Direct Research Journal of Biology and Biotechnology

Direct RESEARCH JOURNALS

Vol. 3(4), pp. 67-77, September 2017
ISSN 4372-2608
DOI: https://doi.org/10.26765/DRJBB.2017.5701
Article Number: DRJA1973865701

http://directresearchpublisher.org/journal/drjbb
Research Paper

Comparative Analysis of Microbes Associated with Biogas Generation by using Dry and Fresh Cow Dung

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Received 17 August 2017; Accepted 26 September, 2017

The comparative study of biogas production from the fresh and dry domestic cow dung was carried out and investigated. Freshly and dry cow dung was collected from the house of Alhaji Yusuf in Shama village located in Udus permanent site, Sokoto state, Nigeria. The method used in biogas production was water displacement method and the biogas was collected through a pipe connected to the digester which was inserted into a measuring cylinder filled with water and inverted in a container with water and each of the samples were replicated into three. A total average biogas of 737.14 cm^3 and 4374.2 cm^3 were recorded by 1:1 and 1:6 ratios of 200 g of fresh and dry respectively. The daily biogas yield from the digester was monitored for 49 days. The amount of biogas produced was recorded at 12 noon on weekly basis. The production temperature ranges from 29°C to 34.3°C. The pH of the slurry before and after the biogas production was determined. Initial and final pH of each digester was (7.8 and 5.5), and (8.5 and 5.6). Statistical analysis (t-Test) was applied to further investigate whether the results differ significantly. The result revealed that there is significance difference between the two samples (1.782 < t Critical two-tail of 2.178). The bacteria and fungi associated with the production were determined. The result obtained were *bacillus megaterium*, *Proteus vulgaris*, Micrococcus *luteus*, *Bacillus laterosporus, Yersinie entrocolitica, pseudomonas auregenosa, Bacillus licheniform, klebsiella sp, Citrobacter fruendi, Staphylococcus aureus, Aspergillus niger, Mucor mucedo, Penicillium notatum*. However, proximate analysis was done to determine the physicochemical parameters of each dried sample. Proximate analysis was done and the parameters determined from both fresh and dry cow dung were Moisture (18.96 and 20.02%), Ash (23.66 and 33.35%), phosphorus (0.216 and 0.196), potassium (92 and 12), Nitrogen (1.5 and 2.4) and organic carbon (13.5 and 10).

Keywords: Bacteria and fungi, biogas, cow dung; micrococcus *luteus*

INTRODUCTION

The production of biogas from renewable resources is becoming a prominent feature of most developed and developing countries of the world (Akinbami *et al*., 2001). Despite the variability of international opinion on this technology, it is agreed that it plays an important role in the domestic and agricultural life of the rural dwellers in the countries like India, China, Korea and Malaysia. Biogas is used for cooking, crop drying and soil fertilizing (Meena and Vijay, 2010). Nigeria is an energy rich source country in terms of both fossil fuels such as crude oil, natural gas, coal and renewable energy resources like solar, wind and biomass. In Nigeria identified feedstock substrate for an economically feasible biogas production includes, water lettuce, water hyacinth, dung, cassava leaves and processing waste, urban refuse, solid (including industrial) waste, agricultural residues and sewage (Akinbami *et al.,*1996, 2001; Okagbue, 1988; Ubalua, 2008). In Nigeria, the use of waste from organic matter, though important, has been relegated to the background, there are abundant agricultural residues and municipal solid waste, whose potentials are yet to be fully tapped for energy generation, and the possibility of using such wastes for biogas production should be explored. The raw materials used in commercial methane generation include plant residues, animal waste like cow dung and various urban wastes which are available in Nigeria.

Biogas technology has advantages which include the following: generation of storable energy sources, production of a stabilized residue that can be used as a

fertilizer, an energy efficient means of manufacturing Nitrogen containing fertilizer, a process having the potential for sterilization which can reduce public health hazards from faecal pathogens, and if applied to agricultural residues, a reduction in the transfer of fungal and plant pathogens from one year's crop to the next. The gas is typically composed of 50-70% methane, 30-40% carbon dioxide, 1-10% hydrogen, 1-3% Nitrogen, 0.1% oxygen and carbon monoxide and trace of hydrogen sulphide. Cow dung has high nitrogen content and due to pre-fermentation in the stomach of ruminant and has been observed that to be most suitable material for high yield of biogas through the study made over the year (Chonkor, 1983).

Biogas production through anaerobic digestion (AD) is an environmental friendly process utilizing the increasing amounts of organic waste produced worldwide. A wide range of waste streams, including industrial and municipal waste waters, agricultural, municipal, and food industrial wastes, as well as plant residues, can be treated with this technology. It offers significant advantages over many other waste treatment processes. The main product of this treatment, i.e., the biogas, is a renewable energy resource, while the by product, i.e. the digester residue, can be utilized as fertilizer because of its high nutrient content available to plants (Ward *et al.,* 2008).

The performance of the AD process is highly dependent on the characteristics of feedstock as well as on the activity of the microorganisms involved in different degradation steps (Batstone *et al.,* 2002). Biogas production may therefore be a profitable means of reducing or even eliminating the menace and nuisance of urban waste in many cities. Cattle manure contains rumen microbes which assist the anaerobic digestion process and thus the biomethanization process is simple and successful. Co-digestion of multiple substrates along with cow manure is also a possible route for enhancement of methane production by creating positive synergistic effect inside the digester (Hashimoto and Varriel, 1978).This research was carried out with a view to determine or compare the efficacy, how much and how long can fresh and dry cow dung can produce biogas. The aims of this research is to compare and analyze, and isolate and identify the microbes associated with biogas generation by using fresh and dry cow dung.

MATERIALS AND METHODS

Samples collection

The fresh and dry cow dung was collected from domestic cow from Alhaji Yusuf house in Shama village located in Udus permanent site in Sokoto, Sokoto State, Nigeria. A clean container with cover was used for collection of the samples and the collected sample was transported to Chemical laboratory of Sokoto energy Research Center (SERC), Usmanu Danfodiyo University Sokoto (UDUS) for biogas production prior to microbiology laboratory for isolation and identification (Baki, 2004).

Sample processing and slurry preparation

The dried cow dung collected was grounded in to powder using pestle and mortar and all unwanted debris from the sample was removed and then 200 g was weighted and mixed with 1200 ml of sterile water in ratio of 1:6 and similarly 200 g of fresh cow dung was also weighted and dissolved in 200 ml of sterile water in ration of 1:1 and the mixture inside each digester was stirred thoroughly to obtain a homogeneous mixture and The initial pH of the mixture was determined (Baki, 2004).

Experimental set up

Six 6 empty peak milk can or tin was cleaned thoroughly and used as digested. A hole was bore on top of each tin (digester) and PVC tube was inserted into the hole and glued using erudite adhesive to prevent the leakage of the gas which convey the gas from digester to a1000 cm^3 measuring cylinder and inverted into a bowl containing water for gas collection using water displacement method. The digester was set up and allows anaerobic digestion for retention period of seven weeks. The volume of water displace is the amount of gas produced and ambient temperature was recorded at 12 noon on daily basis and as well as pH. And digester was labeled as Cf1, Cf2, Cf3, Cd1, Cd2, Cd3; each sample was replicated into three (Baki, 2004).

Determination of pH before and after digestion

The determination of pH, a calibrated pH meter is to be used to measure the pH of each of the sample before digestion. The pH of the digested slurry is to be measured on the last day of the retention period (Bagudo *et al.,* 2008). The optimum pH value for methane production is between 6.6 and 6.7. However, when the pH drop below 6.6, there is significance inhibition of the methane producing bacteria and pH of 6.2 is toxic. Although it has been proven that the optimal range of pH for obtaining maximal biogas yield in anaerobic digestion is 6.5 and 7.5, the range is relatively wide in the plants and the optimal value of pH varies with substrate and digestion technique (Liu *et al.,* 2008).

Proximate analysis

The moisture, ash content and mineral elements of the

cow dung was determined using the method adopted by (AOAC, 2003). The Nitrogen content was analyzed as described by (Khanadainal and Malidi, 1981). The volatile solid (Vs) was determined by subtracting the percentage of moisture and ash content from 100% (Garba and Atiku, 1992) .

Determination of moisture content

Moisture was determined by oven drying method. 2 g of well-mixed sample was accurately weighed in clean, dried crucible (W_1). The crucible was allowed in an oven at 100-105 C for 6-12 h until a constant weight was obtained. Then, the crucible was placed in the desiccator for 30 min to cool. After cooling, it was weighed again (W_2). The percent moisture was calculated by following formula:

$$\% \text{ moisture} = \frac{W_1\text{-}W_2 \times 100}{W_1\text{-}W_o}$$

Where
W_o = weight of empty dish
W_1= weight of dish + wet sample
W_2= weight of dish + dry sample

Determination of ash content

For the determination of ash, clean empty crucible was placed in a muffle furnace at 600°C for an hour, cooled in desiccator and then weight of empty crucible was noted (W_o). 2 gram of each of one sample was taken in crucible (W_1). The sample was ignited over a burner with the help of blowpipe, until it charred. Then the crucible was placed in muffle furnace at 55°C for 2-4 h. The appearances of gray white ash indicate complete oxidation of all organic matter in the sample. After ashing furnace was switch off. The crucible was cooled and weighed (W_2).
Percent ash was calculated by following formula:

$$\% \text{ Ash} = \frac{W_2 — W_O \times 100}{W_1\text{-}W_O}$$

Where
W_o = weight of empty crucible
W_1 = weight of crucible + wet sample
W_2 = weight of crucible + dry sample

Organic matter content

The percentage organic matter content was obtained by subtracting the sum of percentage ash and moisture content from 100. That is using the formula:
%organic matter = 100- (%ash + %moisture)

Determination of mineral elements

The mineral elements that can determine in the samples are nitrogen, potassium and phosphorus. The nitrogen can be determined using kjeldahl method. Potassium and phosphorus can be determined using atomic absorption spectroscopy (AOAC, 2003).

Determination of potassium

The flame photometer machine was switched on and allowed to stabilize. The filter was placed in position and the machine was set to zero galvanometer by aspirating distilled water and to 100% deflection by aspirating the 10ppm solution. The working solution of 2,4,6,8 and 10 ppm were separately aspirated into the machine and their corresponding galvanometer deflection was recorded. The appropriate sample solution was then aspirated into the flame photometer and the galvanometer deflection was recorded (AOAC, 2003) .

Concentration of potassium (mg/kg) = DF1xDF2

Where

DF1 = first dilution factor
DF2 = second dilution factor

Determination of phosphorous

Two (2 cm^3)of already prepared solution of 2 g ashed samples as prepared for the determination of potassium were pipette and transferred in to a 50cm^3 volumetric flask. 2cm^3 of phosphoric extraction solutions were added in each followed by 2cm^3 ammonium monohydrate solutions. The mixture were diluted and distilled with to about 48 cm^3, The 1cm^3 freshly prepared diluted stannors chloride solution were added which reduced the yelling of co - coir of the solutions to blue. The mixture was then shaken thoroughly for 2-5 minutes and distilled water was added to each to the marked of the 50cm^3 volumetric flask. Then, the colour intensity of the calibration solution (2,468 and 10 ppm) and the sample were measured using a section photometer at 660nm, the absorbance of the solution was read and recorded. The phosphorus content was determined by considering all the dilution factors (Bray and Kurtz, 1945).

$$\% \text{ phosphorous} = \frac{\text{Absorbance x Concentration x Dilution factor} \times 100}{\text{Atomic weight of phosphorous}}$$

Determination of nitrogen content

0.5g of the sample was transferred in to micro kjedahl flask. 10cm^3 of sulphuric acid were added followed by one digested tablet, the flask was then placed on the digestion rack until the color of the samples cleared (usually 2 h) and the digest was allowed cooled and diluted and distilled water to the mark of 50cm^3 volumetric flask and 20cm^3 of 40%NaOH solution was added. The mixtures was then connected to micro-kjedahl apparatus and heated to distil out ammonia. The distillatory was then connected to 20cm^3 of boric acid indicator in the form of green colour. The distilled was then titrated with 0.01m H_2SO_2 until the colour change from green to pink (Khanadainal and Malidi, 1981).

Microbial analysis

The media used for the microbiological analysis of sample (cow dung) for biogas production were Nutrient agar (NA) and sabouraud Dextrose agar (SDA). From sample labeled Cf (fresh cow dung) and Cd (dry cow dung). 1 ml of each organic waste placed in a macCarney bottle containing 9ml of sterile distilled water, and shaken well to homogenize the suspension (this is 10^{-1} dilution). Therefore, 1 ml of aliquot from this 10^{-1} dilution is to be measured into another bottle containing 9 ml of sterile distilled water and obtained dilution 10-^{2} dilution. Further dilution carries out till a dilution level of 10-^{7} marked on the bottle as follow (10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6}, and 10^{-7}). 1 ml of the suspension from the dilution 10^{-7} pipette and to be dispensed into 9 centimeter diameter sterile disposable Petri dish labeled NA and SDA. The isolates was purified by sub culturing of isolate in to fresh and SDA which were inverted and incubate at 37°C for bacteria and 28^{o}C for fungi, therefore the Petri plate were examined for colony formation after 24 h for bacteria and 3-5 days respectively for fungi growth. The number of colonies per Petri plate and per dilution is recorded (Angelidaki *et al.,* 2002).

Isolation of fungi

0.1 milliliter aliquots of the serially-diluted samples were introduced into culture plates containing sterile sabouraud Dextrose Agar (SDA), with Chloramphenicol at a concentration of 0.05mg/ml to inhibit bacterial growth (Holt *et al*., 1994). The samples were uniformly spread on the surface of the medium with a sterile glass rod. Incubation was carried out in an inverted position at 28^{o}C for five days for the development of the fungal colonies.

Characterization and identification of the fungal

Isolates the colonial and microscopic characteristics of the fungal isolates were determined using the lacto phenol cotton blue staining method.

Lactophenol cotton blue staining

A solution of lactophenol cotton blue was prepared. Using a straight wire, a fragment of a fungal isolate was placed on a clean grease-free slide. Two drops of the lacto phenol cotton blue solution were added and the stain allowed penetrating. The slide was then viewed under the microscope. The isolates were identified following the description of (Oyeleke and Manga, 2008).

Purification and maintenance of the microbial Isolates

The bacterial colonies which developed on the plates were randomly picked and purified by sub culturing on nutrient agar (NA) plates before transferring onto NA slants. The fungal isolates were also purified using the same method as for the bacterial isolates but they were sub cultured onto SDA plates before transferring onto SDA slants. These isolates were stored at 4^{0}C in the refrigerator as stock cultures for characterization and identification (Holt *et al*., 1994).

Characterization and identification of the bacteria

Media preparation

Nutrient agar

The Nutrient agar medium prepared according to manufacturer instruction that is 28 g of nutrient agar suspended in 1000 ml of distilled water and mixed thoroughly and heated on hot plate to dissolve completely. The preparation was autoclaved at 121°C for 15 min, and then allowed to cool at 45°C and then dispensed into sterile petri dish for isolation of bacteria (Cheesbrough, 2006).

Gama staining

A thin smear of bacterial isolates was prepared on a glass slide, air dried and then fixed by passing over a burner flame atleast three Times. The smear was covered with crystal violet stain for 1 minutes and then washed, it was then covered with Lugosi' iodine and washed after 1 minutes, and then decolorized with acetone and washed immediately for few Seconds. The smear was covered with safaranin and washed after 1 min. The Back of slide was wiped out with cotton wool and allowed to air dried; the dried smear was then examined microscopically with oil immersion and viewed

with a microscope using x 100 objective lens (Cheesbrough, 2006).

Microscopy

The back of the glass slide is wiped Clean and a drop of oil' (glycerin) was applied on the smear which was examined microscopically with x 100 objectives lens for the observation, of gram reaction and morphological characteristics of the bacteria cells. Gram positive bacteria appear purple in colour, while gram negative cells retained the staining colour of safranin and appear pink in colour. After the gram staining and microscopy, the isolate subculture into universal bottle containing nutrient agar in a slant form for subsequent used in biochemical test (Oyekele and Manga, 2008).

Biochemical identification of isolates

The identification of fungi is by microscopic examination of colonies and also microscopic examination after staining with lactophenol in cotton blue. Bacteria isolate identify with the following biochemical test. Motility test, catalase test, voges-proskauer test (Fawole and Osho, 1998; Seeley and Vandemark, 1972).

Sugar fermentation test

Triple sugar ion agar slant in a tube were inoculated with isolates (24 h old culture) using a sterile inoculation needle. The surface was streaked and butt was stabbed 3 times.

The test tubes were incubated at 37^{o}C for 24 h after which they are examined for gas production; glucose fermentation respectively including motility, for a positive reaction, glucose fermentation is indicated by butt becoming yellow, while in lactose, the media appeared yellow. Gas production was determined by appearance of bubbles. Motility was positive due to cloudy medium and line of inoculation not sharply defined (Oyeleke and Manga, 2008).

Catalase test

It is used to differentiate those bacteria that produce enzymes catalase, such as staphylococcus from non catalase producing bacteria such as streptococcus. A drop of hydrogen peroxide was placed on a slide and a 24 h growth culture was emulsified with drop of hydrogen peroxide on the slide. Immediately it was observed for the presents of bubbles as indication for positive reaction, absence of bubbles indication indicates negative result (Cheesebrough, 2006).

Voges-proskauer (vp)

Five (5) drops of potassium hydroxide (KOH) was added to 48 h old MR-VP medium culture of isolates. 2 to 3 drops of alpha naphthol is added and shaken. The test tube is sloped (slant) and examined for 1 h. Positive test was indicated by strong red coloration while negative result showed no red colour (Oyeleke and Manga, 2008).

Methyl red test

Isolates were inoculated into test tubes and incubated at 35°C for 48 h. Two drop of methyl red indicator was added after the period of incubation. Positive test was by red colour while negative test showed yellow color (Oyeleke and Manga, 2008).

Citrate test

A twenty four 24 h culture isolate is inoculated in to slanted test tubes containing sterile Simmons citrate medium aseptically and incubated at 37^{o}C for 72 h. Observing daily for the presence of growth and color changes, positive reaction was indicated by development of deep blue color (Oyeleke and Manga, 2008).

Indole test

Twenty-four 24 h old culture was incubated into a test tube containing a sterile nutrient broth and incubated at 37^{o}C for 48 h. Five drops of kovaca's indole reagent was added to the 48 h broth culture which was then shaken. A positive reaction was indicated by the appearance of red ring coloration on top of the reagent above the broth medium within 1 min. While in negative reaction, the indole reagent retains it yellow color (Nwankwo. 2014).

Urease test

Urea agar slants in universally bottles were inoculated (streaking) with 24 h old culture and inoculated at 37^{o}C for 48 h. Positive test was indicated by pink colour as a result of an enzymes Urease which hydrolyzed the Urea to carbon dioxide and ammonia, while negative test indicated by absence of pink colour (Oyeleke and Manga, 2008).

Method used in measuring of daily biogas produced was displacement method

The gas production is measured by water displacement method whereas the quality, which is the percentage of methane from the biogas, is estimated by the displacement

of sodium hydroxide, with a process held one next to the other. The gas volume produced in the anaerobic reactor was captured in a bottle filled with water, which was kept under pressure. When a gas bubble entered the bottle with water, the gas replaced the water, which was then forced out of the bottle into an empty bottle. The volume of water in the measuring cylinder thus resembled the gas production in the reactor. The displaced water is collected and then using a measuring cylinder (Veeken and Hamelers, 1999).

RESULTS

The result of bacteria and fungi involved in biogas generation of this research work was identified and recorded. The result of bacterial identification is represented in (Table 1). Similarly, that of the fungal is presented in (Table 2). This result obtained shown that the bacteria and fungi isolated and identified were *bacillus megaterium*, *Proteus vulgaris***,** micrococcus *luteus***,** *Bacillus alvial, Bacillus lentus, Yersinie sp, pseudomonas auregenosa, Bacillus licheniform, klebsiella sp, Citrobacter fruendi, Aspergillus niger*, *Mucor mucedo* and *Penicillium notatum*. The result of the percentage frequency of occurrence of the isolates is presented in (Table 3). The result of the studyl shows that *Bacillus spp*. are the predominant organisms isolated from fresh and dry cow dung with frequency of occurrence (42.8%) and (40%) and other organisms are (14.3%) and 20% while *Aspergillus* was the predominant species in fungi isolated The result of the pH of the digesters before and after the biogas production is presented in (Table 4). The volume of biogas produced in the six digesters on weekly basis of seven weeks is presented in (Table 5) and the statistical analysis (t-test) was applied to further investigate whether the results differ significantly. The results revealed that there is significance difference between the two samples (1.782< t critical two-tail of 2.178). The result of proximate analysis of both digested and undigested is represented in (Table 6).

DISCUSSION

The comparative study of investigation of microorganisms associated with biogas production as well as comparing the efficacy using fresh and dry cow dung. The investigations reveals that biogas production was delayed in the first week of set up, although a day after set up it was observed that one of the fresh sample **(**Cf1) had commence to produce gas. This delay could be traced to the fact that, most cows feed on fibrous materials and microorganisms require a larger time to degrade fibrous materials and this investigation is in conformity to that, from the research of Babatola, (2008) in Akure, and Ukpai and Nnabuchi, (2012) in Abakalaki, both in Nigeria. From the second week all digester begun to produce biogas and the production of biogas was less and gradually increases and this is because the biogas producing microorganisms are in the lag phase of growth where the acclimatization of the cells take place. This report is in consonance to that of Abubakar and Ismail, (2012). Therefore, the biogas production depends on the growth of methanogenes. However, from the third to the fourth week the volume of biogas production was very higher and lager volume was produced. This indicates that the methanogenes are in their exponential stage of growth. The dry cow dung have high production when it begun to produce but it took at least a week before started to producing. However, temperature and pH are the most essential factors that determine the biogas production. In a day, at higher temperature, at 3rd and 4th week, a digester of the dry cow dung displaced about 500 ml and sometimes even 1000 ml of water from the cylinder containing water, which was equivalent to 0.5dm^3 and 1dm^3 of biogas meaning that the volume of water displaced is equal to the volume of biogas produced according to displacement method. While the fresh cow dung production was moderate and slow when compared to the dry. During the first week fresh cow dung produced a cumulative biogas of 260 cm^3 while the dry produced no biogas at 32°C operating temperature. And also at second week of experiment fresh cow dung produced a biogas of 750 cm^3 while dry produced a total of 5800cm^3 at operating temperature. And at the third week shows a greater gas production which shows a cumulative gas production of 1320 cm^3 for Fresh and 12,550cm^3 for dry at 34.3°C. And at fourth week the cumulative biogas produced from fresh was 1820cm^3 and the dry was 9,680cm^3 at 34.1°C operating temperature. At fifth week the production of biogas produced continued to decline in which the fresh produced 690cm^3 and dry produced 1690cm^3 at 31°C operating temperature. While at sixth and seventh week shows lower gas production and later ceased to produced as shown in the (Tables 4 to 5) and the comparative total average of biogas produced from fresh was 737.14 cm^3 and dry was 4374.2 cm^3. This shows that the dry cow dung produced higher average volume of biogas 4374.2cm3 compared to fresh that produced 737.14 cm^3. However considering the work done by (Udochukwu *et al*., 2016) reported that cow dung have (4730.55 cm^3) total volume of biogas produced, which is in compare with the present study, the result can be seen that the reported work was a little bit different, but are within the same range with the volume of dry one, while the average volume of fresh cow dung produced was very low, this different may be due to the nature of fresh cow dung used. Statistical analysis (t-Test) was applied to further investigate whether the results differ significantly. The result revealed that there is significance difference between the two samples (1.782 < t Critical

Table 1. Isolation and characterization of bacterial isolates from the biogas digesters.

Isolate Code	Gram Reaction	Catalase	Glucose	Sucrose	Lactose	Motility	H_2S	Gas	MR	VP	Indole	Urase	Citrate	Organism Isolated
Cf1a	+	+	+	_	_	+	+	_	_	+	_	_	+	*Bacillus megaterium*
Cf1b	+	_	+	+	_	+	_	_	+	_	+	+	_	*Yersinie entrocolitica*
Cf2a	_	+	+	+	+	+	+	_	_	_	+	+	+	*Proteus vulgaris*
Cf2b	+	+	+	+	_	+	+	_	_	+	_	+	_	*Bacillus alvei*
Cf3a	+	+	+	+	_	_	_	_	_	+	_	+	+	*Staphylococcus aureus*
Cf3b	_	+	+	+	_	_	_	+	_	+	_	+	_	*Klebsiella sp*
Cd1a	+	+	+	_	_	+	_	_	+	_	_	+	_	*Bacillus lentus*
Cd1b	+	+	+	+	_	+	_	+	_	+	_	+	+	*Bacillus licheniform*
Cd2a	+	+	+	_	+	+	+	+	+	_	_	_	+	*Citrobacter fruendi*
Cd3a	+	+	+	_	_	_	_	_	_	_	_	+	_	*Micrococcus luteus*
Cd3b	+	_	+	_	_	+	_	_	+	_	_	_	+	*Pseudomonas auregenosa*

Key Cf= fresh cow dung, Cd= dry cow dung.

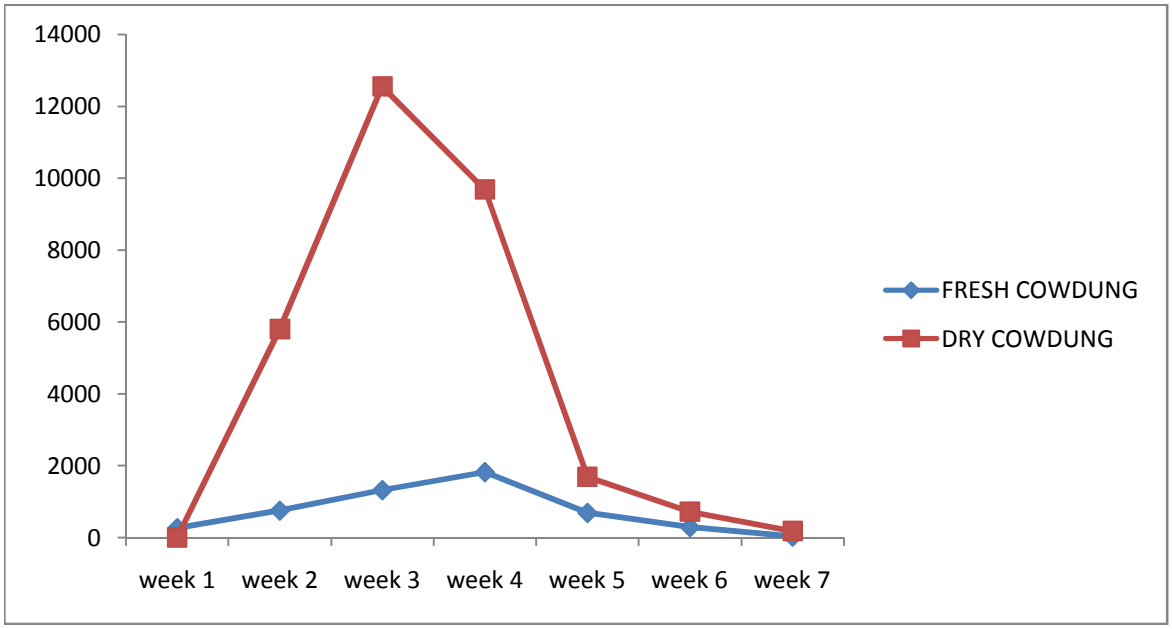

Figure 1. Difference between fresh and dry cow dung of biogas produce.

two-tail of 2.178). The experiment showed that cow dung is better substrates for gas productions especially dry one. The pH of the fresh feed-in was in 7.8. The effluent–out after digestion showed decreased in pH 5.5, while the pH of dry feed-in was 8.5 and later drop to 5.6. And from the Fifth to six week the volume of biogas produced continued to decline and pH of the slurry also decreases and the decreases in the pH may be is as a result of anaerobic fermentation take place and was reported that anaerobic require a natural or neutral environment, (Garba and Atiku, 1992). Thus the optimum pH value for methane production is between 6.6 and 6.7.

Microbial analysis

Tables 2 and 3 show the total bacterial and fungal count was recorded in the study. And the bacteria isolated and identified after the biogas production were *bacillus megaterium*, *Proteus vulgaris*, *micrococcus luteus*, *Bacillus*

Table 2. Characterization and morphology of fungal isolates from the biogas digesters.

Isolate code	Total fungal count (cfu/ml)	Colony top view	Colony reverse view	Isolated organism	Microscopic characteristic
Cf1	6 x 10^7	Black colony with granular surface	Black reverse	*Aspergillus niger*	Septate hyphae, dark brown, large globose conidial head hyaline smooth -walled conidiosphores which turn dark toward the vesicle, conidial heads are biseriate.
Cf2	4 x10^7	Dark green	Cream green	*Penicillin notatum*	Septate hyphae with conidiosphores developing into branched phalides bearing chain with brush like appearance
Cf3	4 x 10^7	Black colony with granular surface		*Aspergillus niger*	Septate hyphae dark brown, large glimpse conidial head hyaline smooth walled conidiosphore which turn dark toward the vesicle, conidial heads are biseriate
Cd1	4 x 10^7	White fluffy	Milk	*mucor mucedo*	Aseptate broad hyphae with large spherical head without rhizoid
Cd2	6 x 10^7	Black colony with granular surface	Black reverse	*Aspergillus niger*	Septete hyphae, dark brown, large globose conidial head Justine smooth walled conidiosphore which turn dark toward the vesicle, conidial heads are biserited
Cf3	4 x 10^7	Black colony with granular surface	Black reverse	*Aspergillus niger*	Septate hyphae, dark brown, large globose conidiosphores which

Key Cf= fresh cow dung, dry= cow dung

Table 3a. Frequency and percentage of bacterial isolate from fresh digester (fresh and cow dung) fresh cow dung.

Organisms	Frequency of occurrence	% Occurrence of bacteria
Bacillus spp	3	42.8
Yersinie entrolitica	1	14.3
staphylococcus aureus	1	14.3
Proteus vulgaris	1	14.3
klebsiella	1	14.3
Total	7	100

Table 3b. Dry cow dung.

Organisms	Frequency of occurrence	% occurrence of bacteria
Bacillus spp	2	40
Pseudomonas eurogenosa	1	20
Micrococcus luteus	1	20
Citrobacter fruendi	1	20
Total	5	100

Table 3c. Colony count of bacteria isolate.

Isolate code	Cfu/ml
Cf1	5.7 x10^7
Cf 2	6.5 x 10^7
Cf3	7.5 x 10^7
Cd1	6.5 x 10^7
Cd2	7.6 x 10^7
Cd3	5.5 x 10^7

Keys Cf= fresh cow dung, Cd = dry cow dung

licheniform, Yersinie entrocolitica, Proteus Bulgaria, Bacillus alvei, staphylococcus aureus, klebsiella pneumoniae,citrobacter fruendi, pseudomonas erougenosa, the fungal isolated are *Aspergillus niger, mucor mucedo, Penicillium notatum,* in both the fresh and dry cow dung, the *Bacillus spp* appeared to be dominant.

Table 4. Average pH of digesters before and after biogas production.

Digester	pH before	pH after
Cf	7.8	5.5
Cd	8.5	5.6

Keys Cf= fresh cow dung, Cd = dry cow dung

Table 5a. Biogas produces for seven (7) weeks of retention.

Days	CF1	CF2	CF3	Cd1	Cd 2	Cd3	Temp. °C
1-7	260	0.00	0.00	0.00	0.00	0.00	32
8-14	290	180	280	1250	2730	1800	33.4
15-21	520	210	590	3750	3900	4900	34.5
22-28	550	650	620	3150	3310	3220	34.1
29-35	180	280	230	430	610	650	31.2
36-42	80	100	110	150	280	290	29
43-49	0.00	10	20	30	70	80	30
Total	1880	1430	1850	8760	10,900	10,940	224

Keys cf= fresh cow dung, Cd= dry cow dung.

Table 5b. Total means of all fresh and dry of biogas produced in seven weeks.

Volume of Biogas Produced (cm^3)			
Days	**Fresh cow dung**	**Dry cow dung**	**Temp. °C**
1-7	260	0.00	32
8-14	750	5800	33.4
15-21	1320	12550	34.3
22-28	1820	9680	34.1
29-35	690	1690	31.2
36-42	290	720	29
43-49	30	180	30

The weekly Average sum of all fresh (cf1, cf2, cf3) and dry cow dung (cd1, cd2, cd3) of biogas Produced.
For Fresh Cow dung (Cf) = 5160/7 = 737.14 cm^3
For Dry Cow dung (Cd) = 30620/7 = 4374.2cm^3

That is, the *Bacillus spp isolated* from fresh had frequency of occurrence 42% and the dry had 40% which are the highest. Where the % frequency of occurrence of others was 14.3% and 20% which is the least as was shown in the (Tables 3a, 3b and 3c) the fungi isolated from both fresh and dry, *Aspergillus Niger was* the dominant. These finding is in line with that reported by (Udochukwu *et al*., 2016) and (Rabah *et al*., 2010) in Abeokuta Ogun and Sokoto state, all are in Nigeria and Oluyega *et al.* (2006) reported that *Bacillus*, *Yersinia* and *Pseudomonas* species were responsible for biogas production in cow dung.

Proximate analysis

The moisture content is one of the parameters that are used to access the viability of biomas for its potentials in biogas generation. Biomass with higher moisture content prove not efficient in biogas generation, from the result obtained in the research work carried out has shown that the percentage moisture content of digested samples of fresh and dry have higher percentage than the undigested sample as shown in (Table 5). Bagudo *et al.* (2008) reported that the moisture content of digested and undigested cow dung was 12.00% and 19.00%. When compared with the result of the present study which are 18.96% and 23.16% as shown in (Table 6a and 6b). While the ash content is a good factor for determining the mineral elements present in the substrate, the higher the ash content in the substrate the higher the percentage of mineral element present, again by considering the work done by (Bagudo *et al.,* 2008) who reported that the % of ash content of cow dung was 24% and 29% of undigested and digested, which was little bit different in compared with the fresh cow dung from the result obtained in this study, but different from dry cow dung as

Table 5c. Biogas produced per day in fresh cow dung (Cf1, Cf2, and Cf3).

Average temperature °C	Mass in (g) of fresh used	Period of Retention day (7x7 =49 days)	Total average of biogas produced cm^3	Biogas Yield (cm^3/g/day)
32	200	49	1880	0.19
32	200	49	1430	0.15
32	200	49	1850	0.19

Table 5d. Biogas produced per day in dry cow dung (Cd1 , Cd2 ,and Cd3).

Average temperature °C	Mass in (g) of dry used	Period of Retention day (49)	Total average of biogas produced cm^3	Biogas Yield Cm^3/g/day
32	200	49	8760	0.89
32	200	49	10,900	1.11
32	200	49	10,940	1.12

Keys cf= fresh cow dung, Cd = dry cow dung.

Table 6a. Undigested substrate (fresh and dry cow dung).

Parameter	Fresh cow dung (Cf) %	Dry cow dung (Cd)%
Moisture(2g)	18.96	20.02
Ash (2g)	23.66	33.35
Nitrogen (N)	1.5	2.4
Phosphorus(P)	0.216	0.196
Potassium (K)	92	12
Organic carbon	13.5	10.0

Table 6b. Digested substrate (fresh and dry cow dung).

Parameters	Fresh cow dung (Cf)	Dry cow dung (cod)
% Moisture (2g)	23.16 %	25.03 %
% Ash (2)	25.72 %	34.32 %
Nitrogen (N)	1.116	2.012
Phosphorus (P)	0.192	0.161
Potassium (K)	51.2	7.61
% Organic carbon	6.31	5.61

shown in the (Tables 6a and 6b). While the % content of Nitrogen, Phosphorus and Potassium are shown in (Tables 6a and 6b). Except in substrate of cow dung where anaerobic digestion does not affect the nitrogen content of cow dung (Bray and Kurt, 1945).

Conclusion

The findings of this study of comparative analysis of fresh and dry cow dung shows that, cow dung could be used as a suitable substrate for biogas production. Biogas production, if carried out at commercial scale, would not only provide an alternative source of energy but would also be a means of waste disposal for Nigeria. Dry cow waste produced comparatively higher volume of gas than fresh waste by 4374.2 cm^3 and 737.14 cm^3. These wastes are readily available in our environment and therefore can be gotten in large quantity for biogas production. Generation of biogas from animal waste reduces environmental pollution and creates an alternative renewable energy source. Finally the most important of biogas produced is methane. Biogas production from large quantities of cow dung especially dry appears to have potential as an alternative renewable energy for many African countries if relevant and appropriate research is carried out to adopt the biogas technology to the local conditions in African countries.

Recommendations

Base on the finding from the experiment carried out the following recommendation are made:

(i) The biogas production provide a means for solving simultaneously the energy demand and the pollution problem in a rural area, therefore its adoption should be encourage by local, state and the federal government.
(ii) Relatively high level of research and development works should be encourage in this area in order to overcome the basic problems associated with the biogas technology.
(iii) Various educational sectors should establish peculiar courses on biogas by introducing the benefits and also the uses of biogas so that people may participate in the process of biogas production and consider it important.
(iv) Government needs to enhance biogas production plant and take major steps by straightening biogas research processes in order to achieve the desired goals of biogas production works and its application.

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