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Biogas Production from Co-Digestion of a Cellulosic Feedstock and Piggery Manure

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ABSTRACT

Nigeria has energy crisis, hence, there is urgent need to diversify the country's energy resources towards the renewable alternatives for economic and social transformation. Biogas generated from the anaerobic digestion of local biomass resources, will increase national energy security, reduce dependence on fossil fuels and as well mitigate the environmental and ecological impacts of the organic wastes polluting our environment. The methane-producing potentials of rice husk co-digested with piggery manure and also using them as single substrate each under anaerobic condition at 35-40oC were studied. The results of the molecular identification of the microorganisms used for the studies based on 16S rRNA gene sequences and biochemical characterization showed that Bacillus, Cellulomonas and Pseudomonas species were the dominant bacterial group while the methanogenic archae were Methanosarcina and Methanobrevibacter species. The result of the proximate composition of rice husk showed that the volatile solids, Carbon, nitrogen, ash content and carbon-nitrogen ratio were 68.5%, 42.5%, 0.84%, 19.20% and 51:1 respectively. Five hundred (500) gram of rice husk was pretreated by steaming at 120oC for 30 minutes, dried at 80oC and ground into powder with a disc mill prior to anaerobic digestion. The result of co-digestion of rice husk with piggery manure at 50% ratio showed that the methane yield was 30.50 cm3/g VS at hydraulic retention time of 35 days while the methane yield using rice husk and piggery manure as single substrate each were 18.4 cm3/g VS and 24.4 cm3g VS at hydraulic retention time of 45 and 38 days respectively. The results of the studies have shown that co-digestion of rice husk and piggery manure has higher biogas yield than using them as single substrates. The one-way analysis of variance of biogas yield results showed that there was significant difference between the biogas yield obtained from co-digestion and that obtained from using both substrates as single substrates at 95% confidence level.

Keywords: Biogas, Co-digestion, Rice husk, Piggery manure, Methanogens

1. Introduction

Biogas is renewable fuel produced through the anaerobic digestion of organic substrates by a consortium of microorganisms (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%, traces of siloxanes may also be found (Zhao *et al.* 2010).

Biomethane is a colourless and odourless gas that burns with a clear blue flame and has autoignition temperature of 650 to 750^oC, 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 (Appels *et 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. 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 biomass (rice husk and other 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 maize straw, sawdust 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).

Rice husks 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, air and water pollutions. The volume of rice husk in Nigeria has continued to increase due to the current rise in rice production to meet up the growing demands for local rice which is considered cheaper and more nutritious than imported rice. Africa is yet to exploit fully the potentials of recycling wastes especially plant residues. Rice husk in Africa is considered as waste and therefore is indiscriminately incinerated making significant contributions to the greenhouse gas emissions with the concomitant effects on global warming and climate change. Rice husk has the potentials of being transformed into biomass energy essentially, methane for domestic and industrial uses. Rice husk 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.

Common products from rice husks are solid fuel (loose forms, briquettes and pellets), carbonized rice husk and furfural. The loose form of rice husk is used mostly for energy production such as combustion and gasification. Gasification is the process of converting biomass materials to syngas in a gasifier reactor with a controlled amount of air. Syngas can be used as fuel for drying and cooking or in a cogeneration system to produce electricity. Densified rice husk (briquettes and pellets) are mainly used in industrial boilers as a substitute for fossil fuel. The high silica content of rice husk ash makes it a good additive for the steel and concrete industries. To a lesser degree, rice husk ash is used as soil conditioner, activated carbon and insulator. More recently, creation of electrical power on a small to medium scale up to 5 megawatts using rice husk has been piloted throughout Asia, with some promising approaches but also some demonstrated limits (Jeng *et al*. 2012).

Vivekanandan and Kamaraj, (2011) studied anaerobic digestion of rice crop residues: rice chaff, rice straw and rice husk as co-substrate with cow dung. The cumulative gas production obtained from the three substrates was 3.8m^3, 3.4m^3 and 1.5m3 respectively. The low performance of cow dung and rice husk was attributed to the high lignin content which contained unfavourable non-lignin carbon to nitrogen ratio (70:1).

According to Madej, (2014), pig manure alone is a poor substrate for biogas production, because of its excessive nitrogen content relative to available organic carbon. In addition high nitrogen content may result in toxic level of

ammonia. Thus, additional substrates with high organic carbon must be added. The most promising is straw, which is available from adjacent biogas plant cultures. However, the abundant lignocellulosic biomass of wheat straw undergoes slow decomposition, and only a fraction of the chemical energy can be converted into biogas. Increased lignocellulosic biomass conversion may be achieved by pretreatment methods such as liquid hot water (LHW) and steam explosion (SE).

Different thermal, chemical and biological pretreatment methods have been reported to enhance anaerobic digestion of lignocellulosic biomass to increase methane yield (Zhong *et al.* (2011); Jorgensen *et al*. (2007); Aliyu and MdZahangir, (2016)). Pretreatment of feedstocks can increase solubilization, biogas production and volatile solids reduction (Tiehm *et al.* 2001). Pretreatment 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 pretreatment method is to make the available nutrients accessible to most microbial species which speed up biomass utilization during anaerobic digestion process (Patil *et al*. 2016). The use of pretreatments is particularly useful in the digestion of biomass feedstocks, as these tend to be high in cellulose or lignin. Pretreatment can break down these recalcitrant polymers physically, chemically and sbiologically.

Nigeria has energy crisis, hence, there is urgent need to diversify the country`s energy resources towards renewable alternatives for economic and social transformation. Biogas generated from the anaerobic digestion of local biomass resources such as rice husk and other plant residues, will increase national energy security as well as reduce dependence on fossil fuels.

2. Materials and Methods

2.1 Source of Materials

The rice husk used for the research work was collected from a rice mill at Ugboka community of Nkanu, West, local Government, Area of Enugu State while the piggery manure was collected from ALPHA Farms LTD. Emene, Enugu.

The equipment, reagents and media used for the proximate, biochemical and microbiological analysis were provided by Applied Microbiology Department, Enugu State University of Science and Technology, Enugu, Nigeria.

2.2 Experimental Design

2.2.1 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 Gopinath *et al.* (2014); Pandian *et al*. (2012); and Mezes *et al*. (2015).

Molecular identification of microorganisms was carried out based on the method of Stephen et al. (1997). The 16S rRNA target region of the organism 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`).

Isolation of Fungi

0.1 gram of white rot fungus-infected rice husk 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.

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 *Methanobrevibacter* spp 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.

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

Gram Staining

The bacterial isolates were Gram`s stained adopting the methods of Sharma, (2007) using the following reagents: Crystal violet, Lugol`s iodine, Absolute alcohol and dilute Carbofuchsin

Microscopy of stained cells:

The stained cells were examined using Olympus Light Microscope. The bacterial cells were viewed using oil immersion objective lens (×100). The morphology and Gram`s reaction of the cells were recorded in the table of results.

2.2.2 Morphological and Biochemical Characterization

The morphological characters of the isolates determined were Gram reaction, Shapes and Motility while the biochemical characters were sugar fermentation, citrate, methyl red, Vouges proskauer, 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 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.

One loopful of the culture was inoculated into 5ml glucose phosphate broth and incubated at 37°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. 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% $MgSO_47H_2O$, 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°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 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°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.

4. Proximate Analysis of Rice Husk

4.1 Determination of the Moisture Content

The moisture content of rice husk duplicate sample 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 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

4.2 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-Loh *et 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.

4.3 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^{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*).*

4.4 Determination of Total Nitrogen

The nitrogen content of the rice husk duplicate-samples were determined based on the method described by Dioha *et 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.

Hydrothermal Pretreatment
500 gram of dry rice husk and piggery manure was wetted with 1,000 ml of water and wrapped with aluminium foil. The wrapped sample was placed in autoclave and steam-heated at 100^{o}C for 30 minutes at a pressure of 15 PSI to soften the β-glycosidic and ether-ester bonds of the lignocellulose. The steam-treated sample was dried at 80^{o}C for 12 hours, cooled and milled to a particle size of 0.50 mm prior to anaerobic digestion.

Biological Pretreatment Of Rice Husk:

Thirty (30) ml broth culture of *Phanerochaete species* was added into 3,500ml of feedstock solution containing 500 gram of rice husk powder and piggery manure in 5-litre glass digester and subjected to submerged fermentation at 35^{o}C for 72hours in a shaker incubator for the extracellular hydrolysis of lignocelluloses. *Phanerochaete species* is a cellulase-producing fungus which attacks 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.

Preparation of the Consortium as inoculum for A.D Process

Mixed cultures of the hydrolytic, acidogenic and methanogenic bacteria were prepared by inoculating each of the isolate into 1000 ml of 2% yeast extract broth to form the consortium for anaerobic digestion.

4.5 Anaerobic Digestion

Each batch of organic slurry (3,500 ml) containing 250 gram of pretreated rice husk powder and 250 gram of piggery manure and 3% (84 ml) consortium was subjected to anaerobic digestion to produce biogas at mesophillic condition (37-40^{o}C).

Anaerobic digestion was equally carried out on 500 gram of Single substrate (rice or piggery manure).The hydraulic retention time and pH for gas production was noted for each batch of feedstock sample.

4.6 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 for each batch of organic slurry sample.

4.7 Evaluation of the Methane-producing potential of the feedstock samples

The methane-producing potentials of each batch of organic slurry sample were evaluated based on the one-way analysis

5. Results and Discussion

The methane-producing potentials of rice husk co-digested with piggery manure under anaerobic condition at 35-40^{o}C were studied.

The result of the biochemical characterization of the bacterial isolates showed that the dominant species were *Cellulomonas*, *Bacillus* and *Pseudomonas* species while the dorminant methanogenic *archaea* were *Methanosarcina* and *Methanobrevibacter*. *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.

Methanosarcina spp. is acetoclastic methanogen which is a key player in anaerobic conversion of acetate to methane while *Methanobrevibacter* spp. is a hydrogentrophic methanogen capable of converting hydrogen to methane in presence of carbon dioxide.

The percentage nitrogen of 0.84% 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 51:1 shows that rice husk has too high carbon but low nitrogen. There should be a balance in carbon and nitrogen content of a substrate intended for anaerobic digestion for improved yield of biogas as reported by Yadvika *et al*. (2003); Dana, (2010) and Igoni *et 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.

The pig manure has nitrogen content of 3.72% which is too high to be used as single substrate hence the low yield of biogas.
The same thing applies to rice husk which has high carbon content and could not yield enough biogas when used as single substrate.

The comparative result of co-digestion of rice husk with piggery manure and using the feedstock as single substrates have shown that co-digestion had higher methane yield than using them as single substrate. The methane yield from piggery manure was higher than the yield from the rice husk. This could be attributed to moderate nitrogen, carbon and mineral content of piggery manure. The higher result of methane yield from co-digestion of the two substrates was in agreement with the report of Lehtomaki et al. (2007) and Dana (2010) that co-digestion of high carbon substrate with a moderate nitrogen feedstock provides buffering capacity and balances the C/N ratio of the blend thereby reducing the risk of ammonia inhibition in the anaerobic digestion process and enhancing the yield of biogas.

Pretreatment of feed stocks prior to anaerobic digestion such as hydrothermal and chemical treatment 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 pretreatment method is to make the available nutrients accessible to most microbial species which speed up biomass utilization during anaerobic digestion process (Patil *et al*. 2016). The use of pretreatments is particularly useful in the digestion of biomass feedstocks, as these tend to be high in cellulose or lignin. Pretreatment can break down these recalcitrant polymers physically, chemically and biologically.

Conclusively, the results so far have shown that co-digestion of rice husk and piggery manure has better potential for biogas production than using them as single substrates.

Recycling of rice husk and piggery and other wastes littering our environment through AD process will help to mitigate their environmental and ecological impacts and as well provides a renewable energy resource for domestic and industrial purposes.
As lignocellulosic substrates, rice husk should be pretreated prior to anaerobic digestion to disrupt the β-glycosidic and ether-ester linkages of lignocellulose for easy hydrolysis and metabolism by microbial enzymes during AD to enhance the yield of methane.
Nigeria should as a matter of urgency create enabling laws for the adoption of the “waste to wealth” policy like other countries of the world as this will help to sanitize the environment, create jobs, reduce pollution and provide a clean, renewable and environmentally-friendly fuel.

The use of viable bacterial consortia in form of powder or broth will make it possible to produce biogas from any organic substrate and as well reduce the unhygienic problems often associated with animal manure or sewage as sources of inoculum for AD process.

Table 5.1: Biochemical Test of the Isolates

Isolate	*Amylase*	*Cellulase*	*Protease*	*Xylanase*	*Lipase*	*Morphology*
Bacillus *spp*	++	++	++	++	Trace	Gram`s + rods
Burkholderia *spp*	++	++	+	++	+	Gram`s + rods
Cellulomonas *spp*	++	++	+	++	+	Gram`s +
Phanerochaete *spp.*	++	+++	+	++	+	Whitish crust fungus
Methanobrevibacter *spp*	+	+	+	+	+	Gram`s + rods
Methanosarcina *spp*	++	+	+	+	+	Gram`s + cocci in sarcina form
Pseudomonas *spp.*	++	+	++	+	+	Gram`s negative tiny rods

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

Table 5.2: Proximate Composition of Pig Manure and Rice husk

Parameter	Pig manure	Rice husk
%Moisture	12.0	6.2±0.06
%Dry matter	20.25	72.3±0.05
%Ash	17.20	19.20±0.05
%Volatile solids	81.20	68.5±0.20
%Carbon	40.40	42.8±0.10
%Nitrogen	3.72	0.84±.015
C:N ratio	11:1	51:1

Table 5.3: Comparative results of methane yield (cm^3/g VS) from co-digested substrates, Rice husk and Piggery manure using mixed cultures at mesophillic temperature (37-40^oC)

Feed stock sample	Methane yield (cm^3/g VS)	Hydrolytic retention time (in days)
Co-digested substrate	30.5	35
Rice husk	18.4	45
Piggery manure	24.4	38

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