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Comparative Study on Biogas Production Using Cowdung, Eucalyptus
(*Eucalyptus Camaldulensis. Dehnh*) and Mixture Wastes

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ABSTRACT

Eucalyptus camaldulensis. Dehnh trees are widely grown in most forest reserves in Nigeria especially in savanna agro-ecological zone for paper and construction industries. The waste (leave litters) generated during wood processing are often wasted. Therefore, comparative study was carried out to compare biogas production using Cow dung, *Eucalyptus* waste and mixture of the two. Rubber buckets (10 liters) were used as digester, slurry was prepared (3kg dung: 3000ml water, 1kg eucalyptus: 5000ml water and 2kg mixture: 4000ml water), digesters were fed in triplicates and subjected to anaerobic digestion for 6 weeks. Biogas was measured using water displacement method. Bacterial count was monitored by spread plating method using blood agar at 10⁶ dilution factor, incubated anaerobically and viewed using microscope. Cow dung recorded highest volume of biogas 3136cm³ at week 3, eucalyptus 2383cm³ at week 4 and then mixture 2259cm³ at week 3. Highest bacterial counts were 367x10⁶cfu/ml at week 3, 352x10⁶cfu/ml at week 4 and 383x10⁶cfu/ml at week 3 for cow dung, eucalyptus and mixture respectively. The data obtained were subjected to Analysis of Variance (ANOVA) using Statistical software (SAS), biogas yield and Bacterial count were significant at (P<0.05). Caloric values and percentage of some gases were also determined for biogas produced using NDIR Gas analyzer, methane content and caloric values were (53.35%) & (249.58 KJ/m³), (34.79%) & (125.98 KJ/m³) and (34.58%) & (124.60 KJ/m³) for eucalyptus, mixture and cow dung respectively. This research has proven the potentiality of biogas from the samples especially eucalyptus which recorded highest methane contents.

Keywords: Cow dung, eucalyptus, digester, biogas, blood agar

1.0 INTRODUCTION

The use of fossil fuels as primary energy source has led to global climate change, environmental degradation and human health problems (Adeniran *et al.*, 2014). Also, improper waste management is one of the major problems confronting every development. This is because increase in industrial, commercial, agricultural and environmental activities has resulted in the generation of large quantities of wastes (Adeniran *et al.*, 2014). These wastes, when not properly managed contribute to unhygienic environmental conditions that breed pathogenic microorganisms. Apart from the health implications, wastes make an environment unpleasant and unattractive. However, these wastes can be managed properly by conversion into useful and more environmental-friendly forms called

biogas. Biogas typically refers to a gas produced by the breakdown of organic matter in the absence of oxygen (Anunputtikul and Rodtong, 2004). It is a renewable energy source, like solar and wind energy. Furthermore, biogas can be produced from regionally available raw materials and recycled waste and it is environment friendly and carbon IV oxide (CO₂) neutral. Biogas is produced by the anaerobic digestion or fermentation of biodegradable materials such as manure, sewage, municipal waste, green waste, plant material, and crops (Kaygusuz and Kaygysuz, 2002). Renewable energy resources appear to be one of the efficient solutions to the problems resulting from the use of fossil fuels (Hankisham and Sung, 2003). For example, biological wastes which are usually seen as low-valued materials are now being transformed

to usable forms for the production of eco-friendly and sustainable fuels (Gomez *et al.*, 2008). This is because most biological wastes contains high level of cellulose, hemicelluloses, lignin, starch, proteins, and lipids, which provide good options for the biotechnological production of liquid biofuels without interfering with the ever-increasing need for world's food supply (Leandro *et al.*, 2009).

Nigeria is blessed with abundant primary energy resources. These include non-renewable energy sources such as natural gas, crude oil, coal and tar sands; and renewable energy sources such as hydro, biomass, solar and wind. (National renewable energy and energy efficiency policy 2015.). However, the economy has mainly depended on the consumption of oil and gas for commercial energy. The use of hydro-power plants, which entered the Nigerian energy scene in the 1960's, now accounts for the second largest energy resource for electricity generation in Nigeria, contributing approximately 26% of the total installed grid-connected generated energy (National renewable energy and energy efficiency policy 2015.). Biogas is a flammable gas produced when organic materials are fermented under anaerobic condition (Sagagi *et al.*, 2009). It contains methane and carbon (IV) oxide with traces of hydrogen sulphide and water vapor. It burns with pale blue flame and has a calorific value of between 25.9-30J/m³ depending on the percentage of methane in the gas. The gas is called by several other names, such as: dung gas, marsh gas, gobar gas, sewage gas and swamp gas (Sagagi *et al.*, 2009). In biogas production, the conversion of complex organic matter to methane and carbon dioxide is possible mainly by the actions of different group of microorganisms, with the microbial community of biogas comprised essentially of bacteria and fungi and other groups of protozoan. The essential microbial complex is comprised of hydrolytic bacteria, fermenting bacteria, acetogenic bacteria and methanogenic bacteria and these groups of microorganisms have been reported to establish syntrophic relationships where the later members of the food chain depend on the previous for their substrate but may also have significant metabolic products (Kaygusuz and Kaygysuz, 2002). These microorganisms act at the different stages of the anaerobic process to bring about effective

Biogas production and are an integral component of nature's waste management and are commonly found in soils and deep waters as well as land fill sites. Eucalyptus trees are widely grown in most forest reserves in Nigeria for paper and construction industries, the waste generated from the leave litters and cow dung can be used to generate biogas. Therefore, there is need for research to enhance the production of biogas using these substrates.

2.0 METHODOLOGY

2.1 Samples Collection

(i) Cow dung was collected in a clean bag from Gidan Yaba and transported to Sokoto Energy Research Center, Usmanu Danfodiyo University Sokoto, Nigeria.

(ii) *Eucalyptus camaldulensis*. Dehnh waste was collected in a clean bag from savanna forestry research station samaru zaria and transported to Sokoto Energy Research Center, Usmanu Danfodiyo University Sokoto, Nigeria. The sample was sun dried and pulverized into powder using mortar and pestle (Galbe, 2007).

2.2 Pretreatment of eucalyptus waste

The grinded powdered sample of the Eucalyptus waste was put in a polythene bags and buried underground for 14days to mesorize.

2.3 Proximate Composition

2.3.1 Determination of Moisture Content (%)

The sample, (2g) was weighed and placed into a pre- weighed crucible and then placed into oven, dried at 105^oC for 24hours. The crucible was then cooled in desiccators and weighed.

The percentage moisture content was calculated using equation (4). AOAC (1990)

$$= \frac{\text{Loss in Weight}}{\text{Weight of sample taken}} \times 100 - (4)$$

2.3.2 Determination of Ash Content (%)

From the sample (2g) was weighed and placed into crucible and then heated in a muffle furnace at a temperature of 600^oC for three hours. The crucible was then cooled in desiccators and weighed.

The percentage ash content was calculated using equation (7). AOAC (1990)

$$= \frac{\text{Weight of Residue}}{\text{Weight of sample taken}} \times 100 - (7)$$

2.3.3 Determination of Volatiles matter (%)

From the sample (1g) was weighed into crucible and heated at a temperature of 925°C for 7 minutes and allowed to cool before weighed. Percentage volatiles matter was calculated using equation (8). (Garba and Sambo 1992)

$$= \text{Dry weight} - \text{Ash weight} \times 100 - (8)$$

2.3.4 Determination of Percentage Carbon

The percentage Carbon was calculated using the equation (IX). (Bagudo *et al.*, 2008)

$$\text{Carbon (\%)} = 0.5 \times \text{Volatiles matter} - (9)$$

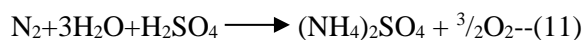
2.3.5 Determination of Carbon to Nitrogen Ratio

Carbon to nitrogen ratio was obtained by calculating the ratio of organic carbon content to that of nitrogen content using equation (10). (Bagudo *et al.*, 2008)

$$\text{C/N Ratio} = \frac{\% \text{ Organic Carbon}}{\% \text{ Nitrogen}} (10)$$

2.3.6 Determination of Total Nitrogen by Kjeldahl Method

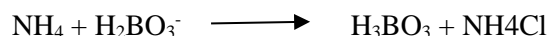
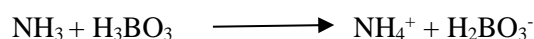
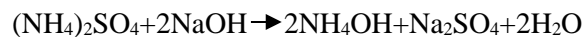
From the sample two gram (2g) was taken and placed into 500cm³ Kjeldahl flask, 20cm³ of water, a tablet of Kjeldahl catalyst (selenium catalyst) and 20cm³ of concentrated sulphuric acid were added. Each flask was set aside for ten minutes and then heated gently in a fume cupboard to a temperature of 500°C. This converted any nitrogen present in the sample to ammonium sulphate as shown in the equation (11). AOAC (1990)



The clear solution was cooled and diluted with 50cm³ distilled water with vigorous shaken. 20cm³ of aliquot was taken into a Kjeldahl's distillation flask, 20cm³ of 40% NaOH was added and the inlet was closed. 20cm³ of boric acid mixed indicator was pipette into a 100cm³ conical flask and placed under the condenser and the receiving end is at 4cm above boric indicator. Steam was then allowed to

pass through the sample under high pressure to NH₃ gas that was received after condensation into a boric acid indicator solution. The distillate was titrated with standard HCl.

The equation for the reactions are shown below



The nitrogen content was calculated from the titer value using the equation below. AOAC (1990)

$$\text{TV} = \text{Titer Value} \quad \text{M} = \text{Molarity of the Acid (0.01M)} \quad \text{V} = \text{Volume of Sample Dilute (50cm}^3\text{)}$$

$$= \frac{\text{TV} \times \text{M} \times 0.014 \times \text{V}}{\text{Weight of sample} \times \text{Volume of aliquot}} \times 100$$

2.3.7 Determination of Potassium Content

The flame photometry method by AOAC (1990) as modified by Egbekun and Ehieze (1997) was used. For the determination of potassium ion, the photometer was set – up and the read out calibrated using potassium standard solution at 5ppm, 10ppm, 15ppm and 20ppm respectively. The meter was set at zero while aspirating blank solution. The meter reading was also set at 100%T while aspirating the standard solution with the highest concentration. All other intermediate standard solutions were then run and percentage transmittances were recorded. A portion of aliquot digest sample solution was aspirated and % T reading was recorded. A standard curve of transmittance versus concentration was plotted. The concentration of K⁺ content of the sample solution was read from the standard curve and the percentage was computed.

2.3.8 Determination of Phosphorus Content

The Vanadate – Molybdate method reported by AOAC (1990) as modified by Egbekun and Ehieze (1997) was used. 10cm³ of the sample was taken and diluted to a volume of 100cm³. Then 10cm³ of aliquot was taken into a 50cm³ volumetric flask and 10cm³ of ammonium molybdate – ammonium vanadate reagent added. This was then diluted to the mark with distilled water and the color was allowed to develop for 20 minutes. For standard curve, 0.2, 0.4, 0.6, 0.8 and 1.0ppm were prepared. The color absorbance of the samples and that of the

standards was determined using spectrophotometer at a wavelength of 400nm using cells of 10mm diameter. The concentration of phosphorus in digested and undigested samples corresponding to the absorbance was obtained from the standard curve.

2.4 Preparations of slurry

2.4.1 Cow dung: Three kilogram (3 kg) of fresh cow dung was mixed with 3000 ml of water, cow dung to water ratio (1:1). The mixture was agitated thoroughly and transferred into the digester and tightly covered with silicon sealant and 4 minutes to create anaerobic condition. This treatment was replicated 3 times. (sagagi *et al.*, 2009).

2.4.2 Eucalyptus: One kilogram (1 kg) of pulverized eucalyptus was mixed with 5000 ml of water, eucalyptus to water ratio (1:5). The mixture was agitated thoroughly and transferred into the digester and tightly covered with silicon sealant and 4 minutes to create anaerobic condition. This treatment was replicated 3 times. (sagagi *et al.*, 2009).

2.4.3 Mixture: One and half kilogram (1.5 kg) of fresh cow dung and 0.5 kilogram of pulverized eucalyptus was mixed with 4000 ml of water in a ratio of (2:4). The mixture was agitated thoroughly and transferred into the digester and tightly covered with silicon sealant and araldites to create anaerobic condition. This treatment was replicated 3 times. (sagagi *et al.*, 2009). pH of the slurries were taken before and after digestion.

2.5 Measurement of Biogas Production

Biogas production was measured using water displacement method. And mean values of weekly Biogas production were taken. (Garba and Sambo 1992)

2.6 Media Preparation

Blood agar was used as a media and was prepared by dissolving 28g of the powdered agar in to 1000ml of distilled water which was heated for the mixture to dissolved fully; the mixture was autoclaved at 121°C for 15minutes and allowed to cool. 10% blood was added and then dispense into Petri-dishes, air bubbles was removed using gas

flame and allowed to solidify. (Cheesebrough, 2000).

2.7 Bacterial counts

Total heterotrophic bacteria counts in the substrates were monitored by spread plate technique using blood agar as a media. A six-fold serial dilution of the substrates was carried out by transferring 1ml each of the substrates into test tubes containing 9ml of sterile distilled water arranged serially in the order $10^{-1} - 10^{-6}$.

Dilutions of 10^{-6} were inoculated using spread plating and incubated anaerobically using anaerobic jar at room temperature for 24 hrs for the enumeration of total heterotrophic bacteria. During the digestion period, samples from the digesters were collected at 24 hrs intervals for 6 weeks, and anaerobic bacteria count was taken. (Cheesebrough, 2000).

2.8 Statistical analysis

The data collected were subjected to ANOVA using statistical analysis software (SAS) and mean differences in total Biogas yield and Bacterial count were separated using least significant difference (LSD) at (5%) level.

2.9 Non Dispersive IR Gas Analysis

Biogas (4000cm^3) were collected using urine bags for each of the three samples and was taken to Department of chemical engineering Ahmadu Bello University, Zaria for NDIR analysis using Gas Analyzer (Gasboard-3100P). Biogas analysis was carried out according to manufacturer's instruction. Biogas sample was pumped into the sample chamber and gas concentration was measured electro-optically by absorption of specific wavelength in the infrared (IR). The IR light was directed through the sample chamber towards the detector. The detector has an optical filter that eliminates all light except the wavelength that the selected gas molecule can absorb. Ideally other gas molecules do not absorb light at this wavelength, and do not affect the amount of light reaching the detector. The detector measures the attenuated signal depending on the amount of gas absorption and it is proportional to the measured gas concentration. Gas Analyzer (Gasboard-3100P) measured concentration of CO, CO₂, CH₄, H₂ and

O₂ in the sample simultaneously and also calculated caloric value automatically.

3.0 RESULTS AND DISCUSSION

Table 1: Proximate Composition of the Substrates Before and After Digestion

Parameter	Cow dung		Eucalyptus		Mixture	
	Undigested	Digested	Undigested	Digested	Undigested	Digested
Moisture (%)	10.5±0.17	9.5±0.03	8.5±0.03	8.0±0.03	9.0±0.00	9.5±0.03
Ash (%)	76.0±0.18	0.5±0.03	24.5±0.03	3.5±0.09	10.50.06	1.0±0.03
Carbon (%)	40.0±0.03	35.0±0.07	37.5.0±0.15	32.5±0.09	39.0±0.00	35.0±0.03
Volatiles matter (%)	80.0±0.03	70.0±0.27	75.0±0.00	65.0±0.15	78.0±0.06	70.0±0.32
Carbon/Nitrogen ratio	88.9.0±0.03	18.4.0±0.12	27.9±0.00	9.0±0.06	26.2±0.09	12.5±0.07

Results are mean ± SD

3.1 Ash and Moisture Content

Results obtained as shown in Table 1 indicate low content of ash in the three digested samples, cow dung (0.5%), Eucalyptus (3.5%) and mixture (1.0%) which is in agreement with the report by Gizegorz *et al.*, (2015) that high ash content (25-35%) reduces the biogas production potentials as only organic matters can be converted to biogas. Therefore, this result shows that most of the organic matters from the three samples are been converted during the production of biogas.

However, with regards to moisture content for undigested samples, as shown in Table 1 lower value was recorded from eucalyptus and high value was recorded from cow dung. The result is in agreement with the work of Bagudo *et al.*, (2008). Lower values recorded from eucalyptus could be as a result of drying and the nature of the substrate and high values from cow dung could be attributed to it nature which was favorable for biogas production. The higher the moisture content, the easier the action by microorganism hence more biogas yields.

3.2 Fixed Carbon

The result of the substrates as shown in Table 1 indicates decrease in carbon content which is in line with the finding of Tambuwal (2005) and Bagudo *et al.*, (2008). This could be due to the action of microbes on organic matter of the substrates. Low organic matter results in low biogas yield and vice versa.

3.3 Volatile matter

Result in Table 1 shows high values from undigested sample for all the three substrates and the result is in agreement with the work of Casebow (1987). The reduction in the values could be due to conversion of the volatiles matter into biogas.

3.3 Carbon to Nitrogen Ratio

Table 1 shows the values of C/N ratio which shows decrease in the values after digestion. It is reported that anaerobic bacteria use up to 30 times carbon faster than nitrogen. This is because bacteria use carbon as food and nitrogen to replenish it tissues. Casebow (1987).

Also the closer to the range of the recommended value of C/N ratio (30:1), the higher the yield of biogas. This could be linked to the fact that low

level of Nitrogen limits growth of bacterial action while excessively high level could be toxic to the bacteria. (Bagudo 2017).

Table 2: Comparison of Different Substrates on Biogas Production

Treatment	Mean Weekly Biogas Production (cm ³)					
	WK1	WK2	WK3	WK4	WK5	WK6
Cow dung	7.4 ^a	54.3 ^a	56.0 ^a	6.0 ^c	0.7 ^c	0.7
Eucalyptus	0.7 ^b	0.7 ^c	26.1 ^c	48.8 ^a	24.9 ^a	0.7
Mixture	0.7 ^b	11.6 ^b	47.5 ^b	41.2 ^b	19.4 ^b	0.7
LSD	0.0453	0.1978	0.3047	0.3547	0.1429	0.0000

Mean followed by same letter(s) in the same column are not statistically different at $P=0.05$ using LSD

The result obtained from the biogas production as shown in Table 2, indicates that highest biogas yield was obtained from cow dung (6376.34cm³) at week 3, followed by eucalyptus waste (2383.33cm³) at week 4 and then mixture (2259cm³) at week 3. This trend shows the production potentials of the samples differ with time, which is in agreement with the work of (Baki, *et al.*, 2004) and (Arinze, *et al.*, 2005). The low biogas yield from eucalyptus waste could be due to high lignin content and the nature of the substrates. However, the low yield observed in the first week for cow dung, first and second week for eucalyptus and mixture could be due to the activity of methanogenic bacteria and at those weeks the

development of such bacteria was not completed. The volume of biogas yield for Mixture at week four start decreasing unlike other samples which could be due to mixing effect from the two different samples.

Similarly, at 5th week of the production, a decline in the biogas yield was observed and this is due to the lower activity of the bacteria which could be due to the shortage of ingredients needed for it survival.

Furthermore, Analysis of Variance (ANOVA) revealed significant difference at $P=0.05$ throughout the six weeks period except week 1, where eucalyptus and mixture are the same and week 6 where all the three samples are the same.

Table 3: Comparison of Different Substrates on Bacterial Count

Treatment	Mean Weekly Bacterial Count (cfug ⁻¹)					
	WK1	WK2	WK3	WK4	WK5	WK6
Cow dung	4795.1 ^a	18787.9 ^a	19209.0 ^b	5099.0 ^c	0.7 ^b	0.7 ^b
Eucalyptus	0.7 ^b	0.7 ^c	11445.3 ^c	18761.3 ^a	12328.8 ^a	7070.1 ^a
Mixture	0.7 ^b	9949.5 ^b	20856.6 ^a	18138.3 ^b	12288.2 ^a	0.7 ^b
LSD	120.29	192.35	195.88	166.34	46.78	163.16

Mean followed by same letter(s) in the same column are not statistically different at $P=0.05$ using LSD

From the table above overall bacterial counts was recorded from cow dung (770.3x10⁶cfuml⁻¹) followed by mixture (1014x10⁶cfuml⁻¹) and lastly eucalyptus (685x10⁶cfuml⁻¹). This is in agreement with the work of (Arinze, *et al.*, 2005). This could

be attributed to the nature and carbon content of each substrate.

Similarly, Analysis of Variance (ANOVA) for bacterial counts, revealed significant difference at $P=0.05$ throughout the six weeks period except

week 1, where eucalyptus and mixture are the same and week 6 where cow dung and mixture are the same.

Table 4: pH of the Slurries Before and After Digestion

Sample	Before digestion	After digestion
Cow dung	7.12±0.01	5.14±0.01
Eucalyptus	7.83±0.01	6.73±0.01
Mixture	6.71±0.01	5.79±0.01

Results are mean ± SD

pH of the sample is another factor that affects biogas production. The optimum pH range for biogas production has been reported as 6.0 – 7.8 before digestion and 5.6 – 7.8 after digestion.

(Garba and Sambo 1992). The result above shows that the samples used in this research were having pH favorable for effective bio digestion

Table 5: Percentage Composition of Some Gases in the Biogas Produced

Sample	Percentage composition (%)			
	CO	CO ₂	CH ₄	H ₂
Cow dung	0.03	9.97	34.58	0.46
Eucalyptus	0.26	11.48	53.35	0.00
Mixture	0.00	10.84	34.79	1.11

From the results above biogas generated from Eucalyptus leaves recorded highest methane content (53.35%) which is the target composition and lowest in cow dung, this is not in agreement to

the work of Joao, *et al.*, (2011). Disagreement could be due to climatic factors, the nature, and composition of Eucalyptus species.

Table 6: Caloric Values of Biogas Production from the Substrates

Sample	Caloric value (KJ/m ³)
Cow dung	124.60
Eucalyptus	249.58

The above results obtained from the NDIR Gas analyzer as shown in Table 4 indicates higher heating values of the biogas produced from *Eucalyptus camaldulensis*. Dehnh (249.58KJ/m³) and lowest from Cow dung (124.60KJ/m³). The Eucalyptus leaves contain highly nutritious rich organic substances that are suitable for sustaining

microbial life in an anaerobic fermentation process, and transform substrates into biogas. These results were in accordance with the work of Joao, *et al.*, (2011). High caloric value is due to high methane content from the biogas produced from Eucalyptus, and this shows the potentiality of biogas generated from Eucalyptus leaves as a cooking gas.

4.0 CONCLUSION

Proximate analysis indicates the substrates used for this research were good candidates for Biogas generation. The highest volume of biogas was recorded from cow dung (9416.34cm³) and lowest come from eucalyptus (3780.00cm³). Higher Bacterial count was recorded in mixture (1014x10⁶cfuml⁻¹) and least come from eucalyptus (685x10⁶cfuml⁻¹). Higher Bacterial count indicates the availability of nutrients and Analysis of variance (ANOVA) indicates significant difference

among Biogas produced and Bacterial counts from the three different samples. Non Dispersive IR using gas analyzer has also proven the potentials and quality of the Biogas produced from eucalyptus over cow dung and mixture of the two substrates. Therefore, generation and utilization of biogas from eucalyptus wastes which has not been exploited prior to this research work should be encouraged. This is because of the novel proven potentials of biogas generation from the wastes.

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