

Determination of the Optimal Alkaline Concentration for the Pretreatment Elephant Grass

Kenekchukwu Theresa Ojiabo^{1*}, Emmanuel Chile Nleonu², Benedict Christopher¹

¹Department of Chemical Engineering, Federal Polytechnic Nekede, Owerri

²Department of Chemistry, Federal Polytechnic Nekede, Owerri

DOI: <https://doi.org/10.36348/sjet.2026.v11i09.008>

| Received: 17.07.2026 | Accepted: 10.09.2026 | Published: 16.09.2026

*Corresponding author: Kenekchukwu Theresa Ojiabo

Department of Chemical Engineering, Federal Polytechnic Nekede, Owerri

Abstract

The need to improve bioethanol yield from elephant grass has made the optimization of the pretreatment process inevitable. This work studied and determined the optimal alkaline concentration for the pretreatment of elephant grass with sodium hydroxide (NaOH) and hot water under the specified conditions. The pretreatment was performed at different concentrations of sodium hydroxide for a period of 1:30 hr. at 97.5°C. Scanning Electron Microscope (SEM) and Fourier Transform Infrared Spectroscopy (FTIR) were used to determine the effects of the pretreatment on the morphology and structure of the elephant grass. The grass hydrolysate was subjected to simultaneous saccharification and fermentation using cellulase enzyme and bakers' yeast. Optimum pretreatment condition was obtained by using 0.25 M NaOH, which yielded 6.778g/L reducing sugar. Bioethanol yield was found to be 8.84% of the available sugar. The SEM and FTIR analysis showed that NaOH pretreatment under the determined optimum conditions successfully disintegrated the recalcitrant structure of elephant grass. Hence, NaOH pretreatment at the concentration of 0.25 M exposed to hot water for 1:30 hr. at 97.5°C is a promising condition for bioethanol production from elephant grass.

Keywords: Bioethanol, Elephant grass, hydrolysis, Simultaneous Saccharification and fermentation.

Copyright © 2026 The Author(s): This is an open-access article distributed under the terms of the Creative Commons Attribution 4.0 International License (CC BY-NC 4.0) which permits unrestricted use, distribution, and reproduction in any medium for non-commercial use provided the original author and source are credited.

1. INTRODUCTION

One of the ways to mitigate climate change and reduce energy dependence on fossil fuel is a shift to renewable energy sources (Borisoot *et al.*, 2025; Kamla, Sushil, and Karmal 2021; Kenekchukwu Theresa Ojiabo, Etonyeama, and Onyewudiala 2024; Shangdiar *et al.*, 2022; da Silva *et al.*, 2025). Lignocellulose biomass (LB) has found application in biorefinery for bioethanol, biogas and biodiesel production due to its abundance from several sources (Blasi *et al.*, 2023; Broda, Yelle, and Serwanska 2022; Espinoza Gil *et al.*, 2023). However, the consistent search for a biomass with high sugar yield and a cost-effective biofuel production process has led to several research on bioethanol production using different lignocellulose wastes, pretreatment, and hydrolysis processes. Elephant grass (EG), one of the predominant plants in Nigeria has also drawn the attention of researchers as a feedstock in biorefinery.

Elephant grass also known as *Pennisetum purpureum* belong to Poaceae family. This plant has

been used both as a forage grass and as an energy crop due to its remarkable growth capabilities and substantial biomass yield (Zhang *et al.*, 2020). The grass also find application in drilling mud additives (Emenike, Osuji, and Onojake 2021), and in synthetic zeolite (Setiadji *et al.*, 2019). The plant can grow as tall as 6 m in height, has robust leaves ranging from 30–90 cm in length and up to 3 cm in width. It can adapt to harsh weather conditions such as intense sunlight, high temperatures, limited water and nutrients such as nitrogen, and carbon dioxide (J *et al.*, 2023). Its ability to withstand varying weather makes it trigger abundantly and available all year round (Ismail *et al.*, 2022) without requiring any form of care. Hence it is cheap and readily available. Similar to other LB, elephant grass also has recalcitrant nature making it unease for enzymes to access the fermentable sugar present in the structure during hydrolysis, hence the need for pretreatment prior to saccharification (Zoghلامي and Paës 2019). Other constituents of a lignocellulose biomass are inorganic matter comprising of macronutrients and micronutrients. The macronutrients which are needed in large

Citation: Ojiabo, K. T., Nleonu, E. C., & Christopher, B. (2026). Determination of the Optimal Alkaline Concentration for the Pretreatment Elephant Grass. *Saudi J Eng Technol*, 11(9), 834-843. <https://doi.org/10.36348/sjet.2026.v11i09.008>

concentration include nitrogen (N), sulfur (S), potassium (K) phosphorus (P), magnesium (Mg), and calcium (Ca), while micronutrients required in a very low concentration include elements such as copper (Cu), manganese (Mn), zinc (Zn), iron (Fe), boron (B), molybdenum (Mo), and chlorine (Cl). Other minerals that may be beneficial to plants, though not essential are nickel (Ni), sodium (Na), selenium (Se), vanadium (V), cobalt (Co), silicon (Si), and aluminum (Al). The elements perform different functions in plants, such as photosynthesis reaction, the production of amino acids, ATP, protein synthesis, nucleic acids, enzyme activation, energy household, and cell wall structure (Awoyale, Lokhat, and Okete 2021).

Several pretreatment methods have been used in the treatment of lignocellulose biomass. However, according to (Ojiabo *et al.*, 2026), a synergistic effect of a combinative pretreatment is essential for optimum reducing sugar yield. However, the process parameters and the choice of which method precedes the other in a two-stage process need to be optimized in order to reap the benefits from the tandem process. It was also noted that sugarcane bagasse has a greater yield when pretreated under liquid hot water assisted pretreatment method. Pretreatment methods involving hydrothermal and alkaline delignification has been reported to produce cellulose rich liquid fractions from native grass (Kommoji *et al.*, 2021). Hydrothermal pretreatment and sulfuric acid combination has been applied by (Ismail *et al.*, 2022) to pretreat elephant grass at 3% acid, and 30 min exposure time of steam explosion at 190 °C.

In this study, different concentration of NaOH was used for the pretreatment of elephant grass at a specific temperature and time. The aim was to identify the optimal concentration of NaOH for fermentable sugar yield from elephant grass. The work in each pretreatment step involved the use of different concentration of NaOH under the same temperature and time to determine the best suitable conditions for EG pretreatment. Despite various pretreatment methods available, the combined chemical and hydrothermal pretreatment of mechanically ground elephant grass was chosen due to its operational simplicity. NaOH was chosen as common chemical, and has also been reported by many researchers to be effective in the treatment of grass. A suitable hydrolysis condition was determined to obtain the pretreatment process that released the highest sugar from the grass. Ethanol production was investigated through simultaneous saccharification and fermentation (SSF) method using a commercial yeast and cellulase enzyme.

2.0 MATERIALS AND METHOD

2.1 Chemical reagents

Elephant grass was locally sourced, Sodium hydroxide (Cas No: 1310-73-2), distilled water, commercial yeast (*saccharomyces cerevisiae*), cellulase (Kaypeeyes Biotech PVT Ltd), boiling water bath (HH-

W600, Thermostatic Water Cabinet), 0.5% w/v sodium sulfate, 2.0% w/v crystalline phenol, 0.25 mg/mL of 100 mL standard glucose solution, 1% w/v of 60 ml reducing sugar standard saturated in benzoic acid. Sodium potassium tartrate solution (prepared by dissolving 300 g of salt in 500 mL distilled water) DNS acid solution (10 g in 200 mL 2 mol/lit sodium hydroxide).

2.2 Sample preparation and composition analysis

The elephant grass (EG) sample was harvested in World Bank Housing Estate, Owerri, Imo State, Nigeria. The EG was sliced into smaller parts, weighed and dried in an oven at 60°C until a constant weight was obtained. The dried EG was cut into small pieces, grinded using a high-speed grinder and then sieved to obtain a particle size fraction of 2:00 mm and then stored in an airtight plastic container to prevent moisture absorption prior to analysis. Compositional analysis of the biomass prior to the pretreatment was performed using gravimetric method as described below.

To remove the hemicellulose content, about 10 g of biomass was refluxed at 100 °C with 500 mL of 0.5 M NaOH solution for 2 hours. The residue, which is the cellulose and lignin content, was filtered and washed with a plentiful amount of distilled water until pH 7 was obtained and dried in an oven at 60°C until the sample was free of moisture. The lignin content was estimated from the dried residue by boiling in 100 mL 0.5 M H₂SO₄ for 2 hours and allowed to remain soaked for 24 hours at ambient temperature. The undissolved residue was washed with a plentiful amount of water several times until pH 7 was attained. The residue was dried at 60°C for 4 hours, cooled in a desiccator, and weighed, and the cellulose content was calculated from equation (1)

$$C_m = B_m - H_m - L_m \quad (1)$$

The percentage composition was calculated using equations (2), (3) and (4) for hemicellulose, cellulose and lignin respectively.

$$\%H_m = \frac{B_m - R_m}{B_m} \times 100 \quad (2)$$

$$\%C_m = \frac{R_m - L_m}{B_m} \times 100 \quad (3)$$

$$\%L_m = \frac{L_m}{B_m} \times 100 \quad (4)$$

Where;

C_m = mass of cellulose content, B_m = mass of biomass, L_m = mass of lignin, H_m = mass of hemicellulose, R_m = mass of residue ($C_m + L_m$)

2.3 Selection of the Optimal Pretreatment Conditions

Different concentrations of NaOH were prepared and used for the pretreatment of EG, while time and temperature were kept constant. Seven (7) sample were pretreated according to the following conditions: reaction time of 1:30 hr., temperature of 97°C and NaOH concentration of (0.25, 0.375, 0.5, 0.625, 0.75, 0.875, and 1.00 M respectively). 30 g of dry elephant grass was

weighed into seven different beakers, mixed with a specific amount of NaOH, placed in a preheated water bath (HH-W600, Thermostatic Water Cabinet) at the temperature of 97°C, and was allowed to heat for 1:30 hr. After heating, the samples were removed from the bath and allowed 20 mins to cool to 29°C. The content of the reactors was separated into solid and liquid fraction using a funnel lined with Whatman filter papers (Whatman PLC, Brentford, UK). The obtained prehydrolyzates were stored at room temperature for sugar analysis. The solids were washed severally with 4 L of water to remove the residual NaOH, sugars and inhibitors, dried in an oven at 60°C for a period of 24 h and stored in plastic bags at room temperature until they are ready to be used. The condition with the highest sugar yield was selected as the optimum pretreatment parameter.

2.4 Estimation of fermentable sugar

The method which was previously reported by (K. T. Ojiabo *et al.*, 2024) was used to estimate the reducing sugar available in the pretreated EG. An aliquot

of the hydrolysate of about 3 mL was mixed in a test tube with 1 mL of the DNS reagent. A blank solution was prepared in a separate tube by adding 1 mL of DNS reagent to 3 mL of distilled water. During the tubes incubation in a boiling water bath for a period of 5 min, a red-brown color was developed. After cooling the mixture at room temperature, 1 mL of sodium sulfate solution was added to the tube to obtain a stable color. The content was analyzed with a UV-visible spectrophotometer at an absorbance rate of 540 nm against the blank sample. A standard solution was prepared and used to determine the concentration of reducing sugar. This was achieved by preparing different concentrations of sugar and having their absorbance determined using a UV-spectrophotometer. After the standard process, the absorbance of the fermentable sugar determined was substituted into the regression equation (equation 5) from the standard graph (Fig. 1).

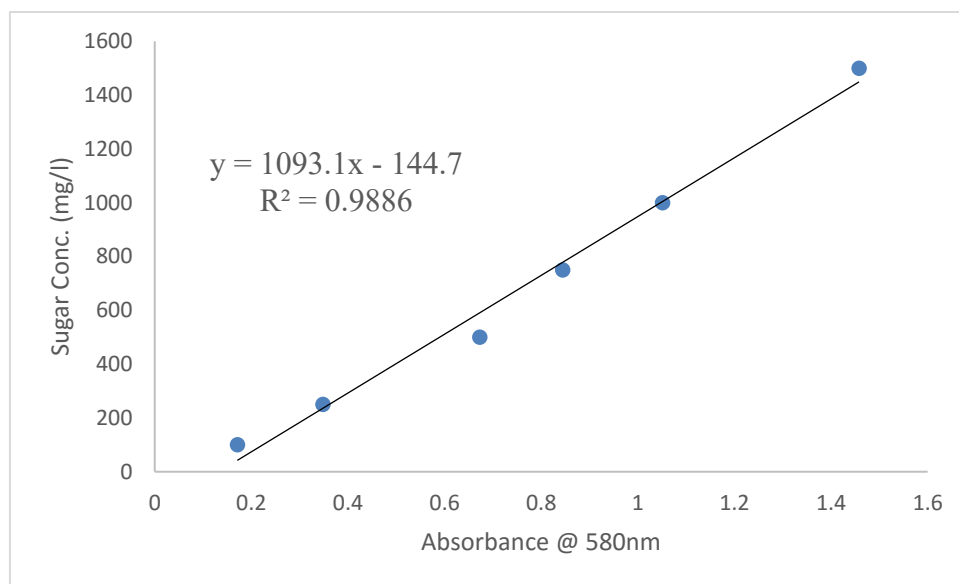
$$y = 1093.1x - 144.7 \quad (5)$$


Figure 1: Absorbance curve for determining the fermentable sugar

2.5 Morphological and Structural analysis of the solid samples

The morphology of both the treated and untreated EG was analyzed using scanning electron microscopy coupled to energy dispersive X-ray spectroscopy (SEM-EDX). SEM/EDX analysis were performed on SEM (Thermoscientific Instrument) equipped with X-ray microanalyzer (BC43 Benchtop Confocal Microscope, Oxford Instrument). The analytical conditions were as follow: full back scattered electrons detector (BSD), electron beam voltage of 15kV, work distance of 7.310 to 7.390 mm, pressure of 60 Pa, and temperature of 20 °C. The FTIR analysis of both samples was determined by (Cary 630 Spectroscopy, Agilent Technologies, Scientific, CA, USA).

2.6 Simultaneous Saccharification and fermentation

The method describe by (Pessani *et al.*, 2011) was adapted for SSF process. Prior to SSF of the pretreated EG sample, yeast fermentation medium was prepared in a 250 ml beaker by adding 5 g of yeast extract, 20 g KH₂PO₄, 10 g of MgSO₄·7H₂O, 20 g of (NH₄)₂SO₄, and 1 g of MnSO₄·H₂O to 1 L of deionized water. SSF with enzyme loading of 0.7 ml/g was performed in 250 ml flasks sealed with a rubber stopper fitted with a one-way air valve to maintain an anaerobic environment. Each fermentation flask contained 10 ml of YFM, 5 ml of 1 M sodium citrate buffer at pH 5.5 and 8 % pretreated and washed EG (w/w). The total mass in each flask was 100 g. All the flasks were incubated at 40°C in a water bath for 48 hrs. Samples were taken to determine the concentration of ethanol produced using a refractometer.

3.0 RESULTS AND DISCUSSIONS

3.1 Composition of Elephant Grass

The compositional analysis result (table 1) shows that elephant grass possesses a high cellulose content (43.12%), moderate hemicellulose content (19.38%), and relatively low lignin content (14.51%). The composition is within the range reported by other researchers (Dresch *et al.*, 2025; Johannes, Minh, and Xuan 2024). However, factors such as the location where biomass is produced, conditions during growth, harvest time and period, and the characterization methods can influence biomass composition (Johannes *et al.*, 2024). This composition is characteristic of a promising lignocellulosic feedstock for bioenergy and biomaterial applications. The high structural carbohydrate fraction enhances its potential for fermentable sugar production, while the moderate lignin concentration may facilitate more efficient pretreatment and conversion processes compared with highly lignified biomass sources.

Table 1: Composition of the Elephant grass

Property	Concentration
Cellulose (%)	43.12
Hemicellulose (%)	19.38
Lignin (%)	14.51

3.2: Sugar and ethanol concentration of the pretreated Elephant Grass

Table 2 shows the sugar and ethanol yield from EG pretreated with several concentration of NaOH. It was noticed that pretreatment conditions influenced the sugar concentration of the substrate. The results indicate that the optimum sugar yield was achieved at 0.25 M NaOH under the exposure time of 1: 30 hr. and temperature of 97°C. Meanwhile, the concentration of the fermentable sugar decreased with increase in alkaline concentration which is in agreement with (Madyira and Olatunji 2025); a higher NaOH concentration with a shorter retention time is more suitable for biomass pretreatment.

Table 2: Fermentable Sugar and Ethanol yield of the pretreated samples

Sample ID	Sugar Conc. (g/L)	Brix x 1.3 (%)	Ethanol yield (%)	Ethanol yield (g/L)
A	6.778	6.8	8.84	0.599
B	2.753	6.1	7.93	0.218
C	2.657	5.7	7.41	0.151
D	2.043	5.5	7.15	0.112
E	1.922	4.2	5.46	0.081
F	1.357	3.9	5.07	0.053
G	0.998	3.8	4.94	37.90

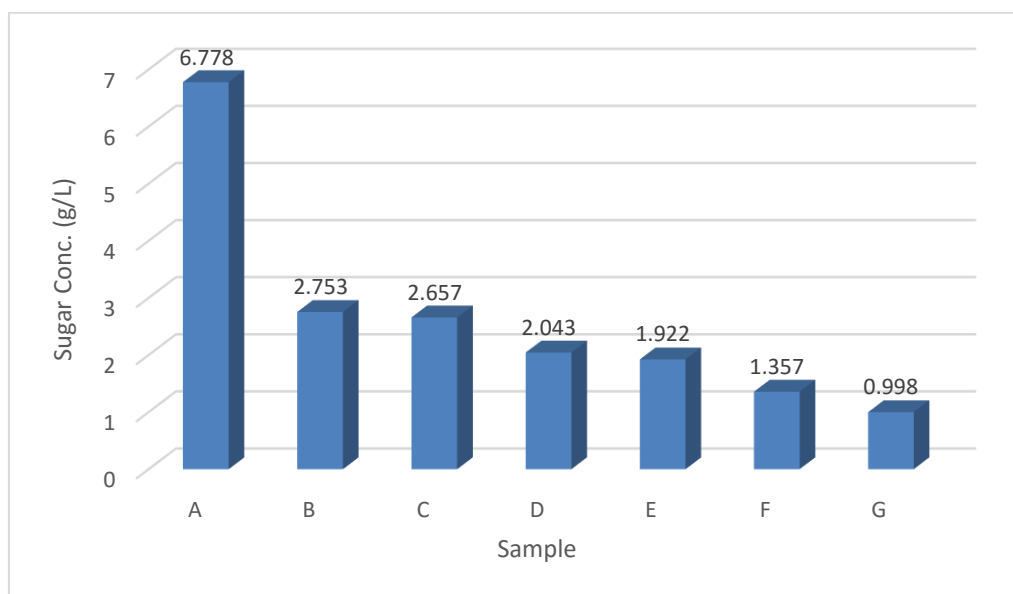


Figure 2: Sugar and ethanol concentration of the pretreated Elephant Grass

As the concentration of the alkali increases, figure 2 shows the decline in sugar concentration among the treated elephant grass samples. Sample A exhibited the highest sugar concentration (6.778 g/L), indicating high sugar accessibility and hydrolysis efficiency following the pretreatment. There was a progressive decline in sugar concentration from samples B to G, with

sample G recording the lowest value (0.998 g/L). These findings suggest that the pretreatment conditions strongly influenced the degree of biomass deconstruction and subsequent sugar release. The elevated sugar yield obtained in Sample A demonstrates its potential as the optimum pretreatment condition for maximizing fermentable sugar production and, consequently,

bioethanol yield from elephant grass biomass. However, the decline in the fermentable sugar yield with increased sodium hydroxide concentration can be attributed to severity of the pretreatment condition and possible formation of inhibitors during the pretreatment exposure, validating the need for a mild NaOH concentration for a prolonged pretreatment. The SSF converted only 8.8% of the available sugar, which is equivalent to ethanol yield of 0.599g/L. According to (Menegol *et al.*, 2016) ethanol concentration (6.1 g/L) was obtained using 16% (w/v) solids. Furthermore, the sugar conversion recorded here is low, which could be attributed to several factors such as fermentation period, possible counter interaction between the yeast and enzymes used for the SSH (Leite, Andrietta, and Franco 2026; Tang *et al.*, 2024).

3.3. Microstructural Arrangement of Elephant Grass

Figures 3 (a, b and c) are the high-resolution images of the cellular structure of untreated EG at 500, 1000 and 2000 magnification levels respectively. The morphologies of the sample comprised of heterogenous complex surface structure peculiar to a lignocellulose biomass. The untreated sample exhibits relatively intact, compact, and well-organized fibrous structures. There are cylindrical bundles representing plants vascular bundles, varying sizes of rough, porous flakes and smaller particulate matter, and a non-uniform, porous topography. The bundles appear tightly bound together with surface deposits and impurities attached to the fiber walls. The smooth and continuous appearance suggests the presence of natural binders such as lignin, hemicellulose, waxes, and other extractives that encapsulate the cellulose microfibrils. Such morphology is typical of untreated lignocellulosic biomass where non-cellulosic components still occupy the fiber surface.

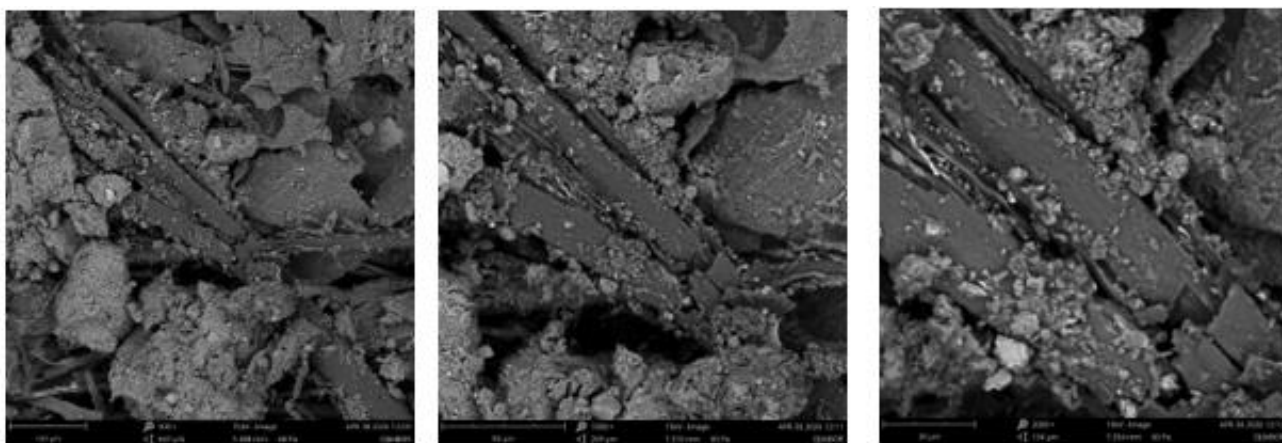
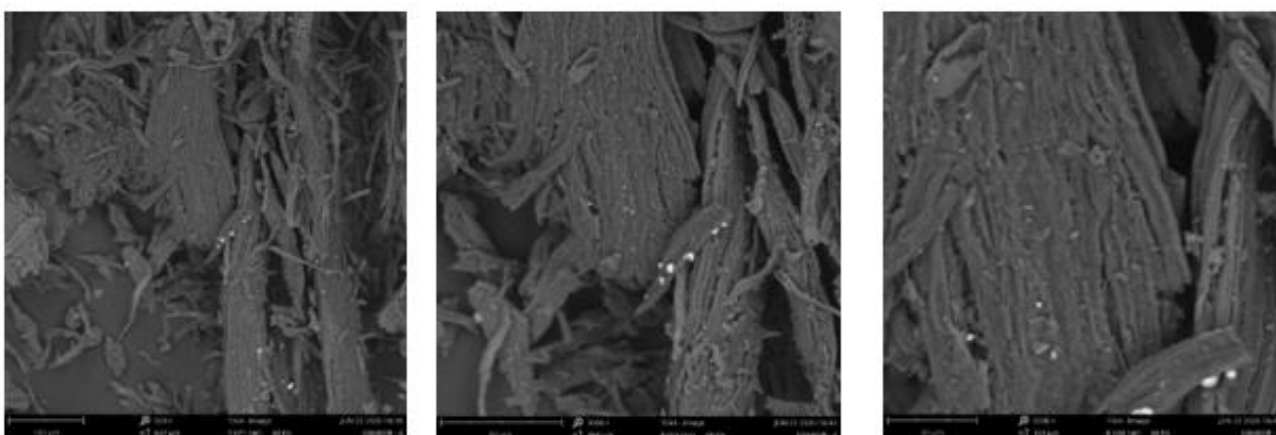


Figure 3 a, b, c: SEM images for Untreated EG at 500, 1000 and 2000 magnification levels



Figures 4 a, b, c: SEM images for Untreated EG at 500, 1000 and 2000 magnification levels

Only the sample with the optimum pretreatment conditions was subjected to SEM analysis. Figs 4 displayed the physical modification caused by the alkali and hot water pretreatment. The treated sample shows a much rougher and more fragmented surface, appearing disrupted, separated, and partially fibrillated, with detached particles visible throughout the structure. This

roughened morphology indicates that sodium hydroxide treatment removed portions of lignin, and hemicellulose, exposing more cellulose-rich regions necessary for enzymatic hydrolysis. The pretreatment of *Arachis hypogaea* shells with NaOH showed similar results (Olatunji *et al.*, 2022). The SEM images imply that higher exposure time with lower alkali concentrations

cause higher lignin and hemicellulose removal thereby enhancing cellulose accessibility when digested. This is in concord with the result gotten from the pretreatment of *Xyris capensis* with NaOH (Madyira and Olatunji

2025) and the pretreatment of Napier grass with NaOH (Khathapon *et al.*, 2022).

3.4 Elemental Composition of Elephant Grass

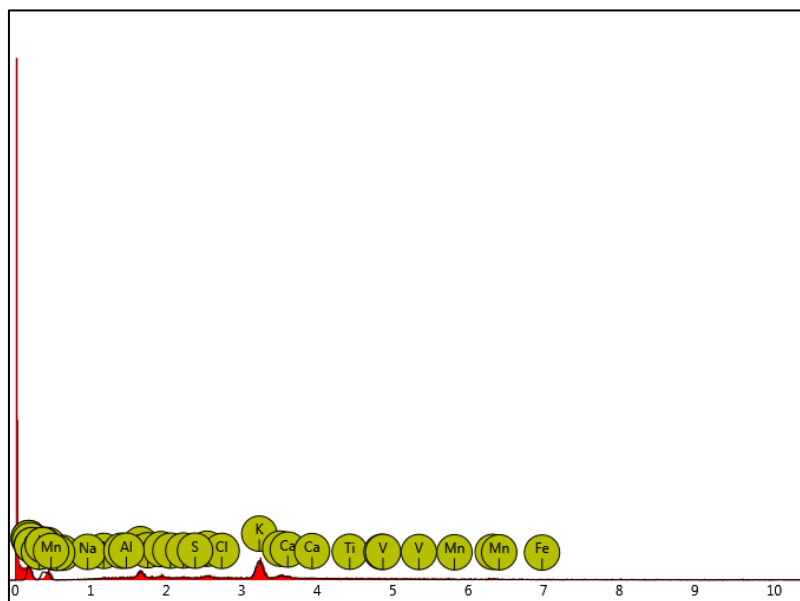


Figure 5: EDX of the untreated Elephant Grass

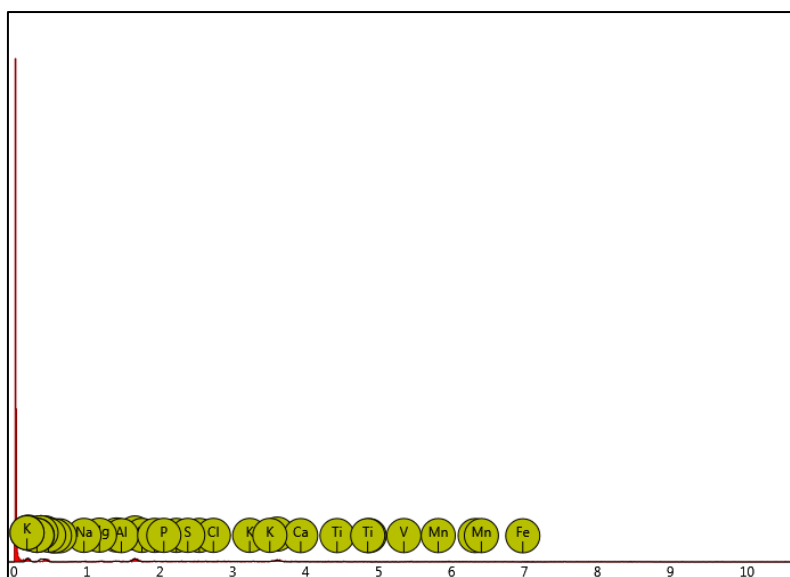


Figure 6: EDX of the pretreated Elephant Grass

The EDX (figs 5 and 6) determined the elemental composition of the untreated and treated biomass. Fig 6 shows how effective the pretreatment at the optimum conditions is in removing undesired elemental components after pretreatment (Emenike *et al.*, 2021). The concentrations of the components are recorded in table 3. The untreated sample contains substantial mineral content, particularly: carbon (C), potassium (K), silicon (S), iron (Fe), chlorine (Cl), calcium (Ca), phosphorus (P), and Magnesium. These minerals are commonly associated with plant ash, soil contamination, and naturally accumulated inorganic constituents in grasses.

According to table 3, carbon represents about 54.28 wt%, indicating a relatively high concentration of organic materials on the fiber surface prior to the pretreatment. The carbon content reduced to 24.89% after exposure to NaOH pretreatment. The observed reduction in carbon is attributed to the removal of lignin, partial solubilization of hemicellulose, and thermal degradation of carbonaceous compounds. During pretreatment, these fractions were dissolved or converted into soluble and volatile products, leading to a loss of carbon from the solid biomass. This phenomenon is consistent with the known effects of alkaline pretreatment, which disrupts the lignocellulosic matrix

and promotes delignification, thereby altering the elemental composition of the biomass (Bioprocess n.d.; Madyira and Olatunji 2025; Olabisi *et al.*, 2023). There is complete removal of potassium, iron, chlorine and

phosphorus. The alkaline pretreatment left a residual sodium in the treated sample, which is in concord with (Awoyale *et al.*, 2021; Emenike *et al.*, 2021).

Table 3: Elemental Composition of Elephant Grass according to EDX analysis

Untreated EG			Treated EG	
Elemental compositions in % weight				
Elements	Atomic conc	Weight conc.	Atomic conc.	Weight conc.
Carbon	78.84	54.23	46.67	24.89
Potassium	12.93	28.95	-	-
Silicon	3.40	5.46	19.72	24.59
Iron	1.42	4.54	-	-
Chlorine	1.25	2.53	-	-
Calcium	0.99	2.27	14.78	26.30
phosphorus	0.96	1.71	-	-
magnesium	0.22	0.31	6.04	6.52
Vanadium	-	-	2.97	6.71
Aluminum	-	-	5.39	6.46
Sodium		-	4.44	4.53

The pretreatment recorded increase in the concentration of Silicon, Calcium, and Magnesium. An increase in elemental concentration (wt%) after alkali pretreatment is usually caused by relative concentration effects on increased analytical surface exposure, rather than an actual gain in total elemental mass. The presence of Aluminum, and Vanadium may be attributed to concentration effect or possible contamination during handling. In line with (Kucharska *et al.*, 2018; Xu *et al.*, 2020), alkaline pretreatment selectively solubilizes and extracts large amount of lignin, hemicellulose, extractives and organic acids. As the total dry mass of the biomass solid residue decreases significantly, non-soluble or partially retained inorganic elements like Si, Ca and Mg account for a large percentage of the remaining mass. Moreso, (Madyira and Olatunji 2025) reported that untreated biomass has an intact, dense outer recalcitrant layer that hides internal minerals, but when subjected to alkaline pretreatment, the lignocellulose

matrix and surface wax are disrupted thereby exposing previously buried salts directly to surface characterization techniques such as SEM-EDX or XRF.

3.5 Structural Composition of Elephant Grass

The effect of the pretreatment on Elephant Grass for the functional group was examined with FTIR spectra. The spectra peak assignment of untreated and pretreated EG are shown in figs 7 and 8 respectively. The spectrum of raw elephant grass (fig. 7) shows major absorption bands at approximately 3438, 1638, 1417, 1101, and 619 cm^{-1} . The broad and intense O-H band at 3438 cm^{-1} indicates the highly hydrophilic nature of untreated elephant grass due to the abundance of hydroxyl-containing lignocellulosic components (cellulose, hemicellulose, lignin, and absorbed moisture).

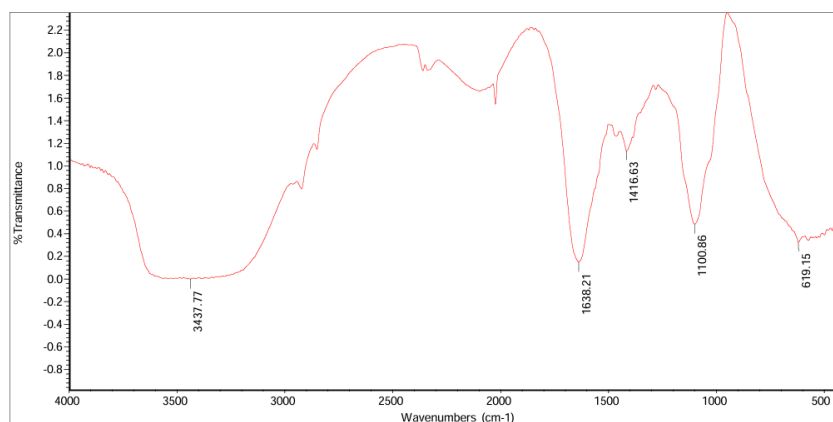


Figure 7: FTIR for Untreated Elephant Grass

The at peak 1639 cm^{-1} band shows the presence of bound water and oxygenated groups in lignocellulosic materials. The bending at peak 1417 cm^{-1} shows the

characteristic cellulose band. The stretching at peak 1101 cm^{-1} shows the polysaccharide structure of cellulose/hemicellulose. The spectrum confirmed that

the sample is a lignocellulose material by showing peaks similar to those of a lignocellulose spectrum.

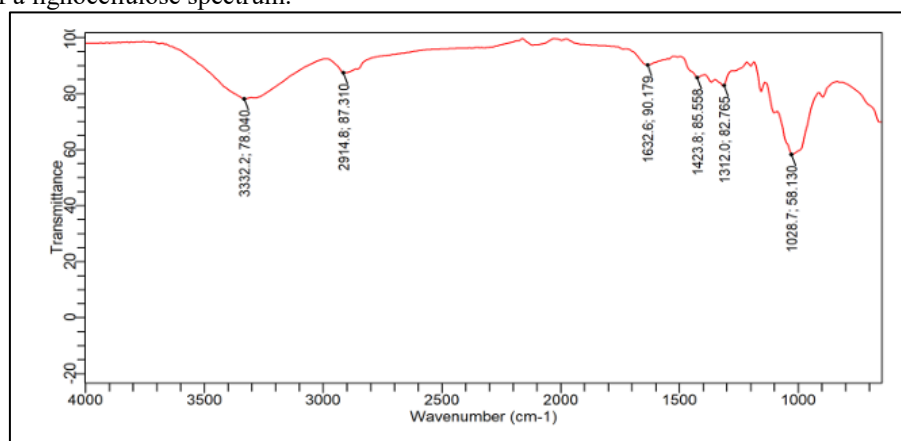


Figure 8: FTIR of the pretreated Elephant Grass

The pretreated sample (fig. 8) exhibits prominent bands at approximately 3332, 2915, 1633, 1424, 1312, and 1029 cm^{-1} . It was observed that the peaks were affected by NaOH pretreatment since they were either decimated or redistributed due to the pretreatment (Madyira and Olatunji 2024). The O-H stretching peak shifts from approximately 3438 cm^{-1} in the raw sample (fig. 7) to 3332 cm^{-1} after pretreatment (fig. 8). This shift indicates alteration of the hydrogen-bonding network within the biomass, suggesting structural rearrangement of cellulose during alkaline treatment. The pretreated sample (fig. 8) displays a clearer band near 2915 cm^{-1} , which is more characteristic of cellulose-rich materials. This suggests that the pretreatment exposed cellulose by removing surface impurities and amorphous constituents. The untreated spectrum (fig. 7) contains broader features typical of a lignocellulosic matrix, whereas the pretreated spectrum (fig. 8) is dominated by cellulose-associated peaks. This is consistent with alkaline delignification and partial solubilization of hemicellulose, which commonly occur during NaOH treatment at elevated temperatures. The stronger cellulose-associated bands at 1424 cm^{-1} , 1312 cm^{-1} , and 1029 cm^{-1} indicate greater exposure and possibly higher relative cellulose content after treatment (fig. 8). These changes are frequently interpreted as evidence of improved cellulose purity and crystallinity. It can be deduced that the pretreatment will lead to high biofuel yield since the cellulose bond has been broken, which increases microbial accessibility (Madyira and Olatunji 2025). This agrees with the findings of (Awoyale and Lokhat 2021) on the effect of pretreatment methods on cellulose O-H bonding.

4.0 CONCLUSION

This study investigated the effect of NaOH and hot water pretreatment on elephant grass for enhanced bioethanol production and identified the optimum pretreatment condition for fermentable sugar release and ethanol generation. 0.25 M NaOH produced the highest fermentable sugar concentration of 6.778 g/L and a corresponding ethanol yield of 8.84%. SEM and FTIR

analyses confirmed substantial morphological and structural modifications after pretreatment. EDX analysis showed considerable changes in the elemental composition of the biomass after pretreatment. Overall, the combined pretreatment at the stated optimum conditions effectively reduced the recalcitrance of elephant grass, enhanced cellulose accessibility, and improved fermentable sugar production, thereby demonstrating the effectiveness of the method in releasing fermentable sugar from elephant grass.

RECOMMENDATIONS

1. Further studies should investigate the combined optimization of alkaline concentration, temperature, and pretreatment time using statistical experimental designs such as Response Surface Methodology (RSM) to obtain global optimum conditions and understand parameter interactions.
2. Future work should evaluate enzyme loading, yeast strain selection, fermentation duration, and solid loading during simultaneous saccharification and fermentation to maximize ethanol productivity beyond the yield obtained in this study.
3. Additional characterization techniques such as X-ray diffraction (XRD) and thermogravimetric analysis (TGA) are recommended to further evaluate changes in crystallinity and thermal stability resulting from pretreatment.

Authorship Contribution Statement

Kenechukwu Theresa Ojiabo: Writing – original draft, Investigation, Funding acquisition, Analysis, Conceptualization. **Emmanuel Chile Nleonu:** Writing – review & editing, Investigation, Analysis. **Benedict Christopher:** Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

ACKNOWLEDGEMENT

The authors would like to acknowledge the tertiary education trust fund (TETFund) for supporting this research through financial provision (TETF/ES/DR&D/CE/POLY/NEKEDE/IBR/2025/VO L.1

REFERENCES

- Awoyale, Adeolu A., and David Lokhat. 2021. "Experimental Determination of the Effects of Pretreatment on Selected Nigerian Lignocellulosic Biomass in Bioethanol Production." *Scientific Reports* (0123456789):1–16. doi:10.1038/s41598-020-78105-8.
- Awoyale, Adeolu A., David Lokhat, and Patrick Okete. 2021. "Investigation of the Effects of Pretreatment on the Elemental Composition of Ash Derived from Selected Nigerian Lignocellulosic Biomass." *Scientific Reports* (0123456789):1–15. doi:10.1038/s41598-021-00672-1.
- Bioprocess, Celignis. n.d. "Alkali Pretreatment of Biomass - Bioprocess Development."
- Blasi, Alessandro, Alessandra Verardi, Catia Giovanna Lopresto, Sarah Siciliano, and Paola Sangiorgio. 2023. "Lignocellulosic Agricultural Waste Valorization to Obtain Valuable Products: An Overview." *Recycling* 8(4):1–46. doi:10.3390/recycling8040061.
- Borisoot, Kanjanapon, Khundej Suriarn, Somchai Maneewan, Chantana Punlek, Amnart Suksri, and Tanakorn Wongwuttanasatian. 2025. "Maximizing Yield with Novel Napier Grass Breed for Tropical Region: A Case Study of Thailand." *Environmental Technology and Innovation* 40(September):104551. doi:10.1016/j.eti.2025.104551.
- Broda, Magdalena, Daniel J. Yelle, and Katarzyna Serwanska. 2022. "Bioethanol Production from Lignocellulosic Biomass — Challenges and Solutions." *Macromolecules* 27(8717).
- Dresch, Aline Perin, Kaillany Eduarda Gonclaves Lipes, Siumar Pedro Tironi, Odinei Fogolari, Guilherme Martinez Mibielli, João Paulo Bender, and Joel Gustavo Teleken. 2025. "Analysis of Biomass Yield and Chemical Composition of Elephant Grass at Different Growth Stages." *Research, Society and Development* 14(7):1–17. doi:10.33448/rsd-v14i7.49300.
- Emenike, I. U., C. Osuji, and M. C. Onojake. 2021. "Synthesis and Characterization of Carboxymethyl Cellulose from Eichhornia Crassipes and Pennisetum Purpureum as Potential Source of Sustainable Drilling Mud Additives." *Asian Journal of Physical and Chemical Sciences* 9(3):29–45. doi:10.9734/AJOPACS/2021/v9i330139.
- Espinoza Gil, Mijael, Octavio Loera-Corral, Rosa Olivia Cañizares-Villanueva, Ricardo Aguilar-López, and Maria del Carmen Montes-Horcasitas. 2023. "A Critical Review of Challenges and Advances to Produce 2G Biodiesel with Oleaginous Microorganisms and Lignocellulose." (November).
- Ismail, Ku Syahidah Ku, Yuki Matano, Yuri Sakihama, Kentaro Inokuma, Yumiko Nambu, Tomohisa Hasunuma, and Akihiko Kondo. 2022. "Pretreatment of Extruded Napier Grass by Hydrothermal Process with Dilute Sulfuric Acid and Fermentation Using a Cellulose-Hydrolyzing and Xylose-Assimilating Yeast for Ethanol Production." *Bioresource Technology* 343. doi:10.1016/j.biortech.2021.126071.
- J, Maldonado-Méndez José de, Guerra-Medina E. Cándido, Avendaño-Arrazate H. Carlos, Luis A. Gálvez-Marroquín, and Rafael Ariza-Flores. 2023. "Morphological Characterization of Elephant Grass (*Pennisetum Purpureum* Schumach) Ecotypes Collected in Chiapas, Mexico." *Agro Productividad* 16(1):33–43. doi:10.32854/agrop.v16i1.2366.
- Johannes, Lovisa Panduleni, Tran Thi Ngoc Minh, and Tran Dang Xuan. 2024. "Elephant Grass (*Pennisetum Purpureum*): A Bioenergy Resource Overview." *Biomass (Switzerland)* 4(3):625–46. doi:10.3390/biomass4030034.
- Kamla, Malik, Ahlawat Sushil, and Malik Karmal. 2021. "Nanotechnology : A Sustainable Solution for Bioenergy and Biofuel Production." *Journal of Nanoscience and Nanotechnology* 21(June):3481–94. doi:10.1166/jnn.2021.19001.
- Khathapon, Pinpatthanaponga, Boonnorat Jarungwit, Glanpracha Naraporn, and Rangseesuriyachai Thaneeya. 2022. "Biogas Production by Co-Digestion of Sodium Hydroxide Pretreated Napier Grass and Food Waste for Community Sustainability." *Energy Sources, Part A: Recovery, Utilization, and Environmental Effects* 44(1):1678–92. doi:https://doi.org/10.1080/15567036.2022.2055232.
- Kommoji, Satish, M. Gopinath, Polinati Satya Sagar, D. Yuvaraj, J. Iyyappan, A. Jaya Varsha, and Varsha Sunil. 2021. "Lipid Bioproduction from Delignified Native Grass (*Cyperus Distans*) Hydrolysate by *Yarrowia Lipolytica*." *Bioresource Technology* 324:124659. doi:10.1016/J.BIORTECH.2020.124659.
- Kucharska, Karolina, Piotr Rybarczyk, Iwona Hołowacz, Rafał Łukajtis, Marta Glinka, and Marian Kamiński. 2018. "Pretreatment of Lignocellulosic Materials as Substrates for Fermentation Processes." *Molecules* 23(11):1–32. doi:10.3390/molecules23112937.
- Leite, Lorena M. F., Silvio R. Andrietta, and Telma T. Franco. 2026. "Industrial Strategies to Minimize Glycerol Formation in Ethanol." *Biofuels, Bioproducts and Biorefining* 20:1006–20. doi:10.1002/bbb.70074.
- Madyira, D. M., and K. O. Olatunji. 2024. "Investigating the Influence of Different Pretreatment Methods on the Morphological Structure of *Arachis Hypogaea* Shells." *Materials Today: Proceedings* 105(July 2023):72–77.

- doi:10.1016/j.matpr.2023.07.289.
- Madyira, Daniel M., and Kehinde O. Olatunji. 2025. "Alkali Pretreatment of Lignocellulose Feedstock Improves Morphological Structure and Biomethane Yield." *Sustainability* 17(534):1–22. doi:https://doi.org/10.3390/su17020534.
 - Menegol, Daiane, Roselei Claudete Fontana, Aldo José Pinheiro Dillon, and Marli Camassola. 2016. "Second-Generation Ethanol Production from Elephant Grass at High Total Solids." *Bioresource Technology* 211:280–90. doi:10.1016/j.biortech.2016.03.098.
 - Ojiabo, K. T., J. I. Onyewudiala, C. C. Nwanwe, O. C. Samuel, and E. G. Chinaza. 2024. "ENZYMATIC HYDROLYSIS OF AUTOCLAVE PRETREATED SUGARCANE BAGASSE FOR BIOETHANOL PRODUCTION." Pp. 121–33 in *Proc. of 1st International Conference of OAUSTECH Leadership Academy*. Okitipupa: Olusegun Agagu University of Science and Technology (OAUSTECH), Leadership Academy, Okitipupa, Ondo State, Nigeria.
 - Ojiabo, Kenechukwu Theresa, Onyemauche Eleazar Etoneyama, and Julius Ibeawuchi Onyewudiala. 2024. "A Comparative Study on the Effects of Chemical and Hydrothermal Pretreatment Methods on the Valorization of Lignocellulose Agricultural Waste (Sugarcane Bagasse)." *International Journal of Research and Scientific Innovation* XI(2321):1289–97. doi:10.51244/IJRSI.
 - Ojiabo, Kenechukwu Theresa, Chibuzo Cosmas Nwanwe, Eshiorenoya David Ajayi, Julius Ibeawuchi Onyewudiala, and Monday Chuwkwu Morgan. 2026. "Synergistic Effects of Combined Chemical and Physiochemical Pretreatment on Sugar Liberation for Bioethanol Production." *Asian Journal of Advanced Research and Reports* 20(2):72–96.
 - Olabisi, Atunnise Shittu Monsurat, Birnin-Yauri Abubakar Umar, Elinge Cosmos Moki, and MuhammedKangiwa Umar. 2023. "Effect of Alkaline Pretreatment on the Mophology of Biomass Prior To Anaerobic Digestion." *Research Journal of Pure Science and Technology* 6(3):8–22. doi:10.56201/rjps.v6.no3.2023.pg8.22.
 - Olatunji, Kehinde O., Daniel M. Madyira, Noor A. Ahmed, and Oyetola Ogunkunle. 2022. "Influence of Alkali Pretreatment on Morphological Structure and Methane Yield of Arachis Hypogaea Shells." *Biomass Conversion and Biorefinery* 14(2024):12143–54. doi:https://doi.org/10.1007/s13399-022-03271-w.
 - Pessani, Naveen K., Hasan K. Atiyeh, Mark R. Wilkins, Danielle D. Bellmer, and Ibrahim M. Banat. 2011. "Simultaneous Saccharification and Fermentation of Kanlow Switchgrass by Thermotolerant Kluyveromyces Marxianus IMB3 : The Effect of Enzyme Loading , Temperature and Higher Solid Loadings." *Bioresource Technology* 102(22):10618–24. doi:10.1016/j.biortech.2011.09.011.
 - Setiadji, S., C. D. D. Sundari, V. Aprilia, E. Sumiyanto, I. Novianti, and A. L. Ivansayah. 2019. "Synthesis of Zeolite NaX Using Elephant Grass (Pennisetum Purpureum) as a Silica Source and Its Characterization." *Journal of Physics:Conference Series* (1402):1–7. doi:10.1088/1742-6596/1402/6/066016.
 - Shangdiar, Sumarlin, Yuan Chung Lin, Vinoth Kumar Ponnusamy, and Tzi Yi Wu. 2022. "Pretreatment of Lignocellulosic Biomass from Sugar Bagasse under Microwave Assisted Dilute Acid Hydrolysis for Biobutanol Production." *Bioresource Technology* 361:127724. doi:10.1016/J.BIORTECH.2022.127724.
 - da Silva, Guilherme Henrique, Natália dos Santos Renato, Felipe Ferreira Coelho, Thiago Paiva Donato, Alberto José Delgado dos Reis, Marcelo Henrique Otenio, and Juarez Campolina Machado. 2025. "Energy Potential of Elephant Grass Broth as Biomass for Biogas Production." *Scientific Reports* 15(1):1–11. doi:10.1038/s41598-025-91938-5.
 - Tang, Chaojun, Adnan Cavka, Mai Bui, and Leif J. Jönsson. 2024. "Comparison of Simultaneous Sacchari Fi Cation and Fermentation with LPMO-Supported Hybrid Hydrolysis and Fermentation." *Frontiers in Bioengineering and Biotechnology* (July):1–11. doi:10.3389/fbioe.2024.1419723.
 - Xu, Li, Sen-Jia Zhang, Cheng Zhong, Bing-Zhi Li, and Ying-Jin Yuan. 2020. "Alkali-Based Pretreatment-Facilitated Lignin Valorization: A Review." *Ind. Eng. Chem. Res.* 59(39):16923–16938. doi:https://doi.org/10.1021/acs.iecr.0c01456.
 - Zhang, Wenqing, Shengkui Zhang, Xianqin Lu, Can Li, Xingwang Liu, Geyu Dong, and Tao Xia. 2020. "Tissue-Specific Transcriptome Analysis Reveals Lignocellulose Synthesis Regulation in Elephant Grass (Pennisetum Purpureum Schum)." *BMC Plant Biology* 20(528):1–12.
 - Zoghalmi, Aya, and Gabriel Paës. 2019. "Lignocellulosic Biomass: Understanding Recalcitrance and Predicting Hydrolysis." *Frontiers in Chemistry* 7(December). doi:10.3389/fchem.2019.00874.