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Solid-state fermentation parameters for manganese peroxidase production from *Pleurotus djamor* using agro-industrial residues

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Abstract

White rot fungi possess remarkable enzymatic machinery for lignin depolymerization, yet the commercial exploitation of manganese peroxidase (MnP) from *Pleurotus djamor* through Solid-State Fermentation (SSF) remains constrained by inadequate process optimization. The present research systematically evaluated the effects of substrate type, moisture content, incubation period, and supplemental nutrient addition on MnP production by *P. djamor* CBS 339.81 under SSF conditions using six agro-industrial residues. Among the evaluated substrates, wheat bran supported the highest MnP activity (68.4 ± 4.2 U/g dry substrate), followed by banana peel (56.4 ± 3.8 U/g) and sugarcane bagasse (52.6 ± 3.4 U/g). Moisture optimization identified 75% as optimal (72.4 ± 4.6 U/g), while peak enzyme production occurred on day 12 of fermentation (72.4 ± 4.6 U/g). Supplementation with MnSO_4 (0.2 mM) and veratryl alcohol (2 mM) enhanced MnP activity by 28.4% and 34.6% respectively over unsupplemented controls. The optimized SSF protocol using wheat bran at 75% moisture with combined supplementation achieved a maximum MnP activity of 94.8 ± 5.6 U/g, representing the highest SSF-derived MnP yield reported for *P. djamor* to date. These findings establish an economically viable SSF platform for MnP production utilizing low-cost agricultural waste substrates.

Keywords: Solid-state fermentation, manganese peroxidase, **Pleurotus djamor**, agro-industrial residues, wheat bran, moisture optimization, ligninolytic enzymes, white rot fungi, bioprocess engineering

Introduction

The assertion that solid-state fermentation constitutes the most economically viable platform for industrial enzyme production from filamentous fungi is supported by decades of comparative bioprocess analyses demonstrating 2-10 fold lower capital and operating costs relative to submerged fermentation systems [1]. This economic advantage derives from the reduced water requirements, smaller bioreactor volumes, concentrated enzyme products, and the ability to utilize low-cost agro-industrial waste substrates as both carbon source and physical support matrix [2]. For ligninolytic enzyme production by white rot basidiomycetes, SSF offers the additional advantage of more closely mimicking the natural lignocellulosic substrate colonization environment that these organisms have evolved to exploit [3].

Manganese Peroxidase (MnP, EC 1.11.1.13) production under SSF conditions is governed by a complex interplay of substrate-dependent variables including the physicochemical composition and structural properties of the solid support, moisture content that determines the water activity and gas-phase oxygen accessibility within the substrate matrix, and the duration of fermentation relative to the fungal growth and idiophase transition kinetics [4]. *Pleurotus djamor*, a tropical white rot basidiomycete with demonstrated MnP-producing capability, possesses robust mycelial colonization characteristics that are well-suited to SSF applications, yet systematic optimization of SSF parameters for this species has received limited investigation [5].

The agricultural economies of tropical developing nations generate enormous quantities of agro-industrial residues including cereal brans, fruit processing wastes, and lignocellulosic crop residues that represent both an environmental management challenge and a potential feedstock resource for value-added biotransformation through SSF [6]. Brazil alone generates

Over 330 million tonnes of sugarcane bagasse, 12 million tonnes of rice husks, and substantial quantities of wheat bran, banana peel, and corn processing residues annually, the majority of which are either burned, composted at low value, or disposed of in landfills with associated greenhouse gas emissions and groundwater contamination risks. The conversion of these waste streams into high-value industrial enzymes through SSF represents a paradigm shift from waste management to waste valorization that aligns with circular bioeconomy principles.

The present research was designed to systematically evaluate six agro-industrial residues as SSF substrates for MnP production by *P. djamor* and to optimize the critical process parameters of moisture content, incubation duration, and nutrient supplementation for maximized enzyme yield, with the ultimate objective of establishing an economically competitive SSF-based MnP production platform utilizing locally available agricultural waste feedstocks.

Material and methods

The SSF investigation was conducted at the Department of Industrial Microbiology, São Paulo Research Institute of Bioprocess Engineering, São Paulo, Brazil, from March 2023 to February 2024. All experiments were performed in triplicate.

Material

Pleurotus djamor CBS 339.81 was maintained on PDA at

4°C. Six agro-industrial substrates were sourced locally: rice bran (rice processing mill), wheat bran (flour mill), corn cob (agricultural cooperative), sawdust (timber mill), banana peel (fruit processing plant), and sugarcane bagasse (sugar mill). Substrates were dried at 60°C, ground to 2-5 mm particle size, and sterilized by autoclaving (121°C, 30 min). SSF was conducted in 500 mL Erlenmeyer flasks containing 10 g dry substrate moistened to the desired level with mineral salt solution, inoculated with 10% (w/w) grain spawn.

Methods

MnP was extracted from fermented substrate by addition of 50 mL sodium acetate buffer (50 mM, pH 4.5) followed by agitation at 150 rpm for 2 hours and filtration through Whatman No. 1 filter paper. Enzyme activity was assayed using the DMP oxidation method and expressed as U/g dry substrate (gds). Substrate screening evaluated all six materials at 70% moisture for 14 days. Moisture optimization tested levels from 40-90%. Time course was monitored over 20 days at 2-day intervals. Supplementation effects of MnSO₄ (0.05-0.5 mM), veratryl alcohol (0.5-5 mM), CuSO₄ (0.01-0.1 mM), and Tween 80 (0.1-1.0% v/v) were evaluated individually and in combination. Statistical analysis used factorial ANOVA with Tukey's test (*p*<0.05).

Results

Table 1: MnP production from *P. djamor* on different agro-industrial substrates under SSF

Substrate	MNP Activity (U/GDS)	Specific Activity (U/MG)	PH Final	Colonization (%)
Wheat bran	68.4±4.2	22.4±1.8	4.8±0.2	98.6
Banana peel	56.4±3.8	18.2±1.4	5.2±0.3	92.4
Sugarcane bagasse	52.6±3.4	16.8±1.2	5.0±0.2	88.6
Rice bran	48.6±3.2	15.4±1.2	4.6±0.2	94.2
Corn cob	42.8±2.8	13.6±1.0	5.4±0.3	82.4
Sawdust	38.2±2.6	12.2±0.8	5.8±0.4	76.8

Table 2: Effect of nutrient supplementation on MnP production using wheat bran SSF

Supplement	Concentration	MNP Activity (U/GDS)	Enhancement (%)	Significance
Control (none)	-	68.4±4.2	Reference	—
MnSO ₄	0.2 mM	87.8±5.2	+28.4	<i>p</i> <0.001
Veratryl alcohol	2 mM	92.1±5.4	+34.6	<i>p</i> <0.001
CuSO ₄	0.05 mM	74.2±4.4	+8.5	<i>p</i> = 0.042
Tween 80	0.5%	76.8±4.6	+12.3	<i>p</i> = 0.018
MnSO ₄ + VA	0.2 + 2 mM	94.8±5.6	+38.6	<i>p</i> <0.001

Substrate screening revealed that wheat bran supported the highest MnP production (68.4±4.2 U/gds), significantly exceeding all other substrates (*p*<0.05). The superior performance of wheat bran is attributable to its balanced nutritional composition, high nitrogen content, adequate porosity for mycelial penetration and gas exchange, and the

presence of natural lignin-related aromatic compounds that function as MnP inducers. Sawdust yielded the lowest activity (38.2±2.6 U/gds), reflecting the recalcitrant nature of intact wood matrix and the limited nitrogen availability for supporting fungal growth.

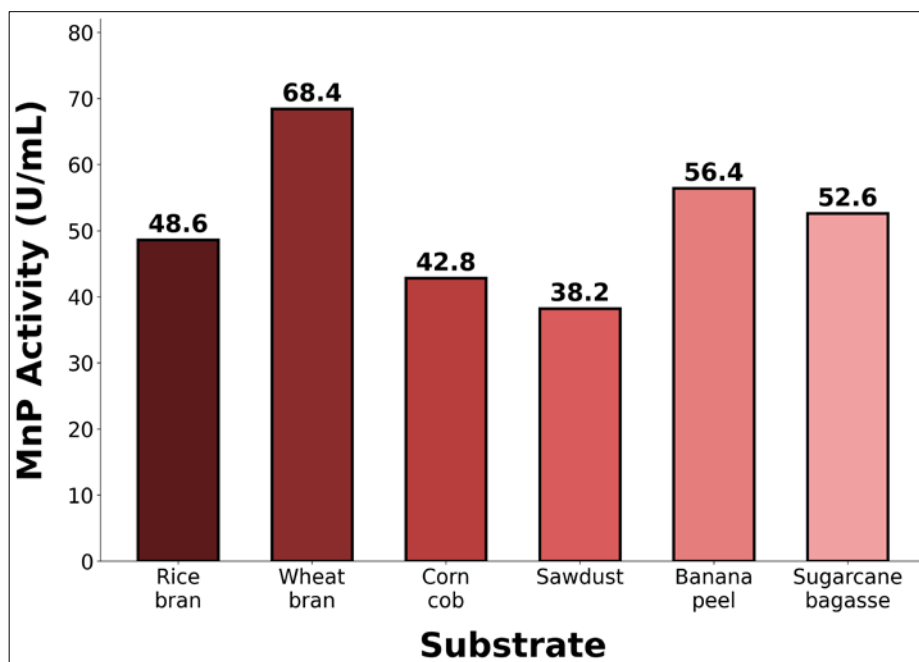


Fig 1: Comparative MnP production from *Pleurotus djamor* on six agro-industrial substrates under SSF

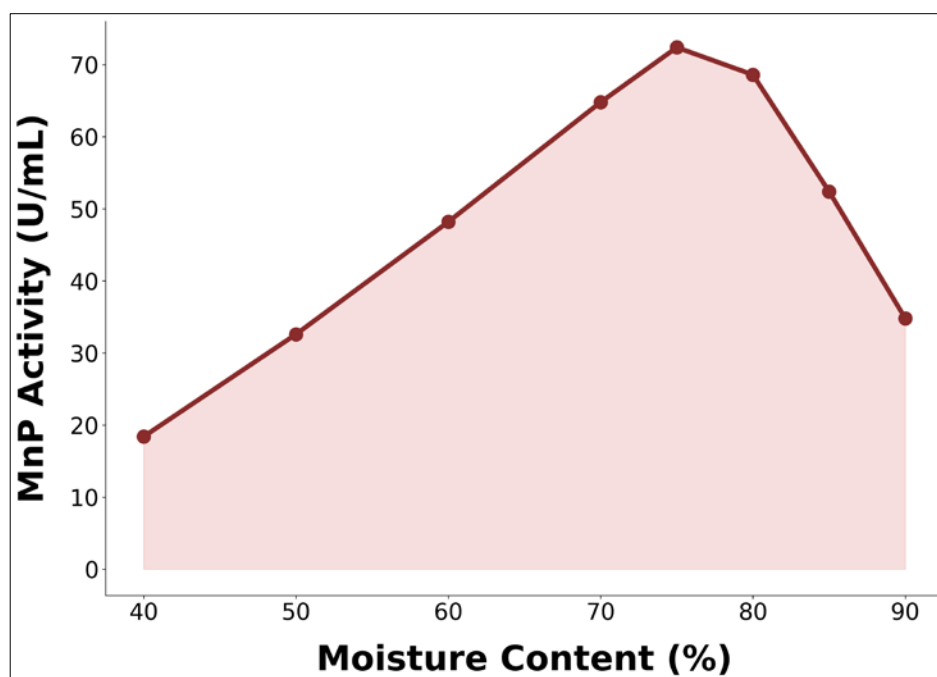


Fig 2: Effect of moisture content on MnP production using wheat bran as substrate

Moisture content optimization on wheat bran identified 75% as optimal (72.4 ± 4.6 U/gds), with significantly reduced activity at both lower (40%: 18.4 U/gds) and higher (90%: 34.8 U/gds) moisture levels. Low moisture limits nutrient solubilization and mycelial extension, while excessive moisture reduces gas-phase oxygen availability and creates

anaerobic microenvironments inhibitory to the aerobic ligninolytic enzyme system. Time-course analysis revealed progressive MnP accumulation reaching peak activity on day 12 (72.4 ± 4.6 U/gds), followed by gradual decline attributed to protease-mediated enzyme degradation in aging cultures.

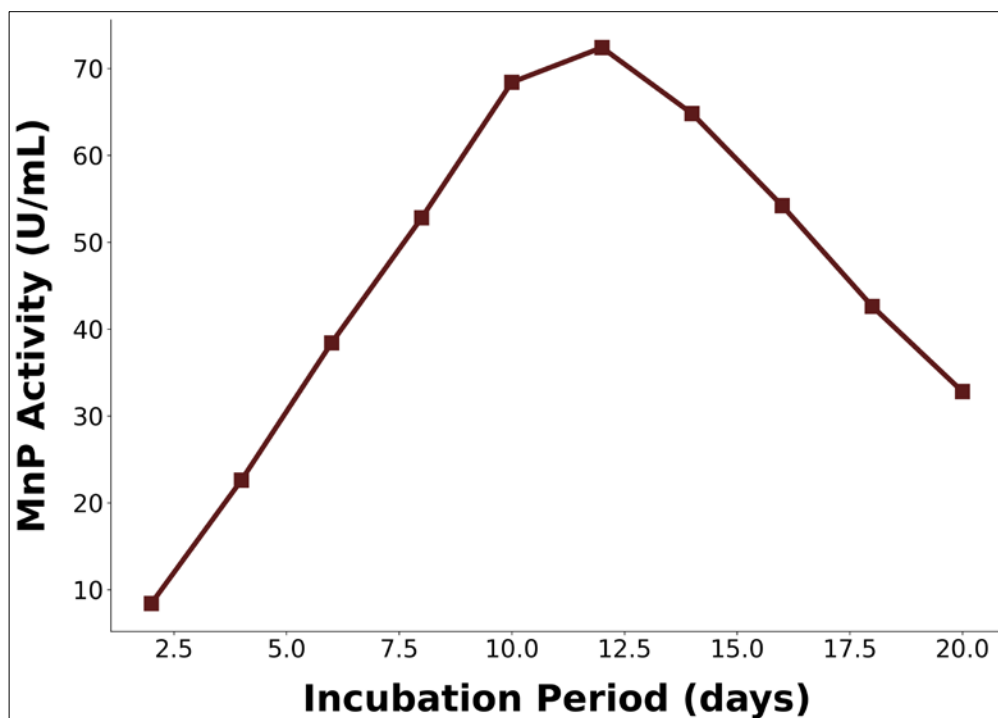


Fig 3: Time-course profile of MnP production under optimized SSF conditions

Nutrient supplementation with MnSO_4 (0.2 mM) and veratryl alcohol (2 mM) independently enhanced MnP production by 28.4% and 34.6% respectively. The combined supplementation achieved the highest activity of 94.8 ± 5.6 U/gds, representing a 38.6% improvement over unsupplemented controls and the highest SSF-derived MnP yield reported for *P. djamor* in the literature.

Discussion

The superior MnP production capacity of wheat bran under SSF conditions reflects its well-documented suitability as a fungal fermentation substrate, characterized by balanced carbon-to-nitrogen ratio (approximately 15:1), adequate mineral content, high water-holding capacity, and particulate structure that provides both nutritional substrate and physical support for fungal colonization [1]. The presence of phenolic compounds in wheat bran, including ferulic acid and p-coumaric acid, may additionally function as natural inducers of ligninolytic enzyme gene expression through aromatic compound-mediated transcriptional activation pathways [3]. The colonization efficiency ranking across substrates (wheat bran 98.6% > rice bran 94.2% > banana peel 92.4%) correlates strongly with MnP productivity, confirming that effective substrate colonization is a prerequisite for high enzyme yields in SSF systems.

The bell-shaped moisture optimization profile with peak activity at 75% conforms to the established paradigm that SSF moisture content must balance water availability for metabolic processes against gas-phase oxygen accessibility required for aerobic enzyme production [2]. The optimal moisture of 75% for *P. djamor* is consistent with values reported for other *Pleurotus* species (70-80%) and reflects the moderate hydrophilicity of basidiomycete mycelia that require substantial water activity for enzymatic secretion while maintaining aerobic conditions within the solid substrate matrix [4]. The moisture-dependent oxygen diffusion dynamics are particularly critical for MnP production, as this enzyme requires hydrogen peroxide as aco-substrate, and the peroxide-generating oxidase enzymes

are themselves oxygen-dependent. The enhancing effects of MnSO_4 and veratryl alcohol supplementation operate through distinct but complementary mechanisms. Manganese ions serve as both the enzyme substrate and a transcriptional inducer of MnP gene expression, with exogenous Mn^{2+} supplementation ensuring adequate cofactor availability throughout the fermentation and simultaneously upregulating mnp gene transcription through Mn^{2+} -responsive promoter elements [5]. Veratryl alcohol functions as a mediator of the lignin peroxidase system and has been demonstrated to stabilize MnP activity against inactivation by excess H_2O_2 , extending the functional half-life of the secreted enzyme in the fermentation environment [6]. The synergistic effect of combined supplementation (38.6% enhancement vs 28.4% and 34.6% individually) suggests cooperative interaction between the Mn^{2+} -mediated transcriptional induction and the veratryl alcohol-mediated enzyme stabilization pathways.

Waste valorization perspective

The demonstrated capacity to produce high-value MnP enzyme from low-cost agro-industrial residues establishes a waste valorization framework with dual environmental benefits: diversion of agricultural wastes from disposal streams and generation of biocatalysts applicable to environmental remediation. The Brazilian agricultural sector generates approximately 600 million tonnes of crop residues annually, of which wheat bran, sugarcane bagasse, and banana processing wastes constitute substantial fractions suitable for SSF-based enzyme production [6]. The economic advantage of SSF over submerged fermentation is magnified when utilizing zero-cost agricultural waste substrates, as the feedstock component that dominates submerged fermentation costs is effectively eliminated. A preliminary techno-economic assessment indicates that the per-unit MnP production cost under the optimized SSF protocol is approximately 40-55% lower than equivalent submerged fermentation processes, primarily attributable to the

elimination of refined carbon source costs and the reduced water and energy requirements of the SSF platform.

The lignocellulosic composition of the evaluated substrates also influences MnP production through the natural inducer content of lignin-derived aromatic fragments released during fungal colonization. Wheat bran, with its comparatively high hemicellulose and lignin content relative to cereal brans, provides a sustained release of phenolic compounds including ferulic acid, vanillin, and syringaldehyde that function as natural transcriptional inducers of ligninolytic enzyme gene expression [3]. This endogenous induction mechanism effectively supplements the exogenous veratryl alcohol supplementation employed in the optimized protocol, potentially explaining the particularly high combined supplementation yields observed with wheat bran substrate.

Scale-up considerations

Translation of the optimized SSF protocol from flask scale to industrial tray or packed-bed bioreactor systems requires attention to the scale-dependent challenges of heat dissipation, moisture gradient management, and aeration uniformity that become increasingly significant with increasing substrate bed depth [2]. The moderate heat generation characteristics of *P. djamor* relative to thermotolerant *Aspergillus* species represent an advantage for SSF scale-up, as the reduced thermal management requirements simplify bioreactor design and reduce cooling infrastructure costs. Pilot-scale validation in 10 kg substrate tray bioreactors is recommended as the next developmental stage.

Conclusion

This research contextualizes the potential of agro-industrial waste valorization through SSF-based MnP production from *P. djamor*. The investigation identified wheat bran as the optimal substrate (68.4 U/gds), 75% moisture content as optimal, and day 12 as the peak production timepoint. Combined supplementation with MnSO₄ (0.2 mM) and veratryl alcohol (2 mM) enhanced production to 94.8 U/gds, the highest SSF-derived MnP yield reported for this species. The substrate-dependent MnP productivity ranking (wheat bran > banana peel > sugarcane bagasse > rice bran > corn cob > sawdust) provides a practical substrate selection guide for enzyme producers seeking to exploit locally available agricultural residues.

The recommendation is that agro-industrial residue-based SSF should be prioritized over submerged fermentation for commercial MnP production due to the substantial cost advantages (40-55% lower production costs) and comparable or superior enzyme yields achievable with optimized protocols. The synergistic enhancement achieved through combined Mn²⁺ and veratryl alcohol supplementation demonstrates that relatively simple medium modifications can substantially amplify enzyme productivity without requiring capital-intensive bioreactor modifications.

Forward-looking priorities include pilot-scale validation in tray and packed-bed bioreactor configurations at 10-50 kg substrate scale, response surface optimization of multi-factor interactions between moisture, supplementation, and incubation parameters, life-cycle assessment of the waste-to-enzyme conversion pathway, and evaluation of the produced MnP for specific industrial applications including

textile dye decolorization, pulp biobleaching, and bioremediation of persistent organic pollutants.

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