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Microorganisms Associated with Biogas Production Using Vegetable (*Telfairia occidentalis*) Wastes, Banana Peel and Pig Dung as Substrates

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Authors' contributions

This work was carried out in collaboration between all authors. Author BEA designed the study, performed the statistical analysis, wrote the protocol, wrote the first draft of the manuscript and managed literature searches. Authors SOI and DRT managed the analyses of the study and literature searches. All authors read and approved the final manuscript.

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ABSTRACT

The research study was aimed at investigating microorganisms associated with biogas production using vegetable (*Telfairia occidentalis*) wastes, banana peel, and pig-dung as substrates. Marian market, Watt market and University of Calabar pig farm were randomly sampled within Calabar metropolis for collection of samples. The study was completed within a period of six month. Standard microbiological methods and anaerobic biodigesters were used to screen the isolates and the wastes substrate for biogas production. Analysis revealed that the temperature of raw substrates ranged between 21℃ and 39℃ while the pH varied bet ween 6.10 and 7.21 during digestion. Highest mean bacterial counts was $8.87\pm3x10^6$ cfu/g and fungal count of 5.67 ± 10^5 cfu/g were obtained in the combined substrates of banana peel, vegetable waste and pig dung (BP + VW + PD) before digestion, as compared to mean bacterial counts of $8.62\pm1.4x10^6$ fu/g and fungal counts of $5.55\pm1.7x10^5$ cf/g obtained during digestion. Anaerobic bacteria isolated were identified as, *Pseudomonas sp*, *Escherichia coli*, *Bacillus sp*, *Salmonella sp*, *Staphylococcus aureus*, *Serratia sp*, *Shigella sp*, *Micrococcus sp, Proteus vulgans, Citrobacter sp* and *Klebsiella sp*, while fungi isolated

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were identified as *Fusarium sp*, *Mucor sp* and *Penicillium sp*. Methanogenic bacteria isolated were identified as *Methanothrix sochngenii, Methanococcoides methylutens* and *Methanoculles bourgense*. The volume of biogas produced and the percentage methane yield varied signficantly ($p<0.05$) between the substrate treatments and the digestion internals (days). However, the study has shown that the role of methanogens and other complementing bacteria and fungi in biogas production is indispensable.

Keywords: Methanogens; biogas; fermentative; digester.

1. INTRODUCTION

The cost and scarcity of improved petroleum products used for industrial, agricultural and domestic fuels are drastically increasing, as this makes it difficult for most people to rise beyond subsistence level especially in developing countries like Nigeria, and these realities have led to a boost in the search for renewable and sustainable alternative to fossil fuels. Researches have shown that biogas, a flammable gas produced when organic materials are fermented under anaerobic condition is one of such alternative [1,2]. Biogas is a readily available energy source that significantly reduces greenhouse gas emission compared to the emission of land fill gas to the atmosphere [2]. Biogas being an alternative energy source is important for generating electricity, car fuelling, cooking as well as other purposes [3].

In biogas production, the conversion of complex organic matter to methane and carbon dioxide is possible mainly by the actions of different group of microorganisms, with the microbial community of biogas comprised essentially of bacteria and fungi and other groups of protozoan [4]. The essential microbial complex is comprised of hydrolytic bacteria, fermenting bacteria, acetogenic bacteria and methanogenic bacteria and these groups of microorganisms have been reported to establish synthrophic relationships where the later members of the food chain depend on the previous for their substrate but may also have significant metabolic products [5]. These microorganisms act at the different stages of the anaerobic process to bring about effective biogas production and are an integral component of nature's waste management and are commonly found in soils and deep waters as well as land fill sites [6]. The anaerobic digestion process of biogas generation is divided into four major stages and these include hydrolysis, acidogenesis, and methomogensis [5]. During hydrolysis, bacteria transform the particulate organic substrate into liquefied monomers and polymers, that is proteins, carbohydrates and fatty acids respectively [6,7]. The biological process of acidogenesis involves further breakdown of the remaining components by acidogenic (fermentative) bacteria. These bacteria transform the products of the first reaction into short chain volatile fatty acids, ketones, alcohols, hydrogen and CO_2 [8].

During acetogenesis which is the third state of anaerobic digestion, the rest of the acidogenesis products are transformed by acetogenic bacteria into hydrogen, CO_2 and acetic acid. Methanogenesis is the terminal stage of anaerobic digestion, in this process, methanogens utilize intermediate products of these proceeding stages and converts them into methane, CO_2 and water, as it is these components that make up the majority of the biogas emitted from the system [9].

2. MATERIALS AND METHODS

2.1 Samples Collection

i) Banana peel: Banana peels were collected in sterile polythene bags from Marian market in Calabar and then transported to the laboratory for analysis.
ii) Vegetable (*Telfairia occidentalis*) waste: Vegetable wastes were collected in large quantity from Watt market in Calabar and placed in sterile polythene bags and then transported to the laboratory for analysis.
iii) Pig dung: Pig dung wastes were obtained from University of Calabar farm and placed in polythene bag and transported to the laboratory for analysis.

2.2 Media Used

The media used in the study were Nutrient agar, Saboroud dextrose agar, MacCokey agar, Simmon's citrate agar, Triple sugar Iron agar (TSI), (all were products of biotech lab Ltd, UK), Motility-Indole Ornithine agar (MIO) (Hardy Diagnostic, USA). All the media were of

analytical grade and were prepared in accordance to the manufacturer's instruction.

2.3 Preparation of Raw Substrates for Microbial Screening

i) **Banana peel:** 10 gram of banana peel was aseptically crushed into powder. Then, it was added into 90 ml of sterile distilled water contained in a 100 ml capacity flask, agitated and allowed to settle.
ii) **Vegetable (*Telfairia occidentalis*) waste:** The leaves and stems of vegetable (*Telfairia occidentalis*) were grounded aseptically and 10 milliliter of the sample was then added in 90 ml of sterile distilled water contained in a 100 ml capacity flask. The mixture was allowed to settle and then used for microbiological analysis.
iii) **Pig dung:** The pig dung wastes obtained were prepared and used for the screening of bacterial and fungal counts. 10 gram of the pig dung was added into 90ml of sterile distilled water contained in a 100 ml capacity flask, agitated and allowed to settle.

2.4 Preparation of Media and Reagents

All the media and reagents used were prepared and preserved according to the manufacturer's specifications.

2.5 Enumeration of Total Heterotrophic Bacteria

Total heterotrophic bacteria in the substrates were enumerated by spread plate technique using nutrient agar. A ten-fold serial dilution of the substrates was carried out by transferring 1ml each of the substrate into test tubes containing 9 ml of sterile distilled water arranged serially in the order 10^{-1} – 10^{-10}. Dilutions of 10^{-1} and 10^{-5} were inoculated and incubated at room temperature for 24-48 hr for the enumeration of total heterotrophic bacteria.

2.6 Enumeration of Total Heterotrophic Fungi

The enumeration of total heterotrophic fungi in substrates was carried out using spread plating technique and Sabouraud dextrose agar. The dilutions of 10^{-3} and 10^{-4} were used. Plates were incubated at room temperature for 48-72 h. After incubation, colonies were counted and expressed as colony forming unit per milliliter of sample (cfu/ml).

2.7 Digester Design

Anaerobic digesters (a batch-types) of about 5 liters each for the digestion of substrates for biogas generation were fabricated locally according to the method described by [10]. 8 empty gas cylinders consisting of an opening through which the substrates were introduced into digester and an outlet tap from where samples were collected for analysis was used.

2.8 Preparation of Slurry and Loading of Digesters

Preparation of substrates for biogas generation was carried out according to the methods described by [5,10].

i) **Banana peel:** 1 kilogram of freshly grinded banana peel was mixed with distilled water in a ratio of 1:3. The mixture was agitated thoroughly and transferred into the digesters and tightly corked with stopper to create anaerobic condition.
ii) **Vegetable waste:** 1 kilogram of grounded vegetable (*Telfairia occidentalis*) waste was mixed with distilled water to give a ratio of 1:3. The mixture was agitated thoroughly and transferred into the digester and tightly corked with stopper to create anaerobic condition.
iii) **Pig dung:** 2 kilogram of pulverized pig dung was prepared in 3 liters of distilled water. The mixture was agitated thoroughly and transferred into the digester and tightly corked with stopper to create anaerobic condition.
iv) **Combination of substrates:** Substrates were prepared in combinations in the order; banana peel and vegetable waste in the ratio of 1:1, pig dung and banana peel in the ratio of 1:1, vegetable waste and pig dung in the ratio of 1:1, and banana peel, vegetable waste, and pig dung in the ratio of 1:1:1 was prepared. Each mixture was loaded into the digesters by mixing with distilled water in the ratio of 1:2

2.9 Sampling of Digester Content for Microbiological Analysis

During the digestion period, samples from the digesters were collected at 24 hr intervals for 30 days, and anaerobic bacteria and fungi were screened using the method described by [10].

Aliquot of 1ml of the dilutions 10^{-4} and 10^{-5} and 10^{-3} and 10^{-4} for bacteria and fungi respectively.

Each sample was incubated by spread plate using nutrients and sabouroud dextrose agar for bacteria and fungi. Plates were incubated anaerobically at 35℃ for 24-72 h. The anaerobic environment was created using anaerobic jar provided with sachets of gas generating kit (gas packs). After enumeration, colonies were sub-cultured and identified using cultural, microscopic and biochemical characteristics.

2.10 Measurement of Gas Production

The method described by [11] was used. Biogas production was measured daily on volume basis in a gasometric chamber by displacement of paraffin oil. The gasometric chamber consists of a graduated burette which the upper-end would be connected to the anaerobic digesters and the lower-end to a glass funnel with paraffin oil. The evidence of biogas production was determined by the displacement of paraffin oil in the graduated burette.

2.11 Measurement of Methane Yield

Methane yield during digestion was determined mathematically by dividing the amount of flammable gas from the total biogas produced according to the equation below. Moreover, flammable gas was detected by lighting a match close to the gas outlet tap to burn off the gas evolved.

Percentage methane (%) =

$$\frac{Flammable\ gas\ evolved}{Total\ biogas\ volume} \times \frac{100}{1}$$

2.12 Statistical Analysis

Statistical analysis of data obtained from the different treatments were carried out using a 2 way analysis of variance (ANOVA) and the means separated using the fishers least significant difference (LSD) at 5% significant level. All data were expressed as means ± standard deviation of triplicate trials.

3. RESULTS

3.1 Temperature Variations of Substrates

Fig. 1 shows the mean temperature variation of the substrates before and during the digestion process. The mean temperature ranged between 27℃ and 29℃ for the raw substrate and between 28℃ and 39℃ during the digestion process.

3.2 pH Variations of the Substrates

Fig. 2 shows the means variation in hydrogen ion concentration of the different substrates treatments before and after digestion. The mean

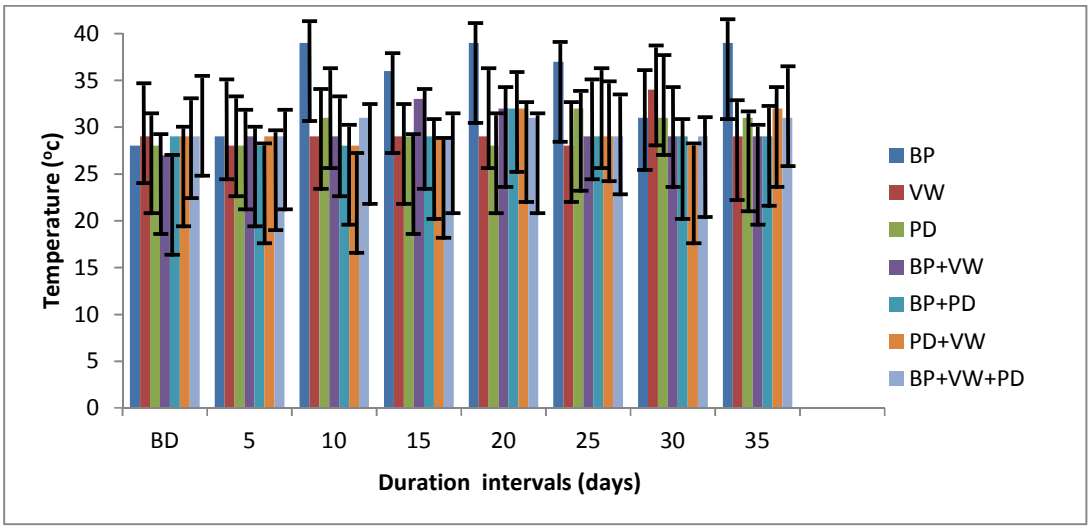

Fig. 1. Mean temperature variations of substrates before digestion (BD) and during anaerobic digestion

Key: BP = Banana peel, VW = Vegetable waste, PD = Pig dung, BP+VW= Banana peel and Vegetable waste, BP+PD = Banana peel + Pig dung, PD+VW = Pig dung and Vegetable waste, BP+VW+PD = Banana peel and Vegetable waste and Pig dung

pH ranged between 6.9 (Vegetable waste) and 7.4 (Banana peel) for the raw substrates before digestion and between 6.1 (Pig dung) within 30 days of digestion and 7.2 (Vegetable waste and Banana peel) within 5 days of digestion. The result showed a marked decrease in the hydrogen iron concentration during the anaerobic digestion of the substrates.

3.3 Total Heterotrophic and Anaerobic Bacteria Counts

Fig. 3 shows the mean heterotrophic and anaerobic bacteria counts in the different substrates treatment before and after anaerobic digestion. The mean heterotrophic bacteria counts before digestion ranged from $4.20\pm1.1x10^6$ cfu/g (vegetable waste) to $8.87\pm1.3 \times 10^6$ cfu/g (Banana peel and Vegetable waste and Pig dung). Simultaneously, the mean anaerobic bacteria counts ranged from $2.78\pm0.1 \times 10^6$ cfu/g (pig dung) within 35 days of digestion to $8.59\pm1.3\ 10^6$ cfu/g (Banana peel and Vegetable waste and Pig dung) within 15 days of digestion. The counts increased between 5 and 20 days of digestion and decreased within 30 and 35 days.

Significant variation ($p<0.05$) in the counts was observed between the substrates and duration of digestion. Higher bacterial counts were observed with the combination of the substrates (Banana peel and Vegetable waste and Pig dung) compared to the substrates.

3.4 Total Heterotrophic and Anaerobic Fungal Counts

Fig. 4 shows the mean heterotrophic and anaerobic fungal counts of the different substrates treatments before and after digestion. The mean fungal counts of the different substrates treatments before digestion ranged between $2.98\pm0.4x10^5$ cfu/g (Pig dung) and $5.67\pm1.2x10^5$ cfu/g (Banana peel and Vegetable waste and Pig dung). Also the mean anaerobic fungi counts of the treatments, ranged between $2.45\pm1.4x10^5$ cfu/g (Banana peel and Vegetable wastes and Pig dung). The mean count varied significantly ($p<0.05$) between the different substrate treatments.

3.5 Characterization and Identification of Isolates

Table 1 presents the biochemical and morphological characterization of bacterial isolates identified, they include; *Bacillus sp, Escherichia coli, Pseudomonas sp, Citrobacter sp, Serratia sp, Salmonella sp, Shigella sp, Staphylococcus aureus, Kelebsiella sp*, and *Proteus vulgaris*.

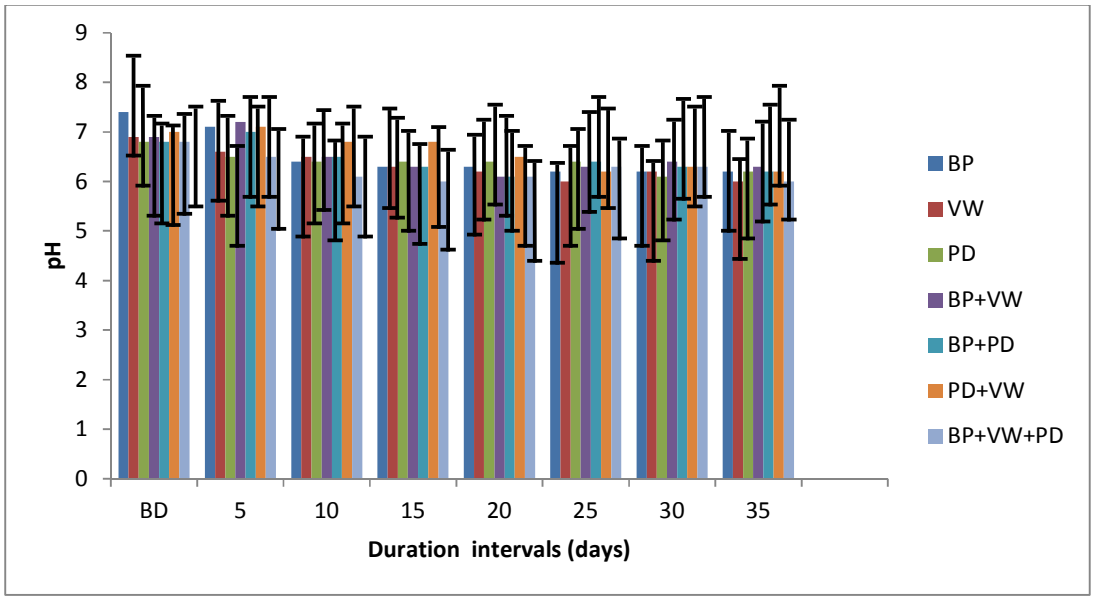

Fig. 2. Mean pH variations of substrates before digestion (BD) and during anaerobic digestion

Key: BP = Banana peel, VW = Vegetable waste, PD = Pig dung, BP+VW= Banana peel and Vegetable waste, BP+PD = Banana peel + Pig dung, PD+VW = Pig dung and Vegetable waste, BP+VW+PD = Banana peel and Vegetable waste and Pig dung

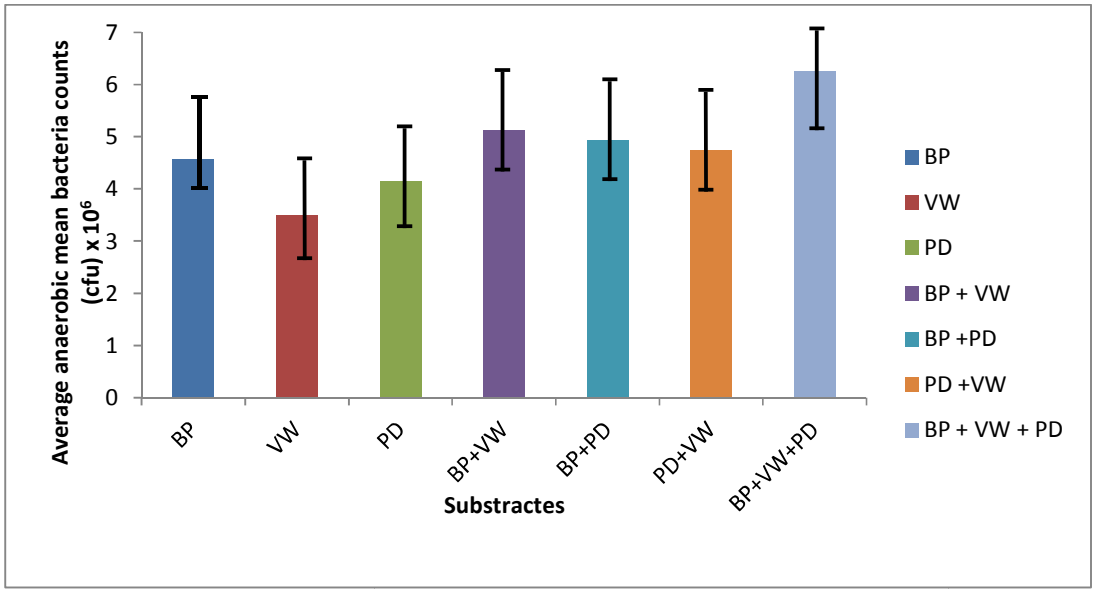

Fig. 3. Average anaerobic bacteria counts after 35 days of digestion

Key: BP = Banana peel, VW = Vegetable waste, PD = Pig dung, BP+VW= Banana peel and Vegetable waste, BP+PD = Banana peel + Pig dung, PD+VW = Pig dung and Vegetable waste, BP+VW+PD = Banana peel and Vegetable waste and Pig dung

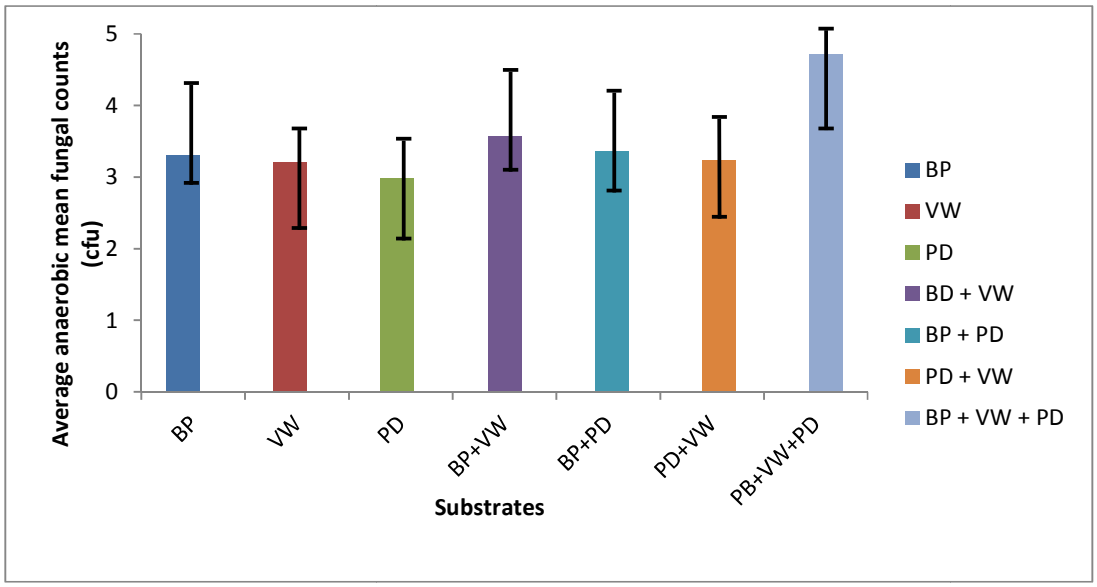

Fig. 4. Average anaerobic fungal counts after 35 days of digestion

Key: BP = Banana peel, VW = Vegetable waste, PD = Pig dung, BP+VW= Banana peel and Vegetable waste, BP+PD = Banana peel + Pig dung, PD+VW = Pig dung and Vegetable waste, BP+VW+PD = Banana peel and Vegetable waste and Pig dung

Table 2 shows the methanogenic bacteria species isolated in this study. The methane producing bacteria include; *Methanothrix sochngenii, Methanococcoides methyilutens* and *Methanoculleus bourgense*.

Table 3 presents the morphological and macroscopic characterization of fungi isolates in this study. Fungi isolates identified include; *Fusarium sp, Aspergillus sp, Mucior sp* and *Penicillium sp*.

Table 1. Biochemical characterization and identification of bacterial isolates

Code	Gram reaction	Morphology	Motility	oxidase	Catalase	Indole	Citrate	Urease	Voges proskaeur	Methyl red	Coagulase	H_2S	Glucose	Lactose	Manitol	Probable organism
BP_1	-	Cocco bacilli	+	+	+	-	+	-	-	-	-	-	-	-	-	*Pseudomonas sp*
BP_2	-	Single rod	+	-	+	+	-	-	-	+	-	-	+	+	+	*Escherichia coli*
BP_3		Straight rods	+	+	+	+	+	+	+	-	-	+	+	-	-	*Bacillus sp*
BP_4	-	Straight rods	-	-	+	-	-	+	+	-	+	-	+	+	+	*Salmonella sp*
VW_1	+	Cocci	-	-	+	-	+	+	-	+	+	-	-	+	+	*Staphylococcus aureus*
VW_2	-	Straight rods	+	-	-	-	+	-	+	-	-	-	+	+	+	*Serratia sp*
VW_3	-	Straight rods	-	-	+	+	+	-	+	+	-	-	-	+	+	*Shigella sp*
VW_4	+	Cocci	-	+	+	-	+	-	-	+	-	-	+	-	-	*Micrococcus sp*
PD_1	-	Straight rods	+	-	+	+	+	-	+	-	-	-	+	+	+	*Proteus vulgaris*
PD_2	-	Straight rods	-	-	+	-	-	+	+	-	+	-	+	+	+	*Salmonella sp*
PD_3	-	Straight rods	+	-	+	-	+	-	-	+	-	-	+	+	+	*Citrobacter sp*
PD_4	-	Straight rods	-	-	+	+	+	-	+	-	-	-	+	+	+	*Klebsiella sp*

Table 2. Methane producing bacteria during the process of biogas production

S/N	Morphology/shape	Grams reaction	Motility	Catabol substrate	Format acetate	pH	Temperature	Isolates
1.	Large sheathed rods	-	-	+	+	7.1-7.8	35-4℃	*Methanothrix sochngenii*
2.	Irregular cocci single in pairs	-	-	+	+	7.0-7.5	30-35℃	*Methanococcoides methylutens*
3.	Irregular cocci	-	-	+	++	7.0	20-40℃	*Methanoculleus bourgense*

3.6 Biogas Yield from Substrates

Fig. 5 shows the volume of biogas yielded from the different substrates treatments. The yield varied significantly ($p<00.5$) between the substrate treatments and the digestion intervals (days). Maximum biogas yield was obtained within 25 days of digestion with the volume of biogas between 45.58 cm^3 (Pig dung) and 58.90 cm^3 (Banana peel and Vegetable waste and Pig dung). Fig. 6 presents the overall volume of biogas produced from each substrate treatment over the digestion period of 35 days ranged between 194.58 cm^3 (pig dung) and 380.29 cm^3 (Banana peel and Pig dung and Vegetable waste).

3.7 Percentage Methane Yield from Substrates

Fig. 7 shows the percentage of methane yielded from the different substrates treatments. The percentage yield ranged between 25.10% (Pig dung and Vegetable waste) and 49.% (Banana peel and Vegetable waste and Pig dung). The methane yield evolved between the fourth and fifth weeks (30-35 days) of digestion.

Table 3. Characterization and identification of fungal isolates

Colony code	Colour of hyphae	Macroscopic features	Probable organisms
BP_5	Pink wooly	Narrow septate hyphae, conidiophores occur singly and in groups, multicellular crescent shaped conidia	*Fusarium sp*
VW_5	White fluffy	Aseptate broad hyphae with large spherical head without rhizoid	*Mucor sp*
VW_6	Black velvety	Septate and broad hyphae, with large head entirely covered with chains of conidia	*Aspergillus sp*
PD_4	Greenish velvety	Septate hyphae, with conidiophores developing into branched phalides bearing chains with brush like appearance	*Penicillium sp*

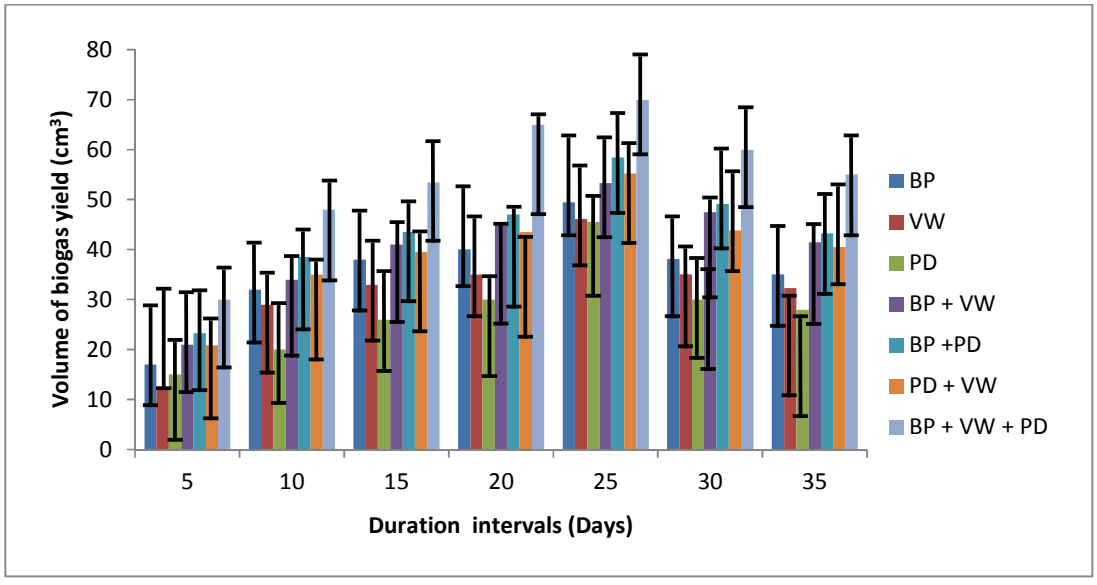

Fig. 5. Mean biogas yield during anaerobic digestion of substrates at varying retention time (5-35 days)

Key: BP = Banana peel, VW = Vegetable waste, PD = Pig dung, BP+VW= Banana peel and Vegetable waste, BP+PD = Banana peel + Pig dung, PD+VW = Pig dung and Vegetable waste, BP+VW+PD = Banana peel and Vegetable waste and Pig dung

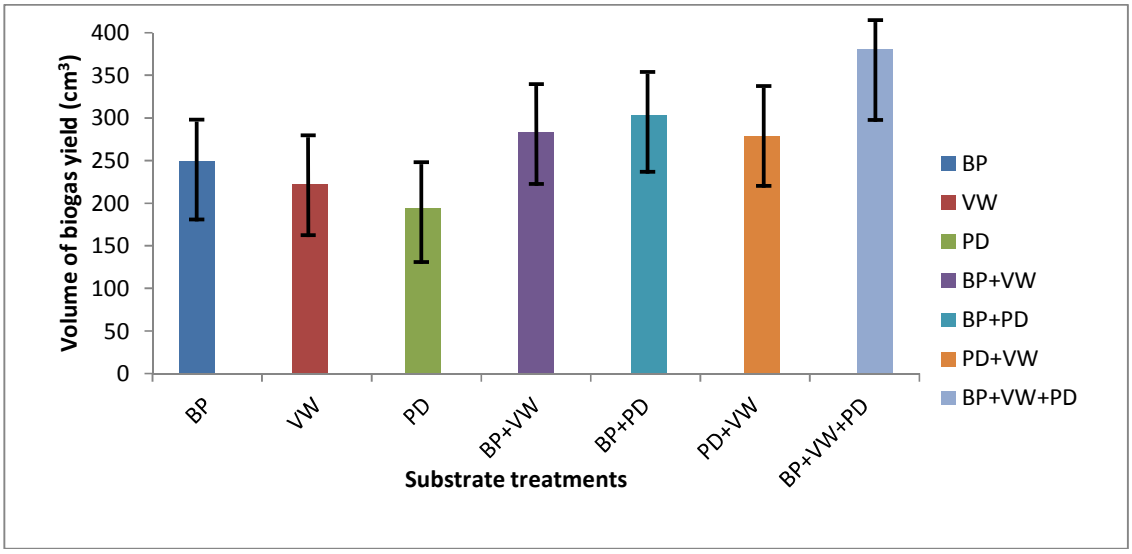

Fig. 6. Total biogas yield during anaerobic digestion of substrates over the retention time of 35 days

Key: BP = Banana peel, VW = Vegetable waste, PD = Pig dung, BP+VW= Banana peel and Vegetable waste, BP+PD = Banana peel + Pig dung, PD+VW = Pig dung and Vegetable waste, BP+VW+PD = Banana peel and Vegetable waste and Pig dung

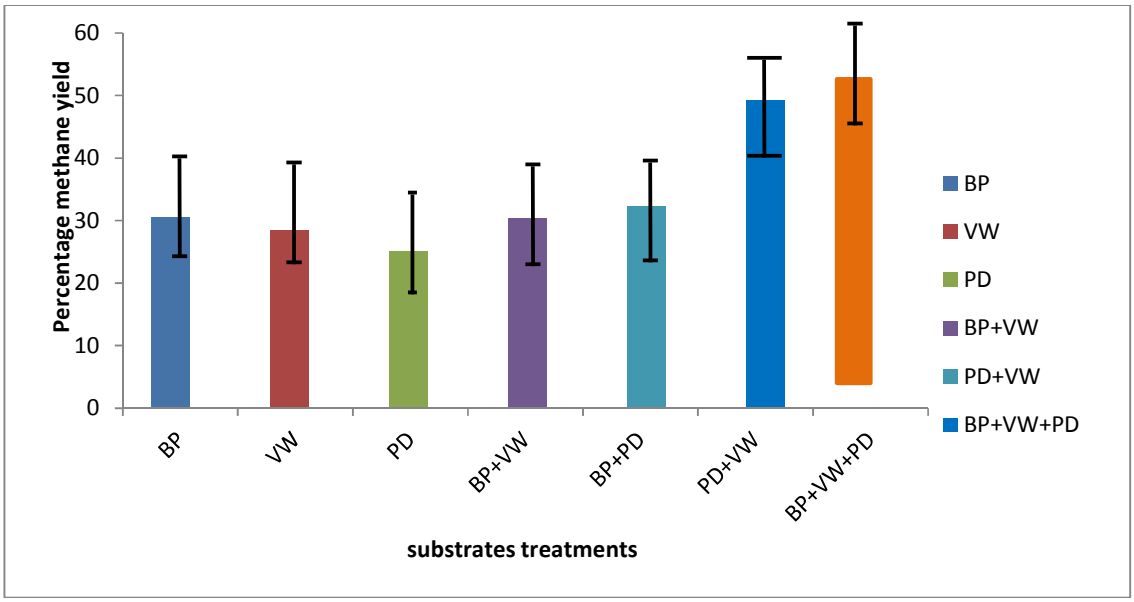

Fig. 7. Percentage methane yield during anaerobic digestion of substrates at varying retention time

Key: BP = Banana peel, VW = Vegetable waste, PD = Pig dung, BP+VW= Banana peel and Vegetable waste, BP+PD = Banana peel + Pig dung, PD+VW = Pig dung and Vegetable waste, BP+VW+PD = Banana peel and Vegetable waste and Pig dung

4. DISCUSSION

The study to investigate microorganism associated with biogas production using Vegetable. (*Telefairaia occidentalis*) waste, Banana peel and Pig dung as substrates was carried out. Significant ($p<0.05$) variations in temperature was observed between the different

substrate treatments before during the anaerobic digestion process. During the anaerobic digestion process, the mean temperatures within the digester ranged between 28℃ and 39℃ compared to the ambient (temperature before digestion) temperature. This observation is in agreement with that reported by [12], who carried out research on biogas production using anaerobic biodigester from cassava starch effluent. Also the result tallied with the temperature ranges reported by [13,14] on similar studies. According to [15], the digestion temperatures within the range are favourable to the hemophilic bacteria populations and well tolerated by anaerobic bacteria for maximum biogas production. pH is an important factor that affects anaerobic digestion process of biogas production. The pH of the slurries (substrates) was observed to have decreased in all the digesters, as a pH range between 6.0 at the end of digestion and 7.4 at the beginning of the digestion was recorded. The observation was not surprising as similar study by [1] on anaerobic digestion of cow dung for biogas production, also reported a decrease in the process pH in the first few days of digestion. Also study by [16] on comparative study of mesophilic biogas production potentials of selected agro-wastes reported a decrease in pH value of the investigated agro-wastes after digestion. The drop of pH observed in this study could have been as a result of the production of metabolites such as acetate, hydrogen gas, carbon dioxide and few other volatile fatty acids such as propionic acting on the substrates in the digesters [16]. Also, the decrease in pH may also be due to the action of acetogenic methanogens which breaks down sulphur containing organic and inorganic compounds as well as the formation of fatty acids dung anaerobic fermentation [17]. Moreover, according to [18], methanogenic bacteria grow and proliferate at a pH range of 6.0-8.2. Also research by [19] reported that there was a perfect link of the acidogenic and methanogenic phases when the pH remained at the range of 6.0-7.4 during anaerobic digestion of substrates.

The results of the total heterotrophic and anaerobic microbial counts in the different substrate treatments before and after anaerobic digestion showed a steady variation in the anaerobic bacteria and fungal counts as fermentation progressed. The count varied significantly ($p<0.05$) between the substrate treatments and the digestion intervals. The counts increased within the first weeks (5-25 days) of digestion but decreased towards the last weeks (30-35 days) of digestion. The observation corroborates with report by [10] on similar studies. This initial increase in bacterial load of the slurries (substrates) may be due to the fact that large populations of anaerobic and facultative anaerobic organisms are usually involved in the hydrolytic and acidogenic phases of methane biogenesis [18]. The highest anaerobic bacterial and fungal counts were recorded in the digester fed with combination of banana peel, vegetable waste and pig dung while the lowest were obtained from the digesters fed with vegetable waste. This might be attributed to the nature of the substrate fed in the digester as this obviously determines the type and extent of fermentative bacteria and fungi present in the digester and the subsequent biogas yield [20]. There are many microbial diversity of biogas digesters which either act singly or synergistically to achieve high production of biogas, as interestingly the microbial species that play crucial role in biogas production are substrate-specific [21]. Different substrate contain varying amount of nutritive contents which the microbes feed and this could also probably be the underlying reasons responsible for the high microbial (anaerobic bacteria and fungi) counts before and after digestion as rerecorded in the digesters fed with combination of Banana peel, Vegetable waste and Pig dung (PB + VW +PD) as compared to other substrate treatment.

According to [22], members of methanogens frequently dominate methanogenic sub-communities in different anaerobic digester systems. Interestingly, it is observed that methanogenic sub-communities within biogas producing consortia are crucial in the anaerobic degradation process for synthesis of methane [23]. Methane producing bacteria identified during the process of biogas production were *Methanothrix sochngenii*, *Methanococcoides methylutens* and *Methanoculleus bourgense*. This observation corroborates with report by [21], who identified *Methanococcoides methylutens*, *Methanobacter SA* and *Ruminatim GIT*, Methanogenium *cariaci VSA GIT* during the investigation of microbial analysis and biogas yield of water hyacinth, cow dung and poultry dropping fed anaerobic digesters.

The result from this study also showed that certain species of bacteria and fungi appeared to extend over one stage of the digestion period to another, suggesting a succession in species of

anaerobic bacteria and fungi during the process of biogas production. The species of bacteria isolated from the substrate before digestion were *Micococcus sp, Klebsiella sp, Shigella sp, Escherichia coli, Pseudomonas sp, Staphylococcus aureus and Citrobacter sp*, while fungal species included *Fusarium sp, Aspergillus sp*, *Penicillium sp* and *Mucor sp*. These organisms were re-isolated during the first week (5-10 days) of digestion in the second week (between 15-20 days) of digestion, species such as *Salmonella sp*, *Serratia sp, proteu, vulgaris* and *Mucor sp*, together with those isolated in the first week except *Klebsiell*a and *Fusarium sp* were isolated. Similarly, the succession of species during the third and fourth weeks (25-30 days) of digestion included *Staphylococcus aureus*, *Micrococcus sp, Pseudomonas geruginosa, Bacillus sp, Escherichia coli, Citrobacter* and *Mucor sp.* These organisms except *Bacillus sp*, *Pseudomonas aeruginosa* and *Mucor sp* were exclusively succeeded by the aforementioned methanogens identified as *Methanothrix sochngenii*, *Methanococcoides methylutens* and *Methanoculleus bourgense.*

These findings are in line with that of [24], who reported that the microbiology of anaerobic transformation of organic wastes is a process which involves many different groups of bacteria such as hydrolyzing, acidifying, acetogenic and methanogenic bacteria which in the final stage produce CO_2 and methane, which is the main products of the digestion process. The marginal increase in the volume of biogas in the second week (5-10 days) and its peak in the third week (20-25 days) indicated the acclimatization of the biogas producing microorganisms after the hydrolysis of the substrates by the hydrolyzing organisms. The action of these biogas producing organisms (mainly methanogens) started declining and these may be due to several factors such as the decrease in pH and increase in temperature of the medium and deposition of microbial metabolites, gradual exhaustion of available nutrient from the substrates and the replacement by organisms that tend to utilize some of the producs of their actions. This probably explains the rationale behind the continued decline in the volume of biogas produced in the fourth (25-30 days) and fifth week (30-35 days) of the digestion process. These observations were in conformity with those reported by [2].

Evidence of methane production was observed between the fourth and fifth weeks (30-35 days) of digestion. The percentage yield of methane also varied significantly ($p<0.05$) between the different treatments. The highest percentage yield of methane was observed in the co-digestion of banana peel, vegetable waste and pig dung and this may be due to the varying proximate composition of the substrate and perhaps the varying number of methanogens isolated from the different substrate treatments.

5. CONCLUSION

The result of this research has shown that organic waste materials such as banana peels, vegetable waste and pig dung can be utilized by microorganisms for biogas production. The utilization of these substrate for this purpose could be possible due to their accessibility by hydrolytic, acetogenic, methanogenic bacteria and fungi. Hence, these afore-mentioned microorganisms play a vital role in biogas production from organic wastes.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Baba S, Shedu U, Abubakar I, Nasir I. Anaerobic digestion of cow dung for biogas production. ARPN Journal of Engineering and Applied Sciences. 2012;7:169-172.
2. Ezeohu S, Dioha I, Eboatu N. Daily biogas production from different wastes and identification of methanogenic bacteria involved. Nigerian Journal of Solar Energy. 2005;15:80-85.
3. Madu C, Sodeinde O. Relevance of biomass in the sustainable energy development in Nigeria. Proceedings of the national engineering conference and annual general meeting of the Nigerian Society of Engineers. 2001;220-227.
4. Garcia J, Patel B, Olivier B. Taxonomic, phylogrnetic and ecological diversity of methanogenic archaeo. Anaerobes. 2000; 6:105-226.
5. Sagagi B, Graba B, Usman N. Studies on biogas production from fruits and vegetable wastes. Bayero Journal of Pure and Applied Science. 2009;2:115-118.
6. Cirne D, Lentomaki A, Blackhall L. Hydrolysis and microbial community analysis in two stage anaerobic digestion

of energy crops. Journal of Applied Microbiology. 2007;103:526-536.
7. Ding S, Crowley M, Hummel E. A biophysical perspective on cellulosome. New perspective for biomass conversion. Current Opinion in Biotechnology. 2008; 19:218-227.
8. Doi R. Cellulases of mesophillc microorganisms. Annual New York Academy of Science. 2008;112:267-279.
9. Verma V, Sigh Y, Rai J. Biogas production from plant biomass used for phytoremediation of industrial wastes. Bioresource Technology. 2007;98:1664-1669.
10. Asikong B, Epoke J, Agbo B, Antai E, Eja M. Four potential of biogas yield from cow dung. European Journal of Experimental Biology. 2013;3:273-282.
11. Olukemi A, Ugoji E. Production of biogas from starchy wastes. Journal of Science and Research Development. 2010;12:34-45.
12. Surnaso S, Siswo S, Budiyono Y. Biogas production using anaerobic biodigester from cassava starch effluent. International Journal of Science and Engineering. 2010;1(2):33-37.
13. Aremu M, Agarry S. Comparison of biogas production from cow dung and pig dung under mesophilic condition. International Referred Journal of Engineering and Science. 2012;4:16-21.
14. El-Mashed H, Zeeman G, Loon W, Bot G, Lettinga G. Effect of temperature fluctuation on thermopholic anaerobic digestion of cattle manure. Bioresources Technology. 2003;95:213-221.
15. Adelekan B, Bamgboye A. Comparison of biogas productivity of cassava peels mixed in selected ratios with major livestock waste types. African Journal of Agricultural Research. 2009;4:571-577.
16. Tsunatu D, Yavini U, Usman H, Taura N, James M. Comparative study of mesophilic biogas production potentials of selected agro-wastes. International Journal of Engineering and Science. 2014;3(2):1-6.
17. Roa M, Sigh S, Sodha M. Bioenergy conversion studies of the organic fraction if MSW assessment of ultimate bioenergy production potential of municipal garbage. Applied Energy. 2000;66:76-78.
18. Viswanath P, Devi S, Ivand K. Anaerobic digestion of fruit and vegetable processing waste for biogas production. Bioresource Technology. 1992;40:43-48.
19. Anuputtikul W, Rodtong S. Investigation of the potential production of biogas from cassava tuber. 2004;70.
20. Nagamiani B, Rasmasamy K. Biogas production technology from utilisinia peels and some animals wastes. International Journal Physical Science. 2003;4(7):398-402.
21. Asikong B, Udensi O, Epoke J, Eja M, Antai E. Microbial analysis and biogas yield of water hyacinth and cow dung digesters. British Journal of Applied Science and Technology. 2014;4(4):650-661.
22. Jaenicke S, Zakzewski M, Ander C, Bekel T. Comparative and joint analysis of two metagenomic data sets from a biogas fermenter by 454-phyro sequencing. Plus One. 2011;6(1):14-19.
23. Dhevagi P, Ramasamy R, Oblisami G. Biological nitrogen fixation and biogas technology. Bioresource Technology. 1992;6:14-23.
24. Demirel B, Scherer P. The roles of acetotrophic and hydrogenotrophic methanogens during anaerobic conversion of biomass of methane. Review of Environmental Science and Technology. 2008;7:73-901.

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