

EFFECTS OF FUNGAL, BACTERIAL AND ALKALINE AUGMENTATIONS ON THE BIOGAS COMPOSITION OF SELECTED PLANT-BASED SUBSTRATES

ABSTRACT

Lignocellulosic residues are interesting sources of renewable energy, if only their biomass recalcitrance could be reduced through appropriate pretreatment technologies and augmentations, to enhance anaerobic digestion. This study aims at assessing the effects of bacterial, fungal and alkaline augmentations on the biogas composition of selected plant-based substrates, namely: maize cob (MC), rice straw (RS) and water hyacinth (WH). Standard methods were adopted; the substrates were mechanically pretreated, loaded in single, dual and composite combinations, into five 52 L capacity metallic batch anaerobic digesters, codigestion was encouraged with the addition of cow rumen waste. The set ups were allowed to run for 42 days under mesophilic conditions, while stirring daily. The biogas composition namely: methane, carbon IV oxide and hydrogen sulfide concentrations were measured on the 42nd day. The results showed that the composite with the combined treatments showed the highest concentration of methane (87%), followed by the composites with fungal augmentation (80%), bacterial (77%) and the alkaline (75%) augmentations. The least percentage of methane (9%) was recorded in the composite without treatment and no cow rumen waste added, which also had the highest percentages of hydrogen sulphide and carbon IV oxide. To improve the yield and quality of biogas generated from a lignocellulosic biomass, appropriate pretreatment strategies and augmentations are required.

Keywords: lignocellulose biomass, biogas , bioaugmentation, alkaline augmentation, gas analysis.

INTRODUCTION

Power generation is a major indicator of a nation's economic advancement. Fossil fuels, a non-renewable form of energy contribute the vast source of energy supply and poses tremendous environmental hazards, due to the generation of green house gases, etc. Lignocellulose biomass holds considerable potential to meet the current energy demand of the modern world. This is also essential in order to overcome the excessive dependence on non-renewable energy, and evidently curb the menace of pollution. Depolymerization, followed by solubilization of the polymers, is the first step in anaerobic digestion of solid wastes. Subsequently, cellulose degradation products can be converted to methane and carbon dioxide through acidogenesis, acetogenesis, and methanogenesis processes [1]. Pretreatment of biomass is a key step both technically and economically, in the bioconversion of lignocellulose biomass for bioenergy production, irrespective of the type of biomass [2]

Biogas is a renewable energy source generated through the anaerobic digestion of organic materials. It is mainly composed of methane (40-75%), carbon IV oxide (25-50%) with minor impurities such as hydrogen sulfide, ammonia, etc. [3]. The methane content of biogas represents its quality and energy value.

MATERIALS AND METHODS

Samples collection

The samples used as feedstock for this study were, maize cobs (MC), rice straw (RS), water hyacinth (WH) plant (*Eichhornia crassipes*) and waste from cow rumen (CR). They were respectively collected from World Bank and Ihiagwa markets in Owerri, Onicha Uboma in Ihitte-Uboma of Imo State, Nun River in Bayelsa State and Obinze Abattoir in Owerri West, Imo State, Nigeria. They were aseptically collected using clean sack bags and transported to the laboratory prior to preparation and loading of the digesters.

Digesters Design

Five (5) metallic batch anaerobic digesters of approximately 54 litres capacity each, were locally fabricated by the Centre for Industrial Studies (CIS), FUTO, Nigeria.



Plate 1: Locally fabricated biodigesters

Sample preparation and Loading of digesters

The samples were prepared and loaded into the digesters, with a slight modification of the standard method described by Asikong *et al.* [4] and Sagagi *et al.* [5]. The substrates were

reduced in size, sundried for 3 days and milled. Each of the milled substrate was weighed out in a 1:1 ratio, and then mixed with clean portable water in a 1:20 ratio. Two (2) kg of cow rumen waste was dissolved in 4 liters of clean water and added to the required set ups. The substrates were loaded in lone, dual and composite combinations. Separate batches were appropriately augmented with 10% NaOH (AA), 1000 ml broths each of ligninolytic bacteria (BA) comprising *Bacillus* species and fungi (FA) comprising *Rhizopus sp*, *Penicillium notatum*, *Aspergillus flavus* and *Aspergillus niger* isolated from *Macrotermes bellicosus* and decaying wood [6, 7, 8, 9, 10, 11].

Determination of Biogas Composition

This was carried out on the 42nd day, by means of a standard Aero-qual 500 series gas Analyzer, by measuring the Methane (CH₄), Carbon IV oxide (CO₂), and Hydrogen sulphide (H₂S) concentrations, using specific probes for each gas [12].

Statistical analysis

The test of hypothesis with $\alpha=0.05$ was conducted to test for statistical difference among treatments.

RESULTS

The methane (CH₄), carbon IV oxide (CO₂) and hydrogen sulfide (H₂S) concentrations at retention time of 42 days are represented respectively in Figures 1, 2 and 3.

The highest value of methane (9869 ppm, up to 87 %, assuming the biogas comprised of only the three components investigated in this study) was obtained in the composite with all the treatments (*MC+RS+WH+CR+BA+FA+AA*), This was followed by the composites augmented

with fungi (9700 ppm, up to 80%), bacteria (7795 ppm, up to 77%), alkali (6670 ppm, up to 75 %) the untreated (842 ppm, up to 50%) respectively, all with cow rumen. The least concentration of methane (70 ppm, that is 9.2%) was obtained in the composite without treatments and no addition of cow rumen (MC+RS+WH). In the dual combinations, with respect to the various treatments, MC+WH performed best, followed by WH+RS and then MC+RS. Considering the lone substrates, water hyacinth took the lead, followed closely by maize cobs and finally rice straw. Values of CO₂ ranged from 688 ppm (giving as high as 90% of the composition in the untreated composite without cow rumen) to 2414 ppm (that is 24%, in the fungi-augmented RS+MC+CR). The least H₂S was recorded in the composite with cow rumen waste and all the treatments (0.02 ppm) while the highest concentration was recorded in the untreated composite, without cow rumen waste (2.6 ppm).

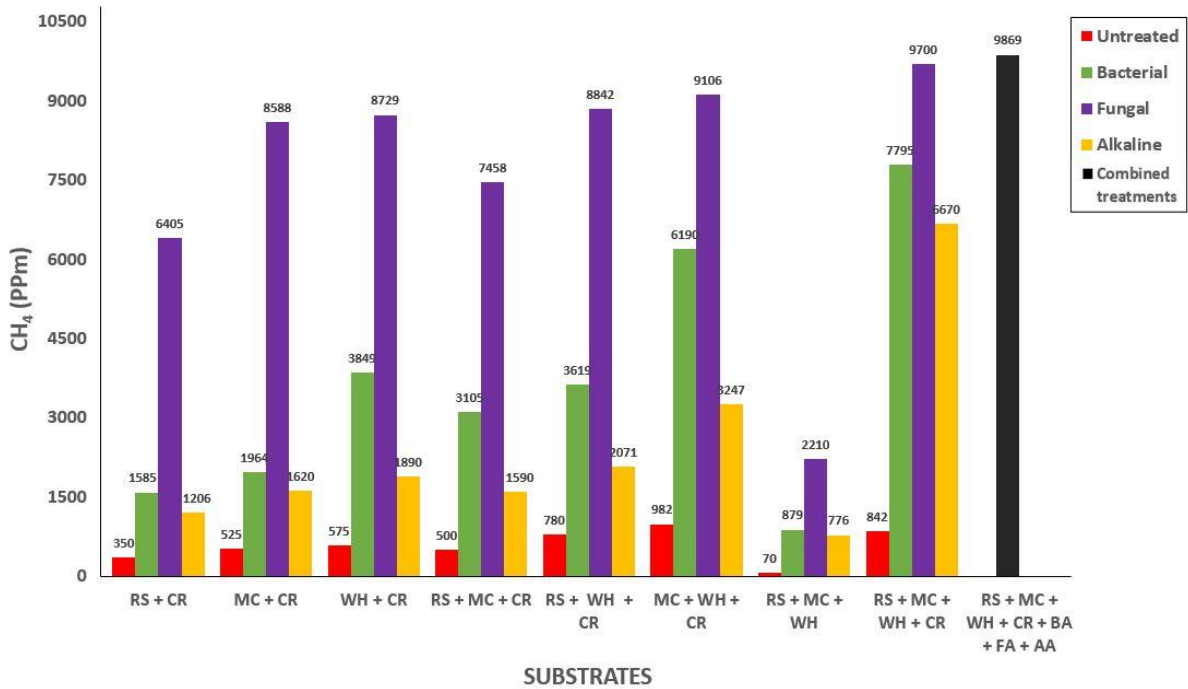


Figure 1: Methane concentration (ppm) of the setups on the 42nd day.

Legend: RS = rice straw, MC = maize cob, WH = water hyacinth, CR = cow rumen waste, AA = alkaline augmentation, BA = bacterial augmentation, FA = fungal augmentation

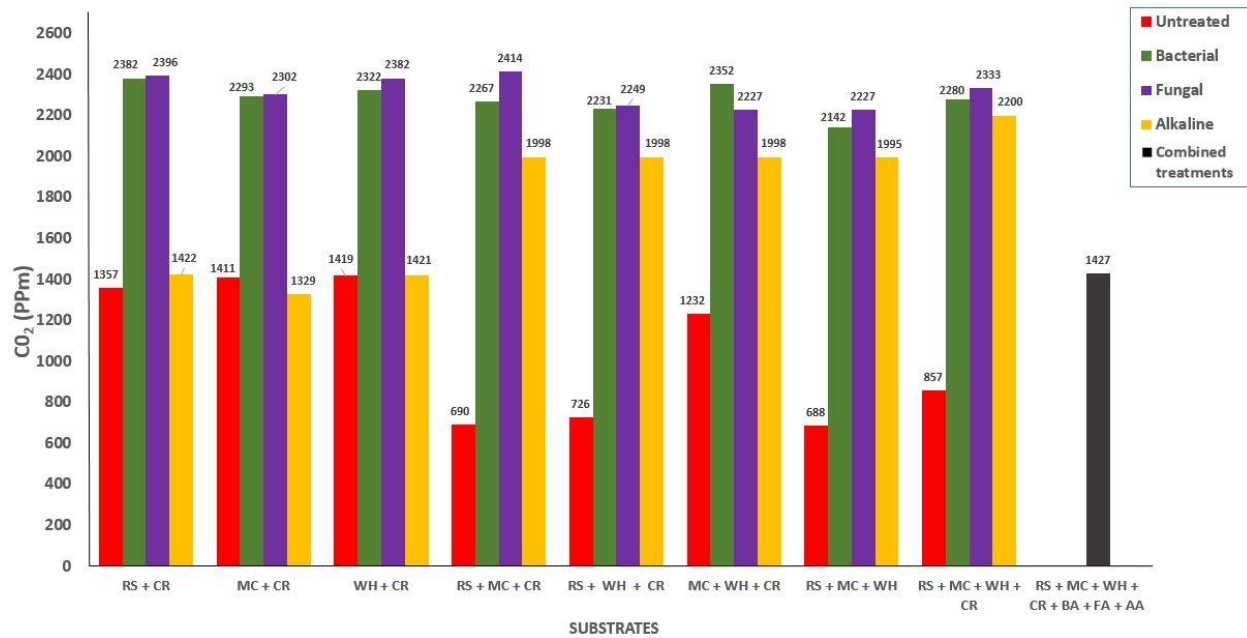


Figure 2: Carbon IV oxide concentration (ppm) of the setups on the 42nd day.

Legend: RS = rice straw, MC = maize cob, WH = water hyacinth, CR = cow rumen waste,
 AA = alkaline augmentation, BA = bacterial augmentation, FA = fungal
 augmentation

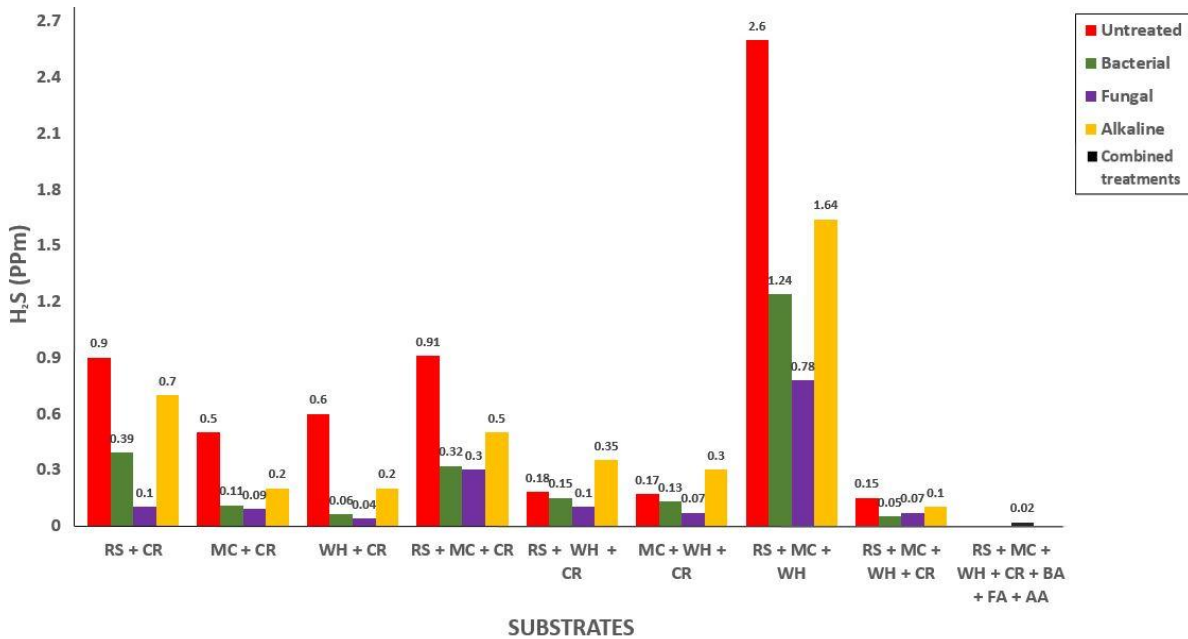


Figure 3: Hydrogen (H₂S) sulfide concentration (ppm) of the setups on the 42nd day.

Legend: RS = rice straw, MC = maize cob, WH = water hyacinth, CR = cow rumen waste, AA = alkaline augmentation, BA = bacterial augmentation, FA = fungal augmentation

DISCUSSION

Biogas comprises a mixture of methane (40-75%), carbon IV oxide (25-50%), with minor impurities such as hydrogen sulfide, ammonia, etc [3]. The methane content of biogas represents its quality and energy value. The process of upgrading removes the impurities in biogas thereby concentrating the methane level. However, carbon IV oxide, which is the second major component can be sequestered and used to produce chemicals of industrial importance [13]. The

percentage of methane, CO₂, and other components of biogas varies with the nature/maturity of feedstocks, temperature, water content, pH, organic loading rate and microbial actions [14]

Methane content

The reports of previous studies [15, 16, 17] lend credence to the highest concentration of methane recorded in the composite with the combined treatments relative to lone treatments. In addition, the low rate of hydrolysis and reaction time in a bioaugmentation process must have been averted with a combination of other treatments, such as physical and chemical as applied in this study, thereby improving the process productivity [16]. The astounding performance of the fungal augmented substrates is in agreement with the report of Liu et al [18] who recorded up to 100% increase in methane yield using fungal pretreatment. Whereas Shah and Ullah [19] reported a 407.1% increase in methane yield of wheat straw using selected strains of fungus producing laccase and lignin peroxidase. The fungal augmentation may have performed better as they are the major degraders of lignin and had more of such ligninolytic species, when compared to the bacterial counterpart with only *Bacillus* species on display. Alkaline augmentation shows high efficiency [20] especially in the delignification process [21]. However to take full advantage of these effects, critical process parameters such as alkaline loading, reaction temperature, and concentration must be optimized [22]. In this study, the experiment was run under mesophilic conditions using 10% w/v NaOH without controlling any operations parameter besides organic loading rate. In addition, various researchers [23] have reported highest methane yield at 2% w/v of NaOH. This may have contributed to the lower performance of the alkaline augmentation relative to the bioaugmentation processes in this study.

Carbon IV oxide content

The CO₂ content of biogas is one of the contributors to the poor combustion of biogas [15]. The highest percentage recorded in the composite without treatments and cow rumen waste, clearly depicts that codigestion and augmentation(s) are key factors to achieving value-added and combustible methane.

Hydrogen sulfide content

The very low concentrations (0.02 – 2.6 ppm) of H₂S recorded in these substrates depicts they are attractive for biogas production, provided proper pretreatment methods and augmentations are employed. A study by Pan-in and Sukasen [24] recorded up to 58.33 ppm of H₂S after 30 days of dry digestion using cow dung. This study however used cow rumen waste, which performed better than cow dung in the preliminary studies. Another reason for improved performance owing to the reduced H₂S content may be attributed to wet digestion, nature of substrates, pretreatment/augmentations, as well as retention time. Ugwu *et al.* [25] reported that the lignocellulose, proximate and physicochemical compositions of the substrates utilized in this study prove that they are good for biogas production.

Statistical analysis

The p-values for all the substrates were less than 0.05, therefore we reject the null hypothesis and conclude that the treatments used were of different effects at 0.05 level of significance.

CONCLUSION

Successful anaerobic biodegradation of lignocellulosic biomass enhances biogas production owing to the fact that the depolymerization and solubilization release the monomeric units thereby enhancing microbial enzymes' access and utilization of the substrates, to yield the required products. Pretreatments and augmentations are major approaches to achieving this feat. Several pretreatment methods abound, however, a combination of various treatment methods proves the best option, followed by bioaugmentation, whose efficiency can highly be enhanced by physical pretreatment. Alkaline augmentation (NaOH) under mesophilic conditions is less

effective, and therefore requires adequate control of its parameters for optimal yield. These treatment approaches improve the quality of biogas by increasing the methane concentration while reducing the concentration of the hazardous hydrogen sulphide produced during the process.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

REFERENCES

1. Lynd, L. R., Weimer, P. J., Van Zyl, W. H. & Pretorius, I. S. (2002). Microbial cellulose utilization: Fundamentals and Biotechnology. *Microbiology and Molecular Biology Reviews*, 66(3): 506–577.
2. Hernandez-Beltran, J.U., Hernandez-Delira, I.O., Cruz-Santos, M.M., Saucedo-Luevanos, A., Hernandez-Teran, F. & Balagurusamy, N. (2019). Insight into pretreatment methods of lignocellulosic biomass to increase biogas yield: current state, challenges and opportunities. *Applied Sciences*, 9: 3721. Doi: 10.3390/app9183721
3. Parsaee, M., Deh Kiani, m.k & Karimi, K. (2019). A review of biogas production from sugarcane vinasse. *Biomass and Energy*, 122: 117-125.

4. Asikong, B., Agbo, B. E, James, E. & Eja, M. (2013). Four potentials of biogas yield from cow dung. *European Journal of Experimental Biology*, 3(3): 273-282.
5. Sagagi, B.S., Garba, B. & Usman, N.S. (2009). Studies on biogas production from fruits and vegetable waste. *Bayero Journal of Pure and Applied Sciences*, 2(1): 115-118
6. Chandra, R., Singh, S., Krishna-Reddy, M.M., Patel, D.K., Purohit, H.J. & Kapley, A. (2008). Isolation and characterization of bacteria strains *Paenibacillus* sp and *Bacillus* sp. For kraft lignin decolorization from pulp paper mill waste. *Journal of General and Applied Microbiology*, 54: 399-407.
7. Cheesebrough, M. (2000). *District Laboratory Practice in Tropical Countries*. Part 2, Cambridge University Press, UK. pp 35-38, 62-69.
8. Buchanan, R.E & Gibbons, N.E (1974). *Bergey's Manual of determinative bacteriology*. 8th Ed. The Williams and Wilkins Co Baltimore. 47-842.
9. Patil, N.P & Chaudhari, B.L. (2010). Production and purification of pectinase by soil isolate *Penicillium* sp. and search for better Agro residue for its SSF. *Rec Res Sci Technol*, 2: 36-42
10. Coll, P.M., Fernandez-Abalos, J.M., Villanueva, J.R., Santamaria, R. & Perez, P. (1993). Purification and characterization of a phenoloxidase (laccase) from the lignin-degrading basidiomycete PM1 (CECT 2971). *Applied Environmental Microbiology*, 59(8): 2607-2613.
11. Nagamani, A., Manoharachary, C. & Kunwar, I.K. (2006). *Handbook of soil fungi*. I.k international publishing house pvt. Ltd New Delhi. Pp 496.
12. Aljaradin, M. & Persson, K.M. (2016). The emission potential from municipal solid waste landfill in Jordan. *The Journal of Ecological Engineering*, 17(1): 38-48.
13. Barbera, E., Menegon, S., Banzato, D., D'Alpaos, C. & Bertucco, A. (2019). From biogas to biomethane: A process simulation-based techno-economic comparison of different upgrading technologies in the Italian context. *Renewable Energy*, 135: 663-673.

14. Bari, S. (1996). Effect of carbon IV oxide on the performance of biogas/diesel duel-fuel engine. *Renewable Energy*, 9(1-4): 1007-1010. Doi: 10.1016/0960-1481(96)88450-3
15. Ponnusamy, V. K., Niguyen, D. D., Dharmaraja, J., Shobana, S., Banu, J. R., Saratale, R. O., Chang, S. W & Kumar, G. (2019). A review on lignin structure, pretreatment, fermentation reactions and biorefinery potential. *Bioresource Technology*, 271: 462-472
16. Shirkavand, E., Baroutian, S., Gapis, D. J. & Young, B. R. (2016). Combination of fungal and physicochemical processes for lignocellulosic biomass pretreatment- A review. *Renew. Sustain. Energy Rev.* 54: 217-234
17. Olatunji, K.O., Ahmed, A.N. & Ogunkule, O. (2021). Optimization of biogas yield from lignocellulosic materials with different pretreatment methods- A review. *Biotechnology for biofuels*, 14: 159-193. <https://doi.org/10.1186/s13068-021-02012-x>
18. Liu, X., Hiligsmann, S., Gourdon, R. & Bayard, R. (2017). Anaerobic digestion of lignocellulosic biomasses pretreated with *Ceriporiopsis subvermispota*. *Journal of Environmental Management*. 193: 154-162.
19. Shah, T. A. & Ullah, R. (2019). Pretreatment of wheat straw with ligninolytic fungi for increased biogas productivity. *Int. J. Sci. Technol.* 1-12.
20. Liu, Y.Y., Xu, J.L., Zhang, Y., Liang, C.Y., He, M.C., Yuan, Z. & Xie, J. (2016). Reinforced alkali pretreatment for enhancing enzymatic hydrolysis of sugarcane bagasse. *Fuel Process Technol.* 143: 1-6.
21. Park, Y.C. & Kim, J.S. (2012). Comparison of various alkaline pretreatment methods of lignocellulosic biomass. *Energy*, 47: 31-35.
22. Kim, I. & Han, J.I. (2012). Optimization of alkaline pretreatment conditions for enhancing glucose yield of rice straw by response surface methodology. *Biomass Bioenergy*, 6: 1-8.
23. Jiang, D., Ge, X., Zhang, Q. & Li, Y. (2016). Comparison of liquid hot water and alkaline pretreatment of Giant Reed for improved enzymatic digestibility and biogas energy production. *Bioresource Technology*, 216: 60-68.
24. Pan-in, S. & Sukasem, N. (2017). Methane production potential from anaerobic co-digestion of different animal dungs and sweet corn residuals. *Energy Procedia*, 138: 943-948.
25. Ugwu, T.N., Nwachukwu, A.A., Ogbulie, T. E & Anyalogbu, E. A. (2021). *Journal of Advances in Biology and Biotechnology*, 24(3): 1-6.