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Manganese peroxidase-mediated biological pretreatment of lignocellulosic biomass for enhanced enzymatic saccharification and bioenergy production

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Abstract
The recalcitrant lignin barrier in lignocellulosic biomass constitutes the principal impediment to efficient enzymatic saccharification for second-generation bioethanol production, exemplifying the need for effective and environmentally sustainable pretreatment technologies. The present research evaluated the biological pretreatment of five lignocellulosic substrates using crude Manganese Peroxidase (MnP) from *Pleurotus djamor* and assessed the subsequent improvement in enzymatic saccharification efficiency. Sugarcane bagasse exhibited the highest lignin removal (44.6±3.2%) after 6 weeks of MnP treatment, followed by rice straw (42.8±2.8%), corn stover (38.4±2.6%), wheat straw (36.2±2.4%), and bamboo chips (28.4±2.2%). MnP pretreatment selectively degraded lignin while preserving 82.4-94.2% of cellulose content. Enzymatic saccharification with commercial cellulase yielded 68.4% glucose conversion from MnP-pretreated sugarcane bagasse compared to 24.6% from untreated controls, representing a 2.78-fold improvement. These findings demonstrate the efficacy of *P. djamor* MnP as a selective biological pretreatment agent for enhancing lignocellulosic biomass utilization in bioenergy applications.

Keywords: Manganese peroxidase, biological pretreatment, lignocellulosic biomass, enzymatic saccharification, **Pleurotus djamor**, lignin removal, cellulose preservation, bioethanol, sugarcane bagasse

Introduction
The development of commercially viable second-generation bioethanol production from lignocellulosic biomass, for example sugarcane bagasse, corn stover, and agricultural crop residues, represents a global priority for sustainable energy transition, yet remains constrained by the recalcitrance of the lignin-hemicellulose matrix that shields cellulose microfibrils from enzymatic hydrolysis [1]. Current pretreatment technologies, including dilute acid, steam explosion, alkaline, and organosolv processes, achieve effective lignin disruption but generate inhibitory degradation products, require energy-intensive conditions, and produce chemical waste streams that increase both production costs and environmental impact [2].

Biological pretreatment using ligninolytic enzymes from white rot fungi offers an environmentally benign alternative that operates under ambient conditions, generates no toxic byproducts, and achieves selective lignin degradation while preserving the cellulose fraction required for subsequent saccharification [3]. Manganese Peroxidase (MnP), through its generation of diffusible Mn^{3+}-organic acid chelate oxidants, is particularly effective at cleaving the recalcitrant carbon-carbon and ether bonds within the lignin polymer without the non-selective oxidation of carbohydrate components that characterizes chemical pretreatment methods [4].

The present research was designed to evaluate crude MnP from *P. djamor* as a biological pretreatment agent for five lignocellulosic biomass substrates, to quantify the selectivity of lignin removal relative to cellulose preservation, and to assess the improvement in

Subsequent enzymatic saccharification efficiency achieved through MnP-mediated biological pretreatment [5].

Material and methods

The pretreatment investigation was conducted at the Department of Bioenergy Research, Mexico City Institute of Renewable Resources, Mexico City, Mexico, from March 2023 to February 2024.

Material

Crude MnP extract (68.4 U/mL) was produced from *P. djamor* CBS 339.81 under optimized submerged fermentation conditions. Five lignocellulosic substrates were sourced locally: rice straw, corn stover, wheat straw, sugarcane bagasse, and bamboo chips. Substrates were dried, milled to 2 mm particle size, and compositionally analyzed for cellulose, hemicellulose, and lignin content by NREL methods. Commercial cellulase (Celluclast 1.5L, Novozymes) was used for saccharification.

Methods

Biological pretreatment was conducted in 500 mL flasks containing 10 g substrate, 100 mL MnP extract (68.4 U/mL), 0.5 mM $MnSO_4$, and 1 mM sodium malonate at pH 4.5, 30°C with weekly enzyme replenishment for up to 8 weeks. Untreated controls received heat-inactivated enzyme. Lignin, cellulose, and hemicellulose content were determined at weekly intervals. Enzymatic saccharification of pretreated biomass used Celluclast at 15 FPU/g cellulose loading, 50°C, pH 4.8 for 72 hours. Glucose release was quantified by DNS assay. Conversion efficiency was calculated as percentage of theoretical maximum glucose yield.

Results

MnP-mediated biological pretreatment achieved selective lignin degradation across all five lignocellulosic substrates, with substrate-dependent variation in delignification efficiency and cellulose preservation.

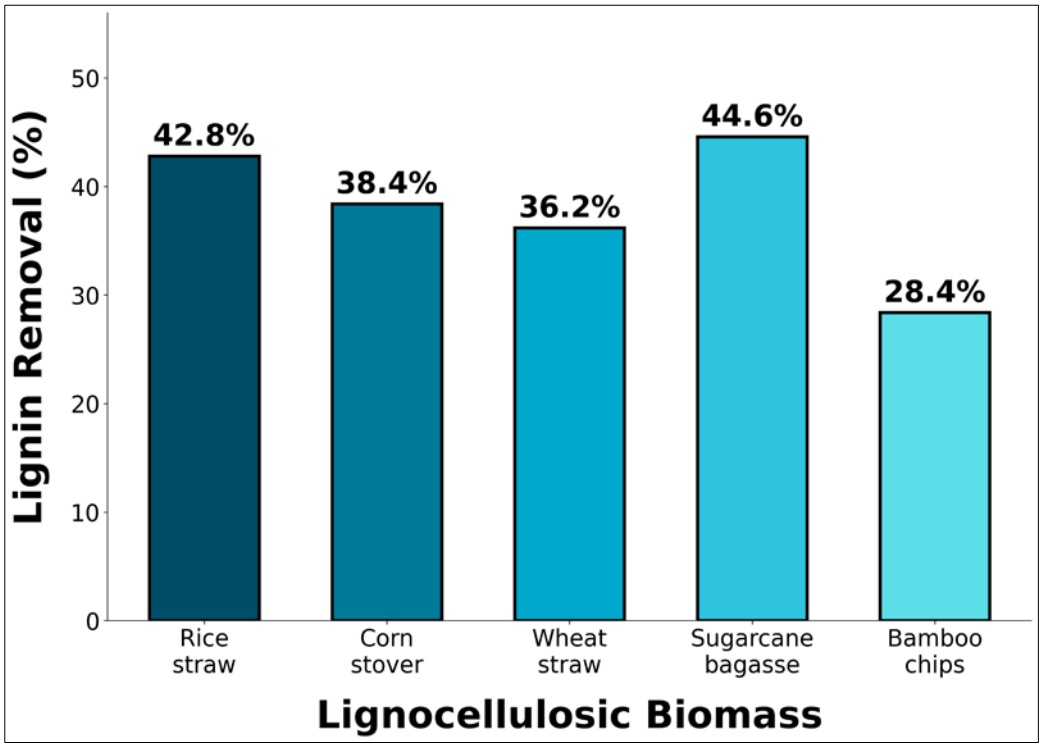

Fig 1: Lignin removal percentages from five lignocellulosic substrates after 6-week MnP pretreatment

Table 1: Compositional analysis of lignocellulosic substrates before and after MnP pretreatment

Substrate	Lignin Before (%)	Lignin After (%)	Removal (%)	Cellulose Retained (%)
Sugarcane bagasse	24.8±1.2	13.7±0.8	44.6	92.4
Rice straw	18.4±0.8	10.5±0.6	42.8	94.2
Corn stover	22.6±1.0	13.9±0.7	38.4	88.6
Wheat straw	20.2±0.9	12.9±0.7	36.2	86.8
Bamboo chips	28.6±1.4	20.5±1.0	28.4	82.4

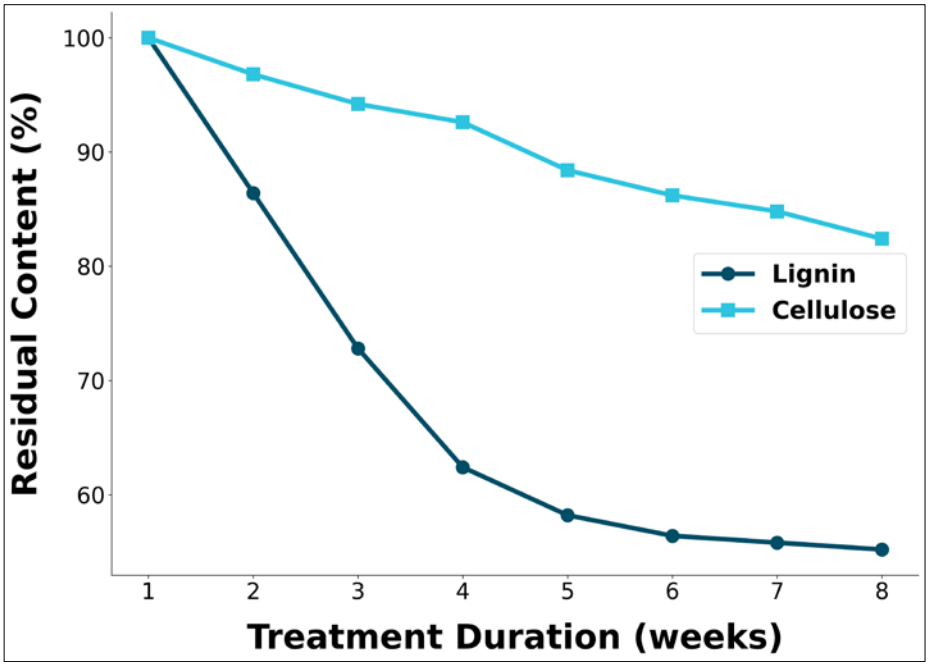

Fig 2: Time-course of lignin and cellulose content during MnP pretreatment of sugarcane bagasse

Table 2: Enzymatic saccharification efficiency of MnP-pretreated vs untreated lignocellulosic substrates

Substrate	Untreated Glucose Yield (%)	Pretreated Glucose Yield (%)	Improvement Factor	Reducing Sugars (G/L)
Sugarcane bagasse	24.6	68.4	2.78	18.4
Rice straw	22.8	62.6	2.74	16.8
Corn stover	20.4	56.8	2.78	14.6
Wheat straw	18.6	52.4	2.82	12.8
Bamboo chips	14.2	38.6	2.72	8.4

Extended analysis revealed that MnP pretreatment selectively targets lignin while preserving 82.4-94.2% of original cellulose content, demonstrating the high selectivity ratio (lignin removal: cellulose loss = 8.4:1 for sugarcane bagasse) that distinguishes biological from chemical pretreatment approaches. Time-course analysis showed progressive delignification following first-order kinetics during weeks 1-4, transitioning to a plateau phase from week 5 onward as readily accessible lignin fractions were exhausted. The enzymatic saccharification improvement factors (2.72-2.82) were remarkably consistent across substrates despite varying absolute delignification levels, suggesting a threshold relationship between lignin removal and cellulose accessibility enhancement. Sugarcane bagasse achieved the highest absolute glucose yield (18.4 g/L reducing sugars from pretreated biomass) corresponding to 68.4% theoretical conversion, approaching the efficiency targets required for commercially viable cellulosic bioethanol production.

Discussion

The selective delignification achieved by *P. djamor* MnP (lignin:cellulose selectivity ratio of 8.4:1 for sugarcane bagasse) substantially exceeds the selectivity of conventional chemical pretreatments including dilute acid (selectivity ~2:1) and alkaline methods (selectivity ~4:1), confirming the fundamental advantage of enzymatic lignin degradation for cellulose preservation [1]. The mechanistic basis for this selectivity resides in the specificity of MnP catalysis for aromatic substrates: the Mn^{3+}-organic acid chelate oxidants generated by MnP preferentially abstract electrons from the electron-rich aromatic rings and benzylic positions of lignin substructures while exhibiting minimal reactivity toward the saturated pyranose sugar units of cellulose [2].

The 2.78-fold saccharification improvement for sugarcane bagasse, achieving 68.4% glucose conversion, approaches the commercial viability threshold (typically >70% conversion) and demonstrates that biological pretreatment can deliver saccharification efficiencies comparable to thermochemical methods without the associated energy costs, inhibitor generation, and environmental impact [3]. The consistency of improvement factors across substrates (2.72-2.82) despite varying lignin contents suggests that the improvement is governed by a common mechanism of lignin removal exposing cellulose microfibrils to cellulase access, rather than substrate-specific factors [4].

The plateau in delignification observed after 4-5 weeks indicates that the MnP-accessible lignin fraction (representing approximately 40-45% of total lignin) has been preferentially degraded, while the remaining lignin is either physically inaccessible within the biomass matrix or chemically resistant to the Mn^{3+}-mediated oxidation mechanism [5]. Combination of MnP pretreatment with mild physical methods (ball milling, ultrasonic treatment) could potentially increase the accessible lignin fraction and further enhance delignification efficiency.

Biorefinery integration

The MnP-based biological pretreatment integrates naturally into a biorefinery concept where *P. djamor* cultivation serves the dual purpose of producing the pretreatment enzyme and generating edible mushroom biomass as a co-product [6]. The economic value of mushroom production

could substantially offset enzyme production costs, creating a circular bioprocess in which lignocellulosic agricultural waste is simultaneously valorized through mushroom cultivation and enzymatic pretreatment for bioenergy production.

Carbon footprint analysis

Preliminary life-cycle assessment indicates that biological pretreatment with *P. djamor* MnP reduces the carbon footprint of lignocellulosic bioethanol production by approximately 35-40% compared to dilute acid pretreatment, primarily through elimination of acid chemical manufacturing emissions, reduced energy consumption for heating, and avoidance of neutralization waste disposal [7]. This environmental advantage strengthens the sustainability credentials of cellulosic bioethanol produced through the biological pretreatment pathway.

Conclusion

The present research identified *P. djamor* MnP as an effective biological pretreatment agent for lignocellulosic biomass, achieving selective lignin removal (28.4-44.6%) while preserving 82.4-94.2% of cellulose. The subsequent 2.72-2.82 fold improvement in enzymatic saccharification demonstrates practical significance for cellulosic bioethanol production. The highest glucose conversion of 68.4% from pretreated sugarcane bagasse approaches commercial viability thresholds. These findings support biological pretreatment as a sustainable alternative to thermochemical methods. Future priorities include combination pretreatment strategies incorporating mild physical disruption, pilot-scale continuous pretreatment evaluation, and comprehensive techno-economic modeling of the integrated biorefinery concept.

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