# 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 54 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 dioxide and hydrogen sulfide concentrations were measured on the 42nd day. The results showed that the compositewith 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 dioxide. To improve the yield and quality of biogas generated under mesophilic conditions, 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]. Shah and Ullah [3] compared the outcome of microbial pretreatment versus enzymatic pretreatment in anaerobic digestion process, and reported that microbial treatment demonstrated much better outcomes, due to high functional diversity and tolerance to environmental factors. Another study by Chandra *et al*. [4] evaluated the effect of particle size on wheat and rice straws, and recorded an increase in methane yield.

Biogas is a renewable energy source generated through the anaerobic digestion of organic materials. It is mainly composed of methane (40-75%), carbon dioxide (25-50%) with minor impurities such as hydrogen sulfide, ammonia, etc. [5]. 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.* [6] and Sagagi *et al.* [7]. 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% w/v 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[ 4, 8, 9, 10, 11, 12].

### 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 ($CH_4$), Carbon dioxide ($CO_2$), and Hydrogen sulphide ($H_2S$) concentrations, using specific probes for each gas [13].The readings for each of the components on each set up, was obtained after proper standardization of the machine with the required probe, each time.

### Statistical analysis

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

Hypothesis:

H0: there is no significant difference between treatments for each of the substrates

H1: there is significant difference between treatments for each of the substrates

## RESULTS

The methane ($CH_4$), carbon dioxide ($CO_2$) and hydrogen sulfide ($H_2S$) 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 $CO_2$ 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 $H_2S$ 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).

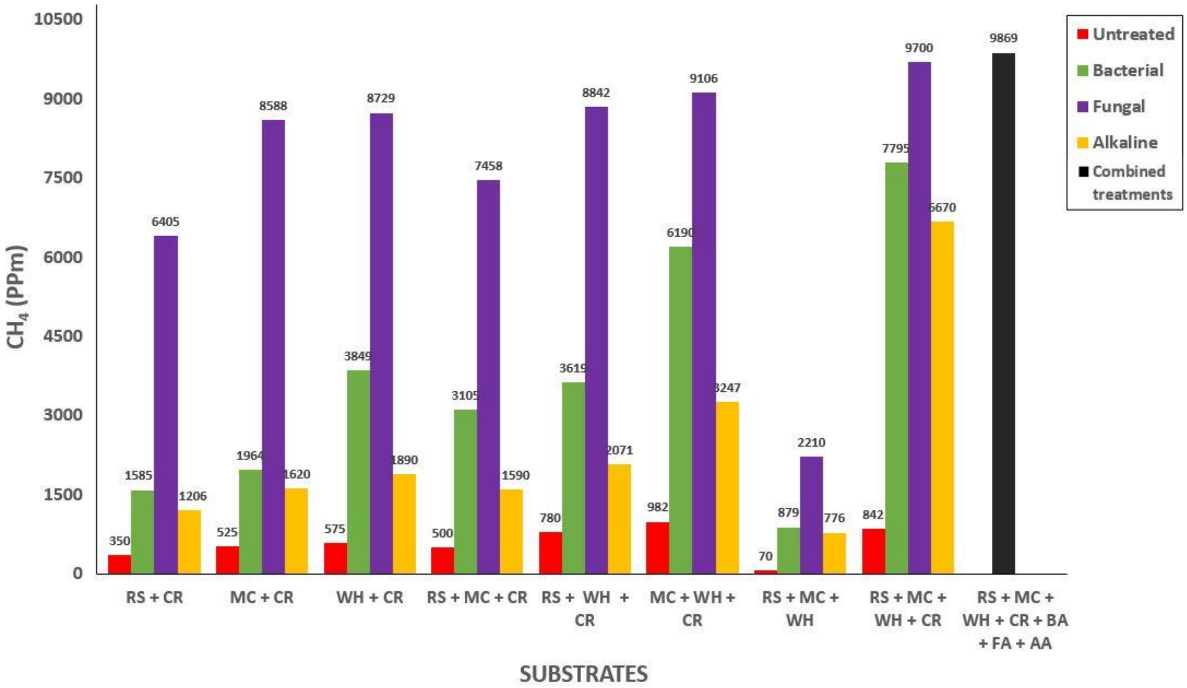

**Figure 1: Methane concentration (ppm) of the setups on the 42nd day.**

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

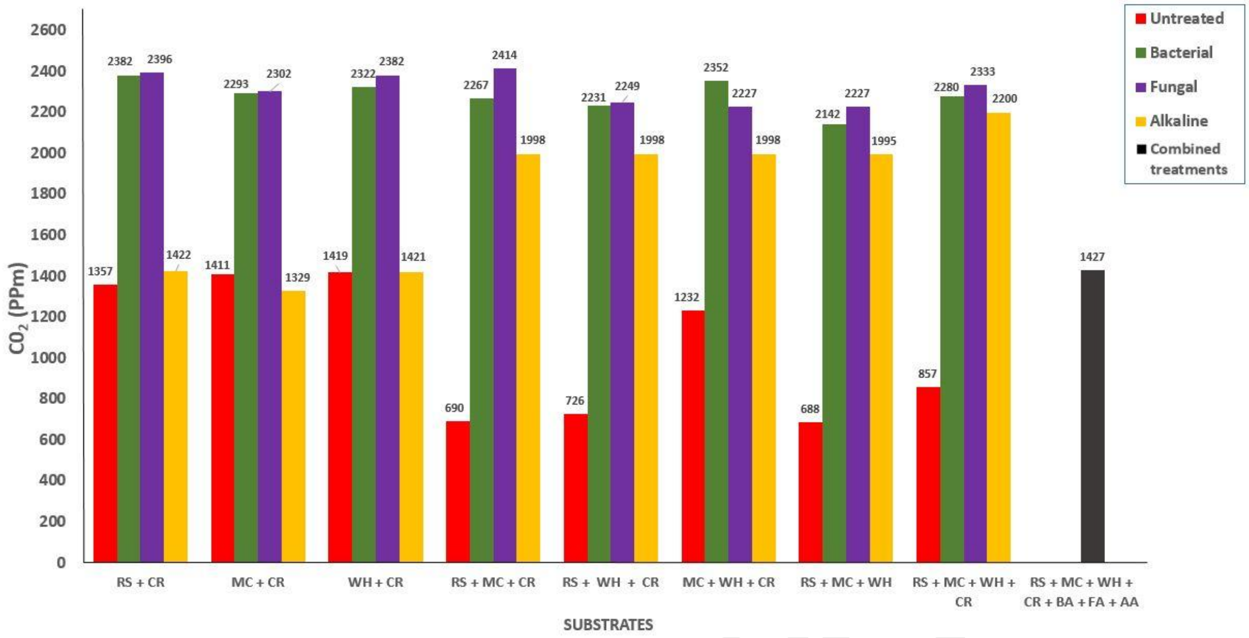

**Figure 2: Carbon dioxide concentration (ppm) of the setups on the 42nd day.**

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

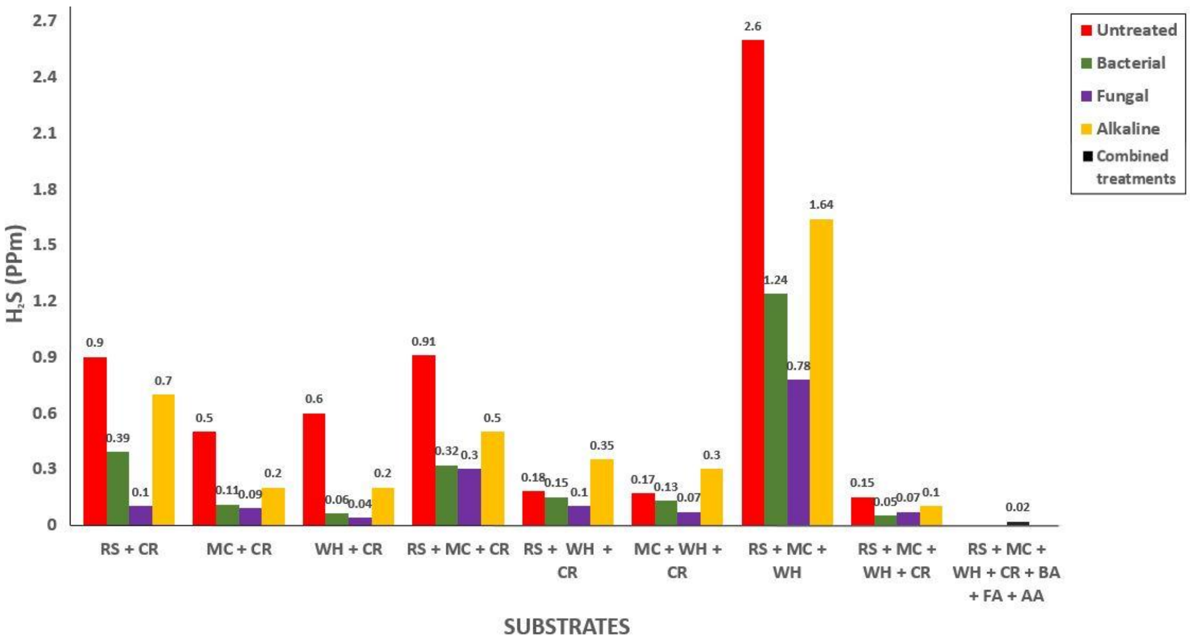

**Figure 3: Hydrogen ($H_2S$) sulfide concentration (ppm) of the setups on the 42nd day.**

**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 dioxide (25-50%), with minor impurities such as hydrogen sulfide, ammonia, etc [5]. 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 dioxide, which is the second major component can be sequestered and used to produce chemicals of industrial importance [14]. The percentage of methane, $CO_2$, and other components of biogas varies with the nature/maturity of feedstocks, temperature, water content, pH, organic loading rate and microbial actions [15]

### Methane content

The reports of previous studies [16 17, 18] 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 bioagumentation 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 [17]. The astounding performance of the fungal augmented substrates is in agreement with the report of Liu et al [19] who recorded up to 100% increase in methane yield using fungal pretreatment. Whereas Shah and Ullah [3] 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 asides 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 dioxide content

The $CO_2$ content of biogas is one of the contributors to the poor combustion of biogas [16]. 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 biogas.

### Hydrogen sulfide content

The very low concentrations (0.02 – 2.6 ppm) of $H_2S$ recorded in these substrates depict that 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 $H_2S$ 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 $H_2S$ 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 different treatments used were of significant 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. Preatreatments 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 used 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.**

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