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Thermophilic Anaerobic Co-digestion of an Alkali Treated Musa sapientum Plant Waste (MSPW) and Cattle Dung for Biogas Production and its Statistical Analysis: a Case of One Factor Response Surface Methodology (RSM)

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Abstract This study investigates thermophilic anaerobic Co-digestion of an alkali treated M*usa sapientum* plant waste (MSPW) and cattle dung for biogas production and its statistical analysis using a one factor response surface methodology (RSM). Proximate compositions of slurry and analysis of microbial count load were carried out with the aids to determine the potential use of spent slurry. Results show highest experimental biogas yield of $2.915\times 10^{-2}m^3$/day on 19th day. Statistical analysis show the predicted biogas yield of $2.834\times 10^{-2}m^3$/day on 19th day. Using this predicted day, the experiment was validated in triplicate and the average optimum biogas yield was $2.82\times 10^{-2}m^3$/day. Analysis of variance (ANOVA) of regression equation shows that the coefficient of determination (R^2) of was 63.81%.Temperature of the slurry measured during the fermentation was within mesophilic temperature (30-40 ℃). Proximate compositions of the substrate and digestate obtained in this study shows a decreasedin ammonia nitrogen, total phosphate, total alkalinity, potassium, dissolved oxygen (DO), iron/10 and total copper after the anaerobic digestion while the pH, aluminium, calcium, magnesium, zinc/35TT, total solid and carbon content increased after the anaerobic digestion process. Microbial load count results showed 6.3×10^{-2}Cfu/ml digestate, 4.6×10^{-2}Cfu/ml of substrate and 5.5×10^{-3}Cfu/ml inoculum. Hence, it can be concluded that an alkali treated MSPW mixed with cattle dung at a 1:1 w/w ratio, showed an increase in biogas production and the spent slurry of this process can improve organic manure for agricultural production.

Keywords Musa sapientum plant waste (MSPW), cattle dung, biogas, statistical analysis, microbial analysis, proximate composition.

Introduction

In the 21st century, energy is an important driving factor to social and economic development. Its impact has effects on virtually all aspect of human endeavors such as the industrial sector, agricultural sector, health sector, power sector, educational sector, and transportation sector among others. The major fuel source in Nigeria and other countries are petroleum based fuels, which is used in the transportation sector in most of the developing nations. Petroleum fuels combustions give emissions that are not environmentally friendly, it is hazardous and very dangerous to the human health, and it is also claimed to be responsible for climate change and global warming. Also, petroleum fuels are non-renewable i.e. its exhaustible, which is the reason for the depleting and dwindling nature of the fuel, increases of the price of petroleum fuels and recurrent scarcity of fuel has led to a search for alternative fuel. An alternative fuel must be technically feasible, economically competitive, environmental acceptable and readily available [1]. The renewed interest to the quest for greener fuel's sources is typical issues that

gain wide societal and political interest especially for its reduced greenhouse emissions, biodegradability, sustainability as well its competitive nature to fossil fuels [2-3].

In developed countries, the disposal of waste is regulated by the law; all waste must be collected and sent to the various facilities for its processing. In Nigeria, a significant amount of waste is thrown out every day from farmlands, households, restaurants, and food processing facilities. From an ecological point of view, management of waste poses a serious problem; waste not disposed properly has adverse effects on the environment, it is a risky waste. Also, most of the wastes generated are still going down the drain which causes enormous problems like repulsive smell, build-ups and blockages in municipal sewer pipes. These quantities of wastes produced daily can be essential sources of feed stock for biofuel such as biogas production. Biogas production from waste, help in solving the problem of the production of environmentally friendly fuel and the disposal, the utilization of biogas converts it from a waste into an energy source that is renewable. However, biogas is produced from the fermentation or digestion of biodegradable materials through anaerobic digestion and hereby has the characteristics such as; renewability, sustainability, non-toxic and environmentally friendly.

Anaerobic digestion is a process by which a complex mixture of symbiotic microorganisms transforms organic materials under oxygen free conditions into biogas. In practice, anaerobic digestion is the engineered methanogenic decomposition of organic matter in reactor vessels that are relatively simple to construct and operate. [4], defined anaerobic digestion as a process which breaks down organic matter in simpler components without oxygen. According to [5], anaerobic digestion (AD) is a biological process that transforms the initial substrate (ingestate) into the desired product (biogas) and into a solid-liquid (digestate). Anaerobic digestion is a series of natural biological processes whereby co-digestion of organic waste material (feedstock) is broken down by microorganisms and converted into energy (biogas) in the absence of oxygen.

Co-digestion is a process in which two or more organic waste materials are digested together in a reactor, thereby improving anaerobic digestion. It shows a higher degradation of organics than the individual processes. Co-digestion improves biogas yield due to the positive synergism established in the digestion medium and the supply of missing nutrients by the co-substrates [6]. Solid wastes are transformed into loadable slurries when mixed with watery manure, which enables easier handling both in the digestion process and afterwards. Co-digestion can help achieving a better NPK ratio by blending different organic wastes. The value of the digestate as a fertilizer is thus enhanced [4]. It was indicated that a mixture of three substrates: cheese whey, glycerin, and corn silage fraction, resulted in the biogas production rate of 1.8 (L/L/d) and the highest methane content equal to 61 % [7]. It was investigated the co-digestion of mixtures of primary sludge and fruit and vegetable fraction of municipal solid wastes, and the results were that the co-digestiongave a higher yield than the digestion of primary sludge alone [8]. It was investigated fertilizer and sanitary quality of digestate bio-fertilizer from the co-digestion of food waste and human extract [9]**.** Silicon compound in biogas was determined from wastewater treatments plants, landfills, and co-digestion plants [10]. The biogas production from leather fleshing waste was carried out by co-digestion with MSW and its optimization [11].

Plantain, *Musa sapientum* (*M. sapientum*) is one of the cultivated varieties of the genus *Musa* whose fruit is also known as the banana. Plantain leaves can exceed two meters in length. They are similar to banana leaves, but are larger and stronger, thus reducing waste in cooking. Mineral composition of *M. sapientum* leaves includes; sodium, manganese, calcium zinc, copper, nitrogen, potassium and iron [12]. Cattle dung, also known as cattle pats, cattle pies or cattle manure, is the waste product of bovine animal species. These species include domestic cattle, bison, yak and water buffalo. Cattle dung is the undigested residue of plant matter which has passed through the animal's gut. The resultant faecal matter is rich in minerals. Its uses include agricultural fertilizer, fuel, mosquito's repellant, thermal insulator, and manufacture of mud brick, to mention but view [13].

In view of these, this research work combined *M. sapientum* plant waste (MSPW) with cattle dung for biogas production and its statistical optimization while computing proximate compositions as well as the microbial count load of the substrate and digestate.

Materials and Methods

Materials

M. sapientum plant wastes (MSPW) were obtained from a garden in a residential compound of Progress Estate, Ogun State, Nigeria. The MSPW were dried in a U Clear model DHG-9053A drying oven at 90 ^{o}C for 30 min to remove moisture content and easy for grinding (Fig. 1). MSPW is a lignin agricultural material; the methanogens (bacteria) cannot digest or process this substance easily, and hence, the chemical pretreatment was done to make the substrate suitable for loading. MSPW were treated with 1% NaOH and then covered in a bucket for 7 days to increases biogas production during anaerobic digestion [14]. After 7 days, alkali treated MSPW was mixed with a

fresh cattle dung obtained from the Cattle Farm in Landmark University Teaching and Research Farm, Omu-Aran, Kwara State, Nigeria, at a 1:1 w/w ratio, and then milled to semi-fine particles using a Delmar R175A diesel engine hammer mill machine to increase its surface area for microbial action [15].The milled alkali treated MSPW with cattle dung (substrate)were collected in a cleaned bucket for further processing.

Figure 1: Dried M. sapientum plant wastes (MSPW)

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.

Methods

Biogas Reactor (Digester) Design with Gas Collection System

A 25 Lconical shape biogas reactor of height 50 cm and diameter 25 cm was fabricated 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 conical shape was adopted to enhance better mixing and stability. 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 bottom 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 connect the digester to the 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 gas holder was designed, built and operated by the methods used by [16] with slight modifications. The base area of gas collector as well as the biogas volume was computed using Eq. (1) and Eq. (2):

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

Where D = diameter of gas holder tank = 25 cm = 0.025 m

$A = \frac{3.142 \times 25^2}{4}$ = 490.94 cm^2 = 0.04904 m^2

$$Volume\ of\ biogas\ produced\ per\ day\ \left(\frac{m^3}{day}\right) V = A \times h \quad (2)$$

Where h = height of gas collector (m)

Preparation of Slurry

The milled alkali treated MSPW with cattle dung kept in a cleaned bucket called substrate was mixed with ionized water in a ratio 1:1 w/win a reactor mix bucket to make the slurry, and the slurry was thermally pretreated using the method earlier stated by [15]. 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[16].Since pH plays an important role when considering the growth of microbial life during digestion, anaerobes prefer a pH close to neutral, in the range of 6.8-7.2 [17]. The pH of the slurry was checkedusing an HANNA, HI 2210 pH meter and was found to be 7.1, which was well within the range earlier reported for growth of microbial life.

Experimental Procedure

Before feeding the digester, the rubber hose connecting the gas outlet from the digester to the gas holder was disconnected, such that the gas outlet was left open. This was done to prevent negative 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 $8.30cm$ as space for gas production. The inflow was directed downward to cause the solids to accumulate at the bottom of the tank for easy removal after digestion. The contents of the digester were gently and manually stirred daily through a stirring rod attached to the digester at 10 am, 2 pm and 6 pm, respectively. The gas was collected by water displacement method and the fermentation process was monitored for 30 days, after which the digestate sample was collected for analysis. During this period, daily ambient temperature within the mesophilic temperature range and the height of the gas holder were measured. Daily biogas volume produced was computed based on aforementioned Eq. (2).

Statistical Analysis of Biogas Production

In order to optimise the volume of biogas produced per day and the temperature, Stat-Ease\Design-Expert 9\DX9.exe" software, a Response Surface Methodology (RSM) with a one factor design was used, the model setting was quadratic, the minimum level was set to be 1 while the maximum level was set to 30, the center points was set to 24 which produced 30 experimental runs. The experiment was randomized to minimize the effects of unexpected variability in the experimental responses (biogas volume and Temperature). In order to optimise the model, sequential model sum of square, lack of the fit test, the R-square and the RMSE values, ANOVA for response surface quadratic model were used.

Proximate Compositional Analysis of Substrate and Digestate

Proximate compositional analysis of the substrate and digestate such as aluminium, ammonia nitrogen, ash content, carbon content, moisture content, nitrogen content, calcium, dissolve oxygen (DO), pH, phosphorus, potassium, total alkalinity, total kjedahl nitrogen, total phosphate, total solids and volatile solids were carried out. Detailed procedures of some of the analysis were described below:

Ash Content (%)

An empty crucible was fire polished in a muffled furnace and allowed to cool in a desiccator 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 ^{o}C for 3 h. The crucible was removed from the furnace, placed in desiccator 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 Eq. (3):

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

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

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

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.

Kjeldahl Nitrogen

A representative sample was prepared and 1g 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 ^{o}C) 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):

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

where, T = titration volume for sample (ml), B = titration volume for blank (ml), N = normality of acid to 4 places of decimal, M.W of Nitrogen.14.007

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, aluminium, potassium, copper, iron, magnesium, calcium, zinc and COD (Chemical Oxygen Demand).

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 opening 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 Eq. (5);

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

Results and Discussion

Daily Biogas Production

Table 1 shows the results of daily biogas production which was taken 12.00 pm every day. It was observed that on the 1st and 2nddays of the anaerobic digestion the volume of biogas computed was 2.70 x 10^{-2} m^3/day, then a decrease in biogas production from the 3rdto the 6th day, a somewhat constant rate for the next 9thdays. The maximum yield of biogas was attained

Table 1: Relationship between the volume of biogas yield per day (m^3/day), temperature and the retention time

Day	Volume of biogas yield per day (m^3/day)	Temperature (°C)
1	0.02696	36
2	0.02696	35
3	0.0261849	35
4	0.027634	33
5	0.027634	32
6	0.0281395	30
7	0.027297	33
8	0.0270611	31
9	0.0270611	31
10	0.026623	33
11	0.0267915	33
12	0.02696	37
13	0.02696	36
14	0.027297	34
15	0.027971	35
16	0.0284765	32
17	0.0291505	38
18	0.029319	40

19	0.029319	40
20	0.027634	39
21	0.027971	39
22	0.0281395	40
23	0.027971	35
24	0.027634	38
25	0.0278025	40
26	0.027971	36
27	0.0281395	38
28	0.0284765	30
29	0.028982	38
30	0.027971	34

on the 19th and 20thdays with a value of 2.915× 10^{-2} m^3/day. [14], attributed the higher biogas yield to the alkali treatment of MSPW combined with manure at a 1:1 w/w ratio, while [18], attributed it to the low carbon-nitrogen ratio. The lowest yield of biogas was on the 6th to 7th days with a value of 2.618× 10^{-2} m^3/day. Observation shows 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 [19], that biogas production rate in batch condition is directly proportional to specific growth rate of methanogenic bacteria in the bio-digester.

Temperature Variation

Also shown in Table 2 are the results ofdaily temperature reading. It was observed that throughout the duration of the digestion process, the temperature range from 30 ℃ and 40℃. However, there are three temperature ranges selected for different bacteria. The pyschrophilic range is less than 30℃, mesophilic is between 30−40 ℃, and thermophilic is between 50 and 60 ℃. Anaerobic bacteria are most active in themesophilic and thermophilic range [20]. The temperature ranges obtained in this study shows that the thermophiles deliver a lower quality effluent and frequent energy to maintain the higher temperature [21].

pH Variation

Since, pH plays an important role when considering the microbial life growth during digestion. Anaerobes prefer a pH close to neutral, in the range of 6.8-7.2 [22]. The weekly pH during anaerobic digestion in this study was taken to be 7.1 (neutral) for the first week, and it dropped to 6.81 (less neutral) for second week, an increased to 8.45 (slightly alkali) in the third week showing the alkali medium and a gradual dropped to 7.9 (high neutral) on the fourth week. This observation support what was earlier reported [23], that optimum biogas production is achieved when the pH value in the digester is between 6 and 7. Furthermore, it was reported that low pH value inhibits methanogenic bacteria and methanogenesis [24]. Observation on the pH fluctuation in this study could be attributed to the nature of the feed within the digester [25-28].

Statistical analysis by Response Surface Methodology (RSM) and Artificial Neural Network (ANN).

Data obtained from biogas experimental yield were statistical analysis using a one factor response surface methodology.Results show R^2 which plays an important role during model checking and also explained the proportion of variability that is explained by the model was 63.81%. RMSE (root mean square error) which is the measure of the unexplained variability was obtained as 0.513. The highest predicted biogas volume by one factor response surface methodology was 2.834 x $10^{-2}$$m^3$/day on 30 day with desirability value of 0.688 [Fig. 2(a and b)]. Using this predicted day, the experiment was validated in triplicate and the average optimum biogas yield was established as 2.82× $10^{-2}$$m^3$/day. Furthermore, the predicted mesophilic temperature was 38.74 ^oC(Table 2).

Table 2: Statistical optimization results

Parameter	**RSM**	**Experimental**
Coefficient of determination (R^2)	63.81%	-
Root mean square error (RMSE)	0.513	-
Predicted biogas volume (m^3/day)	2.834 x 10^{-2}	2.915 x 10^{-2}
Days	30	19
Desirability	0.688	Un specify
Predicted temperature (^oC)	38.74	40

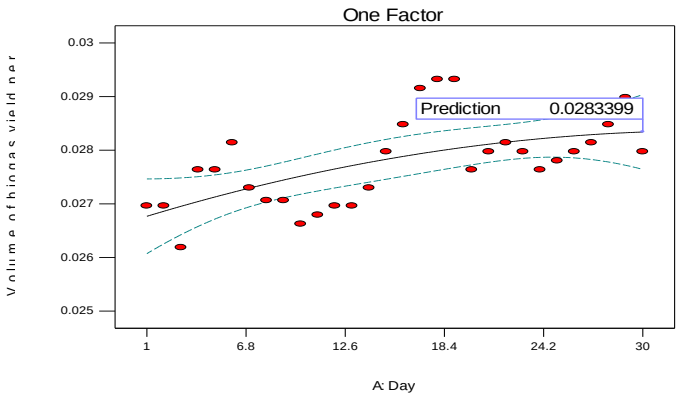

Figure 2 (a): Plot of predicted biogas yield

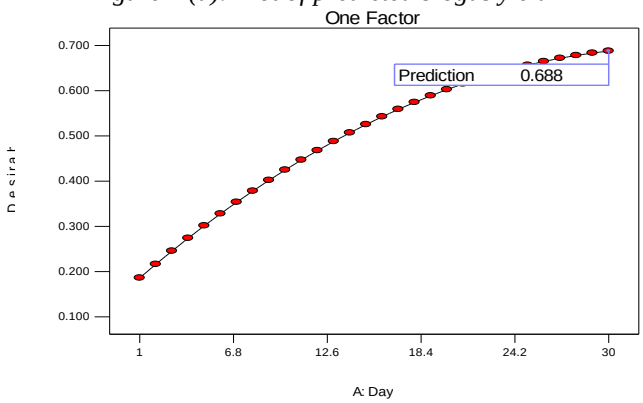

Figure 2 (b): Desirability plot

Proximate Compositions Analysis of Substrate and Digestate

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 digestionis through the determination of the total solids of the wastes. Table 3 shows the proximate compositions of the substrate and digestate before and after the anaerobic digestion while Fig. 3 shows a 3-D column bar chart plot of proximate parameter variation in substrate and digestate.

Table 3: Proximate Compositions

Parameters	**Substrate**	**Digestate**
Ammonia nitrogen (mg/L N)	0.78	0.43
Total phosphate(mg/L P)	6.90	3.86
Total alkalinity(mg/L $CaCO_3$)	470	390
pH	7.47	7.95
Aluminum(mg/L Al)	0.29	0.34
Potassium(mg/L K)	5.20	4.70
Iron/10(mg/L Fe)	7.00	5.80
Total copper(mg/L Cu)	3.90	3.20
Magnesium(mg/L Mg)	90	100
Calcium(mg/L Ca)	4	14
Zinc/35 TT(mg/L Zn)	24.00	25.00
DO (mg/L O_2)	5.00	1.90
Total Solids (%)	4.00	4.30
Ash content (%)	4.50	2.70
Volatile solids (%)	95.50	97.30
Carbon	13.20	79.20
Nitrogen	2.59	7.77
Phosphorus	15.01	39.26

Conductivity	17.39	27.22

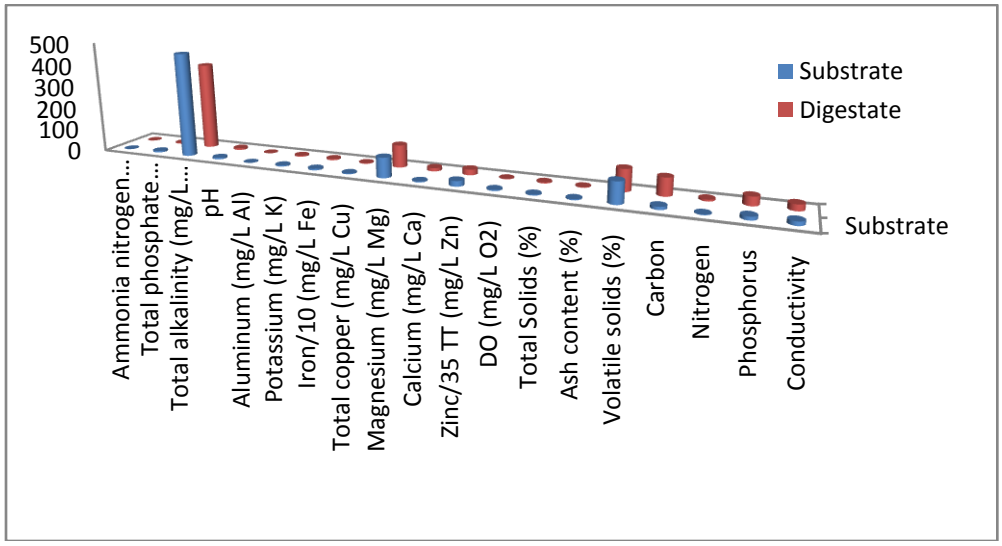

Figure 3: A 3-D column bar chart plot of proximate parameter variation of substrate and digestate.

The values obtained for ammonia nitrogen, total phosphate, total alkalinity, potassium, dissolved oxygen (DO), iron/10 and total copper showed a decreased after the anaerobic digestion while pH, aluminium, calcium, magnesium, zinc/35TT, total solid and carbon content increased after the digestion process. This observation is in line with what was earlier reported by [9, 15].The percentage total solids and volatile solids in this study are therefore within the ranges earlier reported for biogas production [29]. The amounts of methane to be produced depend on the quantity of volatile solids present in the waste and their digestibility. Higher ash content also corresponds with higher volatile solids content. Cattle dung has a higher potential for organic manure compared with MSPW because of its higher percentage ash content. The value changed obtained in conductivity shows that the substrate and digestate are good energy carrier. However, the high values of nitrogen, phosphorus and potassium in the digestate indicate that the end product will be good for fertilizer application.

Microbial Analysis Results

Since the substrate and digestate contains the methane producing bacteria called methanogens, hence, there is a need to carry out the microbial load count analyses.Fig. 4 shows aplot of ternary microbial load count obtained which account for 4.6×10^{-2}Cfu/ml substrate, 5.5 $\times 10^{-3}$Cfu/ml inoculum and6.3×10^{-2}Cfu/ml digestate. The increase in microbial load count for digestate during the digestion of substrate was due to growth of the microbes that aided the completion of the anaerobic reaction as well as biogas production during the digestion.

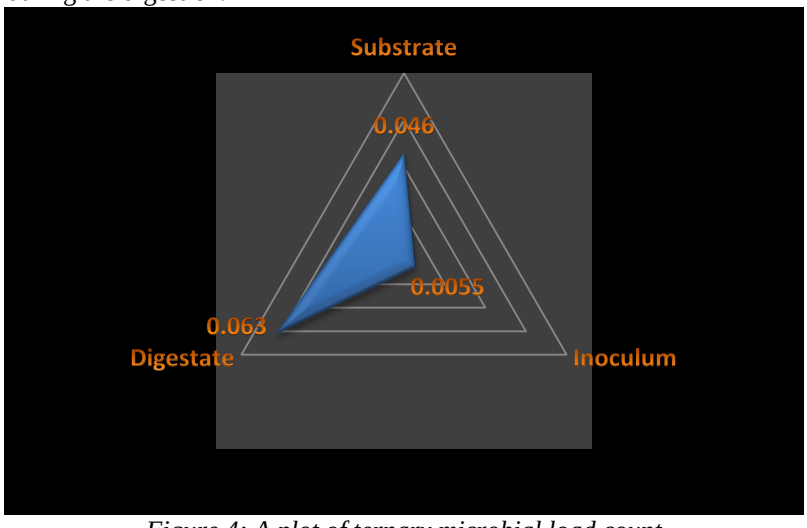

Figure 4: A plot of ternary microbial load count

Conclusion

Co-digestion of an alkali treated *Musa sapientum* plant waste (MSPW) and cattle dung for biogas production and its statistical analysis: a case of artificial neural network (ANN) and response surface methodology (RSM) was carried out and the following conclusions were drawn:

i. The maximum experimental yield of biogas obtained was $2.915\times 10^{-2}m^3$/day on the 19^{th} and 20^{th}day.
ii. The temperature of the slurry measured during the fermentation period was within the mesophilic temperature ranges (30 – 40 ℃).
iii. The pH of the slurry increased from 7.1 to 7.95 in the space of 4 weeks.
iv. Statistical analysis showed predicted biogas yield of $2.834 \times 10^{-2}m^3$/day on 30^{th}day. Using this predicted days, the experiment was validated twice and the optimum yield obtained was $2.82 \times 10^{-2}m^3$/day
v. The R^2 obtained was 63.81%, implies that three quarter of the variability is explained by regression model function.
vi. Proximate compositions of the slurry in the digester showed variation before and after anaerobic digestion.
vii. Microbial load count results showed 6.3×10^{-2}Cfu/ml digestate, 4.6×10^{-2}Cfu/ml of substrate and 5.5×10^{-3}Cfu/ml inoculum.
viii. The spent slurry of this process can improve organic manure for agricultural production.
ix. MSPW treated with alkali at 1% NaOH for 7 days mixed with cattle dung at a 1:1 w/w ratio, showed a double fold increase in biogas production.

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References

1. Meher LC, Vidya SD, Naik SN.Technical aspects of biodiesel production by transesterification-a review.Renew. Sustainable Energy Rev. 2004; 10: 248-268.
2. Amish PV, Subrahmanyam N, Payal AP. Production of Biodiesel through Transesterification of Jatropha oil using KNO_3/ Al_2O_3. Fuel.2009; 625-628.
3. Mushtaq A, Sofia R, Mir AK, Muhammad Z, Shazia S, and Sobia G, Optimization of base catalyzed transesterification of peanut oil biodiesel. Afr. J. Biotechnol. 2009; 8: 441-446.
4. Monnet P. An Introduction to Anaerobic Digestion of Organic Wastes.Final Report Thesis. Remade Scotland. 2003.
5. Checchi F, Traverso PG, Mata-Alvarez J, Clancy J, Zaror C, State-of-the-art of R&D in the anaerobic digestion process of municipal solid waste in Europe. Biomass Bioenergy.1988; 16: 257-284.
6. Gupta P, Singh SR, Vidyarthi A, Gupta A.A re-appraisal on intensification of biogas production. Renew. Sustainable Energy Rev. 2012;16:4908-4916.Doi:10.1016/j.rser.2012.05.005.
7. Kacprzak, Anna, Krzystek, Liliana, Ledakowicz, Stanislaw. Co-digestion of agricultural and industrial wastes. Chem. Papers.2010; 64: 127–131.
8. Go´mez X, Cuetos MJ, Cara J, Mora´n A, Garcı´a AI. Anaerobic co-digestion of primary sludge and the fruit and vegetable fraction of the municipal solid wastes: Conditions for mixing and evaluation of the organic loading rate. Renewable Energy.2006; 32: 17–24.
9. Owamah HI, Dahunsi SO, Oranusi US, Alfa MI. Fertilizer and sanitary quality of digestate bio-fertilizer from the co-digestion of food waste and human excreta. Waste Management.2014;34: 747-752.
10. Rasi S, Jenni L, Jukka R. Determination of silicon compound in biogas from wastewater treatments plants, landfills, and co-digestion plants. Renewable Energy.2010; 35: 2666-2673.
11. Shanmugam P, Horan NJ. Optimizing the biogas production from leather fleshing waste by co-digestion with MSW.Bioresource Technology.2002;100: 4117-4120.
12. Ighodaro OM. Evaluation study on Nigerian species of *Musa paradisiacal* Peels: Phytochemical screening, proximate analysis, Mineral Composition and Antimicrobial Activities. Sciencepub.net/researcher.2012; 4(8).
13. Dittrich ADK, Helden AJ. Experimental sward islets: the effect of dung and fertilization on Hemiptera and Araneae. Insect Conserv Diver.2012; 5:46-56.
14. Dar H, Tardon S. Biogas Production from Pretreated Wheat Straw, Lantana Residue, Apple and Peach Leaf Litter with Cattle Dung. Biological Waste.1987; 21.

15. Adepoju TF, Oni OO, Dahunsi SO. Optimization investigation of biogas potential of *Tithonia diversifolia* as an alternative energy source. *IJCPR.* 2015;*(In press).*
16. Fountoulakis MS, Drakopoulou S, Terzakis S, Georgakia E, Manios T. Potential for methane production from typical Mediterranean agro-industrial by-products. Biomass Bioenergy.2008; 32: 155-161.
17. Karim K, Hoffman R, Klasson T, Al-Dahhan MH. Anaerobic Digestion of Animal Waste; Effect of Mode Mixing.Water Resources.2005; 39.
18. Fulford D. Running a Biogas Programme: A Handbook. *The Biogas Technology in China.* 1988.
19. Nordberg A. Edstrom M. Co-digestion of Energy Crops and the Source-Sorted Organic Fraction of Municipal Solid Waste.Water Science Technology.2005; 52: 217–222.
20. El-Mashad H, Zeeman G, Loon W, Bot G, Lettinga G. Effect of Temperature Fluctuation on Thermophilic Anaerobic Digestion of Cattle Manure. Bioresource Technology. 2003;Pp: 95.
21. HarikishanS, Sung S. Caste Waste Treatment and Class A Biosolid Production Using Temperature-Phased Anaerobic Digester. Advanced Environmental Resources.2003; 7.
22. Yadvika A, Santosh A, Sreekrishan TR, Kohli S, Rana V. Enhancement of Biogas Production from Solid Substrates Using different Techniques. Bioresource Technology.2004; 95.Energy Information Administartion. http://www.eia.doe.gov/emeu/cabs/usa.html. November, 27, 2005.
23. Garba B. Effect of Temperature and Retention Period on Biogas Production from Lignocellulosic Materials. Renewable Energy.1996; 9: 634-641.
24. Vicenta M, Pacheco G, Alamis MLA, Anglo PG, Tan BV, Silverio CM. A Study of Some Factors Affecting Biogas Production from Pineapple Peelings. In: Bidin, R.., Chong, C. N., Wang, C. W., 1984. Proceedings of the second ASEAN Workshop on biogas production applied to the management and utilization of food waste materials. Kaula, Terengganu, Malaysia.189-202.
25. Ojolo SJ, Dinrifo RR, Adesuyi KB. Comparative study of Biogas Production from Five Substrates.Advanced Material Resource Journal. 2007;18:519-525.
26. Ahmadu TO, Folayan CO, Yawas DS. Comparative Performance of Cow Dung and Chicken Droppings for Biogas Production.Niger Journal.2009; 16:154-64.
27. Abubakar BSU, Ismail N. Anaerobic Digestion of Cow Dung for Biogas Production. ARPN JEAS. 2012; 7(2): 169-172.
28. Alfa IM, Dahunsi SO, Iorhemen OT, Okafor CC, Ajayi SA. Comparative Evaluation of Biogas Production from Poultry Droppings, Cow Dung and Lemon Grass.BioresourceTechnology.2014; 157: 270-277.
29. Ofoefule AU, Uzodinma EO, Anyanwu CN. Studies on the Effect of Anaerobic Digestion on the Microbial Flora of Animal Wastes: Digestion and Modelling of Process Parameters. Trends in Applied ScienceReserch.2010; 5: 39-47.