ISSN: 0067-2904

Production of Biogas from Mono- and Co-Digestion of Agricultural Waste (Cow Dung, Chicken Dropping, and Rice Husk)

Musa O. Arekemase, Isaac Aweda
Department of Microbiology, Faculty of Life Sciences, University of Ilorin, Kwara State, Nigeria

Received: 16/12/2020 Accepted: 15/3/2020

ABSTRACT

This study focused on waste to energy technology that utilized mono- and co-digestion of cow dung (CD), chicken dropping (ChD), and rice husk (RH). The fabricated digesters were assessed for the influence of temperature and pH on biogas production from the materials used. The total aerobic bacteria and fungi counts for the mono- and co-digestion of cow dung with chicken droppings had highest number on day zero ($1.5x10^7$cfu/ml, $1.6x10^5$cfu/ml and $1.4x10^8$cfu/ml, $1.2x10^5$, respectively), while the lowest counts were recorded on the 35^{th} day ($1.3x10^1$cfu/ml, $1.0x10^1$cfu/ml and $1.1x10^1$cfu/ml, $1.0x10^1$cfu/ml, respectively). The highest count of the acetogenic organisms was $1.8x10^5$cfu/ml on the 18^{th} day whereas no count was observed on the 35^{th} day. Methanogenic bacteria had a count ranging from $1.0x\ 10^1$ cfu/ml to $3.4x10^4$cfu/ml on the 18^{th} day. pH was within the range of 5.3 – 8.5 in the digesters. Cow dung (100% CD) showed the highest cumulative gas production of $41.65m^3$ compared with chicken droppings (100% ChD) and rice husk (100% RH) which showed values of 8.91 m^3 and 0 m^3, respectively, within temperature. Furthermore, the co-digestion of 75% CD + 25% ChD, 50% CD + 50% ChD, 25% CD + 75% ChD, 50% CD + 50% RH, and 50% ChD + 50% RH produced biogas values of $20.1m^3$, $15.13m^3$, $7.51m^3$, $5.1m^3$, and $2.09m^3$, respectively, at the same temperature range of 36.2^OC - 41.7^OC.

The assay for nitrogen (N), phosphate (P), potassium (K) and sulphate (S) to find the major plant nutrient from the digestate showed that 100% CD was richer in N (1.8mg/l), P (0.5mg/l), and S (0.5mg/l) than the other biomass types, whereas 50% CD 50% ChD had the highest content of P. The present study suggests that the digestion of cow dung, chicken droppings, and rice husk can be an effective means of waste management, pollution control, and generation of renewable energy (biogas) and fertilizers, thereby further strengthening the role of agriculture in the area of food security.

Keywords: Dungs, rice husk, methanogens, biogas, digestate

INTRODUCTION

The increased discharge of solid wastes and the associated environmental pollution and lack of access to adequate energy resources are some of the major challenges facing the human populace in Sub Saharan Africa [1, 2]. Mshandete and Parawira [3] reported that out of 21 Sub-Saharan African countries, less than 10% have access to energy. Therefore, there is a serious need to search for alternative and renewable energy sources from locally available resources in the quest for human survival and national development in Africa [4]. Besides, there is a need for the adoption of appropriate and economically feasible technologies for the effective management of solid and liquid wastes and energy recovery from them.

Energy is a basic tool for national and international development. Developing countries like Nigeria face added dilemmas regarding environmental protection due to their heavy dependency on

*Email: arekemase.com@gmail.com

biomass and fossil fuel. An earlier study [5] opined that Nigeria is endowed with huge resources of conventional energy resources (crude oil, tar sands, natural gas, and coal) as well as reasonable amount of renewable energy resources (e.g. hydro, solar, wind, and biomass). The global quest for environmentally friendly, ecologically balanced, and sustainable energy has been on the increase over the last few decades and this has forced the world to search for other alternate sources of energy [6]. However, the new alternative energy sources demand immense economic investment and technical power to operate, which makes it relatively difficult for a country like Nigeria. Currently, energy from biogas is a reliable, accessible and economically feasible source of alternative and renewable energy which can be generated using agricultural, domestic, and industrial materials by employing simple technology [7]. The prospect of this technology is bright because Nigeria is rich in fossil and other renewable fuels [3]. The technology can be utilized to provide energy for households, rural communities, farms, and industries [4].

Anaerobic digestion is a naturally occurring process of decay and decomposition, in which organic matter is broken down into its simpler chemicals components under anaerobic conditions. According to Monnet [8], anaerobic microorganisms digest the organic materials in oxygen deficient regions to produce methane and carbon dioxide as end products under ideal conditions. The biogas produced in anaerobic digestion plants usually contains small amounts of hydrogen sulphide (H_2S) and ammonia (NH_3), as well as trace amounts of other gases. Acid formers previously isolated from biogas digesters include species of *Escherichia, Citrobacter, Bacillus, Pseudomonas, Klebsiella, Clostridium, Bacteroides, Salmonella, Aspergillus, Mucor, Rhizopus,* and *Penicillium* while methane formers previously implicated include species of *Methanococcus* and *Methanosarcinae*. The science underlying anaerobic digestion can be complex. Thus the process is best understood if split into four main stages. The full process of anaerobic digestion occurs in the following four stages [8, 9].

- hydrolysis, in which complex molecules are broken down to constituent monomers;
- acidogenesis, in which acids are formed;
- acetogenesis, or the production of acetate, e.g. by *Acetomicrobium* sp.; and
- methanogenesis, the stage in which methane is produced from either acetate or hydrogen, e.g. by *Mathanosarcina* sp. and *Mathanospirillum* sp.

The aim of this research was to generate biogas and biofertilizers from mono- and co-digestion of cow dung, chicken droppings, and rice husk, using a consortium of microorganisms from cattle's rumen content, and to compare the substrates for maximum production of methane.

MATERIALS AND METHODS

Sterilization of materials

All the glassware was washed using disinfectants and sterilized according to the standard of APHA [10]. All media used were sterilized according to the manufacturer instructions.

Design of Pilot Scale Anaerobic Digesters

The design used for this investigation combines the Ajoy Karki's kitchen waste biogas model [11] and a gas holder system that is separate from the digester tank. The digester's shape was cylindrical in order to ensure adequate substrate mixing. The separate gas holder system was incorporated into this design to allow for ease of measurement of gas volume at atmospheric pressure. The theory behind the design is simply "downward delivery and upward displacement". The slurry on fermenting in the digester produces gas which is detected by the pressure gauge reading present on the digester, as shown in Figures- 1 and 2. The operating volume used in this work was 20 liters according to an earlier design [12]. The digester was designed to operate within the mesophilic temperature range (20-44^{o}C). This was achieved by natural heating from the sun. An absorptive surface was required for the digester; this was to absorb solar radiation during the day time. An insulating material was required for the digester at night in order to retain the heat within and keep temperature fluctuations within manageable limits.

The indirect approach method was used to measure the quantity of gas produced in the digestion tank. The ideal gas equation was uses to quantify the volume of the gas yield;

$$\frac{V_1P_1}{T_1} = \frac{V_2P_2}{T_2}$$

where

$V_{1=}$ V_O (operational volume)

P_1 = atmospheric pressure
T_1 = temperature at 9:00am
P_2 = pressure reading on the digester (pressure gauge)
T_2 = temperature at 3:00pm
V_2 = volume of gas yield

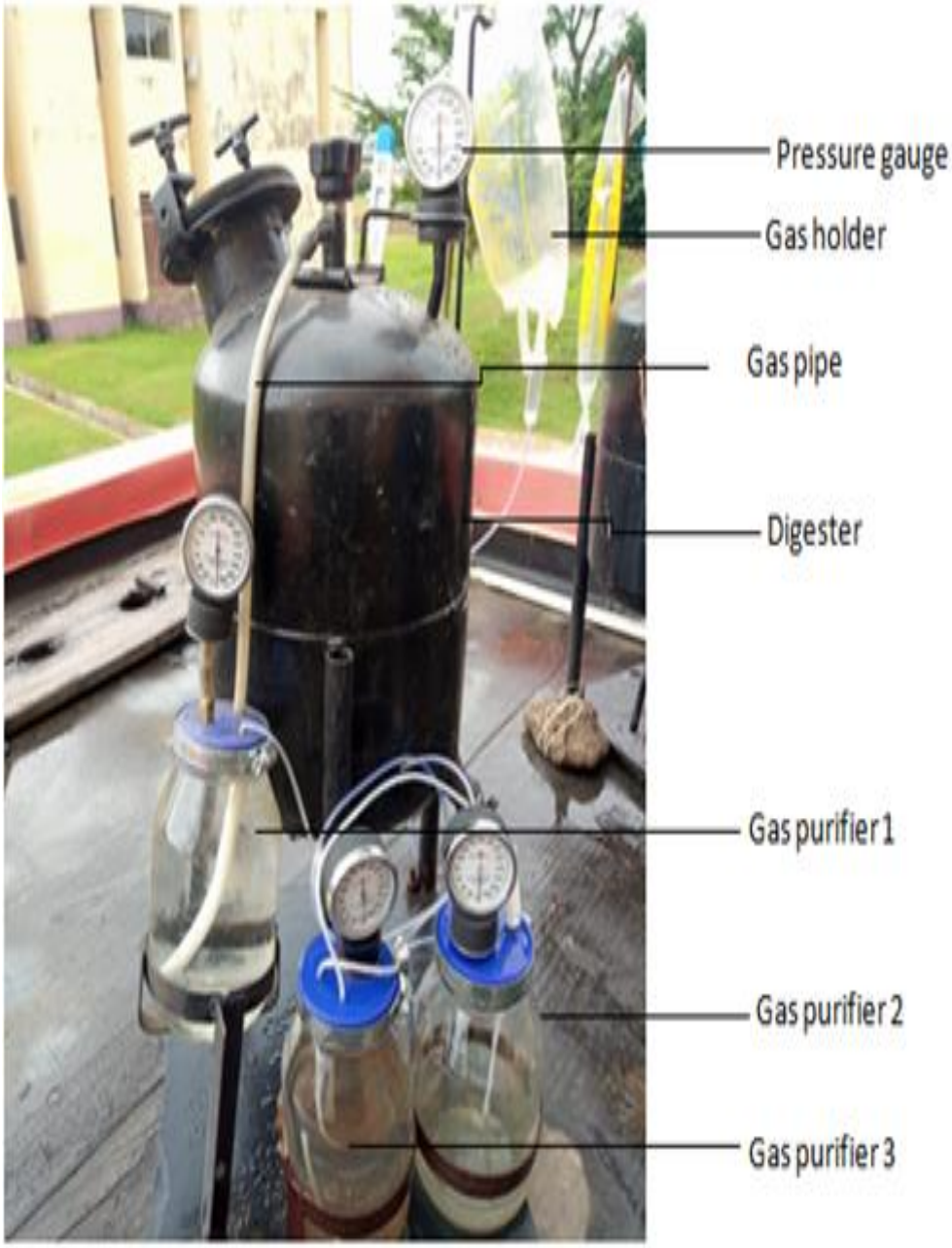

Figure 1-Anaerobic digester

Figure 2-Experimental set up

Collection of samples

Ten kg of each of the substrates (cow dung, chicken droppings, and rice husk) were collected at Ilorin abattoir, Hope farm Jebba, and Jebba rice mill, respectively, located in Kwara state, Nigeria. Substrates were collected from this source due to the availability and accessibility of large volume of agro-industrial waste generated by a number of farmers in this region.

Anaerobic digestion of substrates

Sets of fabricated digesters (labeled 1-8) were used for digestion. Each digester contained organic waste, with co-digestion at different percentage ratios of 25%, 50%, 75%, and 100% which was equivalent to 0.75kg, 1.5kg, 2.25k, and 3kg, respectively. Eight digesters (D1 to D8) contained, respectively, 100% cow dung (CD), 100% chicken droppings (ChD), 50% CD 50% ChD, 25% CD 75% ChD, 75% CD 25% ChD , 100% RH, 50% CD 50% RH, and 50% ChD 50% RH. Different percentage ratios of the substrates selected as mentioned above were mixed with water to form slurry in the ratio of 1:1 by volume, which was separately introduced into each digester through an inlet pipe at the top of the digester tank. The slurry was allowed to occupy three quarters of the digester space leaving a clear height which served as space for gas production. The inflow was directed downward to cause the solids to accumulate at the bottom of the tank so that, after digestion, they can be easily removed. The gas was collected from the digester through a flexible hose connected from the digester to the bottom of the gas collection system. The collected gas was allowed to pass through water, iron filings, and slaked lime, respectively, as scrubbers.

Technical Evaluation of the Anaerobic Digestion Process

The digesters were monitored for 35 days retention time in the following areas: microbial succession assessment, measurement of gas production, effect of operating parameter (temperature and pH) on biogas production, analysis of biogas constituents, and analysis for total nitrogen, phosphorous, potassium and sulphate in the digestate, as well as to ascertain it suitability as organic fertilizer.

Microbial succession assessment

The succession studies of the diversity of microorganisms at each stage of fermentation were carried out by periodically isolating and identifying organisms from all the digesters at an interval of five days from day zero day to day 35.

Aerobic total bacteria count

The microorganisms at each stage of fermentation were periodically isolated and identified from day zero to the 35^{th} day. Total Aerobic Plate Count (TAPC) enumeration in the fermenting materials was carried out according to the method of APHA [10].

Cellulose Degrading Bacterial Population

Nutrient agar enriched with yeast extract, Congo red indicator, and carboxyl methyl cellulose as substrate for cellulose degradation, was prepared according to specification of Ahmadu[12] and inoculated with the sample from the anaerobic digester using pour plate technique.

Acetate producing bacterial populations

This test was carried out to determine species involved in the convection of volatile acids produced by acidogens to acetate (substrate) that can be used for methanogens. Acetogenic bacteria were isolated through the use of nutrient agar, based on a modified method of Manimegalai *et al*. (2014). Briefly, sample collected from the anaerobic digestion tank was serially diluted in sterile physiological saline (0.1% w/v bacteriological peptone, 0.85% w/v NaCl), homogenized, and plated on the sterile compounded medium. The plates were inoculated in duplicates and incubated for 48 h at 37^{o}C in an anaerobic jar.

Methanogenic bacterial populations

For methanogens, the enriched mineral medium for methanogenic bacteria evaluation described by Manimegalai *et al*. (2014) was compounded and used in this study using the serial dilution method.

Molecular characterization and identification of bacterial isolates

Molecular analysis based on 16sRNA PCR technique was performed at the Bioscience department, International institute of tropical agriculture (IITA), Ibadan, Nigeria for further identification at genomic level .

Fungi counts

Potato dextrose agar (PDA) was use to enumerate the Total Fungi Count (TFC) in the fermenting materials according to the method of APHA for each fermentation stage (10).

Daily Monitoring of Operational Parameters

In order to determine the most feasible local environmental conditions to optimally operate the biogas facilities, various operational parameters were periodically evaluated in order to assess the stability of the digesters.

Temperature

Temperature provides the kinetic energy of atoms or molecules. It was measured to determine the feedstock influence on temperature and consequently, the metabolism of the bacteria. Thermometers were used to measure the temperatures of the digesters. The digester temperatures were taken twice daily, i.e. 09:00hrs and 16:00 h. Black polythene nylons were used to cover the digesters at night so as to eliminate the possibilities of heat loss.

pH

pH was measured to determine the feedstock influence on the acidity/alkalinity and, consequently, the metabolism of the bacteria. Samples were analyzed at ambient temperature with a pH meter. The meter was calibrated twice per a week and the analyses were carried out immediately after sampling to avoid loss of carbon dioxide from the sample.

Analysis of the digestate for nitrogen, phosphorus, potassium and sulfate

The digestates were analyzed for their proximate composition according to the standard methods in order to ascertain the sludge suitability as biofertilizer. Biofertilizer parameters were evaluated in the soil science research laboratory of the University of Ilorin, Nigeria. In all samples, estimations of total

nitrogen, phosphorus, sulphates, potassium, and magnesium were conducted using the Pallintest Advanced Digital Readout Photometer (Model 7500 PHOT.1.1.AUTO.75, Camlad, Cambridge, United Kingdom).

Gas Analysis

Methane (CH_4) and carbon dioxide (CO_2) content of biogas were determined by Gas analyzer [13].

RESULTS

Total Aerobic, Anaerobic, Methanogenic Bacteria, and Fungal Populations

The results of microbial evaluation and succession in the anaerobic digestion of 100% cow dung are presented in Table-1. The bacterial population was fluctuated over time in the bioreactor vessel, with the highest mean total aerobic plate count (TAPC) recorded at day zero (1.5×10^8 cfu/ml), while the lowest count was 1.1×10^2cfu/ml at the end of day 35. Acidogenic bacteria count was the highest (3.8×10^3 cfu/ml) on day zero, while the lowest count (1.2×10^1 cfu/ml) was observed on day 35, with no growth recorded for days 24 and 30. The highest total plate count (TPC) of acetogenic bacterial was 1.8×10^5cfu/ml on day 18. The highest total fungal count (TFC) of 1.5×10^5 cfu/ml was recorded on day zero, while the value was reduced steadily till day 35 (1.0×10^1 cfu/ml). The highest TPC of methanogenic bacterial was 3.4×10^4 cfu/ml on day 18.

According to the results of the mono-digestion poultry droppings (100% ChD) presented in Table-2, the total aerobic bacterial counts were within the range of 1.5×10^7cfu/ml to 1.3×10^1cfu/ml. The highest TPC of acidogenic bacterial was 9.6×10^4cfu/ml on day zero. No growth for the acetogenic bacteria was recorded for days 0 and 35. The highest TPC of acetogenic bacteria was 3.4×10^4 cfu/ml on day 18, while the highest methanogenic TPC was recorded on day 18 (1.7×10^4 cfu/ml). The highest TFC was 1.3×10^4 cfu/ml on day zero.

Table 1-Microbial succession in the anaerobic digestion of 100% cow dung

Retention Time (Day)	Bacterial Population				Fungi (CFU/ml)
	Total bacterial (CFU/ml)	Acidogenic Bacterial (CFU/ml)	Acetogenic Bacterial (CFU/ml)	Methanogenic Bacteria (CFU/ml)	
0	$1.5x10^8$	$3.8 x10^3$	0	$1.0 x10^1$	$1.5 x10^5$
6	$1.7 x10^5$	$3.9 x10^2$	$1.1 x10^2$	$1.2 x10^1$	$2.6 x10^4$
12	$2.5 x10^3$	$4.5 x10^1$	$1.5 x10^3$	$1.5 x10^3$	$1.1 x10^3$
18	$3.5 x10^2$	$2.0 x10^1$	$1.8 x10^5$	$3.4 x10^4$	$4.8 x10^2$
24	$2.3 x10^2$	0	$1.7 x10^4$	$1.5 x10^3$	$4.1 x10^2$
30	$1.8 x10^2$	0	$1.0 x10^2$	$1.3 x10^3$	$3.1 x10^2$
35	$1.1 x10^1$	$1.2 x10^1$	$2.1 x10^1$	$1.5 x10^2$	$1.0 x10^1$

Table 2-Microbial succession in the anaerobic digestion of 100% chicken dropping (ChD)

Retention Time (Day)	Bacterial Population				Fungi (CFU/ml)
	Total bacterial (CFU/ml)	Acidogenic Bacterial (CFU/ml)	Acetogenic Bacterial (CFU/ml)	Methanogenic Bacteria (CFU/ml)	
0	1.5×10^7	9.6×10^4	0	0	1.3×10^4
5	1.3×10^5	8.0×10^2	1.1×10^1	1.2×10^1	1.8×10^3
10	1.2×10^2	7.7×10^2	1.5×10^2	1.9×10^1	7.0×10^2
15	1.1×10^2	4.0×10^2	1.0×10^3	1.7×10^4	3.4×10^2
20	2.3×10^1	3.6×10^2	1.0×10^1	1.6×10^3	2.8×10^2
25	1.8×10^1	3.0×10^1	1.0×10^1	1.3×10^2	1.4×10^2
30	1.3×10^1	2.3×10^1	0	1.7×10^1	1.1×10^1

The microbial succession pattern in the co-digestion is presented in Tables-3 and 4. A similar trend was also observed within the tables similar for the mono-digestion. The populations of aerobic bacteria and fungi attained their highest number during the 1st week and gradually reduced by the 2nd week till the end of the 5th week. However, the populations of facultative anaerobes increased gradually till it reached highest count on the 3rd week, while those of methanogens experienced a steady increase and were later reduced towards the end of the experiments.

The results of co-digestion of cow dung (CD) 75% with chicken dropping (ChD) 25%, presented in Table-7 showed that the highest TAPC was recorded on day zero (1.4×10^8 cfu/ml), while the lowest count was 1.1×10^1cfu/ml at the end of day 35. The TPC for acetogenic bacterial was 1.2×10^3cfu/ml on day 18. The highest TFC was 1.2×10^5 cfu/ml on day zero, while the value was reduced steadily till day 35 (0 cfu/ml). The highest TPC of methanogens was 1.7×10^3 cfu/ml.

The results of co-digestion of 50% cow dung (CD) with 50% chicken dropping (ChD) are presented in Table-4. TAPC was within the range of 2.5×10^7cfu/ml to 1.1×10^1cfu/ml. The highest TPC of acidogenic bacterial was 1.5×10^2cfu/ml on day zero. No growth was recorded for day 35. The highest TPC of acetogenic bacteria was 1.3×10^3 cfu/ml on day 18 and the highest methanogenic TPC was recorded on the same day (1.7×10^4 cfu/ml). The highest TFC was 2.1×10^4 cfu/ml on day zero.

Table 3-Microbial succession in the anaerobic co-digestion of 75% cow dung (CD) with 25% chicken dropping (ChD)

Retention Time (Day)	Bacterial Population				Fungi (CFU/ml)
	Total bacterial (CFU/ml)	Acidogenic Bacterial (CFU/ml)	Acetogenic Bacterial (CFU/ml)	Methanogenic Bacteria (CFU/ml)	
0	1.4×10^8	4.5×10^3	1.1×10^1	1.2×10^1	1.2×10^5
5	1.3×10^5	2.0×10^2	1.3×10^1	1.7×10^2	2.1×10^3
10	2.8×10^3	1.7×10^2	1.4×10^2	1.9×10^2	1.0×10^2
15	3.9×10^2	5.0×10^1	1.2×10^3	1.7×10^3	1.6×10^1
20	1.8×10^1	3.6×10^1	1.7×10^2	1.5×10^3	1.2×10^1
25	1.5×10^1	1.2×10^1	1.0×10^1	1.5×10^2	1.0×10^1
30	1.1×10^1	0	0	1.5×10^2	0

Table 4-Microbial succession in the anaerobic co-digestion of 50% cow dung (CD) with 50% chicken dropping (ChD)

Retention Time (Day)	Bacterial Population				Fungi (CFU/ml)
	Total bacterial (CFU/ml)	Acidogenic Bacterial (CFU/ml)	Acetogenic Bacterial (CFU/ml)	Methanogenic Bacteria (CFU/ml)	
0	2.5×10^7	1.5×10^2	1.6×10^1	1.2×10^1	2.1×10^4
5	1.5×10^4	1.4×10^2	1.2×10^1	1.1×10^2	1.1×10^4
10	4.0×10^2	1.9×10^1	1.0×10^2	1.0×10^3	1.0×10^3
15	1.1×10^2	1.5×10^1	1.3×10^3	1.7×10^4	1.2×10^2
20	1.8×10^1	1.0×10^1	1.8×10^2	1.6×10^4	1.9×10^1
25	1.3×10^1	0	1.4×10^1	1.5×10^2	1.3×10^1
30	1.1×10^1	0	0	1.6×10^1	1.1×10^1

Measurement of gas production for mono- and co-digestion of the substrate

At the end of 35-days retention period, the biogas production values for both mono- and co-digestion of the substrates were at the peak between the late 2nd and 3rd week, followed by a steady reduction in the biogas yield.

The results of mono-digestion of the cow dung (100% CD), chicken dropping (100% ChD), and rice husk (100% RH) are shown in Figure-3. 100% CD and 100% ChD produced more biogas compared with RH which was regarded as failed digester, because of its inability to produce tangible

biogas. 100% CD produced the highest daily and cumulative biogas values of 2.129m^3 and 42.848m^3, respectively.

Figures-3 and 4 show the daily biogas production and cumulative values of the co-digestion of CD, ChD, and RH at different ratios. It was observed that the co-digestion of 75% CD 25% ChD produced the highest daily biogas, while the least was recorded with 50% ChD 50% RH. Also, 25% CD 75% ChD produced the lowest cumulative co-digestion of 4.5m^3 while 50% ChD 50% RH produced the lowest value of 3.913m^3, as shown in Figure-4.

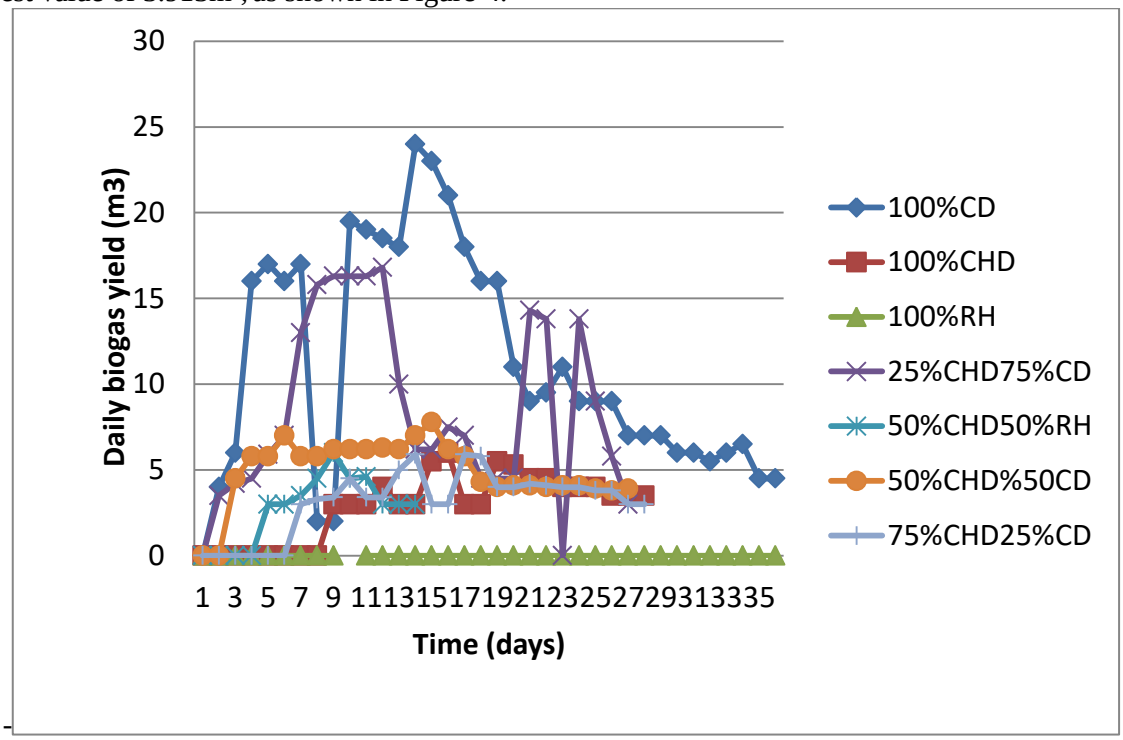

Figure 3-Daily biogas production of mono-digestion of cow dung (CD), chicken droppings (ChD), and rice husk (RH) at different percentage ratios

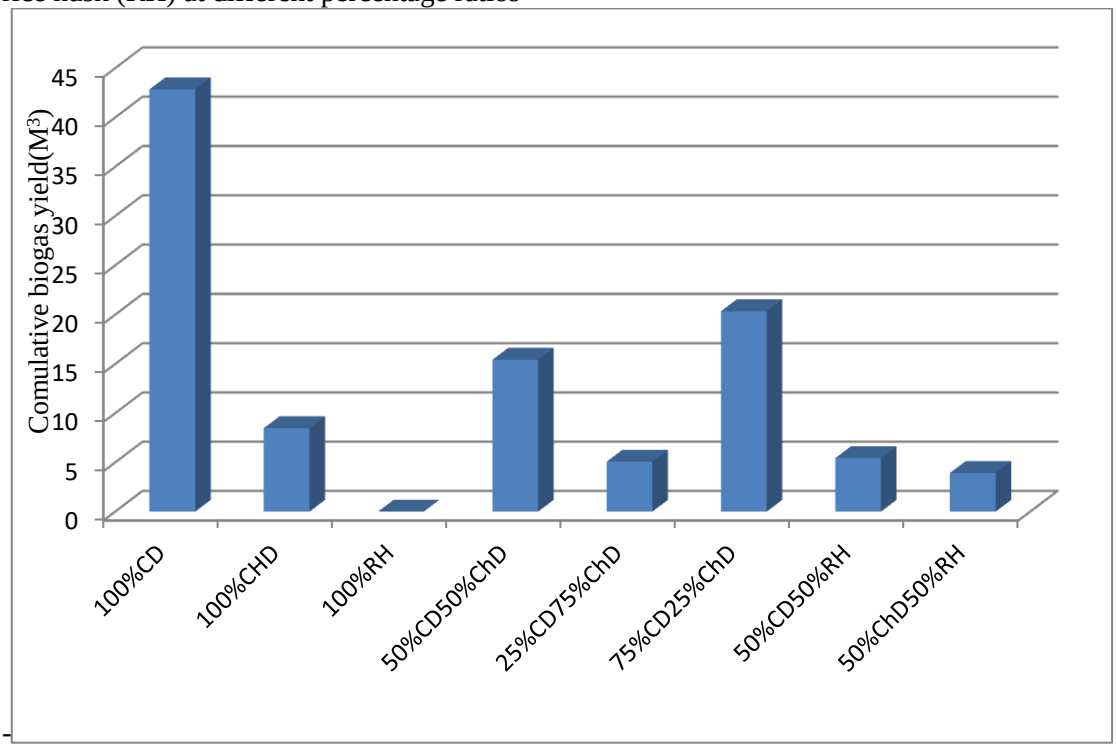

Figure 4-Cumulative biogas production of the different substrates

Daily Monitoring of Operational Parameters

Average temperatures of the digesters

The digesters average temperatures were taken twice daily (09:00hrs and 16:00hrs) during the investigation period. The average temperature within the respective digesters fluctuated optimally between 32.0°C and 43.7°C (Table-5), which is conformed to the mesophilic range required for the micro-organisms for the production of biogas. The peak of gas production was obtained at an average temperature of 40.1^{o}C.

Measurement of the pH of the digester

The pH readings of the mono- and co-digestion of the agricultural wastes were monitored twice in a week (Mondays and Thursdays) during the digestion period, as presented in Table-6. It was observed that the feed stocks showed a general increase in pH with a minimal fluctuation which was within the optimal limits of methanogenic bacteria (pH 6.5 - 8.2). However, there was a decrease in the pH of the 100% ChD as the investigation period increased.

Table 5-Average digester temperatures for the mono and co-digestion (°C)

Substrate	Temperature (°C)
100%CD	41.7±2[ab]
100%ChD	41.0±3 [a]
100%RH	40.0±1 [a]
50%CD50%ChD	41.0±2 [c]
50%CD50%RH	36.2±3 [b]
50%ChD50%RH	37.8±3 [ab]
75%CD25%ChD	38.0±2 [b]
25%CD75%ChD	35.6±3 [a]

[abc] Means with different superscripts in a row are significantly different (p<0.05). Values are means of three replicate determinations (±SD)

Table 6-Substrates pH during the Retention Period

Substrate Day	100%CD	100%ChD	100%RH	50%CD50%CHD	75%CD25%ChD	75%ChD%25CD	50%CD50%RH	50%ChD50%RH
0	7.5±0.21	6.8±0.11	7.4±0.12	6.9±0.37	6.9±0.16	6.3±0.18	6.3±0.51	6.9±0.76
4	7.9±0.11	6.0±0.18	7.0±0.51	6.4±0.23	6.5±0.81	7.4±0.21	6.1±0.53	6.9±0.50
8	8.0±0.51	5.3±0.26	6.1±0.23	6.0±0.18	7.2±0.73	8.3±0.52	6.5±0.1	7.1±0.21

12	8.2±0.32	5.9±0.36	6.0±0.52	6.7±0.15	7.5±0.52	8.1±0.14	7.5±0.51	7.3±0.23
16	8.0±0.51	6.1±0.59	5.8±0.51	6.7±0.21	8.2±0.31	7.2±0.37	7.9±0.12	7.0±0.21
20	7.8±0.23	6.0±0.23	6.2±0.41	6.4±0.41	8.5±0.11	7.3±0.13	8.2±0.31	7.3±0.41
24	7.8±0.50	5.6±0.32	6.4±0.51	6.6±0.35	8.6±0.42	7.0±0.13	8.5±0.21	7.4±0.31
28	8.0±0.64	5.7±0.31	5.9±0.51	6.7±0.2	8.4±0.5	7.1±0.5	8.4±0.2	7.7±0.51
32	8.1±0.31	5.8±0.41	6.0±0.11	6.9±0.4 8.3±0.5 8.4±0.27		7.5±0.51	8.2±0.31	8.0±0.51
35	8.1±0.21	5.9±0.13	6.0±0.51	6.9±0.36	8.5±0.21	7.2±0.21	8.1±0.51	8.0±0.51

Values represented are means of triplicates ± standard deviation

Table 7-Molecular characterization of methanogenic bacterial Isolates

ISOLATES	ORGANISM	NUMBER OF BASES	IDENTITY (%)	ACCESSION NUMBER
M_1	*Methanobacterium soehngenii*	582	98	AY196685
M_3	*Methanosacina mazei*	547	97	DQ177344

Physiochemical Properties of the digestate \ after digestion

The quantity of each parameter determined during the experimental analysis was computed in terms of the mass of substrates used in the analysis. The values obtained for the substrates, i.e. nitrate, sulphate, phosphate, and potassium after digestions are shown in Figure-5.

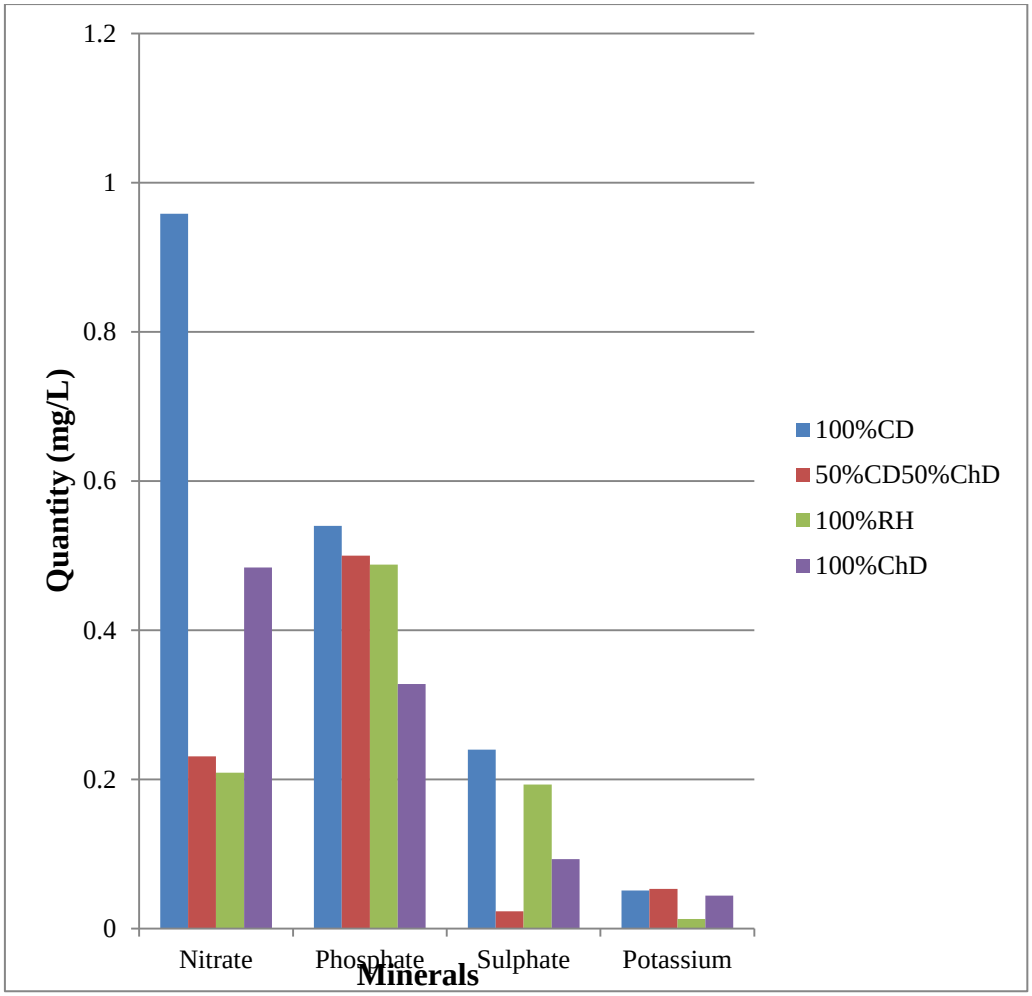

Figure 5- Mineral analysis of the digestate

Discussion

This investigation revealed the inherent ability and the dynamics of microbial communities involved in turning waste (cow dung, rice husk, and chicken droppings) to wealth (biogas) as well as the effects of environmental conditions (temperature and pH) required for optimal production. The work also evaluated the suitability of the digestates as organic fertilizers.

During the batch digestion period of 35 days, the total bacteria populations of aerobic, anaerobic, and methane producing bacteria were monitored. Tables-1 and 2 show a similar trend of an increased microbial population of the aerobes at the zero day, when the pH was at neutrality, followed by a steady reduction as the period of investigation increased, while the anaerobes and methanogens increased within the same period. This was revealed by the changes in population at different days of the period under investigation. These changes in the populations in the digesters might be due to fluctuations in environmental conditions of digestion (i.e. oxygen, temperature, and pH) and excessive volatile fatty acids that had accumulated in the reactor due to degradation. The observed change in microbial populations is in agreement with the study of Jain *et al*. [14] who reported that cellulose-degrading bacteria is reduced due to the release of soluble sugars and the decrease in pH of the reactor, which in turn inhibits both cellular activity and metabolite production. Anaerobic organisms (anaerobes and methanogens) are highly sensitive to extreme values of environmental factors such as pH and temperature, which favored their proliferation at the latter period of the study. Our results are in line with the reports of Mao *et al*. [15] and Mckennedy and Sherlock [16] that temperature and pH were important factors in anaerobiosis, because the various arrays of methanogenic bacteria and archaea responsible for the bioconversion of substrates are known to be efficient at specific temperatures and pH.

The results also showed that mono-digestion of CD (Table-4) had the highest microbial population and diversity as compared to the that of ChD and RH (Tables-(5 and 6) .

This might be attributed to the faster time taken for the bacteria to acclimatize in the digester. Also, the possible presence of sufficient bacterial populations and optimum level of carbon to nitrogen ratio of substrate in cow dung was confirmed by a previous work carried out by Ofoefule *et al*. [17]. These authors showed that cow dung, swine dung, and rabbit dung were better starters or blending wastes for the low-producing waste, such as plant waste, since they contained a diverse group of organisms which encourage quick anaerobic digestion. Rice husk had the least number of microbial numbers and diversity of facultative anaerobe and methanogen. This might be due to lignin content of rice husk, which required longer time and specific organisms to degrade. The mono digestion of cow dung indicated that aerobic bacteria and fungi had their highest populations ($1.5x10^8$cfu/ml and $1.5x10^5$cfu/ml, respectively) during the first week of retention time when the pH was neutral. However, the gradual reduction between days 0 – 8, when the temperature and pH were at optimum levels, might be a result of mechanical deficiency in the stirrer of the digestion, leading to uneven distribution of the bacteria at the time of sampling. Furthermore, oxygen level in the digester and the slightly acidic state of the fermenting material might have supported mainly fermenting aerobes and fungal proliferation. The facultative anaerobe and methanogens thrived most during the later part of the experiments, having their highest populations ($1.8x\ 10^5$ cfu/ml and $3.4x\ 10^4$ cfu/ml, respectively) on the 18th day. This might be due to favorable temperature (41.7°C) and very alkaline nature of the medium (pH 8.1) which led to efficient proliferation and increased activities of this group of microorganisms. The results of Ezeonu and Ezeonu [18] are in disagreement with this finding, since they reported that temperature increases the enzymatic activity of micro-organisms associated with the anaerobic digestion of horse dung.

Similar trends were observed in the co-digestion of the substrates (50% CD 50% ChD, 75% CD 25% ChD, 50% CD 50% RH, 50% ChD 50% RH, and 25% CD75% ChD). There were diverse and high populations of bacterial species, especially members of the genera *Clostridia* ($1.2x10^3$cfu/ml) in the co-digestion of 75% CD 25% ChD, which might have contributed to the pronounced methanogenesis stage. The anaerobic and methanogenic bacteria had minimal populations during the first week because they had not acclimatized to the optimal condition for growth. However, there was an increase in their population as the period of investigation increased.

At the end of 35-days of the investigation period, the biogas production rate was reduced in all the digesters. There was also a significant difference ($P < 0.05$) for all the biogas yield throughout the period of investigation. It was observed that digester with 100% CD (Figure-1) produced the highest cumulative biogas production potential ($42m^3$) followed by 100% ChD ($8m^3$), while 100% RH was regarded as a failed digester because of its inability to produce tangible biogas. This underscores the superiority of 100% CD as a better biogas producer over the waste at different percentages. 100% CD commenced production of gas on the 1st day ($0.4m^3$), while produced the highest volume of gas on the 16th day ($2.3m^3$). These fast rates of production are comparative with the results reported by Ofoefule that the anaerobic digestion of substrates was generally a function of time, whereas biogas production was highly dependent on microbial load. 100% ChD started production on the 7th day and yielded the highest volume of gas of $0.65m^3$ on the 19th day, while stopped production of gas on the 28th day. This might be as a result of the accumulation of ammonia in the digester. The higher biogas production was achieved by 100% CD, followed by 100% ChD and the least was by 100% RH. These results might also be attributed to the high content of carbon, hydrogen, nitrogen, sulfur, phosphorous, potassium, calcium, magnesium and a number of trace elements and the quick proliferation of methane producing bacteria. This result is in agreement with the findings of Ahmadu *et al*. [19] who reported that proliferation of mathanogenic bacteria might be attributed to the faster rate of decomposition of animal intestinal wastes which had undergone a form of digestion in the digestive track of the cows and the horse dung. Therefore, the actions of bacteria on this category of waste (cow dung and chicken dropping) are faster relative to that of the rice husk that had not undergone any form of decomposition, since it has not passed through any digestion during the time this experiment carried out. The works of Gupta et al. [20] and Igboro [18] are in agreement with this finding. They reported that the digestive system of the animal will influence the amount of organic matter in its faeces. In addition, the decrease in the levels of gas production and subsequent peak might be associated to microbial succession in the reactors.

In Figures-3 and 4, the daily and cumulative biogas production in cow dung (CD) and its co-digestion with chicken dropping (ChD) and rise husk (RH) are shown, at various percentage combination (50% CD 50% ChD, 75% CD 25% ChD, 50% CD 50% RH, 50% ChD 50% RH, 25% CD 75% ChD). 75% CD 25% ChD started production on the 2nd day, whereas 50% CD 50% RH started production on the fourth day (Figure-3). High rate of gas production was observed in the 75% CD 25% ChD as compared to RH and ChD. In Figure-4, it is clear that 75% CD 25% ChD produced the highest cumulative volume of biogas (20m^3), followed by 50% CH 50% ChD (15m^3), 25% CD 75% ChD (7m^3), 50% CD 50% RH (5m^3), and 50% ChD 50% RH (4m^3). There were significant differences ($P < 0.05$) among these values. This variation might be due to the nature of organic matters present in the substrates, which is also a function of the feeds the animals were exposed to, in the case of animal wastes, and the slow rate of decomposition of the rice husk. The decrease in levels of gas production and their subsequent peak might be associated to microbial succession in the reactors (Tables-2 and 3). There were faster rates of production relative to those reported by previous studies Ahmadu, [19]. He reported an optimal temperature of 40^{o}c and a very alkaline nature of the medium (pH 8.0) that favors the exponential growth phase of the methanogenic micro-organisms. The work of Mao *et al*. [15] also disagrees with this finding.

Temperature was found to be one of the major driving forces in biogas production in the digesters during the investigation. Biogas production was increased on the day the digester's temperature was relatively high and reduced with the decline in temperature. There was a significant difference ($P < 0.05$) in the operational temperature recorded for all the digesters, ranging 32.6 ^{o}C - 43.7 ^{o}C. The highest biogas production was obtained in the digester containing 100%CD (42m^3) and it had the highest temperature reading (41.7±2 ^{o}C) throughout the investigation period. This is an indication that high temperature promotes rapid biogas yield by enabling quick microbial acclimatization and short lag phase favored by the temperature.

The reduction in the pH of the digester 100% ChD from 6.0 to 5.6 on day 24 negatively affected the microbial population and consequently affected gas yield. The co-digestion of ChD with CD and RH generally demonstrated a slight reduction of pH. This was compactable with the microbial population recorded between these days and also in agreement with the reports of Tamaru *et al*. [21] that the reduction in certain groups of bacteria during anaerobic digestion might be due to the release of sugars and the reduction in pH, which was inhibitory to some microbial cells. Also, 100% CD maintained neutral to slightly alkaline pH (7.5-8.1) throughout the investigation period, which favored the gas yield in the digester. The measured high productivity might be due to the initial neutral pH that might have promoted hydrogenotrophic methanogenesis, during which CO_2 and H_2 are converted into CH_4 and H_2O. Cow dung, chicken droppings, and rise husk produced higher phosphates concentration, precipitation by calcium or magnesium. This might be the reason for the increase in phosphate measured after digestion. This might be attributed to high rate of activity, as microbial action indicated high biogas yield where calcium and magnesium are used by micro-organisms in their cellular metabolism.

As shown in Figure-5, there was an increase of plant usable nitrogen in all the substrates, with 100% CD having the peak value of 0.9584 mg/L, followed by 100% ChD (0.4840 mg/L), 50% CD 50% ChD (0.2310 mg/L) and 100% RH (0.2090 mg/L). There were also significant differences ($P < 0.05$) among the results. It is important that the proper amount of nitrogen is provided in the feedstock, to avoid either nutrient limitation (very low nitrogen) or ammonia toxicity (very high nitrogen). It was also observed that 50% CD and 50% ChD had the highest phosphate (0.5mg/L), while 100% ChD had the least (0.328mg/L). Potassium content in all digesters was generally low, with 50% CD and 50% ChD having the highest composition (0.053mg/L), whereas the lowest was 100% ChD (0.032mg/L). The differences in the organic fertilizer contents of the biomasses might be a result of the varying proportion of organic matter in the biomass undergoing anaerobic digestions, which had an important role on the growth rate of the anaerobic bacteria and the production of biogas. In this investigation, animal wastes were evaluated for their suitability as organic fertilizer at environmental temperature range (36^{o}C – 40 ^{o}C) with no form of physical treatment. Cattle manure was established to have lower available volatile solids, because ruminants extract much of the nutrients from the fodder and the leftover is rich in lignin complexes which are extensively exposed to enzyme action of the four chamber stomach of ruminants. Cestonaro *et al*. [22] work agrees with the findings in this work. They reported that the feeds with increased proportion of concentrate relative to the amount of forage result

in manure with increased digestibility, and therefore, biomass with higher nutrients and carbon level could be easily assimilated. Thus, lower amounts of concentrate in rice husk, when compared to those found in cow dung and poultry dropping in this investigation, might have contributed to the different levels of nutrient found in the organic fertilizers.

Conclusions

The mono- and co-digestion of the biomasses were found by this study to yield biogas/methane. The study also revealed the consortia of organisms involved in the anaerobic digestion of the biomasses. Temperature, pH, and retention time were found to affect bacterial activity and biogas production from cow dung, rice husk, and chicken droppings. The digestates obtained in this study contained nitrogen, phosphorus, potassium, and sulphate, which are essential plant nutrients. Thus, these digestates can be used as organic fertilizer. The present study utilized cow dung, rice husk, and chicken droppings for energy generation. Their abundance in several locations around the world is an indication that a veritable and environmental-friendly usage needs to be sought. Therefore, this study suggests that anaerobic digestion is considered as a viable option in safely reusing this major agro-industrial waste and transforming it to renewable energy (biogas) and organic fertilizers. This will contribute to the sectors of energy and waste management and further strengthen the role of agriculture in the area of food security.

References

1. Abadi, N., Gebrehiwot, K., Techane, A. and. Nerea, **2017**. Links between biogas technology adoption and health status of households in rural Tigray, Northern Ethiopia. *Energy Policy*, **101**: 284–292. Doi: 10.1016/j.enpol.2016.11.015
2. Roopnarain, A. and Adeleke, R. **2017**. Current status, hurdles and future prospects of biogas digestion technology in Africa. *Renewable and Sustainable Energy Reviews*, **67**: 1162–1179. DOI: 10.1016/j.rser.2016.09.087
3. Mshandete, A. M. and Parawira, W. **2009**. Biogas technology research in selected Sub Saharan Africa. *African Journal of Biotechnology*, **8**(2): 116-125. DOI:10.5897/AJB2009.000-9023
4. Giwa, A., Alabi, A., Yusuf, A. and Olukan, T. **2017**. A comprehensive review on biomass and solar energy for sustainable energy generation in Nigeria. *Renewable and Sustainable Energy Reviews*, **69**: 620–641. Doi:10.1016/j.rser.2016.11.160
5. Adaramola, M. S. and Oyewole, M. **2011**. Wind Speed Distribution and Characteristics in Nigeria. *ARPN Journal of Engineering and Applied Sciences*, **6**: 2-9. RePEc:eee: enepol: v:39 :y:2011:i:5
6. Su, H., Liu, L., Wang, S., Wang, Q., Jiang, Y., Hou, X. and Tan, T. **2015**. Semi continuous anaerobic digestion for biogas production: influence of ammonium acetate supplement and structure of the microbial community. *Biotechnology for Biofuels*, **8**(13):1-13. https://doi.org /10.1186/s13068-015-0197-z
7. Kwietniewska, E. and Tys, J. **2014**. Process characteristics, inhibition factors and methane yield of anaerobic digestion process, with particular focus on microalgal biomass fermentation. *Renewable and Sustainable Energy Reviews*, **34**: 491–500. Doi : 10.1016/j.rser.2014.03.041
8. Monnet, F. **2003**. An Introduction to Anaerobic Digestion of Organic Waste, as an alternative source of energy. *Journal of Environmental Management*, 88:881–889
9. Hati, K. M. and Marti, K. K. **2016**. Effect of inorganic fertilizer and farmyard manure on soil physical properties, root distribution, and water-use efficiency of soybean in Vertisols of central India. *Bioresource Technology*, **97**: 2182–2188. Doi:10.1016/j.biortech.2005.09.033
10. APHA, **2012**. Standard Methods for Examination of Water and Waste-water, 22nd ed. American Public Health Association, Washington DC.pp 12-34. https://trove.nla.gov.au/work/16646325
11. Karki, A. B., Shrestha, N. J. and Bajgain, S. **2005**. Biogas as Renewable Energy Source in Nepal. *Renewable and Sustainable Energy Reviews*, **48**: 182–198.
12. Ahmadu T. O. **2009**. Comparative Performance of Cow Dung and Chicken Droppings for Biogas Production, M.Sc Thesis Submitted to the Department of Mechanical Engineering, Ahmadu Bello University, Zaria, Nigeria,.
13. Dahunsi S. O. and Oranusi, U.S. **2013**. Co-digestion of food waste and human excreta for biogas production. *British Biotechnology Journal*, **3**(4): 485-499. DOI : 10.9734/BBJ/2013/4476

14. Jain, S., Jain, S., Wolf, I. T., Lee, J. and Tong, Y. W. **2015**. A comprehensive review on operating parameters and different pretreatment methodologies for anaerobic digestion of municipal solid waste. *Renewable and Sustainable Energy Reviews*, **52**: 142–154. RePEc:eee:rensus: v:52 :y: *2015*:i:c

15. Moa, X., Zhou, B., Gao, W., Qu, Y., Wang, Z. and Zhu, Y. **2015**. A recycling method for the production method of pure silica from rice hull. *Trends in Applied Sciences Research*, **3**(1): 39-47.

16. McKennedy, J. and O. Sherlock, **2015**. Anaerobic digestion of marine macroalgae. *Renewable and Sustainable Energy Reviews*, **52**: 1781–1790. Doi: 10.1016/j.rser.2015.07.101

17. Ofoefule, A. U., Uzodinma, E. O. and Anyanwa C. N. **2010**. Study on the effect of anaerobic digl flora of animal wastes. *Trends in Applied Sciences Research*, **5**(1): 39-47. DOI**:** 10.3923/tasr.2010.39.47

18. Igboro, S. B. **2011**. Production of Biogas and Compost from Cow Dung in Zaria, Nigeria, unpublished PhD Dissertation Presented to the Department of Water Resources and Environmental Engineering, Ahmadu Bello University, Zaria Nigeria 2011.

19. Ezeonu, C. S. and Ezeonu, N. C. **2016**. Alternative Sources of Petrochemicals from Readily Available Biomass and Agro-Products in Africa. *Journal of Petroleum and Environmental Biotechnology*, **7**(5): 12-23. D0I: 10.4172/2157-7463.1000301

20. Gupta, P., samant, K. and Sahu, A. **2012**. Isolation of cellulose-degrading bacteria determination of cellulolytic potential. *International Journal of Microbiology*, **216**: 41-49.http://dx.doi.org/ 10.1155/2012/578925

21. Tamaru, Y., Miyake, H., Kuroda, K., Ueda, M. and Doi, R. H. **2010**. Comparative genomics the mesophilic cellulose-producing *Clostridia cellulovorans* and it application to biofuel production via consolidated bioprocessing. *Environmental technology*, **31**: 889-903. https://doi.org /10.1080/09593330.2010.490856

22. Cestonaro, T., Monica, S., Luiz, A., Marcos, A., Dercio, C., Higor, E. and leocir, J. **2015**. The anaerobic digestion of sheep bedding and 80% cattle manure increases bigas production and improve biofertilizer quality. *Waste Management*, **2069**: 184-190. https://doi.org/ 10.1016 /j.wasman.2015.08.040