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Microbiological potentials of co-digestion of chicken droppings and banana peels as substrates for biogas production

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ABSTRACT

The microbiological potentials of using chicken droppings and banana peels for the production of biogas was investigated. Fermentation digesters were set up using sterilized and unsterilized samples with each having a set treated by heating at 80^0C for 1hour, cooled, filtered and dried and the other set untreated. The volatile fatty acid, organic matter, organic carbon, biogas production and microbial population were determined. The organic acid content was found to be directly proportional to the amount of volatile fatty acid produced hence the quantity of biogas. The microbiological analysis showed that the bacterial and actinomycetes populations increased from $2.4x10^4$ to $6.0x10^7$cfu/g and 1.6 $x10^2$ to $8.0x10^9$cfu/g respectively by the 30^{th} day. There was a reduction in the population of the cellulolytic bacteria. Biogas production was higher using co-substrates compared to when single substrates were used. The sterilized samples yielded no biogas indicative of microbial roles in biogas production.

INTRODUCTION

Biogas represents a mixture of gases produced as a result of the action of anaerobic microorganisms on domestic and agricultural waste [1]. It usually contains 50% and above methane and other gases in relatively low proportions namely CO_2, N_2 and O_2[2]. The mixture of the gases is combustible if the methane content is more than 50% [3]. If the$CO_2$is eliminated from the biogas mixture, the remaining gas often called biomethane has the properties of a natural gas and can be utilized to replace fossil natural gas as transportation fuel, raw material for the chemical industry or on fuel cells which convert it to electricity with high efficiency.

It is a natural process with microorganisms and typical substrates include sewage sludge, foodwaste, residues from agricultural herbs and plants [4]. Biogas technologies commonly apply anaerobic consortia of microbes. The biochemistry involve hydrolysis with the help of hydrolytic bacteria, acid formation with the help of acid forming bacteria (acetogenic bacteria) and methane formation which involves the anaerobic methanogenic bacteria [5]. The composition of the various microbes (microbial consortium) depends on various factors such as substrate ingredients, temperature, pH, mixing or the geometry of the anaerobic digester[6]. Members of Archae,Clostridia, Bacilli, Bacteriocides, Mollicutes, Gammaproteobacteria, Actinobacteria, methanogenes have been implicated in biogas production . Rising cost of fossil fuel, potentially diminishing supplies, environmental problems and desert encroachment, necessitate the need for alternative sources of energy. A wide variety of substrates, animal and plant wastes, food industry waste have been used for biogas production [7][8][9].

An improved yield of biogas is obtained when there is co-digestion of substrates. Co-digestion of several substrates such as banana and plantain peels, spent grains and rice husk, sewage and brewery sludge have produced increased methane yield by as much as 60% compared to that obtained from single substrates [10] [11] [12][13].

The need to address the problems associated with fossil fuels and the need to reduce environmental hazards associated with waste disposal, necessitated the exploration of microbiological potential of biogas production from chicken droppings and banana peels which otherwise would have constituted environmental problems.

EXPERIMENTAL SECTION

About 1.0 kg of chicken droppings was obtained from a farm in Abraka, Delta State, Nigeria while banana peels were obtained from a dump in the main market, Abraka. The droppings were sun-dried and crushed mechanically using a mortar and pestle to ensure homogeneity. The banana peels were also sun dried after which they were ground in a mill and mixed with water to form slurry. The slurry was divided into two parts: 1) untreated and 2) treated which was heated at 80^oC for 1 hour, cooled, filtered and dried to remove the water content. This was sieved and then kept aside.

One part each of the chicken droppings, the untreated and treated banana peels was sterilized in the autoclave at 121^oC for 15 minutes.

Fermentation Design

The prepared chicken droppings and banana peels were loaded into 5 No 1- litre Erlenmeyer flask containing 500ml distilled water in the ratio 250 g: 250 g (A – E) while 500g of only unsterilized poultry droppings and banana peels were placed into 2 flasks respectively (F and G). The content of each flask was thoroughly mixed. Each incubation flask was connected to a 2- litre Marriott bottle filled with $(NH_4)_2SO_4$ by means of a connecting tube and connected to a measuring cylinder by means of a connecting tube on the other side. The flasks were covered with black polythene to prevent light penetration. The digesters were incubated at room temperature that varied between 26 - 29^oC. The volume of water acid displaced was taken as a measure of the volume of biogas produced and was measured every 24 hours (modification of method of Iyagba)[6]. Digesters were agitated twice a day for digestion in the entire medium. The fermentation period was 25 days.

Digester's set- up:
A = Unsterilized poultry droppings + Unsterilized untreated banana peels
B = Sterilized poultry droppings + Sterilized untreated banana peels
C = Unsterilized poultry droppings + Unsterilized treated banana peels
D = Sterilized poultry droppings + Sterilized treated banana peels
E = Unsterilized poultry droppings + Sterilized treated banana peels
F = Unsterilized untreated banana peels only
G = Unsterilized untreated poultry droppings only

Determination of Volatile Fatty Acid

This was determined by High Performance Liquid Chromatography under the following conditions: Solvent 0.1 N H_2SO_4; flow rate 0.8mlmin^{-1}; Column temperature, 41^oC.

Determination of Organic matter

This was done using the loss on ignition method [14]. A known weight of sample was placed in a crucible which was heated to 430^OC overnight. The sample was then cooled in a desiccator and weighed. Organic matter content was calculated as the difference in the initial and final sample weights divided by initial sample weight multiplied by 100%. All weights were corrected for moisture / water content prior to organic matter content calculation.

Determination of Organic Carbon

This was determined by oxidation with K_2CrO_7 in H_2SO_4 according to Yeomans and Bremner[15].

Estimation of Microbial Population in Samples of Poultry Droppings and banana Peels

The homogenized samples of banana peels and chicken droppings were serially diluted and inoculated onto Nutrient agar, Glycerol agar, Cellulose agar and Lactose agar plates using pour plate method for the determination of bacteria, actinomycetes, cellulolytic bacteria, and coliform bacteria respectively. The fungi were inoculated into Saboraud Dextrose Agar plates. The plates were incubated at 28 ± 2^oC for 24-72 hours. Distinct colonies were counted and recorded as cfu/g.

Measurement of Biogas Formed

The biogas formed in the digesters was passed to the flask containing the $(NH_4)_2SO_4$solution as described previously. A pressure is built up which forces the displacement of the solution. The amount of displaced solution is equivalent to the amount of biogas produced.

RESULTS AND DISCUSSION

Biogas production was highest in the unsterilized poultry dropping and unsterilized banana peels (807.8) followed by the unsterilized poultry dropping mixed with sterilized treated banana peels. The least biogas production was got with unsterilized untreated banana peels while the sterilized materials did not yield any biogas(Table 4). The inability of the sterilized materials to produce biogas might be attributable to the destruction of the microorganisms that would have produced the enzymes necessary for the biochemical reactions to produce the biogas. There was an initial lag phase of about 5-10 days (Table 4) before the biogas production. The lag may be due to the complexity of biodegradation involving high lignin content present in banana peels [6]. The lag may also be accounted for by the lag phase of microorganisms present as a result of changes in the environment and nutrient. The organisms may be trying to adapt to the environment or the methanogens were consuming the methane precursors produced. The differences in the quantities of biogas produced in the various set-ups may be as a result of differences in the bio-digestibility of the poultry droppings and banana peels and the concentrations of the volatile fatty acid[16]. The amount of volatile fatty acids has been found to be directly proportional to the biogas produced.

The microbial population and type can affect the biogas production. Similar organisms were isolated from the banana peels and chicken droppings. The organisms include *Actinomycesisraeli, Pseudomonas aeruginosa, Clostridium perfringes, Methylibiumsp, Methylvibriosp, Methanosarcina, Penicilliumsp, E. coli, Fusariumsp, Staphylococcus aureus, Shigellaflexnerii*and*Aspergillus sp*. [11] isolated similar organisms from banana peels. These microorganisms would have contributed to the yield of biogas since biogas technologies have been found to commonly apply natural anaerobic consortia of microbes.

The microbial population utilize the biopolymers to produce volatile fatty acid and hydrogen and the volatile fatty acidsare converted into biogas (CH_4 and CO_2) [17] [18]. The treated banana samples had reduced lag phase (early biogas production) which can be attributed to the lignin content being removed as a result of hydrolysis during the heat treatment thus making cellulose material available for digestion. The isolates which include cellulolytic fungi may have contributed to the biogas production since they sequentially reduce the chain and branch to dimeric and then to monomeric sugar molecules which are then converted to organic acids. Biogas yield in the poultry droppings and banana peels were better enhanced (Table 4). These may be due to the synergistic action of microorganisms of the poultry droppings and banana peels. Biogas production using co-substrate have been found to have higher biogas yield due to positive synergism in the digestive medium and the supply of missing nutrient by co-substrate [19]. Poultry droppings had an organic content of 68.4% and produced higher concentration of volatile fatty acids and the highest quantity of biogas (Tables 3 and 4). This conforms to the fact that biogas from methanogenicactivity, depend on the amount of organic matter content which is decomposed or digestible in the raw materials during fermentation. Poultry droppings have been found to be highly decomposable as they contain little cellulose and hemicellulose fractions which are components of banana peels in addition to lignin which is an indigestible fraction of the plant material [20] and protects cellulose from bacterial action. The decrease in the cellulolytic organisms present in the digester as the experiment progressed may be attributed to a decrease in the amount of cellulose materials in the digesters due to the continuous breakdown of the complex materials to simple organic compounds. There might have also, been some degree of microbial succession in which probably, the fungal, bacterial and cellulolytic organism produced a favourable environment for the rapid growth of actinomycetes[21].

The sterilized substrates gave no biogas yield which indicated that microorganisms played the significant role of biogas production. The poultry droppings with banana peels as co-substrates produced the highest yield of biogas so can be resorted to as cheap and more ecofriendly substitute of the conventional energy sources.

Table 1: Estimation of Microbial Population in the Poultry Droppings and Banana Peels

Type of Organism	Organism's (cfu/g)	LOAD(cfu/g)				
	Day 0	Day 5	Day 10	Day 15	Day 20	Day 25
Bacteria	1.44**	1.32**	2.05**	0.90**	1.81**	3.61**
Actinomyces	0.96*	1.44*	0.72*	2.41*	2.41*	4.82*
Coliforms	1.69**	1.57**	1.93**	1.93**	3.73**	1.20**
Fungi	2.17**	2.53**	1.81**	2.05**	2.53**	2.53**
Cellulolytic Bactreria	0.60**	2.47**	3.61**	2.53**	1.81**	1.51**

P < 0.05
** Not Significant*
*** Significant*

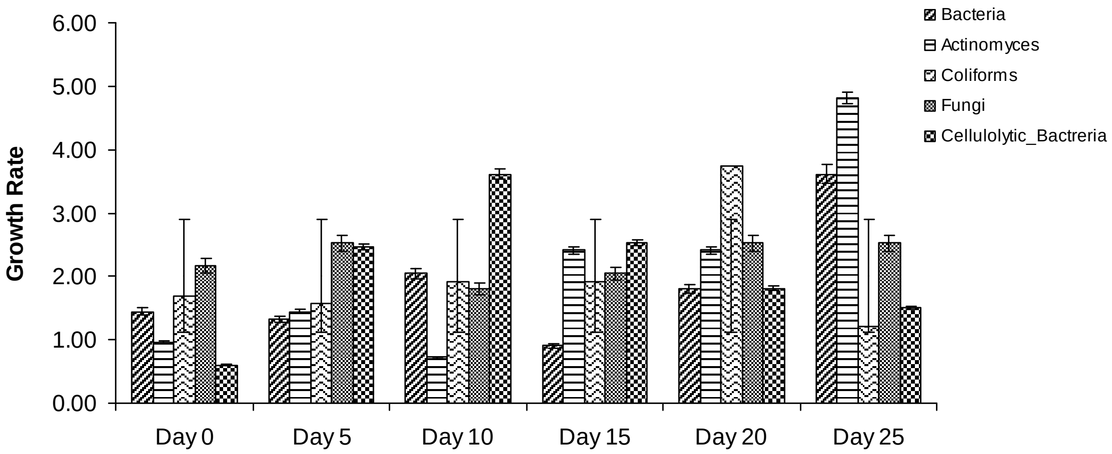


Table 2: Volatile Fatty Acid Contents of Samples Contained in Various set-ups

Incubation period (Days)	Volatile Fatty acid (mg/l)						
	A	B	C	D	E	F	G
0	250	240	350	400	180	169	90
5	725	650	800	680	600	620	120
10	800	680	850	700	620	630	160
15	780	680	920	720	650	640	110
20	560	520	840	550	450	480	90
25	450	320	620	340	320	420	50
30	200	300	410	280	200	210	20

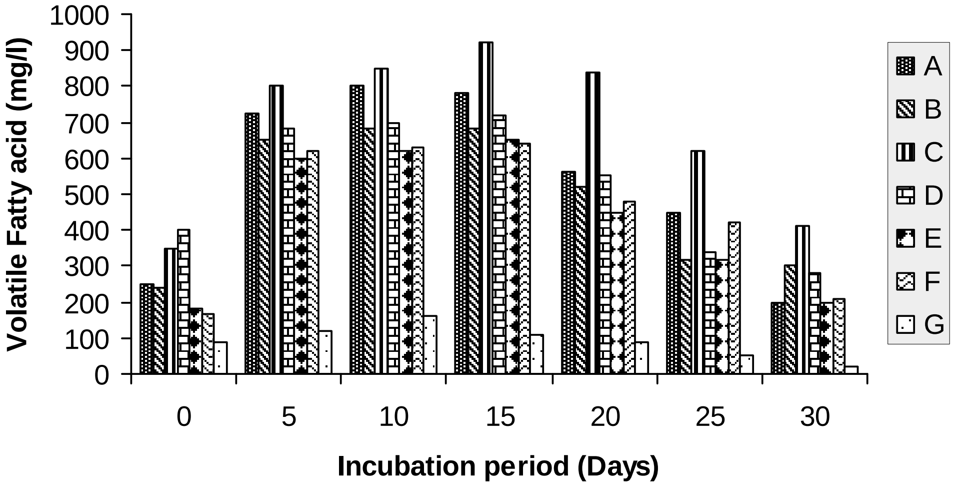


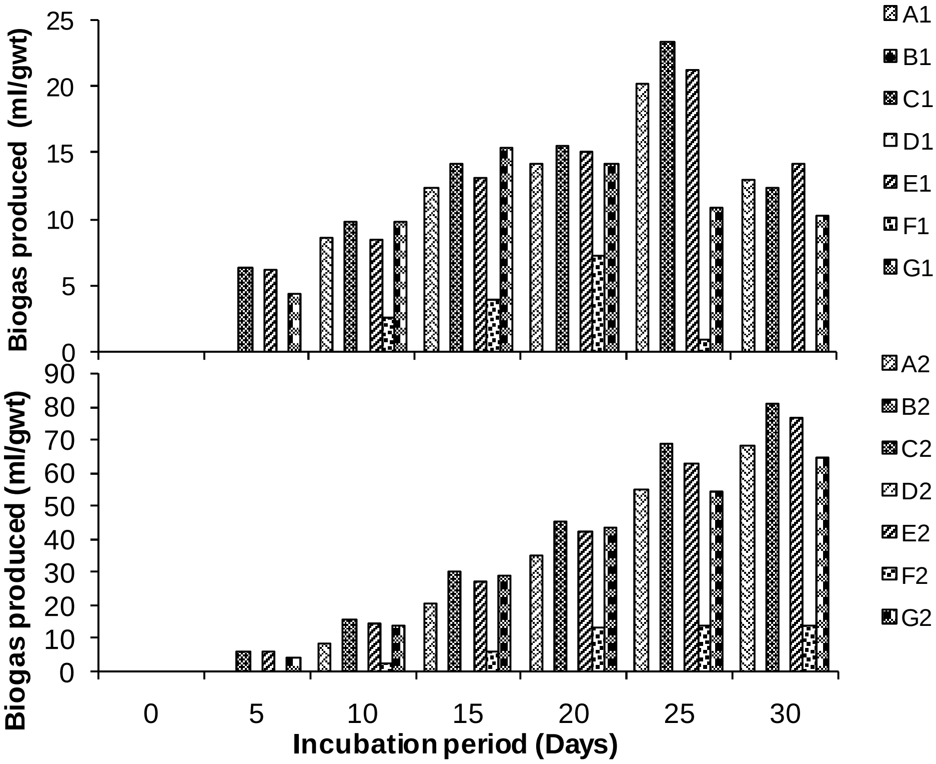
Table 3: Total organic carbon and organic matter contents of various samples used for biogas production

Sample	Total Organic Carbon (%)	Total Organic matter (%)
Poultry dropping	34.6 ± 10.3	68.40 ± 8.10
Banana peels	45.2± 11.20	52.3 ± 10.2

Table 4: Daily and cumulative biogas production

Incubation period (Days)	Biogas produced (ml/gwt)													
	A1	A2	B1	B2	C1	C2	D1	D2	E1	E2	F1	F2	G1	G2
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
5	0	0	0	0	6.25	6.25	0	0	6.03	6.03	0	0	4.26	4.26
10	8.46	8.46	0	0	9.64	15.89	0	0	8.42	14.45	2.42	2.42	9.64	13.9
15	12.27	20.73	0	0	14.02	29.91	0	0	12.96	27.41	3.78	6.2	15.34	29.24
20	14.01	34.74	0	0	15.37	45.28	0	0	15.06	42.41	7.16	13.36	14.01	43.25
25	20.15	54.89	0	0	23.26	68.84	0	0	21.15	62.60	0.8	14.16	10.83	54.08
30	12.93	67.82	0	0	12.24	80.78	0	0	14.04	76.66	0	14.16	10.21	64.21

1Daily biogas production
2 Cumulative biogas productions



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