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JOURNAL OF BIOMEDICAL RESEARCH & CLINICAL PRACTICE

Original Article

Isolation of Microorganisms Associated with Biodegradation of Household Domestic Wastes for Biogas Production in Niger State, Nigeria

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ABSTRACT

This study focused on the isolation of microorganisms associated with biodegradation of domestic wastes in three rural communities (Gbadagbadzu (A), Ndawangwa (B), and Kuchiworo (C)) in Lavun Local Government Area of Niger State, Nigeria, for biogas production. The biogas was produced by anaerobic microbial degradation of different biodegradable household domestic waste aided by fresh rumen of cow. The anaerobic microbial degradation was carried out in a temperature range of 25^0C to 32^0C for a detention time of 39 days for rural biogas production. The results showed the presence of the following bacteria: *Bacillus cereus*, *Sphingobacterium yamdrokense*, *Clostridium perfringens*, *Salmonella typhi*, *Alkaligenes faecalis*, *Pseudomonas aeruginosa*, *Staphylococcus epidermidis*, *Klebsiella pneumoniae* and *Bacillus licheniformis* while fungi isolated were *Muccor pusillus* and *Aspergillus flavus*. The research therefore shows that household domestic wastes have the potential to produce biogas with or without the addition of inoculum.

Keywords*: Biogas, Biodegradation, Domestic waste, Household, Microorganisms.*

INTRODUCTION

2018 Journal Impact Factor: 1.10
Print ISSN: 2636-7378 | Online ISSN: 2651-5865

Energy crisis and climate change are among the major problems drawing much attention all over the globe and renewable energy has been identified as one of the solutions.[1] Biogas is an alternative source of renewable energy, it is clean and environmentally friendly and often produced from organic materials that are first decomposed by microorganisms in an anaerobic environment.[2] A complex microbiological process lies behind the efficient production of biogas.[3] Many different species of microorganisms need to be active in order for biogas to form and these organisms have to work closely together. A disturbance of this teamwork results in reduced biogas production. [4] Controlling the biogas process in an efficient manner requires the knowledge of microbiology that results in reducing pressure on wood as fuel source and improves the environment.[5] Microorganisms require food (substrate) in order to function and grow. The organic waste pre-treated in the biogas process represents the substrate for various microorganisms. These includes sludge from municipal wastewater treatment plants, slaughterhouse waste, waste from the food and feed industries, source-sorted food waste and manure, grease traps, fryer fat, wastes from the dairy and pharmaceutical industries, grass silage, and domestic household wastes.[6,7] Careful removal of agro-industrial/domestic household wastes from the environment and converting them to biogas is a recommended method for development of sustainable healthy environment. Many local communities especially in developing world have no environmentally

friendly ways to dispose such wastes. Generally, large amounts of household and municipal wastes are dumped around human settlements, resulting in disposal problems and methane emissions during its natural decomposition. Some of these wastes are of low density and easily become air borne pollutants.[8] Environmental problems associated with poor wastes management have resulted in increased water borne illness especially typhoid fever, dysentery and diarrhoea.[9,10] These challenges have continued to retard public health improvement programmes of governments and private organizations. Several reports indicated that organic wastes which represent 45-65% of the volume of municipal wastes is a key challenge in waste management.[11,12,13] The aim of this study was to isolate and identify microorganisms associated with biogas production from domestic wastes generated from rural communities in Niger State.

MATERIALS AND METHODS

Collection and processing of samples

The substrates used for this study were domestic household wastes including carbohydrate food wastes (boiled yam, yam peels and products, bread crumps, boiled rice, potato peels, cassava peels, cassava products), maize cobs, groundnut shells, leafy vegetables as well as foods containing proteins (beans and beans products, egg shells, fish crumps). They were collected from three local communities: Gbadagbadzu (A), Ndawangwa (B), and Kuchiworo (C), all in Lavun Local Government Area of Niger State, Nigeria. In each of these communities, ten (10) clean waste bags were distributed to ten (10) household for a period of one month. The waste bags were collected every two days and emptied into two clean waste containers in each of the communities giving a total of six waste containers. All the samples collected were air-dried at room temperature (28 $\pm$ 2°C) for seven days, pounded using a clean mortar and pestle, kept in air-tight containers.

Analysis of substrates for microbiological properties

The microbiological parameters determined were : total aerobic heterotrophic bacterial counts, methanogenic/anaerobic bacteria counts, faecal coliform and non-faecal coliform counts, total salmonella-shigella counts and fungi counts using Nutrient agar (NA), Mac Conkey Agar (MCA), Eosin methylene blue (EMB) agar and Sabouraud dextrose agar (SDA) respectively.[14, 15]

Determination of total aerobic heterotrophic and methanogenic/anaerobic bacteria counts

Substrate homogenate was prepared by dissolving 1g of substrate in 10 mL of sterile distilled water. This was serially diluted and inoculated on Nutrient agar (NA) plates. The plates were incubated at 37°C for 24 hours while plates for anaerobic counts were incubated anaerobically using anaerobic jars at 37°C for 24 – 48 hours. Plates with 30 – 300 colonies were counted (including pin point colonies) and the mean counts calculated factor. The aerobic and anaerobic colony counts were computed as reported by Kiiyukia[14] and is given as

$$N = A \times D \quad (1)$$

where N is the number of colonies per mL of sample, A is the average count per plate and D is the respective dilution factor

Enumeration of coliforms

Samples were serially diluted and the suspension was inoculated into the respective media using pour plating technique. Colonies that grew on the media were sub-cultured repeatedly on the media used for primary isolation to obtain pure cultures. The pure cultures were maintained on agar slants for further characterization and identification using standard biochemical tests.[16]

Enumeration of fungi

The fungi were enumerated using standard methods reported by Kiiyukia[14] and Asikong *et al.*[17] Serially diluted samples were inoculated into sabouraud dextrose agar plates with two vial of chloramphenicol to inhibit the growth of bacteria. The plates were incubated at room temperature (28 ± 2°C) for 3-5 days. Colonies were counted and expressed as colony forming units per gram of sample (cfu/g). Colonies were subcultured repeatedly on media used for primary isolation to obtain pure cultures. The pure cultures were maintained on SDA slants for further characterization and identification.

Identification and characterization of microbial isolates

The bacterial isolates were Gram stained and subjected to biochemical tests including production of catalase, coagulase, indole, oxidase, hydrogen sulphide, methyl–red Voges-prokauer, starch hydrolysis, citrate utilization, sugar fermentation.[15,16] The isolates were identified by comparing their characteristics with those of known taxa using Bergey's

Manual of Systematic Bacteriology.[18] The fungal isolates were characterized based on the colony morphology, nature of hyphae, nature of conidia and shape. A portion of the mycelial mat of the fungi was picked with sterile needle and placed on a clean, grease-free slide containing a drop of lacto-phenol cotton blue stain. The mycelial growth was teased gently to allow it mix with the stain, covered with cover slip and was observed under a low to high power objectives (x10 and x40) of the light microscope. The fungi isolates were identified by comparing their characteristics with those of known taxa using the schemes of Jott *et al*.[18]

Equipment used for the production of biogas

A biodigester capable of producing biogas from household domestic waste was designed and constructed in order to achieve the study objectives. The digester (20 litres capacity) consisted of anaerobic chamber and gas collecting chamber. In between the two chambers was a narrow passage which allowed the flow of gas from anaerobic chamber to gas chamber. As microbial activities began, the emissions were released and in about 21 days it was ready for harvesting. A short valve of 10 mm diameter conveyed the gas from gas chamber to element for burning. In between the burner and gas chamber was a knob which served to regulate the biogas flow as shown in Plate 1.[17]

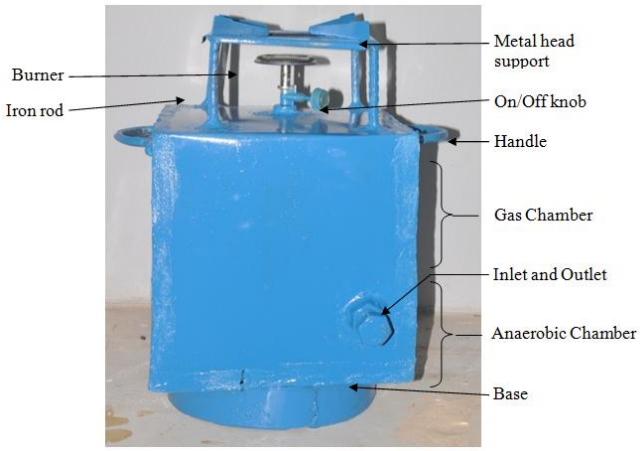

Plate 1: Biogas production design for rural communities

RESULTS

Microbiological properties of organic wastes

The total microbial counts of undigested (UDW) and digested (DGW) wastes respectively, are presented in Table 1. The results revealed that total heterotrophic bacterial counts, total fungi, total faecal coliform and total Salmonella-shigella counts were higher in UDW than DGW samples (Table 1). It was 3.8 x 10^8, 6.7 x 10^8TVC, 1.3 x 10^3, 1.10 x10^3 TFC, 1.4 x 10^5, 2.3 x 10^5 TFCC and 6.0 x 10^6 TSSC for UDW while DGW had bacterial counts of 4.5 x 10^4, 2.45 x 10^6, 1.21 x 10^2 TVC, 1.2 x 10^2, 1.0 x 10^2 TFC, 1.6 x 10^3, 1.2 x 10^3 TFCC and 3.6 x 10^3, 2.5 x 10^6 TSSC respectively.

In the same vein, anaerobic / methanogenic counts were higher in UDW 1.8 x 10^6 and 2.10 x 10^6 than DGW 1.31 x 10^3 and 1.7 x 10^3. The sum total of bacterial counts for AN/MB UDW was 3.9 x 10^{36}as against 3.01 x 10^9DGW respectively (Table 1).

Table 1: Microbial counts of undigested and digested organic waste

	Aerobic bacteria (Cfu/g)				Anae./Methano. Bacteria (Cfu/g)
Sample	TVC	T.FC	TFCC	TSSC	Anae./Methano.
UAL	3.8 x 10^8	1.3x10^3	1.4x10^5	Nil	Nil
UBL	6.7x10^9	1.10x10^3	2.3x10^5	6.0 x 10^6	Nil
UAL	Nil	Nil	Nil	Nil	1.8 x 10^6
UBL	Nil	Nil	Nil	Nil	Nil
DLW(CABCD)	4.5 x 10^4	1.2 x 10^2	1.6 x 10^3	3.6 x 10^3	Nil
DSW(CABCD)	6.0 x 10^6	1.0 x 10^2	1.2 x 10^3	245.0 x 10^6	Nil
DLW(RA, RB, RC, RD)	Nil	Nil	Nil	Nil	1.31 x 10^3
DSW(RA RB RC RD)	Nil	Nil	Nil	Nil	1.7 x 10^3

UAL: Undigested household domestic (organic waste), UBL: Fresh content of the rumen of cow, DGW: Digested organic waste, DSW: Disgested Solid Waste, TVC: Total viable counts, TFC: Total fungi counts, TFCC: Total faecal coliform counts, TSSC: Total *Salmonella–Shigella* counts, Anae./Methano.: Anaerobic/Methanogenic bacteria, CABCD: Communities AB (D the Control), RA, RB, RC, RD: digesters containing waste used for rural biogas production Cfu/g: Colony forming units per gram Note: UAL and UBL = UDW, DLW and DSW = DGW.

Identification of microbial isolates and their frequencies of occurrence

Morphological characteristics from digested and undigested organic waste revealed a total of nine (9) bacterial species. The bacteria were *Bacillus cereus, Clostridium perfringens, Sphingobacterium yamdrokense, Alkaligenes faecalis, Staphylococcus epidermidis, Pseudomonas aeruginosa, Klebsiella pneumoniae, Salmonella typhi, Bacillus licheniformis*. All the bacteria were rods (bacolli) except *Staphylococcus epidermidis* which was cocci.

Macroscopic and microscopic morphology of fungi isolated from digested and undigested organic wastes revealed the presence of *Aspergillus flavus* and *Mucor pusillus*.

Table 2, showed the frequency of occurrence of bacterial isolates in liquid digestate from rural digesters (RA, RB, RC and RD respectively). The highest frequency of occurrence was recorded with *Bacillus cereus* (35.48%) while *Bacillus licheniformis* had the least frequency of occurrence (2.65%) *Clostridium perfringens, Sphingobacterium yamdrokense, Alkaligenes faecalis, Pseudomonas aeruginosa, Staphylococcus epidermidis Klebesiella pneumoniae* and *Salmonella typhi* had 6.19%, 15.04, 5.30%, 3.30%, 6.19%, 15.92% and 7.96% respectively. The results also revealed that rural digester RA had the highest total number of isolates and decreased in the order RA (50), RB (32), RC (19) and RD (12) which is the least, with *Bacillus cereus, Klebesiella pneumoniae* and *Salmonella typhi* found in all the digesters (Table 2).

Table 2: Frequency of occurrence of bacterial isolates from liquid digestate in rural digesters with or without starter culture

	Rural digesters				
	RA	RB	RC	RD*	Total (%)
Bacillus cereus	13(11.50)	11(9.73)	9(7.96)	7(6.19)	40(35.48)
Klebsiela pneumoniae	6(5.30)	3(2.65)	6(5.30)	3(2.65)	18(15.92)
Sphingobacterium yamdrokense	10(8.55)	7(6.19)	0(0)	0(0)	17(15.04)
Salmonella typhi	2(1.77)	1(0.88)	4(3.54)	2(1.77)	9(7.96)
Clostridium perfringens	5(4.42)	2(1.77)	0(0)	0(0)	7(6.19)
Staphylococcus epidermidis	4(3.54)	3(2.65)	0(0)	0(0)	7(6.19)
Staphylococcus aureus	4(3.54)	2(1.77)	0(0)	0(0)	6(5.30)
Pseudomonas aeruginosa	4(3.54)	2(1.77)	0(0)	0(0)	6(5.30)
Bacillus licheniformis	2(1.77)	1(0.88)	0(0)	0(0)	3(2.65)
Total	50(44.23)	33(28.29)	19(16.74)	12(10.61)	113(100)

RD: Liquid digestate without starter culture (control) Values obtained are significantly different at ($p \leq 0.05$)*

Biogas production

Figures 1 and 2 show the rates of biogas production from household domestic wastes with or without starter culture in rural digesters RA, RB, RC and RD. The results indicated that in (39 detention days) rural digester RA had a biogas volume of 98.14 cm^3, rural digester RB had 31.53 cm^3, RC gave 6.21 cm^3 while RD, (control) that was without starter culture gave 4.72 cm^3 within a detention time of 33 days (Fig. 1). Thus, RA gave the highest yield and the yield fluctuated in other digesters in decreasing order giving the least yield in RD. The total volumes were 10539.39 cm^3, 5426.71 cm^3, 2275.93 cm^3 and 124.04cm^3 from rural biogas digesters RA, RB, RC and RD respectively (Figure 2). However, while biogas production fluctuated in the same pattern in RA (98.14) and RB (31.53), the pattern changed slightly for RC and RD with RD (8.12) having higher production than RC (6.21).

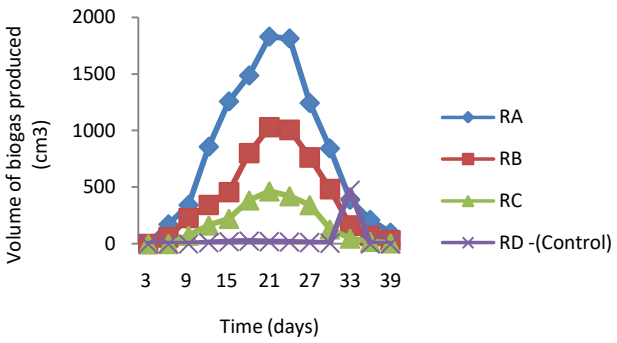

Figure 1: Biogas Production from organic waste in locally designed biodigesters (RA,RB RC and RD)

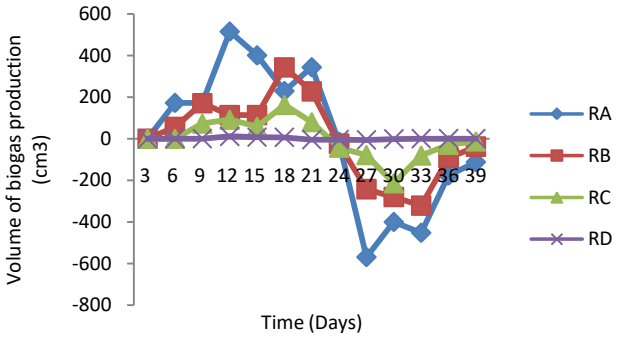

Figure 2: Rate of Biogas Production in locally designed biodigesters (RA, RB, RC and RD)

DISCUSSION

Microbiological counts of the organic waste

The microbial load appeared to be decreasing significantly after 50 days and 39 days of biogas production from the laboratory and rural digesters. This could be due to the production of toxic materials as the end product of metabolism. This agrees with the findings of Farina *et al.*[14] who reported that ammonia stress during thermophilic digestion of poultry droppings had high contents of ammonia. This raises the pH outside the upper minimum range which resulted in the reduction/inhibition of methanogenic organisms. This decrease can also be attributed to the exhaustion of essential nutrients from the digester due to continuous breakdown of complex material to simple organic compounds or could be from the use of different succession of microorganisms participating in the process.[17, 19]

The anaerobic bacteria counts range from 1.8 x 10^6 cfu/g and, 2.10x10^6 cfu/g for UDG (undigested waste), 1.31x10^2, 1.7x10^3 DGW (digested waste) respectively (Table 1). The variation in

the microbial counts might be attributed to complete anaerobic process and stability of the condition in the anaerobic digester especially when there is co-digestion of different organic wastes. This is in line with the findings of El-Mashad *et al.*[20] that digestion of more than one substrate in the same digester can establish positive synergism and the added nutrients can support anaerobic bacterial growth. The investigators also reported that during mesophilic anaerobic co-digestion of cattle manure, fruit and vegetable wastes (FVW) in a continuous stirred tank reactor at 35^0C, increasing the percentage of FVW from 20 ^{0}C to 50^0C leads to increase in methane yield from 230 to 450l/kg. This is also in agreement with Eze and Agbo[21] who reported that increase in total anaerobic counts is due to the fact that conditions are favourable for their growth and development. The differences may also have resulted from the activities of anaerobic methanogenic organisms consuming methane sors produced from the initial activity. [22,23]

The fungal counts (Table 1) showed a decrease from $1.3x10^3$, $1.10x10^3$ undigested waste (UDW) to $1.2x10^2$, $1.0x10^2$ cfu/g digested waste (DGW) respectively. The presence of fungi in anaerobic biogas process may be based on their ability to adhere and penetrate cell walls through which they open the cells for numerous members of bacterial community and speed up the whole decomposition process, while majority may be there as contaminants and when they die, become substrate nutrients.[24] The decrease in fungal counts in the present study is contrary to the finding of Sirohi *et al.*[21] who reported that increase can be traced to the decomposition of lignocellulosic materials. This decrease in microbial counts is also in line with the report of Asikong *et al.*[17].

Identification of bacteria and their frequency of occurrence from biogas produced in the laboratory

Bacillus cereus, *Sphingobacterium yamdrokense* and *Alkaligenes faecalis* were the dominant species. This suggests that the species play a vital role in the production of biogas. The frequency of occurrence of *Bacillus cereus* after digestion must have resulted from microbial succession in which probably the fungal and cellulolytic organisms produce favourable environment for their rapid growth[25,26] or as a result of antagonism that results in the production of secondary metabolites such as antibiotics which inhibited the growth of other microorganisms present in the digester thereby paving way for them to get to the final stage of methanogenesis.[22] Species of *Clostridium*, *Alkaligenes* and *Bacillus* secret hydrolytic enzymes capable of decomposing organic waste in anaerobic digestion and can also overlap from one stage to another during biogas production also suggest a succession in species of bacteria during methanogenesis.[27] The ability of *Bacillus* species to overlap during biogas production and to survive in both liquid and solid digestate were probably due to the fact that the organisms can produce spores which help them to withstand high temperatures, dryness and heat that evolved from biogas production or harsh anaerobic conditions.[22,28] These findings were also in conformity with that of Oluyega[29] and Bagudo *et al.*[30]. This frequency was also attributable to the fact that methanogens live in a synthrophic or complementary relationship with other organisms that breakdown the biomass to simple monomers.[2] Asikong *et al.*[17] reported that the presence of cyanogenic glycosides in cassava peels and other plant peels as in the present study can induce excess acidic production, Nitrogen deficiency and the release of cyanide which is highly toxic to bacteria.

Identification of fungal isolates

The low frequency of occurrence of fungal species owing to the fact that only *Aspergillus flavus* and *Mucor pusillus* were isolated in the present study (Table 3) is contrary to the findings of Getu *et al.*[28] who recorded a high frequency of *Aspergillus niger* to justify the fact that most *Aspergillus* blend well with plant material and are beneficial in Agriculture.[29] It was however observed that fungi count was slightly higher in undigested organic waste than digested organic waste. This was probably due to the ability of fungi to tolerate acidic condition initially than slightly alkaline condition that was later prevalent in some of the sample components such as cassava and orange peels as a result of cyanogenic acid. Furthermore, the reduction in fungi counts after digestion could be due to the inability of the organism to survive in oxygen free environment. This result agrees with the report of Uzodinma *et al.*[24] who observed a reduction in bacterial and fungi counts from various substrates used for digestion. The presence of fungi isolate in organic wastes is an indication of their geotropic nature and possession of extracellular inducible enzymes such as keratinolytic proteases which are crucial for decomposition of protein keratin material in the organic waste.[30]

CONCLUSION

The following microorganisms: *Bacillus cereus*, *Sphingobacterium yamdrokense*, *Clostridium perfringens*, *Salmonella typhi*, *Alkaligenes Faecalis*, *Pseudomonas aeruginosa*, *Staphylococcus epidermidis*, *Klebsiella pneumoniae* and *Bacillus licheniformisMuccor pusillus* and *Aspergillu flavus* were involved in biogas production from domestic wastes. Domestic household wastes from laboratory biogas production had the highest rate and total biogas volume of 183.97 cm^3 while that from rural biogas production gave the highest rate and total biogas volume of 10539.39 cm^3. This implies that domestic household waste could serve as a suitable substrate for biogas production and that the utilization of this substrate for biogas production could solve its disposable problems thus making way for abundant source of sustainable energy.

RECOMMENDATION

It is recommended that, other household domestic waste not used in this study should be harnessed for biogas production. For pathogens like *salmonella* species amongst others to have been found to be associated with biogas process and to survive the anaerobic process to the end in this study, may pose a threat on agricultural industry and thus, the use of solid digestate be preferred to liquid digestate as organic fertiliser or measures that can allow their elimination be adopted before application.

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