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Co-digestion of Citrullus Lanatus Peels and Chicken Droppings for Methane (Biogas) Production and Its Statistical Analysis Using Least Square Method

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Abstract:

In this work, Co-digestion of Citrullus lanatus peels and chicken droppings for methane production was investigated. Data obtained were analyzed statistical software. Proximate compositions of slurry (substrate, digestate) and microbial count load were determined. Results show maximum volume of biogas obtained as 6.8726 X 10^{-3} m^3 on the 33rd day. The thermophilic slurry temperature measured during the fermentation period ranged from 28 - 40 °C (mesophilic temperature).Analysis of the data via statistical analyses showed the determination coefficient (R^2) to be 99.99% with regression parameters β_0 and β_1 as -3.0139 and 1.00, respectively. Based on the proximate composition of the slurry in the digester, Chemical Oxygen Demand (COD/2000 mg/L O_2) contributed a total different of 77%, Total Alkalinity (mg/L $CaCO_3$) contributed 17%, 4% by Total solid, Nitrogen (mg/LN) contributed a total different of 1%. Result of microbial count load showed a 43% of colony forming unit (Cfu/ml) for the substrate, 5% for the inoculum, and 52% for the digestate. Hence, it can be established that the substrates used in this investigation are good materials for methane production and the statistical software used is good for statistical analysis when dealing with curvilinear regression.

Keywords: Citrullus lanatus peels, chicken droppings, methane, proximate composition, statistical analysis, microbial analysis

I. INTRODUCTION

The current increase in population size of human has strained the earth's capability to provide food, shelter, clothing and most especially energy. As the living standard increases in the developing countries, the average consumption and need for energy (methane) has increased drastically and thus, when viewed on a long-run period, it can be established that sooner or later, there will be shortage of sufficient resource and energy in term of methane to meet the ever needs of the populace. Nevertheless, man has hang on to a great extent on wood, sun, and animal dung for their sources of fuel until the discovery and use of fossil fuel in the early 19th century, which has shown a well substitute and relieved the latter source most particularly in the urban sector of the nation. With the research understanding of science and the resultant overview of new technologies, countless application of wood burning and the early sources of energy were slowly dabbed out by the use of fossil fuels. Based on nation consideration, many developing countries are seriously reliant on the importation of fossil fuels, consuming a large part of the often rare foreign exchange. Though there is a vast reserve of crude oil in a developing country such as Nigeria, it has been exposed that year after year, there is an ever growing cost of petroleum-based fuels that serves as a major source of energy in the country. Hence, the search for substitute sources of energy resources has become a main issue to be considered not only by the government but also by individuals [1]. It has been reported that the growth of methane plants in developing countries addressed quite a number of problems such as scarcity of firewood, indoor health problems with cooking on firewood or cow-dung fire, loss of fertilizer from burning cow dung, time consuming firewood gathering is a problem for many women and lack of efficient and affordable light sources for studying through the evenings [2]. However, Production of biogas has been carried out with the use of plant wastes, manures from domestic animals or mixture of the two [3-7].

Citrullus lanatus (C. lanatus) is a plant species belonging to the family of Cucurbitaceae, a vine-like pinnacle plant that was originated from Southern Africa. It is cultured because of its fruit; cultivars include watermelons *(Citrullus lanatus* var. *lanatus*) and citron melons *(Citrullus lanatus* var. *itroides). Citrullus lanatus* peels contained a compound known as citrulline. Citrulline when converted to arginine, an amino acid vital to the heart, vascular system and immune system. Researchers guess that *C. lanatus rind* might relax blood vessels and have a role in treating erectile dysfunction [8]. Some of the benefits of *C. lanatus peels* include nutritional benefits such as vitamin B and C, economical benefits such as food (as in pickles, relishes or jam) and medicinal benefits. [9] reported the composition of *C. lanatus peels* (rind) as moisture content (5.12), dry matter (94.88), ash (3.07), lipid (1.05), crude fibre (2.98), crude protein (7.04) and carbohydrate (80.75).

Chicken droppings or litter is a mixture of bedding materials (rice hulls, sawdust, wood chips, etc.) and animal excreta. The nutrient content of litter differs from households and within the same house conditional on location and

management [10]. Chicken droppings have a higher proportion of biodegradable organic matter than the excrements of any other livestock. However, anaerobic digestion is the maximum apt method for handling of this municipal solid waste (chicken droppings). On the average, faeces, urine and litters which are mainly made up of nitrates are the physical structures of chicken droppings. Nitrate pollution is undesirable because of its potential role in eutrophication, methemoglobinemia and nitrosamines formation [11]. Benefits of chicken droppings include manure usage, energy application and livestock feed for beef animals.

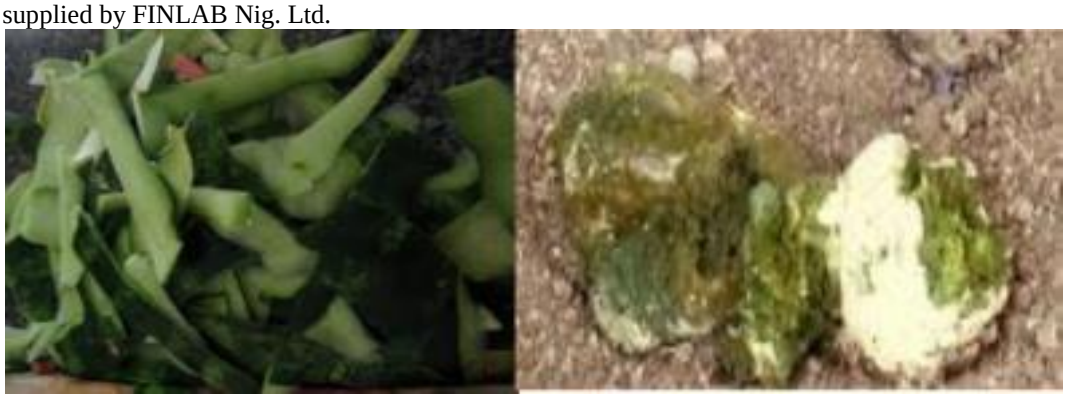
In view of these, this research work utilized the combination of *C. lanatus peels* with chicken droppings for biogas production.

II. MATERIALS AND METHODS

2.1 Materials

Freshly harvested *C. lanatus* was purchased in Omu-Aran market, Kwara State, Nigeria. It was carefully peeled and the peels (Fig. 1a) were washed in ionized water to eliminate the adherent dirt's. The washed peels were milled into semi-fine particles in order to increase its surface area for microbial actions [12]. The milled *C. lanatus* peels were collected in a cleaned bucket for further processing.

Chicken droppings were obtained free of wood shavings from the Poultry Department of the Teaching and Research Farm in Landmark University, Omu-Aran, Kwara State, Nigeria (Fig. 1b). The chicken droppings were sundried for four days and grinded mechanically with a mortar and pestle. All chemical and reagents used were of analytical grades made by GFS Chemicals, Inc., 867 McKinley Ave., Columbus OH 43223 (99.7-100%) and BDH Analar Ltd., Poole England (99%) and supplied by FINLAB Nig. Ltd.



(a) (b)

Fig. 1**:** Citrullus lanatus peels and chicken droppings

2.2 Methods

2.2. 1 Biogas reactor (digester) design with gas collection system.

A 25 *L* methane reactor of dimension 50 *cm* x 25 *cm* was made-up from galvanized steel. Galvanized steel was used as building material because of its strength and durability in acid and basic environments. Three different holes were bored on the lid of the digester for the slurry inlet, the insertion of a thermometer and the gas outlet. The shape was implemented to improve proper mixing. The digester was air tight, painted black and placed above ground level where it was exposed to sunlight for easy absorption. The major unit of the digester is the stirring unit at the top of the digester while, at the lowest of the digester, there is a tap for the slurry outlet. A 12 *L* gas holder tank of height 27 *cm* and diameter 25 *cm* was fabricated from thin sheet metal and used to collect and store the biogas until. Rubber hose was used to attach the digester to the methane (gas) collection system through the water displacement method. The volume of biogas was measured through the height displaced by the gas via the liquid column. The digester and methane gas holder was intended, constructed and run by the methods used by [13] and [14] with little alterations. The base area of gas collector as well as the methane volume was computed using Eq. (1) and Eq. (2):

$$Base\ area\ of\ gas\ collector = {}^{\pi D^2}/_{4} \quad (1)$$

Where the D (diameter of gas holder) = 0.025 m

$$Base\ area\ of\ gas\ collector = \frac{3.142X\,0.025^2}{4} = 0.04904^2$$

$$Biogas\ Volume\ (V) = Base\ area\ of\ gas\ collector\ X\ Height\ of\ gas\ collector \quad (2)$$

2.2.2 Slurry preparation

For preparation of slurry, the substrate (milled ATDCS with goat dung) was mixed with distil water in a ratio 1:1 w/w in a reactor mix bucket (Fig. 1), and the slurry was thermally pretreated using the standard method. Thermal pretreatment has been said to lead to pathogen removal and also improves dewatering performance and reduces viscosity

of the digestate with subsequent enhancement of digestate handling, the pH of the slurry was checked in order to know the degree of acidity of the slurry. $NaOH_{(aq)}$ was added to the slurry to reduce its acidity from 5.4 to 8.8 for organism adaptation during anaerobic digestion.

2.2.3. Experimental Procedure

Before feeding the digester, the rubber hose joining the methane gas outlet from the digester to the gas holder was disconnected, such that the gas outlet was left open. This was done to prevent undesirable pressure build-up in the digester. The slurry was fed into the digester through the inlet and was sealed to prevent air from getting into the digester and gas from escaping. The slurry was allowed to occupy three-quarter of the digester space leaving a clear height of about 6.25 *cm* as space for gas production. The inflow was fixed downward to cause the solids to gather at the lowest of the tank for easy elimination after digestion. The contents of the digester were gently and manually stirred daily through a stirring rod attached to the digester at 12 pm and 4 pm. The methane gas was placid by water displacement method and the fermentation process was observed for 40 days. Daily ambient temperature within the mesophilic temperature range and the height of the methane gas holder were measured during this period. Daily biogas volume produced was computed based on aforementioned Eqn. (2).

2.2.4 Statistical Analysis

The linear regression model and correlation was used to analysis and evaluate the data obtained from the volume of gas production per day. From these, the coefficient of determination (R^2) was obtained and the regression parameters β_0 *and* β_1 were evaluated. To estimate the variation of volume of biogas produced per day, temperature of fermentation against digestion time and pH against digestion time per week, Microsoft Excel Version 2013 was used to plot the graphs.

2.3 Proximate Analysis Of The Substrate And Digestate

Proximate analysis of the substrate and digestate such as aluminum, ammonia nitrogen, ash content, carbon content, moisture content, nitrogen content, calcium, carbon to nitrogen ratio, chemical oxygen demand (COD), dissolve oxygen (DO), pH, phosphorus, potassium, total alkalinity, total kjedahl nitrogen, total phosphate, total solids and volatile solids were carried out. Detailed procedures of the analysis are described as follows:

2.3.1 Ash content (%)

An empty crucible was fire polished in a muffled furnace and allowed to cool in a desiccators containing calcium chloride for 20 min and then weighed. About 2.0 g of dried sample (substrate/digestate) was weighed out into the crucible and transferred into a muffle furnace at 650 ^oC for 3 h. The crucible was removed from the furnace, placed in desiccators and then allowed to cool and then re-weighed to get the final weight. The percentage of ash content of the sample was calculated using Eqn. (3):

$$Ash\,\% = \frac{X-Y}{W} \times 100 \qquad (3)$$

where, X = weight of crucible + ash, Y = weight of crucible

W = weight of sample to be determined in grams before ashing.

2.3.2 Phosphorous content

5 ml aliquot of the soil extract was pipette into a 25 ml volumetric flask and distilled water of 10 ml was added. 4 ml of reagent of phosphorus standard solution was added and made up to volume with distilled water. The blue colour was allowed to develop for 15 min and remain stable for 24 h. Phosphorus content in solution was then determined using Jenway Spectrophotometer at 660 mμ.

2.3.3 Kjeldahl Nitrogen

A representative sample was prepared and 1 g was weighed to an accuracy of 0.1 mg into a digestion tube. Two kjeltabs were added (5 g Na_2SO_4 and 1 g $CuSO_4$. $5H_2O$ and Selenium). 12 ml of concentrated H_2SO_4 was carefully added and shook to wet the acid with the sample. The exhaust system was attached to the digestion tubes in the rack and the water aspirator was set to full effect. The rack was loaded with exhaust into a preheated digestion block (420 ^oC) and contained within the exhaust head. After 5 min, the water aspirator was turned down until the acid fumed. Digestion was continued until all samples were clear with a blue/green solution (normally after 30-60 min). The rack of tubes was removed with exhaust still in place and put in the stand to cool for 15 min. 80 ml of de-ionized water was carefully added to the tubes. The steam valve on the Kjeltec 1002 was opened and distilled for approximately 4 min. At the end of the distillation cycle the steam valve was closed and the distillate was titrated with standardized HCl until the blue/grey end point was achieved and the volume of acid consumed in the titration was recorded. Kjeldahl nitrogen was estimated using Eq. (4):

$$KN = \frac{(T-B)\times N\times 14{,}007\times 100}{weight\ of\ sample\,(mg)} \qquad (4)$$

where, T = titration volume for sample (ml), B = titration volume for blank (ml), N = normality of acid, molar weight of Nitrogen = 14.007

2.3.4 Total alkalinity

1 ml of the sample (substrate and digestate) was diluted with 9 ml of distilled water and then inserted into the tube-hole of the apparatus and covered. Blank test of distilled water was then run and Total alkalinity was determined. This procedure was also used to determined ammonia nitrogen, total phosphate, total solids, aluminum, potassium, copper, iron, magnesium, calcium, zinc and dissolve oxygen.

2.4 Microbial Activities Of Substrate And Digestate

Test tubes and empty petri dishes were laid out and labeled, the lids of the test tubes 0 and 1 were flamed and loosened. A sterile pipette was used to transfer 1 ml of liquid from tube 0 to plate 0 and same pipette was used to transfer 1 ml of liquid from the source culture containing the substrate and digestate separately (tube 0) to tube 1 and the pipette was then discarded. The edge of tube 1 was flamed, then sealed and the content was homogeneously mixed gently. These steps were repeated 5 more times moving along the chain for each source culture. At the end of this process, a conical flask of sterilized nutrient agar was taken from the 45 °C water bath, where it had been kept just above setting temperature. The outside of the conical flask was dried and the top and neck area were then flamed, all these steps were carried out in the flame cupboard. By slightly opened each Petri dish lid, the nutrient agar was poured into the dilution liquid already in the Petri dish, until it covered about two thirds of the area (although this is not critical).The nutrient agar was mixed with the dilution liquid by a gentle swirling action, then the edge of the conical flask was flamed and this step was then repeated for the remaining Petri dishes. The Petri dishes were left in flame cupboard to set for 15 min, and then sealed, inverted, and placed in the laboratory incubator at 37 ^{o}C for 48 h, Petri dishes were then examined without opening. The individual colonies of Petri dishes with dilution factors 10^{-5} and 10^{-6} of each source culture were counted using the colony counter. The results of the counting using the colony counter were recorded and the microbial load count was calculated using Eqn. (5):

$$Microbial\ load\ count\ \left(\frac{Cfu}{ml}\right) = \frac{No\ of\ colony}{dilution\ factor} \times 10\ ml \qquad (5)$$

III. RESULTS AND DISCUSSION

3.0 Daily biogas production

Fig. 2 shows the plot of daily biogas production. Production of biogas started on the 1st day with a value of 3.19085 x 10 $^{-3}$m^3, then a decrease in biogas production from the 3rd to the 6th day, a somewhat constant rate for the next 8 days. The maximum yield of biogas was attained on the 33rd day with a value of 6.8726X 10 $^{-3}$ m^3. [15] attributed the higher biogas yield from the substrates to the presence of native micro flora in the chicken droppings while [16] attributed it to the low carbon-nitrogen ratio. The lowest yield of biogas was on the 6th day with a value of 1.71815 X 10^{-3} m^3. It was also observed that biogas production was slow at the beginning and slightly slow at the end period. This was in line with what was early stated by [17], that biogas production rate in batch condition is directly proportional to specific growth rate of methanogenic bacteria in the bio-digester.

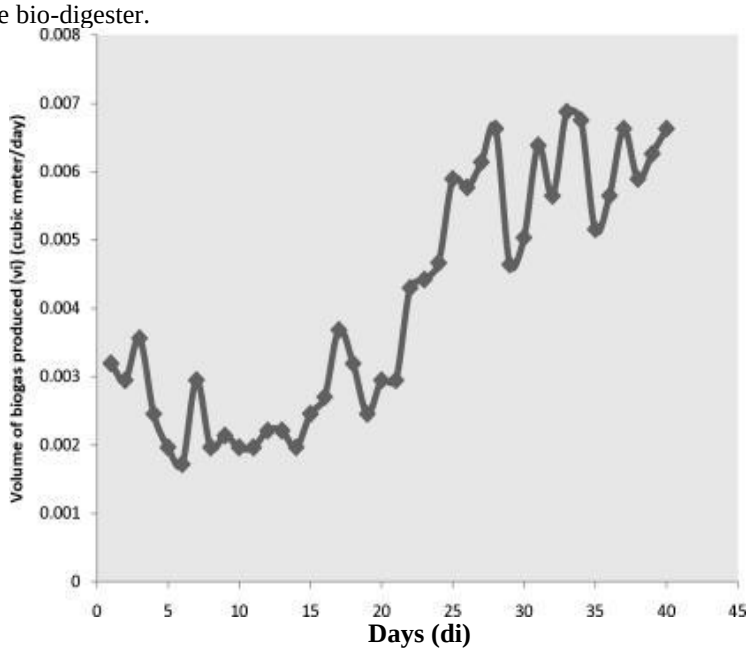


Fig. 2: Daily biogas production

3.1 Temperature Variation

Fig. 3 shows the plot of relationship between temperature and digestion time in days. It was observed that throughout the duration of the digestion process, the temperature was between 20 and 40°C, showing that the digestion process was effective at a mesophilic temperature range. Earlier reports shows that temperature has been observed to be quite critical for anaerobic digestion, since methane producing bacteria operate most efficiently at temperatures 30.0- 60.0 °C [18-19].

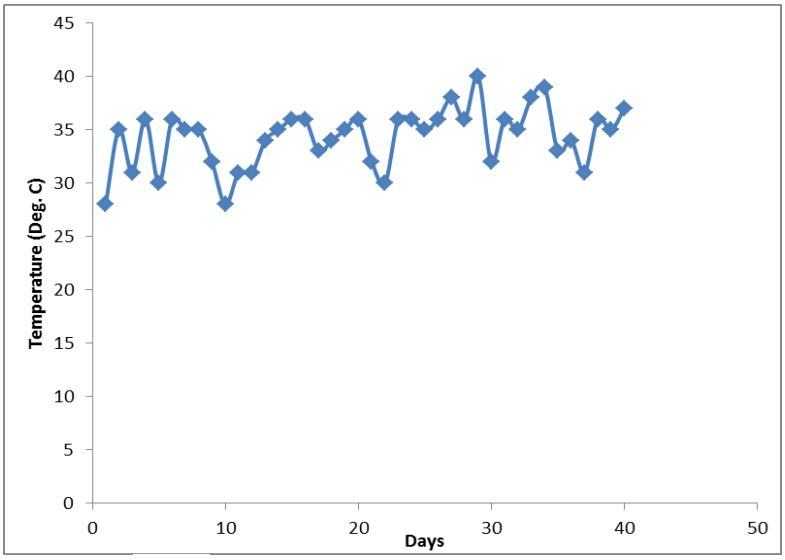


Fig. 3: Plot of temperature variation against days

3.2 pH Variation

Fig. 4 shows a plot of relationship between pH and the digestion time in week. It was observed that the value of pH reduced from 8.8 to 7.65 in the space of 5 weeks. This supported what was earlier mentioned by [20], that optimum biogas production is achieved when the pH value in the digester is between 6 and 7. In the same vein, [21] reported that low pH value inhibits methanogenic bacteria and methanogenesis. The high pH value recorded in this study could be attributed to large ammonia losses resulting from C/N ratio of chicken droppings [22].

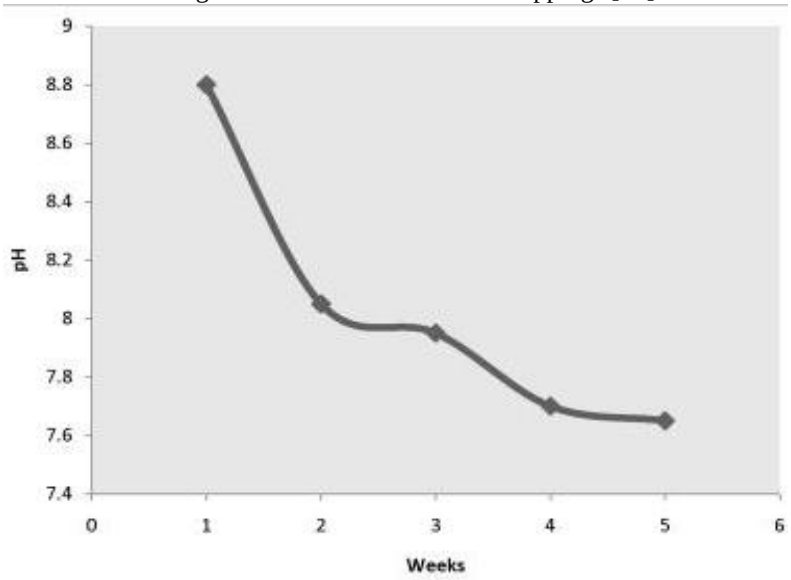


Fig. 4: Weekly pH variation

3.3 Statistical Analysis

Fig. 2 shows the plot of daily biogas production. The scatter plot of (d_t, v_t), indicates clearly that the data points lie on a zigzag curve, so the simple linear regression model is not appropriate. Engineers believe that a curve defined by either $v = ae^{(b/d)}$, or $v = ad^b$ (where 'a' and 'b' are to be determined), provides a good fit to the observed data and are called intrinsically linear because they can be transformed into models by means of a suitable change of variables. Therefore, convert this curve fitting problem to a linear regression problem by taking logarithms as in Eqns. (6) and (7):

$$V = ae^{b/d};\ Then\ \ln V = \ln(a) + b\left(\frac{1}{d}\right) \qquad (6)$$

$$V = ad^{b};\ Then\ \ln V = \ln(a) + b\ln(d) \qquad (7)$$

Thus, to fit the curve defined by Eqn. (7), it was noted that the transformed variables $v = \ln v$ and $d = In\ d$, satisfy the linear equation $y = \beta_0 + \beta_1 x$, $(\beta_0 = \ln a,\ \beta_1 = b)$. Hence, a least square method to fit a straight line to the transformed data, shown in Fig. 5. Thus, the curvilinear regression problem was solved by first fitting a straight line to the transformed data and then the parameters of the curves $V = ad^b$ was estimated using linear transformation. From the graph, the regression coefficients β_0 and β_1 were obtained as -3.0139 and 1.00, respectively. The coefficient of determination R^2 which plays an

important role during model checking and also explained the proportion of variability explained by the model was obtained as 0.9999. This value implied that most of the variability is explained by regression model. The estimated regression function of volume of biogas produced per day is given in Eqn. (8).

$$y = x - 3.0139 \quad (8)$$

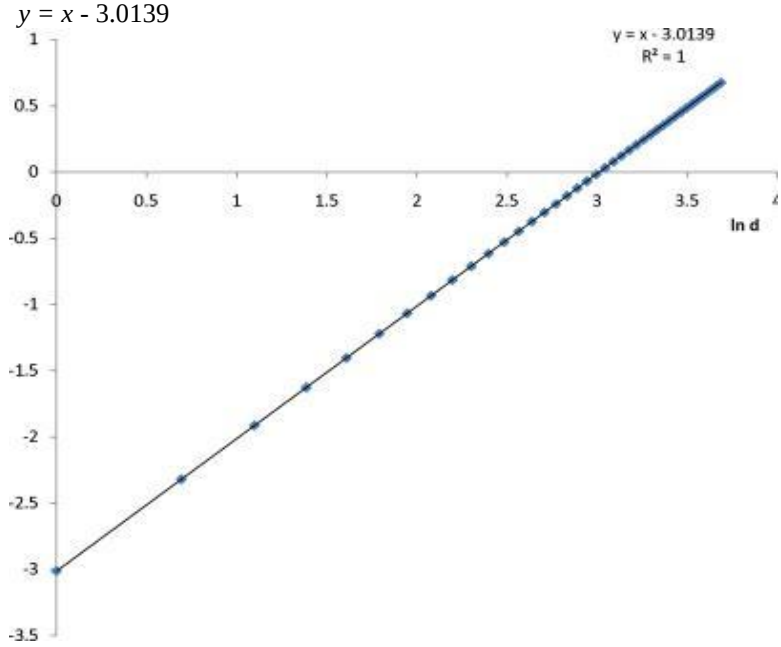


Fig. 5: Least square method to fitting a straight line

3.4 Proximate Compositions Of Substrate And Digestate

The proximate compositions of the substrate and digestate before and after the anaerobic digestion are shown in Table 1 while Fig. 6 shows the percentage variation. Proximate compositions were determined using a digital photometer. An effective way of finding the availability of the amount of nutrients accessible for bacterial action during digestion is through the determination of the total solids of the wastes. The total solids and volatile solids in this study are therefore within the range for biogas production [23]. The amounts of methane to be produced depend on the quantity of volatile solids present in the waste and their digestibility or degradability. Higher ash content also corresponded with higher volatile solids content as can be seen in Table 1. Chicken droppings have a higher potential for organic manure compared with *C. lanatus* peels because of its higher ash content. The values obtained for COD, phosphorus, potassium, total kjedahl nitrogen, total alkalinity, C/N ratio and carbon content showed a decreased after the anaerobic digestion while aluminum, ammonia nitrogen, calcium, DO, % moisture content, nitrogen content and total phosphate increased after the digestion process. This observation is in line with what was earlier reported by [7]. However, the high values of nitrogen, phosphorus and potassium in the digestate indicate that the end by product will be useful for fertilizer application.

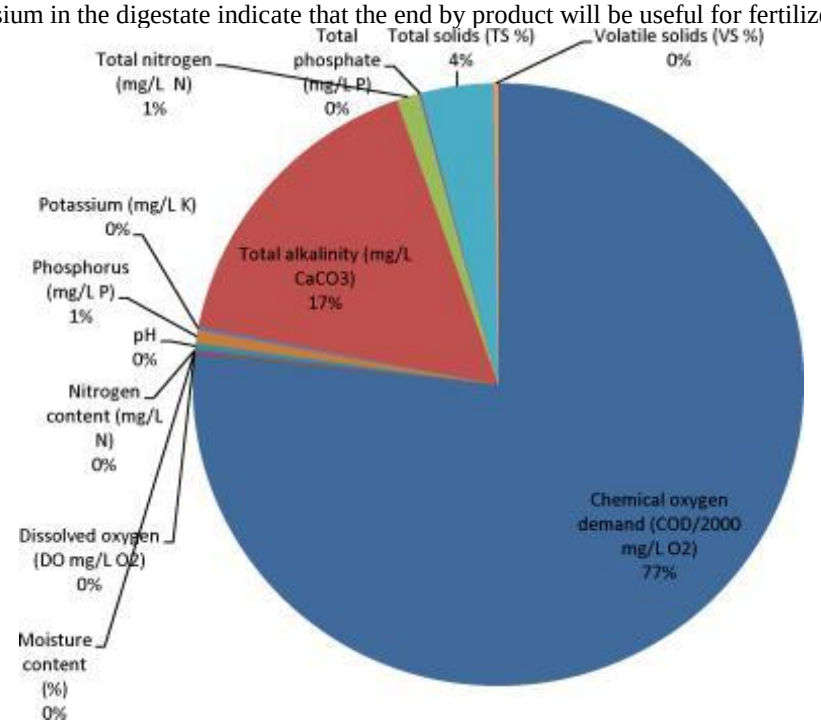


Fig.6: Percentage different conversion of substrate and digestate during anaerobic digestion

3.5 Microbial Analysis Results

The result of microbial analysis of the substrate and digestate contains the methane producing bacteria called methanogens. The microbial load count obtained (Fig. 7) showed that colony forming unit account for 43% substrate equivalent to 7.3 x 10^{-3} Cfu/ml, 5% inoculum equivalent to 7.8 x 10^{-4} Cfu/ml and 52% digestate equivalent to 8.8 x 10^{-3} Cfu/ml. The microbial

Table 1: Proximate composition of substrate and digestate

Parameters	Substrate	Digestate
Aluminum (mg/L Al)	0.13	0.17
Ammonia nitrogen (mg/L N)	0.28	0.38
Ash content (%)	75	25
Calcium (mg/L Ca)	29	34
Carbon content (mg/L C)	16.2	15.4
Carbon/nitrogen ratio (C/N)	5.7:1	4.8:1
Chemical oxygen demand (COD/2000 mg/L O_2)	1900	1040
Dissolved oxygen (DO mg/L O2)	2.2	3.7
Moisture content (%)	0.95	1.85
Nitrogen content (mg/L N)	2.828	3.2
Ph	8.8	7.65
Phosphorus (mg/L P)	16.8	16.2
Potassium (mg/L K)	4.7	4.1
Total alkalinity (mg/L $CaCO_3$)	410	350
Total nitrogen (mg/L N)	29.5	26
Total phosphate (mg/L P)	3.2	3.36
Total solids (TS %)	95	93
Volatile solids (VS %)	5.66	5.26

load count for the digestate increased during the digestion of the substrate due to growth of the microbes which aided the completion of the anaerobic reaction and the production of biogas during the digestion.

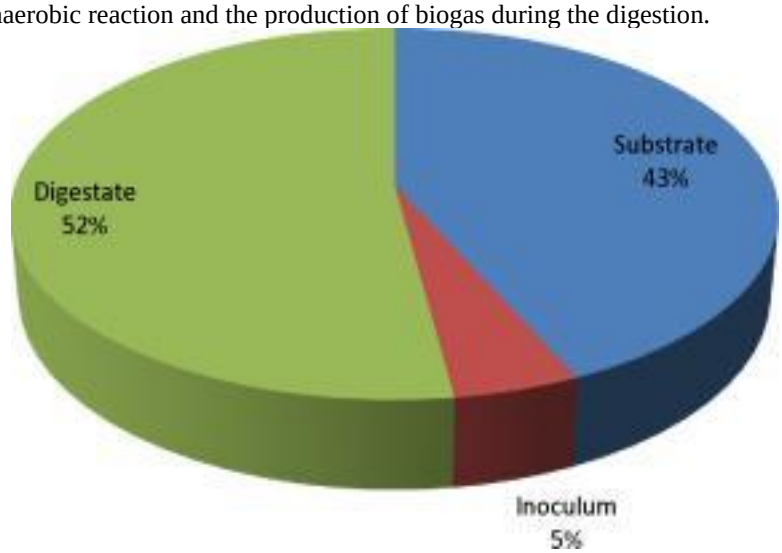


Fig.7: Percentage microbial load count

IV. CONCLUSION

Co-digestion of *Citrullus lanatus peels* and chicken droppings for biogas production was carried out and the following conclusions were drawn:

i. The maximum yield of biogas was obtained as 6.8726 X 10^{-3} m^3 on the 33rd day, followed by 6.749875 X 10^{-3} m^3 on the 34th day and, 6.62715x 10^{-3} m^3 on the 28th, 37th and 40th days, respectively.
ii. The temperature of the slurry measured during the fermentation period ranged from 28 - 40 °C (mesophilic temperature range).
iii. The pH of the slurry reduced from 8.8 to 7.65. This could be attributed to the nature of the feed within the digester.
iv. The coefficient of determination R obtained was 99.99%. This implies that all the variability is explained by regression model function.

v. Based on the proximate composition of the slurry in the digester; aluminum, ammonia nitrogen, dissolved oxygen, moisture content, nitrogen content and total phosphate increased after anaerobic digestion while ash content, calcium, carbon content, chemical oxygen demand, pH, phosphorus, potassium, total alkalinity, total nitrogen, total solids and volatile solids decreased after anaerobic digestion.
vi. The carbon-nitrogen ratio of the slurry was calculated as 5.7:1 before digestion and 4.8:1 after digestion. The lower rate obtained indicates a faster rate of degradation which corresponds to the biodegradability of the substrate during digestion.
vii. Result of microbial load count showed a 43% of colony forming unit (Cfu/ml) for the substrate, 5% for the inoculum, and 52% for the digestate.
viii. The by-products or spent slurry of this process could also be used as fertilizer or improved organic manure for agricultural production.
ix. The substrates used in this investigation are good materials for biogas production and the least square method is good for statistical analysis when dealing with curvilinear regression in energy production.

However, the volume of biogas produced by the substrate concentration is dependent on the nature of the waste material, the time taken to start production, temperature and other environmental factors. This is an indication that fruits and vegetable wastes which are the major constituents of biodegradable domestic waste can be converted to fuel or other sources of energy for domestic and industrial applications. Also, Mathematical models derived using least square method indicated that biogas production of fruit and animal wastes can be predicted based on digestion time. The polynomial function seemed to be more reliable in predicting gas production in anaerobic digestion of wastes. This tool is useful in statistical analysis of biogas production from energy materials, and requires further validation and refinement.

COMPETING INTEREST

Authors declare no competing interest whatsoever.

AUTHORS' CONTRIBUTIONS

The project was carried out by the Authors.

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