RESEARCH PAPER

Effective Optimization of Bacterial and Alkaline Augmented Plants Substrate on Biogas Yield Using Operational Conditions

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Abstract

Biogas technology as an alternative energy source illuminates the need for less dependence on fossil fuel. This study highlights the importance of bacteria and alkaline augmentation on lignocellulose-rich biomass for enhanced biogas production. Three different plant substrates namely: maize cob (MC), rice straw (RS), water hyacinth (WH), were augmented with 10% alkaline (NaOH) and 1000 ml broth culture of isolated bacteria (*Bacillus sp*), while cow rumen (CR) waste served as inoculum. They were formed into three batches as Batch A (maize cob), Batch B (rice straw) and Batch C (water hyacinth). Hydraulic retention time, temperature and pH were monitored during the experiment while biogas production was obtained daily. The results showed that the highest biogas yield was obtained in bacteria augmented MC (626.265 ml/kg TS) at 28 °C and alkaline augmented WH (498.265 ml/kg) at 25 °C. The least biogas production yield was observed in bacteria augmented WH (290.398 ml/kg TS) and untreated MC (311.939 ml/kg TS) at 35 °C and 38 °C respectively. The methane concentrations of the biogas produced were highest in untreated WH and bacteria augmented RS at 3849 ppm and 8558 ppm, the least was observed in bacteria augmented WH at 1130 ppm. The pH of the slurry were within range as the least was 5.4 and the highest recorded was 7.4. The performance of the substrates indicates that plant substrates are impacted by augmentation. However, characteristics and operational conditions are vital irrespective of the required augmentation utilized to enhance production efficiency.

Keywords: lignocellulose, bioenergy, bioaugmentation, biodegradation, biogas

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1. Introduction

The world population was predicted to be about 9 to 10 billion by the year 2050 and among the ten largest countries worldwide, Nigeria is growing the most rapidly. Consequently, the population of Nigeria, currently the world's 7th largest, is projected to surpass that of the United States and become the third largest country in the world shortly before 2050 (United Nations Department of Economic and Social Affairs [UN] [1]) and this population must be provided with energy and materials. Since fossil fuel is non-renewable, it will be depleted and lead to limited supply [2]. Therefore, the need for the world population to transition towards a sustainable energy supply is paramount. One major key to this transition is the increased use of biomass to generate renewable energy. Nigeria as a country is rich in vast vegetation which is useful in agricultural activities. These activities such as composting, bush burning, and manure control indiscriminate disposal of lignocellulose-rich biomass. Lignocellulose biomass approximately constitute about 47% of global anthropogenic methane emission [3]. The involvement of these waste in anaerobic digestion is utilized in biogas production, as it has higher nutrient quality than the usual organic fertilizer and has reduced greenhouse gas emissions [4]. Lignocellulose biomass has been in focus recently, especially in the area of bioenergy, considering its potential.

Rice straw (RS), water hyacinth (WH) and maize cob (MC) as agricultural biomass can be utilized in bioenergy production. The aforementioned plants waste are rich in lignocellulose. The recalcitrant nature of these biomass interferes with its digestibility, mainly due to the presence of lignin. However, different approaches for delignification are available, which include but are not limited to enzyme, acid/alkaline, heat and microbial applications.

The application of enzymes in delignification of lignocellulose biomass as elucidated by Madubuike *et al.* [5] highlighted its relevance. However, the processes of isolating such enzymes is not feasible economically. The prospects of chemical and biological applications are promising but not without its limitations. Exploitation of chemicals for biomass degradation has been studied by several researchers. Chemical treatments of the lignocellulose has increased the availability of mono sugars, indicating its efficiency in degradation of lignin. Consequently, the utilization of alkaline for treatment has yielded improved outcome as some of the mono-lignin obtained from acid treatment are toxic [6].

This toxicity occurs at elevated temperatures; however, utilization of activated carbon or calcium chloride for precipitation can eradicate the toxic substance and thereby reduce the impact on enzyme or microbial community [7]. Some microorganisms can acclimatize to the environment in view of less concentration of the toxic compounds. Moreover, other substances such as nitric acid are formed at improved temperature with the toxic substances, e.g. furfural. Further, alkaline

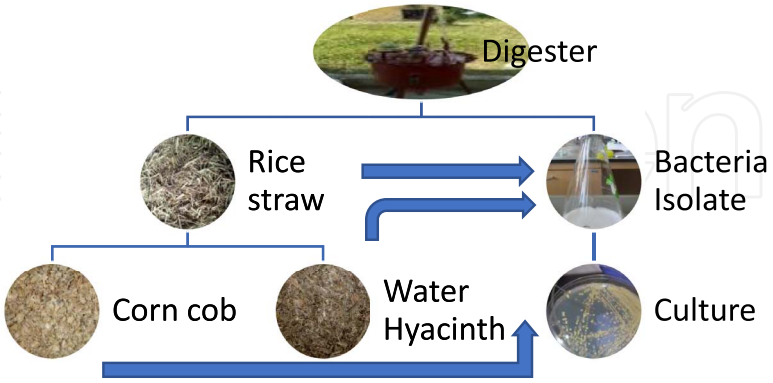
treatment involves the introduction of alkaline to the substrates for hydrolytic and lignocellulose disintegration. Alkaline impacts the structure of lignin, thereby making it components accessible for degradation. The base initiates the enlargement of the substance through reduction in crystalline index and improvement in the specific area of the biomass [8]. Besides, the influence of alkaline treatment is not limited to lignin. Hemicellulose with long-chain polysaccharides is dissolved to make available more mono sugars [9]. The energy imputed for alkaline treatment is low, and some products can be recovered at low temperature when utilizing alkaline, e.g. xylane. However, some alkaline can be recycled during treatment which makes it expedient compared to acid treatment.

Biological degradation of lignocellulose biomass mostly comprises fungi. Different species of fungi have been studied for this purpose. Fungi produce enzymes that cleave lignin, cellulose and hemicellulose either synergistically or separately. Fungi attack the plant cell wall through chemical breakdown which precedes the rot and color change in the substrate. Fungi produce different rots during breakdown which are categorized as white, brown, and corrosive rots and are distinguished with action niche [10].

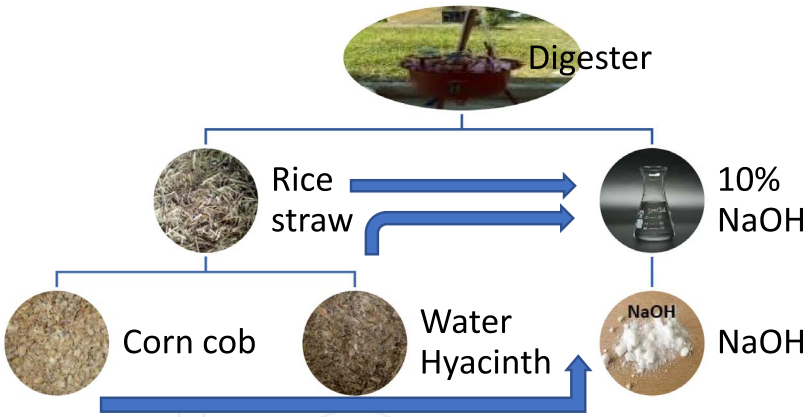
Additionally, the action of fungi in lignocellulose decomposition is time consuming. However, the exploitation of bacteria for depolymerization of lignin is gaining traction as fungi decomposition takes more time and is not economically feasible in terms of enzyme extraction. Although, the efficiency of lignin depolymerization by bacteria is limited, it is still attainable considering the economic advances attributed to it. Conversely, bacteria are efficient in degrading substrates with low quantity of lignin [11]. The application of cow rumen during anaerobic degradation of lignocellulos material is beneficial as it introduces the methanogens to the slurry. Different studies have stressed the impact of inoculum in the start-up digester as it influences the biogas production as well as the degradation rate of the substrates. The abundance of the methanogens is related to the inoculum source, suggesting its tolerance for ammonia [12].

Agricultural residues are abundant in Nigeria and after harvest most of these residues are abandoned as waste. The agricultural residues are rich in lignocellulose biomass, suggesting that they can be channeled to bioenergy production [13]. However, their lignin content is low when compared to the cellulose and hemicellulose content. Therefore, augmentation processes can enhance its efficiency in biogas production. This study intends to examine the impact of bacteria and alkaline augmentation to broaden insight in improving biogas production utilizing agricultural residue and how the operating conditions affect the substrates. The hypothesis tested was that bacteria and alkaline augmentation increases biogas yield in view of the low lignin and high cellulose and hemicellulose content of the substrates. The second objective is to enhance biogas production efficiency

through optimization of operational conditions. Different plants substrates, namely; RS, WH and MC were obtained for analyses. Cow rumen was applied as inocula in the batch digester for anaerobic degradation. Selected operational conditions were evaluated with biogas production level during the investigation.



a: Illustration of Substrates augmentation with Bacteria isolate.



b: Illustration of Substrates augmentation with Alkaline

2. *Materials and methods*

2.1. Collection of substrate samples

RS was collected in sterile polythene bags from Ihite-Uboma in Imo State and Abakaliki in Ebonyi State, both in Nigeria. MC was obtained from different agricultural farms in Owerri whereas WH was obtained from Amassoma river Nun at Bayelsa State, also in Nigeria. All test plant-based substrates were collected using surface sterilized polythene bags and transported to the laboratory for analysis.

2.2. Samples preparation

The plant substrates were all subjected to physical pretreatment by shredding into small sizes of about 2 mm, after which they were sun dried to reduce the moisture content of the waste. The shredded substrates were soaked in big water baths for two days to facilitate the breakdown of cellulose as described by Ofoefule *et al.* [14]. The physical pretreatment was to increase the surface area of the plant substrate and reduce the size for faster degradation. Alkaline augmentation was done by using 10% sodium hydroxide (NaOH) and bacteria augmentation was done using 1000 ml broth culture of *Bacillus sp*. The ligninase degrading bacteria (*Bacillus sp*) were isolated from decaying wood bark and termite gut using the method described by Bandounas *et al.* [15], while standard methods [16] were used for characterization and identification.

2.3. Substrates characteristics

Substrates compositions were determined considering its effect on the biogas production rate. The relevant substrate compositions such as pH, total solid (TS), total volatile solid (TVS), and proximate compositions were determined. The pH was measured using a pocket-size pH meter (Hanna's instrument) with model number 02895 A1, while TS, TVS and proximate compositions were determined using standard methods of Association of Official Agricultural Chemists (AOAC) [17]. Lignocellulose contents of the plant's substrate were measured using volatilization gravimetric method as described by Ugwu *et al.* [13].

2.4. Biogas digester fabrication

Biogas digester capacity of 54.883 L was fabricated with 16 Sheet gauges of cylindrical section of 50.272 L and conical section of 4.608 L, 2 bearings, silicone gum, 2 gate valves, suction pump, brass rods, flux, gasket sheet, bolts and nuts, stirrer shaft, gas discharge valve, manometric gauge, gas seal, gas brazing, electrodes, stand pipe and manometer pipe, and laboratory thermometer. The fabricated biogas digester with a capacity of 54 L was painstakingly cleaned to be deployed.

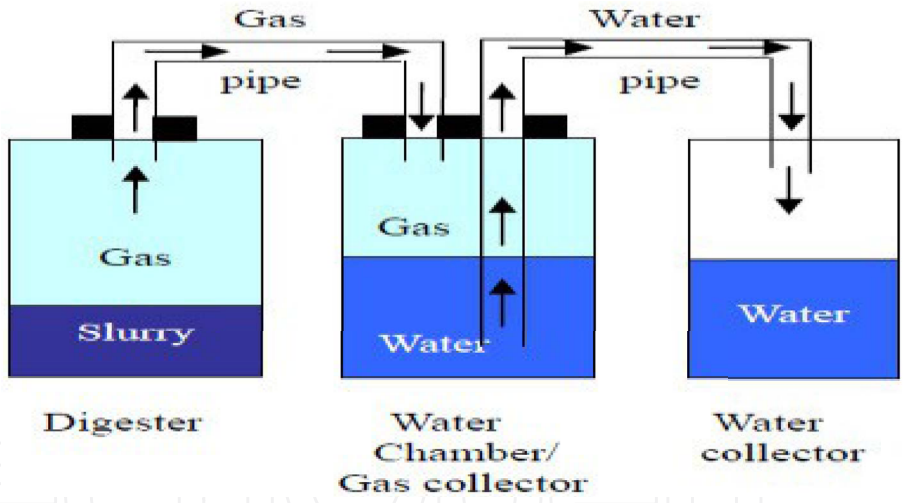
2.5. Experimental set-up

The different plant substrates were prepared in a ratio of 1:1 slurry with MC (4.68 w/v), RS (1.3 w/v), WH (3.64 w/v) and cow rumen waste (4 v/v) (Table 1) to occupy about 30 L volume of digesters each with a gas volume space of 24 L. Bacteria and alkaline augmentation was carried out according to Nwachukwu *et al.* [18]. About 10% alkaline and 1000 ml of isolated *Bacillus* species were introduced into each digester respectively, while each of the control was not augmented with either alkaline or bacteria. About nine fabricated biogas digesters set were utilized for the

Table 1. Plant substrates augmentation.

Substrates	Cow rumen (Cr) (v/v)	*Bacillus sp.* (Ba) (ml)	NaOH (Aa) (%)
Maize cob (MC)	4	1000	10
Rice straw (RS)	4	1000	10
Water hyacinth (WH)	4	1000	10
Control (Untreated)	4	—	—

experiment. The total digester volume was about 54 L. The pH was measured using a pocket-size pH meter (Hanna's instrument) with model number 02895 A1, while the mercury in gas thermometer fitted in a cork of the biogas digester was used to measure temperature. The digesters were monitored under mesophilic conditions for 42 days and water displacement method was utilized to determine volume of biogas produced, while methane concentration was obtained with Aero-Qual gas analyzer model 500 series [19].



c: Schematic biogas digester set-up.

2.6. Data analysis

Statistical analysis of data was done with Minitab software 2017 version (6MX8-OOXO-PEX5-52R27). The operational conditions (retention time and temperature) were analyzed using response surface methodology (RSM) which is a second-order polynomial model to determine the optimum condition by studying the functional relationships between responses and factors which is ascertained by estimating the co-efficient. Central composite design (CCD) was utilized.

3. Results and discussions

3.1. Effect of alkaline and bacteria augmentation on biogas production

Figure 1(a–c) shows untreated, alkaline and bacteria augmented plant substrates while Figure 2 presents methane concentration of the substrates. In Figure 1(a), the highest biogas production rate of 626.265 ml/kg TS was observed in bacteria augmented maize cob (MC + Cr + Ba), while the least was in alkaline treated maize cob [MC + Cr + Aa] (311.939 ml/kg TS). Figure 1(b) presents the highest biogas production rate in bio-augmented rice straw [RS + Cr + Ba] (459.640 ml/kg TS) and the least in untreated rice straw [RS + Cr] (272.1626 ml/kg TS). Untreated water hyacinth (WH + Cr) had the highest biogas production rate at 519.201 ml/kg TS, whereas the least production rate was in bacteria augmented water hyacinths [WH + Cr + Ba] (290.398 ml/kg TS) as presented in figure 1(c).

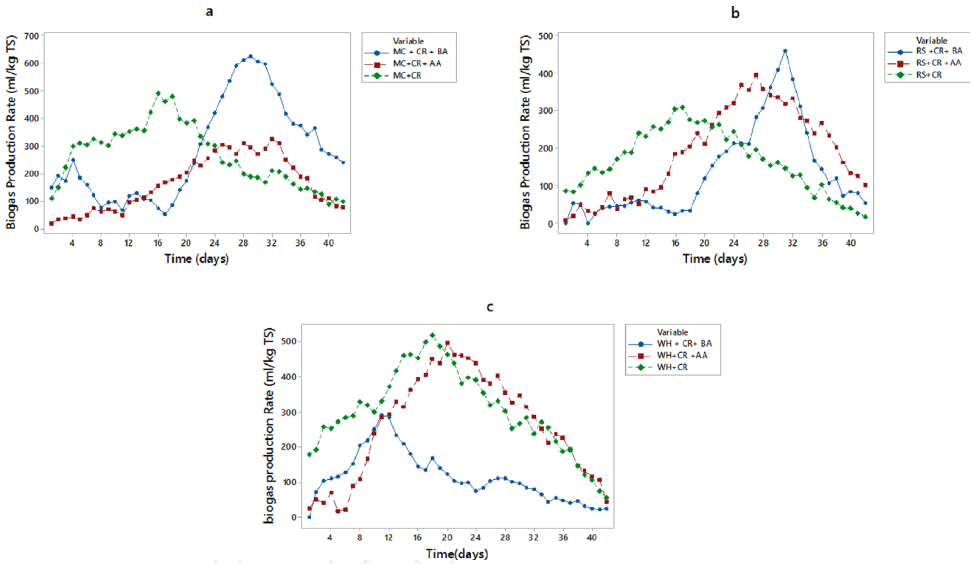


Figure 1. (a) Biogas production from maize cob with different augmentation methods, hydraulic retention time (HRT) 42 days. (b) Biogas production from rice straw with different augmentation methods hydraulic retention time (HRT) 42 days. (c) Biogas production from water hyacinth with different augmentation methods Hydraulic Retention Time (HRT) 42 days.

3.2. Methane concentration (ppm) of substrates in biogas production

The bio-augmented substrates of maize cob (MC + Cr) and rice straw (Rs + Cr) with lignin's degrading bacteria had the highest methane concentration of 2575 ppm and 8558 ppm respectively. However, untreated plant substrates of water hyacinth

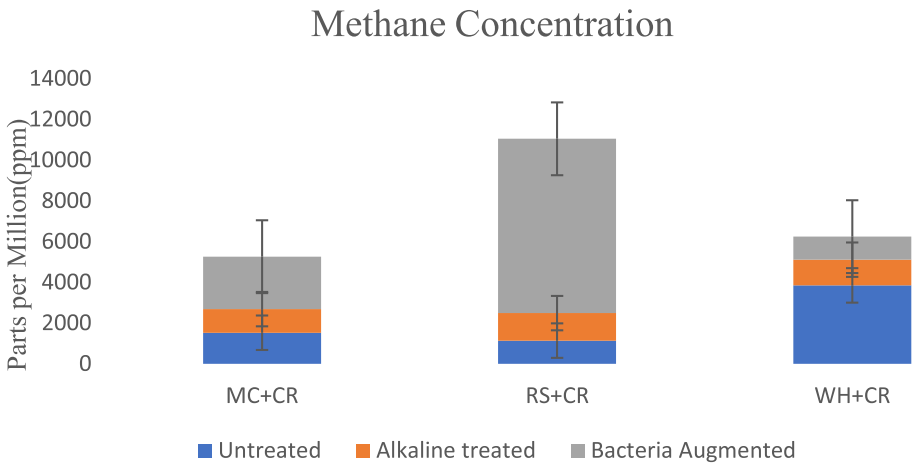


Figure 2. Methane concentration of substrates.

(WH + Cr) had 3849 ppm of methane. Generally, the least concentration of methane was obtained in the alkaline augmented plant substrates.

The attainment of biogas production of different plant substrates utilized is an indication of microbial interaction, operational conditions and substrates characteristics. Generally, the limited biogas production rate of alkaline augmented substrates may be attributed to the toxicity in the anaerobic digestion system. Alkaline treated MC performed the least in terms of biogas yield, while there was an improvement with RS and WH. Nonetheless, generated biogas yield was still less than that of bioaugmented substrates. Though, delignification potential is probable in view of lignin quantity of the substrates. The prospect of Na^+ enrichment might have hindered the biogas yield as sodium ion inhibits the microbial activities. Further, the formation of substances during lignin degradation, mostly phenolic compounds, has the potential of weakening the microbial consortia involved in the anaerobic digestion processes. However, acclimatization of microorganisms continues the degradation processes. Chen *et al.* [20] reported that excess Na^+ restrains interference with their metabolism of micro organisms while, Hierholtzer *et al.* [21] indicated that Na^+ leads to complete dehydration and quickens the osmotic pressure of methanogenic microorganism. Shetty *et al.* [22] remarked that exploitation of high concentrations of NaOH in treating rice straw yielded a lower biogas production compared to low concentration of alkaline. However, plant substrates with bacteria augmentation exhibited an improved biogas production yield than alkaline treated and untreated counterparts. This suggests that the addition of ligninase degrading bacteria to the plant substrates enhanced the biogas production more effectively. This agrees with Magdalena *et al.* [23] who reported that there was an increase in biogas production after bioaugmentation with shorter HRT when compared to control. The observed increase could be attributed to improved microbial activities in the bioaugmented biogas digester. This supports

Duran *et al.* [24] who observed that bioaugmentation with selected strains belonging to *Bacillus sp.*, *Pseudomonas sp.*, and *Actinomycetes sp.* showed a slight increase in biogas production.

However, the trend observed in methane concentration indicates the performance of the augmented substrates. Substrates augmented with ligninase producing bacteria had the most methane concentration, followed by untreated plant substrates. The least concentration of methane was established in the alkaline augmented substrate. This is probably a result of augmented alkaline (NaOH) which improved pH level of the medium above optimal as well as, toxicity probably because of lignification. This supports Duran *et al.* [24] who reported that increased level of NaOH in biogas production impairs the system as its elevate the pH of the medium.

3.3. Effect of hydraulic retention time (HRT) on biogas production

Daily biogas production rate of the plants substrates based on retention time 42 days revealed that the plants substrates attained their peak of production within the period of 15 to 32 days HRT as shown in figure 1. At Day 14, biogas production was on a steady increase, whereas the production rate started dropping towards the end of Day 42. This indicates that short retention time affects digestion of the plants substrates, while longer retention time than the optimum leads to a decline in biogas production from plants substrates. This corresponds to Alepu *et al.* [25] who observed that short HRT results in volatile fatty acid (VFA) accumulation in anaerobic environment and this caused low biogas production yield but, HRT longer than 15 days allows an efficient utilization of substrates in the biogas digester. Sithara & Kiran [26] also reported that retention time above optimum value leads to ineffective degradation of digester's component which attributes to low biogas yield.

3.4. Effect of substrates characteristics on biogas production

The substrates characteristics of MC, RS and WH as shown in figure 3(a) depicts that percentage TS, TVS, moisture, protein, fat, carbohydrate, fiber and ash contents. The highest moisture content of 12.93% was found in MC, while the least with 5.45% was in RS. MC recorded the least (1.54%) ash content while the highest (24.40%) was recorded in rice straw. Carbohydrate, crude protein and fat were all in the range of 24.22–9.21%, 14.58–5.67% and 6.83–0.65% respectively. The fiber content of MC was the highest at 70.0% while that of rice straw was the least at 24.1%. Further, TS and TVS were within the range of 94.55–87.07% and 85.53–70.82% respectively.

3.5. The percentage lignocellulose composition of the test plant substrates

The lignin content of RS was the highest (14.8%) while that of MC was the least (2.73%). There were differences in the values of hemicellulose, cellulose and

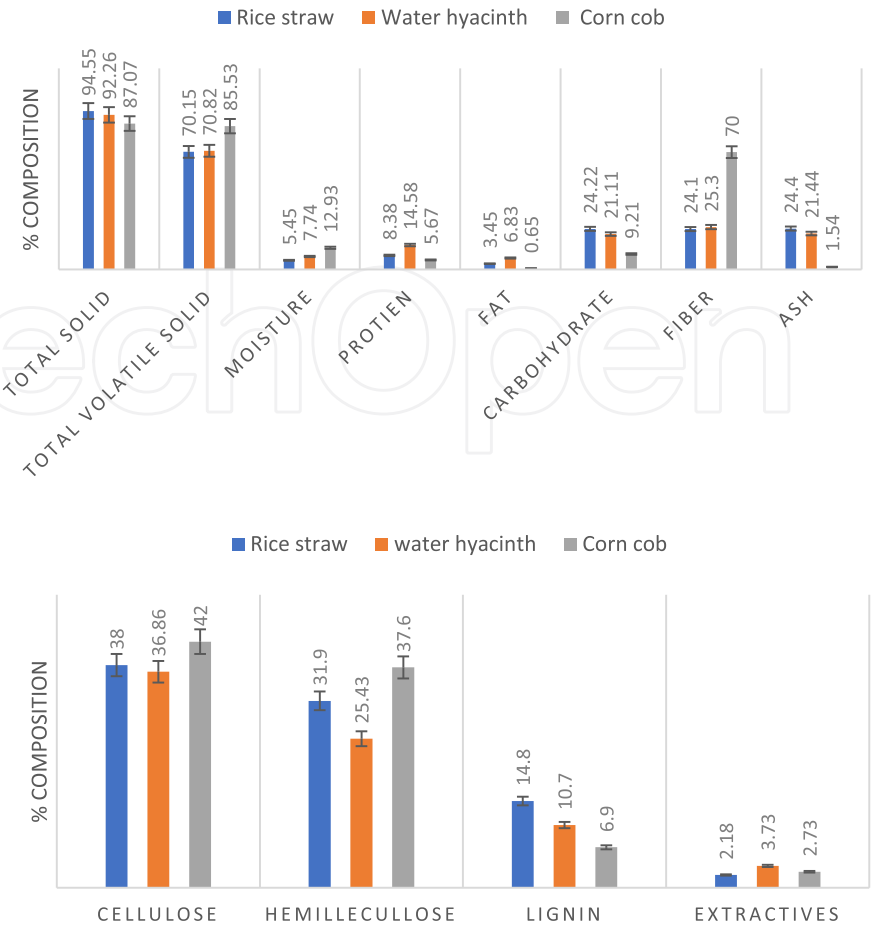


Figure 3. (a) Characteristics of plant substrates. (b) Lignocellulose components of test plant substrates.

extractive of the plant biomass obtained. Hemicellulose and cellulose recorded the highest values of 37.6%, and 42% in MC respectively, while WH had the least values of hemicellulose (25.42%) and cellulose (36.86%) as shown in Figure 3(b).

The high values of TS and TVS in the test plant substrates suggest the quality of plant substrates for biogas production. This supports the result of Igoni *et al.* [27] who reported that abundant TS of municipal solid wastes in an anaerobic digestion process correspond to high yield in biogas production. Moreover, Ofoefule *et al.* [14] in their input reported that in an anaerobic environment the degradation of TS and TVS by micro-organisms translates to increase in biogas production. Furthermore, the moisture content of the plant substrates was low and this was attributed to the fact that the substrates were dried in the sun to reduce water content prior to analysis. This was to preserve the plant substrate from spoilage. In biogas production the moisture content provides greater activity of water as soluble enzymes and coenzymes needed for metabolic activities will help the growth of microorganisms [28]. Therefore, the substrates were properly mixed with adequate

amount of water during loading. In addition, the carbohydrate content of the plant substrates showed that they are effective biogas production material. This supports the results by Osibote *et al.* [29] who reported that substrate rich in carbohydrate content produce more of propionate which removes hydrogen from methane during degradation for improved biogas production, while low level of crude protein further confirms the plant substrates as suitable biomass for production of biogas as protein-rich substrates yield high ammonia level which is toxic to the methanogens [30]. However, the lignin values of the substrates indicate they are not outrageous which makes it advantageous to biogas production with treatment considering its toxicity to methanogens. This agrees with Chen *et al.* (2008) who reported that lignin evaluation on methanogen toxicity using lignin monomers in kraft condensate is based on polarity.

3.6. Effect of pH on biogas production from plants substrates

Figure 4(a–c) shows the pH variations of biogas production of different plants substrates. In figure 5(a), the pH on biogas production of the different untreated plant substrates are shown.The initial pH of untreated plants substrates was between 5.4–6.0, while the final pH was within the range of 7.0–7.3. Initial alkaline substrate pH was observed to be between 7.1 and 7.4 and the final pH falls within 6.9–7.2 as is shown in figure 5(b). Furthermore, the bio-augmented initial pH ranges was 5.7–6.4 and the final pH was 6.9–7.4.

According to Shujun *et al.* [31], pH is critical to anaerobic digestion because it governs the metabolic activities of micro-organisms, and methanogens are affected more by low pH with limited growth rate than the fermentative organisms. An overall pH range of 5.4–7.4 was recorded for optimal biogas production. This was in line with Cavinato *et al.* [32] who reported that the optimal value of pH which maximizes biogas production was in the range of 6.5 to 7.2. Also, Tengku *et al.* [33] in his work showed that pH should be maintained within the range of 6.3–7.8 for better biogas production. Furthermore, the pH of alkaline augmented plants substrate for biogas production was within the range of 7.1–7.4 which is relatively high compared to that of untreated substrates. This may be a result of alkaline augmentation of the substrates before loading into the digester. The plants substrate augmented with bacteria has a pH variation of 5.7 to 7.4. These pH values were favorable for methanogenic activities except for MC and RS, for which the values ranged between 5.7 and 5.8 within the first 14 days of fermentation process but subsequently increased.

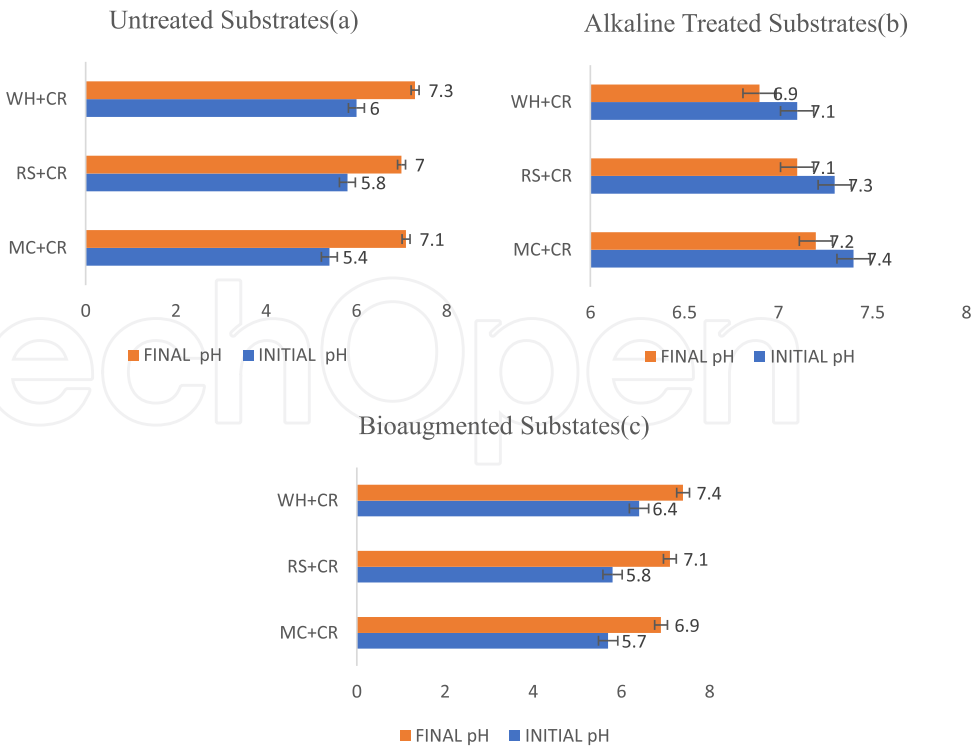


Figure 4. Biogas production pH of plants substrates.

3.7. Temperature effect on biogas production

Temperature is one of the major determinant environmental factors for biogas production. Figure 5 displays the temperature range at which the most biogas yield per day was obtained. Different temperature ranges have been identified in anaerobic process as the reaction tends to be either exothermic or endothermic. However, elevated temperature hastens the degradation process as it reduces retention period. Consequently, the probability of eliciting toxic substance to the organisms is eminent. Moreover, anaerobic degradation reactions have been supported in lower temperatures. Figure 5 demonstrates the temperature range at which the highest biogas yield per day was obtained. The highest biogas yield in untreated substrates was obtained from WH + CR (519.201 ml/kg TS) at 26 °C, whereas the least was in RS + CR (272.162) at 25 °C. Bacteria and alkaline augmented substrates had the highest biogas yield of MC + CR (626.265 ml/kg TS) and WH + CR (498.265 ml/kg TS) at 28 °C and 25 °C while, the least was attained in WH + CR (290.398 ml/kg TS) and MC + CR (311.939 ml/kg TS) at 35 °C and 28 °C respectively.

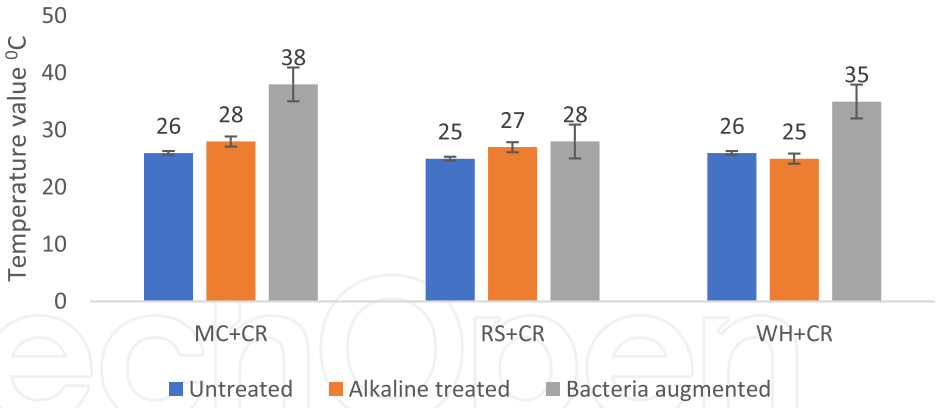


Figure 5. Temperature variation for biogas yield.

The mesophilic temperature variation of the biogas digester set-up exhibited a trend of increase in biogas yield with an increase in temperature except for bioaugmented digesters. This was supported by Manjula *et al.* [34] who observed in his work that increased temperature hastened biogas production rate. However, WH exhibited a negative trend because of temperature influence and this may be attributed to the high level of protein content of the substrates when compared to other substrates. Therefore, ammonia toxicity is imminent as the temperature increases during protein degradation. This agrees with Appels *et al.* [35] who reported that increase in temperature has a positive effect on microbial activities but also corresponds to increase in ammonia.

3.8. Response surface model

This model was applied to obtain the effect of retention time and temperature on the plant substrates used for biogas production. The model shows the single and interactive terms of operational conditions. The surface plots in Figure 6 depicts the influence of each variables (temperature and retention time) on biogas production of the plant substrates. The shape of the surface plot expose the response efficiency of the substrates as regards to the interaction between the two variables. Figure 6(a) shows the three-dimensional plot of retention time and temperature on biogas production. The figure demonstrated that increase in retention time and temperature causes a decline in biogas production. Although, temperature had a greater implication than retention time. Figure 6(b) shows that increase in retention time and temperature increases biogas production. However, temperature had more effect on biogas yield than retention time as the curvature shape of the figure suggests that increase in retention time beyond the midpoint signals a decrease in

the biogas yield. Figure 6(c) shows that as retention time and temperature increases, biogas yield increases. Temperature demonstrated a greater significance in biogas yield than retention time.

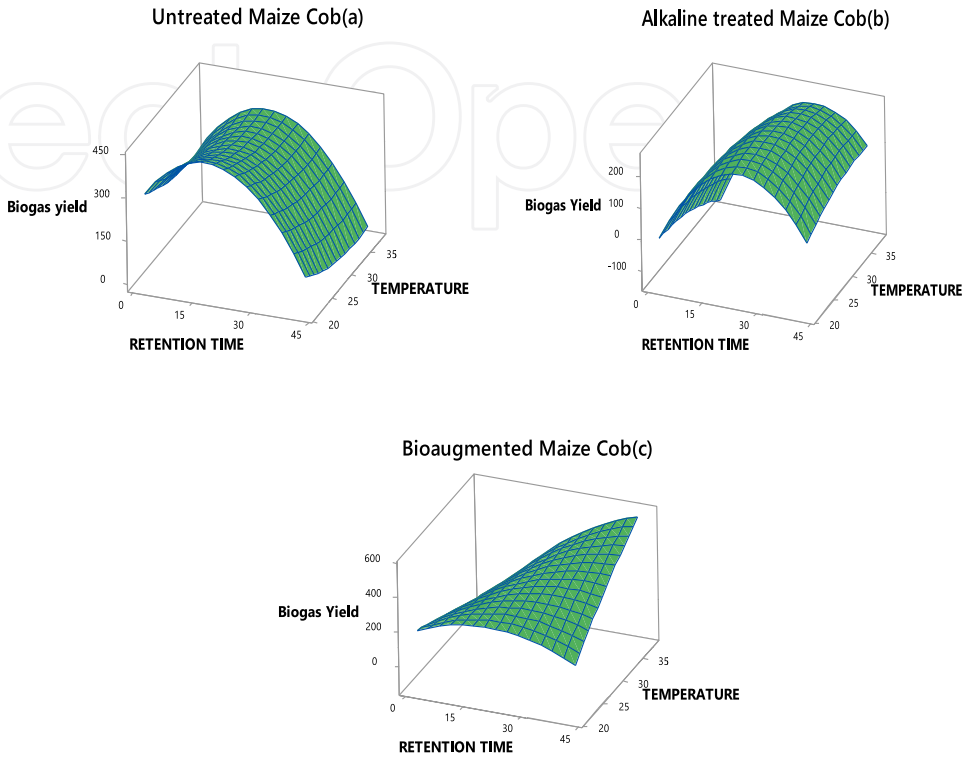


Figure 6. Response surface plot of MC for biogas production.

3.8.1. Optimization of biogas production

Optimization of biogas was conducted on bacteria augmented MC considering its excellent yield. Typically, the intersection of numerical values maximize the desirability function. The choice of variables for process operation ranges between temperature (22–38 °C) and hydraulic retention time of (1–42 days) and was precise for optimum determination. This was displayed by the optimization plots as shown in figure 6 below. The optimum temperature and hydraulic retention time for best yield of biogas production using bioaugmented MC was 42 (days) at 38 °C with desirability function of 0.8889 (see Figure 7).

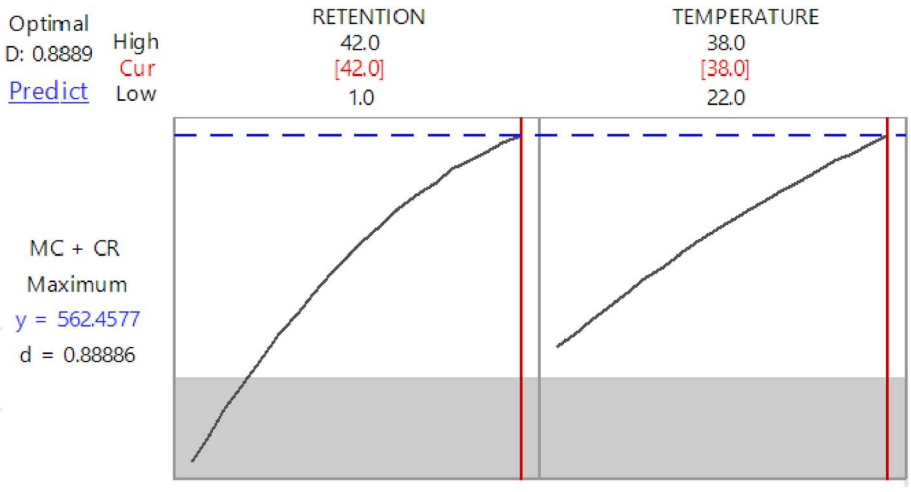


Figure 7. Optimization plot of bioaugmented MC.

4. Conclusion

Biological and chemical treatment approach is a method of delignification of lignin in substrates for bioenergy production. This method is an enhanced technique mainly utilized in lignocellulose substrates to free the degradable materials embedded in it. However, with the contribution of operational and process conditions, lignocellulose materials are characterized with low biogas yield. Therefore, treatment options were engaged and the result showed that alkaline augmentation and bioaugmentation of lignocellulose-rich plants substrate is an efficient method of degrading the lignin content to enhance production of biogas energy. Bioaugmented plant substrates indicates that isolated *bacillus sp* augmentation is effective in lignin degradation as high yield was observed in maize cob and rice straw, with low yield in water hyacinth due to ammonia intolerance of the methanogens. Alkaline augmentation increased degradation but with less biogas yield which may be due to the high concentration of NaOH involved during the study. Hence, the efficiency of NaOH augmentation depends on the concentration of the alkali utilized, the operating parameters and the composition of the plant substrates. However, the methane concentration pattern reflected the efficiency of the biological treatment approach as the bacteria augmented substrates had the highest yield tailed by the untreated substrates, while the alkaline treated substrates had low yield indicating inefficient fermentation or degradation. The treatment approach proved to be highly efficient in degrading lignocellulose materials in capacity of application.

Conflict of interest

The authors declare no conflict of interest.

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