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METAGENOMICS IN-SIGHT INTO THE MICROBIAL STRUCTURAL DIVERSITY OF ANAEROBIC DIGESTER UTILISING FRUIT WASTE AS SUBSTRATE

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ABSTRACT

Anaerobic digester has emerged as a technology of choice in management of waste and production of biogas. However, the microbial ecology of digesters utilizing various substrates are very poorly understood. The ecology of anaerobic digester utilizing *Citrullus lanatus* fruit waste was analyzed using metagenomics. Slurry substrate sample was collected from a functional digester aseptically and anaerobically. Metagenomic DNA was extracted using ZYMO DNA extraction Kit (Model D 6001, Zymo Research, USA) following manufacturer's instruction. Extracted DNA was amplified using the 16S rRNA gene amplicon PCR primers set and sequenced using Illumina MiSeq platform. Taxonomic analysis of the reads was performed using NCBI-BLAST-2.2.24 and CLC bio Genomics workbench v7.5.1. Taxonomic classification of the sequences revealed that bacteria and archae were the top two kingdoms with reads counts of 57,554 and 80, respectively. The top 7 phyla were Unknown, Firmicutes, Proteobacteria, Planctomycetes, Bacteroidetes, Actinobacteria and Euryarchaeota in decreasing order of counts. A total of 30 microbial classes, 43 orders, 79 families and 210 species were further classified. Over half of the 210 species detected were not routinely cultured species in the laboratory, indicating that there is still a relatively wide gap between culturable and non-culturable species in an anaerobic digester. The Acidogens captured in this study were *Clostridium*, *Uncultured rumen bacteria* and *Bacteroidetes species.* Furthermore, we also detected uncultured syntrophic Acetogens such as *Syntrophomonas* species and the hydrogenotrophic thermophile, *Methanothermobacter* sp. The *Syntrophomonas* species is known to breakdown short chain fatty acids, like propionate and butanoate in concert with hydrogenotrophic Methanogens indicating methane generation was via the hydrogenotrophic route. However, the main representative hydrogenotrophic methanogens detected were *Methanoculleus bourgensis* and *Methanoculleus marisnigri*, with the former being more abundant. In addition to the aforementioned species, other species captured were largely classified as unknown or uncultured species and they include Uncultured *species of Clostridium, Syntrophomonas, Synergistetes, Synergistaceae Anaerobic sp, Ruminococcaceae, Rumen sp, Thermomonas, Thermoanaerobacteriales, Bacterium, Compost, Firmicutes, Bacteroidetes, Chloroflexi, Clostriaceae, Acetobacter. Eubacterium, Alpha* and *Aacteroides.* The results of the study revealed that culture-independent approach is better able to capture the anaerobes including both culturable and unknown that dominate anaerobic digesters and are responsible for the bioconversion of organic waste into biogas.

Keywords: Metagenome; 16S rRNA; Illumina MiSeq; Syntrophic Acetogens; Hydrogenotrophic Methanogens

INTRODUCTION

With increasing awareness of the health benefits of fruits, there has been an increased cultivation and consumption of fruits around the world (**Ijah *et al.*, 2015; Sagar *et al.*, 2018**). In most developing countries such as Nigeria, fruit waste disposal is usually not properly handled despite enormous potentials (**Oladepo *et al.*, 2015; Sagar *et al.*, 2018**). Furthermore, the lack of post-harvest technology or preservation techniques for fruits increases the quantity of wastes generated from fruits in Nigeria. Anaerobic degradation of waste is a well-known method of biological waste treatment with concomitant production of biogas such as methane and carbon dioxide using a complex community of microorganisms (**Kleinsteuber, 2018**).

The processes of anaerobic biogas generation are divided into four major stages namely: hydrolysis, acidogenesis, acetogenesis and methanogenesis (**Sagagi *et al.*, 2009**). During hydrolysis, complex organic polymers are broken down into simpler forms (**Cirne *et al.*, 2007**; **Doi, 2008**). If the substrate is not readily degradable, this phase may limit the entire process (**Parawira, 2005**). However, if the substrate is easily digested, then methanogenesis would be the phase that may limit the process in the reactor (**Bjornsson, 2001**). The process of acidogenesis involves further disintegration of the components into compounds such as organic acids, ketones, alcohols and carbon dioxide (**Ding *et al.*, 2008**). Meanwhile, the resulting acidogenic products are converted into hydrogen, carbon dioxide and acetic acid during acetogenesis (**Schink, 1997**). In the methanogenic stage, the acetogenic products are transformed into methane and CO_2 which make up the bulk of the biogas liberated from the process (**Verma *et al.*, 2007).**

Anaerobic digestion (AD) is a very complex biological process. Despite being so widely used around the world, the microbial ecology of this process is very poorly understood (**Nelson *et al.*, 2010; Amha *et al.*, 2018**). Unfolding and proper understanding of the complex structural diversity is very important in understanding functional relationship between the various metabolic groups of microorganisms (hydrolytic, acidogenic, acetogenic and methanogenic). Understanding this synergy will help improve and optimize the process of AD thereby making it more effective (**Manyi-Loh *et al.*, 2013; Amha *et al.*, 2018**).

Most of the microorganisms involved in AD are anaerobes and their cultivation in the laboratory is one of the most challenging areas of microbial research (**Mori and Kamagata, 2014**). Before the advent of molecular tools such as metagenomics, microbial ecology of various environments including those of the anaerobic world were largely elusive (**Edet *et al.*, 2017a; Edet *et al.*, 2017b; Edet *et al.*, 2018a; Edet *et al.*, 2018b; Mori and Kamagata, 2014**). Metagenomics, a culture-independent method allows for the direct examination of microbial community structure and function in an ecosystem using various bioinformatics pipelines (**Edet *et al.*, 2017a; Manyi-Loh *et al.*, 2013**). Application of omics-based studies have revealed a number of things previously unknown to the anaerobic microbial world such as new taxa and their roles in

various anaerobic systems (**Manyi-Loh *et al.*, 2013; Mori and Kamagata, 2014**).
Microbial composition in an anaerobic digester is driven by a number of factors. Prominent amongst the factors is the type of substrate utilized in the AD and free ammonia (**Zhang *et al.*, 2014; Li *et al.*, 2015)**. Other factors include design of the reactor and its operational condition such as hydraulic retention time, organic loading rates pH, temperature and mixing (**Lin *et al.*, 2013; Town *et al.*, 2014; Manyi-Loh *et al.*, 2013**) and even co-substrate (**Sun *et al.*, 2015**). In Nigeria, a number of studies abound on the utilization of anaerobic digestion systems in biogas production and waste treatment but none have utilized metagenomics in the evaluation of microbial structure. Thus, the primary aim of this study was characterization of the microbial communities in an anaerobic digester utilizing water melon fruit waste in biogas production process.

MATERIAL AND METHODS

Sampling of slurry

Slurry sample from an operational digester was collected for characterization of the microbial community composition of the digesters after shaking to achieve a uniform mixture. The samples from the digester were collected from the outlet tap using a sterile capped bottle and transported immediately for immediate analysis. The operational conditions of the digester have been described previously (**Anika *et al.*, 2019**).

Metagenomic DNA extraction

DNA extraction from slurry sample was performed using ZYMO DNA extraction Kit (Model D 6001, Zymo Research, USA) adhering to the manufacturer's instructions.

DNA amplification and electrophoresis

The universal bacterial 16S rRNA gene amplicon PCR primers set **TruSeq tailed 341F** TGACTGGAGTTCAGACGTGTGCTCTTCCGATCTCCTACGGGNGGCWG CAG and **TruSeq tailed 785R** ACACTCTTTCCCCACACGACGCTCTTCCGATCTGACTACHVGGGTATC TAATCC was used to amplify and produce more copies of target region in the extracted metagenome. The reactants (substrates) for DNA amplification were prepared using 2.5 µL (5 ng/µL) microbial DNA, 5 µL (1 µM) amplicon PCR forward primer, 5 µL (1 µM) amplicon PCR reverse primer, and 2× KAPA HiFi Hot Start Ready Mix 12.5 µL (total 25 µL). Using the reaction mixture above, the following protocol was used to run a PCR for the slurry extracted DNAs in a thermal cycler (Applied Biosystems9700, USA) for twenty five cycles: The first cycle was allowed to denature for three minutes at 95 ℃, but for the rest cycles that followed, the DNAs were denatured for five minutes at 95 ℃. Following, the temperature of the reaction mixture was brought down to 55 ℃ for three minutes to allow for DNA annealing and later increased to 72 °C for thirty seconds for elongation to take place, then, another five minutes was allowed at same temperature for a final extension. Exactly 1 µL was run on a bioanalyzer DNA 1000 chip to verify the size of the amplified product (approximately 550bp)

Illumina sequencing

In order to assess all the nucleotide sequences present in the DNAs eluted from digesting slurry samples (to reveal all the microbial communities present), DNA sequencing was carried out using Next Generation Sequencing Technique, following library preparation, using automated PCR cycle- Genome Sequencer™ MiSeq (Illumina). Vecton NTI suite 9 (InforMax, Inc.) was used to analyze and align the sequences (**Salam *et al.*, 2017; Edet *et al.*, 2017a**).

Taxonomic analysis

Following next generation sequencing, quality control and trimming of the sequences was performed as reported previously (**Salam *et al.*, 2017; Edet *et al.*, 2017a**). Taxonomic analysis of the reads was performed using NCBI-BLAST-2.2.24 and CLC bio Genomics workbench v7.5.1 (**Edet *et al.*, 2017a**).

RESULTS

The results of the study are presented in the tables below. Table 1 shows the various kingdom in our studied sample. The various kingdoms classified were Bacteria, Archae, Unknown, Fungi, Plantae, Animalia, and Protozoa in decreasing order of abundance. Their read counts were 57554, 80, 23, 7, 4, 3, and 2, respectively. As can be seen from the read counts, bacteria were the most dominant kingdom representing 99.79% and the followed by archae with 0.14%. Unknown kingdom represented 0.04% while fungi, plantae, and animalia represented 0.01% each. The least abundant kingdom was protozoa with a representation that was less than 0.00%.

Phylum classification revealed a total of 18 phyla with various read counts as shown in Table 2. These were Unknown, Firmicutes, Proteobacteria, Planctomycetes, Bacteroidetes, Actinobacteria, Euryarchaeota, Chloroflexi, Lentisphaerae, Ascomycota, Basidiomycetes, Chordata, Tracheophyta, Ciliophora, Cyanobacteria, Deinococcus-Thermus, Bryophyta, and Crenarchaeota with reads of 53739, 2206, 1177, 186, 186, 116,84, 79, 59, 8, 4, 3, 3, 2, 1, 1, 1, 1 and 1, respectively.
Class classification as presented in table 3, revealed a total of 30 classes including the Unknown and Not Assigned categories. The top 15 classes were Unknown, Clostridia, Gammaproteobacteria, Bacilli, Planctomycetacia, Alphaproteobacteria, Actinobacteria, Bacteroidetes, Chloroflexi, Betaproteobacteria, Halobacteria, Flavobacteria, Methanomicrobia, Lentisphaerae, and Deltaproteobacteria. Their corresponding read counts were 53740, 1792, 1010, 414, 186, 107, 84, 72, 58, 54, 46, 43, 32, 8 and 6.
A total of 43 orders were captured in our sample. The top 30 orders are as presented in Table 4. The top 15 orders were Unknown, Clostridiales, Clostridiales, Bacillales, Planctomycetales, Planctomycetales, Xanthomonadales, Actinomycetales, Bacteroidales, Chloroflexales, Burkholderiales, Halobacteriales, Flavobacteriales, Methanomicrobiales and Rhodospirillales. Their corresponding read counts were 53748, 1792, 908, 401, 186, 401, 186, 85, 79, 72, 58, 54, 46 and 43. Also included in the top 30 orders was an important methanogen order, Methanomicrobiales.
The various families were 79 in total. However, the top 30 orders in our AD sample is presented in table 5. In increasing order of read counts, they were Unknown, Clostridiaceae, Enterobacteriaceae, Listeriaceae, Eubacteriaceae, Planctomycetaceae, Xanthomonadaceae, Prevotellaceae, Halobacteriaceae, Flavobacteriaceae, Not assigned, Bacillaceae, Comamonadaceae, Syntrophomonadaceae, Rhodobacteraceae, Dietziaceae, Acetobacteraceae, Rhodospirillaceae, Phyllobacteriaceae, Sphingomonadaceae, Burkholderiaceae, Pseudomonadaceae, Lactobacillaceae, Caulobacteraceae, Alcaligenaceae, Victivallaceae, Pseudonocardiaceae, Bradyrhizobiaceae, Nocardioidaceae and Micrococcaceae. Their respective read counts were 54160, 1060, 908, 365, 349, 186, 85, 69, 46, 43, 35, 30, 30, 36, 23, 21, 16, 15, 13, 12, 12, 10, 10, 10, 9, 8, 7, 7, 7 and 7. The remaining families are as captured in the supplementary material attached. In addition to the aforementioned families, Methanobacteriaceae and Methylobacteriaceae were also detected in our sample.
Genus/species level classification revealed a total of 210 species majority of which were uncultured species. Selected 45 species are as presented in Table 6. These were dominated by strict anaerobes or facultative anaerobes as well as methanogens and methane utilizing species.

Table 1 Kingdom classification

Kingdoms	Read counts	Percentage (%)
Bacteria	57554	99.79
Archae	80	0.14
Unknown	23	0.04
Fungi	7	0.01
Plantae	4	0.01
Animalia	3	0.01
Protozoa	2	0.00

Table 2 Phyla classification

Phyla	Read count	%
Unknown	53739	93.18
Firmicutes	2206	3.83
Proteobacteria	1177	2.04
Planctomycetes	186	0.32
Bacteroidetes	116	0.20
Actinobacteria	84	0.15
Euryarchaeota	79	0.14
Chloroflexi	59	0.10
Lentisphaerae	8	0.01
Ascomycota	4	0.01
Basidiomycota	3	0.01
Chordata	3	0.01
Tracheophyta	3	0.01
Ciliophora	2	0.00
Cyanobacteria	1	0.00
Deinococcus-thermus	1	0.00
Bryophyta	1	0.00
Crenarchaeota	1	0.00

Table 3 Class classification

Class	Read Count	%
Unknown	53740	93.18
Clostridia	1792	3.11
Gammaproteobacteria	1010	1.75
Bacilli	414	0.72
Planctomycetacia	186	0.32
Alphaproteobacteria	107	0.19
Actinobacteria	84	0.15
Bacteroidetes	72	0.12
Chloroflexi	58	0.10
Betaproteobacteria	54	0.09
Halobacteria	46	0.08
Flavobacteria	43	0.07
Methanomicrobia	32	0.06
Lentisphaerae	8	0.01
Deltaproteobacteria	6	0.01
Mammalia	3	0.01
Agaricomycetes	2	0.00
Saccharomycetes	2	0.00
Leotiomycetes	2	0.00
Heterotrichea	2	0.00
Bryopsida	1	0.00
Sphingobacteria	1	0.00
Lycopodiopsida	1	0.00
Anaerolineae	1	0.00
Methanobacteria	1	0.00
Not assigned	1	0.00
Thermoprotei	1	0.00
Polypodiopsida	1	0.00
Deinococci	1	0.00
Cycadopsida	1	0.00

Table 4 Top 30 orders

Order	Read Count	%
Unknown	53748	93.19
Clostridiales	1792	3.11
Enterobacteriales	908	1.57
Bacillales	401	0.70
Planctomycetales	186	0.32
Xanthomonadales	85	0.15
Actinomycetales	79	0.14
Bacteroidales	72	0.12
Chloroflexales	58	0.10
Burkholderiales	54	0.09
Halobacteriales	46	0.08
Flavobacteriales	43	0.07
Methanomicrobiales	32	0.06
Rhodospirillales	31	0.05
Rhizobiales	28	0.05
Rhodobacterales	23	0.04
Lactobacillales	13	0.02
Sphingomonadales	12	0.02
Caulobacterales	10	0.02
Pseudomonadales	10	0.02
Victivallales	8	0.01
Myxococcales	4	0.01
Alteromonadales	4	0.01
Primates	3	0.01
Thelebolales	2	0.00
Agaricales	2	0.00
Saccharomycetales	2	0.00
Heterotrichida	2	0.00
Chromatiales	1	0.00

Table 5 Top 30 families

Family	Read Count	%
Unknown	54160	93.91
Clostridiaceae	1060	1.84
Enterobacteriaceae	908	1.57
Listeriaceae	365	0.63
Eubacteriaceae	349	0.61
Planctomycetaceae	186	0.32
Xanthomonadaceae	85	0.15
Prevotellaceae	69	0.12
Halobacteriaceae	46	0.08
Flavobacteriaceae	43	0.07
Not assigned	35	0.06
Bacillaceae	30	0.05
Comamonadaceae	30	0.05
Syntrophomonadaceae	26	0.05
Rhodobacteraceae	23	0.04
Dietziaceae	21	0.04
Acetobacteraceae	16	0.03
Rhodospirillaceae	15	0.03
Phyllobacteriaceae	13	0.02
Sphingomonadaceae	12	0.02
Burkholderiaceae	12	0.02
Pseudomonadaceae	10	0.02
Lactobacillaceae	10	0.02
Caulobacteraceae	10	0.02
Alcaligenaceae	9	0.02
Victivallaceae	8	0.01
Pseudonocardiaceae	7	0.01
Bradyrhizobiaceae	7	0.01
Nocardioidaceae	7	0.01
Micrococcaceae	7	0.01

Table 6 Selected genus/species

Top BLAST hits	**Read counts**	**Percentage (%)**
Uncultured bacterium	49712	83.52
Bacterium enrichment	2543	4.32
Uncultured compost	955	1.62
Uncultured clostridium	947	1.61
Enterobacter cloacae	388	0.66
Uncultured synergistetes	355	0.60
Uncultured firmicutes	355	0.60
Iron-reducing bacterium	83	0.14
Uncultured Bacteroidetes	83	0.14
Clostridium species	59	0.10
Uncultured chloroflexi	58	0.10
Listeria innocua	51	0.09
Uncultured clostriaceae	51	0.09
Uncultured rumen sp	27	0.05
Bacillus sp.	27	0.05
Uncultured syntrophomonas	24	0.04
Uncultured anaerobic sp	20	0.03
Clostridium pasterianum	20	0.03
Uncultured eubacterium	15	0.03
Uncultured ruminococcaceae	12	0.02
Uncultured ruminococcaceae	12	0.02
Lactobacillus fermentum	10	0.02
Uncultured acetobacter	5	0.01
Paracoccus denitrificans	5	0.01
Uncultured thermomonas	5	0.01
Enterobacter sp.	3	0.01
Uncultured thermoanaerobacteriales	3	0.01
Uncultured alpha	3	0.01
Methanoculleus marisnigri	3	0.01
Anaeromyxobacter dehalogenans	3	0.01
Maricaulis maris	3	0.01
Methanoculleus bourgensis	2	0.00
Uncultured bacteroides	2	0.00
Propionibacterium acnes	2	0.00
Uncultured synergistaceae	1	0.00
Ethylobacteriumpopuli	1	0.00
Ruminococcaceae bacterium	1	0.00
Marinobacter hydrocarbonlasticus	1	0.00
Bifidobacterium bifidum	1	0.00
Methanothermobacter sp.	1	0.00
Clostridiaceae bacterium	1	0.00
Leuconostoc pseudomesenteroides	1	0.00
Clostridium autoethanogenum	1	0.00
Methylobacterium platani	1	0.00
Methylobacterium nodulans	1	0.00

DISCUSSION

Citrullus lanatus waste is usually composed of the entire spoiled fruits or the seeds and the edible pulp. Studies indicate that the seeds, whole fruits and rind contains mineral, proximate nutrients such as proteins, fat, moisture, fiber and higher amounts of carbohydrate (**Fila *et al.*, 2013; Tabiri *et al.*, 2016; Olayinka and Etejere, 2018**). These nutrients provide an enabling environment for the growth of microorganisms. This has made fruit waste an attractive substrate for anaerobic digestion (AD). In anaerobic digestion, microorganisms function synergistically to achieve high biogas output (**Asikong *et al.*, 2014**). The microbial diversity in a biogas digester is enormous and rarely captured with cultural techniques.

In this study, the structural composition of anaerobic digester utilizing water melon (*Citrullus lanatus*) fruit waste was evaluated. The study revealed the

dominant phyla to include: unknown (53739 read counts), Firmicutes (2206), Proteobacteria (1177 read counts), Planctomycetes (186 read counts), Bacteroidetes (116 read counts), Actinobacteria (84 read counts), Euryarchaeota (79 read counts) and Chloroflexi (59 read counts). The results of microbial composition of the anaerobic digester studied indicate that the digesting slurry of *Citrullus lanatus* waste had unique taxonomic groups and overlapping taxa with other substrate types described previously. The kingdoms taxa identified were predominantly bacteria followed by archae. This confirms the fact that bacteria and archae are the main players in anaerobic digesters.

Li *et al* (2015) detected firmicutes, Bacteroidetes, Proteobacteria, Chloroflexi, Synergistetes, Verrumicrobia, Spirochaetes, Actinobacteria, Tenericutes, Acidobacteria and Planctomycetes as their unique phyla using different animal manures as substrates. Compared to our finding, Tenericutes, Verrumicrobia, Spirochaetes and Acidobacteria were not found. Studies have shown that Tenericutes and Spirochaetes are obligate intracellular parasites **(Ludwig *et al.*, 2010; Gupta et al., 2013)** while Verrumicrobia and Acidobacteria are common to soil (**Navarrete et al., 2015; Kielak, et al., 2016).** Furthermore**,** Deinococcus-thermus and Cyanobacteria amongst others were unique to our sample even though they have been implicated in earlier studies (**Vincent *et al,* 2018; Detman *et al.*, 2018**).

Using metagenomics, a number of taxa have been identified in AD utilizing various operating conditions. These include archae such as Methanomicrobia, Methanobacteria, Methanosarcinaceae, Methanobacteriacea, Methanomicrobiales, and Methanosarcina. Dominant bacterial taxa include Clostridia, Bacilli, Bacteroidetes, Chloroflexi, Proteobacteria, Actinobacteria, Synergistetes and Thermotoga (**Kleinsteuber, 2018).** These taxa and archae were also detected in our study.

It was observed in this study that Firmicutes, Proteobacteria, Actinobacteria Bacteriodetes and Chloroflexi in descending order of abundance were the top five phyla present in our studied anaerobic digester. This agrees with the work of **Kirkegaard *et al* (2017)** who also reported these phyla. Among the phyla, Firmicutes was the most dominant after the Unknown phylum. The class Clostridia was the predominant of the bacterial classes in our digester. This class have been reported by **Kirkegaard *et al* (2017)** and **Jha *et al.* (2012)** to be active bio-degraders of a wide assortment of organic polymers.

In an earlier study, unique operational taxonomic units (OTU) were observed for different substrates even though some phyla did overlap (**Li *et al.*, 2015**). In swine abundant manure sludge sample, Syntrophomonadaceae, Lachnospiraceae, Clostridiales, *Clostridium* XI, and Bacteria were unique OTU. In cattle abundant sample, Sporobacter, Corynebacterineae, Bacteria, Planococcaceae, Firmicutes, *Clostridium sensustricto*, Bacteria, *Anaerovorax and* Ruminococcaceae were unique. These taxonomic groups were also detected in our sample especially the Firmicutes.

In a study utilizing cow dung and straw as substrates, some of the reported dominant bacterial classes were Bacilli, Clostridia, Alphaproteobacteria and Actinobacteria (**Sun *et al.*, 2015**). Compared to our study, all these bacterial classes were also reported but Clostridia had the highest reads followed by Gammaproteobacteria, Bacilli, Planctomycetes, Alphaproteobacteria, Actinobacteria and Bacteroidetes. **Liu *et al* (2018)** reported Syntrophomonas, Clostridium, Lactobacillus, and Pseudomonas as relative abundant in AD and these also detected in our samples.

Microbial community within a digester have been grouped into acidogens, syntropic acetogens and methanogens (**Manyi-Loh *et al.*, 2013**). The acidogens are well known for hydrolysis ability and are often referred to as fermentative bacteria. Reported acidogens in AD include members of the family *Enterobacteriaceae* which in our study was the third most abundant family. *Clostridium*, *Bacteroides*, *Succinivibrio*, *Prevotella* and *Ruminococcus* have also been described as an important genera in the hydrolysis extensively in ruminants and biogas digesters. In our study, the abundance of these genuses *Clostridium*, *Uncultured rumen bacteria*, *Bacteroidetes*, and *Prevotella* confirms their hydrolytic roles in our cellulose rich substrate (**Dowd *et al.*, 2008; Callaway *et al.*, 2010**).

The syntrophic acetogens are usually responsible for the syntrophic metabolisms of C3 to C6 short chain fatty acids, alcohols, and amino acids (**McInerney et al., 2008**). This ability is almost limited to *Syntrophobacter and Syntrophomonas species. In our sample,* uncultured Syntrophomonas was detected. Others groups capable of this metabolism include the thermophilic bacteria (**Manyi-Loh *et al.*, 2013).** These were found amongst our detected genera including the *Methanothermobacter sp*.

Among the archae, the genus *Methanoculleus*is predominant in anaerobic reactors previously studied digesters (**Krober *et al.*, 2009; Feng *et al.*, 2010; Jaenicke *et al.*, 2011; Jha *et al.*, 2012**). This genus was also detected in our study and the two representatives were *Methanoculleus bourgensis* and *Methanoculleus marisnigri*, with the former being more predominant member. This may be because *M. bourgensis* have been reported by **Weiss *et al* (2009)** to easily adjust to unfavorable conditions that typically inhibit the success of an anaerobic digestion operation. The important archaeal community responsible for methane gas production, at the class level were dominated by Methanomicrobia and Methanobacteria. In addition to *Methanoculleus*, *Methanothermobacter* was also detected in our sample.

Using cultural techniques to characterize microbial community in an anaerobic digester failed to reveal important member of significant phyla Firmicutes such as Clostridium species and other species that are largely unculturable due to their anaerobic requirement (**Ofoefule *et al.*, 2010; Asikong *et al.*, 2016**).

CONCLUSION

The result indicates that bacteria and archae dominated the microbial kingdoms in our anaerobic digester utilizing *Citrullus* lanatus fruit waste. Dominant phyla were Unknown, Firmicutes, Proteobacteria, Planctomycetes, Bacteroidetes, Actinobacteria and Euryarchaeota in decreasing order of counts. As expected, classes, orders, families belonging to these top phyla dominated the class, order and family classifications. A total of 210 phylotypes were obtained out of which half of these were not routinely culturable isolates. These findings confirm the fact that AD systems harbor complex microbial communities that are unculturable using routine media. Interestingly, phylotypes involved in key processes of AD such as hydrolysis, acidogenesis, acetogenesis, acetogenesis and methanogenesis were obtained indicating a self-sustaining and synergistic AD.

Conflict of interest: The authors hereby declare that no conflict of interest exist.

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