FUDMA Journal of Sciences (FJS)
ISSN online: 2616-1370
ISSN print: 2645 - 2944
Vol. 9 No. 7, July, 2025, pp 268 – 274
DOI: https://doi.org/10.33003/fjs-2025-0907-3798

INVESTIGATING LIGNIN-MODIFYING ENZYMES FOR SUSTAINABLE PULP AND PAPER PRODUCTION

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ABSTRACT

The pulp and paper industry is under pressure to reduce its reliance on conventional pulping methods that involve harsh chemicals, high energy input and the generation of toxic effluents. This study presents a sustainable alternative using enzyme-assisted alkaline peroxide pretreatment for pulping agricultural residues, specifically corn (Zea mays) husk and pineapple (Ananas comosus) crown. Xylanase and ligninase enzymes produced from Aspergillus niger and Trichoderma reesei grown on agro-waste substrates, were evaluated for their delignification potential. Enzyme activity was measured using UV spectrophotometry while lignin removal post-treatment was quantified chemically. Among the enzymes tested, xylanase demonstrated the highest delignification efficiency. Peak xylanase activity on pineapple crown was recorded at 3.4854 nm after 96 hours, while corn husk showed a maximum activity of 1.9535 nm at 72 hours. These variations underscore the importance of enzyme-substrate compatibility and incubation time for optimal performance. Xylanase pretreatment led to substantial lignin reductions of 64.91% for pineapple crown and 60.61% for corn husk facilitating improved fiber liberation. This enhances pulp yield, fiber bonding, and paper strength making the process suitable for industrial application. By integrating enzymatic treatment with alkaline peroxide pretreatment, this method offers an eco-friendly pulping approach that reduces chemical dependency and environmental burden. Furthermore, the use of agricultural residues promotes waste valorization and supports a circular bioeconomy. This research demonstrates the viability of enzyme-assisted pulping as a green technology pathway, advancing both environmental sustainability and economic efficiency in the pulp and paper industry.

Keywords: *Ananas comosus, Aspergillus niger*, Pulp and Paper, *Ligninase*, Xylanase, *Zea mays*

INTRODUCTION

The increasing environmental and economic pressures associated with traditional chemical pulping methods have catalyzed a shift toward sustainable alternatives in the pulp and paper industry (Mboowa, 2024). Conventional processes often rely on harsh chemicals and high energy inputs resulting in toxic effluent discharge and significant ecological footprints (Abyar, & Nowrouzi 2023). As a response, enzyme-assisted pulping has emerged as a viable green technology offering an eco-friendly route to lignin removal while preserving fiber quality and reducing chemical dependency (Kumar et al., 2022).

Among the diverse classes of enzymes applied in biopulping, xylanases and ligninases have shown particular promise. These enzymes catalyze the breakdown of hemicellulose and lignin respectively, facilitating fiber separation by enhancing pulp yield and strength properties (Yang et al., 2019). The enzymatic depolymerization of lignin not only improves the digestibility of plant fibers but also minimizes the generation of environmentally persistent pollutants. Despite these advantages, there is limited research on the synergistic use of xylanase and ligninase in conjunction with alkaline peroxide chemistry a combination that has the potential to further optimize delignification processes (Ahmad et al., 2023). Agricultural residues such as corn (*Zea mays*) husks and pineapple (*Ananas comosus*) crowns represent abundant and underutilized lignocellulosic feedstocks, especially in regions like Nigeria where these crops are widely cultivated (Adeleke et al., 2023). These residues contain substantial amounts of cellulose and hemicellulose and can serve as promising raw materials for sustainable pulp production (Worku et al., 2023). Their valorization not only supports waste-to-resource strategies but also aligns with global efforts to develop circular bioeconomies.

This study explores a novel biotechnological approach by evaluating the delignification efficiency of xylanase and ligninase enzymes, individually and in combination applied to corn husk and pineapple crown leaves. Uniquely, the same agro-wastes served as substrates for microbial enzyme production making the process both cost-effective and environmentally sound. The enzymes isolated from *Aspergillus niger* and *Trichoderma reesei* were assessed through spectrophotometric assays while lignin removal was quantified post-treatment using chemical analysis. The enzymatic pretreatments were integrated with alkaline peroxide chemistry to enhance lignin degradation. To the best of the authors' knowledge, this is the first study to investigate and compare the performance of xylanase and ligninase on these two specific agro-wastes within an integrated alkaline peroxide pulping system. This research contributes to the development of low-impact pulping strategies and highlights the potential of enzyme technology in valorizing agricultural residues for sustainable industrial applications.

MATERIALS AND METHODS

Chemical Characterization of Corn Husk and Pineapple Crown Leaves

The chemical composition of corn husk and pineapple crown leaves was characterized before and after enzymatic treatment to assess the extent of lignin dissociation. Standard procedures established by the Technical Association of the Pulp and Paper Industry (TAPPI) were followed. Hot water solubility (TAPPI T 207 cm-99), ash content (TAPPI T 211 om-02), acid-insoluble lignin (TAPPI T 222 om-06) and 1% sodium hydroxide solubility (TAPPI T 212 om-02) were determined. Holocellulose content was analyzed using the chlorination method, while cellulose content was estimated by the Kürschner-Höffner method. Both cold and hot water

solubilities were evaluated according to TAPPI T 207 cm-99. These chemical analyses provided insights into the structural modifications induced by enzymatic pretreatment, supporting the evaluation of the material's suitability for pulp and paper applications (Aguilar-Rivera & Olvera-Vargas 2022).

Isolation and Cultivation of Fungal Strains

Fungal isolates were sourced from agricultural wastes, specifically corn husk and pineapple crown leaves. Samples were thoroughly washed, air-dried and crushed aseptically using a sterile mortar and pestle. A 10 g portion of each homogenized sample was suspended in 90 mL of sterile distilled water to create an initial 10^{-4} dilution. Serial dilutions were performed up to 10^{-5} and aliquots (0.1 mL) from the 10^{-2}, 10^{-4} and 10^{-5} dilutions were inoculated onto Potato Dextrose Agar (PDA) plates. The plates were incubated at 28 ± 2°C for 3–5 days to allow fungal growth. Isolation was conducted following the pour-plate method (Abdulmumini et al., 2022).

Morphological Identification of Fungal Isolates

Colonial Morphology

Isolates were sub-cultured onto fresh PDA plates and incubated at 28 ± 2°C for five days. Observations were made on colony color, growth rate, reverse side pigmentation, surface texture and morphological changes over time (Aragaw et al., 2023).

Microscopic Examination

Microscopic identification involved preparing wet mounts stained with lactophenol cotton blue. A portion of fungal mycelium was transferred onto grease-free glass slides, stained, and observed under a microscope at 40× magnification to examine the fungal hyphal and spore structures (Nadodkar et al., 2024).

Enzymatic Screening of Fungal Isolates

Xylanase Activity Assay

The ability of fungal isolates to produce xylanase was evaluated using a minimal agar medium supplemented with 0.5% xylan as the sole carbon source. The medium comprised $MgSO_4 \cdot 7H_2O$ (0.05 g), KCl (0.23 g), $CaCl_2$ (0.005 g), Peptone (2.0 g), $NaNO_3$ (0.005 g), $FeSO_4 \cdot 7H_2O$ (0.009 g), $ZnSO_4$ (0.012 g), $MnSO_4$ (0.012 g), KH_2PO_4 (0.23 g), Agar-agar (18 g), Xylan (5 g), and distilled water (1000 mL). Inoculated plates were incubated at 28 ± 2°C for two days. Enzyme activity was visualized by flooding plates with 0.4% Congo red solution, followed by distaining with 1M NaCl. Clear halos around colonies indicated xylanase production (Dhaver et al., 2022).

Ligninolytic (Ligninase) Activity Assay

Ligninolytic activity was screened using PDA medium supplemented with kraft lignin and 0.04% Remazol Brilliant Blue R (RBBR). The prepared plates were surface-dried at 45°C, inoculated with fungal isolates, and incubated at 28°C for seven days in the dark. Clear zones of discoloration around fungal growth were taken as evidence of ligninolytic enzyme activity (De Sousa et al., 2024).

Screening for Optimal Agrowaste

The enzymatic pretreatment of the corn husk and pineapple crown leaves was carried out according to the procedure reported by Jain et al. (2023) with modifications. The materials were dried to a constant weight in a hot air oven at 50°C, milled using a laboratory ball milling machine and stored in sterile Ziploc bags at 4°C. Milled dried samples (10 g) were weighed individually into four separate 250 mL conical flasks. Treatments were as follows: (i) distilled water (control), (ii) ligninase (LP), (iii) xylanase (XP) and (iv) ligninase and xylanase mixture (LXP). The flasks were incubated at ambient temperature for 144 hours (6 days). At 24-hour intervals, 10 mL aliquots were withdrawn and the optical density (OD) at 600 nm was measured using a UV spectrophotometer. Measurements were recorded for further analysis.

Effect of Xylanase Pretreatment on Chemical Composition

Enzymatic Pretreatment of Biomass

Based on preliminary enzyme screening results, xylanase was identified as the most effective enzyme for delignification exhibiting peak activity at 96 hours for pineapple crown and 72 hours for corn husk. Consequently, xylanase was selected for enzymatic pretreatment of both biomass samples in comparison to ligninase and a ligninase–xylanase combination which demonstrated lower delignification efficiency (Hebal et al., 2021). For the pretreatment, accurately weighed portions of corn husk and pineapple crown substrates were incubated with xylanase under the optimized conditions determined during the enzyme screening phase of 72 hours for corn husk and 96 hours for pineapple crown. Enzymatic reactions were conducted in controlled conditions appropriate for xylanase activity (e.g., optimal pH and temperature) ensuring effective lignin breakdown.

Following incubation, the treated biomass samples were thoroughly washed with distilled water to remove residual enzymes and soluble degradation products. The washed samples were then oven-dried at 60 °C until a constant weight was achieved. Dried samples were stored in airtight ziplock at room temperature prior to subsequent chemical characterization (Dhaver et al., 2022).

Chemical Composition Analysis

Both untreated and xylanase-pretreated samples were chemically characterized following standard TAPPI methods. Parameters analyzed included ash content, 1% NaOH solubility, cold water solubility, hot water solubility, acid-insoluble lignin, extractives and holocellulose content. All analyses were performed in triplicate and reported as mean ± standard deviation. Comparative evaluation emphasized lignin reduction as the primary indicator of delignification efficiency.

RESULTS AND DISCUSSION

Chemical Characterization of Corn Husk and Pineapple Crown Leaves

The chemical composition of corn husk and pineapple crown leaves is presented in Table 1. Both materials exhibited comparable ash contents of 3.87% and 3.88% respectively indicating similar levels of inorganic residue. However, pineapple crown leaves showed greater solubility in 1% NaOH of 12.00% as well as in cold of 4.60% and hot water of 7.00% compared to corn husk of 10.33%, 3.80% and 6.58%, respectively. This suggests a higher proportion of low-molecular-weight carbohydrates and extractives in pineapple leaves, which is further supported by their elevated extractives content of 4.90% compare to corn husk of 3.00%. The result shows that the biomass gave Holocellulose of 89.60 ± 7.72% (corn husk) and 90.40 ± 8.13% (pineapple crown). Conversely, corn husk exhibited higher acid-insoluble lignin of 9.85% compare to hat of pineapple of 7.95% and α-cellulose content of 59.00% with pineapple of 54.00% indicative of a denser, more lignified fiber structure.

Table 1: Chemical characteristics of Corn husk and Pineapple crown

Chemical Parameters	Corn Husk	Pineapple crown
Ash content (%)	3.87 ± 0.90	3.88 ± 0.50
1% NaOH solubility	10.33 ± 0.02	12.00 ± 1.11
Cold water solubility (%)	3.80 ± 0.01	4.60 ± 1.65
Hot water solubility (%)	6.58 ± 0.80	7.00 ± 1.21
Acid insoluble lignin (%)	9.85 ± 0.18	7.95 ± 1.05
Extractive contents (%)	3.00 ± 1.00	4.90 ± 2.35
Holocellulose (%)	89.60 ± 7.72	90.40 ± 8.13
Alpha- Cellulose (%)	59.00 ± 5.57	54.00 ± 1.31

These differences indicate the distinct potentials of the two raw materials. The higher cellulose and lignin content in corn husk suggests greater fiber strength and durability, advantageous for printing paper production (Ratna et al., 2022). Meanwhile, the higher holocellulose and extractives content in pineapple crown leaves may contribute to enhanced fiber flexibility and bonding ability (Mathura, & Maharaj 2024). Both materials demonstrate promising qualities for sustainable paper production with their specific chemical profiles offering complementary advantages.

Identification of Fungal Isolates and Enzymatic Activity Screening

Fungal Isolates

Two fungal species were successfully isolated and identified based on their colonial and microscopic characteristics.

Aspergillus niger

Colonial Morphology: The colonies exhibited rapid growth with a fluffy, velvety texture. Initially, the colonies appeared white due to the presence of aerial mycelium, gradually transitioning to a brown-to-black color with no distinct reverse coloration.

Microscopic Morphology: The conidial heads were large, round, and radiating in structure. The conidiophores were smooth, brown, and often fragmented upon crushing. The vesicles were globose and bore phialides directly on their surface, with metulae distinctly present.

Trichoderma-reesei

Colonial Morphology: The colonies demonstrated rapid cobweb-like growth with irregular clusters of dark green spores and visible reverse pigmentation.

Microscopic Morphology: Conidia were small, oval-shaped, and formed in dense, ball-like clusters along right-angled branches. The conidial apparatus appeared irregular, and the conidia were distinctly green.

Enzymatic Activity Screening

Xylanase-Activity

Aspergillus niger showed significant xylanase production, as indicated by the formation of a clear halo zone around the fungal growth on xylan agar plates, following Congo red staining. This halo formation confirmed the fungal ability to degrade xylan polymers, highlighting its potential for xylanase production (Plate 1)

Ligninolytic-Activity

Trichoderma reesei exhibited prominent ligninolytic activity, evidenced by a clear decolorized zone surrounding the fungal growth on Remazol Brilliant Blue R (RBBR) agar. This decolorization indicates the production of lignin-degrading enzymes, demonstrating T. reesei's potential in lignin biotransformation processes (Plate 2)

Visual Representation of Enzymatic Activity on Culture Plates

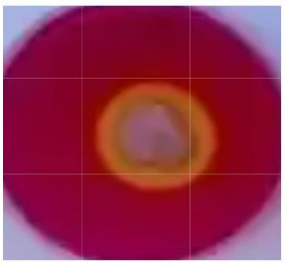

Plate 1: *Culture plate of Aspergillus niger on xylan agar medium.* The plate shows a clear halo around the fungal growth after Congo red staining, indicating xylanase production

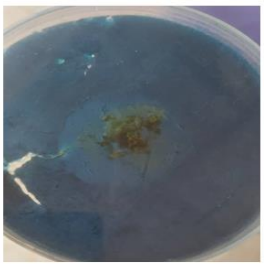

Plate 2: *Culture plate of Trichoderma reesei on Remazol Brilliant Blue R (RBBR) agar.* The fungal growth is surrounded by a decolorized zone demonstrating ligninolytic enzyme production

Enzymatic Activities on Corn Husk and Pineapple Crown Leaves

Lignin Degradability Trends

Figures 1 and 2 illustrate lignin degradability over time for the enzymatic treatments applied to pineapple crown and corn husk. Figure 3 highlights the effect of ligninase, xylanase and their combination on the breakdown of lignin in the substrates. The results demonstrate the effectiveness of these enzymes in degrading the complex lignin-rich plant cell wall structures with noticeably higher activity observed in pineapple crown compared to corn husk at all incubation periods. Enzymatic activity increased steadily over the first 96 hours of incubation. For example, xylanase XP applied to the substrate blend showed a rise in absorbance from 2.04 to

3.48 indicating enhanced lignin breakdown. Similarly, ligninase LP activity on pineapple substrate rose from 2.03 to 2.76 when both enzymes LXP were used in combination a comparable upward trend was also observed especially with the pineapple crown suggesting synergistic effects in lignin degradation. Peak degradability occurred at 96 hours for all enzyme treatments with values ranging from 2.76 to 3.48. However, extending the incubation beyond 96 hours resulted in a decline in enzymatic activity possibly due to enzyme denaturation or substrate exhaustion (Kabir, & Ju, 2023). Among all treatments, xylanase alone showed the highest degradability of 3.4854 at 96 hours as determined by UV spectrophotometry at 600 nm. This indicates xylanase's superior efficiency in breaking down lignin particularly in pineapple crown and underscores its potential as the good effective enzymatic agent for bio-pulping applications (Dukare et al., 2023)

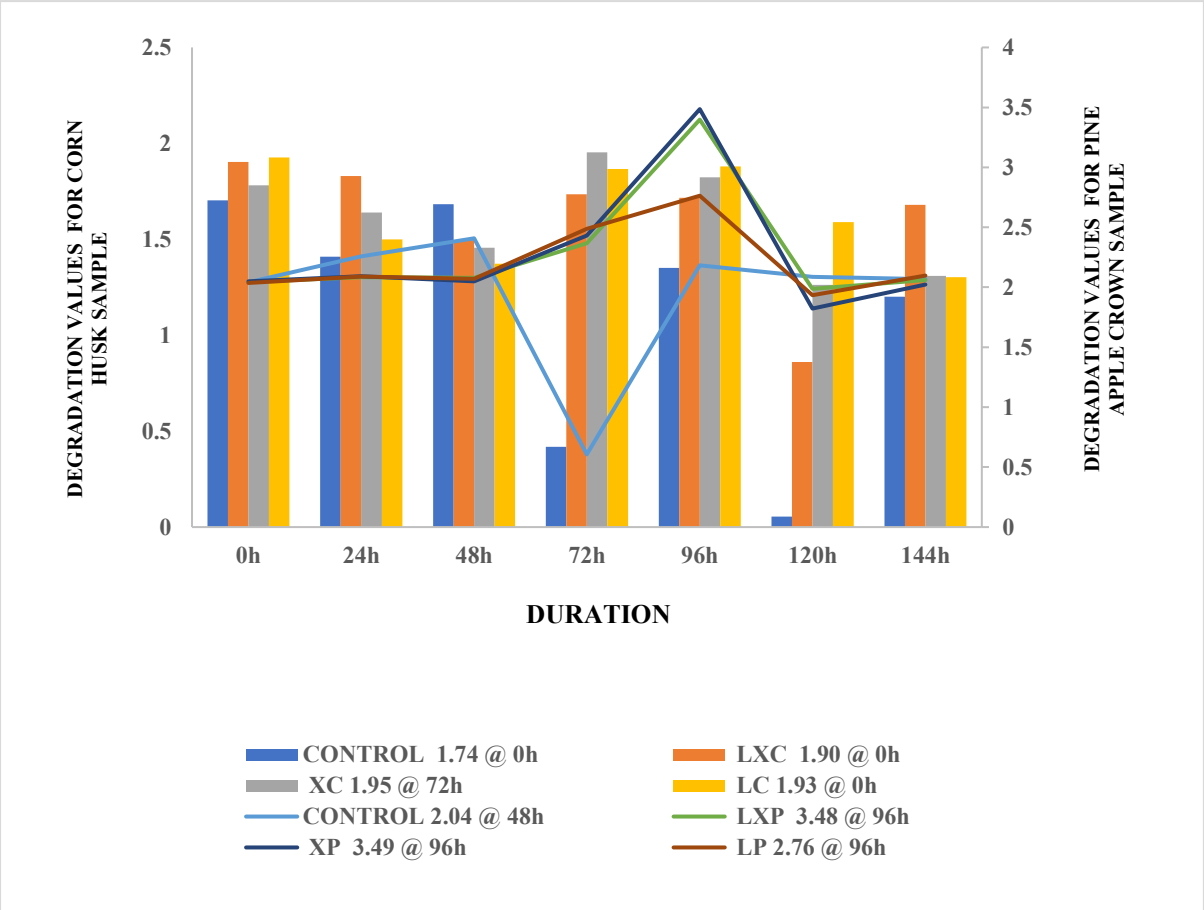

Figure 1: Enzymatic degradability of both Corn Husk Sample and Pineapple crown Sample (NB: Where control portion contains only distill water, LXC and LXP represents the portion with both xynalase and ligninase only (for both corn husk and Pineapple crown); the LC and LP represents the portion with only Ligninase (for both corn husk and Pineapple crown) while XC and XP represents the portion with only Xynalase)

In contrast to the trend observed in *Ananas comosus* crown, the enzymatic degradation pattern of *Zea mays* husk using ligninase (LC), xylanase (XC) and a combination of both enzymes (LXC) exhibited a distinct behavior (Figure 2). At 0 hours, the highest initial degradability values were recorded for ligninase alone (LC) at 1.9367 and for the ligninase/xylanase combination (LXC) at 1.9031 based on absorbance readings obtained via UV-spectrophotometry. These elevated initial values likely resulted from immediate enzyme-substrate interactions before the onset of significant incubation dynamics (Neun et al., 2022). Between 24 and 48 hours a decline in degradability was observed across treatments. Specifically, the degradability value for LXC dropped from 1.8295 at 24 hours to 1.6395at 48 hours, while LC showed a decrease from 1.4955 to 1.4566 during the same period. This reduction may be attributed to transient enzyme inhibition, substrate saturation, or the formation of inhibitory by-products that temporarily suppressed enzymatic activity (Neun et al., 2022).

At 72 hours, a resurgence in enzymatic activity was observed, particularly for the xylanase (XC) treatment, which achieved the highest degradability value recorded across all time points, reaching 1.9500. In contrast, LC and LXC treatments also showed moderate increases, suggesting that structural loosening of lignocellulosic complexes or delayed enzymatic access to previously inaccessible sites facilitated renewed substrate breakdown. However, further incubation up to 96 hours led to a gradual decline in degradability across all treatments, suggesting possible enzyme denaturation, exhaustion of readily degradable components within the corn husk matrix, or accumulation of degradation-resistant residues (Manyi-Loh et al., 2023). Notably, while the control group showed a steady decline over time, XC maintained relatively higher degradability values compared to other treatments, indicating better sustained enzymatic activity. In the case of ligninase (LC) applied in isolation, although an initial decline was evident between 24 and 48 hours, enzymatic activity remained comparatively stable over the

later stages of incubation, with degradability values at 96 hours remaining high at approximately 1.8800. This trend suggests that ligninase may operate optimally with extended incubation, possibly due to its selective affinity for lignin structures that become more exposed as incubation progresses. The different degradability patterns observed between *A. comosus* crown and *Z. mays* husk highlight underlying anatomical and biochemical differences between the two biomasses. Previous studies have shown that *A. comosus* crown contains 80–89% cellulose and 10–14% lignin (Rahaman et al., 2023), whereas *Z. mays* husk contains approximately 50–55% cellulose and 10–15% lignin (Bajpai et al., 2022). The higher cellulose content and potentially more porous structure of *A. comosus* may facilitate faster enzymatic access and breakdown, accounting for the faster degradation observed in earlier experiments (Tong et al., 2024).

Furthermore, xylanase (XC) demonstrated superior lignocellulose degradation efficiency during the 72 to 96hour window particularly for *Z. mays* husk. This observation suggests that xylanase may be more effective in the early to mid-stages of pulping especially for non-woody agrowaste materials with substantial hemicellulose content. Interestingly, the combined application of ligninase and xylanase (LXC) consistently resulted in lower degradability values compared to individual enzyme treatments particularly evident up to 96 hours. The reduced efficiency in the combined enzyme treatment could be attributed to potential enzymatic interference such as substrate competition, active site blocking or non-synergistic interactions (Jain et al., 2023). These show the complexity of enzymatic interactions with different agrowaste biomasses and highlight the importance of tailoring enzyme selection and incubation parameters to the specific structural characteristics of the target substrate.

Table 2: Effect of Xylanase Pretreatment on Chemical Composition of Corn Husk and Pineapple Crown

Parameters	Corn husk			Pineapple crown leaves		
Treatment phases	**Before Xylanase pretreatment**	**After Xylanase pretreatment**	**% Change in pretreatment**	**Before Xylanase pretreatment**	**After Xylanase pretreatment**	**% Change in pretreatment**
Ash content (%)	3.87 ± 0.23	3.19 ± 0.51	- 17.56	3.88 ± 0.9	3.66 ± 1.28	-5.67
1% NaOH	10.33 ±1.15	9.61 ± 0.81	-6.97	12.00 ± 0.21	11.02 ± 0.80	-8.17
Cold water solubility (%)	3.8 ± 0.47	3.6 ± 1.27	-5.26	4.60 ± 2.27	4.63 ± 1.34	0.65
Hot water solubility (%)	6.58± 0.27	5.86 ± 0.93	-10.94	7.00 ± 2.27	7.03 ± 1.34	0.43
Acid insoluble Lignin (%)	9.85 ± 0.19	3.88 ± 0.58	-60.61	7.95 ± 0.52	2.79 ± 0.38	- 64.91
Extractive (%)	3.0	2.43 ± 0.80	- 9.00	4.90	4.18 ± 1.12	-14.69
Holocellulose (%)	89.60	87.36 ± 2.06	-2.50	90.40	87.9 ± 3.01	-2.77
α- Cellulose (%)	59.00	57.1 ± 4.74	-3.22	54.00	52.78 ± 2.12	-2.30

Enzymatic Pretreatment of Biomass

Effect of Xylanase Pretreatment on Chemical Composition

The chemical composition of corn husk and pineapple crown leaves before and after xylanase pretreatment is shown in Table 4. Xylanase pretreatment notably improved the chemical composition of both corn husk and pineapple crown leaves enhancing their suitability for sustainable pulp production. The ash content in corn husk decreased from 3.87% to 3.19%, representing a 17.56% reduction while in pineapple crown leaves it declined from 3.88% to 3.66% corresponding to a 5.67% decrease. The reduction in ash content reflects the removal of inorganic matter and mineral impurities contributing to improved fiber quality and reduced process residues (Kukuruzović et al., 2023).

A slight decrease was observed in NaOH solubility, dropping from 10.33% to 9.61% (6.97% reduction) in corn husk and from 12.00% to 11.02% (8.17% reduction) in pineapple crown. This suggests a partial removal of low-molecular-weight lignin and hemicellulose components resulting in a more purified cellulose matrix (Tanis et al., 2023). Changes in water solubility were minimal. Cold water solubility decreased by 5.26% in corn husk while pineapple crown leaves exhibited a slight increase of 0.65%. Hot water solubility remained almost unchanged; indicating that xylanase pretreatment primarily targeted bound lignin and hemicellulose with minimal effect on water-soluble extractives (Kaur et al., 2024). A significant reduction in lignin content was achieved in both substrates. Lignin content in corn husk decreased from 9.85% to 3.88% amounting to a 60.61% reduction while in pineapple crown it dropped from 7.95% to 2.79% representing a 64.91% reduction. This substantial delignification confirms the selective action of xylanase which facilitates lignin removal by disrupting the hemicellulosic matrix and enhancing lignin accessibility (Zhai et al., 2022).

Extractive content also decreased following pretreatment. In corn husk, extractives reduced from 3.00% to 2.43% (19.00% reduction), and in pineapple crown, from 4.90% to 4.18% (14.69% reduction). Lower extractive content is beneficial for pulping processes, as it mitigates pitch-related issues and improves paper quality (Lehr et al., 2021). Holocellulose content exhibited a slight decline, from 89.60% to 87.36% (2.5% decrease) in corn husk and from 90.40% to 87.90% (2.77% decrease) in pineapple crown. These minor losses may be attributed to partial hydrolysis of hemicellulose while the cellulose fraction remained largely intact underscoring the selective and mild action of xylanase (Baksi et al., 2023).

However, the results show that xylanase pretreatment significantly enhanced the chemical profile of both corn husk and pineapple crown leaves. The substantial lignin reduction, minimal loss of holocellulose, and decreased ash and extractive contents confirm xylanase as an effective, selective and eco-friendly enzyme for improving the pulping potential of non-wood biomass for alkaline peroxide and other sustainable pulping processes. The results from both the pineapple crown and corn husk experiments shows the variable effects of enzymatic treatments on different lignocellulosic substrates. In pineapple crown leaves,

xylanase treatment consistently outperformed ligninase and even the combined enzyme application in terms of lignin degradation. This superiority suggests that xylanase pretreatment effectively disrupted the hemicellulosic network, thereby enhancing lignin accessibility for subsequent chemical delignification (Kabir & Ju, 2023).
In corn husk samples however, ligninase demonstrated a more favorable trend, albeit with delayed action. This might be attributed to the denser and less porous fiber structure of corn husks, which possibly limits initial enzyme penetration. Likewise, the combination of ligninase and xylanase in both biomass sources did not consistently result in synergistic improvements. Rather, enzymatic competition or interference seemed to compromise their combined effectiveness. This outcome aligns with previous studies suggesting that enzyme cocktails must be carefully optimized as simultaneous action does not always guarantee enhanced lignin removal (Manyi-Loh, & Lues. 2023).

Comparative Performance and Implications
Overall, xylanase showed greater effectiveness than ligninase or combined enzymatic applications particularly in pineapple crown leaves. Corn husk samples responded more favorably to ligninase with extended incubation. The lack of synergistic effects in combined treatments highlights the need for enzyme cocktail optimization as simultaneous application may lead to substrate competition (Melati et al., 2019).

CONCLUSION

This study demonstrated the potential of corn husk and pineapple crown leaves as sustainable raw materials for pulp production. Chemical characterization revealed favorable holocellulose and α-cellulose contents in both biomasses with corn husk exhibiting higher lignin and cellulose contents while pineapple crown leaves showed greater solubility and extractives content.
Enzymatic screening confirmed *Aspergillus niger* and *Trichoderma reesei* as efficient producers of xylanase and ligninolytic enzymes respectively. Pineapple crown leaves exhibited superior enzymatic degradability compared to corn husk particularly under xylanase pretreatment. Single enzyme applications were more effective than combined treatments suggesting enzyme-enzyme interactions may affect degradation efficiency.
Xylanase pretreatment significantly reduced lignin, ash and extractive contents in both biomasses enhancing their suitability for eco-friendly pulping processes. Holocellulose and α-cellulose contents remained relatively stable indicating the selective action of xylanase on lignin and hemicellulose fractions without significant cellulose loss.
With this, it confirms that xylanase-assisted pretreatment is a promising, environmentally friendly strategy for enhancing the pulping quality of agricultural residues like corn husk and pineapple crown leaves contributing to the advancement of sustainable paper production technologies.
These confirm that enzyme performance is substrate-dependent and future work should focus on optimizing enzyme sequences, dosages and supplementing with accessory enzymes (e.g., laccases, peroxidases) to enhance the biopulping potential of non-wood agricultural residues.

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