**International Journal of Research in Engineering and Applied Sciences(IJREAS)**
Available online at: http://euroasiapub.org
Vol. 8 Issue 2, February- 2018
ISSN (o): 2249-3905 ISSN (P): 2349-6525 | Impact Factor: 7.19 |

# RECYCLING OF SAWDUST FOR BIOGAS PRODUCTION USING BACTERIAL CONSORTIA

**ANIAKU, VINCENT OLUCHUKWU**

Department of Applied Microbiology and Brewing,

Enugu State University of Science and Technology, Enugu, Nigeria

## ABSTRACT

Recycling of sawdust to biomethane using bacterial consortia were investigated. The Molecular identification of the bacterial and archaeal isolates based on 16S rRNA gene sequences showed that the dominant bacterial species were the Bacillus and Pseudomonas species while Methanosarcina and Methanobrevibacter were the dominant archaeal group. Four bacterial consortia (1, 2, 3 and 4) were formulated by combining the isolates in different ratios. One batch of the organic slurry was biologically-pre-treated with 1.0% broth culture of Phanarochaete chrysosporium for easy hydrolysis of β-glycosidic and ether-ester bonds of lignocellulose while the other batch was not treated. The results obtained on methane production from 1,600 ml of organic slurry containing 200 grams of pre-treated and untreated substrate each shows that consortium 4 containing more of the Methanogens had the highest methane-producing potential followed by consortia 3, 2 and the least consortium 1 at different digestion temperatures. The graphical plot of methane yield against different digestion temperatures using the consortia showed that the optimum temperature was 55$^{o}$C with highest methane yield of 11.80 cm$^{3}$ /g VS and 7.38 cm$^{3}$/g VS produced by consortium 4 for pre-treated and untreated substrate respectively while the maximum temperature was 65$^{o}$C with a decline in methane production. One-way analysis of variance of the results obtained from biogas yield shows that methane production was significantly ($p \leq 0$) dependent on the microbial consortia, nature of substrates and digestion temperature.

**KEYWORDS:** Recycling, Sawdust, Bacterial consortia, Digestion temperatures, Biomethane

## INTRODUCTION

Biogas is a combustible mixture of methane, carbon dioxide and trace of other gases produced by the anaerobic decomposition of biomass using natural or deliberate cultures of microorganisms essentially the Methanogens (Faryyaz, 2014). Methane and carbon dioxide account for about 60-70% and 30-40% respectively of the entire biogas volume while hydrogen sulphide ($H_2S$) makes up to 0.5 to 1.0% and traces of siloxanes may also be found (Zhao et al. 2010). Biogas is a colourless and odourless gas that burns with a clear blue flame and has auto ignition temperature of 650 to 750$^{o}$C,

density of 1.214g/$m^3$and calorific value of 20 to 26MJ/$m^3$ and is less polluting compared to fossil fuels. Biogas is about 20% lighter than air and liquefies at a pressure of 47.4 kg/$cm^2$ at a critical temperature of -82$^oC$ (Appelset al. 2008). Biogas generated from anaerobic digestion of biomass can be a valuable energy resource and is a clean and environmentally friendly renewable fuel. It is important to clean or upgrade the gas to methane to increase its heating value to make it useable in some gas appliances such as engines and boilers (Wellinger and lindberg, 2005; Scott, 2015). The three basic uses of biogas are: production of heat and steam for domestic and industrial purposes, electricity generation and fuel for automobiles.

In today`s energy demanding life style, the need for exploring and exploiting new sources of energy which are renewable as well as eco-friendly becomes imperative and Ogbonna (2011), had suggested that renewable energy sourcing should be focused on those resources that are carbon-neutral or negative to save the biosphere from total collapse.

In rural areas of developing countries, various cellulosic biomasses (sawdust and agricultural residues) are found abundantly and have very high potential to meet up our energy demand especially in the domestic sector. Lignocellulosic biomass such as sawdust, maize straw, and rice husk are considered as the most abundant renewable energy resource with the potentials of making a substantial difference in the supply of biofuels (Zhong et al. 2011). Plant biomass is composed primarily of cellulose, hemicelluloses and lignin in varying amounts in the different parts of the plants and they are intimately associated to form the structural framework of the plant cell wall (Jorgensen et al. 2007).

Sawdusts which are products of timbers, wood mills and furniture works are produced in copious amounts in Africa without adequate means of utilization or disposal; hence they accumulate in the environment to constitute environmental menace such as greenhouse gas emissions, fire hazards, and air and water pollutions. The volume of sawdust from sawmills has continued to increase due to a rise in timber production to meet up the growing demands for wood products. Africa is yet to exploit fully the potentials of recycling wastes especially saw dust. Sawdust heaps in Africa are considered as wastes and therefore are indiscriminately incinerated making significant contributions to the greenhouse gas emissions with the concomitant effects on global warming and climate change. Sawdust has the potentials of being transformed into biomass energy essentially, methane for domestic and industrial uses. Sawdust like any lignocellulosic biomass has substantially lower biogas yield per volatile solids in conventional anaerobic fermentation compared to starch, lipids or protein-rich biomass due to the recalcitrant nature of their β-glycosidic and ether-ester bonds to microbial degradation. Different thermal, chemical and biological pre-treatment methods have been reported to enhance anaerobic digestion of lignocellulosic biomass to increase methane yield

(Zhonget al. (2011); Jorgensen et al. (2007); Aliyu and MdZahangir, (2016)). Pre-treatment of feedstocks can increase solubilization, biogas production and volatile solids reduction (Tiehmet al. 2001). Pre-treatment methods aid in facilitating the anaerobic digestion by increasing the rate of **organic matter** hydrolysis which in effect results in enhanced production of biogas and aids in waste stabilization as well as disposal. The target of any pre-treatment method is to make the available nutrients accessible to most microbial species which speed up biomass utilization during anaerobic digestion process (Patilet al. 2016). The use of pre-treatments is particularly useful in the digestion of biomass feed stocks, as these tend to be high in cellulose or lignin. Pre-treatment can break down these recalcitrant polymers physically, chemically and biologically.

According to the International Energy Agency "Renewable energy resources have entered the main stream and should be developed in synergy among nations to have global impact and not in isolation" (IEA, 2007). African countries should key in into this noble project to ensure sustainable energy security for economic transformation for the present and future generations.

Production of methane from sawdust which is found in copious quantity in Nigeria and other African countries using microbial consortia will help to alleviate our energy problems and reduce its environmental and ecological impacts. In this paper, we assessed the potential of microbial consortia for biogas production from lignocellulosic wastes.

**3.0 MATERIALS AND METHODS**

**3.10 SOURCE OF MATERIALS**

The sawdust used for the research work was collected from Timber Shade, Abakpa Nike, Enugu, Nigeria. The equipment, reagents and media used for the proximate, biochemical and microbiological analysis were provided by Resource Concept Laboratory Limited, Enugu

**3.2.0 EXPERIMENTAL DESIGN**

**3.2.1 ISOLATION OF MICROORGANISMS**

The microorganisms used for methane production was isolated from cow dung collected from cattle slaughterhouse Mami Market, Army Barack, Enugu

The bacteria were isolated according to the methods described by Gopinathet al. (2014); Pandianet al. (2012); and Mezes et al. (2015).

**ISOLATION OF FUNGI**

One gram of fungus-infested sawdust sample was added to 99ml of sterile distilled water, shaken vigorously and diluted serially. An inoculum of the diluted sample was streaked on a freshly prepared sabouraud dextrose agar (SDA). The plates were incubated at 30°C for 72 hours. After incubation, the fungal colonies were subcultured on fresh SDA plates to purify the isolates.

**IDENTIFICATION OF FUNGI**

The fungal isolates were identified based on the cultural features of the colonies and morphological characteristics of the stained cells as described by Barbara et al. (2011

**STAINING AND MICROSCOPY**

A sample of the fungal culture was picked with a sterile forceps and fixed on a clean glass slide with a drop of absolute ethanol. Two drops of lactophenol cotton blue were poured on the smear and covered with a cover slip. The stained cells were viewed using ×10 and ×40 objective lenses of a binocular microscope (Olympus, Italy).

**SELECTIVE ISOLATION OF METHANOGENS**

The Methanogens were isolated using SAB 119 medium containing mineral salts supplemented with yeast extract, vitamins and essential amino acids (tryptophan and cysteine). Sodium chloride (NaCl) (2.6%) was added to one part of SAB Medium for the selective isolation of Methanobrevibacterspp while 3.0% was added in another part for isolation of Methanosarcina spp. (Rea et al. 2007; Thakker and Ranade, 2002).The serially diluted sample ($10^{-8}$) was inoculated on the enriched agar plates using streak plate technique and placed in anaerobic jar containing anaerobic gas kit to maintain anoxic condition at 40$^{o}$C for 48 hours.

**MOLECULAR IDENTIFICATION OF THE BACTERIAL ISOLATES**

The processes involved in molecular identification of the isolates were: Extraction of the organism genomic DNA, Polymerase chain reaction (PCR), Denaturing Gradient Gel Electrophoresis (DGGE), sequencing of the PCR products (amplicons) and BLAST (Basic local alignment search tools).

**EXTRACTION OF ORGANISM GENOMIC DNA**

The genomic DNA of each of the isolate was extracted following the boiling method as described by Maria et al. (2008).

**PROCEDURE**

Four hundred microlitres (400µl) of sterile distilled water was measured into 1.5ml eppendorf tube and 3 loopfuls of each of the isolate was added into the tube, covered and vortexed to dissolve. The tubes containing the isolates were put into the wells of Acublock heater (Dri-Block Techne, model: FDBO3DD R, Mbby Scientific Ltd. U.S.A) and boiled at 100$^{o}$C for 10 minutes. After boiling, the tubes were cooled in ice and centrifuged at 15,000 rpm for 5 minutes. The supernatant of each isolate was decanted into another set of epperndorf tubes as DNA extracts and stored at -20$^{o}$C for PCR (polymerase chain reaction) amplification.

**POLYMERASE CHAIN REACTION**

The 16S rRNA target region was amplified using Dream Taq$^{tm}$ DNA polymerase (Thermo Scientific$^{tm}$) with bacterial primers 16S-27F (5`-AGAGTTTGATCMTGGCTCAG-3`) and 16S-1492R (5`-CGGTTACCTTGTTACGACTT-3`) and archaeal universal primers, Arch $f_2$b (5`-TTCYGGTTGATCCYGCCRGA-3`) and Archr 1386 (5`-GCGGTGTGTGCAAGGAGC-3`) following the methods described by Stephen et al. 1997; Takashi and Yuji, 2011).

**GEL ELECTROPHORESIS**

Gel electrophoresis of PCR products was carried out by INQABA genomic company, South Africa following the method described by Stephen et al. (1997).

Sequencing and BLAST. The purified PCR products were sequenced in the forward and reverse directions on the ABI PRISM$^{tm}$ 350xl Genetic Analyser. Purified sequencing products (Zymo Research, ZR-96 DNA sequencing clean-up kit $^{tm}$) were analysed using CLC main workbench 7 followed by a BLAST search (NCBL).

**3.2.2 MORPHOLOGICAL AND BIOCHEMICAL CHARACTERIZATION**

**3.2.2.1 MORPHOLOGICAL/BIOCHEMICAL CHARACTERISTICS**

The morphological characters of the isolates determined were Gram reaction, Shapes and Motility while the biochemical characters were sugar fermentation, citrate, methyl red, Vougesproskauer, catalase tests and ability to produce hydrolytic enzymes (Amylase, protease, cellulase, lipase and xylanase). These characteristics were determined as described by Sharma, (2007) and Norrell and Messley (2003).

**BIOCHEMICAL CHARACTERIZATION OF THE ISOLATES**

**SUGAR FERMENTATION AND GAS PRODUCTION**

One loopful of the isolate was inoculated into each 50ml culture tube containing sterile peptone water broth with 10% D-glucose. Two drops of phenol red indicator solution were added into the broth and an inverted Durham tube was inserted in the culture tube. The broth was incubated for 24 hours at 37$^{o}$C. Production of acid which is a product of fermentation was indicated by the change of yellow colour to red. Presence of gas was indicated by appearance of gas bubble in the Durham tube. Negative result shows no change in colour or appearance of gas.

**CITRATE UTILIZATION**

One loopful of the isolate was inoculated into Simmon`s citrate agar containing two drops of bromothymol blue indicator and incubated at 37$^{o}$C for 24 hours, positive test was indicated by the appearance of growth with blue colour while negative result shows no change in colour. Positive test is indicative that the organism can utilize citrate as source of carbon.

**INDOLE PRODUCTION**

One loopful of the culture was inoculated into peptone water broth and incubated at 37$^{o}$C for 48-96 hours. 0.50 ml of kovac`s reagent was added into the broth culture and shaked, the appearance of pink colour in the alcohol layer indicates positive indole production while non-appearance of pink or red colour indicate negative indole production. If the isolate possess enzyme tryptophanase, it will degrade amino acid tryptophan to indole.

Voges-proskauer test

**REAGENTS:** 40% KOH (40ml of KOH and 60ml of distilled water, 0.3% creatine ( 0.3 gram in 100ml of ditilled water, 5% solution of α-naphthol in absolute alcohol (5 gm in 100ml of absolute alcohol) and Glucose phosphate broth.

One loopful of the culture was inoculated into 5ml glucose phosphate broth and incubated at 37$^{o}$C for 48 hours. After incubation, 1ml 40% KOH containing 0.3% creatine and 3ml of 5% solution of α-naphthol in absolute alcohol and shaked, appearance of pink colour in 2-5 minutes indicates positive test.

Fermentation of carbohydrates by some bacteria results in the production of acetyl methyl carbinol (acetion). In the presence of alkali and atmospheric oxygen, acetion is oxidized to diacetyl which reacts with peptone of the broth to give a red colour.

Catalase test

One loopful of the isolate was smeared on clean glass slide with a drop of hydrogen peroxide solution. Prompt effervescence indicates catalase production. Catalase is an enyme which can breakdown hydrogen peroxide to liberate oxygen gas. Negative catalase produces no gas.

**3.2.2.2 ENZYME TESTS**

**Test for cellulase:** The isolate was inoculated on mineral medium containing 1.0% peptone, 1.0% carboxymethyl cellulose (CMC), 0.2% $K_2HPO_4$, 2.0% agar, 0.3% $MgSO_4 7H_2O$, 0.25% ( $NH_4)_2SO4$ and 0.2% gelatin per 100ml of distilled water at pH7.0 using streak plate technique and incubated at 37$^{o}$C for 48 hours. After incubation, the cultures were flooded with 1.0% congo- red dye solution and examined for the appearance of clear zones around the colonies.

**Test for Xylanase:** The isolate was inoculated on nutrient agar medium containing 0.5% xylan and incubated at 37$^{o}$C for 48hours. After incubation the cultures was flooded with lugol iodine solution and examined for the appearance of yellow zones around the colonies. Appearance of clear zones indicates the breakdown of xylan by xylanase produced by the organism to sugars. but appearance of blue black colour indicates negative xylanase production.

**Test for Protease:** The isolate was inoculated on skimmed milk agar plate and incubated at 37$^{o}$C for 24 hours. After incubation, the cultures were examined for the appearance of clear zones around the colonies which indicates presence of protease.

**Test for Amylase:** The isolate was inoculated on starch agar plate by streaking and incubated at 37$^{o}$C for 24 hours, The cultures were flooded with Gram`s Lugol iodine solution and examined for appearance of clear yellow zones around the colonies which indicates the conversion of starch to sugars by the amylase produced by the organism. Presence of blue black colour indicates absence of amylase activity.

**Test for Lipase:** The isolate was inoculated on Tributyrin agar plate containing skimmed milk by streaking and incubated at 37$^{o}$C for 24 hours. Appearance of clear zones around the colonies indicates presence of lipase enzyme.

**3.3.0 PROXIMATE ANALYSIS OF SAWDUST**

**3.3.1 Determination of the moisture content:** The moisture content of sawdust sample was determined as described by Cioablaet al. (2012) and Manyi-lohet al. (2015). Five (5) grams of each sample was weighed into a tarred moisture dish and dried in a preheated oven at 105$^{o}$C for 24 hours. Duplicate samples were subjected to the oven drying conditions. After drying, the dried samples were cooled in a dissecator containing activated silica for 3 hours and reweighed. The oven dry weight of the duplicate samples each was noted and the moisture content calculated from the formular:

% moisture = (Ws-Wsd/Ws) × 100.

Where Ws = wt of sample before drying,

Wsd = oven dry wt of the sample

**3.3.2 Determination of total solids (dry matter) content:** The total solids content of each sample was determined from the oven dry weight of the samples as described by Manyi-Lohet al. (2015). Known weight (5gm each) of the duplicate sample was dried at 105$^{o}$C for 24 hours. After drying and cooling, the oven dry weight of the sample was recorded and calculated in percentage as stated:

% Total solids =Wd/Ws × 100, where Wd= dried wt, Ws= sample wt.

**3.2.3 Determination of the volatile solids and ash content:** The total solids and ash content of the duplicate samples were determined as described by Cioablaet al. (2012).

The overnight dry weight of each sample was combusted at 550$^{o}$C in a muffle furnace for 1hour. The sample weight after combustion was calculated in percentage as ash content while the percentage volatile solids was calculated from the difference in weight of the total solids and ash content.

% volatile solids = (Wdm-Wash/ Wdm) × 100, where Wdm= total solids, Wash =wt of ash.

% ash = (Wash/Ws) × 100. Where Wash =wt of ash, Ws=wt of original sample.

Percentage of organic carbon= 58% × wt of volatile solids (dry organic matter), (Tinsely and Nowakowski, 1959).

**3.2.4 Determination of Total Nitrogen:** The nitrogen content of the sawdust duplicate-samples were determined based on the method described by Diohaet al. (2013).

**Extraction of Nitrate:** Nitrate was extracted from 1.0 gram of dry organic matter in a 50ml beaker using 50ml 1M $NH_4Cl_2$ solution for 30 minutes, stirring every 10 minutes interval. During extraction, the nitrate was reduced to nitrite and forms a red-azo dye. The intensity of the red colour produced is proportional to the nitrate level in the sample. The nitrate level was determined using Palintest photometer.

**Procedure:** A round glass test tube was filled to 10 ml mark with the extract. One (1) Nitricol N-tablet was ground in a motar and mixed with the extract solution to dissolve. The solution was allowed to stand for 10 minutes to develop full colour. The nitrate nitrogen was determined at a wave length of 570 nm using the photometer. The nitrate calibration chart was used to find the nitrate nitrogen concentration in the sample.

**BIOLOGICAL PRETREATMENT OF SAWDUST**

Thirty (30) ml broth culture of Phanerochaetechrysosporium was added into 1,600ml of feedstock solution containing 200gram of sawdust powder in 2-litre glass digester and subjected to submerged fermentation at 35$^{o}$C for 72hours in a shaker incubator for the extracellular hydrolysis of lignocelluloses.

**3.3.1 FORMULATION OF STARTER CULTURES FOR BIOGAS PRODUCTION**

The microorganisms used to formulate the starter cultures for biogas production were selected based on their organic matter-degrading potentials. The organisms were combined in different ratios to form different consortia (1, 2, 3 and 4) used for the anaerobic digestion of pre-treated and untreated sawdust to produce biogas at different temperatures (35°C, 45°C, 55°C and 65°C). The starter cultures were formulated based on the methods described by Gopinathet al. (2014).

**3.3.1.1 Digester Units:** Four digester units of 2-litre capacity each were designed, each one representing a consortium made of different organisms as follows:

Consortium 1: This was made up of 1% hydrolytic bacteria, 1% acidogenic/acetogenic bacteria, 1% methanogens.

Consortium 2: This was made up of 1.5% hydrogens, 0.75% acidogen/acetogens and 0.75% methanogens.

Consortium 3: This was made up of 1.5% acidogens/acetogens, 0.75% hydrogens and 0.75% methanogens

Consortium 4: This was made up of 1.5% methanogens, 0.75% acidogens/acetogens and 0.75% hydrogens.

**3.3.2 BIOGAS DIGESTER AND PURIFICATION UNITS**

Four glass digester units of 2-litre capacity each were designed, fabricated and used to produce biogas from pre-treated and untreated sawdust samples. Each digester unit was coupled to 3 purification units containing silica gel to remove water vapour, activated clay to remove hydrogen sulphide and potassium hydroxide to remove carbon dioxide which are considered as impurities in the gas stream.

**3.3.3 FORMULATION OF ORGANIC SLURRY**

Each batch of organic slurry was composed of the following:

Feedstock material= 200gram per batch

Water = 1,400 $cm^3$

3%Consortium = 36 $cm^3$

**3.3.4Anaerobic Digestion**: Each batch of organic slurry (1,600ml) containing 200gram of pre-treated or untreated sawdust powder and 3% consortium was subjected to anaerobic digestion to produce biogas at four temperature regimes (35$^o$C, 45$^o$C, 55$^o$C and 65$^o$C). The hydraulic retention time and pH for gas production was noted for each batch of feedstock sample.

**3.3.5 Purification of biogas and measurement of methane volume:**

The raw biogas stream produced from anaerobic digestion of feedstock sample was subjected to purification processes as described by Wellinger and Lindberg, (2005). The scrubbing processes involved the removal of water vapour, hydrogen sulphide and carbon dioxide with silica gel, activated clay and potassium hydroxide in scrubbing unit 1, 2 and 3 respectively.

The volume of methane produced was measured by downward displacement of water process. The volume of water displaced equals the volume of methane gas produced per unit time by each of the consortium from pre-treated and untreated substrate at a particular temperature.

**BIOGAS PROFILING**

The concentrations of methane and gaseous impurities (carbon dioxide, hydrogen sulphide, water vapour and oxygen) in biogas samples were determined using Infrared gas analyser (Crowcon GAS-PRO (Crowcon Instruments Ltd, Oxfordshire, UK) based on the principle of infrared-gas absorption technique.

**3.3.6 EVALUATION OF THE METHANE-PRODUCING POTENTIAL OF THE BACTERIAL CONSORTIA**

The methane-producing potentials of the bacterial consortia were evaluated based on the one-way analysis of variance (ANOVA) of the volume of biogas produced by each of the consortium from

pretreated and untreated sawdust samples at different digestion temperatures at 95% confidence level.

**4.0 RESULTS AND DISCUSSION**

The methane-producing potentials of different bacterial consortia from sawdust at four temperature regimes were investigated. The result of molecular identification based on the 16S RNA gene sequences of bacterial isolates showed that the dominant species were Bacillus and Pseudomonas which belong to the Phylum Firmicutes and Proteobacteria respectively while the dorminant methanogeni carchaea were Methanosarcina and Methanobrevibacter species. Bacillus, Cellulomonas and Pseudomonas species were reported to possess cellulase, protease, lipase and amylase enzymes which make them special for hydrolysis of lignocellulosic biomass to amino acids, fatty acids and sugars which are essential nutrients for acidogenic and acetogenic microbes. Methanosarcinathermophilla is acetoclastic methanogen which is a key player in anaerobic conversion of acetate to methane while Methanobrevibactersmithii is a hydrogentrophic methanogen capable of converting hydrogen to methane in presence of carbon dioxide.

The result of the proximate composition of sawdust duplicate samples on average showed that the volatile solids, carbon, nitrogen content and carbon-nitrogen ratio was 93.8%, 54.6%, 0.376% and 145:1 respectively. The percentage nitrogen of 0.376% is too low for microbial growth and metabolism as this could result in stunted fermentation and poor yield of methane during anaerobic digestion. The carbon nitrogen ratio of 160:1 shows that sawdust has too high carbon but low nitrogen. There should a balance in carbon and nitrogen content of a substrate for anaerobic digestion for improved yield of biogas as reported by Yadvikaet al. (2003); Dana, (2010) and Igoniet al. (2007). A carbon-nitrogen of 20:1 to 30:1 has been reported to be adequate for a feedstock for a successful AD process and enhanced yield of biogas.

**Table 1: Enzyme test of the isolates**

| Isolate | Amylase | Cellulase | Protease | Xylanase | Lipase |
|---|---|---|---|---|---|
| Geobacillusstearothermophilus | ++ | ++ | ++ | ++ | Trace |
| Burkholderiafungorm | ++ | ++ | + | ++ | + |
| Cellulomonasflavigena | ++ | ++ | + | ++ | + |
| Phanonaerochaetechrysposporium | ++ | +++ | + | ++ | + |
| Bacillus licheniformis | ++ | + | ++ | Trace | + |
| Methanosarcinathermophilla | ++ | + | + | + | + |
| Methanobrevibactersmithii | ++ | ++ | + | + | + |
| Pseudomonas aeruginosa | ++ | + | ++ | + | + |
| Bacillus cereus | ++ | + | ++ | Trace | + |

**+**positive to enzyme production, ++ very positive to enzyme, +++ produces enzyme significantly

**Table 2: Result of proximate composition of sawdust**

| Parameter | Result |
|---|---|
| %Moisture | 5.54±0.06 |
| %Dry matter | 94.65±0.05 |
| %Ash | 1.25±0.05 |
| %Volatile solids | 93.8±0.20 |
| %Carbon | 54.60±0.10 |
| %Nitrogen | 0.376±.015 |
| C:N ratio | 145:1 |

Four bacterial consortia (1, 2, 3 and 4) were formulated by the combination of the hydrolytic, acidogenic and methanogenic microbes in different ratios and used to produce biogas from pretreated and untreated sawdust at four temperature regimes (35°C, 45°C, 55°C and 65°C) under anoxic condition. The biogas stream was subjected to scrubbing processes using calcium chloride, activated clay and potassium hydroxide. The result of methane production at different temperatures using the consortia showed that consortium 4 containing more of the methanogens gave the highest methane production followed by consortium 3 and 2 at various temperatures. The highest methane yield of 11.80 $cm^3$/g VS and 7.38 $cm^3$/g VS was produced by consortium 4 for both pre-treated and untreated substrate respectively at the optimum temperature (55$^oC$) while consortium 3 has methane yield of 9.50 $cm^3$/g and 6.08 $cm^3$/g for pre-treated and untreated substrate respectively at 55°C. Methane production was found to decline at 65°C for both pre-treated and untreated substrates. Biological pre-treatment of the substrate using Phanaerochaetecrysporium was found to have significant effect on methane yield in agreement with the reports of Zhonget al. (2011); Aliyu and Mdzahangir (2016). Phanerochaetechrysosporium is a cellulase-producing fungus which attack the β-glycosidic linkages of cellulose and the ether-ester bonds of hemicellulose and lignin liberating the sugars such as xylose and β-D-glucose

**Table 3: Methane yield ($cm^3$/gVS) from bio-treated sawdust usingfour bacterial consortia**

| Consortium | 35$^oC$ | 45$^oC$ | 55$^oC$ | 65$^oC$ |
|---|---|---|---|---|
| 1 | 4.50±0.55 | 6.13±0.53 | 7.48±0.12 | 4.08±0.47 |
| 2 | 4.23±0.32 | 6.91±0.19 | 8.17±0.19 | 5.19±0.35 |
| 3 | 4.76±0.36 | 8.10±0.15 | 9.50±0.28 | 5.38±0.88 |
| 4 | 7.40±0.17 | 9.62±0.14 | 11.80±1.11 | 5.75±0.12 |

**Table 4: Methane yield ($cm^3$/g VS) from untreated Sawdust using four consortia**

| Consortium | 35°C | 45°C | 55°C | 65°C |
|---|---|---|---|---|
| 1 | 2.90±0.22 | 4.60±0.15 | 4.95±0.28 | 2.29±0.16 |
| 2 | 2.22±0.31 | 5.24±0.27 | 5.80±0.37 | 2.68±0.21 |
| 3 | 3.50±0.14 | 4.90±0.15 | 6.08±0.18 | 3.17±0.16 |
| 4 | 4.26±0.16 | 5.96±0.08 | 7.38±0.18 | 3.63±0.17 |

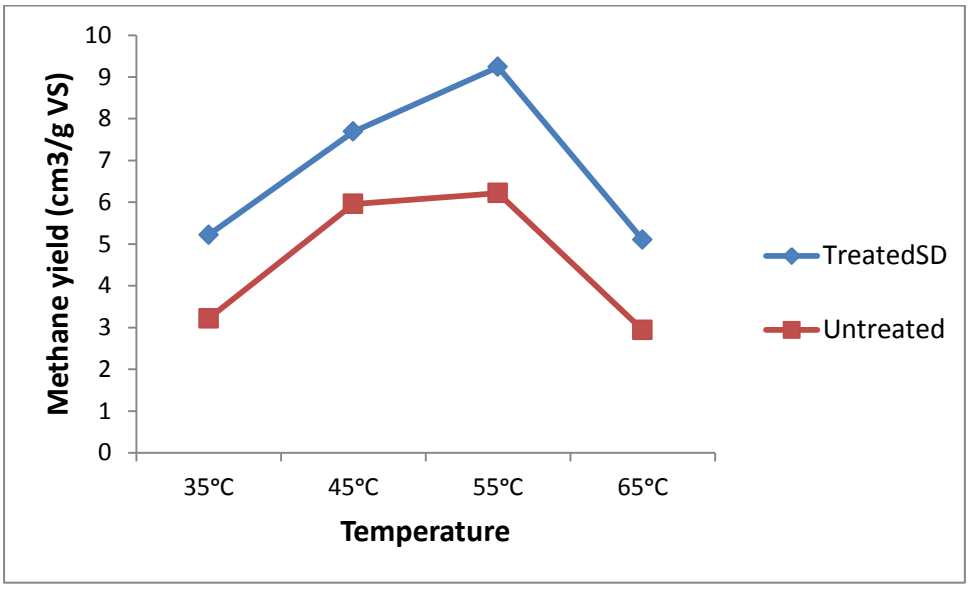


**Fig. 2: Comparative methane yield ($cm^3$/g) from bio-treated and untreated sawdust**

The result of biogas profiling using infrared gas analyzer showed that the methane and gaseous impurity concentrations obtained in biogas samples were higher at thermophillic temperature (55°C) than the values obtained at mesophillic condition (35°C). The hydrogen sulphide content of biogas obtained at both conditions fall within the acceptable limit of ≤ 700 ppm of gas intended for combined heat and power (CHP) generation as reported by Angelidaki and Ahring (2004).

**Table 5: Percentage of methane and gaseous impurities in biogas produced by the consortia from sawdust at thermophilic condition (55°C)**

| Consortium | %$CH_4$ | %$CO_2$ | $H_2S$(ppm) | %$H_20$ | %$O_2$ |
|---|---|---|---|---|---|
| 1 | 69.70 | 29.38 | 520 | 0.16 | 0.05 |
| 2 | 69.98 | 28.8 | 472 | 0.14 | 0.08 |
| 3 | 71.12 | 27.6 | 456 | 0.16 | 0.04 |
| 4 | 72.51 | 26.3 | 389 | 0.12 | 0.02 |

**Table 6: Percentage of methane and gaseous impurities in biogas produced by the consortia from 100% sawdust at mesophilic condition (35°C)**

| Consortium | $\%CH_4$ | $\%CO_2$ | $H_2S$(ppm) | $\%H_20$ | $\%O_2$ |
|---|---|---|---|---|---|
| 1 | 69.22 | 29.66 | 480 | 0.12 | 0.04 |
| 2 | 69.53 | 29.2 | 443 | 0.08 | 0.05 |
| 3 | 70.14 | 27.82 | 424 | 0.06 | 0.03 |
| 4 | 71.88 | 26.93 | 412 | 0.08 | 0.03 |

**Tests Between-Subjects Effects**

Dependent Variable: gas yield

| Source | Type III Sum of Squares | df | Mean Square | F | Sig. | Partial Eta Squared |
|---|---|---|---|---|---|---|
| Corrected Model | 7179275.646[a] | 15 | 478618.376 | 16.087 | .000 | .883 |
| Intercept | 89219260.021 | 1 | 89219260.021 | 2998.711 | .000 | .989 |
| Temperature | 4288800.062 | 3 | 1429600.021 | 48.050 | .000 | .818 |
| Consortium | 2468517.896 | 3 | 822839.299 | 27.656 | .000 | .722 |
| temperature * Consortium | 421957.688 | 9 | 46884.188 | 1.576 | .165 | .307 |
| Error | 952081.333 | 32 | 29752.542 | | | |
| Total | 97350617.000 | 48 | | | | |
| Corrected Total | 8131356.979 | 47 | | | | |

a. R Squared = .883 (Adjusted R Squared = .828)

**P ≤ 0.05**

**CONCLUSION**

It can be deduced from the results of the study that sawdust which is treated as waste can be economically converted to biogas which is a renewable fuel. The consortium containing more of the methanogens is endowed with higher methane-producing potential than others which implies that methanogens are the major key players in anaerobic conversion of organic substrates to methane. Pre-treatment of the substrate prior to anaerobic digestion could enhance methane production as can be confirmed from the results of the study. Anaerobic digestion of recalcitrant substrates such as sawdust and plant residues to renewable fuels through "waste to wealth conversion initiative" will help in environmental management of these wastes.

**REFERENCES**

Aliyu, S and Md Zahangir, A (2016), Pre-treatment methods of organic wastes for biogas production, Journal of Applied Sciences, 16:124-137

Angelidaki, I. and Ahring, B.K. (2004), Thermophillic anaerobic digestion of livestock wastes, the effect of ammonia and hydrogen sulphide, Applied Microbiology and Biotechnology, 38 (4): 560-564.

Appels, L, Baeyens, J, Degreve, J and Dewil, R. (2008). Principles and potentials of anaerobic digestion of waste activated sludge, Progress in Energy and Combustion Science, 234: 755-25.

Barbara H.E, Anna, P.R and Norman J.W (2011). Identification of Fungi, In: Basic Medical Laboratory Techniques 4th Edition, Delmar PUB.CO, Auburn Alabama, United States.

Cioabla, A.E., Lonel, L, Dumitrel, G.A. and Popescu, F (2012), Factors affecting anaerobic digestion of agricultural residues, Biotechnology Biofuels, 5:39-42.

Dioha, I.J., Ikeme, C.H., Nafiu, T., Soba, N.I., Yusuf, M.B.S. (2013), Carbon and Nitrogen content of various biogas feed stocks International Resource Journal of Natural Science, 1(3):1-10.

Fayyaz, A.S, Qaisar M, Mohammad M.S, Arshid P, Saeed A.A (2014), Ecology of anaerobic digesters, The Science World Journal, 2014: 183752-21

Gopinath, L.R, MerlinChristy, P, Mahesh K, Bhuvaneswari, R., Divya, D. (2014). Use of bacterial consortia for biogas production, Journal of Environmental Science, Toxicology and food Technology, 8(3):80-6.

I.E.A (2007). Renewables in global Energy supply; An International Energy Agency (IEA) facts sheet Vol. 34.

Jorgensen, H., Kristensen, J.B., Felby, C. (2007). Enzymatic conversion of lignocelluloses to sugars for biofuel production, Biofuel Bio product Bio refining, 1: 119-134

Manyi-loh C. E., Mamphweli, S.N., Meyer, E. L., Okoh, A. I., Makaka, G and Michael, S. (2015). Investigation into the biogas potential of cattle manure, Journal of Clean Energy Technologies, 3(5):326-331

Maria, I.Q., Juan D.D.C., Manuel, M., Maria, J.B., Pillorm, D.N.A. (2008), Extraction of DNA by boiling method Clinical Vaccine Immunology, 15 (2):293-3.

Mezes, L., Nagy, A., Galya, B., Tamas, J. (2015). Poultry waste recycling possibility as soil nutrients, Eurassian Journal of Soil Science, 4(4)244-252.

Norrel, S.A. and Messley, K.E. (2013). Culturing of microorganisms In: Microbiology Laboratory Manual, 2nd edition, Prentice Hall, India

Ogbonna, J.C. (2011). Renewable energy Resources as better alternatives to fossil fuels, Key note address, Proceedings of the 19thAnnual conference of FADIB held at IMT, Enugu Nigeria.

Pandian, S., Jawahar, S., and Prabakaran, P. (2012). Isolation and identification of feather-degrading bacteria, European Journal of Experimental Biology, 2012; 2(1): 274-8.

Patil, P.N., Goalgate, p.r, Csoka, L., Dregelyi-kiss and Horvath, M (2016). Intensification of biogas production using pre-treatment based on hydrodynamic cavitation, Ultrasonics Sonochem, 30:79-86

Rea, S., Bowman, J.P., Popovski, S., Wright, A.D.G. (2007). Culturing of MethanobrevibacterSpp, Institute of System Evolution Microbiology, 57: 450-456.

Sharma, K. Biochemical characterization of microorganisms, In: Manual of Microbiology, Tools and Techniques, New Delhi India.

Stephen, F.A, Thomas, L.M, Alejandro, A.S, Jinghui, Z, Zheng, Z, Webb, M and David, J.L (1997). Molecular Biology In: Basic Technologies and Applications, Institute of Cell and Molecular Science, UK

Thakker, C.D. and Ranade, D.R (2002). Alkalophilic Methanosarcinaspp isolated from Lonar Lake, Current Science, 82:455-3.

Wellinger, A. and Lindberg, A. (2005), Biogas upgrading and utilization, International Energy Agency Bioenergy, 24:1100-1200.

Zhoa, R., Zhang, Z., Zhang, R., Li, M., Lei, Z., Utsumi, M. and Sugiura, N. (2010). Pre-treatment of lignocellulose biomass for methane production, Bio resource Technology, 101: 990-994

Zhong, W., Zhang, Z., Qiao, W., Fu P. and Liu, M. (2011). Comparison of chemical and biological pre-treatment of corn straw for biogas production, Renewable Energy, 36:1875-1882.