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Microbial Dynamics and Biogas Production during Single and Co-digestion of Cow Dung and Rice Husk

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Abstract

Anaerobic digestion is achieved by the combined effort of hydrolytic, acetogenic and methanogenic bacteria. Microbial dynamics and biogas production during anaerobic digestion of cow dung and rice husk were studied in this research. The experiment lasted for 30 days using a 10 L scale bio-digester. All proximate parameters reduced significantly after digestion for CD (cow dung), RH (rice husk), and CD:RH (cow dung and rice husk) except moisture content, which increased for all substrates. Ash content (1.08-1.67 mg) and crude fibre (1.27-1.96 mg) increased in CD only. The pH ranges for the substrates were CD (7.0-7.5), RH (6.1-7.6), and CD:RH (6.1-7.8). Temperature ranges were CD (27.4 ^{o}C-33.5 ^{o}C), RH (27.2 ^{o}C-33.3 ^{o}C) and CD:RH (27.3 ^{o}C-33.4 ^{o}C). The total biogas production of the substrates and components of each gas produced were, CD (4327.65 cm^3 : 62.4 % CH_4, 37.4 % CO_2, 0.2 % H_2S), RH (150 cm^3 : 100 % CO_2), and CD:RH (4730.55 cm^3 : 73.8 % CH_4, 25.8 % CO_2, 0.4 % H_2S). Percentage distribution of the digester's microflora include aerobes (40.75 %), anaerobes (31.25 %), fungi (25 %) and methanogenic bacteria (3 %). Hydrolytic bacteria and fungi isolated were *Bacillus spp, Enterobacter spp, Pseudomonas spp, Proteus spp, Micrococcus spp, Aspergillus spp, Penicillium spp* and *Streptococcus spp.* Acetogens isolated were *Clostridium spp, Streptococcus spp* and *Pseudomonas spp. Methanococcus spp* and *Methanobacterium spp* were the only isolated methanogens. Rice husk produced the least amount of biogas.

Keywords: Biogas; Cow dung; Rice husk; Hydrolytic bacteria; Acetogens; Methanogens

Introduction

Industrialization, urbanization and population growth give rise to increasing energy demand. Fossil fuels, a non-renewable source of energy is the major source of the world's energy and contributes to climate change. Hence there is an urgent need to find alternative and environmentally friendly energy sources. Guruswamy et al. [1] and Alvarez et al. [2] identified two challenges facing humanity in the 21st century. First, the development and use of renewable energy to decrease our over-dependence on fossil fuels, and second, the management of the waste generated by human activities. According to Nagamiani and Ramasamy and Adeyanju, achieving the Millennium Development Goals (MDGs) in Africa requires a significant expansion of access to modern and alternative renewable energy such as biogas which is of growing interest for the sustainable management of our waste and a major breakthrough in the search for renewable energy [3-4].

Biogas technology is an attractive alternative energy source. Its production from biomass has been identified and found to be environmentally friendly and renewable. Agriculture is a major source of revenue for the Federal Government of Nigeria. Agricultural production is generates organic waste (either as crop residues after harvesting crops, or as manure during livestock production). Northern Nigerian farmers are known for their interest in cattle rearing and rice production. Rice husk is mostly milled to reduce its bulk. In most cases it is burnt, contributing in a major way to environmental pollution by increasing the concentration of carbon monoxide (CO) and particulates in the atmosphere. Environmental pollution arises from offensive odours associated with cow dung and littering of roads with cow dung (an environmental concern) when nomads travel from one geographical area to another, as well as pollution of waterways.

Ezeonu et al. defined biogas to represent a mixture of different gases produced as a result of the action of anaerobic microorganisms on domestic and agricultural waste [5]. Various researchers have co-digested animal waste with plant waste. The biogas system operates via anaerobic digestion. Anaerobic biogas digesters are constructed to hold the waste. Biogas consists of 50-70 % methane, 30-40 % carbon dioxide, 5-10 % hydrogen, 1-2 % nitrogen, 0-3 % water vapour and traces of hydrogen sulphide, carbon monoxide and oxygen. It is colourless, relatively odourless and flammable [4, 6].

The current research explores a productive way to use organic waste to generate biogas. Cow dung, which constitutes a serious environmental threat, is an excellent substrate for biogas production, because it contains the necessary microorganisms (acid formers and methane formers) for biogas production. Rice husk has also been reported to contribute to environmental pollution. The option of using rice husk and cow dung will solve the following problems of reduction in environmental pollution, reduced dependence on fossil fuels and provision of a cheap, affordable and natural source of renewable fuel.

A working knowledge of the successional pattern and abundance of hydrolytic, acetogenic and methanogenic bacteria involved in the biogas process will enhance and improve quantity of biogas produced. Thus, microbial dynamics and biogas production during anaerobic digestion of cow dung and rice husk were studied in this research.

Materials and methods

1) Sample collection

Fresh cow dung was collected from COLANIM farm, Federal University of Agriculture, Abeokuta (FUNAAB), Ogun State, Nigeria. The sample was collected in a sterile polythene bag and transported within 24 h to the laboratory for sample analysis. Milled rice husk was obtained from the Ofada rice mill in Lafenwa Market, Abeokuta, Ogun Sate, Nigeria.

2) Bio-digester design and loading

Figure 1 shows a 10 L laboratory scale anaerobic bio-digester constructed for the research. The bio-digester was constructed using Karki's biogas model as a guide. It was designed with three openings: one for slurry inlet, the second serving as gas outlet while the third was the slurry outlet. The gas produced in the bio-digesters was collected into sterile tyre tubes.

Nine bio-digesters were constructed for the research. An equal slurry to water ratio was ensured in each bio-digester. Approximately 3 L of slurry was fed into the bio-digester along with 3 L of water while the remaining part of the bio-digester accounts for the gas space. A summary of the content of each bio-digester is given below:

- Bio-digester 1: 3 kg cow dung +3 L of water
- Bio-digester 2: 1.5 kg rice husk + 1.5 kg cow dung + 3 L of water
- Bio-digester 3: 3 kg of rice husk + 3 L of water

Three digesters were used for each treatment. The experiment was allowed to run for 30 d in continuous fermentation during and after which the following were recorded:

- The temperature of the bio-digester content and its pH recorded every 3 d.
- Proximate analysis of the bio-digester content before and after the termination of the experiment.
- Collection of samples at 3 d interval for microbial analysis.
- Volume of gas produced upon completion of the study.
- Separation of gas produced into its various components.

Figure 1 A 10 L scale bio-digester.

3) Isolation and assessment of bacterial population

Serial dilution of the wastes was performed by placing 1 g of each waste into a McCartney bottle containing 9 mL of sterile distilled water coupled with shaking to homogenise the suspension (10^{-1} dilution). Thereafter, 1 mL of aliquot from the 10^{-1} dilution was measured into another bottle containing 9 mL of sterile distilled water to obtain a 10^{-2} dilution. Further dilutions were carried out until a dilution level of 10^{-7} was reached. Samples were taken once every 3 d for total heterotrophic counts. For bacterial screening, dilutions 10^{-5} to 10^{-7} of the samples (upon serial dilution) were plated on starch agar, carboxymethyl cellulose agar, egg yolk agar, nutrient-gelatin agar (hydrolytic bacteria media), basal medium (acetogens growth medium), enriched medium and fastidious anaerobic agar (methanogens growth media). Plates were incubated for 24-48 h at 35 ^{o}C. Colony forming units per gram (CFU g^{-1}) of bacterial growth between 30-300 colonies were enumerated. The colonies formed were sub cultured and identified using cultural, morpho-logical, biochemical and molecular methods.

4) Isolation and assessment of fungal population

For screening of fungi, dilutions of 10^{-3} to 10^{-4} were plated on potato dextrose agar and Saboraud's dextrose agar, supplemented with 100 mg mL^{-1} streptomycin and 15 mg mL^{-1} of penicillin (to inhibit bacterial growth). The plates was incubated at 25 ^{o}C for 72 to 96 h. Total fungal counts were enumerated in CFU g^{-1}. The colonies formed were sub-cultured and identified using microscopic, colonial and molecular methods.

5) Characterization and identification of the isolates

Bacterial isolates were identified using standard biochemical tests with reference to Bergey's manual. The fungal isolates were identified based on cultural and morphological characterization with reference to de Hoog et al. and Ellis et al. [7-8]. Molecular characterisation by ribosomal DNA genes analysis (i.e 16S rRNA for bacteria and 18S rRNA for fungi) was also done using the method of Fowora [9].

6) Gas production analysis

A portable hand-held biogas analyser obtained from Beijing Shi'an Tech Instrument Co., Ltd was used to determine volume of gas produced and the percentage of constituents present in biogas.

7) Physico-chemical analyses of cow dung (CD), rice husk (RH) and their combination (CD:RH)

Physico-chemical parameters analyzed were organic carbon, moisture content, total solids, total nitrogen, ash content, carbon/nitrogen ratio, crude fibre, volatile solid, crude protein, crude ash, biochemical oxygen demand (BOD) and chemical oxygen demand (COD) using standard method as described by the 20th edition of Association of Analytical Chemists [10].

Results and discussion

Figures 2 and 3 show the total biogas yield and their constituents from substrates during anaerobic digestion. CD:RH had the highest gas production as shown in Figure 2, producing 4,730.55 cm^3 after 30 d of anaerobic digestion followed by CD with a volume of 4,327.65 cm^3, while RH produced the least amount of biogas after 30 d of anaerobic digestion with a volume of 100 cm^3. The constituents of each gas produced as shown in Figure 3 were CD (62.4 % CH_4, 37.4 % CO_2, 0.2 % H_2S), RH (100 % CO_2), and CD:RH (73.8 % CH_4, 25.8 % CO_2, 0.4 % H_2S).

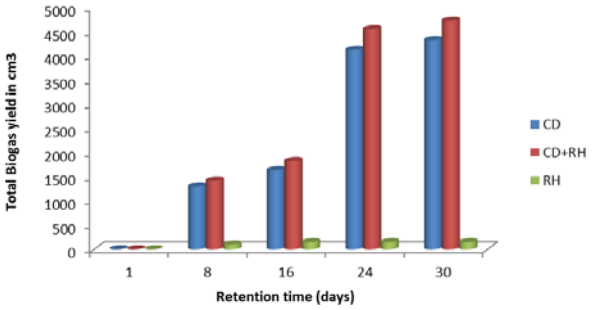

Figure 2 Total biogas produced by each waste at different days of anaerobic digestion.
Note: CD (cow dung), RH (Rice Husk), CD: RH (mixture of cow dung and rice husk)

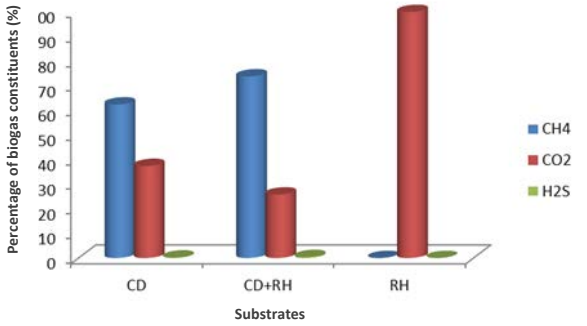

Figure 3 Percentage of biogas constituents produced by wastes after anaerobic digestion.
Note: CD (cow dung), RH (Rice Husk), CD:RH (mixture of cow dung and rice husk)

Figure 4 shows the variations in the temperature of the treatments with time. For all digesters, overall temperatures ranged from 27.2 ^{o}C to 33.5 ^{o}C. The highest overall temperature (33.5 ^{o}C) was recorded in CD at the 21st and 30th day of digestion while the lowest temperature (27.2 ^{o}C) was recorded in RH at the 18th day of

digestion. The overall pH range recorded in all the digesters was from 6.1 to 7.8. The lowest pH measurement (6.1) was recorded after the 27th day of digestion in CD:RH and 30th day of digestion in RH while the highest pH measurement (7.8) was recorded at the 1st day of digestion in CD: RH. The temperature of the digester (27.2 ^{o}C-33.5 ^{o}C) remained constant at mesophilic range. This was similar to that of Dahunsi and Oranusi [11] who reported a temperature range of 22.0 ºC-30.5 ºC [11]. Frequent rainfall during the research period was responsible for the non-steady and lowered temperature readings. However, Dahunsi and Oranusi reported that temperature seems not to have any significant effect on the amount of gas produced daily as daily gas generation tends not to follow a specific pattern and this is indicative of the fact that other parameters apart from temperature could be responsible for the quantity of biogas generated per day [11].

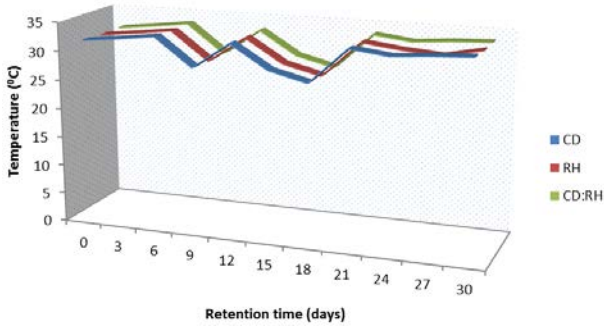

Figure 4 Temperature changes in the digester content during anaerobic digestion.
Note: CD (cow dung), RH (Rice Husk), CD:RH (mixture of cow dung and rice husk)

Figure 5 shows the variations in the pH of the treatments with time. The overall pH range recorded in all the digesters was 6.1 to 7.8. This agrees with reports by Karthikeyan and Farrel et al. who found that methano-genesis occurs best within a pH range of about 6-7.8 [12-13]. Acidic pH level recorded resulted from the activities of aerobes and facultative anaerobes relevant for the production of acidic metabolites and an important precursor for methane production. Acid production (acidogenesis) is an important biogas process responsible for the lowering of pH, thereby hindering growth of organisms unable to thrive at low pH. However, subsequent stages of anaerobic digestion leads to an increased pH as described by the Food and Agricultural Organisation [14].

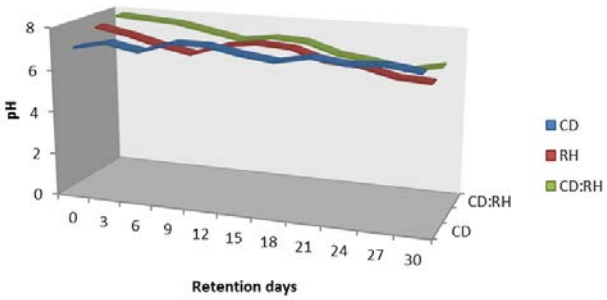

Figure 5 pH changes in the digester content during anaerobic digestion.
Note: CD (cow dung), RH (Rice Husk), CD:RH (mixture of cow dung and rice husk)

Figures 6, 7 and 8 show the variations of microbial counts with time. The total aerobic bacterial count had CD:RH with the highest count of 5.52 x 10^{8} CFU g^{-1} and CD with least count of 1.05 x 10^{8} CFU g^{-1}. The total anaerobic bacterial count ranged from 1.10 x 10^{8} CFU g^{-1} to 3.76 x 10^{8} CFU g^{-1} with CD: RH having the highest anaerobic count while RH had the least count. The total fungal count showed that CD: RH had the highest count of 4.73 x 10^{5} CFU g^{-1} while CD had the least count.

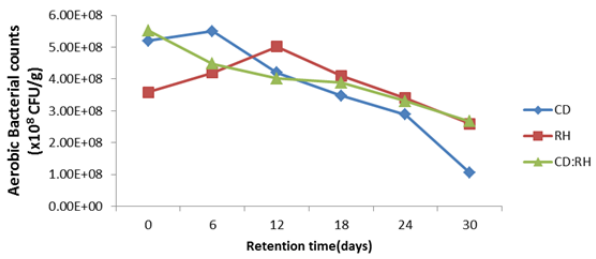

Figure 6 Variation in the aerobic bacterial counts of the treatments with time.
Note: CD (cow dung), RH (Rice Husk), CD:RH (mixture of cow dung and rice husk)

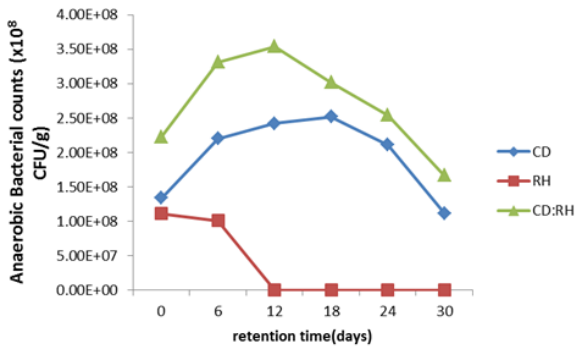

Figure 7 Variation in the anaerobic bacterial counts of the treatments with time.
Note: CD (cow dung), RH (Rice Husk), CD:RH (mixture of cow dung and rice husk)

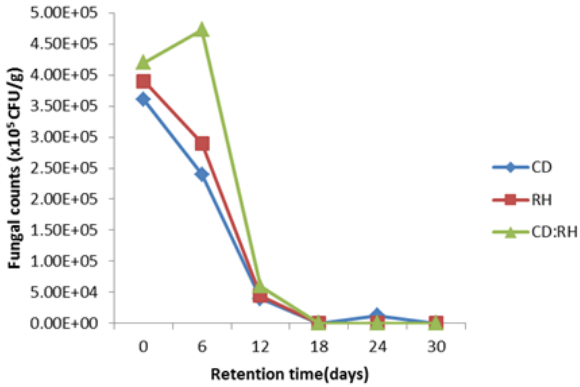

Figure 8 Variation in the fungal counts of the treatments with time.
Note: CD (cow dung), RH (Rice Husk), CD: RH (mixture of cow dung and rice husk)

Table 1 shows the result of the physico-chemical analysis of the substrate with reduction in nitrogen content, carbon content, carbon/ nitrogen ratio, ash content, crude fibre, crude protein, fat content, total solids, volatile solids, biochemical oxygen demand (BOD) and chemical oxygen demand (COD) upon anaerobic digestion except moisture content that increased in all the substrates. Ash content (1.08-1.67 mg) and crude fibre level (1.27-1.96 mg) increased in CD only. The reduction in BOD and COD agrees with the reports by Dahunsi and Oranusi indicating that anaerobic digestion is a potent method of reducing these parameters and pathogens from sludge or wastewater [11]. The carbon to nitrogen ratio (C:N) was also determined. An optimum C:N ratio of between 20:1 and 30:1 has been suggested in previous studies to be adequate for optimum gas production. According to Schnurer and Javis, if the C:N ratio is very high, nitrogen will be consumed rapidly by methanogens to meet their protein requirements and will no longer react on the leftover carbon content of the material, reducing gas production [15]. The reduction in total solids and volatile solids may be due to the utilisation of the waste by the microorganisms. This agrees with the reports of Oyeleke et al. who stated that, the total solids and volatile solids reduce as methane yield increases [16]. The study showed that co-digested CD:RH had the highest biogas production, followed by CD and RH only. RH produced the least amount of biogas. This is corroborated by Kalia et al. and Momoh who reported that the composition of biogas as well as biogas yields depend on the substrates owing to differences in material characterisation in each feedstock [17-18]. Hence, given the high cellulose and lignin content of RH, it is not surprising that it is resistant to enzymatic degradation, as explained by Iyagba [19].

Table 2 shows the isolated group of micro-organisms during 30 d of anaerobic digestion. Three groups of bacteria were isolated from the digester. These include hydrolytic bacteria such as *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Micrococcus luteus*, *Pseudomonas fluorescens*, *Peptostreptococcus spp*, *Pseudomonas putida*, *Bacillus mycoides*, *Bacillus licheniformis*; acetogenic bacteria such as *Streptococcus spp*, *Clostridium spp* and methanogenic bacteria such as *Methanobacterium spp* and *Methanococcus spp*. Hydrolytic bacteria convert organic polymers into monomers, acid forming bacteria convert monomeric hydrolysis products into low molecular weight fatty acids, acetate and simple organic compounds which are then converted by methane producing bacteria to biogas. Of the four groups of bacteria isolated, fungal presence

was only recorded in the hydrolytic stage where *Aspergillus spp, Mucor spp* and *Penicillium spp* were isolated. This agrees with Agunwamba who reported that bacteria are responsible for anaerobic digestion in the biogas production process [20]. Isolated hydrolytic bacteria where divided into four groups: cellulolytic, amylolytic, proteolytic and lipolytic bacteria. Cellulolytic microorganisms were identified as *Klebsiella spp, Pseudomonas spp, Micrococcus spp, Mucor spp, Aspergillus spp* and *Penicillium spp*. Identified amylase-producing bacteria were *Bacillus spp, Enterobacter spp* and *Pseudomonas spp*. This results corroborates the work of Mazzucoteli et al. who isolated *Bacillus, Serratia, Enterococcus*, Klebsiella, Stenotrophomonas, Lacto-coccus, and *Escherichia* genera as cellulose and amylase producing bacteria [21].

Isolated proteolytic bacteria include *Clostridium spp, Pseudomonas spp and Peptostreptococcus spp*. This is supported by the work of Ramsay and Pullammanappallil who claimed that the genera *Clostridium, Peptostreptococcus*, and *Bifidobacterium* are proteolytic bacteria [22]. *Bacillus spp, Pseudomonas spp, Micrococcus spp, and Proteus spp* were the identified lipolytic bacteria. Isolated *Methanobacterium spp* and *Methanococcus spp* (methane producing bacteria) is supported by the work of Dahunsi and Oranusi and Oyewole who isolated *Methanobacterium spp* and *Methanococcus spp* from human waste and chicken droppings, respectively [11-23].

Percentage distribution of digester microflora (aerobes 40.75 %, anaerobes 31.25 %, fungi 25 % and methanogenic bacteria 3 %) was found to be similar to Dahunsi and Oranusi who reported the percentage distribution of aerobic organisms as 40 % followed by anaerobic bacteria and fungi with 28 % and 24 %, respectively, while methanogenic bacteria were the least populated in the digester, representing only 8 % of the total microflora [11].

Table 1 Physico-chemical analysis of CD, RH and CD:RH during 30 d of anaerobic digestion

Parameter	CD		RH		CD:RH	
	Fresh slurry	Digested slurry	Fresh slurry	Digested slurry	Fresh slurry	Digested slurry
Nitrogen (%)	0.31	0.25	0.26	0.23	0.29	0.21
Carbon content (%)	7.79	5.81	6.29	5.16	8.22	5.55
Carbon/nitrogen	25.12	23.22	24.21	22.42	28.33	26.44
Ash (g $100g^{-1}$)	1.08	1.67	2.86	0.12	1.45	0.51
Moisture (g $100g^{-1}$)	80.09	94.00	28.20	99.45	72.60	97.79
Crude fibre (g $100g^{-1}$)	1.27	1.96	4.16	0.21	1.88	0.89
Crude protein (g $100g^{-1}$)	6.86	1.12	27.65	0.14	9.62	0.26
Volatile solid (%)	9.15	0.06	9.11	0.02	9.11	0.02
Total solid (g $100g^{-1}$)	19.91	6.00	71.90	0.58	27.40	2.03
Fat content (g $100g^{-1}$)	0.89	0.12	2.65	0.00	1.27	0.09
BOD (mg L^{-1})	20.54	11.25	18.92	10.36	20.38	11.16
COD (mg L^{-1})	7.30	4.01	7.10	3.89	6.79	3.72

Note: CD (cow dung), RH (Rice Husk), CD:RH (mixture of cow dung and rice husk)

Table 2 Isolated group of microorganisms during 30 days of anaerobic digestion

S/N	Hydrolytic microorganisms				Acetogenic bacteria	Methanogenic bacteria
	Cellulolytic	**Lipolytic**	**Proteolytic**	**Amylolytic**		
1	*Micrococcus luteus*	*Bacillus subtilis*	*Clostridium spp*	*Bacillus subtilis*	*Streptococcus spp*	*Methanobacterium spp*
2	*Pseudomonas aeruginosa*	*Pseudomonas aeruginosa*	*Pseudomonas aeruginosa*	*Enterobacter aerogene*	*Pseudomonas spp*	*Methanococcus spp*
3	*Klebsiella oxytoca*	*Micrococcus luteus*	*Peptostreptococcus spp*	*Pseudomonas aeruginosa*	*Clostridium spp*	
4	*Aspergillus spp*	*Proteus vulgaris*				
5	*Penicillium notatum*					
6	*Mucor spp*					

The five isolates (four bacteria and one fungus) screened were selected for Polymerase Chain Reaction (PCR) amplification, 16S ribosomal and 18S ribosomal sequencing. On the basis of the 16S rRNA gene sequence analysis, the four isolates were identified as *Pseudomonas aeruginosa PA1, Pseudomonas aeruginosa PA7 and Pseudomonas aeruginosa MTB-1* and *Methanococcoides methylutens.* While for 18S rRNA, the isolate identified was *Aspergillus niger.* The nucleotide sequences of the isolates were submitted to the Gen-Bank database and assigned accession numbers. The accession numbers of the bacterial isolates above were NC_022808.2, NC_009656.1, NC_023019.1 and KF999876, respectively. The fungal isolate *Aspergillus niger* had the accession number KF414527. The molecular relatedness of the selected bacteria and fungi is presented in Table 3.

Table 3 The similarity of DNA of sequences with sequences obtained from NCBI database Gene-bank

S/N	Identified organism	Identity (%)	Accession no.
1	*Pseudomonas aeruginosa PA1*	98	NC_022808.2
2	*Pseudomonas aeruginosa PA7*	95	NC_009656.1
3	*Pseudomonas aeruginosa MTB-1*	95	NC_023019.1
4	*Methanococcoides methylutens*	99	KF999876
5	*Aspergillus niger*	95	KF414527

Conclusion

Bacillus subtilis, Pseudomonas aeruginosa, Micrococcus luteus, Pseudomonas fluorescens, Peptostreptococcus spp, Pseudomonas putida, Bacillus mycoides and Bacillus licheniformis Micrococcus luteus, Pseudomonas aeruginosa, Bacillus cereus, Streptococcus spp, Clostridium spp, Methanococcus spp and *Methanobacteria spp* are the organisms involved in biogas production. Also, the research showed that the combination of cow dung and rice husk (CD: RH) is best for biogas production. Also, methanogens surfaced towards the end of the digestion process.

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