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CHARACTERISATION OF AGRO-WASTES AS SUBSTRATE FOR MICROBIAL LACCASE PRODUCTION

***[1]OYEWOLE, O.A [1]TSADO, P. Y[1,2] CHIMBEKUJWO, K.I., [1]ILYASU, U.S.,[1] ABIOYE, O.P. [3] EGWIM, E.C.**

[1]Department of Microbiology, Federal University of Technology, Minna, Nigeria

[2]Department of Microbiology, Modibbo Adama University, Yola, Nigeria

[3]Department of Biochemistry, Federal University of Technology, Minna Nigeria

***Correspondence author:**

Email address: oa.oyewole@futminna.edu.ng

Tel: +234-8140031875

ABSTRACT

Laccase, a widely utilised blue copper oxidoreductase enzyme, catalyses the oxidation of various substrates, including phenolic compounds, while reducing molecular oxygen to water. This study aimed to evaluate the potential of agro-wastes, namely corncob, sugarcane bagasse, and plantain peduncle, as substrates for laccase production, emphasising the effects of alkaline pretreatment using NaOH. Analytical methods, including scanning electron microscopy (SEM), Energy Dispersive X-ray (EDX), X-ray Diffraction (XRD), and Fourier Transform Infrared Spectroscopy (FTIR), were employed to analyse the structural and chemical changes induced in these materials. The SEM-EDX results revealed alterations in elemental composition, showcasing reduced lignin-associated elements and improved exposure of cellulose. The XRD analysis demonstrated an enhanced crystallinity index, indicating the selective removal of amorphous components (lignin and hemicellulose), thus increasing cellulose accessibility. The FTIR analysis identified significant changes in functional groups, such as the disappearance of lignin-related peaks and the amplification of cellulose and hydroxyl signals. These findings established that NaOH pretreatment effectively modifies the physicochemical properties of agro-wastes, rendering them cost-efficient substrates for laccase production.

Keywords: Laccase, X-ray Diffractogram, Energy Dispersive X-ray, Fourier Transform Infrared, Agro-wastes

INTRODUCTION

Laccase is categorised as a benzenediol oxygen reductase (EC 1.10.3.2), which includes a variety of proteins with diverse substrate specificities and biological functions. They are also called urushiol oxidases or p-diphenol oxidases (Arregui *et al*., 2019; Janusz *et al*., 2020). Laccases are notable for their ability to oxidise phenolic and non-phenolic lignin-related compounds and persistent environmental residues. Unlike peroxidases, laccases do not rely on hydrogen peroxide (H_2O_2) for their oxidation reactions. Instead, they can oxidise various chemical compounds, including aromatic amines, diamines, diphenols, polyphenols, and benzenethiols (Bello *et al*., 2021).To facilitate large-scale industrial applications and reduce production costs, agro-wastes have been identified as an effective alternative to synthetic carbon sources in fermentation for laccase production (Han *et al*., 2021). Agro-wastes, being lignocellulosic, are known to enhance laccase production (Narayanan *et al*., 2015). They offer a cost-effective nutrient source for microorganisms, significantly reducing production expenses. Submerged agro-waste fermentation has gained traction due to its dual advantages of providing renewable and inexpensive substrates and mitigating environmental pollution. High lignin content in these substrates makes them ideal for significantly promoting the secretion of lignocellulolytic enzymes (Naik *et al*., 2023). Most agricultural waste is discarded, incinerated, or improperly disposed of, contributing to air pollution and climate change. These practices adversely affect human and animal health and the environment, highlighting the urgency of developing sustainable waste management strategies (Naik *et al.*, 2023). This study focuses on characterising agro-wastes (corncob, sugarcane bagasse, and plantain peduncle) as substrates for laccase production.

MATERIALS AND METHODS

Collection of Samples

Agro-wastes, including sugarcane bagasse, plantain peduncle, and corncob, were collected aseptically in sterile polythene bags from Kasuwan Gwari market in Minna and transported to the Microbiology Laboratory of the Federal University of Technology, Minna.

Pre-treatment of Agro-wastes

Adapted from Makut and Ekeleme (2018), agro-waste were washed, air-dried for five days, milled, soaked(20 g in 200 mL NaOH,1:10) for five minutes, microwaved at 2450 MHz for five minutes, rinsed under running water to neutral pH (measured by pH meter), achieving delignification effectively.

Analysis of Agro-waste for Laccase Production

The substrates were analysed before and after pre-treatment using X-ray diffraction (XRD), Scanning Electron Microscopy (SEM), and Fourier Transform Infrared (FTIR) spectroscopy, following the method described by Abd Halim and Saidin (2020).

Scanning Electron Microscopy (SEM) and Energy Dispersive X-Ray (EDX) Analysis of Agro-waste

The agro-waste sample was mounted on stubs using adhesive carbon and coated in 20 nm carbon using a QUORUM Q150R ES mini sputter coater before being examined using a Phenom PRO-X SEM equipped with an Oxford XMax 50 Silicon Drift Energy Dispersive X-ray detector at 15KV in a high vacuum (Abd Halim and Saidin, 2020).

X-ray Diffraction Analysis of Agro-waste

Agro-waste was ground, mounted on a smooth surface, and analysed using the reflection-transmission spinner stage with Theta-Theta settings. Parameters included 40mA, 45VA, and a 0.026261 step at 8.67 seconds. Diffracted X-rays were continuously recorded with a Gonio Scan, diverging slit, and 5mm mask (Abd Halim and Saidin, 2020).

Fourier Transform Infrared Spectroscopy (FTIR) Analysis of Agro-waste

Untreated and pre-treated agro-wastes were analysed using Agilent's Cary 630 spectrophotometer. Oven-dried samples (105 °C, 4-5 hours) were mixed with KBr (1:200 w/w), vacuum-pressed, and scanned in transmittance mode over the 4000–500 cm-1 range to identify structural differences(Abd Halim and Saidin, 2020).

RESULTS

Scanning Electron Microscopy Structural Characteristics of Corncob

Untreated corncob revealed rough, fibrous structures with small pores. In contrast, NaOH-treated corncob had smoother, uniform morphology, indicating delignification as shown in Figure 1.

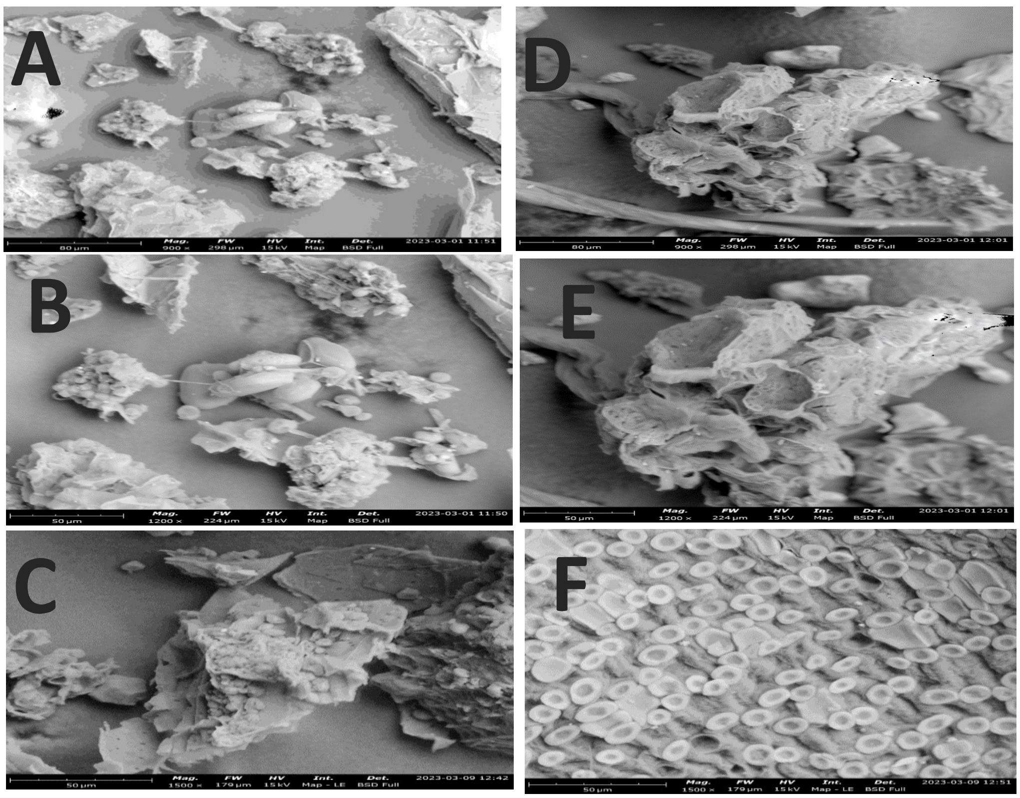

Figure 1: SEM Images of Corncob. Untreated - A: x900, B: x1200,C: x1500.Pre-treated with NaOH for 5 mins- D: x900, E: x1200 and F: x1500 Objective Lens.

Energy Dispersive X-ray (EDX) Morphological Characteristics of Corncob

EDX analysis identified carbon (C), oxygen (O), potassium (K), chlorine (Cl), and molybdenum (Mo) in untreated samples. Treated corncob revealed changes, detecting silicon (Si), calcium (Ca), iron (Fe), and aluminium (Al), showing structural and compositional modifications as shown in Figure 2.

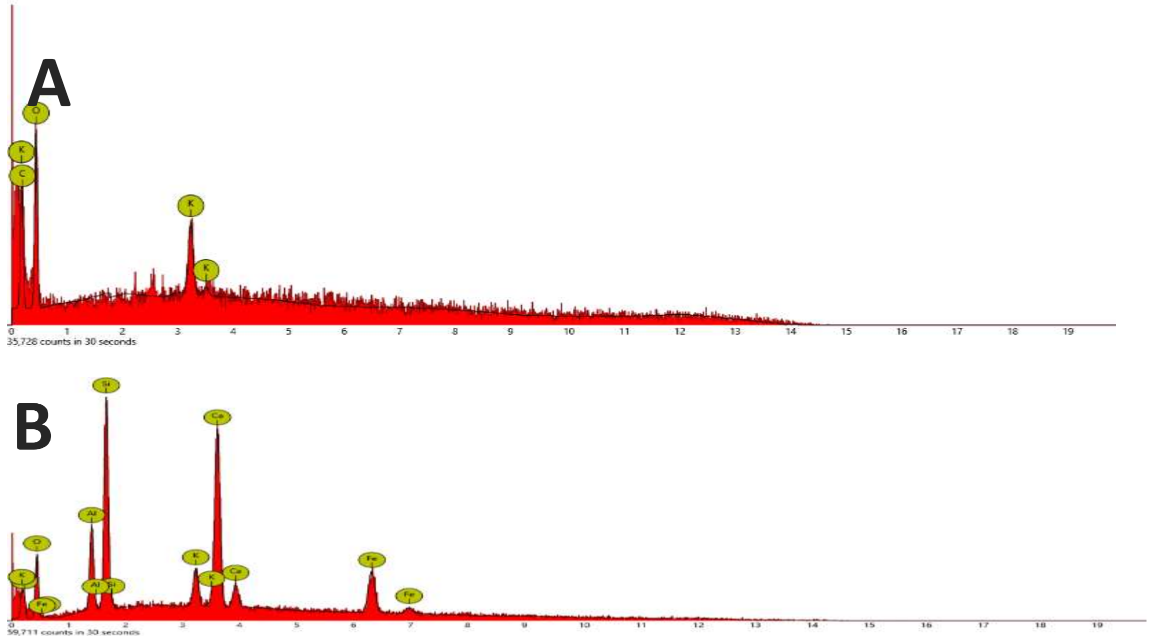

Figure 2: Energy Dispersive X-Ray Graph o f Corncob A=UntreatedCorncob, B= Pre - treated Corncob.

X-ray Diffractogram (XRD) of Corncob

The X-ray diffractogram analysis, illustrated in Figure 3, compares the untreated corncob (A) with the NaOH-treated corncob (B). The untreated corncob contained minerals such as refikite (74.3%), davyne (4.0%), chaoite (7.4%), mellite (4.31%), cristobalite (6.4%), and chlorite (3.6%). In contrast, the NaOH-treated corncob showed refikite (72%), davyne (2.5%), chaoite (9%), mellite (6.3%), cristobalite (7%), and chlorite (3%).

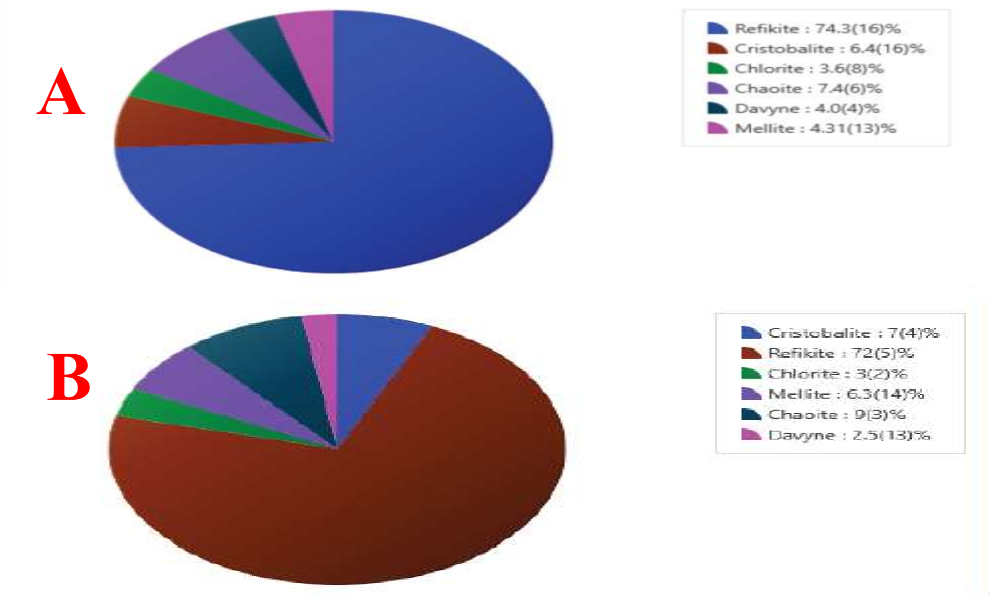

Figure 3: X-Ray Diffractograms of Corncob; A= Untreated B= Pre-treated

Fourier Transform Infrared (FTIR) Spectroscopy of Corncob

FTIR spectra revealed O-H stretching peaks at 3280 cm⁻¹ (untreated) and 3302 cm⁻¹ (treated), indicating cellulose, hemicellulose, and lignin. Peaks shifted in treated samples for C-H stretching (2922 to 2888 cm⁻¹) and C=C stretching (1640 to 1595 cm⁻¹). Notable is the absence of the untreated C-O peak at 1028 cm⁻¹ after NaOH treatment as seen in Figure 4.

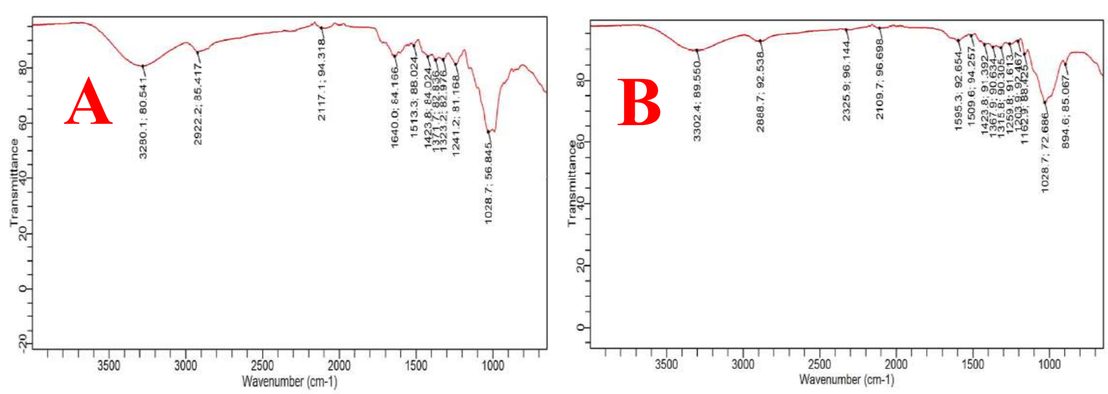

Figure 4: Functional groups in the corncob determined by FTIR. A- untreated corncob, B: pre-treated corncob.

Scanning Electron Microscopy Structural Characteristics of Sugarcane Bagasse

The untreated sample displayed a rigid and compact structure, while the NaOH-treated bagasse exhibited a more disorganized morphology with exposed fibers and increased surface area, as shown in Figure 5.

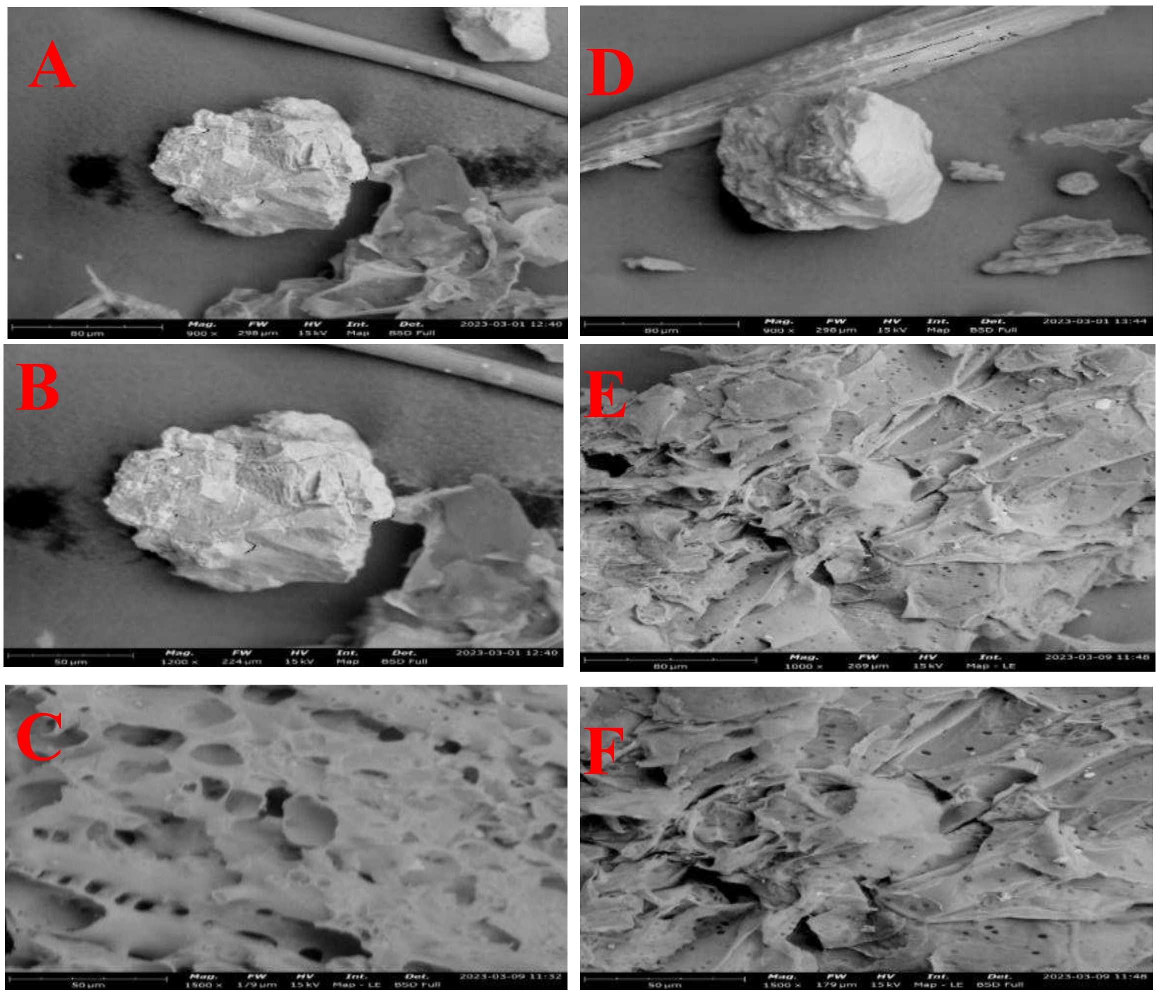

Figure 5: SEM images of sugarcane bagasse. Untreated -A: x900, B: x1200, C: x1500. Pre-treated with NaOH for 5 mins - D: x900, E: x1200 and F: x1500 objective lens)

Energy Dispersive X-ray Morphological Characteristics of Sugarcane Baggase

Untreated bagasse contained carbon (C), oxygen (O), silicon (Si), aluminium (Al), phosphorus (P), and indium (In) in varying concentrations. After NaOH treatment, Peaks for carbon and oxygen remained prominent, while new elements such as iron (Fe) and potassium (K) were detected. Elements like silicon (Si), phosphorus (P), indium (In), and aluminium (Al) were not observed in the treated sample, as shown in Figure 6.

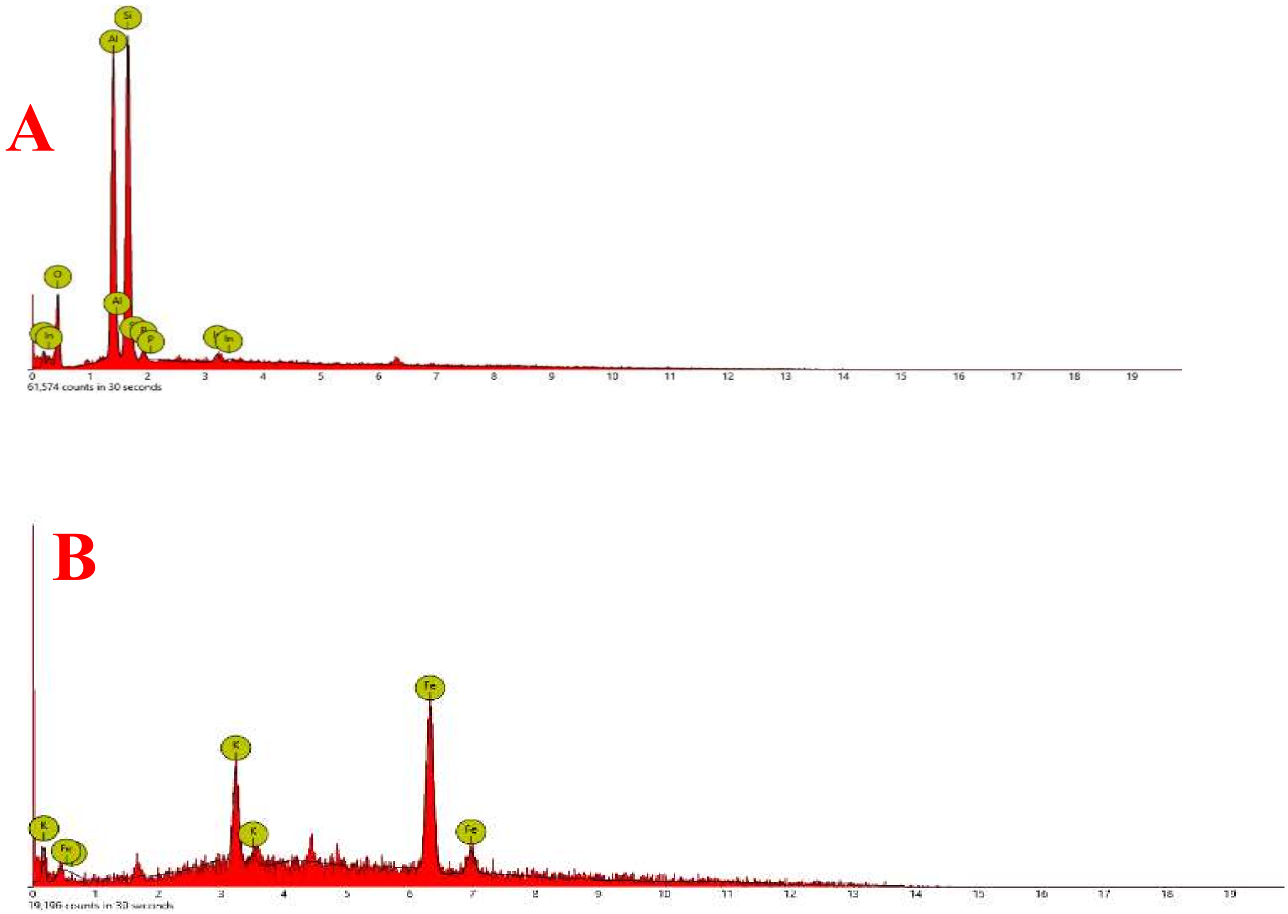

Figure 6: EDX graph of Sugarcane Bagasse. A= untreated bagasse, B= pre-treated bagasse

X-ray Diffractogram of Sugarcane Bagasse

Figure 7 displays the X-ray diffractograms of untreated sugarcane bagasse (A) and NaOH-treated bagasse (B). The untreated bagasse contained minerals such as refikite (50.5%), davyne (13.1%), chaoite (12.3%), mellite (9.2%), cristobalite (7.5%), and chlorite (7.5%). After NaOH treatment, the mineral composition shifted to include refikite (51%), mellite (1%), chlorite (1%), cristobalite (16.2%), chaoite (26.6%), and davyne (4%).

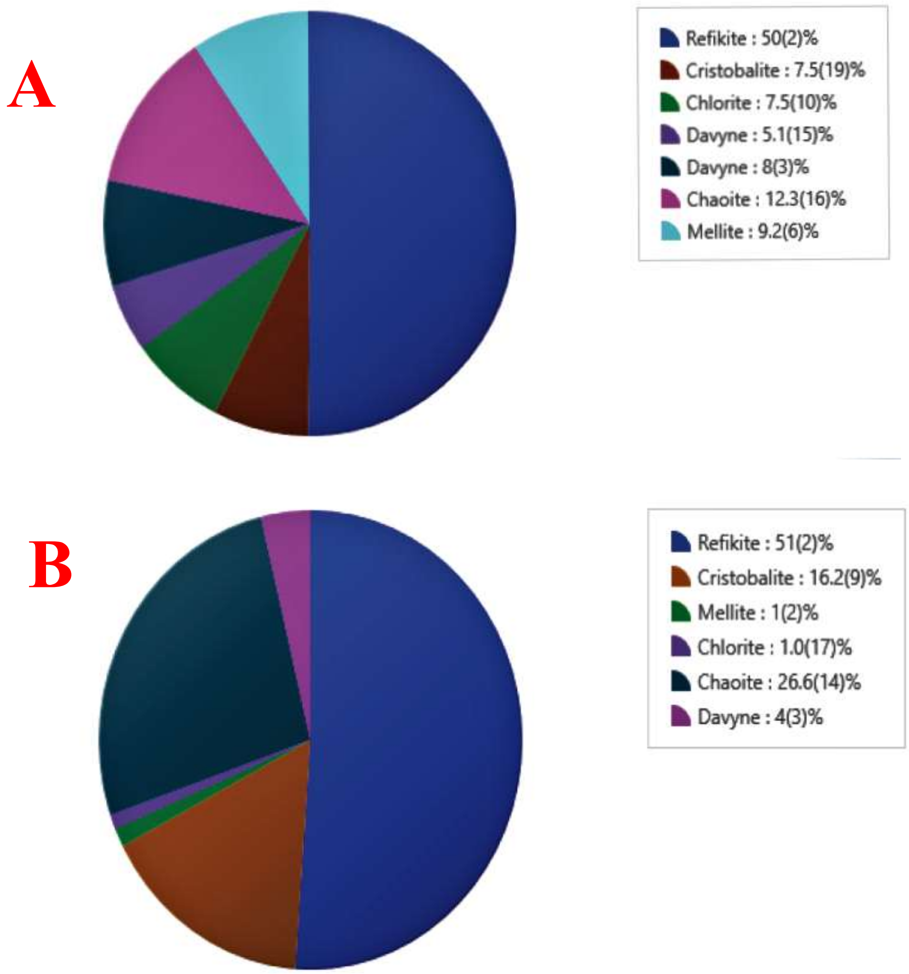

Figure 7: XRD diffractograms of untreated sugarcane bagasse = A, pre-treated sugarcane bagasse =B

Fourier Transform Infrared Spectroscopy of Sugarcane bagasse

Figure 8 presents the FTIR spectra of sugarcane bagasse before and after NaOH treatment. Untreated sugarcane bagasse displayed peaks at 3280 cm^{-1} (O-H), 2892 cm^{-1} (C-H), and 2370 cm^{-1} (C-O). NaOH treatment removed several peaks, while intensifying others like 1371 cm^{-1}. The spectral band at 1423 cm^{-1} highlighted significant cellulose, and the 1423/898 cm^{-1} ratio in treated samples confirmed structural changes and varying crystallinity of cellulose.

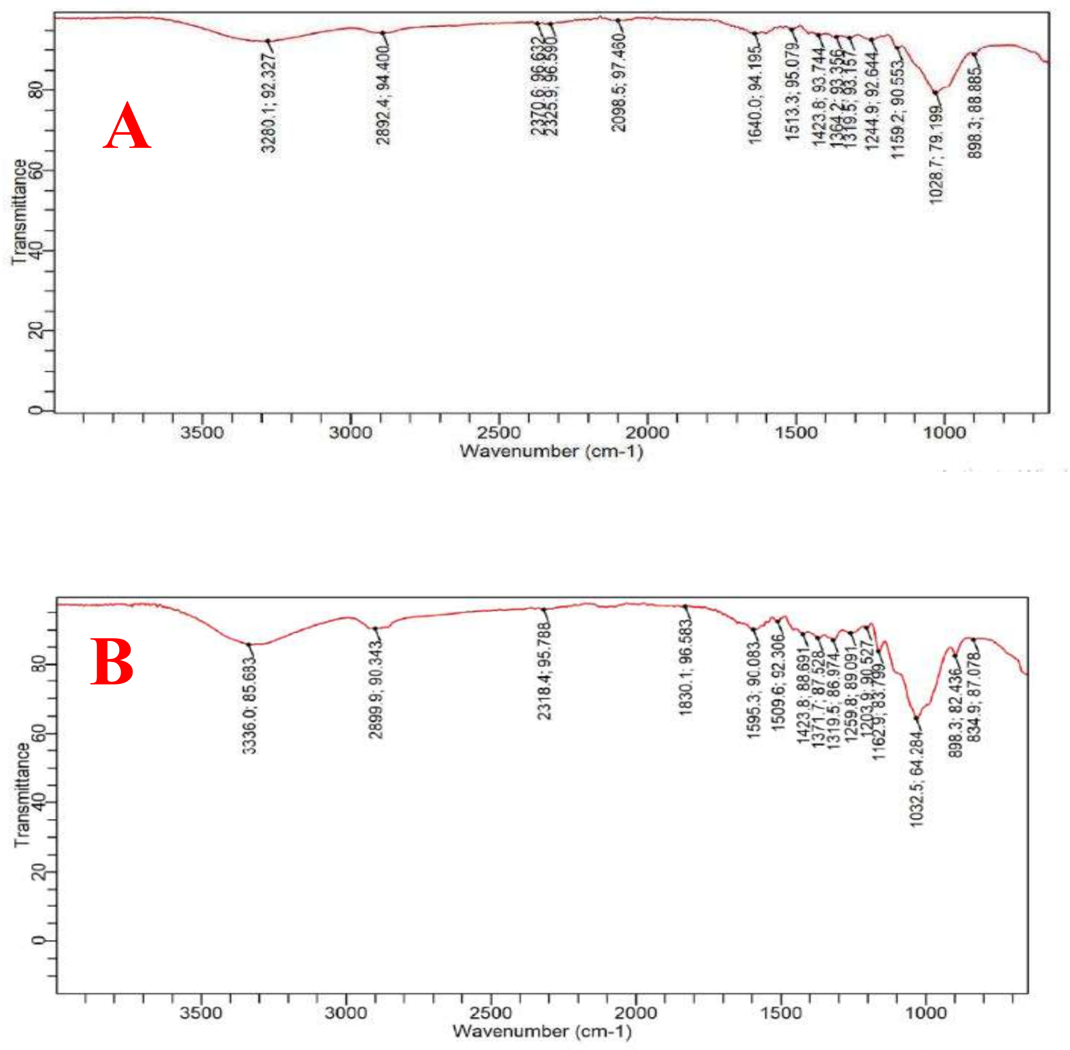

Figure 8: FTIR spectra, A-untreated bagasse, B - pre-treated bagasse.

Scanning Electron Microscopy Structural Characteristics of Plantain Peduncle

Figure 9 depicts the SEM images of the untreated and NaOH-treated plantain peduncle. The untreated sample exhibited a unique surface structure with uneven and asymmetrical roughness. In contrast, the NaOH-treated plantain peduncle showed a gristly and unbundled structure. The gristly components appeared more scattered, with individual fibres displaying enhanced separation and reduced bundling, indicating a breakdown of inter-fibre connections and a loosening of the fibre structure.

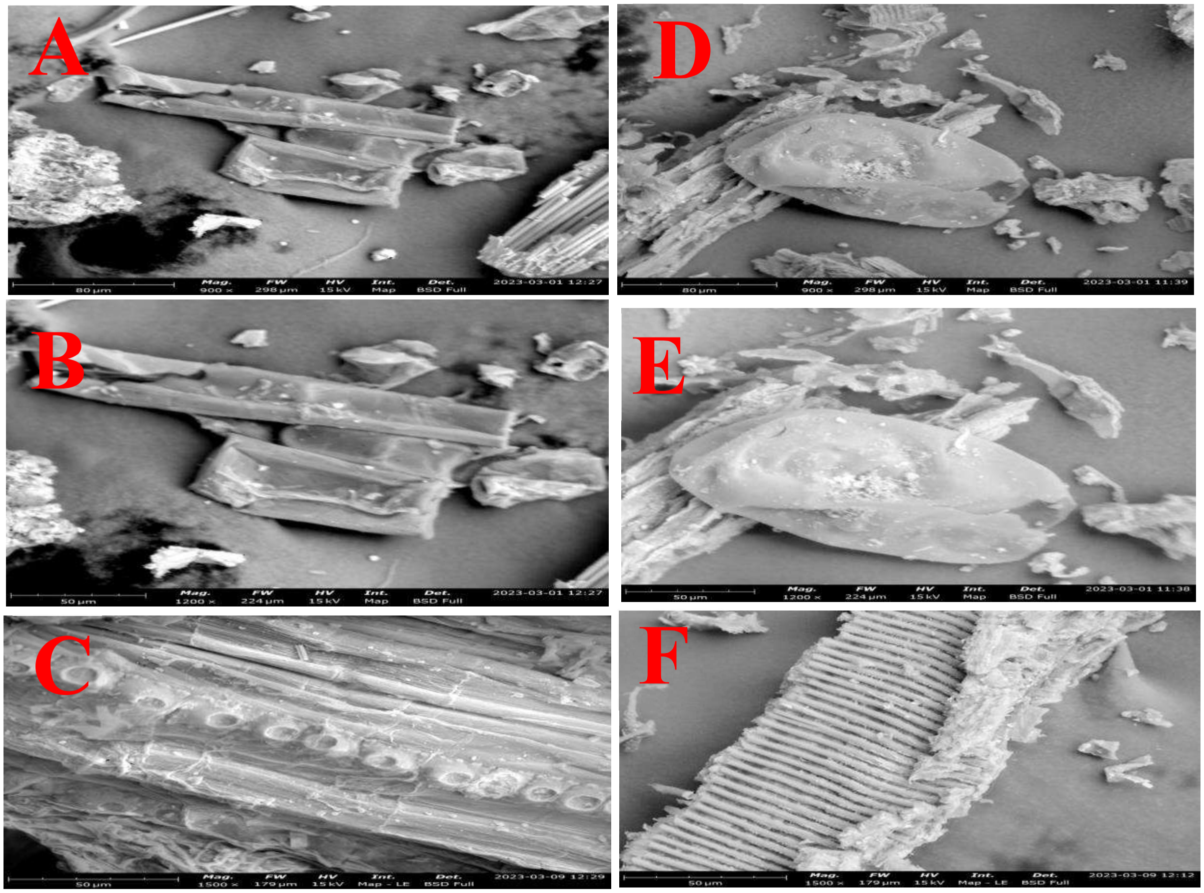

Figure 9: SEM images of plantain peduncle. Untreated - A:x900, B:x1200, C: x1500. Pre-treated with NaOH for 5 mins - D:x900, E:x1200, F: x1500 objective lens)

Energy Dispersive X-Ray Morphological Characteristics of Plantain Peduncle

Figure 10 illustrates the EDX analysis of the untreated and NaOH-treated plantain peduncle. In the untreated sample, the major elements detected included carbon (C), oxygen (O), potassium (K), americium (Am), calcium (Ca), tellurium (Te), and plutonium (Pu), present in varying atomic and weight concentrations.

The NaOH-treated sample retained carbon (C), oxygen (O), and potassium (K), while new elements such as iron (Fe), silicon (Si), chlorine (Cl), strontium (Sr), and bromine (Br) were identified. Conversely, elements like americium (Am), calcium (Ca), tellurium (Te), and plutonium (Pu) were not detected in the treated sample, likely due to the chemical action of NaOH.

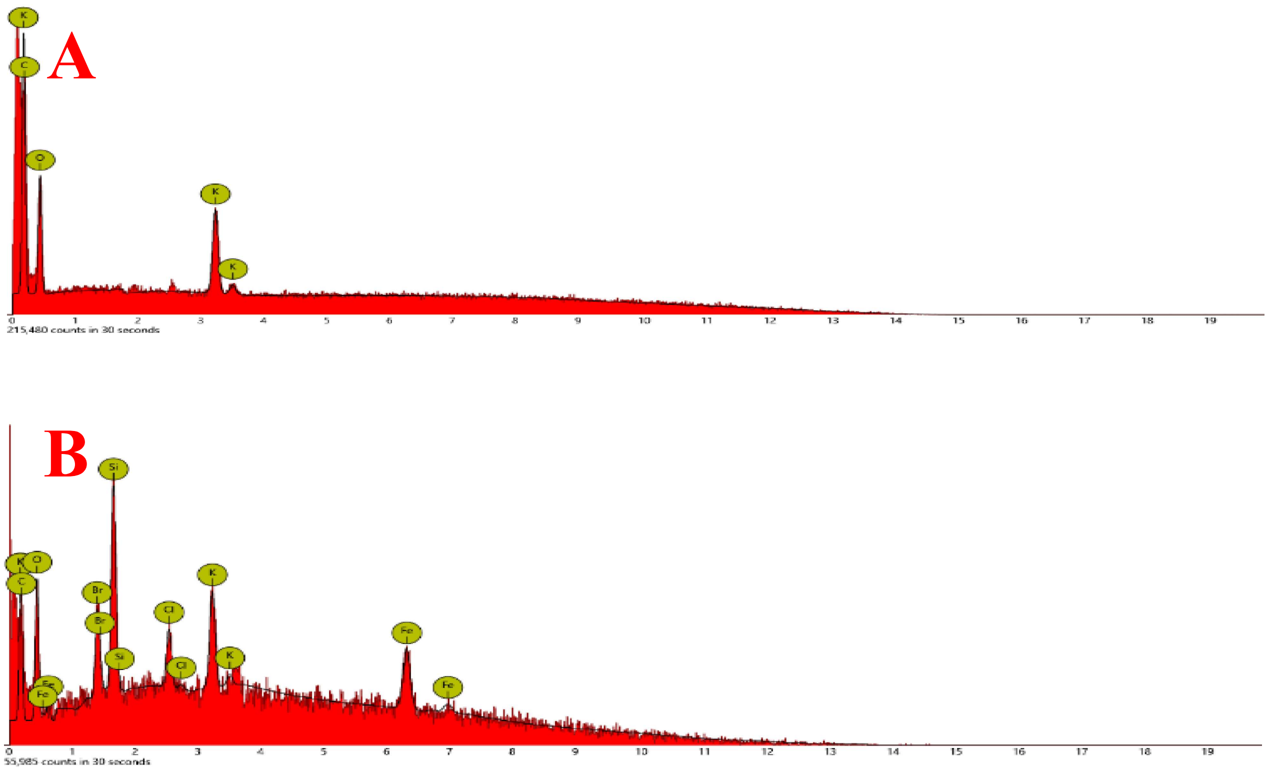

Figure 10: EDX graph of Agrowastes A=untreated plantain peduncle, B= pre-treated Plantain peduncle

X-ray Diffractogram of Plantain Peduncle

Figure 11 presents the X-ray diffractograms of the untreated and NaOH-treated plantain peduncle. The untreated plantain peduncle comprised minerals such as refikite (72%), davyne (2.5%), chaoite (9%), mellite (6.3%), cristobalite (7%), and chlorite (3%). After treatment with NaOH, the mineral composition shifted slightly, with refikite (72%), davyne (4.7%), chaoite (7.2%), mellite (3.4%), cristobalite (7.5%), and chlorite (5%) being identified.

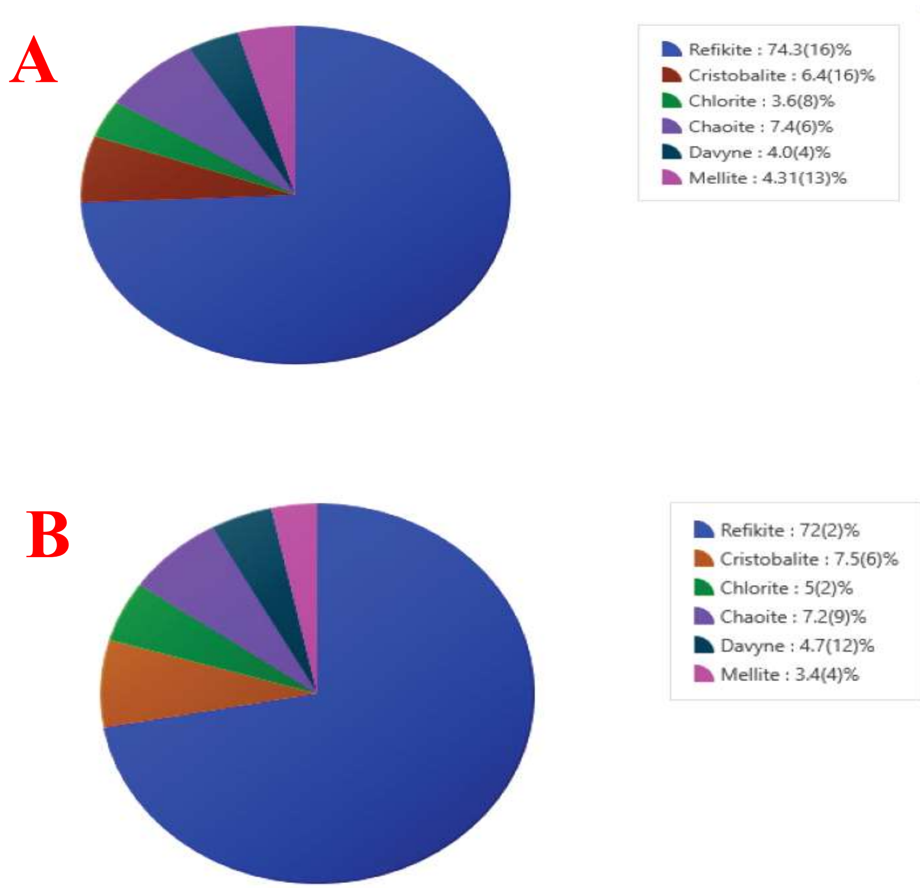

Figure 11: XRD diffractograms of untreated plantain peduncle= A ;and Pre-treated plantain peduncle =B

Fourier Transform Infrared Spectroscopy of Plantain Peduncles

Untreated plantain peduncles exhibited O-H peaks at 3276 cm^{-1}, C-H at 2922 cm^{-1}, and C=C at 1617 cm^{-1}. NaOH treatment introduced changes, including C-O stretching at 1200 cm^{-1} and N-O stretching at 1364 cm^{-1}. Spectral features confirmed aromatic compounds, alkenes, alcohols, and increased crystallinity, reflecting significant structural modifications after-treatment as seen in Figure 12.

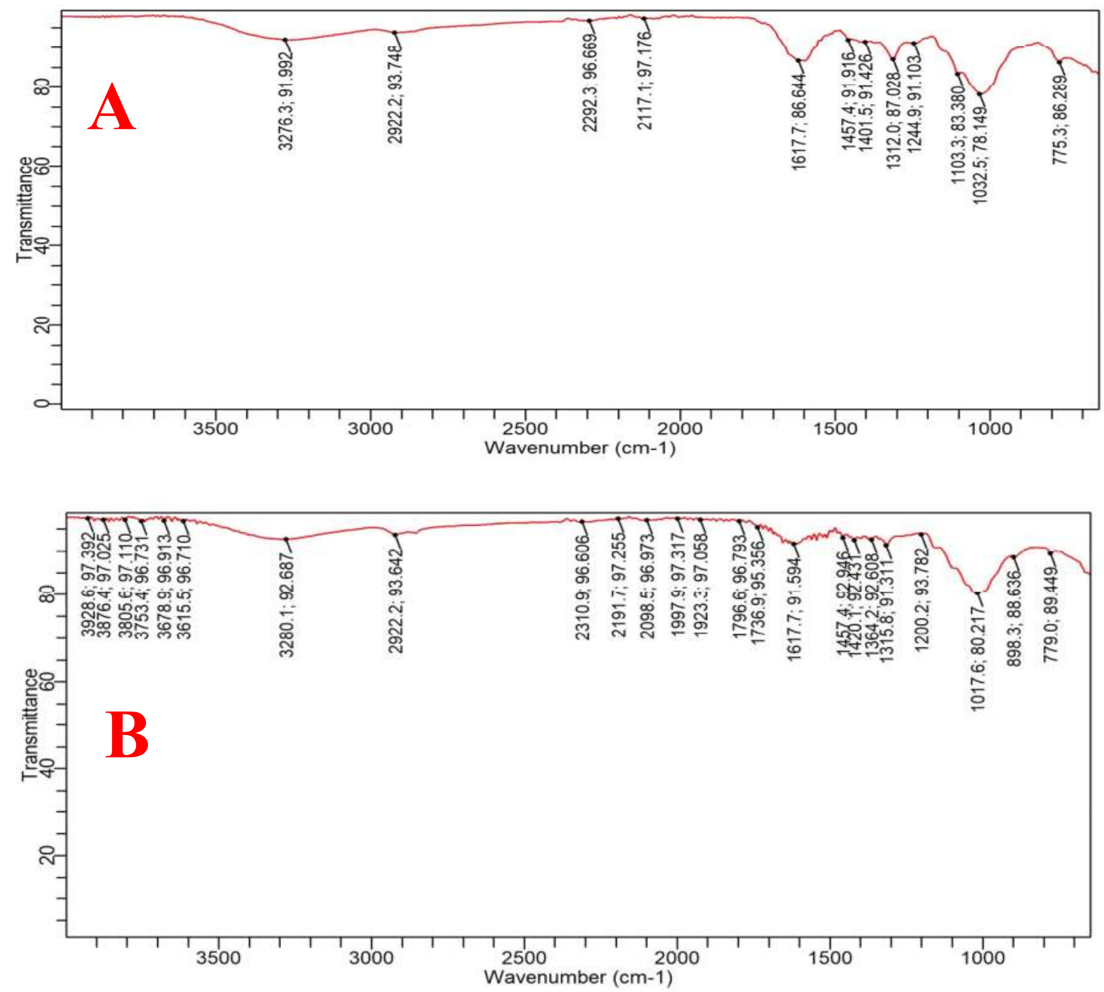

Figure 12: FTIR Analysis of Plantain Peduncles untreated plantain peduncle=A; After Pre-treatment of plantain peduncle=B

DISCUSSION

This study demonstrated that NaOH pretreatment effectively promotes delignification of agro-waste materials. Supporting studies by Binod *et al*. (2010) and Boonsombuti *et al*. (2013) highlight alkali pretreatment as an efficient method to remove lignin and parts of hemicellulose, thereby improving cellulose accessibility to enzymes. Sahare *et al*. (2012) reported that NaOH pretreatment creates a porous, smoother surface by removing roughness, expanding fiber structure, and increasing surface area, facilitating enzyme access and enhancing hydrolytic efficiency. Similarly, Putranto *et al*. (2021) observed that NaOH treatment efficiently reduces lignin and hemicellulose while increasing cellulose content in corncob biomass.

The microstructural changes observed in the pretreated agro-wastes result from alkaline pretreatment, where NaOH degrades the lignin-hemicellulose matrix. As a strong alkali, NaOH disrupts this matrix, weakening cell wall integrity and creating pores and channels in corncob structures (Xu *et al*., 2020). These structural modifications increase enzyme accessibility, promoting cellulose hydrolysis into fermentable sugars (Kaur and Kuhad, 2019).

Alterations in elemental composition between untreated and NaOH-pretreated agro-wastes likely arise from chemical interactions during pretreatment. Consistent with Adeogun *et al*. (2019), the untreated corncob surface mainly contained carbon and oxygen from cellulose, while pretreatment exposed more surface area, enhancing elemental peak intensities. Their study also reported that pulsed electric field-NaOH pretreatment introduced additional atoms, implying residual fibre content in treated samples.

These results highlight NaOH pretreatment's transformative impact on the elemental composition of corncob, sugarcane bagasse, and plantain peduncle, showing potential for selective elemental modification. Changes in elemental profiles stem from the removal of lignin, hemicellulose, and ash, along with possible incorporation of elements from chemicals or processing (Hiloidhari *et al*., 2020; Dey *et al*., 2021; Kumar *et al*., 2023). These modifications enhance carbohydrate accessibility, improving substrate suitability for laccase production.

The XRD analysis of untreated and NaOH-pretreated agro-wastes revealed distinct mineral peaks. While overall mineral profiles remained unchanged primarily, slight shifts in mineral proportions were observed after NaOH treatment, reflecting its selective impact on mineral composition in corncob, sugarcane bagasse, and plantain peduncle. This aligns with Raja *et al*. (2022), who found that alkali pretreatment combined with heat disrupts hydrogen bonds, dissolving lignin, hemicellulose, and amorphous materials. This exposes more crystalline cellulose, increasing the crystallinity index. Removing amorphous components also reduces the surface area masked from crystallinity measurement, further elevating the crystallinity index.

The FTIR spectra of the agro-wastes revealed a carboxyl group band at 3213 cm^1 and a band at 2109 cm^1 attributed to uronate via symmetrical carboxylate stretching (Ntozonke *et al*., 2017). The peak at 1397.8 cm^{-1} corresponded to OH groups, while the 1118.2 cm^{-1} peak was linked to amine N–H bending. Sugar derivatives were inferred from 849 and 928 cm^{-1} absorption bands associated with sugar monomers and β-glycosidic linkages (Agunbiade *et al*., 2018). The presence of hydroxyl and carboxyl functional groups suggests their role in the adsorption of suspended particles, making these agro-wastes suitable for fermentation. These functional groups significantly contribute to the efficient fermentation observed.

Conclusion

This study evaluated the effectiveness of sodium hydroxide (NaOH) alkaline pretreatment in enhancing agro-waste accessibility for microbial fermentation. SEM analysis showed clear structural changes such as increased porosity and fibre separation, improving substrate availability for laccase production. XRD results demonstrated increased crystallinity due to selective removal of amorphous lignin and hemicellulose, further facilitating cellulose accessibility. FTIR spectra confirmed modifications in functional groups, with reduced lignin-hemicellulose signals and enhanced cellulose and hydroxyl-related peaks. These findings validate sodium hydroxide pretreatment as an effective method for disrupting lignin-hemicellulose complexes and improving substrate suitability for fermentation.

Declarations Section

Authors Contribution

Oluwafemi Oyewole Adebayo (OOA) and Evans Egwim Chidi (EEC) conceptualised the study. The study was designed by Abioye Olabisi Peter (AOP) and Oluwafemi Oyewole Adebayo (OOA). Tsado Priscilla Yetu (TPY) participated in fieldwork and data collection. Tsado Priscilla Yetu (TYP) and Oluwafemi Oyewole Adebayo (OOA) performed the data analysis; Oluwafemi Oyewole Adebayo (OOA), Tsado Priscilla Yetu (TYP), Chimbekujwo Konjerimam Ishaku (CKI) and Ilyasu Ummulkhair Salamah (IUS) interpreted the data. Tsado Priscilla Yetu (TPY) prepared the first draft of the manuscript, reviewed by Oluwafemi Oyewole Adebayo (OOA), Abioye Olabisi Peter (AOP) and Chimbekujwo Konjerimam Ishaku (CKI). All authors contributed to developing the final manuscript and approved its submission.

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Disclosure of Conflict of Interest

None

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