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Biogas production using poultry wastes, yam and plantain peels

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Abstract

Current global warming trends are primarily a result of the current energy reliance on fossil fuels and necessitate the provision of alternative sources of energy. Biofuels are one of the many alternatives to Hydrocarbon fuels. This research evaluates the potential of biogas production using poultry wastes, yam, and plantain peels in a ratio of 2:1:1. The organic waste materials were crushed using a sterile mortar and pestle. It was weighed and mixed with distilled water in a sterile bowl and loaded in a bioreactor for 45 days. The proximate analysis was carried out to determine the nutrient composition of the samples using standard procedures. Microbiological analysis was carried out using both general-purpose and differential media before and after loading the samples in the bioreactor. The pH and the temperature were monitored as well. A flame meter was used to compare the quality of the gas produced with that of cooking gas. The percentage moisture content obtained was 21.31, 28.71 and 23.17, % crude protein (6.17, 5.98 and 7.17), % ash content (7.9, 4.37, 12.13), % fat content (2.13, 1.14, 7.24), %crude fiber (19.94, 14.36, 36.89), % carbohydrate (42.53, 39.06 and 13.12) and the calories value of (213.09, 196.43 and 146.36) for plantain peels, yam peels and poultry waste respectively. Three fungal isolates and nine bacterial isolates were obtained. Produced biogas constituted of 310, 1352, and 2264 ppm of Liquified Petroleum Gas, Carbon (II) Oxide, and Smoke respectively. The biogas was observed to be highly flammable and burnt with a bluish flame.

Keywords: Biogas; Biofuels; Organic Wastes; Global Warming; Bacteria Isolate; Fungi Isolate

1. Introduction

Current global warming trends, primarily because of the current energy reliance on fossil fuels, necessitate the provision of alternative sources of energy [1, 2]. Biofuels are one of the many alternatives to Hydrocarbon fuels and can be converted into different forms of renewable energy by employing differing biochemical, thermochemical and physiochemical technology [2]. Biogas is constituted by a mixture of gases, primarily Carbon(iv)oxide (CO_2) and Methane (CH_4) and other gases in minute quantities such as Hydrogen (H_2), Carbon(ii)oxide (CO), Hydrogen Sulfide (H_2S), Ammonia (NH_3) and Nitrogen (N_2) [3].

While the disposal of agricultural waste on farmlands serves the purpose of supplying nutrients to the farmland, when deposited in large quantities, they result in environmental degradation, emission of greenhouse and other toxic gases [3]. The utilization of the Anaerobic Digestion (AD) method of biogas production employs the use of anaerobic organisms that breakdown these waste materials within an enclosed system resulting in the production of biogas [4]. AD offers an advantage as different types of organic substrate easily undergo AD, as against its production using other processes driven by biomass [5], making it one of the widely accepted forms of biogas production. AD also holds significant benefit for the environment as it facilitates reduction in waste materials [6].

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Biogas is primarily obtained from the anaerobic digestion (AD) of organic matter and waste products such as feces and certain industrial waste [2, 3, 5]. The production of biogas from anaerobic digestion of organic waste materials is one of the prominent forms of renewable energy with a significant potential to aid in addressing current energy challenges [2, 7]. Obtained biogas could be used in the production of electricity, as well as transportation fuels thereby facilitating environmental sustainability and addressing socio-economics issues around energy [2, 3].

The scale of energy insecurity has necessitated numerous efforts in the industry, however, it remains common knowledge that challenges; mostly related to efficiency and feasibility of emerging renewable technologies currently complicate the attainment of this key SDG agenda [8]. Production of biogas is usually from organic wastes and other biodegradable resources by different groups of bacteria in an anaerobic condition [9]. The use of different feedstock in bioreactor, such as agricultural wastes and plant residues, food and other domestic wastes, solid and liquid wastes from municipalities and industries for the production of biogas has been demonstrated Slorach *et al*. [10]. Hence, this research evaluates the potential of biogas production using poultry wastes, yam, and plantain peels.

2. Material and methods

2.1. Sample Collection

Yam peels were collected in a sterile polythene bag from the hostel in Federal Polytechnic Ado-Ekiti and then transported to the laboratory for analysis. Poultry waste was obtained from The Federal Polytechnic Ado-Ekiti poultry farm and placed in a polythene bag and transported to the laboratory for analysis. Plantain peels were collected in large quantities from the hostel in Federal Polytechnic Ado-Ekiti and placed in sterile bags and then transported to the laboratory for analysis.

2.2. Feedstock Collection and Preparation

The poultry waste obtained was weighed. 50 grams of the poultry waste was obtained and put in a sterile bowl mixed with distilled water. Yam peel was aseptically crushed in a sterile mortar and pestle. 25 grams were weighed and put in a sterile bowl mixed with distilled water. Plantain peel was aseptically pounded in a sterile mortar and pestle. 25 grams were weighed and put in a sterile bowl mixed with distilled water.

The yam peels and plantain peels grounded were put in a sterile bowl adding the poultry waste, was mixed with 1500mL of distilled water using a sterile turning stick in a ratio of 1:1:2. The mixture was agitated thoroughly and transferred into the bioreactor and tightly corked with a stopper to create an anaerobic condition.

2.3. Feedstock characterization

The proximate nutrient composition of the samples was determined using standard procedures of The Association of Official Analytical Chemists [11]. The moisture content, crude fibre, protein, fat, Ash and carbohydrates were estimated.

2.4. Preparation of medium and reagents

All the media and reagents used were prepared according to the manufacturer's specifications. The media was dissolved in an adequate amount of distilled water. The media was homogenized and autoclaved at 121^{o}C for 24hours.

2.5. Sampling of Bioreactor Content for Microbial Analysis

During the digestion period, samples from the bioreactor were collected using a sterile syringe. Bacteria and anaerobic fungi were screened. Aliquot of 1mL of the dilution 10^{-1}, 10^{-3} and 10^{-5} for bacteria and fungi respectively. The sample was incubated by pour plate method using Nutrient agar. Potato, Mannitol salt and Eosin methylene blue (EMB) also called Levine's formulation for Bacteria plates were incubated. *Staphylococcus aureus* was present in Mannitol Salt agar, *Escherichia coli* was present in Eosin methylene blue (EMB).

Using a sterile pipette, 1mL of each of the 10^{-2}, 10^{-4}, and 10^{-6} dilutions were used to inoculate the nutrient agar, Mannitol salt, EMB, Tryptone soy agar, and *Pseudomonas* cetrimide agar was then inoculated at 37°C and observed daily. Subculture was done on the previously prepared slant to get a fresh culture from the plates, then growth was observed in the incubated plate. The pure cultures were stored in the slant, and kept for Identification.

The pour plate method was used. One milliliter of the serially diluted sample were dispensed into a conical flask containing sterile potatoes dextrose agar (PDA) and two percent (2%) chloramphenicol to inhibit bacterial growth. The

contents were properly mixed and dispensed aseptically into sterile Petri-dishes; incubation was carried out in a non-inverted position at room temperature and observed after 72 hours. The colonies that developed were counted and sub-cultured repeatedly on PDA plates to obtain pure cultures. They were later stored on PDA slants for identification and characterization.

2.6. Characterization and identification of the isolates

The pure cultures of the isolates were identified based on their colony growth pattern, conidial morphology and pigmentation using the slide culture technique and microscopic examination. The bacterial isolates were identified by using morphological and biochemical tests such as coagulase test, catalase test, and sugar fermentation tests.

2.7. Microscopic examination

Lactophenol cotton blue solution was used; a drop of the solution was placed on a clean grease-free slide. A fragment of the mold was emulsified in the solution after which the slide was covered with a coverslip, avoiding bubbles. The slide was thereafter viewed under the microscope.

2.8. Biogas production and determination of biogas composition

After 45 days, the experiment was terminated. The quantity of biogas was determined. The composition of the biogas generated was compared with the normal cooking gas and conventional gas lighter.

3. Results

Physiochemical analysis of the properties of the bioreactor feedstock evaluated to determine the availability of digestible nutrients in the feedstock, accessible by the bacterial consortia in the course of anaerobic digestion and biogas production emanated in the following results presented in Table 1 and Figure 1.

Nine (9) bacterial isolates were identified, which include *Staphylococcus aureus*, *S. epidermis*, *Bacillus cereus*, *Yersinia* spp., *Bacillus brevis*, *Bacillus lentus* and *Bacillus licheniformis*, all gram positive. *Escherichia coli* and *Salmonella* spp. and were also gram negative. *Staphylococcus aureus*, *S. epidermis*, *Bacillus cereus* were catalase positive. *Bacillus cereus*, *Staphylococcus aureus*, and *Yersinia* spp. were coagulase positive. Table 2 below provides a summary of their biochemical properties.

Three fungi isolate, *Trichoderma* spp., *Fusarium* spp. and *Aspergillus niger*, were identified and are shown in Table 3. Cultures of *Trichoderma* spp. strain growing on potato dextrose agar shown in Figure 2 indicates that the white areas do not contain spores, while the green areas are covered with dense masses of spores (conidia). Cultures of *Fusarium* spp. hyphae (Figure 3), shows conidiophores occurring singly and in groups of multicellular crescent-shaped conidia. Black velvety Septate and broad hyphae, with large head entirely covered with chains of conidia hosts the *Aspergillus niger* (Figure 4).

The gas detector findings were applied on biogas, cooking gas, and gaslighter, comprising three contents Liquefied Petroleum Gas (LPG), Carbon monoxide (CO), and Smoke. Table 4 and Figure 5 shows the result obtain using the gas detector from liquefied petroleum gas, carbon monoxide, and smoke. The bioreactor ranges from 310ppm to 2264ppm, for Biogas, 1010 PPM to 8874 PPM for cooking gas and 1110240 PPM to 1123467 PPM for lighter gas. Liquified.

Table 1 Proximate Analysis of Feedstock Samples

	Moisture (%) (Dry wt)	**Crude protein (%)**	**Ash (%)**	**Fat (%)**	**Crude fiber (%)**	**CHO (%)**	**Calorie Value (KCAL)**
A	21.3121	6.1701	7.9122	2.1313	19.9434	42.5314	213.0852
B	28.7122	5.9814	4.3712	1.1415	14.3612	39.0634	196.4314
C	23.1722	7.1766	12.1315	7.2433	36.8912	13.1156	146.3585

Key: A = Plantain waste; B = Yam waste; C = Poultry waste

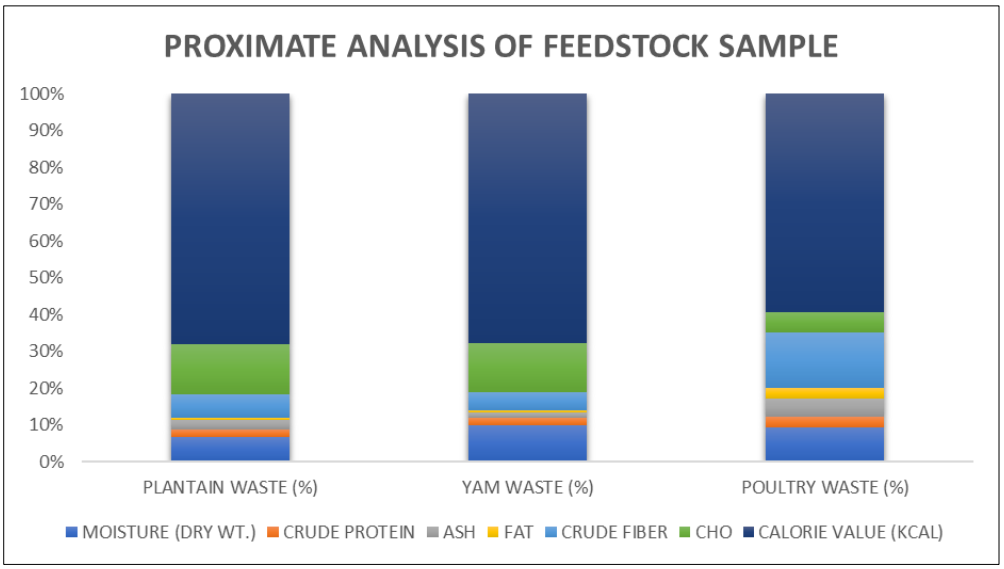

Figure 1 Proximate analysis of Feedstock Samples

Table 2 Biochemical characteristics of bacteria isolated from different poultry waste, yam peel, plantain peel

Isolate	Grams reaction	Catalase	Coagulase	Lactose	Glucose	Gas	Suspected organism
1	+	+	+	+	+	–	*Staphylococcus aureus*
2	+	+	–	+	+	+	*S. epidermidis*
3	+	+	–	–	+	+	*Bacillus cereus*
4	–	+	–	+	+	–	*Escherichia coli*
5	+	-	+	–	+	–	*Yersinia sp.*
6	–	–	–	–	+	–	*Salmonella sp.*
7	+	+	+	–	+	-	*Bacillus lentus*
8	+	+	-	ND	+	+	*Bacillus licheniformis*
9	+	+	–	–	+	+	*Bacillus brevis*

+ = Positive; – = Negative; ND = Not detected

Table 3 Characteristics and Identification of Fungi Isolates

Colour of hyphae	Macroscopic features	Probable organisms
Black velvety	Septate and broad hyphae, with large head entirely covered with chains of conidia	*Aspergillus niger*
Greenish velvety	Septate hyphae, with conidiophores developing into branched phalides bearing chains with brush-like appearance	*Trichoderma sp.*
White	Narrow septate hyphae, conidiophores occur singly and in groups, multicellular crescent shaped conidia	*Fusarium sp.*

Figure 2 Cultures of *Trichoderma sp*

Figure 3 Cultures of *Fusarium sp*

Figure 4 *Aspergillus niger*

Table 4 Obtained Gas Composition for Biogas, Cooking Gas and Lighter Gas

	Liquified Petroleum gas (LPG) (ppm)	Carbon(II)Oxide (CO) (ppm)	Smoke (ppm)
Biogas	310.00	1353.00	2264.00
Cooking Gas	1010.00	116118.00	8874.00
Gas Lighter	1110240.00	2346781.00	1123467.00

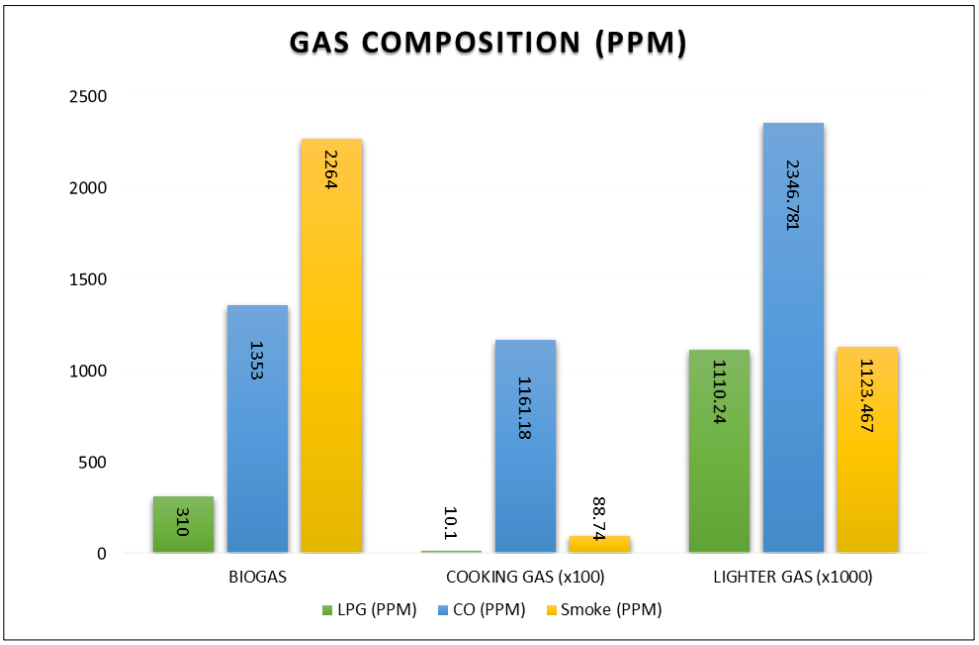

Figure 5 Obtained Gas Composition for Biogas, Cooking Gas and Lighter Gas

Table 5 Absorbance at 490 nm with different concentration of working standard of glucose solution

Table no.	Blank	1	2	3	4	5
Glucose sol. (in ml)	0	0.2	0.4	0.6	0.8	1
Distil water (in ml)	1	0.8	0.6	0.4	0.2	0
5% phenol sol. (in ml)	1	1	1	1	1	1
96% sulphuric acid sol. (in ml)	5	5	5	5	5	5
Table no.	Blank	1	2	3	4	5
Glucose sol. (in ml)	0	0.2	0.4	0.6	0.8	1
Distil water (in ml)	1	0.8	0.6	0.4	0.2	0
OD (490nm)						

4. Discussion

The above results obtained for moisture content crude protein and ash content are comparable to the values reported by Deressa *et al*. [12]. The crude fiber of wastes in each digester ranged from 14.3612% in the yam waste, to 19.9434% in the plantain waste to 36.8912% in the poultry waste which conforms with the optimum crude fiber range for biogas production [13], with a degradation process initiated with the first 1-15 days of set-up.

The Biogas production process was affected by temperature, pH, volatile and total solids [14], all of which were closely monitored to ensure the effective quality and quantity of the produced biogas. Recorded pH levels within the digester which rose from 6.21 on day one to 7.11, and subsequently 7.5 on the seventh day of fermentation indicate that microorganisms in the anaerobic digesters were not affected by the pH of the slurry in the digester, therefore no inhibition of biogas production was caused by the effect of pH [15]. The temperature in the digester ranged from 26 – 32^{o}C which happens to be in the range of mesophilic 25 – 45^{o}C which is allowed for the production of biogas [16]. This is in agreement with the findings of previous researchers, which holds that at the initial stages of the overall process of biogas production, acid-forming bacteria produce Volatile Fatty Acids (VFA) resulting in declining pH and diminishing growth of methanogenic bacteria and fungi [14, 17].

The experimental setup for Biogas was held for 45 days, of which at the end, the gas collection tube was filled. In the first 10days, there was no gas produced in the digester, most likely because of the lag phase of the inoculums which often results from changes in the environment or richness of the medium. During this period, the inoculums are trying to adapt to their new environment or probably due to the methanogens undergoing a metamorphic growth process by consuming methane precursors produced from the initial activity. After 12 days yielding of gas started. After 25 days yielding of gas started forming at a high rate, the production of gas was at a peak level between the 35th to 45th days. The duration of biogas production process aligns with studies by Deublein and Steinhauser [18]. The amount of biogas produced as a result of anaerobic fermentation of the substrate, measured by the volume of water displaced from each bio-digester is shown in Table 4. The difference in waste feedstock utilized, size of digesters, volumes of slurry and gas collection apparatus accounts for the difference in gas volume yields from this study which is relatively lower than the 2500 cm^3 of biogas generated from the anaerobic digestion of the contents of sheep colon reported by Wahyudi *et al*. [19] and obtained from the sole use of Poultry waste by Bagudo *et al*. [20] at a volume of 8772 cm^3.

Results from this work showed that biogas was produced at different retention times. After the first week, there was a low volume of biogas, and in the second week, the increase in the volume of biogas produced was not that much. However, from the third to the fourth week the volume of biogas produced increased rapidly. Thus, it can be deduced that the increase in the fourth week indicated the acclimatization of the biogas producing microorganisms after the hydrolysis of the waste in the first week by the hydrolyzing organisms during the third and fourth week. This conformed to the findings of Bagudo *et al*. [20] in which 8772.50 cm^3 of biogas was produced from poultry waste. Wahyudi *et al*. [19] also reported the production of 2500 ml of biogas from the content of poultry waste at four weeks' retention time.

The *Bacillus* species overlap from one stage to another during biogas production, suggesting a succession in species of bacteria during the process of gas production. But some species such as Bacillus were found to be present throughout the process of gas production [21]. The result obtained from this study indicates that Bacillus species were the most common bacteria isolated and identified during the research, suggesting that the species plays a vital role in the microbial activities for the production of biogas. It should be noted that *Bacillus cereus, and Bacillus licheniformis* were isolated. However, *Staphylococcus epidermidis, Staphylococcus aureus*, and *Bacillus brevis* were isolated. The ability of *Bacillus* species to overlap during the production was probably due to the fact the organisms can produce spores which helps them to withstand the harsh anaerobic condition or heat evolve during the biogas production [21]. These findings are in line with that of Oluyega *et al*. [22] in which Bacillus and Yersinia species were found to be responsible for biogas production.

The analysis of three parameters – Liquefied Petroleum Gas (LPG), Carbon (II) Oxide and Smoke obtained from the produced biogas we compared to those of cooking gas and gas lighter as shown in Table 4 and Figure 5. The produced Biogas had the lowest LPG because it is pure and it is from natural waste and cooking gas follows after LPG and gas lighter have the highest LPG because it is not derived from natural gas but is derived from natural gas processing. LPG is better than natural gas because it has higher energy content, it is portable and available everywhere, and in many cases, it is now less expensive. Recorded Carbon (II) Oxide (CO) contained in the Biogas was the lowest, compared to its significantly higher amounts of CO carbon concentration. The Smoke content was also recorded to be significantly lower for Biogas relative to cooking gas and lighter gas. Produced biogas was tested and it was confirmed that the biogas was combustible. This was tested by connecting a hose to the cylinder and to the biogas burner and lighting the burner. The produced gas burnt with bluish flame.

5. Conclusion

Production of biogas from wastes is a major step towards harnessing one of the world's most prevalent, yet least utilized renewable energy. Agricultural waste can be utilized in the production of biogas which can serve as a source of energy. By facilitating access to raw materials, the production of biogas yield could be significantly improved alongside the utilization of pre-treatment of feedstock.

Compliance with ethical standards

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Disclosure of conflict of interest

There is no conflict of interest.

References

[1] Litvinenko V. The role of hydrocarbons in the global energy agenda: The focus on liquefied natural gas. Resources. 2020; 9(5): 59.

[2] Tabatabaei M, Aghbashlo M, Valijanian E, Panahi HKS, Nizami AS, Ghanavati H, Sulaiman A, Mirmohamadsadeghi S, Karimi K. A comprehensive review on recent biological innovations to improve biogas production, part 1: upstream strategies. Renewable Energy. 2020; 146: 1204-20.

[3] Onthong U, Juntarachat N. Evaluation of biogas production potential from raw and processed agricultural wastes. Energy Procedia. 2017; 138: 205-210.

[4] Van Lier JB, Tilche A, Ahring BK, Macarie H, Moletta R, Dohanyos M, Hulshoff Pol LW, Lens P, Verstraete W. New perspectives in anaerobic digestion. Water Science and Technology. 2001; 43(1): 1-18.

[5] Montgomery LF, Bochmann G. Pretreatment of feedstock for enhanced biogas production. Ireland: IEA Bioenergy. 2014; 1-20.

[6] Mustafa MY, Calay RK, Román E. Biogas from organic waste-a case study. Procedia Engineering. 2016; 146: 310-317.

[7] Aghbashlo M, Tabatabaei M, Soltanian S, Ghanavati H, Dadak A. Comprehensive exergoeconomic analysis of a municipal solid waste digestion plant equipped with a biogas genset. Waste management. 2019; 87: 485-498.

[8] Awosusi A, Sethunya V, Matambo T. Synergistic effect of anaerobic co-digestion of South African food waste with cow manure: Role of low density-polyethylene in process modulation. Materials Today: Proceedings. 2021; 38: 793-803.

[9] Chuichulcherm S, Kasichan N, Srinophakun P, Saisriyoot M, Thanapimmetha A. The use of ozone in a continuous cyclical swing mode regeneration of FeEDTA for a clean biogas process from a swine farm waste. J. Cleaner Prod. 2017; 142(3): 1267–73.

[10] Slorach PC, Jeswani HK, Cuéllar-Franca R, Azapagic A. Environmental sustainability of anaerobic digestion of household food waste. J. Environ. Manage. 2019; 236: 798–814.

[11] AOAC International. Official methods of analysis of the Association of Official Analytical Chemists. Association of official analytical chemists; 1970.

[12] Deressa L, Libsu S, Chavan RB, Manaye D, Dabassa A. Production of biogas from fruit and vegetable wastes mixed with different wastes. Environment and Ecology Research. 2015; 3(3): 65-71.

[13] Chua KH, Yip CH, Nie WLS. A case study on the anaerobic treatment of food waste and gas formation. In International Conference on the Construction and Building Technology (ICCBT). 2008; 29: 311-316.

[14] Adekunle KF, Okolie JA. A review of biochemical process of anaerobic digestion. Advances in Bioscience and Biotechnology. 2015; 6(03): 205.

[15] Ali S, Hua B, Huang JJ, Droste RL, Zhou Q, Zhao W, Chen L. Effect of different initial low pH conditions on biogas production, composition, and shift in the aceticlastic methanogenic population. Bioresource technology. 2019; 289: 121579.

[16] Westerholm M, Müller B, Isaksson S, Schnürer A. Trace element and temperature effects on microbial communities and links to biogas digester performance at high ammonia levels. Biotechnology for biofuels. 2015; 8(1): 1-19.

[17] Lu J, Gavala HN, Skiadas IV, Mladenovska Z, Ahring BK. Improving anaerobic sewage sludge digestion by implementation of a hyper-thermophilic prehydrolysis step. Journal of Environmental Management. 2008; 88(4): 881-889.

[18] Deublein D, Steinhauser A. Biogas from waste and renewable resources: an introduction. John Wiley & Sons; 2011

[19] Wahyudi A, Hendraningsih L, Malik A. Potency of fibrolytic bacteria isolated from Indonesian sheep's colon as inoculum for biogas and methane production. African Journal of Biotechnology. 2010; 9(20): 34-39.

[20] Bagudo BU, Garba B, Dangoggo SM, Hassan LG. Comparative study of biogas production from locally sourced substrate materials. Nigerian Journal of Basic and Applied Sciences. 2008; 16(2): 269-274.

[21] Baki AS. Isolation and identification of microbes associated with biogas production at different retention time using cow dung. Master of Science Dissertation, Usman Dan Fodio University, Sokoto, Nigeria. 2004.

[22] Oluyega IO, Femi-Ola TO, Opomu D. Anaerobic digestion of Cassava peels mixed with pig waste for biogas and biofertilizer production. Nigerian J Microbiol. 2006; 3: 2-7.