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A comparative study of biogas production from water hyacinth (*Eichhornia crassipes*) and chicken droppings seeded with ruminant microorganisms

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ABSTRACT: A comparative study of biogas production from water hyacinth (*Eichhornia crassipes)* and chicken droppings was undertaken in the laboratory for nine weeks at room temperature of $28^0C \pm 1$. Sterile chicken droppings (200g) and *Eichhornia crassipes* (200g) were individually mixed with 1200mls of sterile distilled water to make a slurry in the ratio 1:6. and fed into the 4000 liters and 10000 liters capacity gallons used as the digesters, while the following organisms *Staphylococcus aureus, Proteus vulgaris, Bacillus subtilis, Shigella dysenteriae, Escherichia coli, Salmonella typhi* and *Methanobrevibacter ruminatum*. isolated from the rumen of cow were incolulated into the digester using sterile needles. The same procedure was carried out for *Eichhornia crassipes.* The mean quantity of biogas increased during the first five weeks reaching a peak ($3060.00cm^3$) on the 5^{th} week, after which a decline was observed for the plant waste- *Eichhornia crassipes.* The total average biogas produced during the 45 days experimental period was $1045cm^3$. In the case of chicken droppings, the highest weekly mean volume of biogas produced was $4860cm^3$ which occurred between 1-5th day which gradually decreases reaching the lowest volume of $58.3\ cm^3$ within $36\text{-}40^{th}$ day. The statistical analysis using Unpaired T- test showed no significant difference ($P > 0.05$) in the amount of gas yield for both chicken droppings and *Eichhornia crassipes*. The proximate analysis of chicken droppings and *Eichhornia crassipes* carried out revealed that crude fat and protein were not significant ($P > 0.05$) different from each other when compared to moisture, ash, crude fibre, crude carbohydrate, nitrogen, sodium, potassium, calcium and magnesium which were significant different from each other ($P < 0.05$) when were subjected to independent T- test. The result of this study suggests that both chicken droppings and *Eichhornia crassipes* are good substrate source for production of biogas. However, chicken droppings is a better substrate for the production of biogas when seeded with ruminant microorganisms.

Key words: Biogas, *Eichhornia crassipes*, Chicken droppings, digesters, rumen, *Methanobrevibacter ruminatum*,

Introduction

Biomass is any organic material that comes from plants, animals or their waste. Biotreatment of wastes can fall into two basic types: Aerobic and Anaerobic. Over the years, aerobic biotreatment has been considered to be more efficient in BOD/COD reduction, better for nitrogen and phosphorus removal, applicable for wide range of wastes and more stable process (Oyeleke, 2011).

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Anaerobic digestion is a natural process that converts biomass to energy. Anaerobic digestion has been used for over 100 years to stabilize municipal sewage and a wide variety of industrial wastes. Most municipal waste water treatment plants use anaerobic digestion to convert waste solids to gas (Burke, 2000). The anaerobic process removes a vast majority of the odorous compounds (Willkie, 2000). It also reduces significantly the pathogens present in the slurry (Lusk, 1995). Over the past 25 years, anaerobic digestion processes have been developed and applied to a wide array of industrial and agricultural wastes (Oyewole, 2010, Oyeleke, 2011).

Anaerobic digestion is preferred for waste treatment process since it produces rather than consumes energy and can be carried out in a relatively small enclosed tanks. The products of this process have value and can be sold to offset treatment costs (Oyeleke, 2011). Microorganisms make use of nutrients as energy source and raw materials for synthesis in order to grow and multiply. There are 3 groups of microorganisms involved in biogas production and these are bacteria, fungi, and protozoans. Of these 3 groups, bacteria are the most important group which takes part in most degradation process (Baker, 1996).

The first step of anaerobic digestion is the break down of particulate matter to soluble organic constituents that can be processed through the bacteria cell wall. Hydrolysis or liquefaction of insoluble materials is a rate limiting step in anaerobic digestion of waste slurries. This step is carried out by a variety of bacteria through the release of extracellular enzymes that live in close proximity to the bacteria. The soluble organic materials that are produced through hydrolysis consist of sugars, fatty acids and amino acids. These soluble constituents are converted to carbondioxide and a variety of short chain organic acids by acid forming bacteria. Other groups of bacteria reduce hydrogen toxicity by scavenging hydrogen to produce ammonia, hydrogen sulfide and methane. A group of methanogens converts acetic acid to methane gas. A wide variety of physical, chemical and biological reaction takes place. The bacteria consortia catalyze these reactions. Consequently, the most important factor in converting waste to gas is the bacterial consortia which are essentially the "bio-enzymes" that accomplish the desired treatment (Burke, 2000).

The objectives of this research include:

- To isolate bacteria present in the rumen of cow
- To generate biogas from chicken droppings and water hyacinth
- To compare the biogas production potential of chicken droppings and water hyacinth

Materials and Methods

Sample Collection and Preparation

Chicken droppings were collected from Emir's poultry farm along Minna road in Bida, while *Eichhornia crassipes* was obtained from River Niger in Nupeko area, Niger State. They were sun dried for 7 days, after which, they were pulverized using mortar and pestle. The two samples were subsequently sterilized and placed in separate sterile containers. The samples were asceptically transported to microbiology laboratories of Federal University of Technology Minna and Usman Danfodio University Sokoto for further analysis.

Isolation and Characterization of Microorganisms

Standard microbiological and biochemical media were used for cultivation, characterization and identification. Fresh rumen of cow was asceptically obtained from Sokoto abattoir and placed inside a sterile container. One gram (1g) of fresh dung from the rumen of cow was weighed using sterile weighing balance. This was transferred into 9 mls of sterile distilled water, stirred and serially diluted using tenfold dilution. 1ml of each of the dilution was plated out on nutrient agar using pour plate techniques. The nutrient agar plates were incubated anaerobically using anaerobic jar at 37°C for 24-48 hours. Colonies differing in colour and shape were picked and subcultured onto nutrient broth and incubated anerobically at 37°C for 24-48 hours.

Colonies from the incubated plates were subjected to microbiological and biochemical tests which include Gram staining, catalase test, coagulase test, motility test, indole test, methyl red voges proskauer test, oxidase test, citrate utilization test, starch hydrolysis, urease test , nitrate reduction , gelatin hydrolysis, hydrogen sulphide (TSI) and sugar fermentation test using the method described by Cheesbrough (2004) and Oyeleke and Manga (2008).

Physico-chemical Analysis

The physico-chemical analysis of powdered chicken droppings and water hyacinth (*Eichhornia crassipes*) was analyzed using the methods of Bakare (1985). The physicochemical properties analysed for were moisture, ash, lipid, fibre, crude protein, crude carbohydrate, sodium, nitrogen, potassium, calcium and magnesium.

The Digestion Process

The digesters used for this study were made up of 4000 and 10000mls capacity gallons with a lid. A hole was made at the side close to the digester lid. A rubber tubing of 8 mm in diameter and 36 cm long was inserted into the hole and firmly glued with a strong adhesive. The whole set-up consisted of 10 digesters, 3 for chicken droppings and 3 for water hyacinth, 2 served as control which were uninoculated (i.e. not seeded with test organisms). The remaining 2 digesters served the purpose of gas collection (Oyeleke *et al*., 2004).

Slurry Preparation and Installation

The digesters were aseptically cleaned with clorex and sterilized using 75% ethanol. Two hundred grams (200g) of the chicken droppings and water hyacinth (200g) were autoclave sterilized and mixed with 1200 mls of sterile distilled water to make slurry in the ratio 1:6. Each of the slurry was fed into the digesters respectively after adjusting the pH to 7.0 with 0.1M sodium hydroxide, or hydrochloric acid. The digesters were sealed tightly using adhesive to seal any hole that exists creating anaerobic condition.

A 2000ml - capacity cylinders were filled with tap water and closed tightly to avoid air bubbles and placed in an inverted position in the bowl containing tap water. The rubber tubes from the digesters were carefully inserted into the cylinders ensuring no formation of air bubbles. The displacement of the water in the inverted cylinders serves as the volume of gas produced (downward displacement of water). Readings of biogas production was taken daily for a period of 45days .In order to collect the gas, two of the digesters (10 liter's gallon) were connected to a plastic bottle containing 1% caustic soda through a rubber tubing to (absorb CO_2), this was further connected to a buchner flask with the aid of another rubber tubing that is provided with an inlet and an outlet. Concentrated sulphuric acid was poured into the flask (to absorb H_2S and other gases). Both the plastic bottle and flask were sealed at the inlet and out let to exclude air getting into them. With the aid of another rubber tubing, the flask was connected through the outlet to a collapsible rubber gas collector with an on/off control tap, switched to "on" position to receive the gas the tap was switched off when the collapsible gas receiver was turgidly inflated and connected to a Bunsen burner through yet another rubber tubing, and tested for its inflammability using a lighter (Oyeleke *et.al*., 2004).

Results

Microorganisms Isolated from Rumen of Cow

The microorganisms isolated from cow rumen were *Staphylococcus aureus*, *Proteus vulgaris*, *Shigella dysenteriae*, *Bacillus subtilis*, *Escherichia coli*, *Salmonella typhi* and *Methanobrevibacter ruminatum*.

Physico-chemical Analysis

Table 1 shows the physicochemical properties of chicken droppings and *Eichhornia crassweipes* There was a significant difference ($P<0.05$) between the mean moisture, ash, crude fiber, crude carbohydrate, nitrogen, sodium, potassium, calcium, magnesium of chicken droppings and *Eichhornia crassipes* but there were no significant differences ($P>.05$) between their mean crude protein and crude carbohydrate .

Table 1: Physicochemical properties of chicken droppings and *Eichhornia crassipes*

Parameter (Mean)	Chicken droppings	Water hyacinth	Level of significance
Moisture	0.67%	63.4%	P<0.05
Ash	50.83%	17.3%	P<0.05
Crude fibre	19.5%	3.5%	P<0.05
Crude fat	2.6%	2.1%	P>0.05
Crude protein	7.32%	10.40%	P>0.05
Crude carbohydrate	19.67%	84.08%	P<0.05
Nitrogen	1.17%	86.80%	P<0.05
Sodium	171.7mg/100g	7.5mg/100g	P<0.05
Potassium	3166.7mg/100g	7.3mg/100g	P<0.05
Calcium	0.065mg/100g	1.5mg/100g	P<0.05
Magnesium	0.19mg/100g	9.0mg/100g	P<0.05

Biogas production from chicken droppings and *Eichhornia crassipes*

Table 2 shows the weekly biogas production from chicken droppings. The highest weekly mean volume of biogas produced from chicken droppings samples A, B and C was 4860cm^3 occurring between 1-5th day with the lowest volume of 58.3cm^3 occurring within 36-40th day. The highest room temperature of 30.2^{0}C occurred in 1-5th day while the lowest was recorded between 11-15th day. There was no significant difference ($P > 0.05$) observed within and between the sample treatments (ABC).

Table 2: Weekly Biogas Production from Chicken Droppings

Period (Days)	Average Room Temperature (^{0}C)	Volume of gas produced without seeding (Control) (cm^3)	Volume of gas produced with seeding (cm^3)			Average volume of gas produced (cm^3)
			A	B	C	
1-5	30.2	0	5440	4260	4880	4860±590.25
6-10	28.8	0	3090	3090	3100	3046.67±83.86
11-15	28.4	0	620	1490	1490	1200.00±502.29
16-20	30	0	390	630	750	590.00±183.30
21-25	29.4	0	250	420	420	363.33±98.15
26-30	28.8	0	210	270	265	248.33±33.29
31-35	29.0	0	110	136	145	130.33±18.18
36-40	29.4	0	50	50	75	58.3±14.43
41-45	28.6	0	0	0	0	0.00±0
Total	262.2	0	10160	10205	11125	1166±0
Mean	29.18± 0.62		1128.89± 1881.82	1133.89± 1506.81	1236.89± 1685.55	

Table 3 shows the weekly biogas production from *Eichhornia crassipes*. The highest weekly mean volume of biogas produced from *Eichhornia crassipes* samples D, E and F was 3060cm^3 which occurred between 21-25th day while the lowest volume of 5.0cm^3 occurred within 41-45th day. The highest room temperature of 30.2^{0}C occurred between 1-5th day while the lowest was recorded between 11-15th day. There was no significant difference (P > 0.05) between the sample treatments (ABC and DEF). There was no effect of temperature on the quantity of biogas generated as mean temperature varied with ± 2 ^{0}C while the control experiment showed no result.

Table 3: Weekly Biogas production from Water hyacinth (*Eichhornia crassipes*)

Period (Days)	Average Room Temperature ^{0}C	Volume of gas produced without seeding (Control) (cm^3)	Volume of gas produced with seeding (cm^3)			Average volume of gas produced (cm^3)
			D	E	F	
1-5	30.2	0	350	470	420	413.33±60.28
6-10	28.8	0	860	1020	1060	980±105.83
11-15	28.4	0	1310	1690	1730	1576.67±231.80
16-20	30	0	2070	2210	2180	2153.33±73.71
21-25	29.4	0	2720	3420	3040	3060.00±350.42
26-30	28.8	0	610	980	960	850.00±208.09
31-35	29.0	0	210	270	305	261.67±48.05
36-40	29.4	0	75	115	115	101.67±23.09
41-45	28.6	0	0	5	10	5.00±5.00
Total	262.2	0	8205	10180	9820	1045
Mean	29.18± 0.62	0	911.67± 948.89	1131.11±1 130.80	1091.11±1 036.83	

Discussion

The organisms isolated and seeded into the digesters were *S. aureus*, *P. vulgaris*, *B. subtilis*, *S. dysenteriae*, *E. coli*, *S. typhi* (hydrolytic bacteria, involved in the hydrolytic, fermentative and acetogenic break down of the chicken droppings and *Eichhornia crassipes* to carbondioxide, alcohols, and higher fatty acids) and *M. ruminatum* (methanogenic bacteria, responsible for the production of methane(CH_4) gas from methanogenesis of carbondioxide, alcohol and higher fatty acids).

The proximate analysis of chicken droppings indicated that it contained moisture (0.67%), Ash (50.83%), Crude fibre (19.5%), Lipid (2.67%), Nitrogen (1.17%), Sodium (171.67mg/100g), Potassium (3166.7mg/100g), Calcium (0.065mg/100g) and Magnesium (0.19mg/100g) while *Eichhornia crassipes* had moisture (63.4%), Ash (17.3%), crude fibre (3.5%), crude fat (2.1%), crude protein (10.4%), crude carbohydrate (84.08%), Nitrogen (86.8%), sodium (7.5mg/100g), potasiums (7.3mg/100g), calcium (1.5mg/100g) and magnesium (9.0mg/100g). All other nutrients are significantly different from each other (P<0.05) except crude fat and crude protein which were not significantly different from each other (P>0/05) when subjected to statistical analysis . The high fibre and carbohydrate content provide substrate for microbial activity while N, K, Na, Ca, Mg, may be essential nutrient for the growth of the microorganisms.

There was a gradual decrease in the mean volume of biogas produced by chicken droppings from 4860cm^3 within 1-5th day to 0cm^3 on 41-45th day. This may either be attributable to exhaustion of essential nutrients, antagonism or utilization of gas by other organisms in close association. The mean volume of biogas produced from *Eichhornia* crassipes sample D, E and F increases gradually to the peak and declined generally with increase in the number of days. This support the work of Eyo and Madu (1990) who carried out a preliminary study on biogas production from *Eichhornia crassipies* and reported that the quantity of biogas generated increased from 50cm^3 in the first week to 82cm^3 in the 2nd week and reaches a peak at 170cm^3 in the 5th week, after which the level of production declined to 80cm^3 in the 9th week. Thus, the lowest gas production occurred in the 9th week. The mean volume of biogas produced from *Eichhornia crassipes* sample D (911.67cm^3) was lower than that produced from *Eichhornia crassipes* Samples E (1131.11) and F (1091.11cm^3) respectively. This may also be due to similar trend reported by Iloeje (1997).

The degradation of *Eichhornia crassipes* which was much slower and gradual throughout the experimental period can be attributed to its complex nature and micro-organism taking time to adjust to growth by synthesizing necessary enzymes needed to metabolize the substrate. This agrees with the work of Audu *et al.*, (2003) who reported that digesters charged with slurry required at least an adaptation period of 24 hours. The total weekly mean volume of biogas produced from *Eichhornia. crassipes* sample D, E, and F are 911.67cm^3, 10,180cm^3,9,820cm^3, respectively. The total mean biogas produced was therefore 1045cm^3. However, the production of biogas terminated from 41 to 45 days. This also is due to accumulation of toxic materials of microbial metabolism. This agrees with the findings of Oyewole, (2010) who reported that the detention period and retention period for biogas production using chicken droppings of eighth days and ten days respectively, may be due to the accumulation of acids, exhaustion of nutrient or production of auto toxic substances by the microbes. There was no significant difference ($P > 0.05$) observed in the level of gas yield in the 3 samples. The control experiment did not produce biogas as it was not seeded with methanogenesis organisms confirming that microbes are the agents of degradation that would lead to the production of methane gas. The average ambient temperature observed throughout the experimental period is between 28±2°C, this also agrees with the work of Audu *et al*,. (2003) who investigated conversion of municipal solid waste for biogas through anaerobic digestion method at mesophilic temperature.

The sludge remaining in the digester could be used as manure. Oyewole (2010) reported an increase in maize plant growth in sludge fortified soil compared to maize plant without sludge. Also, Burke, (2000) stated that the primary advantage of an anaerobic digester is its ability to nearly completely stabilize raw manure and complete retention of fertilizer nutrients (NPK) that were in the raw manure.

The study revealed the capability of both chicken droppings and *Eichhornia crassipes* to produce biogas when seeded with methanogenic organisms. But generally, the degradation of chicken droppings was more rapid initially compared to *Eichhornia crassipes* that was a slow process. Chicken droppings produce more biogas (1166cm^3) compared to *Eichhornia crassipes* (1045cm^3). This implies that the rate of conversion of organic waste to their end products at appropriate temperature depends on the nature of the waste. This agree with the work of Aliyu, *et al.* (1996) who reported that pigeon droppings, mixed with several substrates, together stabilized the fermentation process and promoted gas yield. He also reported an increased biogas production when the ratio of pigeon droppings with water was increased from 1:3 to 1:4 (w/v). Statistically, there was no significant difference ($P>0.05$) in the level of gas yield between the chicken droppings and *Eichhornia crassipes*.

This study therefore, implied that the waste materials can be used successfully for the production of alternative energy sources, especially now when demand for power is constantly increasing and the use of bio-fuel instead of fossil fuel has been on the agenda of national and international development strategies for sustainable energy prodction.

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