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Utilization of Lignocellulosic Waste Hydrolysates as A Low-Cost Carbon Source for Cellulase-Poor Xylanase Production by *Fusarium Oxysporum* Under Submerged Fermentation

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Abstract

Xylanases are industrially important enzymes extensively used in pulp processing, food technology, feed improvement, and biofuel production. Commercial production of these enzymes is often constrained by the high cost of purified xylan used as a carbon source. The present investigation evaluates the potential of hemicellulose-rich hydrolysates derived from agricultural residues as economical substitutes for pure xylan in submerged fermentation by *Fusarium oxysporum*. Sixteen locally available lignocellulosic wastes uch as banana peel, corn cobs, cotton shells, green gram husk, ground nut shells, jowar stalks, maize stalks, oat meal, orange peel, paddy husk, paddy straw, pomegranate peel, sugar cane pulp, wheat bran, wheat straw and wood husk were chosen as substrates for xylanase production in submerged fermentation (SmF) because of their abundance in the local area as agricultural wastes. Among them, maize stalk hydrolysate produced the highest enzyme activity (420 U/mL), closely approaching the yield obtained with purified xylan (440 U/mL). Corn cob and wheat bran hydrolysates also supported significant enzyme production. Only trace cellulase activity (0.06–0.76 U/mL) was detected, indicating the production of cellulase-poor xylanase suitable for pulp bleaching. These findings highlight the feasibility of transforming agricultural residues into value-added bioproducts while lowering production costs and supporting sustainable waste management strategies.

1. Introduction

The growing demand for sustainable industrial processes has intensified research on the bioconversion of lignocellulosic biomass. Lignocellulosic biomass is the most abundant renewable organic resource and consists primarily of cellulose, hemicellulose, and lignin [1,2]. Plant biomass primarily consists of cellulose, hemicellulose and lignin, with hemicellulose representing the second most abundant polysaccharide fraction. Xylan forms the major structural component of hemicellulose and must be hydrolysed into fermentable sugars for industrial utilization. Hemicellulose constitutes 20–35% of plant biomass and is mainly composed of xylan [3]. Complete degradation of xylan requires the synergistic action of endo-β-1,4-xylanase, β-xylosidase and accessory enzymes such as arabinofuranosidase and acetyl xylan esterase [4]. Efficient degradation of xylan requires a coordinated enzyme system composed of endo-xylanases, β-xylosidases and several accessory enzymes that remove side-chain substituents

[Fig.1 &2]. Due to their broad substrate specificity and extracellular enzyme secretion, filamentous fungi are widely recognized as promising producers of hemicellulolytic enzymes.

Microbial xylanases have gained considerable industrial importance. Their application in pulp and paper processing reduces the need for chlorine-based bleaching chemicals. In food and feed sectors, xylanases improve dough quality and nutrient availability, while in biofuel production they contribute to biomass saccharification [5-7]. In the pulp and paper industry, cellulase-free xylanases are especially desirable because they reduce chlorine consumption without damaging cellulose fibers [8]. Filamentous fungi are preferred producers of xylanases due to their extracellular secretion, high productivity, and ability to produce accessory enzymes [6,9]. However, the high cost of pure xylan contributes up to 40% of enzyme production cost [10]. Therefore, replacing pure substrates with agricultural wastes is essential for cost-effective enzyme production [11].

Agricultural residues such as corn cobs, wheat straw, rice husk, and fruit peels are abundant and inexpensive lignocellulosic resources [12,13]. Their utilization not only reduces production costs but also addresses environmental pollution associated with waste disposal [14].

Despite their importance, large-scale production remains expensive, largely because purified xylan is commonly used as the primary carbon source. Agricultural residues represent an abundant and inexpensive alternative. Converting these wastes into fermentation substrates simultaneously addresses environmental concerns and reduces production costs. Therefore, the present work investigates the use of lignocellulosic hydrolysates as alternative carbon sources for xylanase production by *Fusarium oxysporum*.

2. Materials and Methods

Microorganism and Substrates

A locally isolated strain of *Fusarium oxysporum* was selected based on its xylanolytic potential [Fig:5]. Sixteen agricultural residues including cereal straws, fruit peels and husks were collected from local sources and used as substrates [12-16].

Fig. 5: Growth of fungal strains on Malt Extract Agar medium. After 7 days of incubation, the medium was stained in 0.1% (w/v) Congo red. Remarkably sharp outline of each colony was due to deeply stained actively growing mycelia.

Preparation of Hemicellulose Hydrolysate

Autohydrolysis is an effective pretreatment method for solubilizing hemicellulose from biomass [17]. Each dried substrate was mixed with water in a 50:1 ratio and subjected to autoclave pretreatment at 121°C

for 30 minutes. This process released soluble hemicellulosic sugars into the liquid fraction, which was separated by filtration and used as the fermentation medium [Fig: 6].

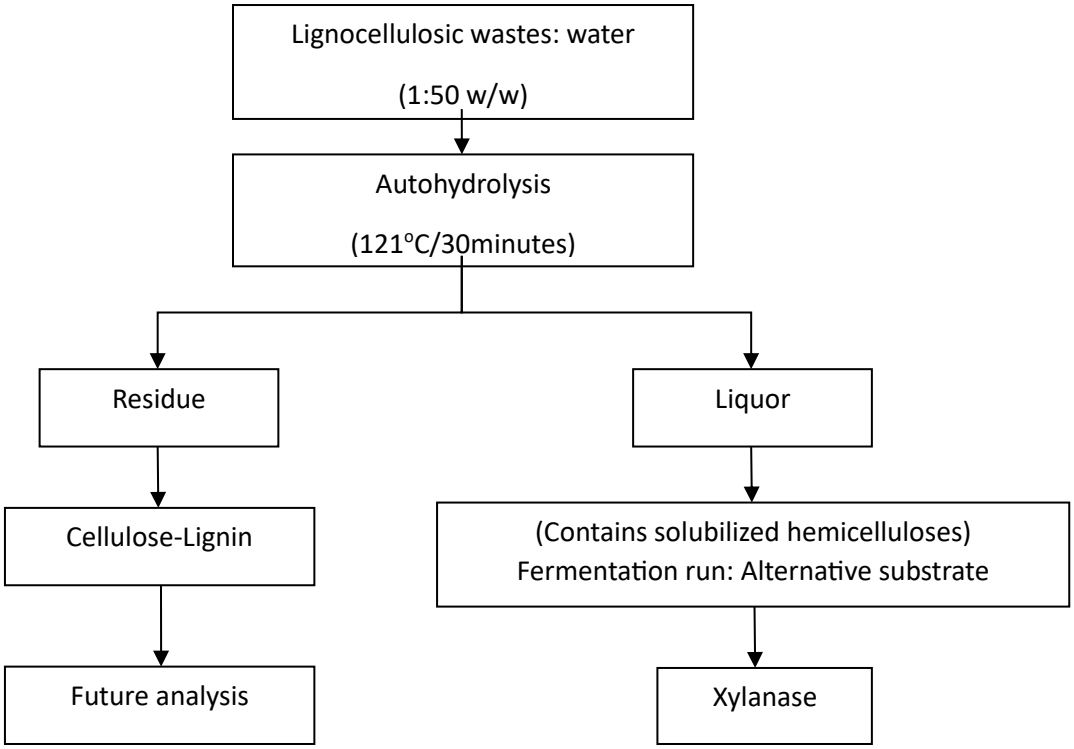


Fig. 6. Scheme of extraction of hemicelluloses fromlignocellulosic wastes

Submerged Fermentation

Fermentation was conducted in Mandels and Sternberg basal medium supplemented with hydrolysate in place of purified xylan [18]. Sixteen lignocellulosic substrates such as banana peel, corn cobs, cotton shells, green gram husk, ground nut shells, jowar stalks, maize stalks, oat meal, orange peel, paddy husk, paddy straw, pomegranate peel, sugar cane pulp, wheat bran, wheat straw and wood husk were chosen as substrates for xylanase production in submerged fermentation (SmF) because of their abundance in the local area as agricultural wastes. Flasks were inoculated with fungal discs and incubated at 28 ± 2°C under static conditions for up to 14 days [Fig. 3 &6].

Fig. 6. Submerged Fermentation of *Fusarium oxysporum* using Autohydrolysis Liquor

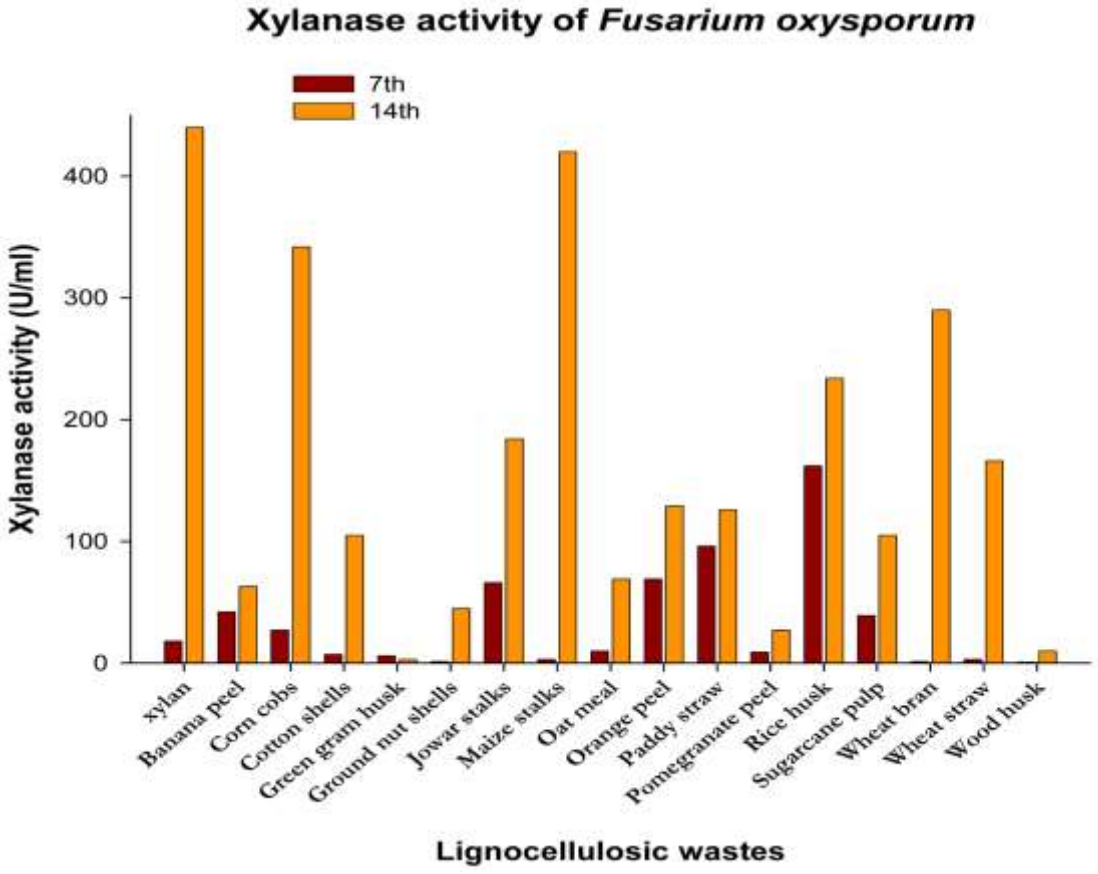
Enzyme Extraction and Assays

After incubation, fungal biomass was removed by filtration and centrifugation. Xylanase activity was measured using birchwood xylan substrate and reducing sugar estimation by DNS reagent [19-20]. Cellulase activity was determined using carboxymethyl cellulose [21]. Protein concentration and fungal biomass were quantified using standard biochemical methods [22].

3. Results and Discussion

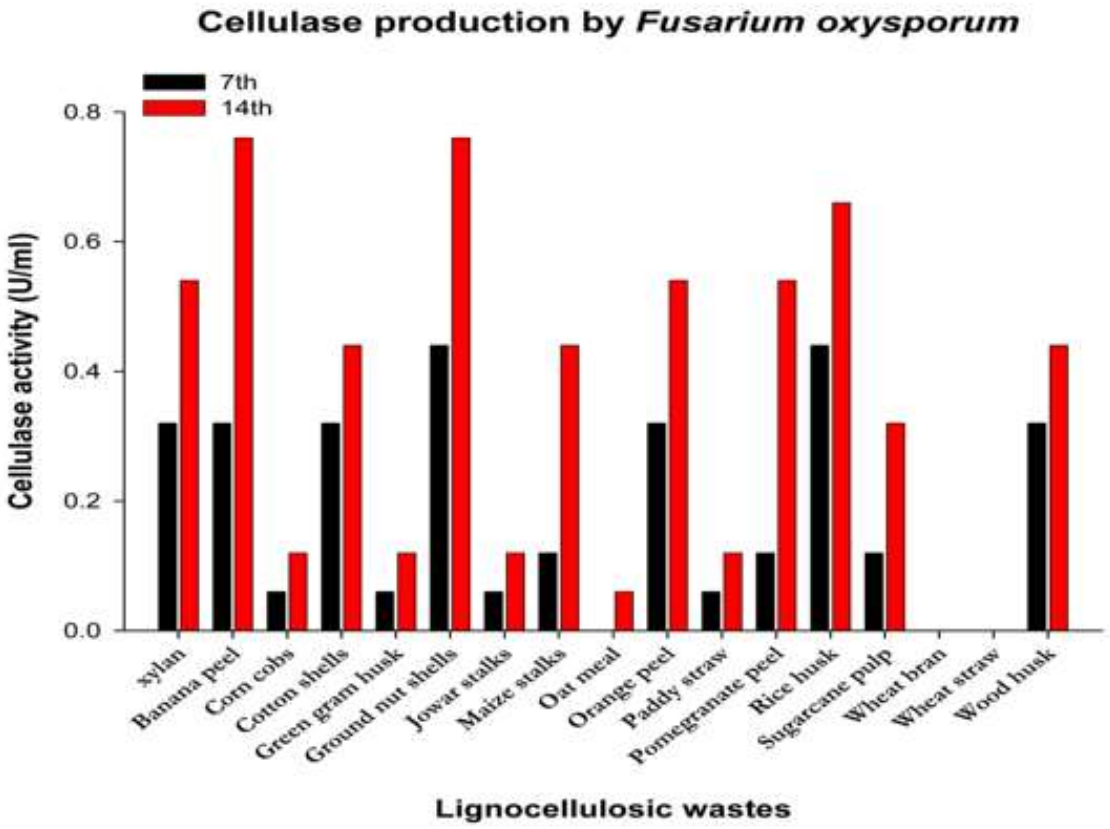
Xylanase Production

Maximum xylanase production occurred on the 14th day of incubation, consistent with previous reports [23]. Maize stalk hydrolysate produced enzyme levels comparable to pure xylan, suggesting the presence of natural inducers in lignocellulosic hydrolysates [24]. Corn cob and wheat bran also supported significant enzyme production. Similar observations were reported for agricultural residues used as substrates for fungal xylanase production [25].



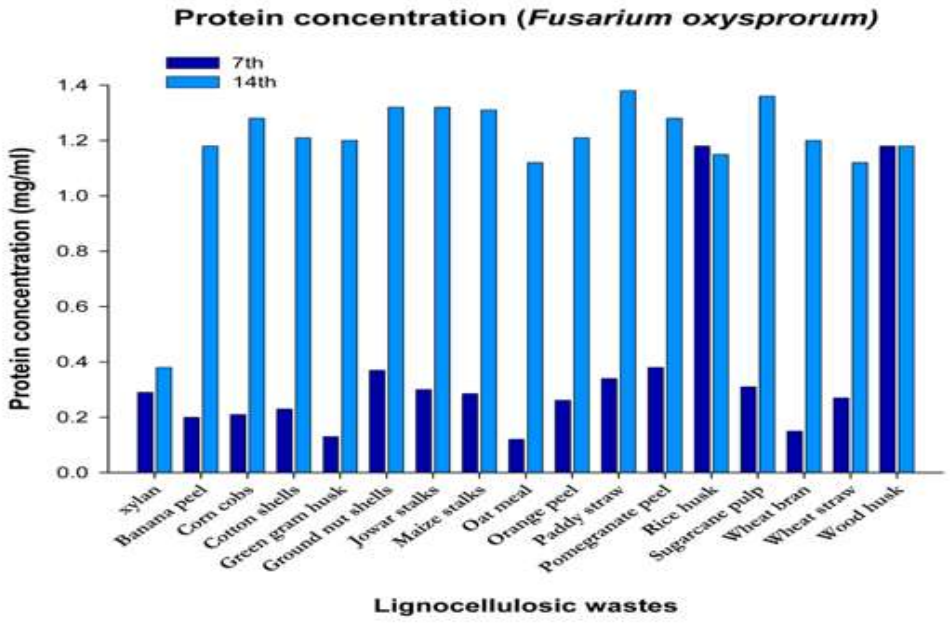
Cellulase Activity

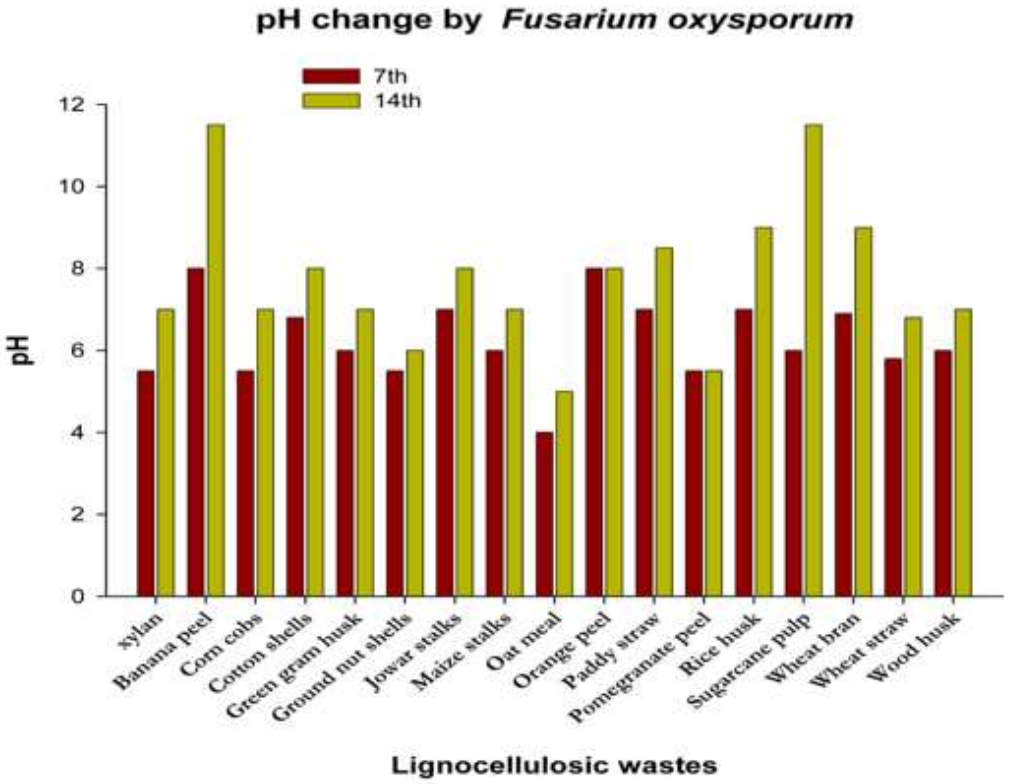
Only minimal cellulase activity was detected across all substrates. This characteristic is advantageous for pulp bleaching applications, where degradation of cellulose fibres must be avoided [8,26].



Protein and Biomass Formation

High protein content and fungal biomass were observed in media containing paddy straw and sugarcane pulp, suggesting efficient fungal growth due to nutrient availability in these hydrolysates [27]. Change in pH in culture filtrate after 7- 14 days of incubation was determined using pH paper.





Industrial Significance

The results demonstrate that agricultural residues can replace expensive purified substrates without compromising enzyme yield. This strategy offers both economic and environmental benefits and supports the concept of circular bioeconomy [28-30]. The overall workflow of the study is summarized in Fig. 4. All figures in this manuscript are original illustrations created by the author. Scientific concepts were adapted from cited literature.

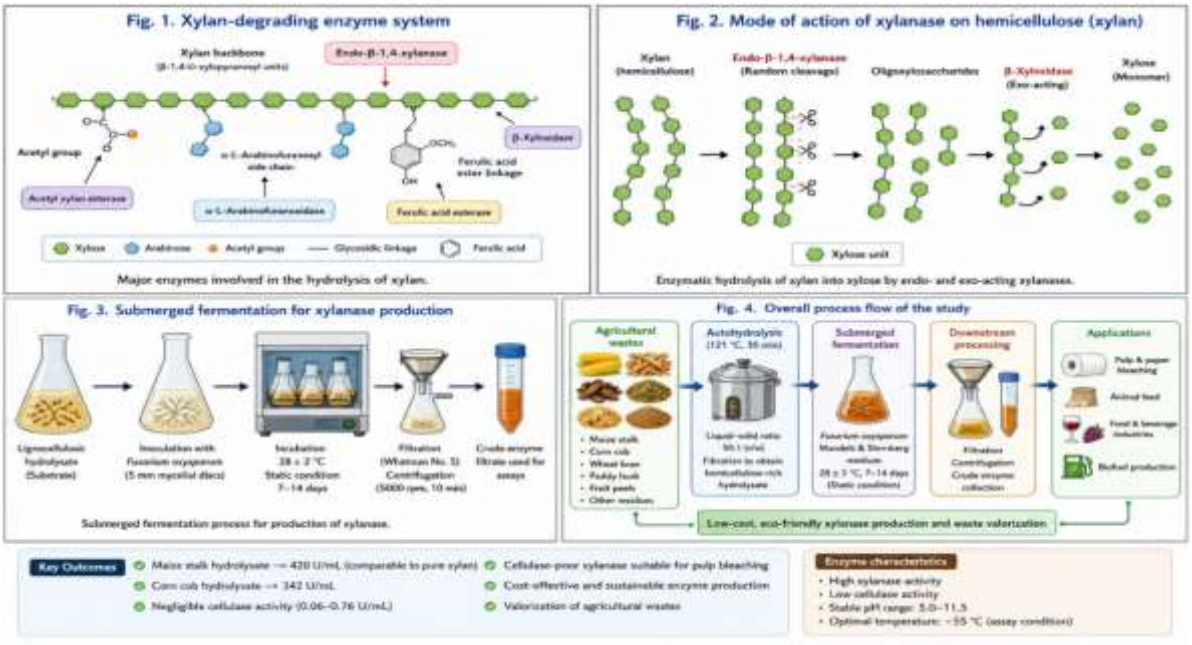


Fig. 1. Major enzymes involved in xylan hydrolysis including endo-β-1,4-xylanase, β-xylosidase and accessory debranching enzymes. *Concept adapted from* [4,6,7]. **Fig. 2.** Enzymatic hydrolysis of xylan into xylose by endo- and exo-acting xylanases. *Concept adapted from* [4,5]. **Fig. 3**. Submerged fermentation workflow for xylanase production by *Fusarium oxysporum*. *Concept adapted from* [18,23]. **Fig. 4.** Process flow of agricultural waste valorization for xylanase production. *Concept adapted from* [12,28,29].

4. Conclusion

The present study confirms that hydrolysates obtained from agricultural wastes can effectively substitute purified xylan for xylanase production by *Fusarium oxysporum*. Among the tested substrates, maize stalk and corn cob hydrolysates yielded the highest enzyme activity. The enzyme produced exhibited negligible cellulase activity, making it suitable for pulp and paper processing. The approach offers a cost-effective and environmentally sustainable strategy for industrial enzyme production.

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