



Evaluation of Ratio Variation of Water Hyacinth (*Eichhornia Crassipes*) on the Production of Pig Dung Biogas

Adegunloye D.V., Olosunde S.Y. and Omokanju A.B.

Department of Microbiology, Federal University of Technology, P. M. B. 704 Akure, NIGERIA

Available online at: www.isca.in

Received 17th January 2013, revised 6th February 2013, accepted 26th February 2013

Abstract

Evaluation of ratio variation of water hyacinth (*Eichhornia crassipes*) on the production of pig dung biogas was investigated. Water hyacinth was blended and mixed with pig dung in different ratio. The blended water hyacinth and pig dung was weighed in ratio 1:1 and 1:3. These were mixed together separately in a sterile container with water to form slurry, they were then poured individually into two digesters. The temperature of the digesting materials and the environment was determined daily; also the pH was measured every two days. The temperature was between 27^oC – 34^oC in the digesting materials while the environmental temperature was between 25^oC – 32^oC. The pH was within the range 4.8 - 6.8. A total of twelve bacteria, five fungi and one yeast were isolated during digestion of materials. The chemical composition of the gases of water hyacinth and pig dung in ratio of 1:1 were 83.40% methane, 0.01% ammonia, 0.03% carbon dioxide, 1.86% carbon monoxide, 5.85% hydrogen sulphide and 8.89% other gasses. The ratio 1:3 were 88.3% methane, 0.04% ammonia, 0.02% carbon dioxide, 1.30% carbon monoxide, 4.10% hydrogen sulphide and 6.20% traces of other unknown gasses. There was increase in the percentage composition of the methane gas when the water hyacinth was just in ratio one and pig dung ratio three, that is the lower the water hyacinth the more will be the methane produced.

Keywords: Water hyacinth, pig dung, digester, pH, temperature, methane gas

Introduction

Water hyacinth is a free-floating perennial aquatic plant, native to tropical South America. With broad, thick, glossy, ovate leaves, water hyacinth may rise above the surface of the water as much as 1 meter in height. The leaves are 10–20 cm across, and float above the water surface. They have long, spongy and bulbous stalks. The feathery, freely hanging roots are purple-black. The seven species of water hyacinth comprise the genus *Eichhornia*. Water hyacinth is one of the fastest growing plants known, reproduces primarily by way of runners or stolons, which eventually form daughter plants. It also produces large quantities of seeds, and these are viable up to thirty years. The common water hyacinth (*Eichhornia crassipes*) is vigorous growers known to double their population in two weeks. Biogas typically refers to as gas produced by the biological breakdown of organic matter in the absence of oxygen. Biogas originates from biogenic material and is a type of biofuel¹. One type of biogas is produced by anaerobic digestion or fermentation of biodegradable materials such as biomass, manure, sewage, municipal waste, green waste, plant material and energy crops. This type of biogas comprises primarily of methane and carbon dioxide. The other principal type of biogas is wood gas which is created by gasification of wood or other biomass. This type of biogas is comprised primarily of nitrogen, hydrogen, and carbon monoxide, with trace amounts of methane. The gases methane, hydrogen and carbon monoxide can be combusted or oxidized with oxygen contain 21% oxygen². The composition of biogas varies, depending upon the origin of the anaerobic digestion

process. Landfill gas typically has methane concentrations around 50%. Advanced waste treatment technologies can produce biogas with 55–75% CH₄ or higher using in situ purification techniques³.

Pigs are primarily rooting animals, feeding on roots they dig up from the soil which are mainly gregarious in nature^{4,5}. Pig manure is rich in potash and when well humified, is best applied to root crops, especially potassium-hungry leeks, celeriac and potatoes⁵. Pig manure contains a variety of pathogen, which can cause disease in man. These pathogens may be bacteria, helminthes, fungi, viruses, or protozoa such as *Salmonella* spp., *Escherichia coli*, *Coxsackie virus*, *Adenovirus*, *Reovirus*, *Candida* spp., *Aspergillus* spp., etc. This study therefore is aimed at determining the types of microorganisms responsible for the digestion of pig dung and water hyacinth, as well as checking the varying temperatures and pH of digestion and composition of biogas produced when mixed pig dung with water hyacinth in different ratios.

Material and Methods

The samples used for this study were pig dung and water hyacinth. Pig dung was collected into a sterile polythene bag from the Department of Animal Production and Health Farm of The Federal University of Technology, Akure, Ondo State. Water hyacinth was collected from sea at Ilaje, Ondo State, Nigeria.

Preparation of slurries: The water hyacinth was reduced to small sizes with the aid of a sterile sharp knife and then blended, experimental and other details are described by^{6,7} to achieve near-homogenous slurry with the dung for easy entry into the inlet pipe of the digester. Two kilograms of the fresh pig dung was weighed into a big clean bowl, using a weighing balance; two kilograms of the blended water hyacinth was also weighed into the same bowl, making the mixing ratio 1:1. Three kilograms of fresh pig and a kilogram of blended water hyacinth were weighed into another cleaned dried big bowl (ratio 1:3), after which same litres of clean borehole water was added to the materials separately to form slurries. Two sterile rods, which had been cleaned with 95% ethanol, were then used for the mixing of the slurries and after the desired consistency had been gotten, the slurries were fed into the digester separately.

Pig Dung and Water Hyacinth Analysis: Microbial population of pig dung and water hyacinth were determined on the first day until the end of the digestion process. The population of microbes mainly bacteria and fungi were determined by using nutrient agar for bacteria enumeration and potatoes dextrose agar for fungi and yeast. The temperature of the pig dung and water hyacinth was measured using a mercury thermometer calibrated in degree centigrade. The temperatures were determined every two days. pH of the pig dung and water hyacinth was determined at the beginning of preparation and subsequently during the digestion process. The pH of pig dung and the blended water hyacinth was determined by using Jenway digital hand pH meter.

Loading of digesters: The digesters were constructed in a mechanical work shop along Federal University of Technology, FUTA Junction, Akure, Ondo State, Nigeria. It is a cylindrical shaped metal container with a chamber, outlet pipe, inlet pipe, gas pipe and stand. The back was coated black to provide solar heating. The slurry was poured into the digester through the inlet pipe until it started coming out from the outlet pipe. The inlet, outlet, and gas pipe were closed tightly and the digester was then left to stand vertically for nineteen days for the gas to be generated. With the digester nearly filled up, an anaerobic environment was created, which is a prerequisite for the activity of methanogenic bacteria. The details of the experiment and analysis of biogas production is described in previous works by⁸⁻¹¹.

Composition of biogas determination: For the collection of the biogas, a small volume of n-Hexane was dispensed in a small glass bottle and the bottle was gently put at the tip of the gas valve of the digester, which was then opened, to collect the biogas sample. The sample was transferred into 20ml capacity vial glass container with no addition of reagents carried out. The vials were capped with hand tool capper made of aluminum materials. The sample in the glass vials were placed in the sample holder of the HP HEADSPACE SAMPLER. The temperature of the operation of the Headspace sampler was 30°C. The Headspace sampler was connected to the Gas

Chromatography (GC) for the injection into the GC column in an automated manner after completing the operational cycle. The standard gas mixture in the glass vials was placed in the Headspace sample hole. The connection with the GC was activated in an automated manner. The standard mixture and samples were analyzed under the same conditions.

Results and Discussion

Microbial population of organic wastes before digestion: Table 1 shows the microbial population of organic waste before digestion. The viable microbial population was counted on pig dung, water hyacinth and pig dung with water hyacinth. Water hyacinth has the lowest bacterial population of 1.2×10^5 cfu/ml, no fungi and yeast was detected on the pig.

Table-1
The microbial population of organic waste before digestion

Organic waste	Bacteria (cfu/ml)	Fungi (sfu/ml)	Yeast (sfu/ml)
Pig dung	1.6×10^5	00	00
Water hyacinth	1.2×10^5	1.4×10^3	1.0×10^3
Pig dung + water hyacinth	1.8×10^5	1.1×10^3	00

Table 2 shows the microbial population of the organic waste materials. The bacteria populations were 2.6×10^5 cfu/ml on the first day, 3.1×10^5 was observed on the seventh day while 3.3×10^5 and 3.6×10^5 cfu/ml were recorded for the ninth and eleventh day respectively. There was gradual falling in bacteria population to 1.7×10^5 cfu/ml, 1.4×10^5 cfu/ml and 1.3×10^5 cfu/ml were observed for the fifteen day, seventeen day and nineteenth day respectively. The fungal population was observed to be 2.3×10^3 sfu/ml on the first day; it was observed that the fungal population decreases to 1.5×10^3 sfu/ml. 1.3×10^3 sfu/ml, 1.0×10^3 sfu/ml and 0.6×10^3 sfu/ml were observed for the seventh day, ninth day and eleventh day respectively. The yeast population was 2.5×10^3 cfu/ml on the first day.

Table-2
Microbial population of waste material during digestion

Days	Bacteria (cfu/ml)	Fungi (sfu/ml)	Yeast (sfu/ml)
1	2.6×10^5	2.3×10^3	2.5×10^3
3	2.7×10^5	1.9×10^3	2.1×10^3
5	2.9×10^5	1.7×10^3	00
7	3.1×10^5	1.5×10^3	00
9	3.3×10^5	1.3×10^3	00
11	3.8×10^5	1.0×10^3	00
13	2.2×10^5	1.1×10^3	00
15	1.7×10^5	1.0×10^3	00
17	1.4×10^5	0.8×10^3	00
19	1.3×10^5	0.6×10^3	00

The bacterial population was noticed to be highest during the digestion on the eleventh day which is the second week of

digestion with a value of 3.8×10^5 cfu/ml though the bacterial population increased at various stages of digestion and after the second week, a decrease in cell population set in as the digestion period increased. The relatively high content of complex organic molecules which is divided into peptides, glycerol, alcohol and the simpler sugars by acidic bacteria could be the reason for this, after which a second type of bacteria starts to convert these simpler compounds into methane. These methane producing bacteria are particularly influenced by the ambient condition, which can slow or halt the process completely if they do not lie within a fairly narrow band. The fungi population was not as much as that of bacterial and this may be due to the fact that the digested mixture favors the growth of bacteria more than fungi^{12,13}. The fungal population as seen in table-1 was observed to be decreasing from the first day of digestion to the last day probably due to the inability of moulds to grow at elevated temperature and utilization of the products from the substrate fermentation. The yeast populations was also observed to follow the same pattern and were not detected after five days this might be due to unfavorable pH and temperature of the medium. During the digestion period, some of the organisms isolated are acid-forming bacteria, whose activities also enhance biogas formation. Acidogenesis is the second stage of methane production. Examples of these are *Escherichia coli*, *Enterobacter aerogenes*, *Proteus morganii*, *Flavobacterium ferrugineum*, and *Klebsiella pneumoniae*. Methanogenesis in microbes is a form of anaerobic respiration¹⁴. It is the final step in the decay of organic matter. During the decay process, electron acceptors (such as oxygen, ferric iron, sulfate, nitrate, and manganese) become depleted, while hydrogen (H₂) and carbon dioxide accumulate. During advanced stages of organic decay, all electron acceptors become depleted except carbon dioxide. Carbon dioxide is a product of most catabolic processes, so it is not depleted like other potential electron acceptors. Only methanogenesis and fermentation can occur in

the absence of electron acceptors other than carbon. Fermentation only allows the breakdown of larger organic compounds, and produces small organic compounds. Methanogenesis effectively removes the semi-final products of decay; hydrogen, small organics, and carbon dioxide. Without methanogenesis, a great deal of carbon (in the form of fermentation products) would accumulate in anaerobic environments. Methanogenic bacteria are grouped into four main genera, which are *Methanobacterium*, *Methanobacillus*, *Methanococcus* and *Methanosarcina*¹⁵.

Temperature of digesting material and environment during digestion: Figure 1 shows the temperature of the digesting material and environment (ambient) during the process of digestion of the organic wastes. It was observed that environmental temperature has an impact on the temperature of the digesting material and the temperature of the digesting material was mostly higher than that of the environment. The highest ambient temperature was 32.67°C, this was observed on the fourteen day, from figure-1 while that in the digester was 34.33°C, on the first day.

The temperature of the environment has an impact on the digesting material and during the process of digestion; the ambient temperature remains about 1 to 3°C lower than that in the digesting material except on few occasions. The temperature range as shown in figure-1 was 27 – 34°C in the digesting material, 26 – 32°C in the environment and the fluctuations in the temperature value was due to the weather condition, which was damp on some days and warm on other days during the experiment. Since, the temperature values remain within the mesophilic range, the growth and activities of methanogens will be enhanced, encouraging the production of biogas.

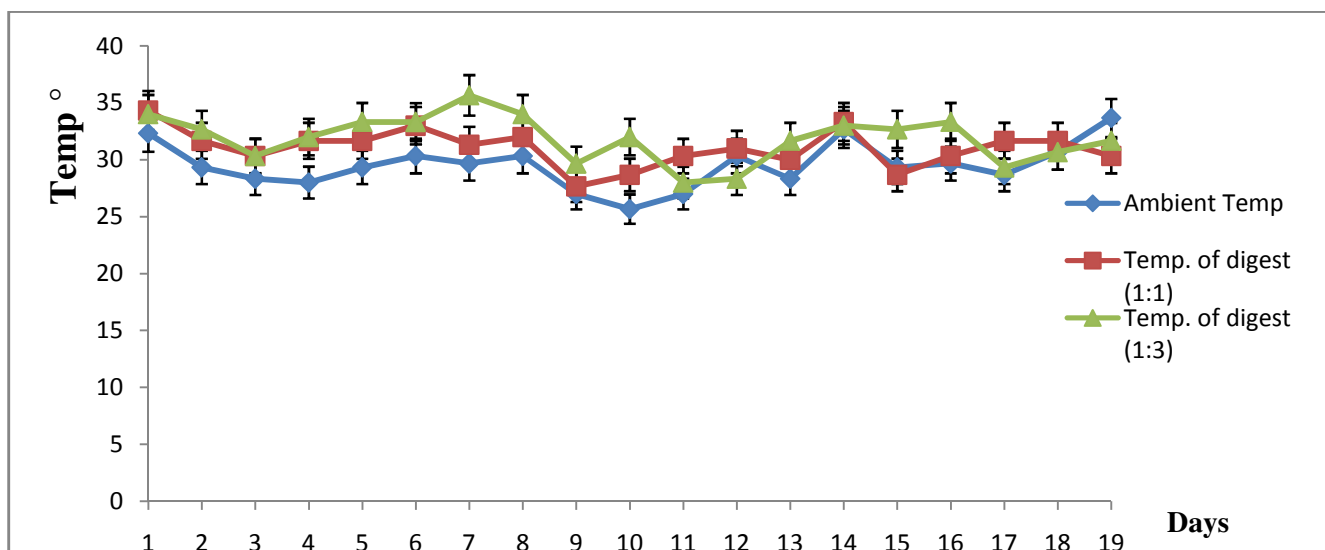


Figure-1
 Temperature (°C) of digesting material and environment (ambient) during digestion for production of biogas
 Keys: Temp. = Temperature, digest. = digesting materials.

pH of digesting materials during digestion: Figure 2, shows the pH of the digesting materials for both ratio 1:1 and ratio 1:3. The value increased from 5.7 to 6.0 and to 5.7. Then increased to 6.8, after which it decreased to 5.4 and 5.1 respectively and then rose to 6.1, 6.4 and then falls back to 6.2. The highest value observed on the seventh day was 6.8 and the lowest value on the eleventh day was 5.1. The initial pH of water hyacinth before the mixture was 6.2. Also, on the zero day of ratio 1:3 the pH value was 6.10 and this increase to 6.2, 6.6 and 6.7 at the third day, fifth day and seventh day respectively. After the initial increase, the pH gradually decreases to 6.0, 4.8 and 5.1 for the ninth day, eleventh day and thirteenth day respectively.

The pH value was increasing and decreasing throughout the digestion and this may be due to the amount of organic acid produced by the acid bacteria at first. Acetate and fatty acid produced during digestion tend to lower the pH of digesting material liquor^{16,17}. High value and low pH observed in figure-2 may be due to function of concentration of ammonia, which increases.

Chemical composition of biogas: Table 3 shows the composition of biogas. It was observed that methane (CH₄) has the highest percentage (88.3%) of gas while carbon dioxide (CO₂) has the lowest percentage (1.3%) of gas for ratio 1:3, the high percentage of methane (83.4%) was produced using water hyacinth and pig dung in the ratio 1:1, 1.86% carbon monoxide, 0.03% carbon dioxide, 0.01% ammonia, 5.85% hydrogen sulphide and 8.89% of other gases.

Table-3
Composition of biogas

Gases (symbol)	Percentage (ratio 1:3)	Percentage (ratio 1:1)
Methane (CH ₄)	88.3	83.3625
Ammonia (NH ₃)	0.004	0.0066
Carbon dioxide (CO ₂)	0.02	0.0327
Carbon monoxide (CO)	1.3	1.8560
Hydrogen sulphide (H ₂ S)	4.1	5.8530
Other gases	6.2	8.8892

From table-3, the biogas produced from the experiment was found to consist of 83.36% methane, 1.86% carbon monoxide, 0.03% carbon dioxide, 0.01% ammonia, 5.85% hydrogen sulphide and 8.89% of other gases for ratio 1:1. The gas produced was high in methane and low in carbon compound and this could be as a result of the activities of methanogens and addition of water hyacinth. The highest gas produced for ratio 1:3 was methane (88.3%) while the lowest gas produced was ammonia (0.004%). Water hyacinth on pig dung (ratio 1:3) biogas has yielded large percentage of methane in the gas production. Water hyacinth has abundant nitrogen content; this makes it suitable as a substrate for biogas production.

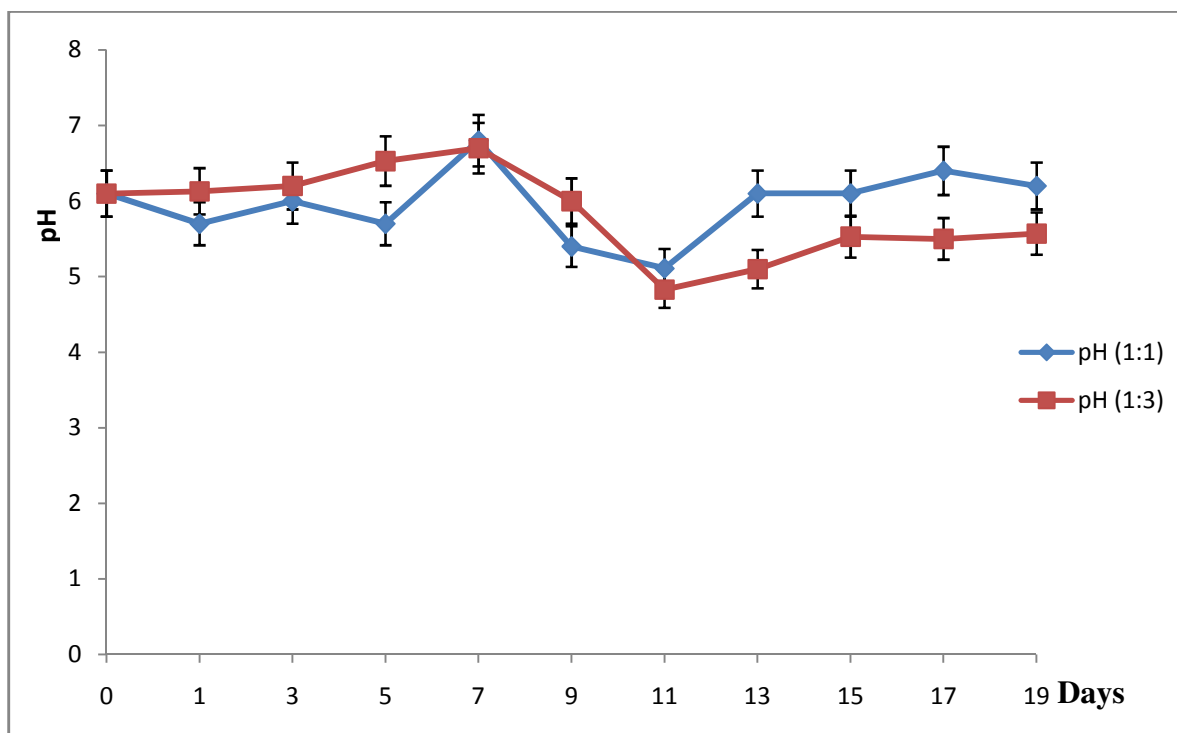


Figure-2
 pH of digesting materials during production of biogas

Conclusion

There was increase in the percentage composition of the methane gas when the water hyacinth was just in ratio one and pig dung ratio three, that is the lower the water hyacinth the more will be the methane produced. The differences in pH and temperature of the pig dung with water hyacinth in the digested materials during digestion affect the microbial population.

Acknowledgement

I want to acknowledge the technologist in The Department of Microbiology, Federal University of Technology, Akure, for the assistance during the experiment in the laboratory.

References

1. Goswami A.P. and Mankodi P.C., Study on Zooplankton of Fresh Water Reservoir Nyari - II Rajkot district, Gujarat, India, *ISCA J. Biological Sci.*, **1(1)**, 30-34 (2012)
2. Tower P., Wetzel J. and Lombard X., New Landfill Gas Treatment Technology Dramatically Lowers Energy Production Costs, *Applied Filter Technology*, Retrieved **2009-04-30 (2006)**
3. Richards B., In situ methane enrichment in methanogenic energy crop digesters, *Biomass and Bioenergy*, 275-274 (1994)
4. Adcock M. and Finelli M., Against nature: The sensitive pig versus the hostile environment of the modern pig farm. *Hsus news Washington*, DC: Human society of the U.S. (1996)
5. Shaziya Bi and Goyal P.K., Anthelmintic effect of Natural Plant (*Carica papaya*) extract against the Gastrointestinal nematode, *Ancylostoma caninum* in Mice, *ISCA J. Biological Sci.*, **1(1)**, 2-6 (2012)
6. Ghosh S., Henry M.P. and Klass D.L., Conversion of Water Hyacinth-Coastal Bermuda grass MSW sludge blends to methane, *Biotechnology and Bioengineering Symp.*, **10**, 163-187 (1980)
7. Somani Vaishali, Quadros Goldin and Pejaver Madhuri K., Occurrence of Rotifers and its Relation to the Water Quality during the Bioremediation process in Lake Kacharali, Thane, MS, India, *ISCA J. Biological Sci.*, **1(3)**, 54-58 (2012)
8. Garba B. and Sambo A.S., Effect of some parameters on biogas production rate, *Nigerian Journal of Renewable Energy*, **3(36)**, 41-42 (1992)
9. Barnett E.A. and Hunter B.H., Illustrated General of imperfecti Third edition, Burgess Publishing Company, Minneapolis, 100-130 (1983)
10. Rhode B. and Hartman G., Introducing Mycology by examples. Schering Aktiengesell Shaft Press Hamburg, 84-121 (2002)
11. Holt J.G., Krieg N.R., Sneath P.H., Stanley J.J and Williams S.T., Bergeys Manual of determinative bacteriology, Wilkins publishers, Baltimore, 5th edition (1994)
12. Adjou Euloge S., Kouton Sandrine, Dahouenon-Ahoussi Edwige, Sohounhloue Dominique C.K., Soumanou Mohamed M., I. Antifungal activity of *Ocimum canum* Essential oil against Toxinogenic Fungi isolated from Peanut Seeds in post-harvest in Benin, *Res. J. Biological Sci.*, **1(7)**, 20-26 (2012)
13. Atlas R.M., Fundamental Approach to Microbiology. 4th edition, New York, 145-290 (1984)
14. Thauer R.K., Biochemistry of Methanogenesis: a Tribute to Marjory Stephenson, *Microbiology*, **144**, 2377-2406 (1998)
15. Balch W.E. Fox G.E., Mergrum L.J., Woese C.R. and Wolfe G., Methanogens; Re-evaluation of unique biological group, *Microbial Rev.*, **43**, 260-296 (1979)
16. Marchaim C.G., Principles and practices for Biogas Systems *World Bank Technical paper*, **49 (1986)**
17. Santhosh Kumar K., Lingaiah Kusuma, Ramachandra N.B. and Nair Vijay Mala, Genetic variations among Ecologically diverse species of Anurans at the level of Genus based on ISSR Marker, *I. Res. J. Biological Sci.*, **1(7)**, 11-19 (2012)