Optimization and Quantification of Biogas Yield by Blending Cow Dung with Three Different Potential Substrates Under Anaerobic Condition Using Proto-Type Chinese Fixed Dome Bioreactor.

UKONU, CHRISTIAN U.[1], MAKANJUOLA, JOHN O.[2], ALABI, NURUDEEN O[3]
[1, 2]*Department of Science Laboratory Technology, Federal Polytechnic, Ilaro, Ogun State.*
[3]*Department of Mathematise and Statistics, Federal Polytechnic, Ilaro, Ogun State.*

Abstract- ***Biogas technology is gaining global recognition as an eco-friendly technology with the potential to meet national needs such as electricity vehicular fuel, cooking gas and adding economical values. This study explores the optimization and quantification of biogas produce through blending cow dung with other potential waste under anaerobic condition. Co-digestions and varying of organic loading rate (OLR) were employed. The biogas process (Hydrophilic retention time) was monitored for 3 weeks; the temperature and the pH were measured using mercury-in-glass thermometer and digital pH meter. The proximate analysis was carried out using AOAC standard method. The gas produced was measured using weighing balance and the quality using portable gas analyser. The result of the proximate analysis revealed the nature of the co-digestion of each bioreactor. The bioreactor four (B4) had higher moisture contents of 94.20% and lowest in biogas produced (3kg), while bioreactor one (B1) had 92.6% as the lest, but with higher production of biogas (7kg). Low value of TFA (0.28%), nitrogen (0.14%) and carbohydrate (3.91%) could contribute to the poor production of biogas in B4. The quality and the flammability time of biogas produced in B3 and B4 were optimized compared to B2. The anaerobic degradation was carried out within the atmospheric temperature of 33.43℃±-0.98℃CC and slurry temperature range of 31.14℃ ±-2.852℃ to 31.43℃±-2.51℃.The pH of the process where also within the range7.51±-0.40 to 7.80±-0.29 which are within the normal biological pH for microorganisms. This implied that the nature of the waste had a direct effect on the production of biogas and co-digestion contribute to the optimization of the retention time and quality of methane gas produced.***

Indexed Terms- ***Biogas, Bioreactor, Degradation, Optimization, Waste***

I. INTRODUCTION

One of the major global challenges of this century is climate change, which has been attributed to the effect of global warming. Global warming is because of the release or presence of gases such as methane, CO_2, CO etc into the atmosphere. This leads to greenhouse gas effect and ozone layer depletion [1]. Emission of methane gas by microorganisms into the atmosphere has been reported to be one of the major contributors of global warning, haven't discovered that about 15 million tonnes of this gas is release through decomposition of waste by microorganism [1]. This huge contribution has stirred the drive by researcher to harness the methane gases, this of course birthed the technology "biogas technology" [2]. Biogas technology is a technology that harness or harvest biomethane and other gases such as CO_2, H_2S and NH_3 from organic wastes, with the help of microorganisms (the decomposers) in a contentment (bioreactors) under anaerobic condition [3]. This technology also has the potential to mitigate indiscriminate disposal of wastes, contamination, pollution etc, And serve as an alternative source of energy for domestic use, generation of electricity, as fuel for vehicle and as an alternative source of organic fertilizer that can be used to improve food security of a nation [4]. Nations such as USA, Finland, China etc have utilized this technology to improve their energy demand in terms of electricity, vehicular fuelling, domestic and industrial needs [4]; [5].

The quality of biogas produced from waste is of paramount impotence. It defines the value of the gas

and the usage. It also highlights standard and safety of the gas. The quality of biogas depends on the purity of the biogas from other gases that includes CO_2, H_2S and NH_3 [6]. The quality of the biogas produced can be optimized or improved using various methods. Many research have been carried out to improve the quantity of biogas using organic loading rate (OLR), co-digestion and pre-treatment. This directly also affect the quality of the gas produced [7]; [8]. The choice of the substrates also have effect on the quality and quantity of the biogas produced as shown by many researcher. Cow dung and poultry dropping have been demonstrated as very good substrate for the production of biogas [2].

The quantity and quality of biogas produced under anaerobic condition is dependent on the nature of the biomass (substrate or waste) used and the ability to maintain the required conditions needed for the optimal production of the biogas. Microorganism are the major players in this process; they feed on the waste as their source of nutrient. With the help of the extracellular enzyme introduce by these microorganism, the waste or substrate are broke down in three major enzymatic stages to generate biogas and other gases in the absence of oxygen [6] following enzyme kinetic reaction, where the enzyme interact with the substrate (waste) forming enzyme substrate complex that result to the production of the product biogas [9].

Substrate + Enzyme $\rightarrow$ k1 Substrate-Enzyme complex $\rightarrow$ k2 E + CH_4 +H_2 S +CO_2

K=Constant, E= Enzymes, CH4=Methane gas. H2S=Hydrogen sulphide. CO2=Carbon dioxide

II. MATERIALS AND METHOD

Materials

The materials used to carry out the process were Pig dung, Cow dung, Poultry dung, Bio reactors Bucket, weighing balance, Sack bags, stirring iron, Plastic bowel, Locust bean, Funnel, bailer, Gloves, Dry leaves, Digital pH meter, Mercury in glass thermometer, and Water. The chemicals used were of analytical standard.

Collection of materials

Cow dung, was collected from an abattoir in Odo eran, Ilaro and Pig dung was also gotten from a pig farm in Ilaro. The Poultry dung was gotten from the federal polytechnic Ilaro poultry farm and the Locust bean from Sayedero market in Ilaro.

Methods

Pretreatment

Cow dung was poured into a bucket and impurities like shaft, bone and pure water sachet were carefully picked out.

Preparation of substrates

After careful selection and sorting, cow dungs were weighed and mixed with water, same with poultry dung and pig dung. The animal wastes and the water were well mixed, and then recharged into an airtight container called the bioreactor.

Charging of Bioreactor(s)

The different weight were mixed thoroughly in a water trough and the mixtures charged into the 200kg galvanized metal prototype of Chinese fixed dome batch bioreactor(s). The waste was charged up to three quarter (¾) of the bioreactor, leaving one-quarter (¼) headspace for gas collection.

Experimental design

Table 1: Composition of the substrate to water recharged in the bioreactors

Bioreactor	Cow dung (kg)	Poultry dung (kg)	Pig dung (kg)	Locust beans (kg)	Water (kg)	Ratio (Water :Waste)
B1	40	-	-	-	119	3:1
B2	-	52	-	-	106	2:1
B3	18.7	18.7		2.6	119	3:1
B4	25.3	-	12	2.6	119	3:1

Determination of slurry pH, atmospheric temperature and slurry temperature

pH meter

The pH of the slurry was measured using a pH meter. Slurry was collected from the bioreactor using a small rubber container. Prior to use, the pH meter was calibrated to ensure accurate measurements. Calibration is crucial for reliable pH readings in similar experimental setups [10].

The mercury in glass thermometer usage
Thermometer-mercury in glass thermometer
To measure the atmospheric temperature: the mercury in glass thermometer was held onto for 3minutes. To measure the slurry temperature after collecting the slurry from the bioreactor the Mercury in glass thermometer was dipped into the slurry for 3 minutes to measure the slurry temperature all of these process was done on daily basis for 3 weeks (morning and evening) [11].

Proximate Analysis
Determination of moisture content
One gram of the sample was weighed in a clean beaker (W1) and placed in an oven, for about 2 hrs at 1050C to a constant weight place in desiccator. Then the beaker (W2) was re-weighed. The difference in weight indicates the amount of water loss contained in the sample ([12].

$$\%\text{Moisture Content}=t=\frac{W1-W2}{W1}\times 100$$

WI-Weight of original sample
W2-Weight of sample of sample after oven dry

Determination of Ash content
An empty crucible (W1) was weighed and 2 g of the sample was weighed into it. Then it was placed in a muffle furnace at 4500C for 4 hrs. The crucible was then removed, placed in a desiccator and reweighed(W2) [12].

$$\%\text{ Ash Content}=\frac{W2-W1}{\text{Weight of sample (2g)}}\times 100$$

WI-Weight of empty crucible
W2-Weight of crucible after ash

Determination of Fatty content (Oil Content)
Method 1-Using separating funnel
Two to three gram of the sample was weighed into a 250 ml beaker, and added 50 ml of distilled water and placed in a hot steam bath for 30 minutes. The beaker containing the solution was removed and its content poured into a 250 ml separating funnel, Using 50 ml of 95 %o di-ethyl ether to separate the oil from the mixture at 25:15:10 ml. into a clean beaker labelled W1. Then the oil was flashed off in oven at 450C, and the weight of the beaker W2 was then taken.

Method 2-Using Soxhlet Extractor
Five gram of the sample was weighed and placed it into a thimble, 100 ml of the solvent (n-hexane) was also placed into a 250 ml round bottom flask. The soxhlet extractor was set up with its refluxing for 4 hrs. An empty beaker WI was weighed, after 4 hrs, the reflux solution was poured into the beaker W1 and then flashed off at 350C, and the weight of the beaker W2 was then taken.

$$\%\text{ Oil Content}=\frac{W2-W1}{\text{Weight of sample}}\times 100$$

WI-Weight of empty Beaker
W2-Weight of beaker plus oil

Determination of crude Fibre
One gram of the sample was weighed into a beaker and 50 ml of 1.5 w/v% sulphuric acid was added and made up to 100 ml of water stirred and allowed to stand for 30mins. The mixture was decanted and added 50 ml of 1.5 w/v% NaOH and made up to 100 ml of water stirred, this allowed to stand for 30 mins. The mixture was then filtered into a pre-weighed crucible W1 and placed into the oven for 1hr at 1050C to a constant weight W2.

$$\%\text{ Crude Fibre}=\frac{W2-W1}{\text{Weight of sample}}X\ 100$$

WI-Weight of empty crucible
W2-Weight of crucible after 1 hr

Determination of Nitrogen and Crude Protein
This involved three stages: digestion, distillation and titration

1. Digestion Stage

Into a round bottom Kjeldahl flask, weighed 0.2 g of the sample was gently added with 25 ml of Concentration of H_2SO_4. Dark brown solution was seen. 0.3 g of Kjeldahl tablet (CuSO4 + Na2SO4 (1:1)) was also added. The mixture was then digested for lhr until a clear colorless solution was obtained. This was then made up to 100 ml with distilled water in a standard flask.

2. Distillation Stage

In a 250ml bottom flask, 10ml aliquot (digest) , 50% of NaOH and anti- bumping agent were added. To another flask, 50 ml of boric acid and screen methyl red indicator were mixed The distillation system was put in place with the outlet tube inserted into the conical flask containing the boric acid for the collection of NH_3 through the condenser. The color turned from red to green giving out NH_3 as nitrogen gas.

3. Titration Stage

The percentage of nitrogen was obtained by titrating with 0.1M HCI

$$\% \text{ Nitrogen} = \frac{TV \times 0.1\ M \times 0.0014 \times 100}{\text{Weight of sample}}$$

TV=Titre Value

0.0014=Molarmass of Nitrogen/ 1000

% Crude Protein =% Nitrogen X 6.25 (factor multiplier for meat)

Determination of Carbohydrate

The sum up of all parameters from 100% was deducted to give percentage carbohydrate

Determination of the quality of biogas produced

The quality of biogas produced was measured using portable biogas analyser (*SNDWAY* SW-7500A)

Determination of quantity of biogas produced

An empty biogas bag was weighed and connected to the bioreactor, thereafter the weight of the biogas produced is measured by subtracting the weight of the empty biogas storage bag from the weight of the biogas plus storage biogas bag. These was carried out daily and the cumulative gas was recorded.

III. RESULT

Table 2: Proximate analysis of the slurry from each bioreactor

PARAMETERS	B1	B2	B3	B4
% Moisture Content	92.6	93.4	93.13	94.20
% Ash Content	0.1	0.30	0.13	0.17
%/ Total Fatty Acid (TFA)	0.31	0.30	0.2	0.28
% Crude Fibre (CF)	0.5	0.46	0.5	0.42
% Nitrogen	0.168	0.171	0.12	0.14
% Crude Protein (CP)-%/ Nitrogen x	1.05	1.07	0.75	0.88
% Carbohydrate	5.27	4.29	5.17	3.91

The table above showed that the moisture content of B4 had the highest value with 94.20% followed by B2 with 93.4%, while the least B1 had 92.6%. Moisture content may promote gas production than others because it contribute to the availability of the nutrient for the microbes [13]. High moisture content is said to increase biodegradability of substrates in anaerobic digestion, because it is a suitable medium for effective activity of microbes. [14] observed that moisture content of substrate above 90% are typical for organic waste, facilitating effective anaerobic digestion.

Volatile fatty acid is represented in total fatty acid, they serve as intermediates in biogas production the result above showed that B1 (0.31) had the highest total fatty acid, followed by B2 (0.30), the least is B3 (0.13). The TFA values obtained implies that samples with lower TFA may lead to higher methane yields due to reduced acid accumulation [15].

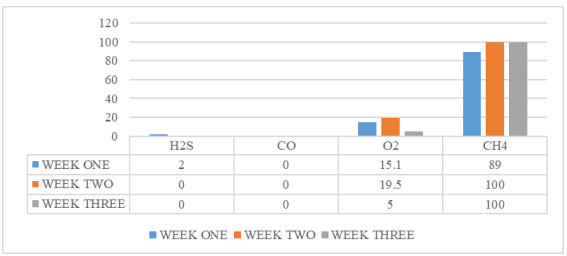
The bioreactor 2 had higher mineral content and other inorganic materials than other bioreactors. This is revealed in the value of ash content of the bioreactors that ranged from 0.1-0.3 %; having the B4 with the highest and the B1 with lowest value. The pattern of the result showing the crude fibre and the carbohydrate

content are the same. They are represented in a descending order of B1>B3>B2>B4. B1 and B4 had the highest CF content (0.5%), while B4 had the lowest (0.41%). Crude fiber plays a role in determining the structure of organic matter in substrates. [16] noted that higher crude fiber content in biogas feedstock could slow down the digestion process due to the difficulty in breaking down fibrous material, thus reducing biogas yield. Also carbohydrates are essential for anaerobic digestion as they are broken down into sugars that microbes convert into methane. [17] found that higher carbohydrate content in substrates leads to increased biogas production due to the availability of simple sugar.

Table 3: The cumulative biogas produced from the bioreactors

Bioreactor	Hydrophilic Retention Time H.R.T	Cumulative biogas produced (kg)
B1	5	7
B2	20	7
B3	10	6
B4	15	3

Bioreactor (B1) and B2 had the highest cumulative biogas yield followed by B3, the list is B4. The flammability time of each of the gas produced also varied. B1 had its HRT at the 5th day, B3 at day 10, while B4 had at day 15 and day 20 for B2. The quantity of biogas produced depends on the nature of the substrate and its combination. These also has a direct effect on the quality of biogas produced and its time of flammability.



H_2S=Hydrogen sulphide. CO= Carbon monoxide. O_2= Oxygen. CH_4= Methane

Figure 1: The quality of biogas produced in percentage (%) for bioreactor one (B1).

The bar chart showed that in week one, there is a low amount of H_2S (µmol), O2 %, no CO (µmol); while there is methane above 80%. Week two and three showed zero for H_2S and CO; while O_2 had a liitle amount in both weeks, CH_4 where both 100%.

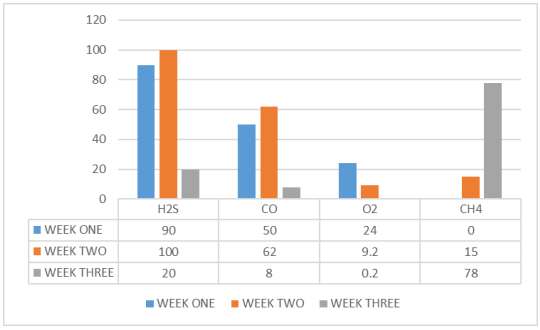


Figure 2: The quality of biogas produced in percentage for bioreactor two (B2).

Figure 2 revealed high value of H_2S, CO, a little amount of O_2 and no value for CH_4 in week one. Week two showed also high value of H_2S, CO, O_2 and CH_4 in a descending order; while the week three had an increase in methane above 70 %.

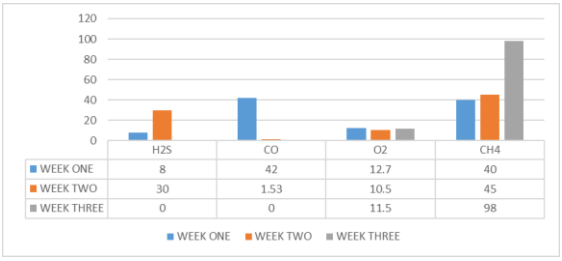


Figure 3: The quality of biogas produced in percentage from bioreactor three (B3).

The quality of methane gas produced in figure 3 revealed an increase progression from week one to week three. The quality was 98% at week three.

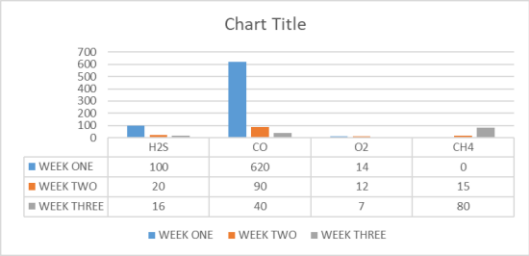


Figure 4: The quality of biogas produced in percentage from bioreactor three (B4).

This figure 4 showed a low quality of methane produced even at week three and high value of carbon monoxide at week two, but improved I week three. This result is a reflection of the effect of the combination of the substrate used on the activities of the anaerobic organisms.

Table 4: Showing the mean±sd of temperature and the pH of the atmosphere and the slurry of the bioreactors (for three (3) weeks).

TIME	Bioreactor	Atmospheric Temperature (℃)	Slurry Temperature (℃)	Slurry pH
MORNING	B1	33.43 ± 0.98	31.29 ±2.50	7.55±0.36
	B2	33.43 ± 0.98	31.14 ± 2.85	7.51 ±0.40
	B3	33.43 ± 0.98	31.43 ± 2.51	7.80 ±0.37
	B4	33.43 ± 0.98	31.43 ± 2.51	7.80 ±0.29
EVENING	B1	32.7 ± 1.17	31.7 ± 1.60	7.73 ±0.48
	B2	32.7 ± 1.17	31.4 ± 1.85	7.63±0.74
	B3	32.9 ± 1.17	31.9 ± 1.60	7.83±0.58
	B4	32.7 ± 1.17	31.9 ± 1.60	7.63±0.58

The table above revealed that the morning atmosphere temperature was within 33.45±0.98 to 33.43±0.98 and evening was within32.7±1.17t032.9±1.17while the morning slurry temperature was between 31.14±2.85 to 31.43 ±2.51 and evening between 31.4±1.85 to 31.9±1.60 and the morning slurry PH between 7.51±0.40 and 7.80±0.37 and the evening between 7.63±0.58 to 7.83±0.5.

IV. DISCUSSION

The characteristics of the Waste in the bioreactors (table 2) revealed the nature of the substrate used.

Optimization of biogas production assumes different dimensions, which includes varying of the organic loading rate (OLR), Co-digestion, inoculation of microorganisms etc. in this study, the optimization of the quantity and quality of biogas produced where assessed by using OLR and Co-digestion of different substrate. The result obtained revealed that bioreactor one (B1) and B2 (7kg) has the highest quantity of biogas produced cumulatively (table 2). The quality of biogas produced in B1 is better than that of B2 (table 2; figure 1 and 2). This also reflect in the period of flammability of the methane gas which is day 5 (B1) and day 20 (B2). B1 contain cow dung only, while B2 contained poultry dung only. However, B4 had high moisture content (94.20%), which is said to promote biogas production than others because of improved availability of nutrient to microbes [13]. High value of nitrogen probably slowed down the rate of the activity of the microbes and consequently decrease the HRT. The high level of CO and H_2S in B4 and B2 respectively affected the quality of the methane gas produced. The effect can be implicated on the nature of the combined waste. This indicate that the quality of biogas produced from cow dung is better than that of poultry dung

The quantity of biogas produced in B3 and B4 were not optimized compared with B1 and B2 that has high quantity. The flammability period of B3 (day 10) and B4 (day 15) were optimized by Co-digestion of cow dung plus poultry plus locust beans and cow dung plus pig dung plus locust bean respectively, when compared with B2 (day 20). The quality of the methane gas produced in B3 and B4 were optimized.

The quality and quantity of methane gas produced in B4 (figure 4 and table 2 respectively) showed lowered values compared with others. This could be because of the nature of pig dung combined with the cow dung. The presence of cassava pills mixed with the pig dung could contribute to high level of cyanide, which had been implicated in the reduction or inhibition of microbial activity anaerobically, hence, affecting the quantity of the methane gas produced.

Temperature and pH directly affect the biodegradation of organic waste anaerobically by microorganism. The anaerobic microbes are thermogenic and therefore are very effective at high temperature (ranging from 30^{o}C-40^{o}C). Very high temperature also affects the activity of microorganisms (GATE & GZT, 2007). The result of the atmospheric and slurry temperature for morning and evening readings for three weeks ranged within 33.43^{o}C and from 31.14+2.85 ^{o}C to 31.43+2.51^{o}C respectively for morning; 32.7+1.17^{o}C-32.9+1.17^{o}C and 31.4+1.85-31.9+1.60 for evening respectively. The temperature range of all the bioreactors were found to be within the normal room temperature. The low temperature affected the productivity of the reaction. It is reported that increase in temperature increases the activity of the anaerobes [9].

pH of a solution or a mixture is one of the determining factors of the productivity or success of that process. Fermentation process like the anaerobic breakdown of organic matter as observed in this study is density to the pH particularly when microorganisms are involved. It has been established that microorganism activity is effective within the pH range of 6.5-8.5 (US Environmental Protection Agency, 2001), Higher acidity or alkalinity of the slurry may either slow down the activities of the extracellular enzymes and microbes involved in the degradation process or permanently inhibit them and consequently short down the process. In this study the pH of the slurry obtained for both morning and evening were at the range of 7.73+0.48-7.82+0.58. The pH in the bioreactors were all within the optimum pH range for the optimum activity of anaerobes. This agreed with the work done by [7].

CONCLUSION

In this study, the quality and HRT (flammability time) of the biogas production were optimized by Co-digestion of cow dung as obtained in B3 and B4. The process, haven monitored for three weeks operated within the optimum pH and normal atmospheric temperature. This implied that combination of cow dung with other substrates could improve the quantity and quality of the biogas produced. Also the nature of the Waste has a direct effect on the process of degradation of the Waste by microbes and a direct consequence on the quality and quantity of the biogas produced.

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