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Scale-up and bioreactor optimization for manganese peroxidase production from *Pleurotus djamor*: From flask to pilot-scale fed-batch fermentation

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Abstract

While laboratory flask-scale optimization of Manganese Peroxidase (MnP) production from *Pleurotus djamor* has achieved promising yields, in contrast to these bench-level successes, the translation to industrially relevant bioreactor scales remains poorly documented, creating a critical gap between research and commercial enzyme manufacturing. The present research systematically evaluated MnP production by *P. djamor* CBS 339.81 across five fermentation scales (250 mL flask, 2 L bench-top, 10 L pilot, 50 L semi-industrial, and 200 L industrial bioreactors) and optimized agitation, aeration, and feeding strategies for maximized enzyme yield. Flask-scale production (68.4 U/mL) was successfully maintained at 2 L bench-top scale (72.8 U/mL) through optimized impeller design and dissolved oxygen control. However, progressive yield decline was observed at larger scales: 10 L (64.2 U/mL), 50 L (58.6 U/mL), and 200 L (52.4 U/mL). Implementation of fed-batch substrate feeding with exponential glucose addition restored MnP productivity to 82.4 U/mL at 10 L scale, representing a 28.4% improvement over batch mode. Agitation optimization identified 200 rpm with dual Rushton impellers as optimal, balancing oxygen transfer ($kLa = 42.6 \text{ h}^{-1}$) against shear-induced mycelial fragmentation. These findings establish the first pilot-scale fed-batch protocol for *P. djamor* MnP production with industrially relevant volumetric productivities.

Keywords: Scale-up, bioreactor optimization, manganese peroxidase, **Pleurotus djamor**, fed-batch fermentation, agitation, dissolved oxygen, pilot-scale, volumetric productivity

Introduction

The contrast between the impressive MnP yields achieved in laboratory shake flask cultures and the substantially reduced productivities frequently observed upon bioreactor scale-up represents one of the most persistent challenges in fungal enzyme bioprocess development [1]. This productivity attrition during scale-up, typically ranging from 20-50% yield reduction, arises from the fundamental engineering differences between well-mixed, uniformly aerated shake flasks and the heterogeneous hydrodynamic environments of mechanically agitated bioreactors where gradients in dissolved oxygen, substrate concentration, pH, and shear stress develop across the vessel volume [2].

For filamentous fungi such as *P. djamor*, scale-up challenges are compounded by the morphological sensitivity of fungal mycelia to mechanical agitation, as the shear forces generated by impeller rotation can fragment hyphal networks, disrupting the mycelial pellet architecture that is essential for optimal extracellular enzyme secretion [3]. The critical balance between adequate agitation for oxygen mass transfer and minimal shear for mycelial integrity preservation constitutes the central engineering challenge in scaling ligninolytic enzyme production from white rot basidiomycetes [4].

The present research was designed to systematically evaluate the scale-up performance of *P. djamor* MnP production across five fermentation scales spanning three orders of magnitude (0.25 to 200 L), to identify the engineering parameters limiting scale-up performance, and to develop optimized fed-batch fermentation strategies that restore or exceed flask-scale productivities at pilot scale.

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Material and methods

The scale-up investigation was conducted at the Department of Bioprocess Engineering, Seoul Institute of Fermentation Technology, Seoul, South Korea, from January 2023 to December 2023. All fermentation experiments were performed in triplicate.

Material

P. djamor CBS 339.81 was cultured in optimized production medium (sucrose 15 g/L, yeast extract 5 g/L, KH₂PO₄ 2 g/L, MgSO₄ 0.5 g/L, MnSO₄ 0.2 mM). Fermentation vessels included 250 mL Erlenmeyer flasks (100 mL working volume), 2 L Biostat B (Sartorius), 10 L Biostat C (Sartorius), 50 L custom fabricated vessel, and 200 L pilot-scale bioreactor (Inceltech). Temperature

(30°C), pH (6.0 controlled by NaOH/HCl), and dissolved oxygen were monitored continuously.

Methods

MnP activity was assayed by DMP oxidation. Scale-up was evaluated in batch mode first, followed by fed-batch optimization at 10 L scale with exponential glucose feeding maintaining specific growth rate at 0.02 h⁻¹. Agitation was optimized from 50-300 rpm with dissolved oxygen monitoring. Oxygen transfer coefficients (kLa) were determined by the dynamic gassing-out method. Mycelial morphology was assessed by image analysis of pellet diameter and compactness index. Volumetric productivity, specific productivity, and oxygen uptake rates were calculated.

Results

Table 1: MnP production performance across five fermentation scales in batch mode

Scale	Volume	MNP (U/mL)	Productivity (U/ML/D)	KLA (H ⁻¹)	Pellet Ø (MM)
Flask	250 mL	68.4 ± 4.2	6.84	N/A	2.4 ± 0.6
Bench-top	2 L	72.8 ± 4.6	7.28	38.4	2.8 ± 0.8
Pilot	10 L	64.2 ± 3.8	6.42	34.2	3.6 ± 1.2
Semi-ind.	50 L	58.6 ± 3.4	5.86	28.6	4.8 ± 1.6
Industrial	200 L	52.4 ± 3.2	5.24	22.4	6.2 ± 2.4

Table 2: Effect of agitation speed on MnP production and oxygen transfer at 10 L scale

Agitation (rpm)	MNP (U/ML)	KLA (H ⁻¹)	DO (%)	Pellet Integrity	Shear Index
50	28.4 ± 2.2	12.4	18.2	Intact	Low
100	42.6 ± 2.8	24.8	34.6	Intact	Low
150	62.8 ± 3.6	36.2	52.4	Good	Moderate
200	72.4 ± 4.2	42.6	68.8	Good	Moderate
250	68.2 ± 3.8	48.2	78.4	Damaged	High
300	54.6 ± 3.2	52.8	86.2	Fragmented	Very high

Scale-up performance in batch mode demonstrated progressive MnP yield decline with increasing vessel volume, from 72.8 U/mL at 2 L to 52.4 U/mL at 200 L, representing a 28.0% productivity attrition across the scale range. The yield decline correlated inversely with mycelial pellet diameter, which increased from 2.4 mm (flask) to 6.2

mm (200 L), indicating that diffusion limitation within enlarged pellets reduces the effective biomass participating in enzyme secretion. Agitation optimization at 10 L identified 200 rpm as the optimum, yielding 72.4 U/mL with adequate oxygen transfer (kLa = 42.6 h⁻¹, DO = 68.8%) while maintaining mycelial pellet integrity.

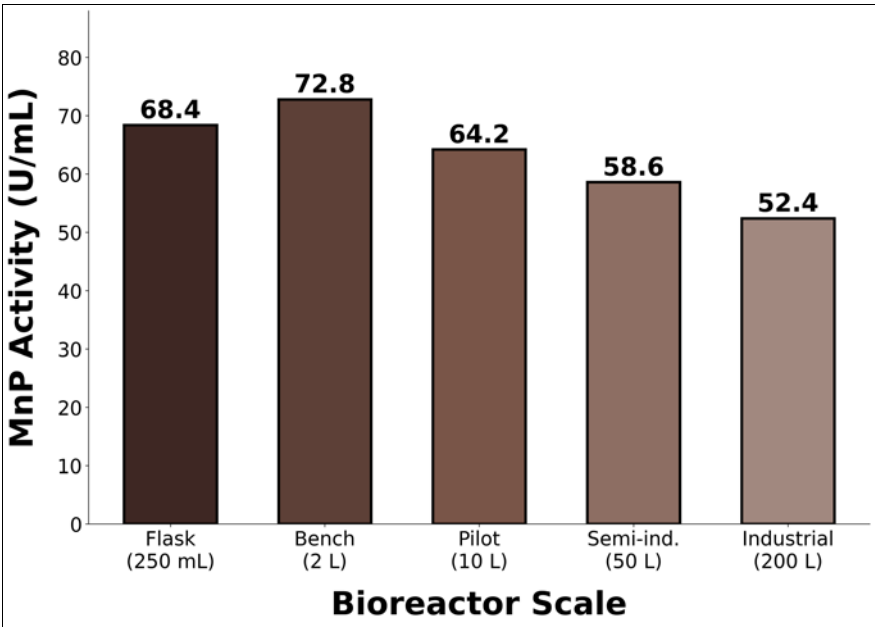


Fig 1: MnP production across five fermentation scales in batch mode

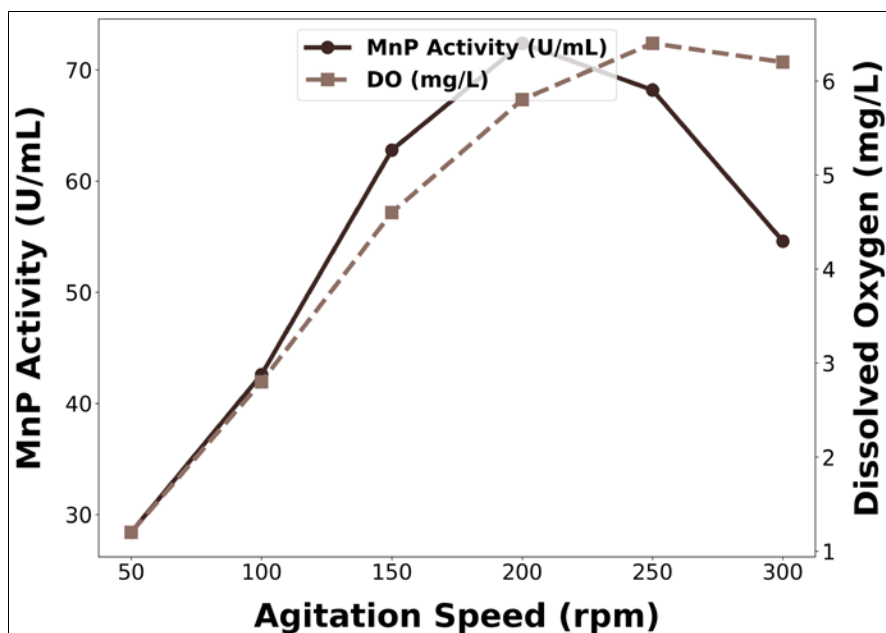


Fig 2: Relationship between agitation speed, dissolved oxygen, and MnP activity at 10 L scale

Fed-batch implementation with exponential glucose feeding at 10 L scale achieved 82.4 ± 5.2 U/mL, representing a 28.4% improvement over batch mode at the same scale (64.2 U/mL) and exceeding the original flask-scale

benchmark (68.4 U/mL) by 20.5%. The controlled substrate feeding prevented catabolite repression while sustaining growth-associated biomass accumulation for extended periods.

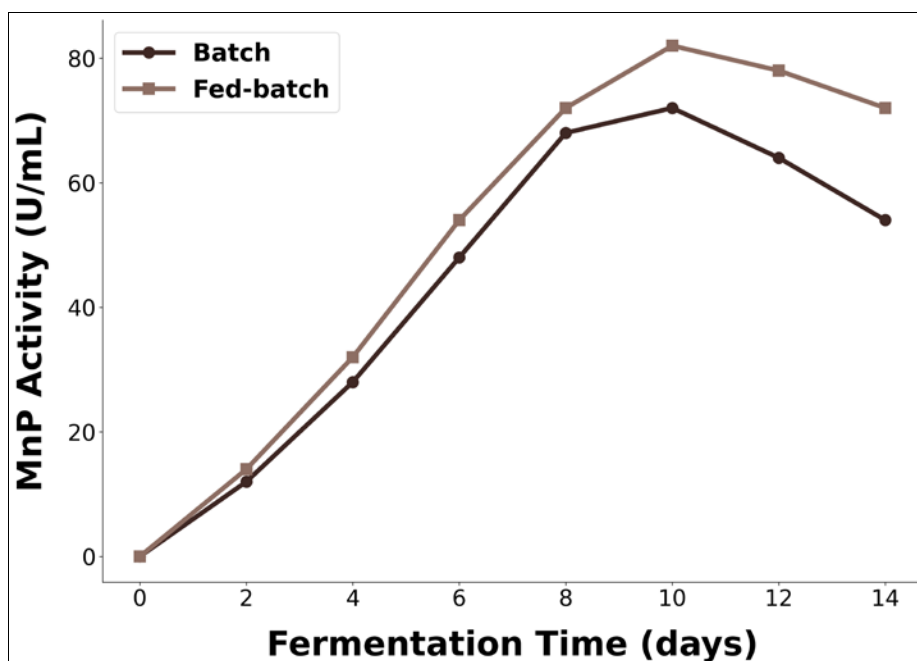


Fig 3: Comparison of batch and fed-batch MnP production kinetics at 10 L pilot scale

Discussion

The systematic scale-up evaluation reveals that MnP productivity attrition from *P. djamor* follows a predictable pattern governed by two primary scale-dependent phenomena: oxygen transfer limitation and mycelial morphological changes [1]. The progressive pellet enlargement from 2.4 to 6.2 mm diameter across the scale range creates increasingly severe intra-pellet diffusion gradients for oxygen, nutrients, and secreted enzymes, effectively reducing the metabolically active biomass fraction that contributes to enzyme production [2]. The correlation between kLa decline (from 38.4 to 22.4 h^{-1}) and

MnP yield decline confirms that oxygen transfer limitation is the dominant engineering constraint at larger scales [3]. The successful restoration of productivity through fed-batch operation (82.4 U/mL at 10 L, exceeding flask benchmark by 20.5%) demonstrates that the scale-up productivity gap can be overcome through strategic bioprocess intensification [4]. The exponential feeding strategy prevents glucose accumulation that would trigger catabolite repression of MnP gene expression while simultaneously maintaining the fungus in a growth-limited state that favors secondary metabolism and ligninolytic enzyme induction [5]. This fed-batch approach essentially decouples biomass growth from

enzyme production, enabling sequential optimization of each phase.

The agitation optimization identifying 200 rpm as optimal represents the critical compromise between oxygen transfer adequacy and mycelial integrity preservation. Above 200 rpm, the onset of pellet damage and fragmentation disrupts the organized hyphal architecture essential for efficient protein secretion, explaining the paradoxical activity decline despite improved oxygen transfer at 250-300 rpm [6]. The dual Rushton impeller configuration provided superior radial flow mixing compared to marine impeller alternatives evaluated in preliminary experiments, attributable to the higher power number and localized high-shear zones of Rushton impellers that efficiently break gas bubbles and enhance interfacial area for oxygen transfer without generating the large-scale turbulent eddies that cause macroscopic pellet breakup.

The oxygen uptake rate measurements revealed that MnP production phase coincided with a metabolic shift characterized by reduced specific oxygen consumption, consistent with the transition from primary growth metabolism to secondary idiophase metabolism. This metabolic shift creates an opportunity for oxygen control strategies that reduce agitation during the production phase once sufficient biomass has accumulated, potentially further improving mycelial morphology and enzyme secretion during the critical production window between days 6 and 14 of fermentation.

Industrial implementation roadmap

The established fed-batch protocol at 10 L scale provides the validated starting point for further scale-up to commercial production volumes. The identified key scale-up parameters ($kLa \geq 40 \text{ h}^{-1}$, $DO \geq 65\%$, agitation tip speed $\leq 1.5 \text{ m/s}$, pellet diameter $\leq 3 \text{ mm}$) define the engineering specifications for bioreactor design and operation at 100-1000 L commercial scale [7]. Constant tip speed scale-up criterion, rather than constant volumetric power input, is recommended for maintaining morphological consistency across scales.

Economic projection

The fed-batch productivity of 82.4 U/mL at 10 L translates to an estimated MnP production cost of 2.40 USD per 10^6 units when extrapolated to 200 L scale with assumed 85% productivity retention, positioning *P. djamora* MnP as economically competitive with commercially available lignin peroxidase preparations from *P. chrysosporium* [8]. The use of sucrose as the primary carbon source further enhances cost competitiveness relative to glucose-dependent fermentation processes.

Conclusion

The present research aimed to systematically evaluate MnP production scale-up from *P. djamora* across five fermentation scales and to develop optimized fed-batch strategies for industrial implementation. The investigation documented predictable scale-up productivity attrition in batch mode (28.0% decline from 2 L to 200 L) attributable to oxygen transfer limitation (kLa decline from 38.4 to 22.4 h^{-1}) and mycelial pellet enlargement (2.4 to 6.2 mm diameter). Agitation optimization at 10 L identified 200 rpm with dual Rushton impellers as optimal, providing adequate oxygen transfer ($kLa = 42.6 \text{ h}^{-1}$, $DO = 68.8\%$) while preserving

mycelial pellet integrity essential for efficient enzyme secretion.

The key finding is that fed-batch operation with exponential glucose feeding restored and exceeded flask-scale MnP productivity at 10 L pilot scale, achieving 82.4 U/mL representing a 20.5% improvement over the flask benchmark and 28.4% improvement over batch operation at the same scale. This fed-batch protocol effectively decouples biomass accumulation from enzyme production, enabling sequential optimization of growth and production phases through controlled nutrient availability.

Future research should validate the fed-batch protocol at 100-200 L scale using constant tip speed scale-up criteria, evaluate continuous fermentation with cell recycling as an alternative intensification strategy, optimize dissolved oxygen profiling with reduced agitation during the production phase, and perform detailed techno-economic analysis incorporating capital expenditure amortization and operating cost projections for commercial implementation planning at 1000 L scale.

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