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Microbial Analysis and Biogas Yield of Water Hyacinth, Cow Dung and Poultry Dropping Fed Anaerobic Digesters

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Authors' contributions

This work was carried out in collaboration between all authors. Authors BEA and JE designed the study, wrote the protocol, and wrote the first draft of the manuscript. Author OUU performed the statistical analysis, interpreted, managed literature searches and wrote the final copy of the manuscript. Authors EME and EEA performed the microbiological analyses and microscopy. All authors read and approved the final manuscript.

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ABSTRACT

Aim: Biogas research and subsequent production is fundamental to mitigating the possible depletion of crude oil and energy crisis, especially in Nigeria. This research paper was aimed at evaluating the biogas production capacity of water hyacinth, poultry droppings, cow dung and their combination.
Methods: One kilogram, two kilograms and three kilograms weight of these substrates were subjected to anaerobic digestion in starter and without starter cultures for 45 days at the interval of 5 days.
Results: Our results showed that heterotrophic bacteria and fungi counts were substrate-specific with poultry dropping fed-digester having the highest. Different bacteria and fungi were isolated including methane-producing bacteria such as *methanolreoibacteria, methanoculleus bourgense, methanogenium cariaci, methanocorpusium parvum, methanoscrcimon barkeri, methanoplanus lunicola, methanococcoides methyluteus* and

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methanothrix sochngenic. There were significant differences in the amount of biogas produced by the different substrates. However there was no significant differences (P>0.05) between the biogas produced by water hyacinth-fed digester (170.41mls) and poultry droppings-fed digester (182.88 mls). Combining all the substrates (WH+PD+CD) yielded the highest biogas (423.80 mls), which was followed by biogas production of cow dung (331.8 mls).
Conclusion: Explicitly, our present report showed that higher biogas yield can be achieved by the combination of different biogas feedstock.

Keywords: Water hyacinth; agro-wastes; biogas production; energy crisis.

1. INTRODUCTION

The ever-increasing price and shortage of fossil fuel, coupled with global concerns on climate change and severe food insecurity has triggered the search for new natural materials that will be amenable to processing during extraction, thus reducing costs drastically and substituting fossil fuel. The anaerobic digestion of these organics has long been used to generate useful resources, which have been harnessed for the use of mankind [1].

Nigeria, indeed Cross River State has abundant, diverse and unexploited renewable energy resources for the production of fuel, which undoubtedly will help in no small measure to solving our energy crisis and poverty [2,3,4]. Importantly, [5,6] identified two challenges in the 21st century: firstly, the development and use of renewable energy to decrease over-dependence on fossil fuel and secondly, the management of the waste generated by human activities. This obviously paints the real picture of the developing countries of the globe such as Nigeria.

Critically, achieving the Millennium Development Goals (MDGs) in Africa requires a significant expansion of access to modern and alternative renewable energy such as biogas, which is of growing interest for the sustainable management of our waste and a major breakthrough in the search for a renewable energy for the reduction in over-dependence on non-renewable fossil fuel [7,8]. Biogas consists of 50 – 70%, methane 30 – 40%, carbon dioxide 5 – 10%, hydrogen 1 – 2%, nitrogen 0 – 3% , water vapour and traces of hydrogen sulphide, carbon monoxide and oxygen. It is colourless, relatively odourless and flammable. It is also stable and non-toxic. It burns with a blue flame and has a calorific value of 4500 – 6000kcal/m^3 when its methane content ranges between 60 – 70% [3,8].

Water hyacinth (*Eichhornia crassipes*) has been reported to serve as organic fertilizer [9], therapeutic agent [10] and recently as a good candidate for the biosynthesis of biofuel. This is however, premised on the fact that it has high cellulose with low lignin content per unit volume of dry matter [11,12], easily degradable and will not compete with arable crops for space, light and nutrients.

The option of using this plant as feedstuff for bio-fuel production will solve the following problems: (a) save the huge sums of money spent in its control annually [10], (b) prevent marshes from becoming breeding grounds for various disease-spreading insects and pests and (c) solve its interference problems with irrigation channels, fishing and recreation activities. Poultry droppings and cow dung though used as fertilizers constitute environmental pollution [13,14] and thus the need to search for an alternative measure for their being converted to other useful ventures.

This research is aimed at determining the potentials of water hyacinth, poultry droppings, cow dung and their combinations in biogas generation.

2. MATERIALS AND METHODS

2.1 Sample Collection and Microbial Screening of Collected Samples

Fresh leaves of water hyacinth were obtained from Itigidi, Cross River State, Nigeria while poultry droppings and cow dung were obtained from University of Calabar poultry farm and abattoirs in Calabar, Nigeria, respectively. Ten kilograms of each sample was put in sterile polythene bags for analysis, which was done within 24 hours. Leaves of water hyacinth were cut into pieces of about 2 to 5mm size, while poultry dropping and cow-dung were well pulverized. Ten grams of each were mixed with 90mls of sterile distilled water in 250mls Erlenmeyer flask. The mixtures were vortexed and agitated thoroughly and allowed to stand for ten minutes.

The supernatant was decanted and one milliliter volumes were prepared in ten-fold serial dilutions. Dilutions of 10^{-5} to 10^{-7} were plated on nutrient agar supplemented with 50µgml^{-1} Nystatin to prevent fungal growth using surface plating. Plates were incubated for 24 – 48 hours at 35ºC. Colony forming units per gram (cfug^{-1}) of bacterial growth between 30 – 300 colonies were enumerated. For screening of fungi, dilutions of 10^{-3} to 10^{-4} of the supernatant were plated on Sabouraud's dextrose agar supplemented with 100mgml^{-1} streptomycin and 15mgml^{-1} of penicillin (to inhibit bacterial growth). This was incubated for 72 to 96 hours. Plates with fungal colonies of between 30 to 300 were enumerated in cfug^{-1}. Methods of [15,16] were employed for isolation and characterization of fungi. All plating was done in triplicates.

2.2 Preparation of Inoculum

The method of [17] were employed. The support activated carbon (charcoal) was washed 5 times with acetate buffer pH (4-5) and finally re-suspended in the buffer overnight. Twenty kilogram weights were placed in storage containers and kept at 10°C in a refrigerator. Twenty kilograms weight of the slurry (residue w/v) of an old but active cow dung digester was mixed with 20kg weight of the pre -treated activated carbon and incubated at room temperature in anaerobic condition for 40 days. The adsorbed cells were used as crude inoculum for all digesting combinations. The advantage of using the activated carbon as support for the immobilisation was that it was relatively cheap and affordable, readily available, mild and posses no problem of cell and enzyme inactivation.

2.3 Preparation of Substrates for Biogas Production

The methods of [18] as modified by [19] was used. The three substrates, respectively were mixed in the ratio of 0.33:0.33:0.33; 0.66:0.66:0.66 and 1:1:1 weights to yield total weights of about 1kg, 2kg and 3kg. The operational mode was the batch method using an operational mesophilic temperature. Respective weights were mixed with water at the ratio of 1:3 and placed in the digesters. Duplicate of each weight were prepared, one without inoculum and the other with 1kg weight of inoculum from an old digester slurry mixed with charcoal. The digesters were tightly corked with rubber stopper to create anaerobic condition and connected to a gasometrical chamber. Biogas was monitored and measured daily over a

period of 45 days using the gasometrical chamber with the displacement of paraffin wax. The total biogas yields were determined by opening the outlet tap of the anaerobic digester and the inlet tap to the graduated burette. The biogas generated was released through the tube which then displaced the paraffin oil in the graduated burette downward. The volume of gas yield was determined by the volume of paraffin oil displaced, i.e gas yield was directly proportional to paraffin oil displaced.

2.4 Data Collection and Analysis

Data on total heterotrophic bacteria and fungi, biogas yield were subjected to analysis of variance using PASW version 18.0 and significant means were separated using Least Significant Difference (LSD).

3. RESULTS AND DISCUSSION

3.1 Results

Our result revealed that the highest bacteria count was obtained in the culture containing poultry droppings- fed digester (without inoculum), which differs slightly from those obtained in the culture of cow dung. There were significant differences ($p<0.05$) observed in the bacteria and fungi counts before and after anaerobic digestion, which was substrate dependent and with or without inoculum –specific (Table 1). The following bacteria species were identified and isolated from the culture; *E.coli, P. aerugenosa, B. cereus, R. dacucus, C. freundi, K. pneumonia, P. vulgaris, S. dysenterae, Y. pestis, S. cholmesuios and S. aureus* while *Fusaruim spp, Aspergllus spp, Penicillium spp, Mucor spp, Aspergillum flavus* were the fungi species that were identified and isolated (Tables 2 and 3).

Additionally, other specific methane-producing bacteria were also identified and isolated such as *methanolreoibacteria, methanoculleus bourgense, methanogenium cariaci, methanocorpusium parvum, methanoscrcimon barkeri, methanoplanus lunicola, methanococcoides methyluteus* and *methanothrix sochngenic* (Table 4).

3.1.1 Biogas yield

There were significant differences in the amount of biogas produced by the different substrates. However there was no significant differences ($P>0.05$) between the biogas produced by water hyacinth-fed digester (170.41mls) and poultry droppings-fed digester (182.88 mls). Combining all the substrates (WH+PD+CD) yielded the highest biogas (423.80 mls), which was followed by biogas production of cow dung (331.8 mls) (Fig. 1). There were also differences recorded ($P<0.05$) among the different substrate inclusions. The biogas yield increased as the quantity of substrate increased. The 3kg weight produced the highest biogas (364.40mls) followed by 2kg (274.59mls) and then 1kg yielded the least (192.68 mls) the substrate type notwithstanding (Fig. 2).

Table 1. Total viable bacteria and fungi counts from substrates slurry before and after anaerobic digestion

	Without inoculum					With inoculum				
	Control (water)	WH	PD	CD	WH+PD+CD	Control (water)	WH	PD	CD	WH+PD+CD
BCBD (cfug^{-1})	5.54 x10^{8}	5.46 x10^{7}	8.83 x10^{7}	8.65 x10^{7}	7.55 x10^{7}	5.54 x10^{8}	6.40 x10^{8}	8.60 x10^{8}	8.45 x10^{8}	7.65 x10^{8}
BCAD (cfug^{-1})	4.40 x10^{5}	3.55 x10^{5}	5.54 x10^{5}	6.45 x10^{5}	4.10 x10^{5}	4.40 x10^{5}	4.55 x10^{5}	6.54 x10^{5}	7.35 x10^{5}	5.25 x10^{5}
FCBD (cfug^{-1})	4.40 x10^{4}	1.46 x10^{4}	3.42x10^{4}	3.55 x10^{4}	2.35 x10^{4}	4.40 x10^{4}	2.46 x10^{4}	4.42 x10^{4}	4.55 x10^{4}	3.35 x10^{4}
FCAD (cfug^{-1})	3.15 x10^{2}	1.20 x10^{2}	2.26 x10^{2}	2.25 x10^{2}	1.20 x10^{2}	3.15 x10^{2}	2.20 x10^{2}	3.26 x10^{2}	3.25 x10^{2}	2.20 x10^{2}

BCBD = Bacteria counts before digestion; BCAD = Bacteria counts after digestion; FCBD = Fungi counts before digestion; FCAD = Fungi counts after digestion; WH = Water hyacinth; PD = Poultry droppings; CD = Cow dung; WH+PD+CD = Combined.

Table 2. Characterization and identification of bacterial isolates

Code	Gram reaction	Morphology	Motility	Odidase	Catalase	Indole	Citrate	Glucose	Lactose	Manitol	Urease	H^2S	Nitraye	V. Proskauer	Methyl Red	Coagulase	O_2 requirement	Probable Isolate
ABE_1	-	Single rod	+	-	+	+	-	+	+	+	-	-	+	-	+	-	±	*Escherichia coli*
ABE_2	-	Coccobacili	±	±	±	-	+	-	-	-	-	-	+	-	-	-		*Pseudomonas aerugenosa*
ABE_3	+	Straight rods	+	+	+	+	+	+	-	-	+	+	-	+	-	-		*Bacillus cereus*
ABE_4	-	straight slightly curved	+	+	+	-	-	+	-	-		+	+		-	-		*Rhizobacter dacucus*
ABE_5	-	straight rod	+	-	+	-	+	+	+	+	-	-	+	-	+	-	±	*Citobacter freundii*
ABE_6	-	straight rod	-	-	+	+	+	+	+	+	NT	-	+	+	-			*Klebsiella pneumonia*
ABE_7	-	straight rod	+	-	+	+	+	+	+	+	NT	-	-	+	-			*Proteus vulgaris*
ABE_8	-	straight rod	+	-	-	-	+	+	+	+		-	+	+	±	-	±	*Seriatia*
ABE_9	-	straight rod	-	-	+	±	-	-	+	+	-	-		+	+	-		*Shigella dysentenae*
ABE_{10}	-	Straight rods	±	-	+	±	-	+	+	+	+	+	-	-	+	-		*Yersinia Pestis*
ABE_{11}	-	Straight rods	-	-	+	-	+	+	+	+	+	-	+	+	-	+	-	*Salmonella choleraesuis*
ABE_{12}	-	Spherical	-	-	+			-	+	+	+	ND	ND	+	ND	+		*Staphylococcus aureus*

Table 3. Characterization and identification of fungal isolates

Colony code	Colour of hyphae	Macroscopic features	Probable organism
ABEF1	Pink wooly	Narrow septate hyphac, conidiophores occur singly and in groups, multicellular crescent shaped macronidia	*Fusarium* spp.
ABEF2	Black velvety	Septate and broad hyphae, large head entirely covered with phalides bearing chains of conidia.	*Aspergillus* spp.
ABEF3	Greenish velvety	Septate hyphae, conidiophore developed into branched phalides bearing chains of conidia brush-like appearance.	Penicillium spp.
ABEF4	White fluffy	Aseptate broad hyphae large spherical head without rhziod	Mucor spp.
ABEF5	Brownish velvety	Septate hypae, narrow hyphae, spherical head entirely covered with phalides bearing chins of conidia	*Aspergillus flavus*
ABEF6	Dack-brown (tan)	Aseptate, round sporangium	*Mucor* spp.
ABEF7	Black velvety	Septate and broad hyphae with large head entirely covered with phalides being chains of conidia	*Aspergillus* spp.
ABEF8	Greenish velvety	Septate hypae, conidiophere developing into branched phalides bearing chains of conidia brush like appearance.	*Pericillium* spp.

Table 4. Methane producing bacteria during the process of biogas production

S/N	Morphology/shape	grams reaction	motility	catabol substrate $H_2 + CO_2$	formate acctate	Organic factor	pH	Temperature of growth	Isolates
1	Short rods in pairs or chain	+	-	+	++		6-7	37-40 ºC	*Methanolreoibacteria SA*
2	Irregular Cocci occurring single	+	-	+	++		6-8	35 – 40 ºC	*Ruminantum GIT*
3	Irrgular cocci	-	-	+	++		7.0	20 – 40 ºC	*Methanoculleus bourgense VSA GIT*
4	Short curved rods	-	+	+	++		6.1 -69	40 ºC	Methanogenium cariaci VSA GIT
5	Small irregular Cocci	-	-	+	++		6-7.5	30 -40 ºC	*Methanocorpuslum Parvum VSA AD*
6	Irrgular sphaeroid alone or group va aggregate		-	+	++		6-7.5	30 -40 ºC	*Methanoscrcinon barkeri VSA*
7	Very Irregular cocci plate shape	-	+	+	++		6.0-7.5	30 -40ºC	*Methanoplanus lunicola* VA AOS
8	Extremely irregular cocci single or in pairs	-	-	+	+		7.0 -7.5	30 – 35 ºC	*Methanococcoides methylutens*
9	large sheathed rods	-	-	+	+		7.1 -7.8	35 -40 ºC	*Methanothrix sochngenii*

Biogas production was also dependent on the use of either starter or without inoculum. The yield of biogas when inoculum was used was significantly higher (319.05 mls) than when inoculum was not used (235.40 mls) (Fig. 3). However, there were no interactions between the substrates, quantity of inclusion and with or without inoculum. It was observed that the yield of biogas was highly dependent on the duration of anaerobic digestion, the substrate notwithstanding. The trend was such that it gradually increased from 5 days of digestion and become optimal in the 15th and 20th day, especially for the combined substrate (653.33 and 695.83mls) before declining significantly from the 25th day to the 40th day (Fig. 4). However, the optimal biogas yield for cow dung was achieved at the 15th day of digestion (610.83 mls).

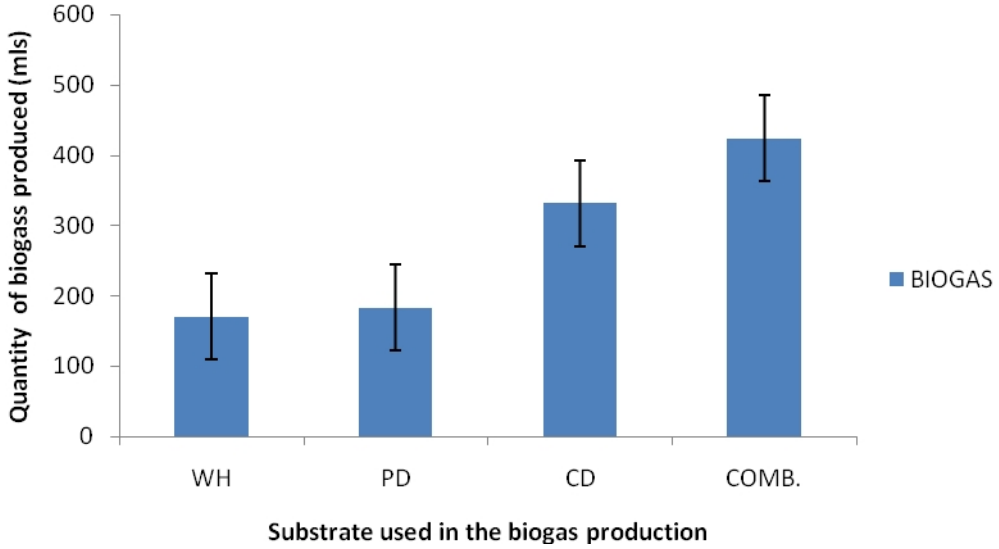

Fig. 1. Effect of substrates on biogas yield

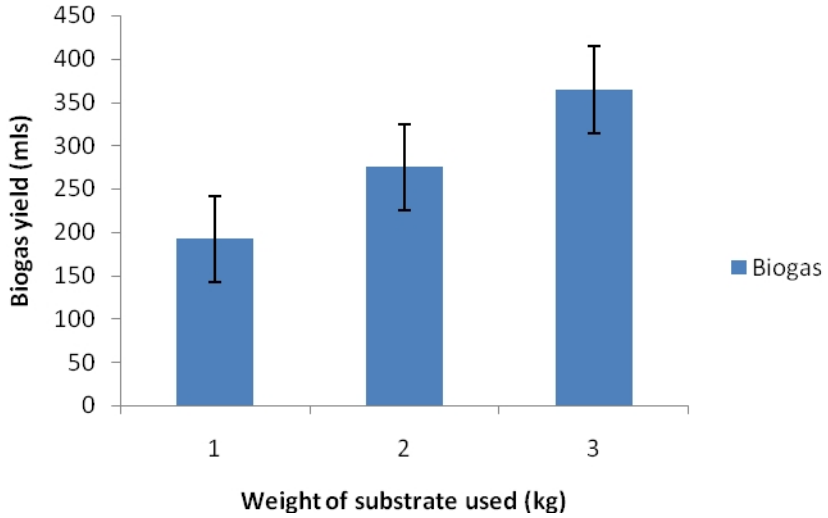

Fig. 2. Effect of quantity of substrates on biogas yield

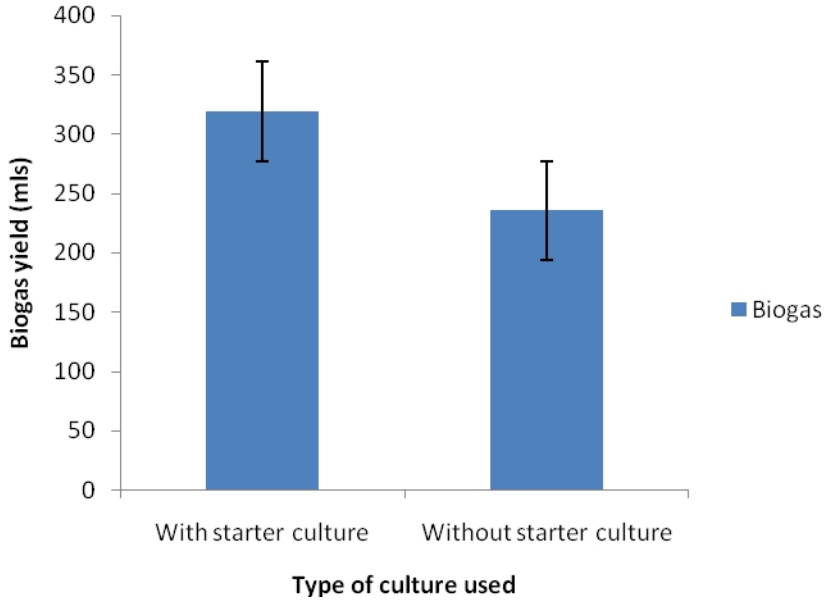

Fig. 3. Effect of with or without inoculum on biogas yield

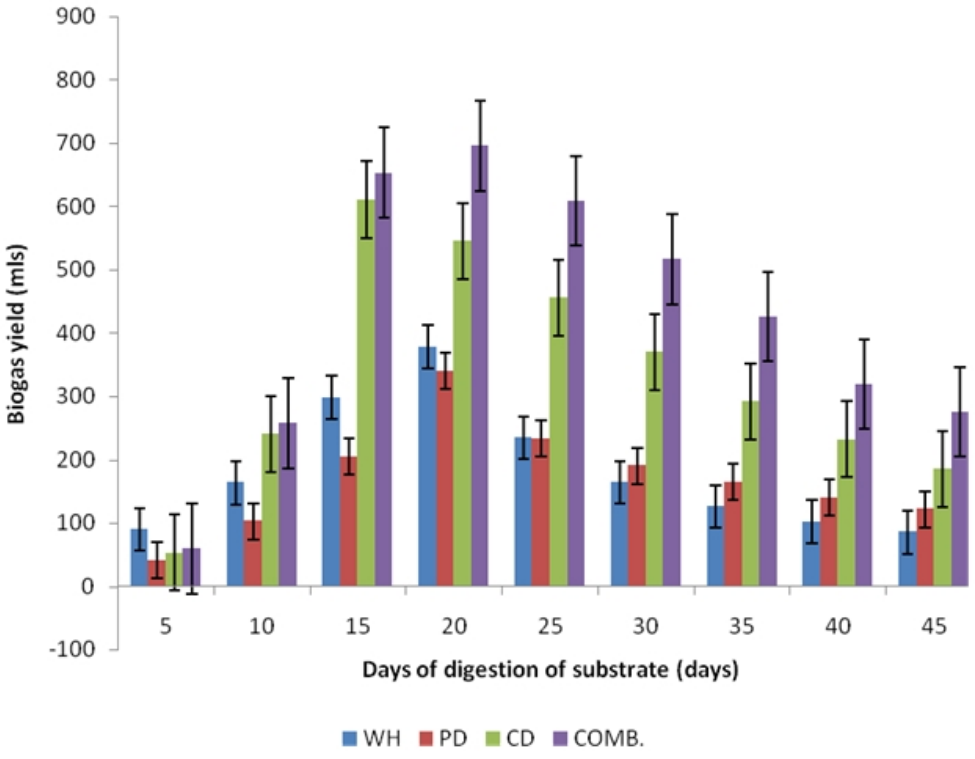

Fig. 4. Effect of digestion duration on biogas yield

3.2 Discussion

The worldwide shortage and rising price of fossil energy, together with climate and environmental protection aims, have led to enhanced development and utilization of alternative energy resources. This is predicated on the fact that the production of biogas from microbial degradation of organic matter is of great importance, since biogas is environmental friendly [20,21]. Regrettably, though biogas production technology has established itself as a technology with great potential, which could exert major influence in the energy scene; however, it has not made any real impact on the total energy scenario despite the presence of about 1.8 million biogas digesters. This might be attributable to the nature of the substrate fed in the digester as this obviously, determines the type and extent of the fermentative bacteria present in the digester[7] and the subsequent biogas yield.

There are many microbial diversity in biogas digesters, which either act singly or synergistically to achieving high production of biogas. Interestingly, the microbial species that play crucial role in biogas production are substrate-specific. Though there was no systematic trend followed as regards bacteria and fungi counts before and after digestion, the counts were substrate-specific. I should be understood that different substrate contain varying amount of nutritive contents, which the microbes feed. This could be the underlying reason responsible for the high microbial counts before and after digestion recorded in the poultry and cow dung-fed digesters, with or without inoculum.

According to [21,22] members of the genus *Methanoculleus* frequently dominate methanogenic subcommunities 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. Many methanogens were identified and isolated in our present study. [23] used different feedstocks like cow dung, buffalo dung, dry animal waste, stray cattle dung, goat waste, and poultry droppings for their biomethanation potential and observed that poultry droppings showed higher gas production. Earlier [24] compared the rates of biogas yield from pig dung-fed and cattle dung-fed digesters and reported that the biogas yield was higher in the former. They attributed this higher biogas yield to the presence of native microflora in the dung. Contrary to the reports of [23,24], the biogas yield of the combined substrate-fed digester notwithstanding, cow dung-fed digester produced the highest quantity of biogas. It is probable that the native microflora present in the dung might be species-specific as the strain of the animal producing the dung might be implicated.

[25] reported higher presence of proteolytic organisms in cow dung-fed digesters and other animal's waste-fed digesters. However, [26] observed that while cow dung-fed digesters supported higher amylolytic microorganisms, poultry waste-fed digesters showed higher proteolytic population. This could answer why cow dung fed-digester gave the highest quantity of biogas singly. It will make sense to affirm that most of the proteolytic bacteria isolated in this report might aid delayed degradation since they produce toxic substances such as phenols, ammonia, etc. Our result revealed that the combined substrate - fed digester produced the highest quantity of biogas (Fig. 1). Undoubtedly, it will be proper to think that the different composites should contribute their integral biogas yield to the combined pool. This was succinctly the case in our report. The main problem in biogas production is the capacity of the substrates to have a methane-producing reservoir that will be subsequently converted into biogas by methanogenic bacteria. It thus implies that cow dung has more of methane producing reservoir comparing water hyacinth and poultry droppings.

It was observed that the time of anaerobic digestion in the substrate-fed digester affected biogas yield. Obviously, biogas production was at its peak within 15 and 20 days of digestion, after which the yield declines, the substrate notwithstanding. This might probably be as a result of the particulate-bound bacteria that predominate up to 20th day of initiation of biogas digester. There is usually active degradation of substrate at the beginning of digestion. However, as the degradation progressed, the source of energy declines, leading to low microbial activity vis-a-vis biogas yield. The implication is that there may not be any need to elongate digestion in the digesters due to the cost effect of running the remaining days. It might rather be necessary to increase the substrate quantity and harvest biogas within these intervals of days. According to our report, increase in the quantity of the substrate fed in the digester resulted to increase biogas yield. This is not unconnected to the number of microbes feeding on the energy source. The implication is the greater the quantity of substrate, the higher the number of microbial community, culminating to increase biogas yield. This was the case in our research.

Presence of some metals influences biogas production. [26,27,28] as they have been indicted for increasing methanogenic population. It seems to suggest that methanogenic bacteria produce enzymes that are metalo-specific, which are involved in biogas production. This might explain the differentials observed in the biogas producing capacity of the substrate used in the current report. Our result also revealed that biogas yield in the digester with inoculum was higher than the digester without inoculum. It thus therefore suggests that priming the degradation with inoculums will enhance biogas yield. The microorganisms identified and isolated, especially the methanogens are of immense importance. This is premised on the fact that they could be genetically manipulated to either increase their capacity to degrade or their genes cloned and moved into other microbes to confer on them the same capacity. It thus suggest that instead of allowing microorganisms that may not have any part in the pathway leading to biogas production to crowd the substrate, there will be more precise and target oriented anaerobic digestion. Worthy of note is the fact that manipulation of the identified and isolated microbes to enhance their capacity to degrade biogas substrates will be a good approach in the right direction.

However, the genetic manipulation of the substrate will undoubtedly, complement this venture for a holistic and improved biogas yield. Unfortunately, of the three substrates used in this research, water hyacinth is the only one that can be manipulated but disappointedly, produced the lowest quantity of biogas. This notwithstanding, the underlying factor(s) leading to the biogas yield recorded against poultry droppings and cow dung-fed digesters could be exploited, harnessed and optimized in water hyacinth genome. This becomes pertinent taking into cognizance the fact that poultry droppings and cow dung as well as other organic-like substrates may not be available in a good quantity that will yield the required biogas if the energy crisis, especially in Nigeria is anything worth solving.

4. CONCLUSION

Explicitly, our results showed that heterotrophic bacteria and fungi counts were substrate-specific with poultry dropping fed-digester having the highest. It also revealed that higher biogas yield can be achieved by the combination of different biogas feedstock. Different bacteria and fungi were isolated including methane-producing bacteria such as *methanolreoibacteria, methanoculleus bourgense, methanogenium cariaci, methanocorpusium parvum, methanoscrcimon barkeri, methanoplanus lunicola, methanococcoides methyluteus* and *methanothrix sochngenic.* However, there are two most important criteria in the choice of selecting feed stocks for biogas production: economic

considerations and biogas yield. It thus suggest that a second consideration should be made on water hyacinth giving its capacity to be manipulated genetically and fortified with metals that could increase microbial population, resulting in higher biogas yield.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Momoh OLY, Nwaogazie LI. Effect of waste paper on biogas production from co-digestion of cow dung and water hyacinth in batch reactors. J. Appl. Sci. Environ. Manag. 2008;12(4):95–98.
2. Itodo IN, Agyio GE, Yusuf P. Performance evaluation of a biogas stove for cooking in Nigeria. J Energy in Southern Africa. 2007;18:(3):14–20.
3. Igoni A. Hikiah Ayotamuno MJ, Eze CL, Ogaji SOT, Robert SD. Designs of anaerobic digesters for producing biogas from municipal solid – waste. Appl Energy, 2008;8:430–438.
4. Mashandete AM, Parawira W. Biogas Technology Research in Selected Subsaharan African Countries – A Review. Afri J Biotechnol. 2009;8(8):116-125.
5. Guruswamy T, Kannan N, Kumar V. Design, Development and evaluation of biogas using selected biomaterials as feedstock. World J Microbiol biotechnol. 2003;84:65.
6. Alvarez R, Gunnar L. The effect of temperature variation on biomethanation. Biores Technol. 2007;99:7278-7284.
7. Nagamiani B, Ramasamy K. Biogas Production Technology: An Indian Perspective Utilisinia) peels with some animal wastes. Intl J Phy Sci. 2003;4(7):398–402.
8. Adeyanju AA. Effects of seeding of wood-ash on biogas production using pig waste and cassava peels. Journal of Engr Appl Sci. 2008;*3*(3):242–245.
9. Gunnarsson CC, Petersen CM. Water hyacinths as a resource in agriculture and energy production: A literature review. Waste Manag. 2007;27:117-129.
10. Bhattacharya A, Kumar P. Water hyacinth as a potential biofuel crop. Elect J Environ Agric Food Chem. 2010;9(1):112-122.
11. Poddar K, Mandal L, Banerjee GC. Studies on water hyacinth (*Eichhornia crassipes*) – Chemical composition of the plant and water from different habitats. Ind. Vet. J. 1991;68:833-837.
12. Gressel J. Transgenics are imperative for biofuel crops. Plant Sci. 2008;174:246-263.
13. Long C. Review and Prospect of Biogas development. China Biogas. 1992;10:104.
14. Lung MS, Anderson SS, Torry-Smith M. Building of flexible biogas. biogas digester in Tanzania, student Report, Technical University of Denmark, Copenhagen; 1996. Available: http://www.fao.org/sp/egdirect/egre0022.htm. 4/20/2007/
15. Ogundero SK. Thermophilic fungi from Nigeria palm produce. Mycologia. 1981;13(1):198–200.
16. Hunter-Cenera JC, Fonda ME, Belt A. Isolation of cultures. In: American Annual Review of Industrial Microbiology and Biotoechnology. 1986;50:1-23.
17. Geluk MA, Norde W, Vankalsbeck HKI, Van't R. Adsorption on lipase from *Candida rugosa* on cellulose and its influence on lipolytic activity. Enzyme Micro. Technol 1992;24:748–754.

18. Chae KJ, Jang AM, Yim SK. The effects of digestion temperature and temperature shock on biogas yields from the mesophilic anaerobic digestion of swine manure. Bioresource Technology 2007;49(4-5):427-434.
19. Asikong BE, Epoke J, Antai EE. Potentials of biogas generation by water hyacinth (*Eichhornia crassipes*) and cassava (*Manihot esculentum*) peels in Cross River State, Nigeria. Global J Environ Sci. 2012;11(1&2):9-20
20. Jaenicke S, Ander C, Bekel T, Bisdorf R, Droge M, Gartemann KH, Junemann S, Kaiser O, Krause L, Tille F, Zakrzewski M, Puhler A, Schluter A, Goesmann A. Comparative and joint analysis of two meta-genomic datasets from a biogas fermenter obtained by 454-pyrosequencing. PLOS ONE. 2011a;6(1):14519.
21. Jaenicke S, Zakrzewski M, Junemann S, Puhler A, Goesmann A, Schluter A. In: Handbook of molecular microbial ecology II: Metagenomics in different habitats. De Bruijn F. T (Ed.) Wiley- Blackwell; 2011b.
22. Zakrzewski M, Goesmann A, Jaenicke S, Janemann S, Eikmeyer F, Szezepanowski R, Al-Soud WA, Sorensen S, Puhler A, Schluter A. Profiling of the metabolically active community from a production-scale biogas plant by means of high through-put meta-transcriptome sequencing. Journal of Biotechnology, 2012;158(4):248-258.
23. Dhevagi P, Ramasamy K, Oblisami G. In: Biological nitrogen fixation and biogas Technology (eds Kannaiyan, S., Ramasamy, K., Ilamurugu, K. and Kumar, K.), Tamil Nadu Agricultural University, Coimbatore; 1992.
24. Yeole TY, Ranade DR. Alternative Feedstocks for Biogas. 2007;1992:10–16.
25. Ramasamy K, Nagamani B, Sahul Hameed M. Tech. Bull. Fermentation Laboratory, Tamil Nadu Agricultural University, Coimbatore. 1990;1:91.
26. Preeti Rao P, Seenayya G. Improvement of methanogenesis from cow dung and poultry litter waste digesters by addition of iron. World J. Microbiol. Biotechnol. 1994;10:211–214.
27. Seenayya G, Rao CV, Shivaraj D, Preeti Rao S, Venkatswamy M. Final report submitted to Department of Non-Conventional Energy Sources, Government of India, New Delhi; 1992.
28. Geetha GS, Jagadeesh KS, Reddy TKR. Nickel as an accelerator of biogas production in water hyacinth (*Eichornia crassipes* solms. Biomass. 1990;21:157–161.

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