Original Research Article

Biogas Production from Palm oil mill effluent (POME) Co-digested with Cassava (*Manihot esculenta*) peels and Cabbage Waste (*Brassica oleracea*)

Abstract

This research work was carried out to produce biogas by co-digesting palm oil mill effluent (POME) with cassava peels (CP) and cabbage waste (CW). The anaerobic digestion in 10L capacity bioreactors charged with three different ratios (3L/ 520g, 3L/ 600g and 3L/ 680g) of POME/CP and POME only (control) was operated under ambient temperature (25-36^{O}C) and pH range of 6.5 - 8.5 for 45 days. Microbial analysis and the characteristics of the bioreactor feeds were estimated by standard methods. The methane content of the produced biogas was determined using Gas Chromatography (GC). The results indicated the presence of *Escherichia coli, Staphylococcus, Pseudomonas sp, Bacillus sp Salmonella sp* among others. Fungal isolates identified include *Saccharomyces, Aspergillus, Rhizopus, Penicillum*, and *Geotrichum* species. The cumulative biogas yield observed in bioreactors charged with POME/ CP 520g, POME/ CP 600g, POME/ CP 680g and POME/CB 520g, POME/CB 600g, POME/CB 680g were 7.08, 5.18, 9.06, 9.13, 9.28 and 8.33 dm^3, respectively, whereas POME alone (control) was 4.64 dm^3. POME/CB 600g exhibited the highest performance in biogas production (9.28dm^3), and the highest percentage methane content (68.80%). Analysis of variance (ANOVA) indicated a significant difference ($P \leq 0.05$) in biogas yield in all the treatments compared to control (POME alone) except in POME/CP 3L: 520g and POME/CP 3L: 600g. The results have shown that biogas production and methane content could be enhanced efficiently via co-digestion process depending on the substrates used as feedstock.

Keywords: POME, kitchen wastes, co-digestion, methane content, Gas chromatography.

1. **INTRODUCTION.**

World population increase has led to the depletion of fossil fuel which is the major source of energy supply. Schleeter [1] reported that Greenpeace Southeast Asia and the Center for Research on Energy and Clean Air (CREA) in their recent research found out that air pollution from burning of fossil fuels have caused approximately 4.5 million deaths each year in the world and estimated global economic losses of about $2.9 trillion each year or approximately 3.3 percent of global GDP, as 230,000 deaths and $600 billion in economic losses annually is linked to the United States alone. Also the use of fossil fuel and wastes generated from industrial, commercial and agricultural activities without proper management practices cause global climate change, environmental degradation/pollution and human health problems because of the emission of greenhouse gases [2, 3]. The recent policy now is to minimize fossil fuel use which is non - renewable and shift to clean renewable energy, as energy has always been a driving force for a successful farming system [4].

Oil palm (*Elaeis guineesis*) is one of the most versatile crops in the tropical world [5]. .It is the most productive oil producing plant in the world, with one hectare of oil palm producing between 10 and 35 tons of fresh fruit bunch (FFB) per year [6, 7]. The production of palm oil, however results in the generation of large quantities of wastewater commonly referred to as palm oil mill effluent (POME) [8]. It contains a mixture of carbohydrate and oil, very high values of chemical oxygen demand (COD) and biochemical oxygen demand (BOD) of about 80,000mg/l [9]. It is an important source of inland water pollution when released into local rivers or lakes without treatment, also causes environmental pollution if not properly disposed.

Cassava (*Manihot esculenta*) is one of the most important crops produced in Nigeria and many sub Saharan Africa and it is known to be the highest source of carbohydrate among other staple crops and can be used in place of maize as an energy source in animal feeds [10]. Cassava roots play an important role in the African diet and they are processed using simple traditional methods into products such as "garri" and "fufu", or flour, some of which are fermented products. As a rough estimate, about 42.5 million metric tons which is estimated to be about 18% of total global production of cassava is processed for garri annually in Nigeria alone [11]. In the processing of fermented cassava products, the roots are normally peeled to get rid of two outer coverings: a thin brown outer covering, and a thicker leathery parenchymatous inner covering. The wastes generated at present pose a disposal problem and would even be more problematic in the future in Nigeria, as there is currently increase in industrial production of cassava products such as in garri production, cassava flour and dried cassava for fufu [12]. These peels are regarded as wastes and only small proportion of it is used as feed for farm animals, this is because the use of cassava peels as feed for non-ruminant animals are influenced by their hydrocyanic acid content which has harmful effect on the growth and development of the animals [13]. Piles of the rest of the wastes generated are usually disposed of indiscriminately or allowed to rot in the environment with offensive odour emanating from it as a result of microbial degradation as the demand increases causing a lot of environmental pollution and health hazards [14, 15].

Cabbage (*Brassica oleracea*) is a leafy green biennial plant grown as an annual vegetable crop for its dense-leaved heads. It is high in nutritional value. The Food and Agricultural Organization of the United Nations (FAO) reported that world production of cabbage and other brassica for 2014 was 71.8million metric tons and approximately 45% of the 60 million tons of cabbage produced in the world is wasted during harvest in the field and when these waste accumulate without proper disposal, they cause environmental pollution and health hazards [11]. The issue of disposal of all these agricultural wastes, sanitation and environmental problems coupled with the high cost of fossil fuel make anaerobic digestion and biogas production a better choice. This is because they offer a renewable and sustainable source of alternative energy at low cost when compared to fossil fuels [16].

The world today is experiencing global warming due to the use of fossil fuel and wood fuel but the use of biogas does not contribute to global warming neither does the methane produced has any effect on the atmosphere [17, 18]. The integration of biogas technology into agriculture popularly called biological or ecological farming will improve the income levels of farmers and facilitate the achievement of effective and low-cost productivity in our agricultural system [19]. Biogas technology can provide the link between animal husbandry and crop farming thus playing an important role in self-sustaining eco-farming. They are easy to manage, eco-friendly and generates an end- product that is used as soil conditioner for improving soil fertility for more yield [20]. The complex biochemical reaction of anaerobic digestion for biogas production is influenced by several factors which include temperature, organic loading rate, C: N ratio, bioreactor design, inoculums, pretreatment methods etc. [21 - 24]. Also the proximate composition of the feedstock has a role to play in the quality of biogas and its cumulative yield, therefore the use of one agricultural waste in anaerobic digestion may not give a better biogas yield hence co-digestion of two or more feedstocks is required to improve biogas yield [25]. This study therefore evaluates the biogas production potential of palm oil mill effluent co-digested with cassava peels and cabbage waste in a batch system bioreactor.

2. Materials and Methods.

The bioreactor feeds used in this study include Palm oil mill effluent (POME), cassava peels (CP) and cabbage waste (CW).The POME used in this study was collected from a palm oil mill industry at Umuagwo in Ohaji- Egbema LGA, Imo State, Nigeria. The sample, after collection was filtered to remove the debris.

The cassava peels (CP) was collected from a cassava processing plant for 'garri' production and cabbage waste (CW) from a fruit salad market in Obinze, Owerri West LGA, Imo state, Nigeria. The samples were sorted to remove unwanted materials and thereafter sun-dried to a moisture content of 11.48% and11.15%, respectively, milled to reduce the particles size, sieved and subsequently stored in an air-tight polythene container to preserve the substrates. The samples were used as digester feeds when required.

Cow rumen waste was used as source of the inoculum to stabilize wastes. It was filtered in cheesecloth and stored in air-tight container in order to maintain the anaerobic environmental condition required by the microorganisms (methanogens) needed for methane production.

2.1. Proximate Analysis of the Bioreactors Feeds.

Proximate analysis of Palm oil mill effluent (POME), Cassava peels (CP) and Cabbage waste (CW) were carried out using standard methods [26 to determine the Total Solid (TS), Volatile Solid (VS), Carbon to Nitrogen (C: N) ratio, Ash content, Moisture content etc.

2.2. Experimental Design.

The experimental design and set-up of Opurum *et al.*, [27] was adopted in this study but with some modifications. Ten (10L) capacity batch bioreactor systems were used for the anaerobic digestion of the substrates. Each bioreactor was equipped with a thermometer for measuring the temperature and an outlet for gas passing to the gas collecting system. The hose from the bioreactor was connected to a 13L transparent bucket and 3L transparent bucket inverted in it which served as a gas collector. A short hose was attached on the 13L bucket for collection of displaced water. The experimental set up which was in triplicate is shown in Figure 1.The bioreactors were charged at three different ratios with POME/CP and POME/CW each; 3L: 520g, 600g and 680g, respectively and 3L of POME as control.

Freshly strained cow rumen waste (20% of the total slurry volume) was used as the inoculum which provided the source of methanogens. Digestion of the substrates under anaerobic condition was at room temperature which varied between 25^{O}C- 36^{O}C. Each bioreactor was manually subjected to periodic agitation to prevent sedimentation/ stratification and for even distribution of substrates, enzymes and microorganisms. The agitation also helps to promote heat transfer and facilitates the release of produced biogas from the bioreactor contents [28]. The daily biogas yield for each bioreactor was recorded by adopting the downward water displacement method [29]. The volume of biogas yield was measured and the mean values recorded on daily basis at every 24hr. The pH was adjusted at the range of 6.8 to 8.0 with Sodium hydroxide (NaOH) and Hydrochloric acid (HCl). The experiment was monitored for 45 days hydraulic retention time.

2.3. Microbiological analysis.

Samples for microbial analysis were collected in sterile bottles immediately after the digesters were set up. The various bacterial and fungal species in the digesting slurry were determined by the use of various culture media using the spread plate technique as described by Bergey's Manual of Determinative Bacteriology [30]. The inoculation of each prepared medium was done after 10 fold serial dilution. From 10^3 dilution of the sample, 0.1ml aliquot was inoculated by spread plate technique onto Nutrient agar (NA), Eosin Methylene Blue agar (EMBA), MacConkey agar (MCA), Salmonella-Shigella agar (SSA) and Potato Dextrose agar (PDA) prepared according to manufacturers' instructions. Microscopic examination, biochemical tests and Gram staining were carried out by adopting the methods described by Cappucino and Cherman [31].

2.4. Gas Chromatography (GC) analysis

The flammability test was also carried out on daily basis to determine the flammable biogas which was collected and the substrate ratios with the highest biogas yield were analyzed using Gas Chromatography (GC), GC-TCN (M910) with helium as a carrier gas at 5psi, with a flow rate of 20ml per minute to ascertain the biogas composition.

2.5. Statistical Analysis of Data

The cumulative biogas yield in all the treatments and control were statistically analyzed using analysis of variance (ANOVA) implemented via IBM SPSS version 20.0.

3.0. Results

3.1. Characteristics of the Bioreactor Feeds.

The characteristics of the POME, CP and CW were evaluated to determine the availability of digestible nutrients in the feedstocks that could be accessed by the microorganisms during the anaerobic digestion process and the results are shown in Table 1.The POME had a Carbon to Nitrogen (C/N) ratio of 10:1, CP 46:1, and CB 17:1.It was observed that cassava peels has a higher C: N ratio than POME and cabbage waste. It is therefore necessary to co-digest the substrates to enhance biogas production.

3.2. Anaerobic Digestion and Biogas production.

This study shows that co-digestion of POME/CP and POME/CW in the ratios of 3L:520g, 600g and 680g respectively had higher cumulative biogas yield of (7.08dm^3), (5.18dm^3),(9.055dm^3) and (9.13dm^3), (9.28dm^3), (8.33dm^3) respectively than POME alone that had cumulative biogas yield of 4.64 dm^3(Table 2).Co-digestion of these substrates was capable of improving the efficiency of biogas production.

The bioreactors charged with POME/CP 3L: 520g, 3L: 600g and 3L: 680g ratios biogas productions started on day 2. Flammability test indicated that flammable biogas production in 3L: 680g started on day 11, 3L: 520g ratio on day 13 and 3L: 600g on day 14.POME/CP 3L: 680g started biogas production on the2nddayand flammable gas on the 11th day, the peak of gas production was recorded on the 13th day with biogas yield of 1.89dm^3 and cumulative biogas yield of 9.055dm^3 as shown in table 2. In POME/CW 3L: 520g, 3L: 600g and 3L: 680g biogas production started on day 2 and flammability test indicated that in3L: 600g ratio, flammable biogas production started on day 5 while 3L: 520g and 3L: 680g was flammable on day15.POME/CW 600g which had the highest biogas yield and percentage methane content, started biogas production on the 2nd day, the peak recorded on the 11th day with biogas yield of 1.55dm^3 and cumulative biogas yield of 9.28dm^3. In the control (POME alone) biogas production started on the 2nd day, flammable biogas production started on the 4th day, the peak was recorded on the 5th day with biogas yield of 1.21dm^3 and cumulative biogas yield of 4.64dm^{3}which was one of the lowest biogas yield had in the study.

3.3. Gas Chromatography.

The result showed that the highest percentage methane yield was achieved from co-digestion of POME/ CW (3L:600g) as shown in Table 3.The methane level achieved was 68.80%for POME/CW (3L:600g), 65.28% for POME/CP (3L: 680g) and 56.53% for POME (control). From this study, the mixing ratio of POME/CW (3L:600g) has proved to be suitable combination for biogas production as well as methane content.

3.4. Microbial Analysis

The bacterial species isolated from the digesting slurry were: *Escherichia coli, Staphylococcus, Pseudomonas, Bacillus, Enterococcus, Enterobacter, Salmonella and Micrococcus* species. The fungal isolates identified were *Saccharomyces, Aspergillus, Rhizopus, Penicillum,* and Geotrichum species.

3.5. Statistical Analysis

Comparative analysis of the means of maximum cumulative biogas yield using the Post-Hoc Duncan test showed that there is significant difference between POME alone and all other treatments except POME/CP 3L:520g and 3L: 600g.

4.0 Discussion.

The result of the proximate analysis of the substrates showed that cassava peel has a higher C: N ratio (46:1) than POME (10.13:1) and cabbage waste (16.90:1), hence there is the need for co-digestion with suitable substrate to improve the C/N ratio. The C/N ratio of the POME (10.13) is in agreement with that reported by Adela *et al.,* [32] with C/N ratio of 10.58%.

Carbon to Nitrogen ratio is one of the important factors that influence biogas production and this must be put into consideration in the course of co-digestion of substrates to enhance biogas production from feedstock [33]. When the C: N ratio of a substrate is very high, the methanogenic bacteria will rapidly use up the nitrogen to meet their protein needs resulting to imbalance in C: N ratio which causes low biogas production. Also if the C: N ratio is low, excess nitrogen will be liberated during microbial metabolism and this accumulates in the form ammonia which increase the pH value of the feedstock above 8.5 and resultant effect being the exertion of toxic substances on the methanogens causing low biogas yield [34].

The total solids (TS) and volatile solids content of POME is low (13.93 and 13.39%) and this may be attributed to the low biogas yield while CP and CB are sufficiently high (88.52; 81.75 and 88.85; 65.04 %), indicating that both substrates are suitable for biogas production. The increase in biogas yield of Cabbage co-digested with POME could be attributed to the supplementary effects (synergism) of the nutrient contents of the individual feedstock. Esposito *et al*., [35] reported that co-digestion of different organic substrates have shown a synergic effect of the combined treatments as it was observed that the biodegradability of the mixture was higher than that of the single substrates. The cassava peels used in this study with regards to the total solid (TS) of 88.52% and moisture content of 11.48% supports the report of Nkodi *et al*.,

[36]. The proximate composition of feedstock has great influence on the quality biogas and its yield. Bolaji and Adebayo [37] reported that wastes from plant materials such as crop residues are not easily digestible as animal wastes because of difficulty in hydrolysis of cellulose, hemicelluloses and lignocellulosic constituents.

The quality of biogas (methane content) and its cumulative yield highly depends on the characteristics of the feedstock hence to efficiently enhance biogas yield, co-digestion of substrates has to be adopted [38, 39]. (Similarly in this study, biogas yield from POME co-digested with other substrates was higher than that of POME alone, this could be attributed to the low volatile solid (TS) concentration of 13.39% which is the amount of the POME convertible to gaseous element and providing nutrients to the microorganisms for their function, as well as the low C: N ratio of 10.13%. Improvement of the characteristics of the feedstock and other operating conditions of the bioreactor is required for optimum microbial activities in anaerobic digestion and efficiency in biogas production [40 - 42].

The cumulative biogas yield of the co-digested substrates; POME/CP and POME/CB, 3L: 520g, 3L: 600g and3L: 680g had higher cumulative biogas yield of (7.08 dm^3), (5.18 dm^3), (9.055dm^3) and (9.13dm^3), (9.28dm^3), (8.33dm^3) respectively than POME alone that had cumulative biogas yield of 4.63dm^3(Table 2). This shows that co-digestion of these substrates was capable of improving the efficiency of biogas production. Previously effluents have been shown to improve biogas yield and methane content while mono-substrate digestion was found to be mostly unstable [43]. (Similar result was observed by Sawyerr *et al*., [25]. Also Ibrahim *et al*., [44] reported that anaerobic digestion of POME alone affected its methanogenic process which is very important in anaerobic digestion as it is the final stage of biogas production.

The microorganisms isolated from the digesting slurry in this study were also reported by Asikong *et al*., [45]. The result also shows that the highest percentage methane yield was achieved from co-digestion of POME/CB (3L: 600g) as shown in Table 3. The methane level achieved was 68.80% for POME/CB (3L: 600g), 65.28% for POME/CP (3L: 680g), and 56.53% for POME (control). The remaining percentages constitute the following: Carbon-dioxide, Carbon-monoxide, and trace elements namely; hydrogen (H_2), oxygen (O_2), nitrogen (N_2) and water (H_2O). From this study, the mixing ratio of POME/CB (3L: 600g) has been recognized as suitable mixture for biomethane production. The study also showed that only 56.53% of methane was produced from the anaerobic mono-digestion of POME. The enhancement of biomethane production through co-digestion of substrates is once more proven as the production level was elevated from 56.53% to much as 65.28and 68.80 %. Similar result was observed by Aragwa *et al.*, [46] who reported that co - digestion of different feedstock substantially enhanced the biogas yields by 24 to 47% over the control (organic kitchen waste and dairy manure only).

Table 1. Proximate Composition of substrates

Parameters (%)	**Substrates**		
	POME	CP	CB
Fat content	8.23	3.94	3.83
Fibre content	-	11.07	13.26
Ash content	0.54	6.77	23.81
Crude Protein	3.25	6.778	14.76
Organic carbon	48.90	49.87	39.54
C:N Ratio	10.13	46	16.90
Nitrogen	5.12	1.084	2.361
Moisture content	86.08	11.48	11.15
Carbohydrate	1.90	-	-
Volatile Solids (VS)	13.39	81.75	65.04
Total Solids (TS)	13.93	88.52	88.85
pH	4.06	5.91	6.84

Table 2. Cumulative biogas Yield from the different substrate ratios (dm^3)

Treatment	**3L:520g**	**3L:600g**	**3L:680g**
POME/CP	7.08	5.18	9.06
POME/CW	9.13	9.28	8.33
POME control	4.64	4.64	4.64

Table 3. Percentage Biogas Composition of bioreactors with the highest methane yield.

Treatment	**Percentage composition (%)**		
	Methane (CH4)	Carbon dioxide (CO_2)	Carbon Monoxide (CO)
POME/CP 3L/680g	54.28	38.80	3.22
POME/CW 3L/600g	68.80	28.50	2.09
POME control	56.54	30.92	0.45

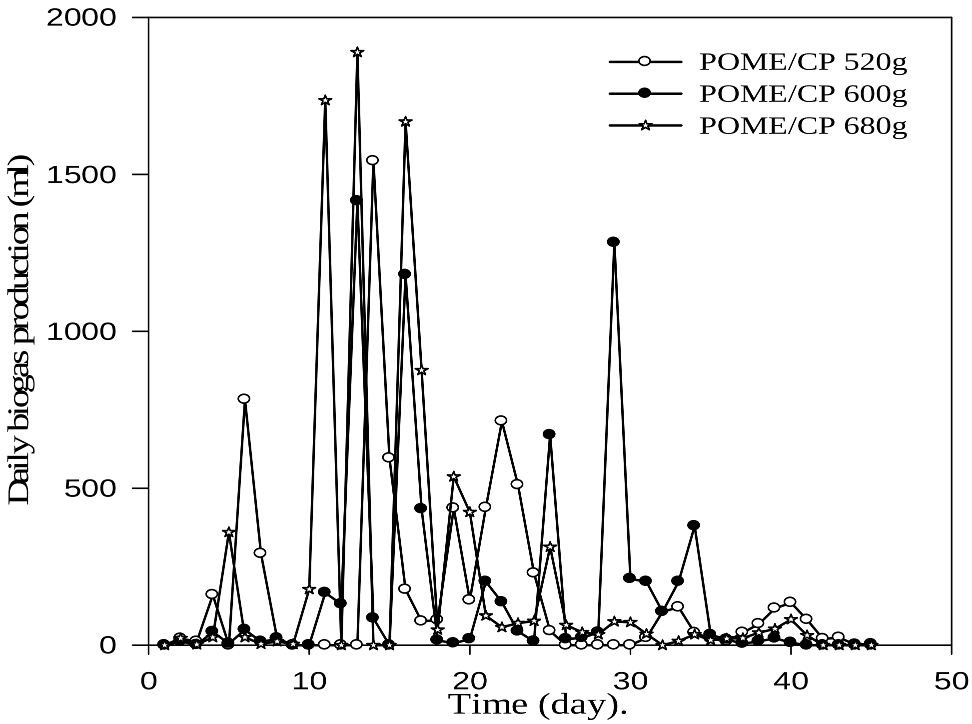

Figure 1: Profile of Daily Biogas Production from Mixtures of POME/CP

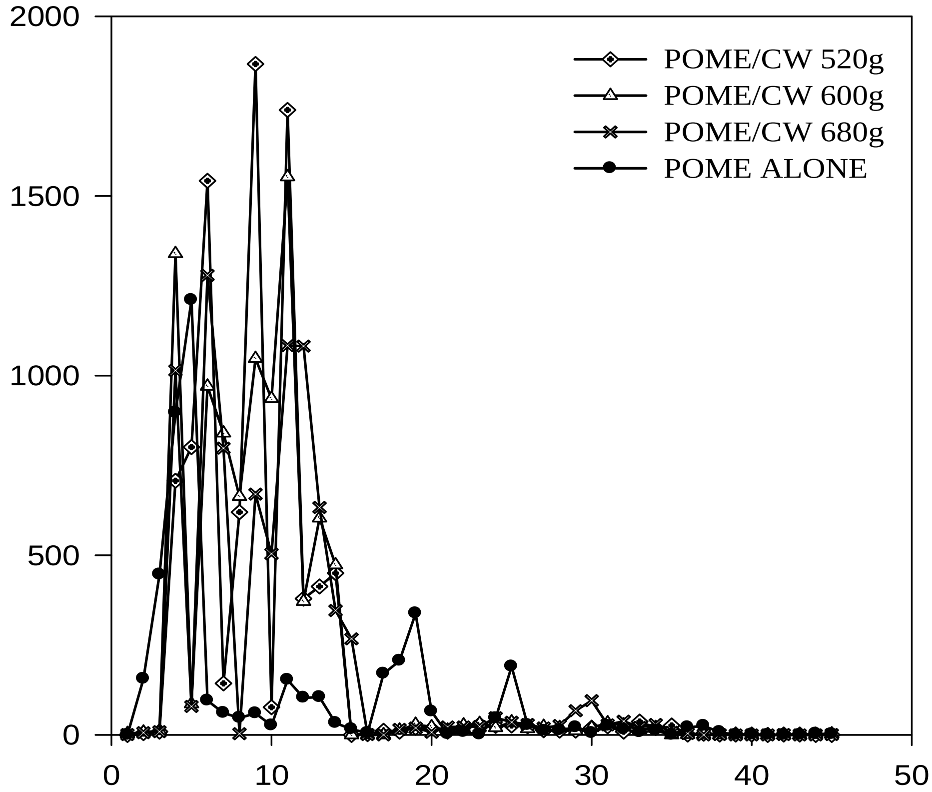

Figure 2: Profile of Daily biogas production from Mixtures of POME/CW and POME alone

5.0. Conclusion.

This study has shown that anaerobic co-digestion of palm oil mill effluent with cassava peels and cabbage is capable of significantly enhancing biogas yield. The best performance in biogas production was noted in bioreactor charged with POME/CB 600g, followed by POME/CP 680g. The feedstock characteristics indicated their potentials as suitable substrates in biogas production. Co-digestion of palm oil mill effluent with cassava peels and cabbage could be adopted in large scale production of biogas as this could help in the production of renewable energy, proper management of agro-wastes and mitigation of greenhouse gas emission which causes global warming.

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