



**Original Paper**

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## Proximate and compositional assessment of pretreatment methods on selected lignocellulose biomass for biogas production

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Received: 03-05-2023

Accepted: 16-08-2023

Published: 31-08-2023

### ABSTRACT

The efficient conversion of lignocellulosic biomass into biogas is a critical avenue in sustainable bioenergy production. Pretreatment enhances the accessibility of lignocellulose components for subsequent enzymatic hydrolysis and biogas production. This study evaluated the proximate and compositional changes induced by various pretreatment techniques on lemon grass and fluted pumpkin stalk, with a focus on optimizing biogas yield. Alkaline, hydrothermal, and combined hydrothermal pretreatment processes were applied on the feedstocks. The biomass surface characteristics were determined through proximate and compositional analysis. The characterization of the untreated biomass showed that lemon grass and fluted pumpkin stalk recorded; dry matter, moisture content, fixed carbon, total kjeldahl nitrogen and ash content in the ranges of 94.49%-94.56%, 5.44%-5.51%, 26.35%-28.95%, 1.33%-1.932% and 6.00%-12.00% respectively. The cellulose values of the untreated biomass ranged from 34.04% for fluted pumpkin stalk to 38.66% for lemon grass. However, the content of the cellulose increased with pretreatment to values ranging from 44.20% to 51.06%. The results of this analysis showed that the selected lignocellulose biomass has properties that are consistent with past literature stating them as suitable substrates for biogas production. Nonetheless, the potential for increased biogas yield from anaerobic digestion is greater when combined hydrothermal and alkaline pretreatment is used. © 2023 International Formulae Group. All rights reserved.

**Keywords:** Anaerobic; energy; physicochemical; pumpkin; renewable.

### INTRODUCTION

Individuals and policy makers have recently expressed tremendous concern over the usage of conventional fossil fuels for human activities and the implications this has had on the environment. This recent eco technological trend in the world has resulted in the gradual shift from the conventional fossil fuels to the use of clean sources of energy. A

wide range of alternative and renewable energy sources such as; solar, wind, geothermal, hydropower and biomass are currently being employed to address the growing environmental issues associated with the use of fossil fuels (Yoo and Pan, 2015). Biogas technology is one of such eco technological solutions, which can help meet our energy needs and dramatically lower the greenhouse

gas emissions associated with the energy production from conventional fossil fuels (Wei, 2016). Biogas can be generated from the anaerobic digestion (AD) of a variety of feedstock's such as organic waste from animals, lignocellulose biomass, municipal garbage, and crop residues. Numerous research have demonstrated the ability of municipal, industrial, slaughterhouse, and agricultural wastes to produce biogas through anaerobic co-digestion or direct digestion (Kpata-Konan et al., 2011; Nikiema et al., 2015; Traoré et al., 2016). The gas produced from the anaerobic digestion of the feedstock's comprises of approximately 50-70% methane, 30-40% carbon dioxide, traces of nitrogen and hydrogen sulphide (Michael and Ben, 2018; Karuppiah and Azariah, 2019). Lignocellulose biomass is natural resource produced from waste products from the forestry sector, agricultural waste, or bioenergy plants like Switch grass, Napier grass, or Miscanthus (Aftab et al., 2019). Plant substrates such as fluted pumpkin stalk and lemon grass are also examples of lignocellulose biomass that can be used for biogas production. The primary components of lignocellulose biomass are cellulose and hemicelluloses, which are kept together by a network of interconnected substances known as lignin. Lignin physically protects the cellulose and hemicellulose portion of the cell structure from enzymatic hydrolysis (Aftab et al., 2019). The first phase (hydrolysis) of the anaerobic digestion occurs when bacteria breaks down complex polymers of the biomass into simpler compounds such as fatty acids, amino acids and sugars (Cotana et al., 2015). Nevertheless, due to the recalcitrant nature of the lignin, enzymatic hydrolysis of the lignocellulose biomass is difficult. Therefore, for the effective enzymatic hydrolysis, one or two or more pretreatment procedures need to be done on the lignocellulose biomass to enable the microorganisms gain access to the cellulose and hemicellulose component of the plant structure. There are other processes that results in the hydrolysis of the lignocellulose biomass, these include changing of the lignin structure, expanding the porosity and surface area, minimizing cellulose crystallinity, elimination of both the lignin and hemicellulose, as well as,

part depolymerization of the hemicellulose structure (Harmsen et al., 2010). Pretreatment also provides microorganisms in the anaerobic digester with a larger surface area to increase and speed up the degradation process of the lignocellulose biomass (Mood et al., 2013; Alaswad et al., 2015; Rodriguez et al., 2017). Many pretreatment techniques have been created in the recent years and are generally categorized into physical, chemical (alkaline, acid or organosolv), biological and combined pretreatments. Pretreatment of lignocellulose biomass is a crucial aspect of any successful anaerobic digestion process. Dahunsi et al. (2018), states that the application of pretreatment procedures on the lignocellulose biomass would help improve the accessibility of microorganisms to the cell structure thereby improving biogas yield. In this study, alkaline, hydrothermal and combined (hydrothermal and alkaline) pretreatments were implemented to improve the digestibility of lignocellulose biomass, lemon grass and fluted pumpkin stalk. The effects of these pretreatment techniques on the proximate and compositional properties of the selected lignocellulose biomass were also evaluated.

## **MATERIALS AND METHODS**

### **Materials and biomass preparation**

The lemon grass was harvested during October from the Ecological Garden of the Petroleum Training Institute, Effurun, Delta State, Nigeria and the stalk of the fluted pumpkin was obtained in bulk on market days from Effurun Market, Uvwie Local Government Area of Delta State, Nigeria. The selected biomasses were thoroughly washed to remove any trace impurities and sundried for 10days. The dried biomasses were pulverized using a hammer mill and sieved to a less than 2 mm particle sizes to remove unwanted particles sizes. Analytical grade Sodium Hydroxide pellets were used for the alkaline pretreatment of the biomass. 100g each of lemon grass and fluted pumpkin stalk were measured and used for this analysis.

### **Pretreatment techniques**

In this study, three pretreatment techniques namely; alkaline (Sodium Hydroxide), hydrothermal and combined

(hydrothermal and alkaline) pretreatment were employed.

### **Alkaline pretreatment**

For this investigation, the approach utilized by Dahunsi et al. (2018) was somewhat modified. The already ground and sieved (physically prepared) biomass was subjected to an alkaline pretreatment using NaOH. For this, 3 g of NaOH pellets per 100 grams at 55°C for 24 hours with a solid loading of 35 g TS/L<sup>-1</sup> was used (Dahunsi et al., 2018). NaOH was selected in light of earlier publications stating that it is one of the best alkalis for lignocellulose's alkaline pretreatments (Dahunsi et al., 2018). After the pretreatment, the mixture was washed with distilled water to get rid of the extra alkali and bring the pH level back to 7-8. The substrate was stored in a sampling container and used for analysis.

### **Hydrothermal pretreatment.**

In this procedure, the lignocellulose biomass was thoroughly mixed with water and placed in a pressure pot and heated at 170°C for one hour (Awoyale and Lokhat, 2021). The temperature was chosen because prior research has shown that heating lignocellulose biomass to higher degrees (above 180°C) can cause inhibitory chemicals to develop, which could hinder the anaerobic digestion process (Dahunsi et al., 2018). The heated vessel was afterwards allowed to cool in a desiccator, and its contents were filtered and kept in a sampling container. The filtrate from the hydrothermal process of lemon grass, on the other hand, can be ingested due to its therapeutic and antioxidant characteristics (Alfa et al., 2012).

### **Combined hydrothermal and alkaline pretreatment.**

This method involves combining the procedures of the hydrothermal and alkaline pretreatment methods stated above. However, at the end of the hydrothermal pretreatment, the biomass was further subjected to alkaline pretreatment (NaOH) using the procedure stated in the alkaline pretreatment. Subsequently, the sample was stored in airtight sampling container for further analysis (Awoyale and Lokhat, 2021).

### **Surface characterization of untreated and pretreated lignocellulose biomass**

Surface characterization was carried out on the untreated and pretreated samples at the central laboratory of the Department of Soil Science, Ahmadu Bello University (ABU), Zaria, Kaduna State, Nigeria to determine the proximate and compositional properties of the biomass.

### **Proximate analysis**

The moisture and ash content were analyzed using ASTM D 1037 and ASTM E1755- II method as stated in Efetobor et al. (2022) and Awoyale and Lokhat, (2021). The total solids (Dry matter) content were determined using the ASTM D2974 method as stated in Iweka et al. (2021). The carbon content was ascertained using ASTM D3174-76 (Efetobor et al., 2022) while the nitrogen content was determined using Kjeldahl method as described by Mukhtar et al. (2022).

### **Compositional analysis**

This was carried out to give more insight on the chemical composition of the substrates. The hemicellulose, cellulose and lignin composition was analyzed based on NREL, Laboratory Analytical Procedure (LAP 02, 03) as stated in Patinvoh et al. (2017) and Ugwu et al. (2021).

### **Extractive**

Soxhlet extractor was used to determine the percentage of extractives in the samples. This was ascertained by the addition of 150 ml of acetone to 1.0 g of the biomass and heated for 3 hours at 80°C. Afterwards the sample was dried in an oven at 107°C until a constant weight was achieved (Efetobor et al., 2022). The extractives were calculated from the equation;

$$\text{Extractive}(E) = A - B \quad (1)$$

Where;

A= Mass of sample substrate

B=Mass of sample residue after drying

E= Amount of extractive (g)

### **Lignin content**

2.0 g of the material were hydrolyzed in a water bath at 30°C for 60 minutes using 72%

Sulfuric acid (H<sub>2</sub>SO<sub>4</sub>). To maintain homogeneity, the sample was mixed every 5 minutes during this procedure. Subsequently, 4% H<sub>2</sub>SO<sub>4</sub> was used in an autoclave for 1 hour at 121°C to perform a second hydrolysis of the sample. The sample was dried at 105°C and heated at 575°C at a muffle furnace to determine the acid insoluble lignin (Ugwu et al., 2021). Similarly, the acid soluble lignin was achieved by measuring the absorbance of the acid hydrolyzed samples at 320nm. The amount of lignin present in the sample was determined by adding the amounts of soluble and insoluble lignin

### Hemicellulose

The hemicellulose (H-cell), lignin, cellulose components were determined through thermo gravimetric method as described by Ugwu et al. (2021); Awoyale and Lokhat (2021) and Efetobor et al. (2022). The hemicellulose content was ascertained by adding 500mol/m<sup>3</sup> of NaOH to 150 ml of distilled water containing 2.0 g of the sample. To neutralize the sample, this combination was boiled for 90 minutes, sieved, and rinsed with distilled water. The sample was then dried in an oven at 105°C to 107°C until a consistent weight was obtained. The amount of hemicellulose is determined by the equation below;

$$\% \text{ Hemicellulose} = \% B - \% C \quad (2)$$

Where,

B = initial weight of free extractive

C = weight of content after heating

### Cellulose

The cellulose of the lignocellulose biomasses was determined using the method described by Ugwu et al. (2021). The cellulose content is calculated by using the equation;

$$\begin{aligned} \text{Holocellulose} &= \text{Cellulose} \\ &+ \text{Hemicellulose} \\ \text{Cellulose} &= \text{Holocellulose} - \\ &\text{Hemicellulose} \quad (3) \end{aligned}$$

## RESULTS

### Proximate analysis of the lignocellulose biomass

The results of the proximate analysis of untreated and pretreated biomass are presented in Tables 1 and 2. The moisture content values of the untreated biomass varied from 5.44% for lemon grass to 5.51% for pumpkin stalk. The highest moisture content of the pretreated samples was recorded in alkaline pretreated pumpkin stalk (7.86%), whereas, combined pretreated lemon grass had the lowest moisture content of 3.83%. The dry matter values of the samples ranged between 92.14% and 96.17% in alkaline pretreated pumpkin and combined pretreated lemon grass respectively. The ash content of the untreated biomass was 6.00% for lemon grass and 12.00% for pumpkin stalk. Similarly, the values of the ash content of the pretreated samples were within the range of 0.20% (combined pretreated pumpkin stalk) to 14.00% (hydrothermal pretreated pumpkin). Furthermore, C/N ratio of the samples ranged from 14.98 for untreated pumpkin stalk to 54.59 for hydrothermal pretreated lemongrass.

### Compositional analysis of lignocellulose biomass

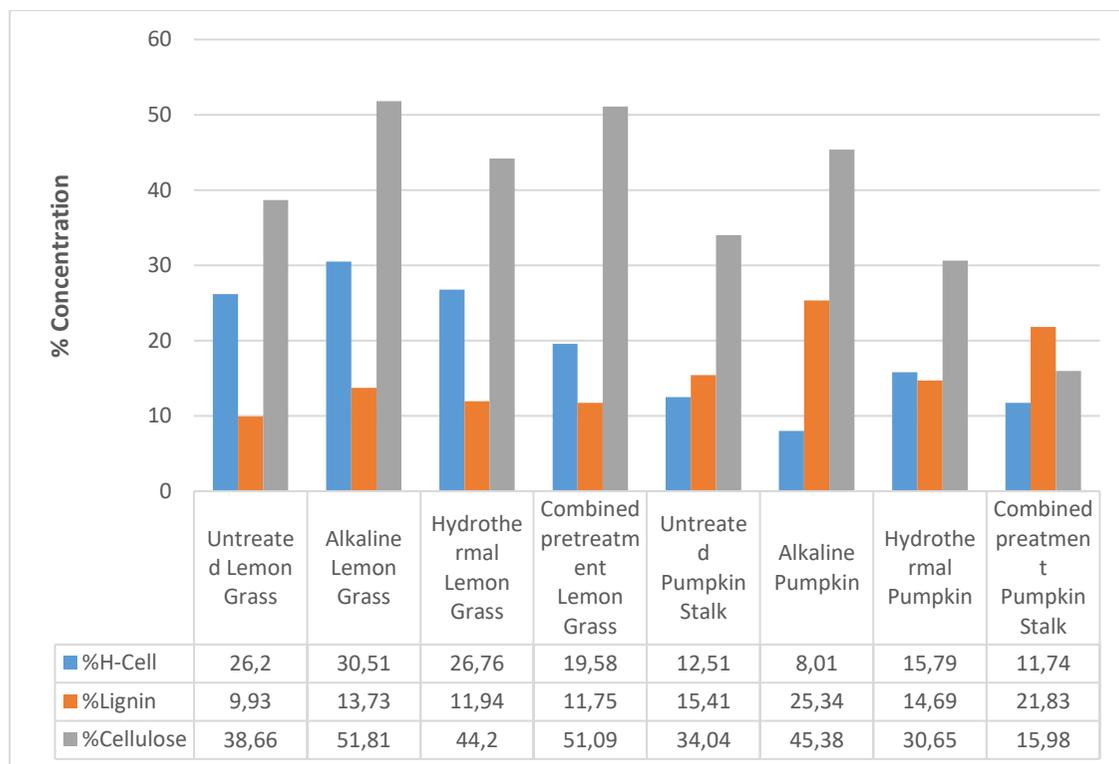
The results of the surface characterization of the different samples (untreated and pretreated lignocellulose biomass) are presented in Figure 1. The compositional analysis of untreated lemon grass revealed a 26.20%, 9.93% and 38.66% hemicellulose, lignin and cellulose content respectively while, the untreated pumpkin stalk had a hemicellulose, lignin and cellulose value of 12.51%, 15.41% and 34.04% respectively. The cellulose values of the pretreated biomass ranged from 15.98% for combined pretreated pumpkin stalk to 51.81% for alkaline pretreated lemon grass while the lignin content varied between 11.75% (combined pretreated lemon grass) and 25.34% (alkaline pretreated pumpkin stalk). Similarly, alkaline pretreated pumpkin stalk had the lowest hemicellulose value of 8.01% as against the 30.51% alkaline pretreated lemon grass that recorded the highest.

**Table 1:** Proximate analysis of the untreated (raw) lignocellulose biomass.

Substrate description	%DM	%MC	%Carbon	%N	C/N ratio	%Ash
Untreated lemon grass	94.56	5.44	26.35	1.330	19.81	6.00
Untreated pumpkin stalk	94.49	5.51	28.95	1.932	14.98	12.00

**Table 2:** Proximate analysis of the pretreated lignocellulose biomass.

Substrate description	%DM	%MC	%CARBON	%N	C/N RATIO	%ASH
Alkaline lemon grass	93.98	6.02	27.47	0.532	51.83	0.20
Hydrothermal lemon grass	93.87	6.13	37.67	0.686	54.59	10.00
Combined hydrothermal and alkaline pretreatment lemon grass	96.17	3.83	31.55	0.784	39.99	4.00
Alkaline pumpkin	92.14	7.86	26.91	1.246	21.53	4.00
Hydrothermal pumpkin	95.27	4.73	36.93	1.540	23.98	14.00
Combined hydrothermal and alkaline pretreatment pumpkin	94.08	5.92	33.78	0.728	46.27	0.20



**Figure 1:** Compositional analysis of untreated and pretreated lignocellulose biomass.

## DISCUSSION

### Effect of pretreatment on the proximate composition of the lignocellulose biomass

The composition of the different biomass before and after pretreatment is given in Tables 1 and 2. The results revealed a 42.65% increase in the moisture content of the alkaline pretreated pumpkin stalk as compared to the untreated pumpkin biomass. Alfa et al. (2014) in their study on the production of biogas from lemon grass, revealed the moisture content of 11.42%, which is higher than the value (7.86%) obtained in this study. The reason for the low moisture content in this study can be attributed to the drying of the substrates to reduce the water content before analysis and preserve them from spoilage (Ugwu et al., 2022). Moisture content is a crucial element in anaerobic digestion since it is required for metabolic processes and promotes the growth of bacteria that produce biogas. Nevertheless, the low moisture content recorded in some samples are not of significant concern as the substrates were mixed with adequate amounts of water to form slurry before loading into the anaerobic digester (Ugwu et al., 2022). The results showed that combined pretreated lemon grass had the highest dry matter content of 96.17%. The viscosity of the substrates in the digester can be impacted by high total solid content (dry matter), which can clog the digestion and slow down mass transfers inside the anaerobic digester thereby resulting in low biogas yield (Asri et al., 2020).

The result of the ash content obtained from the untreated lemon grass is in tandem with the ash content obtained by Abid and Bashir (2019). Furthermore, the ash content (12.00%) obtained in untreated pumpkin stalk was higher than the value (3.5%) obtained by Uzodinma et al. (2015). Nevertheless, Abid and Bashir (2019) went on to say that, among other things, the environmental factors that influenced the growth of the plants and the species that were employed in the experiments can account for differences in the proximate

analysis results when compared to previous literatures. In addition, lignocellulose biomass inherent qualities also heavily rely on organic components (lignin, cellulose, and hemicellulose), and that the makeup of these elements varies according to the species, climatic circumstances, origin, etc (Deshpande et al., 2022).

### Effect of pretreatment on the carbon/nitrogen ratio of the lignocellulose biomass

One of the crucial factors that impacts how well microorganisms perform during an anaerobic digesting process is the carbon nitrogen (C/N) ratio. Tables 1 and 2, respectively, show the C/N ratios of the samples of lignocellulose biomass. Low C/N ratio substrates hinder methane synthesis by causing the generation of ammonia, whereas high C/N ratio substrates cause the formation of proteins and have an impact on the structure and growth metabolism of the microorganisms (Deublein and Steinhauser, 2008). Odejobi et al. (2022) suggest that the ideal C/N ratio for AD should be between 20.1 and 30.1. Therefore, the C/N ratio was high in combined pretreatment pumpkin, combined pretreatment lemon grass, alkaline lemon grass, and hydrothermal lemon grass with values of 46.27, 39.99, 51.83, and 54.99 respectively. Mukhtar et al. (2020) noted that a high C/N ratio causes the methanogens to rapidly consume the nitrogen content. As a result of their quick nitrogen intake to satisfy their needs, there won't be any leftover nitrogen to react with the remaining carbon content, which is necessary for microbial growth, resulting in minimal methane generation. On the other side, a low C/N ratio will cause nitrogen to be released and accumulate as ammonia. This phenomenon raises the pH of slurry and inhibits microbial activity in the anaerobic digestion process. Nevertheless, the co-digestion of a nitrogen-rich inoculum and a lignocellulose biomass can assist stabilize the C/N ratio during the AD process and make it

favorable for microbial activities. The best C/N ratios, according to Vogeli et al. (2014), can be achieved by combining diverse feedstock materials with high and low C/N ratios, such as lignocellulose biomass and animal manure. The anaerobic co-digestion of animal manure with lignocellulose biomass will boost nutrient and buffer capacity of the slurry. This in turn stabilizes the C/N ratio by reducing ammonia deficit and improving the biogas yield (Singh, et al., 2017).

### **Effect of pretreatment on the compositional properties the lignocellulose biomass**

The characterization of the lignocellulose biomass is essential for in determining its suitability for biogas production. Heterogeneity is an innate characteristic of lignocellulose biomass thus it is vital that the biomass is characterized to understand the physiochemical properties and its suitability for bioconversion to sustainable products (Deshpande et al., 2022). The results of the surface characterization of the different samples (untreated and pretreated lignocellulose biomass) are presented in Figure 1. In alkaline pretreated biomasses, reduction in the cell composition was only evident in hemicellulose content of alkaline pretreated pumpkin stalk. There was a 35.97% reduction in the hemicellulose content as compared to the untreated pumpkin stalk. On the other hand, the alkaline pretreatment of pumpkin stalk resulted in an increase in lignin and cellulose fraction by 64.4% and 33.31% respectively. Similarly, all the cell components of hemicellulose, lignin and cellulose present in lemon grass increased by 16.45%, 38.26% and 34.01% after pretreatment with NaOH. In the alkaline pretreated biomasses (Figure 1), reduction in the cell composition was only evident in hemicellulose content of alkaline pretreated pumpkin stalk. There was a 35.97% reduction in the hemicellulose content as compared to the untreated pumpkin stalk. On the other hand, the alkaline pretreatment of pumpkin stalk resulted in an increase in lignin and cellulose fraction

by 64.4% and 33.31% respectively. Similarly, all the cell properties of hemicellulose, lignin and cellulose present in lemon grass increased by 16.45%, 38.26% and 34.01% after pretreatment with NaOH. Although, lignin content can be removed with some decent efficiency by alkaline pretreatment, this may also increase the biomass' cellulose content (Olatunji et al., 2021). Alkaline pretreatment technique results in the swelling of the cell fiber thus, creating large surface area for microorganisms to access the cell structure. This technique also reduces the bond between the lignin and the carbohydrate thereby breaking the lignin structure. Nonetheless, in this investigation, the alkaline pumpkin stalk's decreased hemicellulose content was noticeable, with a 36% decrease in the measured hemicellulose value as compared with the untreated pumpkin stalk. Although, lignin content can be removed with some decent efficiency by alkaline pretreatment, this may also increase the biomass' cellulose content (Olatunji et al., 2021).

Alkaline pretreatment technique results in the swelling of the cell fiber thus, creating large surface area for microorganisms to access the cell structure. This technique also reduces the bond between the lignin and the carbohydrate thereby breaking the lignin structure. Nonetheless, in this investigation, the alkaline pumpkin stalk's decreased hemicellulose content was noticeable, with a 36% decrease in the measured hemicellulose value as compared with the untreated pumpkin stalk. Nonetheless, the findings of this research are consistent with a study by Mirahmadi et al. (2010) on the use of alkaline pretreatment on pine trees and spruce wood for biogas production, which indicated that the lignin content of spruce wood treated with NaOH was not altered significantly. He et al. (2008) and Chen et al. (2010) found something similar, showing that NaOH pretreatment of rice straw decreased the lignin concentration while increasing the concentration of other cell components (cellulose and hemicellulose).

Similar findings were made by Olugbemide et al. (2021), who found that the lignin fraction of maize stover's increased after pretreatment. The author attributed the increment to the formation of pseudo-lignin during the pretreatment process. The same author also noted an increase in the cellulose crystallinity of some samples of corn stover and explained that this was caused by the removal of the sample's amorphous portions and may not necessarily indicate a rise in the crystallinity of the sample.

Consequently, Zborowska et al. (2022) pretreated maize cobs with NaOH, which led to an increase in holocellulose content. The loss of other cell components including lignin and extractives, according to Zborowska et al. (2022), is the cause of this rise in holocellulose content after NaOH pretreatment. The pretreatment process breaks down the hemicellulose into its monomers and subcomponents (arabinose, mannose, galactose, glucose, and xylose), some of which are easily converted to furfural in the presence of high temperatures and/or chemical reactions. As a result, some of the subcomponents (monomers) of these cell components (lignin, hemicellulose, and lignin) are added to the other cell components during pretreatment, which either results in a decrease in certain cell components and an increase in others (Zborowska et al., 2022). Due to its efficiency in penetrating lignocellulose biomass to remove hemicellulose, some lignin, and some of the cellulose fraction of the biomass, hydrothermal pretreatment has recently attracted attention on a global scale (Hernández-Beltrán et al., 2019).

In this work, compared to the untreated pumpkin stalk, the hydrothermal pretreatment of pumpkin stalk resulted in a 4.69% reduction in the lignin percentage and a 9.95% reduction in the cellulose fraction. In contrast to the hemicellulose concentration of the untreated pumpkin stalk, the hydrothermally processed pumpkin saw a 26.22% rise in hemicellulose content. This outcome is consistent with a

study by Xue et al. (2020), who attributed the increase in the hemicellulose content of miscanthus after hydrothermal pretreatment to the dissolution of cellulose and lignin content in hot water which resulted in the increase in the hemicellulose content. Figure 1 shows that the content of lignin, hemicellulose and cellulose content of untreated lemon grass increased after hydrothermal pretreatment. This pretreatment technique used on lemon grass resulted in 2.14%, 20.24%, and 14.33% increase in the hemicellulose, lignin and cellulose content respectively as compared to the result obtained in untreated lemon grass. A similar finding was obtained by Chen et al. (2016) who reported that the hydrothermal pretreatment of wheat straw resulted in an increase in lignin content as compared with the untreated wheat straw. The researcher further reported that the lignin yield increased proportionately with the increase in severity of the hydrothermal pretreatment.

According to Ahmed et al. (2019) and Fan et al. (2016), the complexity of the cellulose structure makes it difficult for it to degrade by biological, chemical, or even high-temperature boiling water. Consequently, Fan et al. (2016), stated that hydrothermal pretreatment can disrupt hydrogen bonds, thereby changing the cellulose's crystalline structure. Furthermore, temperature is a key factor in hydrothermal pretreatment; Sakaki et al. (2002) reported that the cellulose component of biomass starts to break down at 230°C; however, in this work, the hydrothermal treatment was conducted at 170°C. It's also crucial to keep in mind that a rise in temperature may cause the creation of inhibitory compounds which affects the effectiveness of the anaerobic digestion process. The combined (hydrothermal and chemical) pretreatment of the substrates had different effects on their cell structure. The application of this pretreatment technique on lemon grass, resulted in reduction of the hemicellulose content by 25.27% however, the lignin and cellulose content increased by

18.32% and 32.30% respectively as compared to untreated lemon grass. Additionally, this pretreatment technique resulted in an increase in the lignin fraction in pumpkin by 41.66% on the contrary, this also led to a 6.16% and 53.05% reduction in the hemicellulose and cellulose content respectively as compared to sample untreated pumpkin. This result obtained from the combined pretreatment of pumpkin stalk is in agreement with the study by Dahunsi et al. (2018). Gomez-Tovar et al. (2012), also reported that oat straw's hemicellulose, cellulose, and lignin were significantly solubilized after pretreatment with moderate HCl and biological pretreatment. Using two or more pretreatment methods, such as physical, hydrothermal, and chemical pretreatment, can improve the lignocellulose biomass's capacity to be digested while also lowering operational costs. The effectiveness of combined pretreatment method is evident as this is the only pretreatment technique that reduces the hemicellulose content of the two biomass employed in this study (combined pretreated lemon grass and combined pretreated pumpkin stalk). Therefore, the results of this study agree with those of Mustafa et al. (2017), who stated that combined pretreatments, such as chemical pretreatment with biological or mechanical procedures, are more effective than single pretreatments of chemical, biological, or mechanical pretreatment alone.

## Conclusion

The suitability of lignocellulose biomass such as lemon grass and fluted pumpkin stalk as a feedstock for bioconversion into renewable energy (biogas technology) was evaluated. However, for the effectiveness of this bioconversion process, the lignocellulose biomass must be pretreated prior to anaerobic digestion of the feedstocks. The primary objective of pretreatment is to disrupt the cellulose's crystalline structure and break down the lignin's structure in order to increase the accessibility of the microorganisms to the

cellulose during the enzymatic hydrolysis step of AD. The selected biomasses were pretreated using alkaline (NaOH), hydrothermal and combined hydrothermal and alkaline pretreatment techniques. The untreated and pretreated biomass was characterized using proximate and compositional analysis. The moisture content, dry matter, and ash content of the untreated lemon grass and pumpkin stalk ranged from 5.44% to 5.51%, 94.56% to 94.49% and 6.00% to 12.00% respectively. The C/N ratio of pretreated biomass ranged from 21.53% for alkaline pumpkin stalk to 54.59% for hydrothermal pumpkin. In addition, the compositional analysis of the pretreated biomass gave values in the range 8.01% - 30.51% for hemicellulose, 11.75%-25.34% lignin and 15.98%-51.81% for cellulose. Based on available literature, proximate and compositional analysis of the selected lignocellulose biomass shows that the results meet the requirements of a suitable feedstock for biogas production. However, the use of combined pretreatment shows a greater potential in enhancing the cell properties of the selected lignocellulose biomass thus more likely to improve the biogas yield. It is therefore recommended that combined pretreatment techniques should be encouraged, to achieve better enzymatic hydrolysis and hence improve biogas yield.

## COMPETING INTERESTS

The authors declare that they have no competing interests.

## AUTHORS' CONTRIBUTIONS

AON, the principal author, collected the data, interpreted the results and prepared the manuscript. IEA supervised the work and manuscript preparation. Both authors read and approved the final manuscript.

## ACKNOWLEDGEMENTS

The authors wish to thank the Department of Environmental Management and Toxicology, Federal University of

Petroleum Resources (FUPRE), Effurun, Delta State, Nigeria and the Department of Industrial Safety and Environmental Technology, Petroleum Training Institute (PTI), Effurun, Delta State, Nigeria.

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