	12.00	15.00	19.00	34	15.33
12	17.00	18.00	21.00	33	18.67
13	22.00	20.00	25.00	31	22.33
14	20.00	23.00	26.00	34	23.00
15	27.00	29.00	27.00	32	27.67
16	30.00	31.00	30.00	30	30.33
17	35.00	40.00	38.00	35	37.67
18	23.00	31.00	21.00	32	25.00
19	10.00	14.00	9.00	31	11.00
20	7.00	10.00	5.00	32	7.33
21	6.00	8.00	3.00	30	5.67
Total	247	278	258		

Key: **N1** = Cow Dung Content Chamber 1; **N2** = Cow Dung Content Chamber 2, **N3** = Cow Dung Content Chamber 3

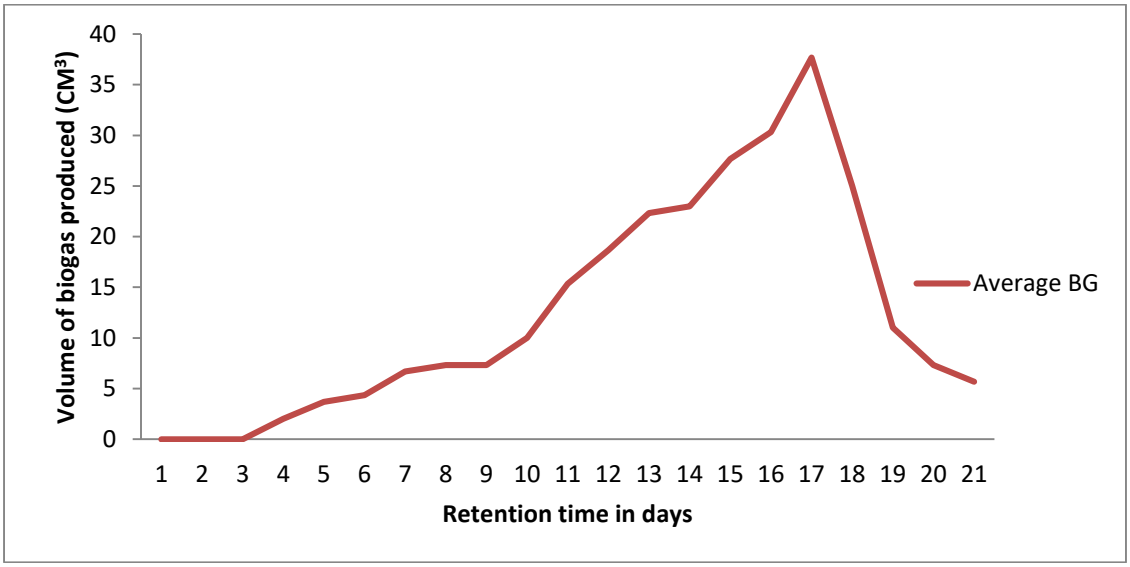

Figure 2: Average volume of biogas production of the digester against retention time (in days). BG means Biogas generated.

Table 2: Species of bacteria isolated during biogas generation based on morphological and biochemical characteristics

Isolate	Biochemical tests											
	G^1	**C^1**	**U**	**MR**	**VP**	**I**	**C^2**	**H_2S**	**C^3**	**L**	**M**	**G^2**
Y. entrocolitica	+	-	+	+	-	-	-	-	-	-	+	+
P. vulgaris	-	+	+	-	-	-	-	+	+	-	+	+
E. coli	-	+	-	-	-	+	-	-	-	-	+	+
P. aeruginosa	+	+	-	+	-	-	-	-	+	-	+	-
S. aureus	-	+	+	-	+	-	+	-	+	-	-	+
B. megaterium	-	+	-	-	+	-	+	+	+	-	+	+
Salmonella Spp	-	+	-	+	-	-	-	+	+	-	+	+
B. lintus	+	+	+	-	+	-	+	-	+	-	+	+

Key: G^1:Gram reactions,C^1: Coagulase test, U: Urease test, MR: Methyl red, VP: Voges proskuer test, I: Indole test, C^2: Catalase test, H_2S: Hydrogen sulphide production test, C^3: Citrate Utilization test, L: Lactose test, M: motility test, G^2: Glucose test

The Digester pH of biogas production before and after was also analyzed, as presented below (Table 4).

Table 3: Percentage of occurrence of bacteria isolated from the cow dung

Bacteria	Number Isolated	Percentage of occurrence (%)
Bacillus sp.	15	47
Yersinia enterocolitica	3	7
Proteus vulgaris	4	14
Escherichia coli	4	10
Pseudomonas aeroginosa	2	6
Staphylococcus aureus	4	13
Salmonella sp.	1	3
Total	33	100

Table 4: pH of digesters before and after biogas production

Digesters	Initial pH	Final pH
N1	7.17	6.40
N2	7.22	6.39
N3	7.24	6.44

Key: N1= Cow Dung Content Digester 1; N2= Cow Dung Content Digester 2; N3= Cow Dung Content Digester 3

When biogas is used, many advantages arise. In North America, the utilization of biogas would generate enough electricity to meet up to three percent of the continent's electricity expenditure (Rabah *et al.,* 2010). In addition, biogas could potentially help reduce global climate change. Normally, manure that is left to decompose releases two main gases that cause global climate change: nitrogen dioxide and methane. Nitrogen dioxide (NO_2) warms the atmosphere 310 times more than carbon dioxide and methane 21 times more than carbon dioxide (Bagudo, 2012). By converting cow manure into methane biogas via anaerobic digestion, the millions of cows in Nigeria would be able to produce one hundred billion kilowatts of electricity, enough to power millions of homes across the country. In fact, one cow can produce enough manure in one day to generate three kilowatt-hours of electricity; only 2.4 kilowatt-hours of electricity are needed to power a single one-hundred-watt light bulb for one day. Furthermore, by converting cow manure into methane biogas instead of letting it decompose, global warming gases could be reduced by ninety-nine million metric tons or four percent (Richards *et al.,* 1994).

This research showed *Bacillus* species appear to overlap from one stage to another during biogas production, suggesting a succession in species of bacteria during the process of gas production. As reported by Baki *et al.* (2004), some species such as Bacillus were found to be present throughout gas production. The result obtained from this study indicates that *Bacillus* species were the most common bacteria isolated and identified, 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 vulgaris* and *Escherichia coli* isolated during the second week (day 8 – day 14) were able to produce about 297cm^3 of biogas, while *Bacillus firmus, Proteus vulgaris, Pseudomonas aeruginosa* and *Bacillus alvei* were isolated in the third week (21days) and produced 434cm^3 of biogas. The ability of *Bacillus* species to overlap during the production was probably because the organisms can produce spore, which help them to withstand the harsh anaerobic condition or heat evolve during the biogas production (Baki *et al.*, 2004). These findings were in line with that of Ahmadu *et al*. (2009), in which *Bacillus, Yersinia*, 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 chambers (Table 4). It is not surprising as the decrease in pH may result from anaerobic fermentation taking place. pH is an important factor that affects biogas production. It was reported that anaerobic bacteria required a neutral environment (Garba *et al*., 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 sulphur-containing organic and inorganic compounds as well as the formation of fatty acids. Some microorganisms also evolved later in the process, while others died off midway. This may be explained in terms of Shellford'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.

The slurry temperature varies from 30– 35^{o}C,these temperature ranges signify that biogas production is

achieved within mesophilic temperature range (25 – 45ºC). The maximum biogas produced from each digester was attained on day 17th, where the temperature of these days was 35ºC, which is in agreement with the work of Ukapai and Nnabuchi, (2012).

5.0 Conclusion

Biogas generation from cow dung was achieved; the production of the gas started on the fourth (4th) day and reached its peak on the seventeenth day (17th). A decrease in pH and an increase in the slurry digester's temperature were observed in the second week of production. It should be noted that *Bacillus megatarium, Bacillus licheniformis, Proteus vulgaris* and *Escherichia coli* isolated during the second week were able to produce about 297cm^3 of biogas, while *Bacillus firmus, Proteus vulgaris, Pseudomonas aeruginosa* and *Bacillus alvei* were isolated in the third week (21days) and produced, 434cm^3 of biogas gas. The results of the research signify that Cow Dung in abattoir can serve as a suitable substrate for the production of biogas. Biogas generation, if carried out at a commercial scale, would provide an alternative source of energy and be a means of waste disposal in Nigeria.

Declarations

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Ethics approval and consent to participate

Not Applicable

Consent for publication

All authors have read and consented to the submission of the manuscript.

Availability of data and material

All data are presented in the report.

Competing interests

All authors declare no competing interests.

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