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CHARACTERIZATION OF BACTERIA ASSOCIATED WITH BIOGAS PRODUCTION USING COW DUNG AND RICE HUSKS

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Abstract

This study explored the production of biogas, a renewable energy source, through anaerobic digestion of rice husk (RH) and cow dung (CD). The research used 10-litre bio-digesters over a 30-day period to examine the process. A mixture of three litres of slurry and water was added to the digesters, and temperature and pH levels were monitored every three days. Samples from the digesters were taken to assess bacterial growth, and bacterial species were identified using standard microbiological methods. Additionally, the plasmid profiles of the isolated bacteria were determined, while a proximate analysis of the digester contents was performed before and after digestion. The temperature within the digesters ranged from 24.1°C to 36.7°C, while the pH varied between 5.5 and 8.0. The bacterial count increased over time in CD and the CD mixture, while it decreased in RH alone. Eight bacterial species were isolated, including Proteus vulgaris and Escherichia coli, with plasmids detected in four species, indicating their genetic potential for effective biogas production. The co-digestion of RH and CD yielded the most biogas (5200 cm³), followed by CD alone (4500 cm³), while RH produced the least (110 cm³). The gas from the CD mixture contained 83.2% methane (CH4), whereas RH alone produced only carbon dioxide (CO2). The findings suggest that combining cow dung with rice husk is an efficient, low-cost method for generating biogas, providing a solution to both waste management and renewable energy production.

Keywords: Biogas, Bacteria, bio digester, Cow dung, Rice husk, PH, Temperature

Introduction

Background to the study

Rice husks are the hard protecting coverings of grains of rice. In addition to protecting rice during the growing season, rice hulls can be put to use as building material, fertilizer, insulation material, or fuel (Wallheimer, 2010). Rice husk is an agricultural waste which remains after the processing

of the crop (Iyagba et al., 2009).Rice husk is hard to eat or swallow and mostly indigestible to humans because of its enriched fibre components (Wallheimer, 2010).

Rice husks can be transformed either by chemical and/or biological means (Vigil et al., 2003). The biological process may be accomplished either aerobically or anaerobically, depending on the availability of oxygen (Iyagba et al., 2009). Due to high content of cellulose, rice husk can be considered as source of biogas substrate after pretreatment of lignin removal. Lignin content can inhibit the production of biogas because microorganisms are hard to degrade the rice husks (Hashfi et al., 2018).

Cow dung can be defined as the undigested residue of consumed food material being excreted by herbivorous bovine animal species. Being a mixture of faeces and urine in the ratio of 3:1, it mainly consists of lignin, cellulose and hemicelluloses (Garg & Mudgal,2007). Cow dung also contains 24 different minerals like nitrogen, potassium, along with trace amounts of sulphur, iron, magnesium, copper, cobalt and manganese (Garg and Mudgal 2007; Randhawa and Kullar, 2011). Cow dung harbours a rich microbial diversity, containing different species of microorganisms (Bacillus species, Corynebacterium species and Lactobacillus species), protozoa and yeast (Saccharomyces and Candida) (Nene,1999; Randhawa & Kullar, 2011).

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 (Akintokun et al., 2017). Guruswamy et al. (2003) and Alvarez et al. (2010) identified two challenges facing humanity in the 21st century. First, the development and use of renewable energy to decrease overdependence on fossil fuels, and second, the management of the waste generated by human activities. According to Nagamiani and Ramasamy (2003) and Adeyanju (2008), 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 waste and a major breakthrough in the search for renewable energy.

Biogas is a term used to represent a mixture of different gases produced as a result of the action of anaerobic microorganisms on domestic and agricultural waste (Mclnerney & Bryant, 1981; Ezeonu et al., 2005). It usually contains 50% and above in methane and other gasesin relatively low proportions namely, carbon dioxide, hydrogen, nitrogen and oxygen (Milono et al., 1981; Kalia et al., 2000). The mixture of the gases is combustible if the methane content is more than 50% (Agunwamba, 2001). Biogas production involves three steps: (i). Hydrolysis: which converts organic polymers into monomers (with the help of hydrolytic bacteria). (ii). Acid formation: Which involves conversion of monomers into simple compounds such as acetic acid, propionic acid, CO2, NH3 and H2, using a group of acid forming bacteria (acetogenic bacteria). (iii). Methane formation: Involving conversion of simple compounds into methane CH4 and CO2, utilizing anaerobic methanogenic bacteria.

Co-digestion is the simultaneous digestion of more than one type of waste in the same unit (Agunwamba, 2001). Advantages of co-digestion include better digestibility, enhanced biogas production/methane yield arising from availability of additional nutrients, as well as a more efficient utilization of equipment and cost sharing (Agunwamba, 2001; Mshandete & Parawira, 2009; Parawira et al., 2004). Studies have shown that co-digestion of several substrates, for example, banana and plantain peels, spent grains and rice husk, pig waste and cassava peels, sewage and brewery sludge, among many others, have resulted in improved methane yield by as much as 60% compared to that obtained from single substrates (Ezekoye & Okeke, 2006; Ilori et al., 2007; Adeyanju, 2008; Babel et al., 2009). Results of co-digestion of food waste and dairy

manure in a two-phase digestion system conducted at laboratory scale showed that the gas production rate (GPR) of co-digestion was enhanced by 0.8 - 5.5 times as compared to the digestion with dairy manure alone (El-Mashad & Zhang, 2007).

Aim and Objectives of the Study

Characterization of bacteria involved in biogas production using cow dung and rice husks.
The specific objectives of this study were to:
1. Construct a 10 litre digester for the production of biogas from rice husks and cow dung.
2. Determine the total bacterial count of all the treatments (rice husk, cow dung and rice husk: cow dung) at the peak of the anaerobic digestion.
3. Isolate and identify bacteria species from thrice husks, cow dung and their combination (rice husk and cow dung) after the anaerobic digestion.
4. Carryout the physic-chemical analysis of the rice husk, cow dung and their combination before and after anaerobic digestion.
5. Monitor the temperature and pH variation in the digester content during anaerobic digestion process.
6. Determine the volume of gas produced for each of the substrates and their combination.
7. Determine the plasmid profile of the bacteria species isolated from the digester after digestion.
8. Determine the quantity of biogas produced during anaerobic digestion.
9. Determine the percentage constituents present in the biogas produced by the substrates

Methods

Sterilization

Glassware was sterilized in a hot air oven at 121°C for one hour to eliminate contaminants.

Sample Collection

Milled rice husks were sourced from Umara Rice Mill, while fresh cow dung was collected from Somachi Slaughter House. Samples were transported to the Microbiology Department at FUTO within 24 hours.

Bio-digester Design

A 10-liter anaerobic bio-digester was constructed based on Karki's Biogas model. The design included three openings for slurry inlet, gas outlet, and slurry outlet, with nine digesters built for experimentation.

Figure 1: 10 litre scale bio-digester

Loading of Bio-digesters

Each bio-digester was loaded with 3 liters of slurry and 3 liters of water. Variations included:
Bio-digester 1: 3 kg cow dung
Bio-digester 2: 3 kg rice husk
Bio-digester 3: 1.5 kg rice husk + 1.5 kg cow dung
The experiment lasted 30 days, monitoring temperature, pH, bacterial counts, and gas production at three-day intervals.

Media Preparation

Agar media were prepared by dissolving specified amounts in distilled water, sterilizing them by autoclaving, and allowing them to solidify before use.

Inoculation and Incubation

Serial dilutions of samples were prepared in sterile distilled water, followed by inoculation on nutrient agar plates. The plates were incubated at 30°C for 24 hours to observe colony development.

Pure Culture Preparation

Isolated cultures were purified by re-streaking on fresh agar plates.

Cultural and Morphological Characterization

Bacterial morphology was assessed through Gram staining, motility tests, and biochemical tests, including catalase, coagulase, and sugar fermentation tests.

Physico-Chemical Analyses

Parameters analyzed in cow dung and rice husk included organic carbon, total solids, nitrogen content, and biochemical oxygen demand (BOD).

Gas Production Analysis

A portable biogas analyzer measured gas volume and constituent percentages.

Plasmid Profile Analysis

Plasmid DNA was extracted and analyzed through agarose gel electrophoresis to assess the genetic basis for biogas production potential.

Statistical Analysis

Data were analyzed using Microsoft Excel and SPSS, with results presented in tables and graphs to summarize findings.

Results

Enumeration of the Total Bacterial Count from the Substrate

The total bacterial count of rice husk (RH) decreased with an increase in retention day (RH = 1.3 x 10^8 cfu/g – 0) (Figures 2). Increase in total bacterial count of cow dung (CD) was observed from Day 3 to Day 21 (1.5 x 10^8 cfu/g – 3.1 x 10^8 cfu/g) and decreased from Day 24 to Day 30 (2.7 x 10^8 cfu/g – 1.4 x 10^8 cfu/g) as shown (Figure 2). Increase in total bacterial count of RH:CD was observed from Day 3 to Day 18 (2.1 x 10^8 cfu/g – 3.1 x 10^8 cfu/g) and decreased from Day 21 to Day 30 (2.9 x 10^8 cfu/g – 1.8 x 10^8 cfu/g) (Figure 2).

Morphological and Biochemical Tests of Bacterial Species Isolated

The result of the morphological and biochemical tests of bacterial species during 30 days of anaerobic digestion showed that eight bacteria were isolated. These bacterial species included *Proteus vulgaris*, *Bacillus subtilis*, *Klebsiella oxytoca*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Streptococcus pyogenes* and *Micrococcus luteus* (Table 1).

Physico–chemical Analysis of Rice Husk and Cow Dung Before and After Anaerobic Digestion

The result of the physico-chemical analysis of the substrate upon anaerobic digestion (Table 2) showed a 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) except moisture content that increased in all the substrates. Increase in ash content (1.10-1.71 mg) and crude fibre level (1.28-1.94 mg) was observed with cow dung only.

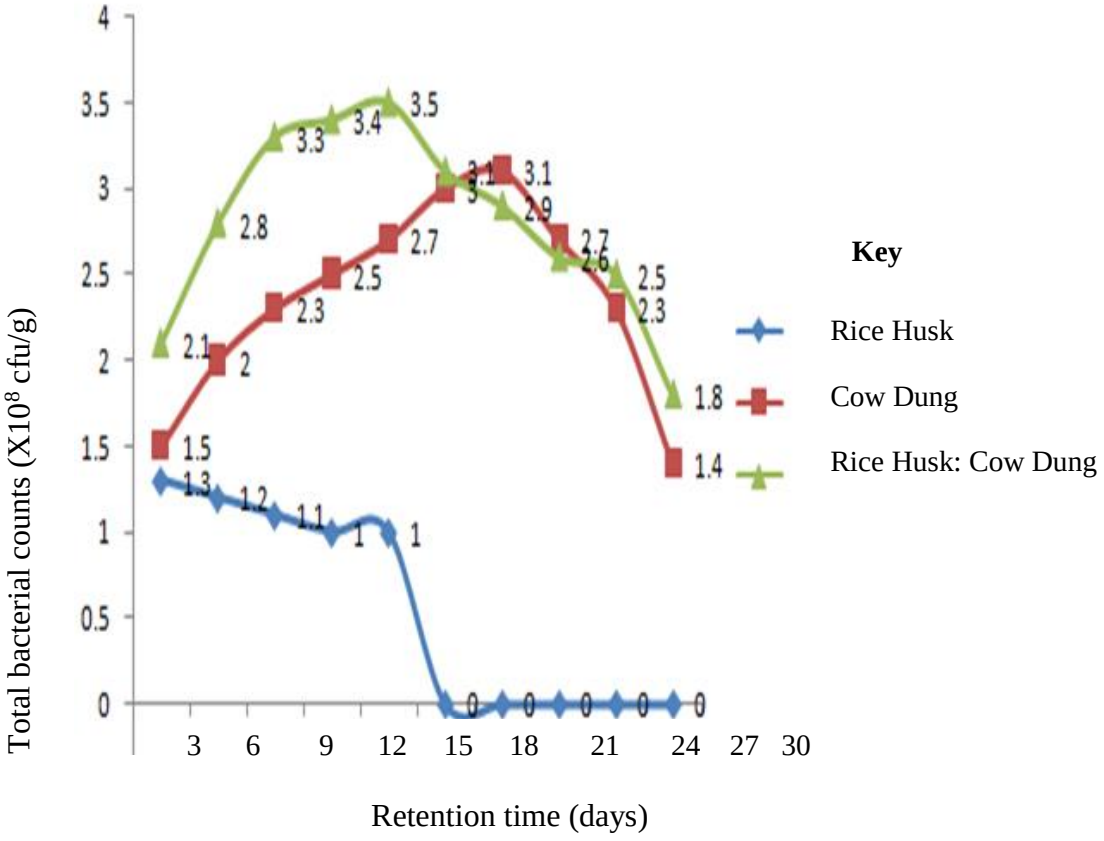


Figure 2: Variation in the total bacterial counts of the treatments with time

Table 1: Morphological and biochemical tests of bacteria species isolated during the anaerobic digestion

Colony Characteristics			Gram staining	Biochemical Tests										Sugar Fermentation Tests					Suspected Organisms
Colour	**Consistency/ Texture**	**Shape**	**Gram Reaction**	**Cell Morphology**	**Motility Test**	**Catalase Test**	**Citrate Test**	**Methyl Red Test**	**Oxidase Test**	**Coagulase Test**	**Urease Test**	**Hemolysis Test**	**Indole Test**	**Voges Proskauer**	**Glucose**	**Maltose**	**Lactose**	**Galactose**	**Fructose**	
Creamy	Raised/ smooth edge	Circular	-	Rod	+	+	+	+	-	-	+	+	-	-	+	-	-	-	+	*Proteus vulgaris*
Grayish	Small round colony	Circular	+	Rod	-	-	+	-	+	-	+	+	-	-	+	+	-	-	-	*Bacillus subtilis*
Creamy	Raised/ smooth edge	Circular	+	Cocci	-	+	-	+	-	+	+	+	-	-	+	+	-	+	-	*Staphylococcus aureus*
Light yellow	Slightly raised	Circular	-	Rod	-	+	+	-	+	-	-	-	-	-	+	-	-	-	-	*Pseudomonas aeruginosa*
Greenish	Rough surface	Circular	-	Rod	-	+	+	+	-	-	-	-	+	-	+	-	+	-	-	*Escherichia coli*
Pink	Mucoid colonies	Circular	-	Rod	-	+	+	-	-	-	+	+	-	+	+	+	+	-	+	*Klebsiella oxytoca*
White-greyish	Small round colony	Circular	+	Cocci	-	-	-	+	-	-	-	+	-	-	+	+	+	+	+	*Streptococcus pyogenes*
Yellow pigment	Entire/convex	Circular	+	Cocci	-	+	-	-	+	-	+	-	-	-	-	-	-	-	-	*Micrococcus luteus*

Key: + = Positive , - = Negative

Table 2: Physico-chemical analysis of RH, CD and RH:CD during 30 days of anaerobic digestion

Parameter	RH		CD		RH: CD	
	Fresh Slurry	Digested Slurry	Fresh Slurry	Digested Slurry	Fresh Slurry	Digested Slurry
Nitrogen (%)	0.25±1.2[a]	0.22±0.5[a]	0.33±0.2 [a]	0.24±1.2[a]	0.28±2.2[a]	0.22±1.2[a]
Carbon content (%)	6.31±0.5[a]	5.13±2.0 [a]	7.80±0.3[a]	5.83±0.3[b]	8.21±1.3[a]	5.54±1.1[b]
Carbon/Nitrogen	23.98±0.2 [a]	22.38±0.3[a]	25.20±1.0	23.21±0.3	28.33±3.1[a]	26.40±1.4[b]
Ash (g/100 g)	2.78±1.1[a]	0.11±0.1[b]	1.10±0.3	1.71±1.2	1.46±0.1[a]	0.52±0.3[a]
Moisture (g/100 g)	27.96±0.4[a]	98.75±0.4[b]	80.10±2.3[a]	93.88±0.8[b]	72.61±2.3[a]	97.81±2.3[b]
Crude fibre (g/100 g)	4.20±0.3[a]	0.22±1.4[b]	1.28±1.2[a]	1.94±1.1[a]	1.87±1.2[a]	0.88±0.2[a]
Crude protein (g/100 g)	27.58±0.4[a]	0.15±1.1[b]	6.79±0.4[b]	1.13±1.3[b]	9.70±0.3[a]	0.24±1.2[b]
Volatile solid (%)	9.30±2.1[a]	0.02±1.0[a]	9.16±1.3[a]	0.05±0.4[b]	9.13±0.4[a]	0.02±0.1[b]
Total solid (g/100 g)	72.01±1.3[a]	0.61±2.1[a]	19.93±0.4[a]	6.01±1.2[a]	27.41±1.2[a]	2.03±1.0[b]
Fat content (g/100 g)	2.71±1.5[a]	0.00±0.0[b]	0.91±1.1[a]	0.11±3.2[a]	1.31±1.0[a]	0.08±0.3[a]
BOD (mg/L) COD	19.11±0.3[a]	10.41±1.1[b]	20.53±2.1[a]	11.27±1.0[b]	20.37±1.1[a]	11.15±1.1[b]
COD (mg/L)	7.32±2.1[a]	3.73±1.5[b]	7.41±1.0[a]	4.02±1.1[b]	6.78±0.3[a]	3.71±0.2[b]

Key: Values were mean ± standard deviation (SD), Values with different superscripts within the same row are significantly different from each other at $p < 0.05$, RH = Rice husk, CD = Dow dung, RH:CD = Rice husk: Cow dung BOD= Oxygen demand and COD = Chemical oxygen demand

Temperature Variation in Digester Content During Anaerobic Digestion CD, RH and RH:CD

Figures 3 shows temperature in digester content during anaerobic digestion of CD, RH and RH:CD. The overall temperature range of all the digesters was from 24.1 ^{0}C to 36.7 ^{0}C. The highest overall temperature (36.7 ^{0}C) was recorded in RH:CD at the 21st day of digestion while the lowest temperature (23.2 ^{0}C) was recorded in CD at the 21st day of digestion.

PH Variation in Digester Content During Anaerobic Digestion CD, RH and RH:CD

Figures 4 shows pH in digester content during anaerobic digestion of CD, RH and CD:RH. The overall pH range recorded in all the digesters was from 5.5 to 8.0. The lowest pH measurement (5.5) was recorded on the 30th day of digestion in RH and while the highest pH measurement (8.0) was recorded at the 3rd day of digestion in RH:CD.

Total Biogas Produced by Substrates during Anaerobic Digestion

Figure 5 shows the total biogas produced by substrates (RH, CD and RH:CD) during anaerobic digestion increased with days during digestion. RH:CD had the highest biogas production (5298.3 cm^3) as observed in figure 5 after 30 days of anaerobic digestion followed by CD with a volume of 4577.9 cm^3 while RH produced the least amount of biogas (110.6 cm^3) after 30 days of anaerobic digestion.

Constituents of Gas Produced Anaerobic Digestion

Figure 6 shows the constituents (CH_2, H_2S and CO_2) of gas produced after 30 days of anaerobic digestion. RH:CD showed the highest CH_2 (83.2 %) produced followed by CD (70.8 %) and none was recorded with RH (0.0). RH:CD also produced the highest H_2S (0.5 %), followed by CD (0.2 %) and none was recorded with RH (0.0). RH showed the highest CO_2 (100 %) produced, followed by CD (35.3 %) and RH:CD recorded the lowest (27.7 %).

Plasmid Profile of Bacteria Species Isolated After Anaerobic Digestion

The number, electrophoretic mobility and corresponding molecular weight of plasmid DNA analyzed are presented in Table 3. Out of the eight isolates (Pa, Sp, Pv, Ko, Ml, Ec, Sa and Bc) analyzed, only four isolates (Pa, Ko, Ml and Bc) showed the presence of plasmids.

Bc revealed the presence of 4 plasmids with mobility of 9 mm, 14 mm, 21 mm and 18 mm and the corresponding molecular weight of 7.2 kbp, 5.3 kbp, 2.2 kbp and 2.4 kbp respectively; Pa showed the presence of 3 plasmid with mobility of 11 mm, 15 mm and 23 mm with the corresponding molecular weight of 6.1 kbp, 4.7 kbp and 2.0 kbp respectively; Ml showed the presence of 3 plasmids with mobility of 15 mm, 22 mm and 9 mm with corresponding molecular weight of 4.8 kbp, 1.9 kbp and 7.1 kbp respectively; While Ko showed the presence of only 2 plasmids with mobility of 9 mm and 13 mm with corresponding molecular weight of 10.1 kbp and 6.5 kbp respectively.

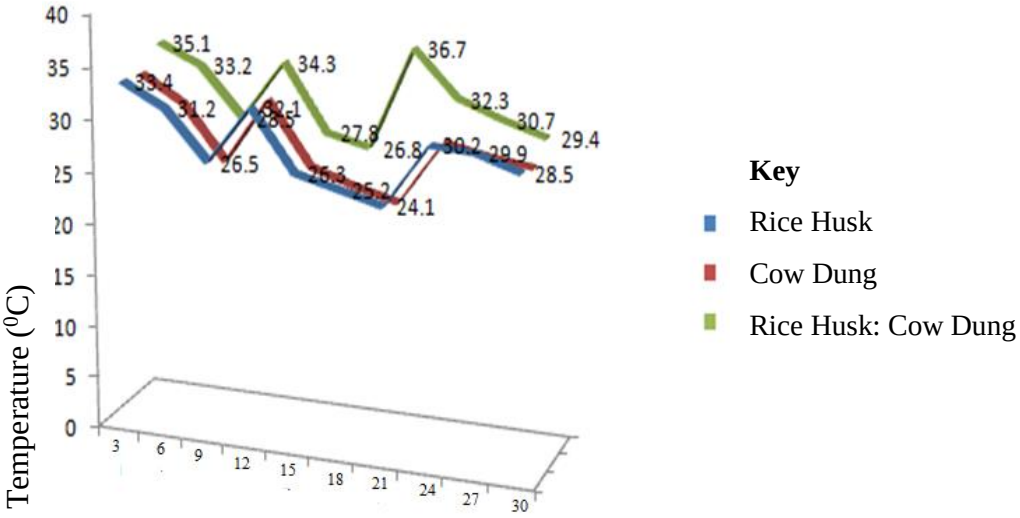
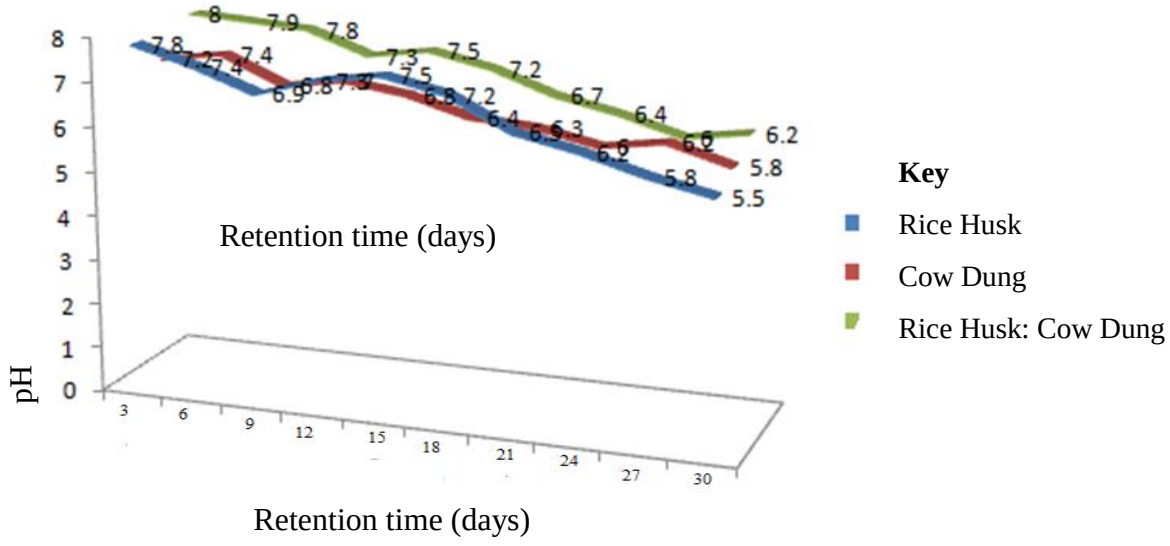


Figure 3: Temperature changes in the digester content during anaerobic digestion



Retention time (days)

Figure 4: pH changes in the digester content during anaerobic digestion

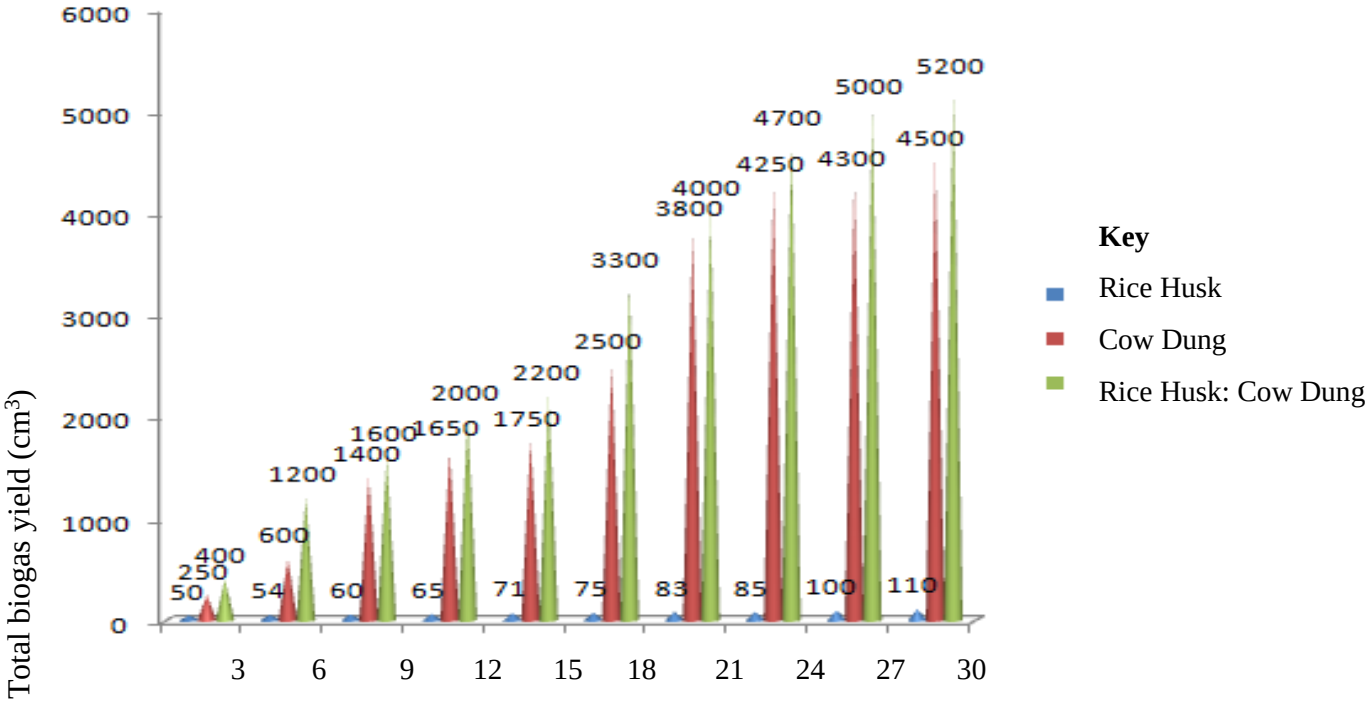


Figure 5: Total biogas produced by each waste at different days of anaerobic digestion

Retention time (days)

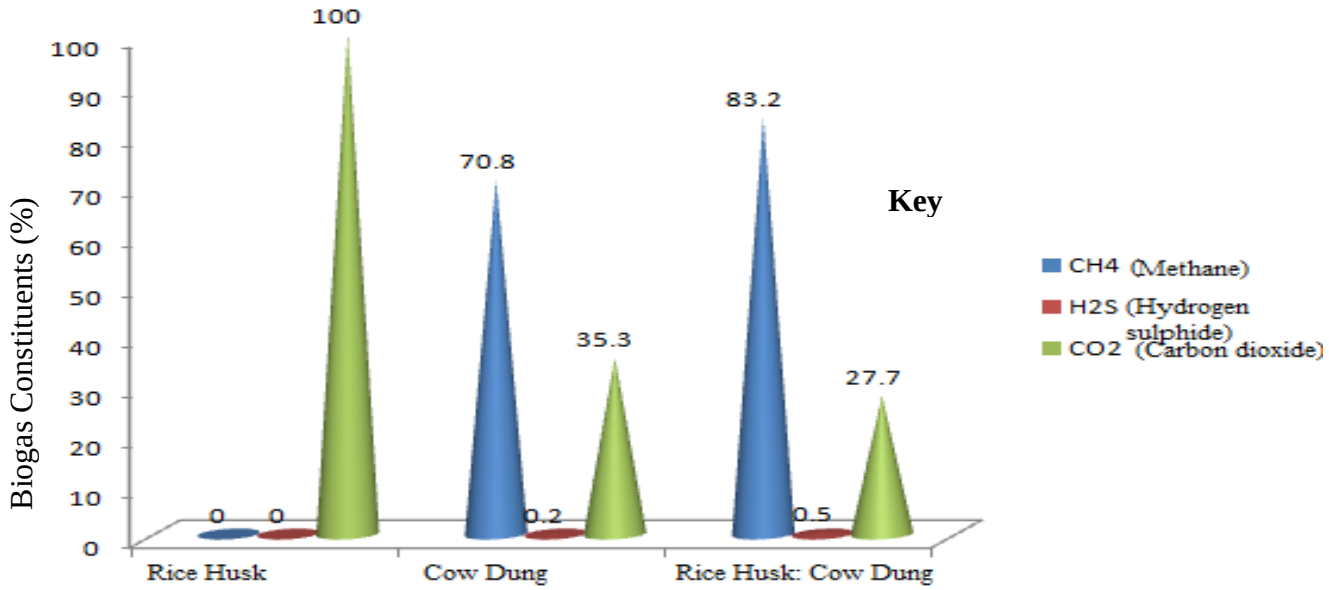


Figure 6: Percentage of biogas constituents produced by wastes after anaerobic digestion

Table 3: Plasmid profile of the bacteria species during anaerobic digestion

Bacteria Code	Number of Plasmids Detected	Mobility (mm)	Molecular Weight (kbp)
Bc	4	9,14,21 and 18	7.2, 5.3, 2.2 and 2.4
Ec	Nil	Nil	Nil
Ko	2	9 and 13	10.1 and 6.5
Ml	3	15, 22 and 9	4.8, 1.9 and 7.1
Pa	3	11,15 and 23	6.1, 4.7 and 2.0
Pv	Nil	Nil	Nil
Sa	Nil	Nil	Nil
Sp	Nil	Nil	Nil

Source: Field work, 2022

Key: Pa = *Pseudomonas aeruginosa*, Sp = *Streptococcus pyogenes*, Pv = *Proteus vulgaris*, Ko = *Klebsiella oxytoca*, Ml = *Micrococcus luteus*, Ec = *Escherichia coli*, Sa = *Staphylococcus aureus* and Bc = *Bacillus cereus*.

Summary of Findings, Conclusion, and Recommendation

Discussion

Anaerobic digestion relies on hydrolytic, acetogenic, and methanogenic bacteria to convert organic waste into biogas, promoting a cleaner environment. Biogas systems utilizing animal and human excreta can meet local energy needs while benefiting ecosystems. The study measured total bacterial counts across different substrates: rice husks (RH), cow dung (CD), and their combination (RH), all showing counts suitable for biogas generation. Eight bacterial species were isolated, indicating diverse microbial activity essential for breaking down organic matter into biogas.

Physico-chemical analysis revealed reductions in key parameters such as nitrogen, carbon, and volatile solids, signifying effective digestion. Notably, an optimal carbon-to-nitrogen (C) ratio (20:1 to 30:1) is crucial for maximizing gas production, as excess nitrogen can hinder methane generation. The digestion process maintained a temperature range conducive to biogas production and a pH level optimal for methanogenic activity. Biogas production increased significantly over the digestion period, with co-digested RH yielding the highest volume of biogas due to the synergistic effects of combined substrates, enhancing nutrient balance and reducing toxic effects. The methane content was also highest in the co-digestion scenario, aligning with expected biogas compositions.

Plasmid analysis showed that certain bacterial isolates possessed plasmids, which may enhance their biogas production potential by facilitating degradation processes and conferring advantageous traits. The plasmid profiles reveal important aspects of the bacteria's genetic makeup, which can significantly influence their metabolic capabilities and efficiency in anaerobic digestion. Bacillus cereus, with four detected plasmids, indicates a strong genetic diversity that may enhance its ability to break down complex organic materials, leading to increased biogas production. The presence of multiple plasmids often correlates with traits such as antibiotic resistance, stress tolerance, and metabolic versatility, which are crucial in the fluctuating conditions of anaerobic environments.

In contrast, the absence of plasmids in bacteria like Escherichia coli, Streptococcus pyogenes, and Proteus vulgaris suggests that these species may lack the necessary genetic tools for effective biogas production in this context. Understanding which bacteria carry plasmids can help researchers identify key species that contribute significantly to methane generation. This knowledge can ultimately guide the optimization of biogas production processes, such as selecting appropriate microbial communities for co-culturing or bioaugmentation, enhancing efficiency and sustainability in bioenergy production from agricultural waste. Additionally, the results can inform future genetic studies aimed at manipulating these bacteria for improved biogas yields, thus contributing to more efficient renewable energy solutions.

Conclusion

The co-digestion of rice husks and cow dung enhances biogas production, demonstrating the efficacy of rice husks as a substrate. This method not only provides a solution to feedstock shortages for biogas production but also contributes to energy security and environmental sustainability. The resulting methane content meets standards for domestic biogas applications.

Recommendations

1. Conduct molecular characterization of isolated bacterial species to understand their roles better.
2. Scale up the study to assess co-digestion effects in larger systems.
3. Optimize physicochemical conditions further to enhance biogas yields.
4. Explore co-digestion potential with other animal and lignocellulosic waste to diversify energy sources.

References

Agunwamba, J. C. (2001). Biogas production from co-digestion of agricultural waste. Journal of Renewable Energy, 22(4), 665-676.

Adeyanju, A. A. (2008). The role of renewable energy in sustainable development in Africa. Energy for Sustainable Development, 12(4), 19-25.

Alvarez, J. A., Mendez, F. J., & Rodriguez, P. (2010). Challenges of renewable energy in the 21st century. Renewable and Sustainable Energy Reviews, 14(1), 43-54.

Babel, S., Kumar, S., & Rattan, R. (2009). Co-digestion of organic waste: A review. Waste Management, 29(1), 285-298.

Ezekoye, V. A., & Okeke, C. A. (2006). Enhanced methane yield from co-digestion of organic wastes. Environmental Technology, 27(1), 51-57.

El-Mashad, H. M., & Zhang, R. (2007). Biogas production from co-digestion of food waste and dairy manure. Waste Management, 27(2), 223-228.

Ezeonu, S. C., Mclnerney, M. J., & Bryant, M. P. (2005). Anaerobic digestion of agricultural waste. Applied and Environmental Microbiology, 71(12), 8356-8360.

Garg, S. K., & Mudgal, V. (2007). Cow dung as a sustainable resource. Journal of Environmental Management, 86(3), 597-608.

Guruswamy, L., Marzouk, S. S., & Wang, Y. (2003). Renewable energy in the 21st century. Energy Policy, 31(10), 1099-1107.

Hashfi, M., Kargbo, D., & Jin, H. (2018). The effects of lignin on biogas production. Renewable Energy, 123, 503-509.

Iyagba, E. T., Akpan, U. E., & Essien, J. P. (2009). Utilization of rice husk as agricultural waste. International Journal of Environmental Science and Technology, 6(3), 419-426.

Ilori, M. O., Omosanya, T. A., & Oyekunle, J. A. (2007). Co-digestion of organic wastes for biogas production. Journal of Applied Microbiology, 103(5), 1972-1981.

Kalia, V. C., Kaushik, R., & Gupta, R. (2000). Biogas: A renewable energy source. Environmental Studies, 29(1), 51-56.

Mclnerney, M. J., & Bryant, M. P. (1981). Anaerobic microbiology and the generation of methane. Microbiology Reviews, 45(2), 245-271.

Milono, S., Onofrio, R., & Scrocco, P. (1981). Composition of biogas from different substrates. Biomass, 1(1), 57-68.

Mshandete, A. M., & Parawira, W. (2009). Biogas technology research in Africa. Renewable and Sustainable Energy Reviews, 13(5), 1240-1246.

Nagamiani, R., & Ramasamy, S. (2003). Renewable energy in the context of Millennium Development Goals. Energy Policy, 31(4), 379-385.

Nene, Y. (1999). The microbial diversity of cow dung. Microbiology Today, 26(1), 10-14.

Parawira, W., Mshandete, A. M., & Zukali, J. (2004). Co-digestion of agro-wastes. Biomass and Bioenergy, 27(4), 331-337.

Randhawa, S., & Kullar, J. S. (2011). Nutritional and mineral content of cow dung. Journal of Animal Science, 89(3), 32-39.

Vigil, M. F., Santarita, G. M., & Aquilino, V. (2003). Biological treatment of agricultural wastes. Waste Management, 23(2), 101-108.

Wallheimer, J. (2010). Agricultural waste management. Agricultural Engineering International: CIGR Journal, 12, 1-12.