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# Analysis of Bio-gas Production from Cow Dung by Indigenous Microbial Consortia

**V. O. Ifeanyi[1*] and I. W. Ossai[1]**

*[1]Department of Microbiology, Anambra State University, Anambra State, Nigeria.*

***Authors' contributions***

*This work was designed and carried out in collaboration between the both authors. Author VOI wrote the protocol, designed and supervised the study. Author IWO managed the literature searches and wrote the first draft of the manuscript. Both authors collaborated during the analyses of the study, read and approved the final manuscript.*

***Original Research Article***

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## ABSTRACT

The study analyzed quantitatively and qualitatively the biogas produced from cowdung by indigenous microbial consortia. Four 20L bioreactors were used for the study. The bioreactors were constructed to imitate the fixed batch prototype. The study lasted for six months and it was carried out at the Microbiology Laboratory of Anambra State University, Anambra State, Nigeria. Slurry was prepared in bioreactors. The substrates in the bioreactors were water and manure (WM), rumen fluid and manure (RM-1 and RM-2), medium and manure (MM). The pH, the total solids (TS), volatile solids (VS) and total volatile fatty acid (VFA) characteristics of the substrate before and after digestion were determined using standard method. Quantification and qualitative analysis of biogas production was by liquid displacement and gas chromatography methods respectively. The microbial analysis of the substrate was carried out using spread plate method. The results of the TS, VS and VFA were 400 mg/l, 92mg/l and 16.7 mg/l respectively in the predigested samples and 92 mg/l, 17.4mg/l and 28.3mg/l respectively in the post digested samples. The quantity of biogas produced at fourth month was 60 ml, 128ml and 220ml from WM, MM and RM-1 respectively. The qualitative analysis showed that the prominent biogas produced was methane. The cultural morphology revealed Gram positive rods with creamy irregular edges. The average heterotrophic counts at the end of each month for a period of four months were $18.5x10^5$cfu/ml, $21.1 x10^5$cfu/ml and $26.7x10^5$cfu/ml for WM, MM and RM respectively. The results of the research concluded that high quantity of biogas can be produced using cow dung. Approaches and technology for more efficient biogas producing consortia are proposed.

**Corresponding author: Email: ifehos@yahoo.com;*

*Keywords: Biogas; methane; microbial consortia; bioreactor; cow-dung; slurry; analysis; digestion.*

## 1. INTRODUCTION

Fossil fuels had for long become the major source of global energy. These fuels are generally used as sources of energy in combustion engines and in some instances as raw materials for the petrochemical industries. Although, fossil fuels play a key role in the global economic and political situations, they have numerous challenges such as environmental pollution, global warming, oil spills and gas flares [1]. The production of biogas from renewable resources is becoming a prominent feature of most developed and developing countries of the world. It is agreed that biogas plays an important role in the domestic and agricultural life of the rural dwellers in countries like India, China, Korea and Malaysia. It is used for cooking, crop drying and soil fertilizing [2]. It also has the advantage of contributing to the solution of environmental problems, because it substitutes fossil fuels [3]. The local manure from animal herds, other agricultural and industrial wastes that are largely generated in Nigeria on a daily basis could be employed as raw material for both small and large-scale biogas production. Nigeria produces about 227,500 tons of fresh waste each day [4]. Biogas technologies commonly apply natural anaerobic consortia of microbes. The use of biomass is one of the most promising technological generations [5,6]. Anaerobes can be divided into two groups: Acidogens and methanogens.

The pH of manure slurries is largely determined by the strength and equilibrium of carbonic acid-bicarbonate buffers, volatile fatty acid and ammonia [7]. In deep storage tanks for slurries, pH would also be a function of depth because of an increasing solubility of carbon dioxide under increasing hydrostatic pressure. Decreasing VFA concentrations would tend to increase pH [7]. Methanogenic bacteria are seriously inhibited at pH below 6.5 and pH ranging from 6.4-7.2 is required for optimum biogas production [8,9]. Sharma [10] reported that cattle dung substrate increases susceptibility to microbial degradation. He overcome problem of acidification in onion storage waste (OSW) resulting to drastic reduction in pH due to rapid acidification of onion by mixing cattle dung with OSW in a suitable ratio so that medium is well buffered to take care of acid accumulation.

The biogas composition varies depending on the substrate [11,12]. However, biogas main constituents are methane and different percentages can be obtained by using various raw materials [11]. Biogas from sewage digesters usually contains from 55-65% methane, 35-45% carbon dioxide, <1% nitrogen, biogas from organic waste digesters usually contains from 60-70% methane, 30-40% carbondioxide, and <1% nitrogen, while in landfills methane content is usually from 45-55%, carbondioxide from 30-40% and nitrogen from 5-15% [13]. Jain and Mattiasson [14] (1998) found that above pH 5.0, the efficiency of $CH_4$ production was more than 75%.Mattsson et al. [15] reported various methane contents from agricultural feed stocks as follows cow slurry; 75-85%, whey 80-95%, leaves 90%, garden wastes 90%.

Numerous studies had been conducted by several researchers in order to increase yield in anaerobic digestion. Some of the approaches involved using two continuously stirred tank reactors (CSTR) in series [16], selectively retaining the solids within the reactor by holding mixing prior to effluent removal and pretreatment of manure by separating solids from digested material in order to improve biodegradability and accessibility [17], In addition, an effort to increase biogas yield has been done by improving contact between bacteria and substrate using stirring [18].

Exploitation of animal dung for production of biogas in Nigeria is in its infancy. Hence, this research was carried out using batch intermittently stirred operation mode. This research aimed at determining quantitatively and qualitatively biogas production from cow dung by indigenous microbial consortia

## 2. MATERIALS AND METHODS

### 2.1 Sample Collection and Preparation

Two weeks old cow dung and fresh rumen fluid were collected from a slaughter house at Uli, Anamrba State and transported to the Microbiology Laboratory of the Anambra State University, Anambra State, Nigeria. At the laboratory, a series of experiments using four 20 liter bioreactors were performed in batch operation mode. Slurry was prepared in four 20 liter bioreactors in the ratio of 1:2 by taking two kilograms of cow dung to 4L of liquid in the order; water and manure (WM), rumen fluid and manure (RM-1 and RM-2), medium and manure (MM). These mixtures were homogenized by mixing the substrates manually. The liquid medium was nutrient broth composed of peptone; 5g/l, meat extract; 1g/l, yeast extract; 2g/l, sodium chloride; 5g/l as outlined by the manufacturer.

#### 2.1.1 Experimental design

Bioreactors used for the study were made from polyethylene bottle that were constructed to imitate the fixed batch prototype as also has been used by previous studies [19,20]. The WM, RM-1 and MM bioreactors were constructed to have inlet and outlet valves. The RM-2 had only an outlet valve which was connected to a sealed collapsible tube. All valves were plugged with tightly rubber plug and made air tight with araldite adhesive. The outlet valves were connected with long delivery tubes which convey the gas from the digester to the water displacement set-up where they were inverted. The digesters were stirred to homogeneity five times per day and were allowed to undergo anaerobic digestion for a retention period of four weeks before analysis started. The pH of the RM-1 was recorded immediately after preparation and repeated weekly throughout the study. The study was carried out under the room temperature.

### 2.2 Pre and Post Digestion Characteristics of the Cow Dung

The pH determination, pre and post digestion analysis of the total solid (TS), volatile solid (VS) and total volatile fatty acid (VFA) of the substrate were carried out using standard method as described by American Public Health Association(APHA) [21]. All the tests were conducted in duplicates and the mean values were reported.

#### 2.2.1 Quantification analysis of biogas

Quantification of biogas production was analysed by liquid displacement method as described by Andersen et al. [22] and Walker et al. [23]. Quantification of biogas in the WM, RM-1 and MM bioreactors was carried out every month after the retention period for a consecutive period of four months.

### 2.2.2 Qualitative analysis of biogas

The qualitative analysis was carried out at the Analtrace Environmental Consultants and Laboratories, Warri, Delta State, Nigeria. The cumulative biogas production during the period of six months was subjected to gas chromatography for the qualitative analysis [22]. Syringe injection method was used in Buck Scientific, model 910, and channel 4 GC–TCD detector, column: restek 30 meter mxt-1 at 5 minute retention at 80ºC, helium carrier at 10 PSI. Integration; peaksens=95.0, Base sens=60.0, Min area=100.00, Standard 1.000, sample = 1.000, Tangents = off. Firstly, a methane standard was run in the gas chromatography.

## 2.3 Microbial Analysis

The microbial analysis of the substrate was carried out using spread plate method. The cultural morphology and the heterotrophic counts of bacteria were determined by making a decimal serial dilution of samples from MM, RM-1 and WM bioreactors. Spread plating was carried out in duplicate using 0.1ml of $10^{-5}$ on nutrient agar plates. The plates were incubated for 36 hours at 28ºC. Gram reaction, colony morphology and heterotrophic count were determined.

## 3. RESULTS

## 3.1Pre and Post Digestion Characteristics of the Cow Dung

The TS and VS characteristics of the cow dung showed high values in the pre digestion analysis with TS giving 400mg/l. The results of the post digested samples showed reduction which denotes adequate utilization of the substrate. The TS was reduced to 92mg/l (Fig. 1). The VFA increased in the post digested sample as against the predigested in which 18.7mg/l and 28.3mg/l were reported respectively. The average pH of the substrate during the period of the study was 6.7.

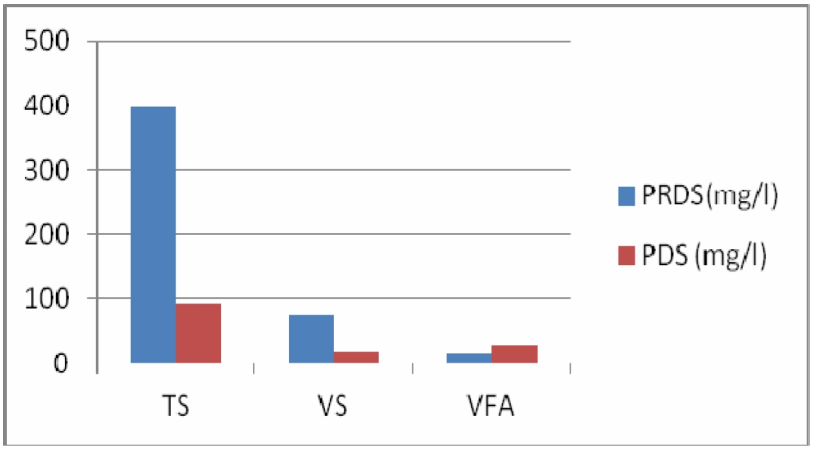


**Fig. 1. Pre and post digestion characteristics of cow dung**
*Key: PRDS = predigestion sample, PDS = post digested sample*

## 3.2 The Quantity of Biogas

A change in volume was noted over the test period. The bioreactors produced different quantities of biogas. The quantity of biogas produced at fourth month was 60ml in WM bioreactor, 128ml in MM and 220ml in RM (Fig. 2). Biogas production was very slow at the beginning. This is predicated upon the fact that biogas production rate in batch condition directly corresponds to microbial growth at the lag phase. The yield in the RM-1 bioreactor at the fourth month was higher than the other two bioreactors. The result indicated that liquid rumen enhanced biogas production. It suggests that anaerobic bacteria content in liquid rumen augments the degradation of organic substrate from manure. It was observed that biogas production was slow initially. During the first week of observation, there was less biogas production. This was predicated to the lag phase of microbial growth whereas within 2 to 4 weeks, biogas production increased substantially due to exponential growth of methanogens.

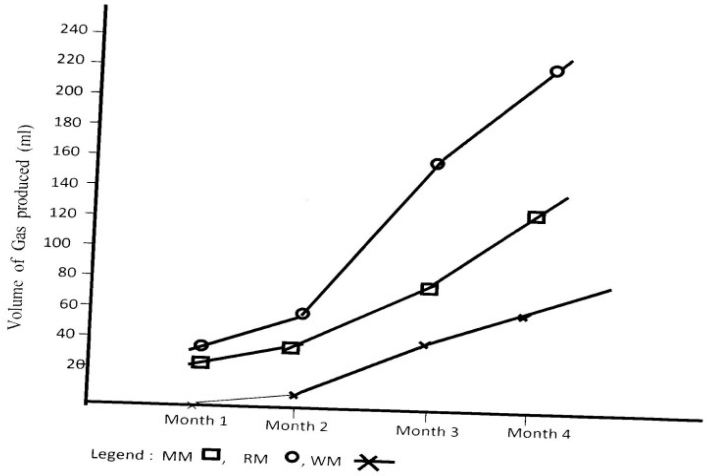


**Fig. 2. The quantity of biogas produced**

## 3.3 The Quality of Biogas

The qualitative analysis of the biogas showed that methane was the prominent biogas produced during the period of study. This declaration was made because the only gas detected by the Thermal Conductivity Detector (TCD) Gas Chromatography used in the study was methane (Fig. 3). It was only the peak formed by methane that was observed in the sample. A Thermal Conductivity Detector Gas Chromatography as against Flame Ionization Detector (FID) Gas Chromatography is used for detecting Hydrogen, Carbon dioxide, Carbon monoxide and carbon numbers $C_1$-$C_6$. Flame Ionization Detector is specific for detecting substances that contain only carbon and hydrogen bonds. It might be proclaimed that close to 100% methane was produced. Other gases produced might be below detectable limit of the instrument. The standard had the same retention time and area as the methane produced in the study (Fig. 4).

### 3.4 Microbial Analysis

The cultural morphology and Gram reaction result revealed colonies with creamy irregular edge and Gram positive rods. The heterotrophic count result is presented (Fig. 5). Multiplication of bacteria was slow initially and gradually increased in the second month. Growth entered into exponential phase in the third month and the highest growth was recorded in the RM-1 bioreactor.

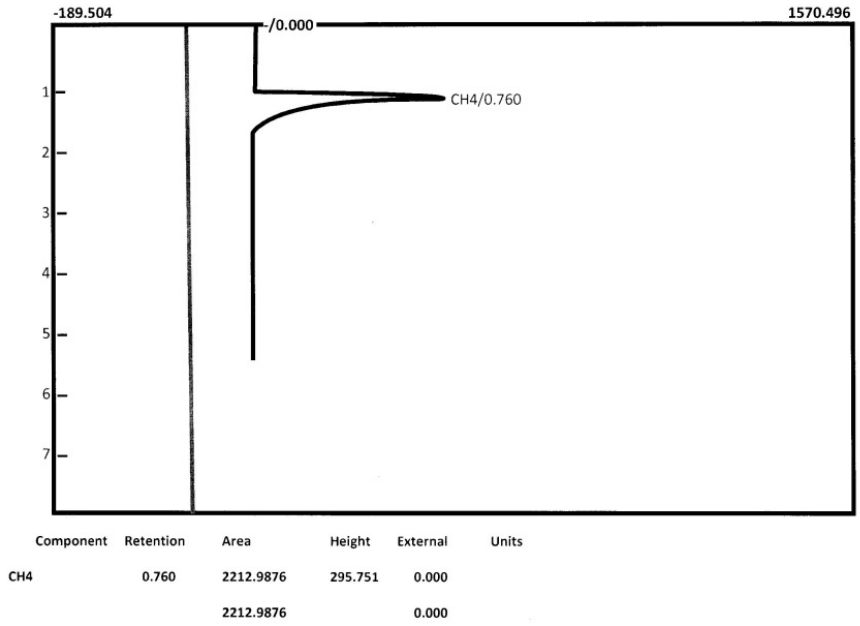


**Fig. 3. Qualitative analysis of biogas**

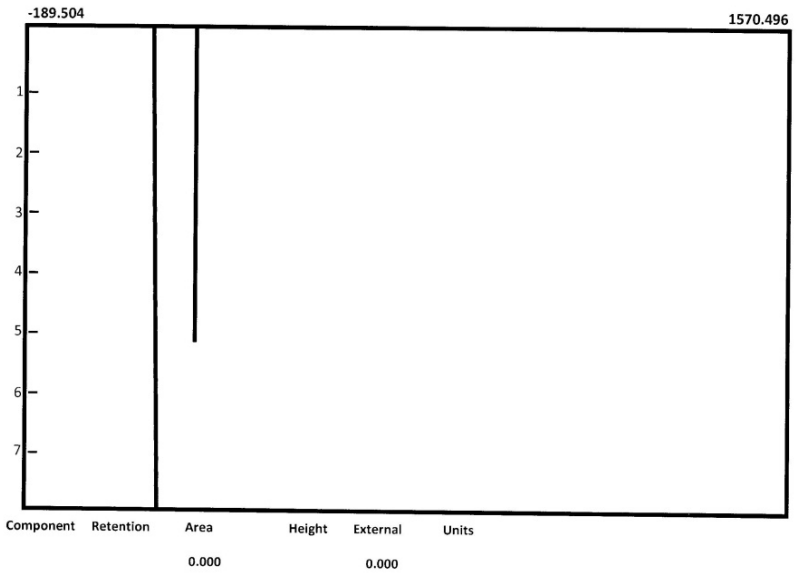


**Fig. 4. The methane standard**

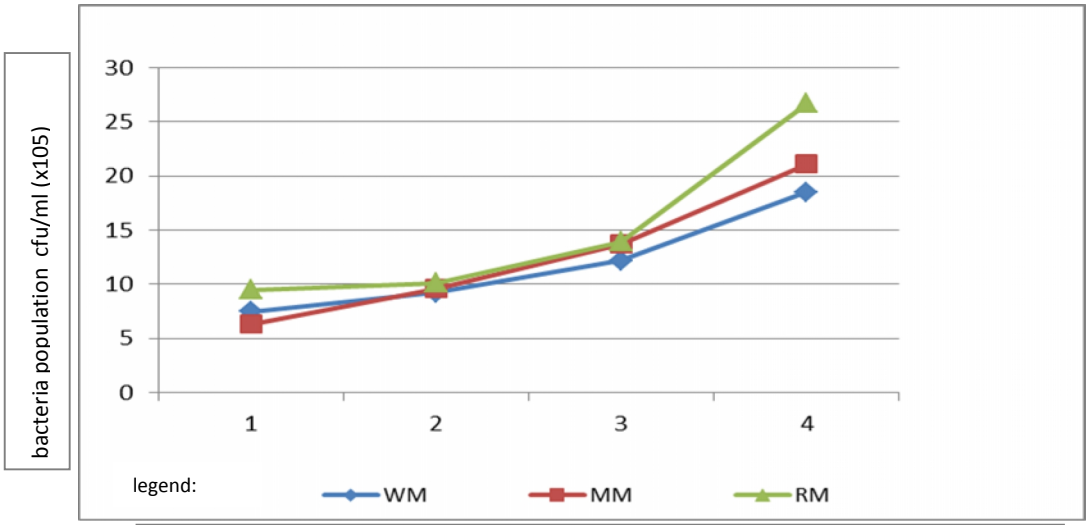


**Fig. 5. The heterotrophic count in months**

## 4. DISCUSSION

The growth of microbes during anaerobic fermentation is affected by pH. The amount of carbon dioxide and volatile fatty acids produced during the anaerobic process affects the pH of the digester contents. The average pH observed in the study is in line with the report of Abubakar and Nasir [24], Sawyer and McCarty [8], Mah [9] and Jain and Mattiasson [14].

The content of biogas varies and depends on the material being decomposed, the solids present in the waste, their digestibility or degradability and the environmental conditions involved. The solids reported in the study were digestible and degradable; hence, the values of the TS and VS in the post digested sample (PDS) were much lower than the predigested sample (PRDS). These findings were similar to the report of Abubakar and Nasir [24]. The results on total solids were correlated to the quantity and quality of biogas in the cow dung. This observation is correlated to the report of Budiyono et al. [19] who reported higher quantity of biogas yield from reactors with TS 7.4% and 9.2% as against rectors with TS 2.65%, 4.6% and 6.2%.

Potentially all organic waste materials contain adequate quantities of the nutrients essential for the growth and metabolism of the anaerobic bacteria in biogas production. However, the chemical composition and biological availability of the nutrients contained in these materials vary with species and factors affecting growth. The result of the research on biogas composition agrees with previous researchers [13,14] that biogas composition varies and depends on sources of organic manure. The 100% biogas composition reported in the study is closely related to the report of Mattsson et al. [15].

Different quantities of biogas have been produced from various sources and can be affected by various factors. This research observed various quantities of biogas from three different bioreactors. The findings were in line with the report of Budiyono et al. [19], Mattsson et al. [15] and Pratiksha and Gireesh [12]. According to Aurora [25], rumen of the ruminant animals contains highly anaerobic bacteria dominated by cellulolytic bacteria able to biodegrade cellulose material from manure. This is in consonance with other results of

researchers that amount of biogas produced seemed proportional to the initial inoculums [19,26]. Highest volume of biogas was produced in the bioreactor supplemented with liquid rumen fluid which corroborates with the research of Lope et al. [27] and Budiyono et al. [19] which concluded that inoculum has a high influence on the rate and cumulative biogas production. The observation of the study also collaborates with the report ofAbubakar and Nasir[24] who reported low biogas production at the starting and the end of observation in an anaerobic digestion of manure.

The increase recorded in the heterotrophic count co-relates with the amount of biogas produced which was higher starting from the second month and this agrees with the report of previous researchers [24,27,28]. Abubakar and Nasir [24] reported slow biogas production at the starting and end of study period which was predicted because biogas production rate in batch condition is directly equal to specific growth of methanogenic bacteria. Highest biogas production was recorded in the study at the exponential phase of microbial growth. This report was similar to the report of Abubakar and Nasir [24] and Budiyono et al. [19].

## 5. CONCLUSION

The study concluded that high quantity of biogas can be produced from cow dung by indigenous microbial consortia. Cow dung is largely generated in Nigeria on a daily basis and could be employed as raw material for both small and large-scale biogas production. The high quantity of methane produced from cow dung can be technologically harnessed and made a viable renewable energy source especially for developing countries.

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## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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