American Journal of Applied Sciences and Engineering | *ISSN 2766-7596*
Published by AIR JOURNALS | *https://airjournal.org/ajase*
12011 WestBrae Pkwy, Houston, TX 77031, United States
airjournals@gmail.com; enquiry@airjournal.org

Research Article

Production of Biofertilizer and Biomethane from Co-Digestion of Lignocellulosic and Keratinolytic Substrates

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Abstract

Environmental pollution from organic wastes such as poultry feathers and rice husks is on the increase in Nigeria with their attendant environmental impacts and health implications. The need to harness the economic potentials of essential wastes from the agro-allied sectors under waste to wealth initiative in Nigeria cannot be overemphasized. Production of biofertilizer and biogas from the anaerobic digestion of poultry feather and rice husk using 5% cow dung as source of microbial consortia was investigated. The constituent microbial consortia used for anaerobic digestion were identified molecularly as Bacillus, Cellulomonas, Pseudomonas, Methanosarcina and Methanobrevibacter species based on their 16S rRNA gene sequences. The result of the proximate analysis of rice husk showed that the volatile solid content, carbon, nitrogen, ash and carbon-nitrogen ratio (C/N) were 68.50%, 42.50%, 0.840% 1.92% and 51:1 respectively while poultry feather had volatile solids and carbon-nitrogen ratio of 96.50% and 4:1 respectively. The feed stocks were hydrothermally treated with steam under pressure, dried at 80oC and milled to a size of 10 µm prior to anaerobic digestion. Anaerobic digestion of the rice husk powder and poultry feather powder at 1:1 ratio under mesophilic condition (35-40oC) at feed stock water ratio of 1:7 lasted for 38 days using four different consortia. The methane yield from rice husk and poultry feather as single substrate were 18.50cm3/g and 14.67cm3/g respectively while the methane yield as combined substrate (1:1) was 30.72cm3/g. The result of compositional analysis of biogas stream obtained by infrared-absorption technique showed that methane and carbon dioxide had average value of 72% and 27% using consortium 4 followed by consortium 3, 2 and 1. The result of digestate (spent slurry) analysis after anaerobic digestion showed that the digestate was rich in micronutrients essentially, Phosphorus (0.16g/l), Nitrogen (2.52 g/l), Calcium (0.93 g/l), Potassium (0.37 g/l) and Magnesium 0.78 g/l). The liquid digestate had pH of 7.30 and total solids content of 16.50%. The use of digestate as fertilizer for enriching the soil for agricultural purposes should be encouraged as the digestates are rich in bioactive agents and mineral elements which help in conditioning the soil. The analysis of variance of the results on methane yield shows that biomethane production was significantly (P≤ 0.05) dependent on the bacterial consortia, substrate constitution and their pair wise interactions.

Keywords	*Biofertilizer and Biomethane; Poultry Feather; Anaerobic Digestion; Production of Biofertilizer; Biomethane Micronutrients*
Citation	Aniaku, V. O. & Anoikete, T. C. (2023). Production of Biofertilizer and Biomethane from Co-Digestion of Lignocellulosic and Keratinolytic Substrates. *American Journal of Applied Sciences and Engineering, 4(2) 1-15.* https://doi.org/10.5281/zenodo.8248356

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Introduction

A biogas plant is not only a supplier of energy. The digested substrate, usually named digestate, is a valuable soil fertilizer, rich in nitrogen, phosphorus, potassium and other micronutrients, which can be applied on soils with the usual equipment for application as liquid manure. Compared to raw animal manure, digestate has improved fertilizer efficiency due to higher homogeneity and nutrient availability, better carbon to nitrogen (C/N) ratio and significantly reduced odors, (Ezemagu et al., 2021). Most coagulation and anaerobic digestion processes have been designed on the premise of treatment and fermentation of waste material and can be used for wastewater treatment and the resultant sludge management. Most of the times, these methods generate sludge and digestate respectively which are disposed as waste products (Ejimofor et al., 2020; Ezemagu et al. 2020; Ezemagu et al., 2021). These treatment methods are associated with serious challenges such as environmental pollution arising from discharge of the generated waste after treatment. The discharge needs to negative impact on soil, eco-diversity, and safety of the environment (Ejimofor et al., 2021a). Consequently, both coagulation and anaerobic digestion processes ought to maximize the use of 3 (three) Rs of reduce (treat), reuse and recycle approach to achieve a greener environment. Specifically, residues obtained after coagulation and anaerobic digestion of wastewater processes (such as sludge and digestate) ought to be used to deliver sustainable benefits for humanity in terms of energy and biofertilizer/soil amendment; which will lead to improvements in the economic, social and industrial developments of the society.

The sludge from wastewater treatment can be anaerobically digested to obtain biogas leaving behind secondary waste termed digestate (Ezemagu et al., 2020; Ejimofor et al., 2021b; Ezemagu, 2020; Li et al., 2011). Digestate is normally in liquid or slurry form. The secondary waste termed digestate has unpleasant characteristics such as viscosity, high content of volatile fatty acids (VFA), high humidity and odor (Ezemagu, 2020; Tampio et al., 2016)**.** Therefore, the digestate is not suitable in its basic form to be applied directly in agricultural soil (Walker et al., 2009). Digestate (obtained from anaerobic digestion of post coagulation sludge) when discharged to the environment results in environmental pollution. Hence, the need to recover and recycle the digestate for environmental sanctity and economic benefits becomes pertinent. Digestate when recovered and recycled by process of composting will result in a value-added product because it is reach in plant macro nutrients (nitrogen, phosphorus, potassium and sulfur), micro nutrients and organic matter (Ezemagu, 2020; Bernhard et al.,2015). This digestate when composted to produce biofertilizer and when put to use by agriculturist will replace the over dependence on chemical fertilizers and its associated problems. The peculiar demerits associated with chemical fertilizer when applied in soil includes soil structure degradation, soil pollution and pollution of underground water resulting in eutrophication; and also, it is very costly to procure (Ezemagu 2020). Recently, researchers encourage the usage of alternative fertilizer (in this case biofertilizer) in place of chemical fertilizer in order to counteract these demerits associated with chemical fertilizer by improving the fertility of soil. Conversely, advantages of digestate when composted as biofertilizer are odor reduction, plant pathogen reduction and reduction in gaseous emission into the atmosphere (Ezemagu, 2020). The need to recover and utilize the waste termed digestate for agricultural purposes (in this case as biofertilizer) envisages the blending of the substrate termed digestate and co-substrate (in this case saw dust-SD) through composting technique.

Composting is an attractive technology as it will be used to control the process of oxidation of heterogeneous mass and microbial decomposition of organic matter in the digestate and saw dust in order to produce biofertilizer (Ezemagu, 2020; Maryam et al., 2015). Hence, composting of digestate and saw dust offers the possibility of waste disposal control and waste recovery in the form of biofertilizer/soil

improver. Also, composting of digestate and saw dust will lead to soil enrichment by retaining moisture and suppressing plant diseases; thereby addressing environmental concerns associated with this waste.

Studies have shown that performance of composting process is largely affected by temperature, nutrient balance (Carbon: Nitrogen ratio), pH of materials, aeration, moisture content, porosity, bulk density, and free air space (FAS) etc. (Ezemagu, 2020; Crechula de Cristina, 2011; Bernal et al., 2009). One of the merits of composting technique is that organic matter will be turned into a stabilized product, which can be applied as a form of returning organic matter to soils. The soil will act as a carbon sink when the biodegradable waste (in this case digestate and saw dust) are subjected to composting in order to reduce carbon dioxide concentration in the atmosphere (Ezemagu, 2020).

Methanogenesis is a complex, redox biochemical reaction occurring under anaerobic condition. Under the symbiotic effects of various anaerobic bacteria, multimolecular organic substances are decomposed into simple, chemically stabilized compounds such as methane and carbon dioxide (Naik e*t al.,* 2010). Generally, this process consists of liquefaction and hydrolysis of insoluble compounds and gasification of intermediates (Fayyaz *et al*., 2014). The anaerobic digestion (AD) occurs in three main stages which include hydrolysis during which complex organics are broken down into soluble components such as sugars, amino acids and fatty acids through the extracellular microbial enzymes (cellulase, xynalase, amylase, lipase, protease etc.). Most of the bacteria in this group are strict anaerobes such as Bactericides, Clostridia and Bifidobacterial (Gerrardi*et al.* 2003). However, some facultative groups such as Streptococci and Enterobacteriaceae are also involved.

The second stage is acidogenesis during which acidogenic bacteria metabolize the monomers to alcohols, lactic acid, acetic acid, ammonia and volatile fatty acids (Metcalf and Eddy 2004). The third stage involves the acetogens which covert the higher volatile fatty acids into acetate and hydrogen. Members of this group are obligate hydrogen-producing bacteria which include *Acetobacteriumwoodii* and *Clostridium aceticum.* The final stage is methanogenesis during which these intermediate products are metabolized to methane, carbon dioxide and trace of other gases by the methanogens (Keri *et al.* 2008). Methanogens, the key microorganisms for biogas production belong to the domain *Archaea* and are known to produce methane under high anoxic condition (Guo*et al*., 2005). The methanogens produce methane from acetate, hydrogen or carbon dioxide. Methanogenic bacteria are strict anaerobes and require a lower redox potential for growth than other anaerobes. Only a few species are able to degrade acetate into methane and carbon dioxide and they include *Methanosarcina* spp (*M.barkeri*)*, Methanococcus mazei, Methanosaetae spp* and *Methanotrix soehgenii* while all methanogens are able to utilize hydrogen to form methane (Schink, 2007). The methanogenic biogas production rate is sensitive to changes in feed stock material, pH, temperature, organic loading rate and hydraulic retention time. These groups of organisms in the microbial community work in syntropy to produce biogas.

The volume of rice husk in Nigeria has continued to increase due to the current rice in local rice production following the restriction on importation of foreign rice by government. Rice husk in Nigeria is considered as a waste and therefore is indiscriminately incinerated making significant contributions to greenhouse gas emissions with the concomitant effects on global warming and climate change. Rice husk like any lignocellulosic biomass has substantially lower biogas yield per volatile solids in conventional anaerobic digestion compared to starch, lipids or protein biomass due to the recalcitrant nature of their β-glycosidic and ether-ester bonds to microbial degradation (Aniaku et al., 2021). Poultry feather is made up of hydrogen disulphide bond which makes it difficult for microbial degradation. Chemical and thermal pre-treatment these wastes prior to anaerobic digestion will make them amenable to microbial decomposition for production of methane and digestate.

Rice husk which is rich in carbon and poultry feather rich in proteins when co-digested have the potentials of being transformed into biomass energy and digestate essentially, methane and biofertilizer respectively, for industrial and agricultural uses. The Benefits of Anaerobic digestion include: Biogas resources are sustainable, reduces greenhouse gas emissions and mitigation of global warming, reduces the importation of fossil fuels, contribution to energy resources of a country, waste reduction and improvement in sanitation, and job creation etc. Researchers have reported on composting of digestate formed after anaerobic digestion of agricultural residues, organic fraction of municipal solid wastes, and animal manure to produce biofertilizer (Crechula de Cristina, 2011). Despite scientific investigation on composting of digestate to produce biofertilizer; there is no report from literature on co-digestion of poultry waste and rice husk to produce biofertilizer and biomethane. This is a major gap this work attempts to close.

Objectives of the Study

The objectives of the study are to:

i. To isolate the constituent microorganisms to be used as microbial consortia for anaerobic of poultry feather and rice husk as combined or single substrate
ii. Produce biofertilizer and biogas through anaerobic digestion of rice husk and poultry feather as single or combined substrate under mesophilic condition
iii. Determine the percentage of the component gases in biogas and the mineral elements in the digestate
iv. Statistical analysis of the result of methane obtained from single and combined substrate at 95% confidence level

Material and Methods

The research on production of biogas and biofertilizer from the blend of sawdust and chicken feather was carried out at BIORESOURCE RESEARCH LABORATORY, Enugu, Nigeria. The poultry feather and rice husk samples were collected from Artisan market poultry slaughter house and a rice mill factory at Ugboka in Nkanu west local government, Enugu respectively. The fresh cow dung used as source of microbial inoculums was sampled from Ogbete main market abattoir, Enugu North. All the chemicals and reagents used in Analysis which are of analytical grade were supplied by Conraws, Nigeria LTD, Enugu.

Isolation and Identification of Microorganisms

Isolation Techniques

The bacteria groups used for biofertilizer and biogas production were isolated from fresh cow dung sample according to the methods described by Gopinath *et al*. (2014); Pandian *et al*. (2012) and Mezes *et al*. (2015). One (1.0) gram of fresh cow dung sample and soil sample (composite) from poultry dump sites were respectively dissolved in 99ml double-distilled water each and serial dilutions with double-distilled water were made up to 10^{-8} folds. An inoculum from $10^{-8\text{-fold}}$ dilution was inoculated on nutrient agar medium by streak plate technique and incubated at 37°C for 24 hours. After incubation, different colonies were subculture into fresh nutrient agar medium to purify the isolates.

Selective cultivation using selective media was applied for fastidious organisms. Eosin-methylene agar was used for members of *Enterobacteriaceae*, Pseudomonas Selective medium was used for Pseudomonas species, Streptococcus agar for Streptococcus species, and Brain heart infusion medium for Clostridium species and Bushnell Haas Medium (BHM) supplemented with 1% Carboxymethyl cellulose (CMC) for Cellulomonas species. Keratinase-producing Bacillii were isolated based on the methods described by Williams *et al.* (1990) and Lakshmi *et al*. (2013). The serially diluted feather dump-site soil sample (10^{-8}) was heated at 80°C for 20 minutes and inoculated on nutrient agar medium by feather baiting technique and incubated at 37°C for 24 hours. Potential keratinolytic isolates were re- inoculated on Basal Mineral

Volume 4, Number 2 | 2023 | 1-15| DOI: https://doi.org/10.5281/zenodo.8248356

Medium containing 0.5% NH_4Cl, 0.5% NaCl, 0.3% K_2HPO4, 0.4% KH_2PO4, 0.1%$MgCl.6H_2O$, 0.1% yeast extract and 1% feather powder at pH 7.50. All the plates containing the different bacterial inocula were incubated at 37°C for 24 to 48 hours.

Selective Isolation of Methanogens

Methanogens were isolated using the methods described by Rea *et al.* (2007) and Thakker and Ranade (2002). The Methanogens were isolated using SAB medium containing mineral salts supplemented with yeast extract, vitamins and essential amino acids (tryptophan and l-cysteine), 2.6% sodium chloride (NaCl) was added to one part of SAB Medium for the selective isolation of *Methanobrevibacter* spp and *Methanomicrobium* spp while 3.0% sodium chloride was added into another part for isolation of *Methanosarcina* spp., 1.0% methanol was added into another part for isolation of *Methanosarcina barkeri and Methanobacterium ruminantum.* 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 incubation temperature of 40°C for 5 days.

After incubation both the bacterial and Archaeal isolates were subjected to morphological, biochemical and enzyme-producing tests to determine their morphology, biochemical characters and ability to degrade organic matters (carbohydrates, proteins and lipids).

Morphological Characterization of the Isolates

The morphological characteristics of the isolates determined were Gram reaction, Shapes and Motility while the biochemical characters were sugar fermentation, citrate, indole production, Voges-proskauer, catalase tests, triple sugar iron test 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).

Gram Staining of the Isolates

The isolates were subjected to gram staining test to determine their gram reaction.

Gram staining reagents: Crystal violet solution, lugol iodine solution, ethanol (95%) and dilute carbofuchsin.

Gram staining procedure*:*

i. A loopful of the colony of an isolate was picked with a sterile wire loop and a smear of it was made on a clean glass slide with a drop of distilled water.
ii. The smear was heat-fixed on the slide by flaming.
iii. Two drops of crystal violet solution were poured on the smear and allowed to react for one (1) minute with the cell after which it was rinsed-off with running water.
iv. Two drops of lugol iodine were poured on the smear and allowed to react for one minute and rinsed-off with water
v. The smear in inclined position was flooded with 95% ethanol solution to decolourize the stain and rinsed-off with water
vi. Two drops of dilute carbofuchsin were poured on the smear as counter stain and allowed to react for 30 seconds after which the excess stain was rinsed-off with water.
vii. The smears were air-dried on a rack for microscopy.

Microscopic Examination of Stained Cells

The stained cells were examined to ascertain their gram reaction using oil immersion objective lens (×100) of a light binocular microscope.

Biochemical Characterization of the Isolates

Sugar fermentation and gas production: One (1) loopful of the isolate was inoculated into each 50ml culture tube containing sterile peptone water broth with 10% D-glcose. 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^oC. 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^oC 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^oC 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 indicates negative indole production. If the isolate possesses 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.

Procedure
One loopful of the culture was inoculated into 5ml glucose phosphate broth and incubated at 37^oC 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.

***Triple Sugar Iron Test (TSI)**:* This test was carried out to determine the ability of the isolates to metabolize three sugars namely: glucose, lactose and sucrose as energy sources and also produces hydrogen sulphide (H_2S) as a bye-product.

Procedure: Thirteen (13.0) gram of Triple Sugar iron Agar (biolab, Gauteng) containing phenol red acid indicator was measured into 200ml of double-distilled water in a 250ml conical flask and shaked to dissolve. The agar solution was sterilized in autoclave at121^oC and pressure of 15.0 P.S. I for 15 minutes. After cooling to 45^oC, the agar was dispensed into sterilized test tubes and inclined to form agar slants on cooling. An inoculum of each isolate was collected with a sterilized inoculating needle and stabbed at the centre of the medium into the bottom of the tube and then streaked on the surface of medium after stabbing. The tubes were loosely caped and incubated at 35^oC for 24 hours. The tubes were examined for colour change from red to yellow or black or both on the surface of the slant and the butt. The presence of yellow and black colour indicates positive triple sugar iron test while presence of deep red or pink colour indicates negative TSI. Appearance of deep red or pink colour indicates presence of ammonia from

peptone metabolism. The processes applied 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 based on the methods of Stephen *et al*. 1997; Maria *et al*. (2008), Takashi and Yuji, 2011). The 16S rRNA target region of the isolates was amplified using Dream Taq^{tm} DNA polymerase (Thermo Scientific^{tm}), Universal bacterial primers 16S-27F (5`-AGAGTTTGATCMTGGCTCAG-3`) and 16S-1492R (5`-CGGTTACCTTGTTACGACTT-3`) and archaeal universal primers Arch f_2b (5`-TTCYGGTTGATCCYGCCRGA-3`) and Archr 1386 (5`-GCGGTGTGTGCAAGGAGC-3`) following the methods described by Stephen *et al*. 1997; Takashi and Yuji, 2011).

Proximate Analysis of the Feedstocks and Digestate

A.O.A. C (1992) methods of analysis were applied for the proximate analysis of poultry feather and rice husks while Atomic Absorption Spectrometry (AAS) methods as described by Elarina et al., (2014) were applied for the determination of the trace elements in the digestates while the constituent gases in biogas stream was determined by infrared-sensory absorption technique using Crowcon GAS-PRO IR gas analyser (Crowcon Instruments Ltd, Oxfordshire, United Kingdom).

Determination of the Moisture Content

The moisture content of rice husk and poultry feather powder samples was determined as described by Cioabla*et al*. (2012) and Manyi-loh*et al.* (2015), Five (5) grams of each sample was weighed into a tarred moisture dish and dried in a preheated oven at 105°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 sample each was noted and the moisture content calculated from the formula:

% moisture = (WS-WSd/WS) × 100.

Where WS= wt of sample before drying,

WSd = oven dry wt of the sample

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-Loh *et al*. (2015). Known weight (5 gm each) of the duplicate sample was dried at 105°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/W S ×100, where WD= dried wt, WS= sample wt.

Determination of the Volatile Solids and Ash Content

The total solids and ash content of the duplicate samples were determined as described by Cioabla *et al*. (2012). The overnight dry weight of each sample was combusted at 550°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*).*

Determination of Total Nitrogen: The nitrogen content of the feed stocks was determined based on the method described by Dioha*et al.* (2013).

***Extraction of Nitrate*:** Nitrate was extracted from 1.0 g 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.

Calculation of carbon-nitrogen (C: N) ratio

The carbon-nitrogen ratios of rice husk, poultry feather and the 50% combinations of rice husk and poultry feather samples were calculated based on the formular:

% Carbon / % Nitrogen

Enzyme Test of the Isolates

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% $MgSO_47H_2O$, 0.25% $(NH_4)_2SO4$ and 0.2% gelatin per 100ml of distilled water at PH 7.0 using streak plate technique and incubated at 37°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°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 the organism to sugars but appearance of blue-black colour indicates presence of xylan and absence of xylanase.

***Test for Protease*:** The isolate was inoculated on skimmed milk agar plate and incubated at 37°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°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°C for 24 hours. Appearance of clear zones around the colonies indicates presence of lipase enzyme.

Anaerobic Digestion of Rice Husk and Poultry Feather

The feed stocks were hydrothermally treated at 100°C under steam pressure, dried at 80°C and milled to powder (10 µm). Anaerobic digestion of the rice husk and chicken feather was carried out using 0.5kg rice husk and 0.5kg chicken feather applying feedstock to water ratio of 1:7. Digestion was carried out under mesophilic condition (35-40°C) using 5% microbial consortium. Anaerobic digestion lasted for 38 days in a 20-litre metallic digester.

Measurement of Gas Volume and Purification of Biogas
The volume of gas generated from both the single and combined substrate was determined by the downward displacement of water technique. The gas on exit from the digester was purified by silica gel, potassium hydroxide and activated carbon in three different compartments.

Compositional Analysis of Biogas in the Gas Stream

The presence of methane, carbon dioxide and hydrogen sulphide, water vapour and oxygen in the gas stream produced at mesophillic (35°C) temperature was detected and quantified by infrared-sensory absorption technique using Crowcon GAS-PRO IR gas analyzer (Crowcon Instruments Ltd, Oxfordshire, United Kingdom). The biogas stream was connected to the equipment through an inlet hose and when the equipment was switched-on, the presence of methane and other gases was signaled by an alarming sound followed by the display of the concentrations of methane, carbon dioxide, hydrogen sulphide, water vapour and oxgen in the gas stream which were recorded.

Principle of Infrared Absorption
When an infrared source illuminates a volume of gas that enters the measurement chamber of the equipment, the gas absorbs some of the infrared wave lengths as the light passes through it. The amount of the absorbed light is proportional to the concentration of the gas in the gas stream which is measured by a set of optical detectors. The detectors quantify the concentration of the different gases and send signals to the microprocessor which computes and reports the concentration of the component gases in percentage.

Statistical Analysis of Data
The results generated on the volume of methane produced from the anaerobic digestion of rice husk and poultry feather as single and combined substrates at mesophilic condition were subjected to one-way analysis of variance (Gelman, 2005) and Duncan`s multiple range test (Post Hoc multiple comparison) and regression analysis to determine the significant differences in methane yield from single and combined substrate at 95% (P≤0) confidence level.

Results and Discussion
The need to exploit the economic potentials of wastes generated from the agro-allied sectors under the waste conversion to wealth initiative cannot be over emphasized. Lignocellulosic and keratinolytic wastes such as rice husk and poultry feathers have economic value if properly harnessed. The result of the proximate analysis of the complex media sources showed that rice husk had the volatile solid content and carbon-nitrogen ratio (C/N) of 68.50% and 51:1 respectively while poultry feather had volatile solids and carbon-nitrogen ratio of 96.50% and 4:1. Anaerobic digestion of equal weight of rice husk and poultry feather slurry using microbial consortia from cow dung at feedstock to water ratio of 1:7 under mesophilic condition (35ºC) lasted for 38 days. The major bacterial consortia used for anaerobic digestion from cow dung were *Bacillus, Cellulomonas, Pseudomonas* species while the methanogens were *Methanosarcina* and *Methanobrevibacter* spp.

The result of methane production from the co-digestion of the two substrates was 30.72 cm^3/g VS at 35ºC and hydraulic retention time of 38 days and pH of 7.30. The result of compositional analysis of biogas stream by infrared-absorption technique showed that methane and carbon dioxide had average value of 72% and 27% using consortium 4 followed by consortium 3 respectively. This result is in agreement with the reports of Fayyaz et al. (2014) that methane and carbon dioxide were the major gaseous constituents of biogas.

The result of solid digestate analysis after anaerobic digestion showed that the digestate was rich in micronutrients essentially, phosphorus (0.16g/l), Nitrogen (2.52 g/l), calcium (0.93 g/l), potassium (0.37 g/l) and Magnesium 0.78 g/l). The liquid digestate had pH of 7.30 and total solids content of 16.50%. The improvement in methane yield using the two substrates at 50% ratio was in agreement with the reports of Forgacs *et al*. (2013); Aniaku *et al*. (2018) that moderate quantity of nitrogen in feedstocks is essential element for the synthesis of amino acids for microbial growth and when in excess is converted to ammonia which as a strong base neutralizes the volatile acids produced by anaerobic bacteria and thus maintains neutral pH for optimum activities of methanogens for enhanced yield of biogas.

The available results from the characterization of the liquid digestate obtained after co-digestion of rice husk and poultry feather has shown that it can be used as a biofertilizer. The results from digestate analysis are in agreement with the reports of Ezemagu *et al*. (2021); Vaneeckhaute *et al*. (2013); Bernal et al., (2009) that rice husk when co-digested with poultry feather can yield measurable quantity of micronutrients needed for plant growth.

Conclusion and Recommendation

The concentration of Zn, Cr, Cu, Cd, K and P shows that the digestate can be used for agricultural purposes as biofertilizer, hence, converting waste to wealth. The use of digestate as biofertilizer is viable and sustainable in remedying the challenge of digestate discharged directly into the environment. The use of digestate as fertilizer for enriching the soil for agricultural purposes should be encouraged as the digestates are rich in bioactive agents and mineral elements which help in conditioning the soil. The solid digestate can be dried and used as fodder for fishes and farm animals. The use of digestate as soil fertilizer and conditioner is harmless to both humans and animals unlike chemical fertilizer which leaves residues in the plants that had been claimed to cause cancer in humans and animals.

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APPENDIX I

Fig. 1: Measurement of Methane Volume by Downward Displacement of Water Technique

Table 1: Proximate Composition of Sawdust and Chicken Feather

Feedstock	*%Moisture*	*%Dry matter*	*%ASH*	*%Volatile solids*	*%Carbon*	*%Nitrogen*	*C:N ratio*
Rice husk	6.2±0.06	72.3±0.05	1.92±0.2	68.50±0.2	42.80±0.1	0.84±0.015	51:1
poultry feather	2.56±0.09	96.60±.0.04	1.560±0.095	96.50±0.5	56.50±0.5	14.50 ±0.05	4:1

APPENDIX II

Table 2: Results of The Morphological and Biochemical Characterization of Isolates

Isolate	*Morphology Characteristics*	*Cat*	*MR*	*Cit*	*Gluc*	*Sucr*	*Lact*	*Ind*	*Vp*	*TSI*
Bacillus stearothermophilus	Gram+ve motile bacillus (Firmicutes) Facultative	+ve	+ve	+ve	+ve	+ve	+ve	-ve	+ve	+ve
Cellulomonas flavigena	Gram+verod, (Actinobacterium)	+ve	+ve	+ve	+ve	-ve	-ve	-ve	+ve	+ve
Bulkhoderia fungorum	Gram-ve,motile bacillus(proteobacterium)	+ve	+ve	+ve	+ve	+ve	-ve	-ve	+ve	+ve
Bacillus licheniformis	Gram+ve, spore-forming bacillus(Firmicutes)	+ve	+v	+ve	+ve	+ve	+ve	-ve	+ve	+ve
Pseudomonas aeruginosa	Gram–ve rod, motile gamma proteobacterium	+ve	+ve	+ve	+ve	+ve	+ve	-ve	+ve	+ve
Streptococcus salivarius	Gram +ve ovoid-shaped, appear in chains,non-motile facultative anaerobe (Firmicutes)	-ve	+ve	-ve	+ve	+ve	+ve	-ve	+ve	+ve
Methanosarcina thermophilla (aceto	Green and purple colonies, gram+ve cocci in sarcina arrangement	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve
Methanobrevibacter smithii (hydrogenotr)	Gram +ve fat, ovoid bacillus	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve

Table 3: Biomethane Yield from Single Substrates and Equal Ratio of Rice Husk and Poultry Feather

Sample	***Biogas yield (cm^3/g)***	***Hydraulic c retention time (days)***
50% rice husk and 50% Poultry feather	30.72	38
Poultry feather	14.67	45
Rice husk	18.50	43

Table 4: Percentage of Methane and Gaseous Impurities in the Gas Stream Produced from Equal Ratio of Rice Husk and Poultry Feather at 35°C

Consortium	***%CH_4***	***%CO_2***	***H_2S(ppm)***	***%H_20***	***%O_2***
1	69.80	29.80	720	0.14	0.06
2	71.26	28.20	744	0.09	0.05
3	71.83	27.40	712	0.07	0.08
4	73.94	26.12	583	0.05	0.06

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APPENDIX III

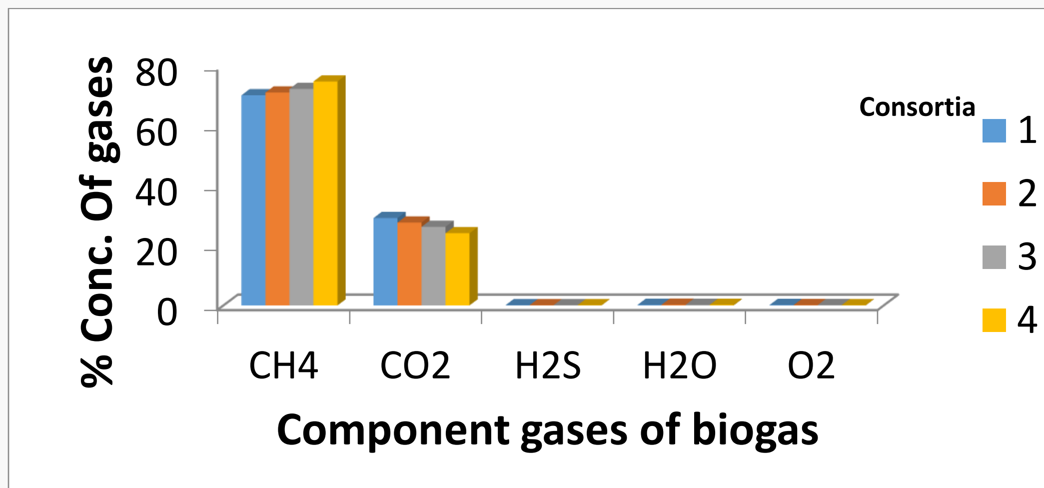

Table 3: Results of the Physicochemical Analysis of Digestate

Parameter	***Result***
pH	7.30
Electrical conductivity (dsm^{-1})	0.4
Total solids g/l	18.2
P g/l	0.16
Nitrogen g/l	2.52
Al g/l	9.80
Ca g/l	0.93
Mg g/l	0.78
Na mg/l	0.32
K g/l	0.37
Mn g/l	0.60
Zn mg/l	145.3
Cu mg/l	90.3
Fe g/l	1.60