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PRODUCTION OF BIOGAS USING ABATTOIR WASTE AT DIFFERENT RETENTION TIME.

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ABSTRACT

The biogas production potential of abattoir waste at different retention time was investigated and the bacteria associated with the production as well as the pH of the slurry before and after the biogas production was determined. The result revealed the presence of *Bacillus megaterium, Bacillus licheniformis, Bacillus pumilus, Bacillus brovis, Bacillus alvei, Bacillus lentus, Yersinia enterocolitica, Proteus vulgaris, Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus* and *Salmonella* sp from the spent slurry. It also indicated a slight shift from a neutral medium to a slightly acidic environment in all the digesters. The highest volume of biogas (2240cm^3) was obtained in week 2 while the least volume (1820cm^3) was obtained in week 4.Significant difference ($p < 0.05$) was observed in the volume of biogas produced in the first and second week as well as to that of third and fourth week. However, no such difference ($p < 0.05$) was observed in the volume of biogas produced in the third and fourth weeks.

Keywords: Abattoir, Biogas, Production, Retention, Time, Waste

INTRODUCTION

The most fascinating features of any civilized communities are the abundant availability of energy for domestic, agricultural and industrial purposes (Baki, 2004). Energy is the source of economic growth. Energy consumption reflects the state of development of a nation. Renewable energy effectively uses natural resources such as sunlight, wind, rain, tides and geothermal heat, which may be naturally replenished. Renewable energy technologies range from solar power, wind power, hydroelectricity, micro-hydro, biomass and biofuels for transportation. In 2006, about 18 percent of global final energy consumption came from renewable, with 13% coming from traditional biomass, like wood-burning. Hydropower was next largest renewable source providing 3% (Oyeleke *et al.,* 2003).

The production of biogas from renewable resources is becoming a prominent feature of most developed and developing countries of the world. Despite the variability of international opinion on this technology, it is agreed that it plays an important role in the domestic and agricultural life of the rural dwellers in countries like India, China, Korea and Malaysia. It is used for cooking, crop drying and soil fertilizing (Meena & Vijay, 2010).

Biogas is produced when bacteria degrade biological materials in the absence of oxygen, in a process known as anaerobic digestion (Garba & Atiku, 1992). Anaerobic treatment is the use of biological processes, in the absence of oxygen, for the breakdown of organic matter and the stabilization of these materials by conversion to methane and carbon dioxide gases and a nearly stable residue (Cassidy *et al*., 2008). Animal wastes can be use as sources of nutrient, feed ingredients to microorganisms and as fuel energy source, they contain high level of organic matter that could be converted into energy as supplement for fossils. Animal wastes are abundant all over the world with Nigeria producing about 227,500 tons of fresh waste each day, (Oyeleke *et al.,* 2003) that 1kg of fresh animal waste produce about 0.03m^3 of gas per day. This shows theoretically that Nigeria can produce 6.8 million M^3 of biogas daily, which in terms of energy is equivalent to about 3.9 million liters of petroleum. The use of biogas is capable of providing a special impetus in both rural and urban areas. Biogas plant can be built by using materials which are locally available in most developing countries (Baki, 2004).

The anaerobic digestion of municipal waste can have positive environmental value since it can combine waste removal and stabilization with net fuel (Biogas) production. The solid or liquid residue can further be used as feed or as biomass briquette for cooking (Baki, 2004). Therefore, the objective of the research is to screened abattoir waste for biogas production at different retention time with a view to solving its disposal problems.

MATERIALS AND METHOD

Sample collection and Preparation: Fresh rumen content of cattle was collected from the Sokoto central abattoir in Sokoto metropolis. A clean container with cover was used for the collection of the waste. The sample was collected when the animals were being slaughtered. The container was placed in a cool box and transported immediately to the Energy research centre laboratory at Usmanu Danfodiyo University, Sokoto.

Slurry Preparation: Two hundred grams (200g) of the sample was weighed and mixed with 400cm^3 of distilled water in a beaker to give a ratio of 2:1. The initial pH of the mixture was determined.

Experimental Set-up: Four sets of 500g capacity tins each containing four tins were used as digesters. The digesters were labeled A, B, C and D and each set replicated three times to give a triplicate sample. Equal concentration of the slurry was poured in to the digesters.

The digesters were sealed with araldite adhesive to cover leakages and connected with delivery tube which conveys the gas from the digester to a 1000cm^3 measuring cylinder and inverted into a bowl containing water for gas collection using water displacement method. The digesters were set up and allowed to undergo anaerobic digestion for a retention period of four weeks (one month). The amount of gas produced was recorded at 12 noon on weekly basis and the amount of gas as well as pH recorded.

Microbial analysis: Serial dilution of the fresh sample and the digested slurry sample were carried out up to 10^6 tube. 0.5ml was obtained using sterile syringe from the 10^5 tube and inoculated onto already prepared nutrient agar plates by spread plate method of inoculation. The plates were replicated three times. Modified Mackintosh and Fildes pattern of anaerobic jar was used to incubate the plates. The residual oxygen (O_2) in the anaerobic jar was evacuated by placing a kindled match stick, which quenched immediately the left-over oxygen was exhausted. The jar was incubated for a period of 72 hours at 37 ^{o}C.

Bacterial colonies that emerge on the plates, were counted and recorded as colony forming units per milliliter (cfu/ml) of the sample. The colonies were also subcultured repeatedly on fresh plates to obtain pure isolates. The pure bacterial isolates were gram-stained and subjected to different biochemical tests which

included production of cogulase, catalase, urease, oxidase, methyl red, Voges-Proskaeur, citrate utilization test, H_2S production and carbohydrate fermentation as described by Cheesebrough (2006). The bacterial isolates were identified by comparing their characteristics with those of known taxa using the schemes of Cowan & Steel (1993).

RESULTS

The result of the bacterial identification was presented in Table 1**.** The result obtained shows that the bacteria isolated and identified were *Bacillus megaterium, Bacillus licheniformis, Bacillus pumilus, Bacillus brovis, Bacillus alvei, Bacillus lentus, Yersinia enterocolitica, Proteus vulgaris, Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus* and *Salmonella* sp.

The result of the percentage frequency of occurence of the isolates was presented in Table 2. Different varieties of *Bacillus* species isolated had the highest frequency of occurence of 46 %. This was followed by *Proteus vulgaris* and *Staphyllococcus aureus* with 17% each. The least frequency of occurence of 4% was obtained in *Yersinia enterocolitica, Pseudomonas aeruginosa* and *Salmonella* sp respectively. The result of the pH of the digesters before and after the biogas production was presented in Table 3. The result indicated a slight shift from a neutral medium to a slightly acidic environment in all the digesters. The volume of biogas produced in the four digesters on weekly basis was presnted in Fig. 1. The highest volume of biogas (2240 cm^3) was obtained in week 2 while the least volume (1820 cm^3) was obtained in week 4.

TABLE 1. ISOLATION AND CHARACTERIZATION OF BACTERIAL ISOLATES FROM THE BIOGAS DIGESTERS

Isolate code	Gram reaction	Catalse	Glucose	Sucrose	Motility	Indole	Urease	H_2S	Gas	Methyl red	Lactose	Citrate	Voges proskeur	Spore	Bateria isolated
BBG1	+ve	-	+	+	+	-	+	-	-	+	-	-	-	-	*Yersinie enterocolitica*
BBG2	+ve	+	+	-	+	-	-	+	-	-	-	+	+	+	*Bacillus megaterium*
BBG3	+ve	+	+	+	+	-	+	-	+	-	-	+	+	+	*Bacillus licheniformis*
BBG4	-ve	-	+	+	+	Wk	+	+	-	Wk	-	+	-	-	*Proteus vulgaris*
BBG5	-ve	-	+	-	+	+	-	-	+	Wk	-	-	-	-	*Escherichia coli*
BBG6	-ve	+	+	+	+	-	-	-	-	-	-	+	+	+	*Bacillus pumilus*
BBG7	-ve	+	+	+	+	-	-	-	-	+	-	+	-	+	*Bacillus brovis*
BBG8	+ve	-	-	-	+	-	-	-	-	+	-	+	-	-	*Pseudomonas aeruginosa*
BBG9	+ve	+	+	+	+	+	+	+	-	-	-	-	+	+	*Bacillus alvei*
BBG10	+ve	+	+	-	+	-	+	-	-	+	-	-	-	+	*Bacillus lentus*
BBG11	-ve	-	+	-	+	-	-	+	-	+	-	+	-	-	*Salmonella* sp
BBG12	+ve	+	+	+	-	-	+	-	-	-	-	+	+	-	*Staphylococcus aureus*

TABLE 2. PERCENTAGE FREQUENCY OF OCCURRENCE OF BACTERIA ISOLATED FROM BIOGAS DIGESTERS

Bacteria	% occurrence of isolate
Bacillus sp	46
Yersinia enterocolitica	4
Proteus vulgaris	17
Escherichia coli	8
Pseudomonas aeroginosa	4
Staphylococcus aureus	17
Salmonella sp	4

TABLE 3. pH OF DIGESTERS BEFORE AND AFTER BIOGAS PRODUCTION

DIGESTER	BEFORE	AFTER
A	7.26±0.01	6.82±0.70
B	7.21±0.01	6.35±0.03
C	7.16±0.03	5.80±0.01
D	7.10±0.01	5.66±0.01

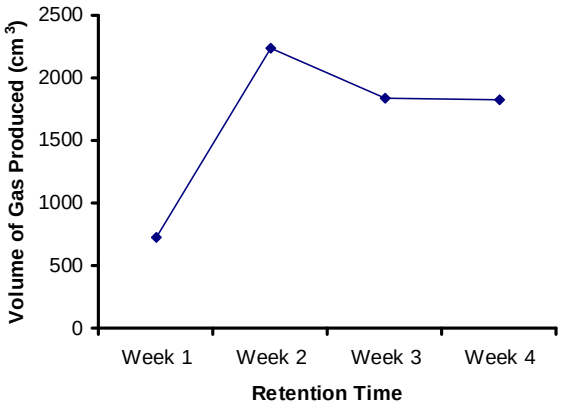

FIG 1. VOLUME OF GAS PRODUCED (CM³) AGAINST RETENTION TIME

DISCUSSIONS

The results from this study showed *Bacillus* species apperas to overlap from one stage to another during biogas production, suggesting a succession in species of bacteria during the process of gas production. But some species such as *Bacillus* where found to be present throughout the process of gas production (Baki, 2004). The result obtained from this study indicates that *Bacillus* species were the most common bacteria isolated and identified during the research, suggesting that the species plays a vital role in the microbial activities for the production of biogas. It should be noted that *Bacillus megatarium, Bacillus licheniformis Proteus vulgaris* and *Escherichia coli* were isolated during the first week and were able to produce about 720 cm^3 of biogas, while *Bacillus pumilus, Proteus vulgaris, Bacillus brovis, Pseudomonas aeruginosa* and *Bacillus alvei* were isolated in the second week (14 days) and produced 2240 cm^3 of biogas gas. *Bacillus lentus, Bacillus pumilus, Proteus vulgaris* and *Salmonella* sp occurred in the third week (21 days) and were able to produce 1840 cm^3 of biogas. However, *Staphylococcus epidermidis, Staphylococcus aureus* and *Bacillus brovis* were isolated from the fourth week (28 days) and produced 1820 cm^3 of biogas. The ability of *Bacillus* species to overlap during the production were probably due to the fact the organisms can produce spore which help them to withstand the harsh anaerobic condition or heat evolve during the biogas production (Baki, 2004). These findings were in line with that of Oluyega *et al.,* (2006) in which *Bacillus, Yersinia,* and *Pseudomonas* species were found to be responsible for biogas production from cow dung.

The pH of the slurry appeared to be decreasing in all the digesters. This is not surprising as the decrease in pH may be as a result of anaerobic fermentation taking place. pH is an important factor that affects biogas production. It was reported that anaerobic bacteria required a natural environment (Garba & Atiku, 1992) and thus, pH ranging from 6.4-7.2 is required for optimum biogas production. Also, the decrease in pH may be due to the action of acetogenic methanogens as they break down sulphur containing organic and inorganic compounds as well as the formation of fatty acids. It was reported by Oyeleke *et al.,* (2003) that biogas produced at pH of 5 is greater than that of pH 10. Some microorganisms also evolved later in the process while others died off midway through the process. This may be explained in terms of Shellford's law of tolerance that the occurence of any organism in any environment is determined not only by availability of nutrients but also by various physicochemical factors. Therefore, as the medium tend to become acidic, non-acid tolerance organisms were replaced by acid tolerant organisms.

Results from this work showed that biogas was produced from the abattoir waste at different retention time. After the first week, there was a sharp increase in the volume of biogas produced in the second week. However, from the third to the fourth week the volume of biogas produced continued to decline. Therefore, it can be deduced that the increase in the second week indicated the acclimitization of the biogas producing microorganisms after the hydrolysis of the waste in the first week by the hydrolyzing organisms. The biogas production reached its peak in the second week and the action of biogas producing organisms decline and were replaced by organisms that tend to utilized some of the products of their actions. This probably explained the continued decline in the volume of biogas produced in ther third and fourth week.

Significant difference ($p < 0.05$) was observed in the volumes of biogas produced in the first and the second week of biogas production. Also, the volume of biogas produced in the second week differed significantly ($p < 0.05$) to that of the third and fourth week. However, no such significant difference ($p > 0.05$) was observed in the volume of biogas produced in the third and fourth weeks. This was in conformity to the findings of Bagudo *et al.,* (2008) in which 8772.50 cm^3 of biogas was produced from cow dung. Wahyudi *et al*., (2010) also reported the production of 2500 ml of biogas from content of sheep colon at two weeks retention time.

CONCLUSION

The results of the research indicated that abattoir waste could serve as a suitable substrate for biogas production. The utilization of this substrate for biogas production could eliminate its disposal problems and create another abundant source of sustainable energy.

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