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Anaerobic Digestion of Biodegradable Domestic Wastes by Microorganisms

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Authors' contributions

This work was carried out in collaboration between all authors. Author CKW designed the study. Author EUE performed the statistical analysis, wrote the protocol, wrote the first draft of the manuscript and managed the literature searches. Authors EUE and AOO managed the analyses of the study. All authors read and approved the final manuscript.

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ABSTRACT

The aim of this study was to demonstrate that biogas can be generated from biodegradable domestic wastes and to determine the bacterial succession involved in the anaerobic decomposition of the wastes. Ten kilogram (10 kg) of biodegradable domestic waste was made into slurry with tap water. The slurry was fed into a batch system biodigester and left at room temperature for 12 weeks. Metagenomic method was used to determine the bacterial and archaeal species involved in the anaerobic digestion. MULTIRAE PGM 50 was used to confirm the presence of the generated biogas from the slurry. Serial dilutions of the slurry was made on alternate days and the appropriate dilutions were inoculated onto nutrient agar plates for bacterial isolation and incubation was at 35℃ for 48 hrs. Potato dextrose agar was used for fungal isolation, and incubation was at ambient temperature for three days. Pure isolates of representative communities were maintained on agar slants at 4℃. Triplicate samples from various tubes were cultured and

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the average count was used. Fungal growth occurred on the PDA plate only on the first day of incubation. The mean total bacteriaial count was highest on the second day (1.3 x10^7 cfu/ml); it decreased with increasing incubation time and became constant from the 23rd day to the end of the experiment (1.0 x10^1 cfu/ml). The microorganisms involved in the biodegradation were found to be *Lactobacillus rapi strain LA1165, Clostridyum tyrobutyricum, Ralstonia pickettii, Methanoculleus marisnigri*, *Methanosarcina acetivorans* C2A, *Clostridium acetobutylicum* EA 2018, *Clostridium tyrobutyricum* 5S, *Halothermothrix oremii* H168, *Lactobacillus rapi strain* LA1165, *Lactobacillus buchneri, Solobacterium moorei* W540, *B. vulgatus ATCC* 8482. *Rhizopus spp* and *Aspergillus spp* were isolated only on the first two days of incubation. The result from this study proves that, it is possible to generate biogas from domestic wastes and diverse species of microorganisms are involved in anaerobic digestion of biodegradable domestic wastes.

Keywords: Biogas; domestic waste; biodigester; anaerobic decomposition; slurry.

1. INTRODUCTION

Improper waste management is one of the major problems confronting every development. This is because increase in industrial, commercial, agricultural and environmental activities has resulted in the generation of large quantities of wastes [1]. However, these wastes can be managed properly by conversion into useful and more environmentally friendly forms called biogases [2]. Biogas refers to a gas produced by the biological breakdown of organic matter in the absence of oxygen. It is a renewable, alternative and sustainable form of energy [3]. A *biogas plant* is the name often given to an anaerobic digester that treats wastes. During the process, an air-tight tank transforms biomass waste into methane, producing renewable energy that can be used for heating, electricity, and many other operations [4].

The rising cost of petroleum and allied products most especially in Nigeria, has triggered a need to develop alternate sources of energy, one of which is biogas production. In Nigeria, majority of the population are rural dwellers without access to gas or electricity and therefore, depend on firewood for cooking and lighting [5]. Unfortunately, this has contributed immensely to the rapid rate of deforestation and desert encroachment. The establishment of biogas plants in these communities would greatly ameliorate these problems and help preserve the environment [6].

Nigeria is blessed with a variety of energy resources (both conventional and non-conventional) [7]. The reserves for animal waste alone which is a viable source for biogas production as at 2005 was estimated to be 61 million tonnes/yr and crop residue was put at 83 million tonnes/yr. However, 400 MW of electricity is targeted to be generated from biomass by 2025 [6]. Not only does biogas technology help to produce an alternative energy source, it also helps in maintaining the environment and improves health conditions [8].

The biogas formed after the decomposition of organic wastes is channeled or transported to homes for use, for cooking, running engines, electrical power generation and heating, with virtually little or no pollution at all. This gas is now used in large scale in many countries [9]. The use of anaerobic digestion as waste-to-energy technology has been employed in the treatment of different organic wastes [10].

Three major groups of bacteria (hydrolytic, acidogens/acetogens, and methanogens) are responsible for breaking down the complex polymers in biomass wastes to form biogas at anaerobic conditions [11]. Any organic matter with the exception of mineral oil can be used as feedstock for anaerobic digestion to produce biogas. Consequently, through the interactions of the microbes, a lot of diversity exists in the biogas system just as in the digestive system of ruminant animals [12]. It has been reported that seventeen fermentative species have played important roles in the production of biogas [13]. However, the nature of the feedstock determines the type and extent of fermentative bacteria present in the digester. Bori et al. [11] reported that the population distributions of the microflora in anaerobic digestion of banana and plantain peels consist mainly of *Micrococcus luteus, Bacillus subtilis, Escherichia coli,* and *Clostridium perfringes* while the methanogens identified belonged to the genera Methanobacterium, Methanococcus, and Desulfovibrio. Some researchers also observed higher amylolytic microorganisms in cow dung-fed digester system but found higher proteolytic population in poultry dung- fed digester systems.

The major components of this gas are methane (55-70%) and carbon dioxide (20-24%) with traces of other gases like nitrogen, ammonia, hydrogen sulphide, hydrogen, carbon monoxide, and water vapour [12,6]. Thus, this biogas contributes significantly to global greenhouse gas emission. Increase in the quantities of green house gases in the atmosphere as a result of various human activities causes anthropogenic climate change.

The composition of biogas-producing microbial communities commonly is determined via construction of 16S-rDNA clone libraries and subsequent sequencing of 16S-rDNA amplicons [14,15,16,17].

Wastes have been effectively used as biogas materials by various studies. Ilaboya et al. [3] studied biogas generation from agricultural wastes. Ojolo et al. [18] examined the biogas potential of municipal solid wastes. Babalola, [19] investigated biogas production from animal and household wastes. Ozor et al. [2] investigated biogas production from cow dung.

Since no investigation has been carried out in the potential area of biogas production using biodegradable domestic wastes in Nigeria, the need to study this area is justified. The choice of Port Harcourt is guided by the abundance of waste generation in the municipal, which poses serious threat to health and the environment.

2. METHODS

2.1 Collection of Domestic Waste

The domestic wastes used were collected from fruit garden in the D Line area and left over food from the student's canteen at the Rivers State University of Science and Technology Oroworukwo Port Harcourt.

2.2 Production of Biogas from the Domestic Wastes

The kitchen waste (10 kg) was pre-treated by macerating in a laboratory mortar and loaded into the biodigester, 21 litres of tap water was added to the macerated wastes to make a slurry. The slurry was fed into a batch system biodigester made from a 25 litre plastic container lagged with flexi glass. The biodigester had two outlet pipes one at the lid for harnessing the gas formed and the other at the base for sample collection. It also has an agitator fitted on the digester lid. The biodigester was fitted with an automatic metering device to measure the volume of gas generated at any given time. The lid was closed tightly and further sealed with plasticine to create an anaerobic environment. The biodigester was well supported to aid stirring while agitating its contents. The digester content was rigorously agitated intermittently at two days interval. The set up was left at room temperature for twelve weeks. The biodigester was tested for biogas production after three days (Fig. 1).

2.3 Isolation and Assessment of Microbial Species

Samples of the slurry collected on alternate days from the outlet pipe of the biodigester were put into sterile universal sample containers. The samples were divided into two parts; 1 ml of the slurry was made into serial dilution with 9 mls of sterile distilled water contained in different test tubes covered with sterile cotton wool, 1 ml from the last tube was discarded. 0.1 ml aliquots of the 6^{th} tube (1^{st} day), 8^{th} and 9^{th} tubes (3^{rd} to 5^{th} day), 4^{th} and 5^{th} tubes (7^{th} to 11^{th} day), and 2^{nd} tube 13^{th} to 42^{nd} day were inoculated into MacConkey, and nutrient agar plates containing 0.015% (w/v) nystatin (to inhibit fungal growth) for bacterial isolation. Potato dextrose agar (PDA), to which 0.05% (w/v) chloramphenicol was added (to inhibit bacteria growth) was used for fungal isolation. One plate each of nutrient and MacConkey agar were incubated aerobically at 37℃, while one plate each of nutrient agar and MacConkey were incubated in an anaerobic jar containing a gas generating kit (Oxiod BR, Basrugstoke England) at 37℃ for 3 days. The plates were inoculated in triplicates and the mean total count determined. The colonies were enumerated by standard plate count technique. The inoculated PDA plates were left at room temperature for one week.

2.4 Total Community DNA Preparation from the Slurry

The second part of the sample was stored in entirely filled, screw capped bottles and sent to Nigerian Institute of Medical Research (NIMR) for genomic DNA extraction. The extracted community DNA was sent to *GS FLX Titanium Sequencing Service* South Africa for the shotgun DNA sequencing.

To analyse the biogas-producing microbial community residing in the biogas fermenter in

terms of the role of specific organisms for biogas formation, a metagenomic approach using the ultrafast 454-pyrosequencing technology and the Genome Sequencer FLX System (Roche Applied Science, Mannheim, Germany) were used. Approximately 4 µg of the DNA-preparation was used to generate a whole-genome-shotgun library according to the protocol supplied by the manufacturer. Standard bioinformatics tools such as BLAST (Basic Local Alignment Search Tool), and ENA (European Nucleotide Archives) were used.

Fig. 1. Showing biodigester and a) Surface pump, (b) Gas cylinder

In the case of fungal isolates, the microscopic and macroscopic features of the hyphal mass, morphology of cells and spores, nature of the fruiting bodies (if any) were used for identification. A portion of the colony was emulsified in a clean grease-free glass slide and a drop of lactophenol cotton blue was added. This was examined under the microscope using X40 objective lens. The microscopic feature of the hyphal mass was used for identification.

2.5 Classification of Metagenome Single Reads According to COG Categories

To characterize the gene content of the biogas reactor sample, all reads were functionally annotated by means of the Clusters of Orthologous Groups of proteins database (COG) [20,21] COGs were identified in the biogas reactor sample based on a BLASTx search of reads vs. the COG database using the -w15' frameshift option and an E-value cut-off of 10^{-8}. Reads were assigned to the COG category of their best BLAST hit.

3. RESULTS

Biogas was obtained from the waste slurry in the biodigester from the first week of incubation. The gas yield was highest on the third week (21st day). Gas generated for the first three days was quite low though an increase in production was observed daily. There was a gradual reduction in the volume of gas produced after it has reached the peak value on the 21st day. There was scanty fungal growth on the PDA plate mainly *Rhizopus spp* and *Aspergillu spp*. Growth on the PDA plates disappeared after the first day of incubation. Bacterial growth appeared on the MacConkey plates only on the first day. There was profuse bacterial growth on the nutrient agar plate (incubated aerobically) especially in the first week.

The mean total count colony count was highest on the third day, and started to decrease from the 5th day, it remained constant from the 23rd day till the end of the experiment.

The isolated species from the gene sequencing of the slurry showed nine representative bacteria species from three different orders and two archaeal species from two different orders.

The frequency of occurrence of each of the representative species was determined by the best BLAST hit approach.

4. DISCUSSION

The mechanical pre-treatment of the wastes by maceration increased the amount of soluble organics; the reduced size of the wastes gave the microorganisms access to a greater surface area, so reducing retention time. Diluting the waste with water also allowed the microorganisms easier movement in the digester. The biodigester was agitated mechanically by mixing. The benefits of mixing digester content during anaerobic process prevents scum formation inside the digester, ensures uniform distribution of microorganisms and substrate throughout the mixture and intestifies contact between them, prevents stratification within the digester, and helps to release gas from the mixture [22]. The lag period of three days observed before the commencement of biogas production was the time required for the bacteria to build up to population large enough to ferment the wastes. This lag period agrees with the 15-27 days for chopped water hyacinth [23].

The use of microbial inoculum as cultures is a common practice during biogas production. Lawal et al. [24] observed rapid gas production when digester feeds-tocks were seeded with adequate bacterial isolates from previous digester production. The initial high bacterial load of the slurry may be due to the fact that large populations of aerobic and facultative anaerobic organisms are usually involved in the hydrolysis stage and acidogenic phase of methane biogenesis whereas only strict or obligate anaerobes are involved in the methanogenesis stage [25]. This suggests that starchy waste biomethanation may have a potential for economically viable waste treatment technology through anaerobic digestion. The decrease in mean total microbial count (Table 1) may also be due to the fact that the biodigester is a batch system and anaerobic. The available oxygen in the digester is exhausted and only anaerobes can survive in the anaerobic biodigester. Available nutrients decreased with time and toxic wastes from the microorganisms also increased, this combined effect will reduce the microbial population.

Table 1. Volume of gas produced per day

Day	Volume of gas produced /m^3
1 -2	Nil
3	0.1
5	0.125
7	0.3
9	0.4
11	0.7
13	0.9
15	1.2
17	1.5
19	1.9
21	2.5
23	2.0
25	1.6
27	0.9
29	0.6
31	0.4
33	0.2
35	0.2
37	0.1
42	0.1

The maximum daily gas yield was observed on the 21st day, this is lower than 36 days reported by Lucas and Bamgboye [26] and higher than 16 days reported by Zuru et al. [22] but in accordance with the report of Hughes and. Christy [23] that increased conversion of organic carbon to methane and carbon dioxide results in greater gas production over a shorter time period in a municipal solid waste bioreactor. As the methanogenic bacteria activities increase, more biogas is produced in the digester. There was a gradual reduction in the volume of gas produced, after it has reached the peak value of gas production. This is due to the fact that the microorganisms responsible for biogas production have consumed a large amount of the substrate and hence subsequent drop in activity.

Fig. 2. Generated biogas used to light a Bunsen burner

Table 2. Mean total bacterial count of the slurry

Day	Mean total count (CFU/ML)
1	$1.8x10^5$
3	$1.3 x10^7$
5	$9.0x10^6$
7	$3.0 x10^5$
9	$4.0x10^4$
11	$1.5x10^3$
13	$9.0x10^2$
15	$7.0x10^2$
17	$4.0x10^2$
19	$2.0x10^2$
21	$2.0x10^2$
23	$1.9x10^1$
25	$1.8x10^1$
27	$1.0x10^1$
30	$1.0x10^1$
42	$1.0x10^1$

Table 3. Lists of organisms isolated

Species	Phlum	Class	Order
Methanoculleus marisnigri JR1	Euryarchaeota	Methanomicrobia	Methanomicrobiales
Methanosarcina acetivorans C2A	Euryarchaeota	Methanomicrobia	Methanosarcinales
Clostridium acetobutylicum EA 2018	Firmicutes	Clostridia	Clostridiales
Clostridium tyrobutyricum 5S	Firmicutes	Clostridia	Clostridiales
Clostridium botulinum	Firmicutes	Clostridia	Clostridiales
Halothermothrix oremii H168	Firmicutes	Clostridia	Halanaerobiales
Lactobacillus rapi strain LA1165	Firmicutes	Bacilli	Burkhololderiales
Lactobacillus buchneri	Firmicutes	Bacilli	Burkhololderiales
Solobacterium moorei W540	Firmicutes	Bacilli	Burkhololderiales
Ralstonia pickettii	Firmicutes	Bacilli	Burkhololderiales
B. vulgatus ATCC 8482	Bacteroidetes	Bacteroidetes	Bacteroidales

A total of 1,159 contigs could be affiliated to specific microbial genome sequences based on a best BLAST hit approach. Counting of best-hit species entries for all contigs led to the bar chart shown in Fig. 3. This approach led to the allocation of contigs to eleven different microbial species. Among the identified species those belonging to the class Clostridia (50%) dominate, followed by Bacilli (14%) and Methanomicrobiales (9%) (Fig. 3). Dominance of members of Clostridia, Bacilli and Bacteroidetes was also described for the microbial community of a biogas-producing reactor fed with fodder beet silage as mono-substrate [27]. The categories of organisms isolated revealed a genetic profile characteristic of an anaerobic microbial consortium conducting fermentative metabolic pathways. Isolation of the archaeal methanogen, *Methanoculleus marisnigri* JR1 suggest that species related to those of the genus *Methanoculleus* play a dominant role in methanogenesis in the analysed fermentation sample. This supports the report of Andreas et al. [25]. Also, *Ralstonia pickettii* which has been shown to have a wide range of biodegradative abilities Ryan et al. [28] confirms the biodegradation occurring in the sample under study. The clostridial genomes isolated indicates that clostridia are important for hydrolysis of cellulosic plant biomass in the biogas fermenter under study [25]. *Halothermothrix orenii* H 168 could play a role in starch degradation. Likewise, other species identified by BLAST analysis of contig sequences potentially are capable of producing metabolites that indirectly or directly contribute to biogas formation from domestic waste *Lactobacillus rapi* and *Lactobacillus buchneri* are examples.

The metabolites produced by some secondary fermenters may feed methanogenic Archaea. Species closely related to those of the genus Methanoculleus are dominant among the methanogens. Methanoculleus species are known to produce methane via the hydrogenotrophic pathway [29]. Methane formation from hydrogen and carbon dioxide is of importance in the analysed fermentation sample. The hydrogenotrophic methanogenesis pathway presumably is accompanied by syntrophic acetate oxidation leading to the formation of hydrogen and carbon dioxide which in turn can be converted to methane by hydrogen utilizing methanogens. Thus, the acetate pool indirectly feeds biogas production [29]. The isolation of some of the species does not necessarily mean that these species represent part of the analysed biogas-producing community. It is rather very likely that bacteria closely related to the respective reference species belong to the microbial consortium residing in the bioreactor, an example is *Staphylococcus aureus*. The odour of the substrate was found to be less offensive after the experiment than before commencement. The biodegradability of the substrate was also confirmed from the physical observation before and after the experiment. There is a great reduction in the fibre content of the feedstock and particles size of the waste components after the experiments. The fungal species isolated only on the first day may have died due to the anaerobic condition in the biodigester, and the increasing generation of biogas which is toxic to them.

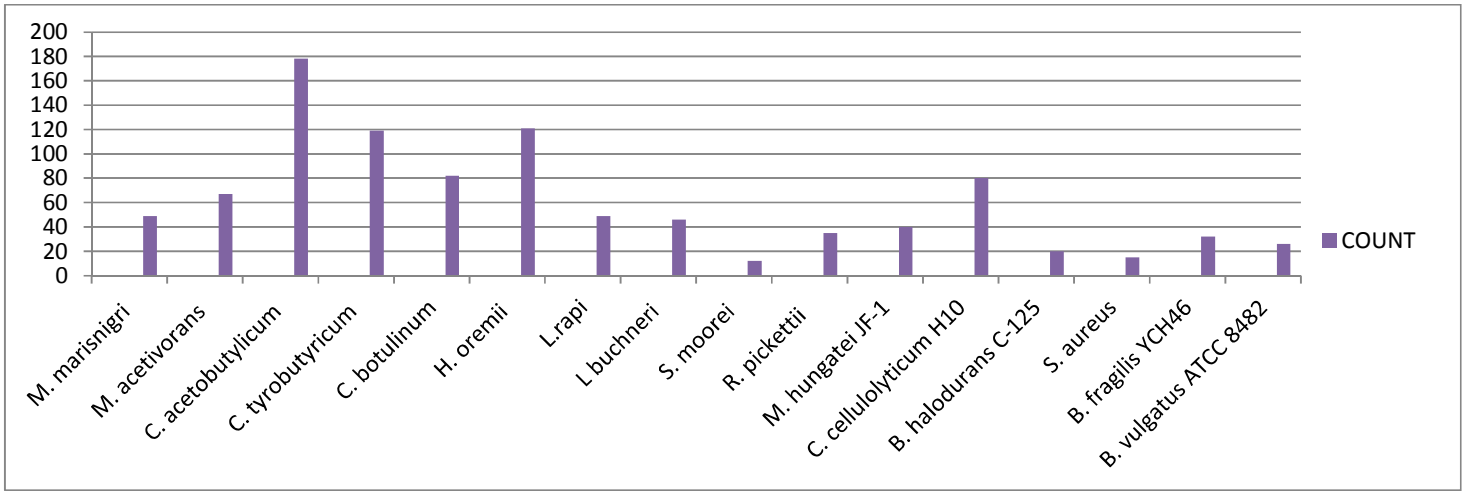

Fig. 3. Frequencies of matches for the most abundant species

5. CONCLUSION

This result of this study has proved that biogas can be generated from biodegradable domestic wastes. The search for alternative energy sources such as biogas when intensified would help arrest the problem of municipal waste disposal. In addition, it will elimination and/or control the spread of harmful insects and rodents that feed on the domestic wastes prior to their removal by waste disposal companies. It should be noted however, that development of biogas does not eliminate wastes, but it does make them easier to manage. In conclusion, in developing countries like Nigeria, where electricity and heat are sparse and biological waste is profuse, the anaerobic digestion process could be of help in addressing these issues.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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