Study of water hyacinth (*Eichhornia crassipes*) on the quality of cow dung biogas

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Abstract

The objective of this research was to know the effect of water hyacinth (*Eichhornia crassipes)* on the quality of cow dung biogas. Water hyacinth (*Eichhornia crassipes*) is a perennial aquatic herb, which belongs to the family Pontedericeae. It is usually found floating freely on the surface of fresh water or can be anchored in mud. Using water hyacinth as a biogas is one of the ways to limit the disadvantageous effects of the plant and to provide a low-cost gas. Seventy five percent cow dung was mixed thoroughly with 25% blended water hyacinth (3:1) and cleaned water was added to form slurry, it was poured through the inlet orifice of a digester. Bacteria and mould counts of the materials were determined before and during digestion. Temperature, pH, of the digesting materials, biochemical and morphological characterization of the isolates were carried out to know the microorganisms responsible for the digestion of the materials. A total of eight (8) bacteria and four (4) fungi were isolated during digestion. The bacteria identified were *Escherichia coli*, *Methanococcus mazei, Enterobacter aerogenes, Clostridium welchii, Methanobacterium ruminantium, Lactobacillus delbrueckii, Methanothrix sochngenii, Klebsiella pneumoniae* and the fungi isolated included *Aspergillus flavus, Aspergillus niger, Aspergillus fumigatus* and *Mucor mucedo.* The pH of the digesting material was between 5.40 and 7.28. The temperature was from 30°C to 39°C. The result of the biogas analysis was 91.10% methane (CH_4), other traced gases was 6.43%, while ammonia (NH_3) was not detected. Twenty five percent (25%) water hyacinth added to the seventy five percent cow dung in ratio 3:1 showed that water hyacinth could be mixed with cow dung to improve the methane quality of the biogas. This could contribute to the reduction of environmental pollution.
Keywords: assessment, biogas, cow dung, environment, quality, methane, pH, temperature, water hyacinth.

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1 Introduction

Water hyacinth (*Eichhornia crassipes*) is a perennial aquatic herb, which belongs to the family Pontedericeae. It is usually found floating freely on the surface of fresh water or can be anchored in mud. Using water hyacinth as a feed is one of the methods to limit the disadvantageous effects of the plant and to provide a low-cost ingredient in animal diets. Thus, water hyacinth is considered to be a plant for hunger and poverty alleviation in several developing countries. Pollutants from urban, industrial and agricultural activities provide essential nutrients for the growth of this aquatic macrophyte. In Tanzania the plant has been identified in rivers Pangani and Sisi, Lake Victoria and in the Mtera hydro-electric dam (Joyce [1]). Leaves are deep green, large and erect. Roots are variable in length from about 10 to 90 cm long (Reza and Khan [2]). The rhizomes are generally 1 to 25 cm long, occasionally producing internodes. The plant is luxuriant in growth and multiplies very rapidly. The average height of the plant is about 45 cm in mature stage but generally ranges from 30 to 70 cm (Reza and Khan [2]). The plant is characterized by formation of large floating mats that normally cover the water surface. When allowed to propagate, it quickly colonizes vast areas of water masses causing a number of problems. Some examples of detrimental effects include loss of fishing ground, provision of habitats for mosquito and bilharzias breeding, occlusion of waterways for navigation, interference with hydroelectric power sources and suppression of other useful aquatic life (Hentges *et al.* [3] and El-Serafy *et al.* [4]). In Lake Victoria the menace caused by water hyacinth has prompted East African governments' action to control the spread of the plant by biological methods (Wulf and Andjelic [5]).

Cow dung is the undigested residue of herbivorous matter which has passed through the animal's gut. The resultant faecal matter is rich in minerals. Colour ranges from greenish to blackish, often darkening in colour soon after exposure to air. The composition of cow dung gas is approximately 55–60% methane, 5–10% hydrogen and 30–35% carbon dioxide (Subba Rao [6]). Microorganisms have been known to digest organic materials such as solid wastes under strictly anaerobic conditions, to generate combustible gas (biogas) with manure or other materials being produced contemporarily as a by-product. Millions of cubic meters of methane in the form of swamp gas and biogas are produced every year by the decomposition of organic matter, both animal and plants. It is almost identical to the natural gas pumped out of the ground by the oil companies used by many for heating in houses and for cooking. The idea for the manufacturing of gas brought to the UK in 1895 by producing wood gas from wood and later coal. The resulting biogas was used for gas lighting in street lamps and homes. Biogas typically refers to a gas produced by the biological breakdown of organic matter in the absence of oxygen. Biogas originates from biogenic material and is a type of biofuel. 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 (Anaerobic digestion factsheet). This type of biogas comprises primarily methane and carbon dioxide.

This study aims to investigate the possibility of utilising water hyacinth and cow dung to improve the quality of the methane of biogas and to determine the microbial population, identify the microorganisms responsible for digestion, determine the optimum temperature and pH under which the gases are produced by microorganisms.

2 Materials and methods

The samples used for this work were cow dung and water hyacinth. Fresh cow 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 coastlines at Ilaje, Ondo State, Nigeria.

2.1 Preparation of slurries

The water hyacinth was chopped, blended, 1kg was weighed and added to 3kg of fresh cow dung this was mixed with appropriate volume of clean water to form slurry. A sterilized rod was used for proper mixing of the slurry; this was fed into the digester through the inlet orifice. The slurry was poured into the digester (tilted to facilitate loading) 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 twenty one days for the gas to be generated.

2.2 Cow dung and water hyacinth analysis

Microbial population of cow dung and water hyacinth were determined separately on the first day before mixing and during 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.

2.2.1 Microbial isolation

One gram (1g) of each sample was macerated in 9ml of sterile physiological saline and diluted serially. Then 1ml and 0.1ml was pipette from each dilution factor unto sterile Petri-dishes. Thereafter, 20ml of nutrient agar and acidified potato dextrose agar was cooled to 45°C and poured separately onto each of the plates in triplicate and the plates were gently swirled and allowed to solidify. The nutrient agar plates were incubated in an inverted position at 37° ± 2°C for 24 hours (bacteria) while potato dextrose agar plates were incubated at 28° ± 2°C for 72 hours (fungi). The viable colonies were sub cultured from mixed culture plate to obtain a pure culture. Bacteria cultures were characterized and identified using various morphological and biological tests such as Gram stain, spore stain, motility, catalase, coagulase, indole, urease, citrate, oxidase and sugar fermentation. Pure cultures of each isolate were obtained by streaking the specific colonies on suitable media and incubated appropriately; these were maintained in an agar slant in McCartney bottles. The identification of the microbial isolates was based on classification Scheme proposed by Harrigan and McCance [7], Collins and Lyne [8] and

Holt *et al.* [9]. The identification was based essentially on morphological and biochemical reactions. The isolated fungi were then identified with reference to Barnet and Hunter [10], Rhode and Hartman [11] and Frazier and Westhoff [12].

The temperature of the cow dung and blended water hyacinth mixture was measured using a mercury thermometer calibrated in degree centigrade. The temperatures and pH were determined every two days. The pH of cow dung and the blended water hyacinth was determined by using a pH meter. The details of the experiment and analysis of biogas production was as described in previous works by Garba *et al.* [13]. The compositions of biogas produced were analyzed with gas chromatography.

3 Results

Table 1 represents the population of microorganisms in the materials used for the production of biogas water hyacinth, cow dung and slurry produced from mixture of the materials used just before digestion.

Table 1: Microbial population of materials before digestion.

Materials	Bacteria (cfu/ml)	Fungi (sfu/ml)	Yeast (cfu/ml)
Water hyacinth	7.4×10^4	5.2×10^4	4.2×10^3
Cow dung	3.5×10^4	2.8×10^4	2.5×10^3
Slurry	7.8×10^4	5.6×10^4	4.1×10^3

4 Discussion

Anaerobic digestion is a simple process that can greatly reduce the amount of organic matter which might otherwise be destined to be land filled or burnt in the incinerator. Almost any organic material can be processed with anaerobic digestion. This includes biodegradable waste materials such as waste paper, leftover food, sewage and animal wastes. The gases produced are methane, carbon dioxide, carbon monoxide, hydrogen sulphide and other gases in traces. The population of the microorganisms before the anaerobic digestion in Table 1 shows the bacteria population in water hyacinth and cow dung to be 3.5×10^4 and 7.4×10^4 cfu/ml respectively while the fungal population were 2.8×10^4 and 5.2×10^4 sfu/ml respectively. There was extremely limited growth of yeast in the water hyacinth which was 2.5×10^3 cfu/ml and cow dung 4.2×10^3. At the initial stage before digestion, the bacteria load of cow dung and cow slurry were 7.8×10^4 and 5.6×10^4 respectively.

Table 2 shows the population of microorganisms contributing to the anaerobic digestion at an isolation interval of 3 days, the digestion period ranges from day 1 to the day 21. The fungal load decreases as the digestion days increases while the yeast growth was not detected at the later days of the digestion. At the beginning of the digestion process, the bacteria load of the slurry was 1.8×10^5cfu/ml; the fungal load was 4.1×10^4sfu/ml while the yeast was 1.3×10^3cfu/ml. As the digestion proceeds, the bacterial load increased and later dropped towards the end of the digestion. The fungal load decreased from the beginning to the end of the digestion while the yeast was no longer detected from the 4th day to the end of the digestion. This might be as a result of the anaerobic condition of the organisms which did not favour their growth.

Table 2: Microbial population contributing to the digestion of biogas.

Days	Bacteria (cfu/ml)	Fungi (sfu/ml)	Yeast (cfu/ml)
1	1.8×10^5	4.1×10^4	1.3×10^3
3	3.6×10^5	3.8×10^4	2.8×10^4
5	5.2×10^5	3.5×10^4	0.5×10^3
7	5.1×10^5	3.0×10^4	0
9	4.7×10^5	2.7×10^4	0
11	4.5×10^5	2.5×10^4	0
13	3.8×10^5	1.7×10^4	0
15	2.6×10^5	1.3×10^4	0
17	2.1×10^5	0.9×10^4	0
19	1.8×10^5	0.5×10^4	0
21	1.7×10^5	0.4×10^4	0

Figure 1 shows the temperature of the environment and the digester during the digestion process. The initial temperature of the digester, ambient and cow slurry were 33°C, 33°C and 32°C. The increase and decrease in the temperature range was due to the environmental condition. The temperature ranges from 30°C to 38°C throughout the digestion process. The temperature obtained was within the mesophilic range with an average temperature of 33°C. Methanogens are inactive in extremely high or low temperature and when ambient temperature is 33°C or less, the average temperature is within the digester remains not higher 4°C above the ambient temperature (Lund [14]).

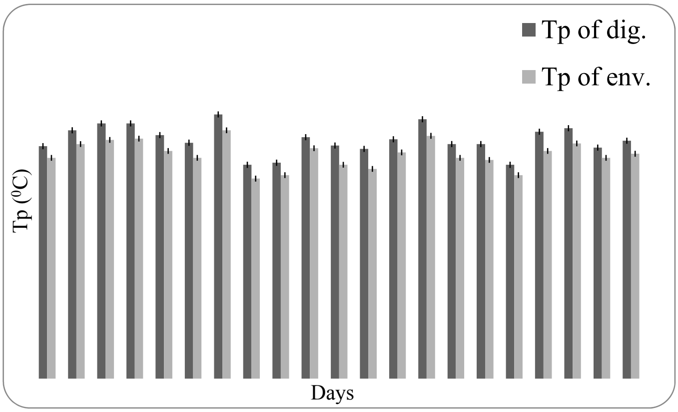

Figure 1: Temperature changes of the digesting materials and the environment. (Key: Tp = temperature, dig. = digesting materials, env. = environment.)

The pH of the anaerobic digestion was shown in Table 3. The initial pH of the cow dung and the slurry were 6.97 and 6.90 respectively. The pH ranges from 5.40 to 7.28 and at the initial stage of anaerobic digestion; there was low production of biogas due to the low pH at the beginning of the digestion process. This might be as a result of the large amounts of organic acids produced by acid forming bacteria. Anaerobic digestion will occur best within the pH range of 6.8 to 8.0, more acidic or basic mixtures will ferment at a lower speed. The introduction of raw materials will often lower the pH thereby making the mixture more acidic. This may be a function of the retention time and may also be due to concentration of ammonium which increases as a result of digestion of nitrogen which can increase the pH value (Marchiam [15]).

The suspected organisms isolated were *Escherichia coli*, *Methanococcus mazei*, *Enterobacter aerogenes*, *Clostridium perfringens*, *Methanobacterium ruminantium*, *Lactobacillus delbrueckii*, *Methanothrix sochngeni, Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Bacillus subtilis* and *Staphylococcus aureus*. All these bacteria were able to survive the mesophilic temperature of digestion. *Klebsiella pneumoniae*, *Enterobacter aerogenes* and *Bacillus subtilis* survived and enhanced the production of biogas even though they are non-methanogens. These can withstand mesophilic temperature range of the digesting materials and affect the pH of the anaerobic digestion since they are known to be acid forming bacteria, thereby contributing to the initial stage of the digestion to produce organic acids, peptides, glucose and other monosaccharides which will be utilized during fermentation leading to gas production. *Pseudomonas aeruginosa* is an extremely adaptable organism that utilizes organic compounds for growth, it is widely distributed in nature as saprophytes and they contribute to the decomposition of the mixture of cow dung and water hyacinth to produce biogas and the by-products. The fungi isolated were: *Aspergillus fumigatus*, *Aspergillus niger*, *Rhizopus stolonifer*, *Mucor mucedo*, *Aspergillus flavus* and *Cladosporium cladosporoides*. These are capable of utilizing a variety of substance.

Table 3: pH changes of the digesting materials during digestion.

Days	pH
1	$7.04^{g} \pm 0.01$
3	$6.95^{e} \pm 0.01$
5	$6.45^{d} \pm 0.12$
7	$5.67^{b} \pm 0.01$
9	$5.40^{a} \pm 0.01$
11	$6.20^{c} \pm 0.01$
13	$6.45^{d} \pm 0.01$
15	$6.91^{d} \pm 0.01$
17	$7.15^{h} \pm 0.01$
19	$7.28^{i} \pm 0.62$

Values that are followed by similar alphabets along the same column are not significantly different:

pH of cow dung = 6.97
pH of water hyacinth = 6.20
pH of slurry before digestion = 6.9

The addition of water hyacinth to cow dung for the biogas production boosted the quality of methane produced. Cow dung gas is usually composed of 55–65% methane, 30–35% carbon dioxide, 0–5% hydrogen sulphide with some hydrogen, nitrogen and other traces (Subba Rao [6]). In a previous study by Adegunloye and Oladejo [16], the addition of crop wastes to the poultry dung (1:4) for the production of biogas greatly increase the methane value to 74.81% and reduce CO_2 value to 8.14%. Table 4 shows an increase in the production of methane (CH_4), to be 91.10%, other gases were in extremely low amount compared to extremely high amount of methane produced and there was no production of ammonia (NH_3). This result indicated that the quality of methane gas is better than when only proportions of cow dung were used without the water hyacinth.

Table 4: Percentage composition of biogas produced from water hyacinth and cow dung.

Name of gas	Percentage (%)
Methane (CH_4)	91.10
Ammonia (NH_3)	0.00
Carbon monoxide (CO)	1.34
Hydrogen sulphide (H_2S)	1.11
Carbon dioxide (CO_2)	0.02
Trace gases	6.43

5 Conclusion

This study clearly showed that, the production of biogas with high methane content can be enhanced or increased by the addition of water hyacinth. The water hyacinth is a plant that has abundant nitrogen content and used as substrate for biogas production and the sludge obtained from the biogas. The cow dung is a biodegradable waste as it is cheaply available and found all year round in our environments and their utilization will generate biogas energy for domestic uses such as cooking, heating and refrigeration. This generated energy will save labour and reduce the stress of buying or gathering fire woods, thus helping to ameliorate deforestation problem in Nigeria.

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