



HYDROLYTIC ENZYME SYSTEMS OF *Trichoderma* spp.: FERMENTATION STRATEGIES, STRAIN ENGINEERING, AND SUSTAINABLE LIGNOCELLULOSIC VALORIZATION

Sistem Enzim Hidrolitik *Trichoderma* spp.: Strategi Fermentasi, Rekayasa Strain, dan Valorisasi Lignoselulosa Berkelanjutan

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ABSTRACT

Hydrolytic enzymes produced by *Trichoderma* spp. play a central role in lignocellulosic biomass conversion due to their high secretion capacity and catalytic versatility. This review provides a critical and integrated analyzes of enzyme systems, fermentation strategies, and bioprocess engineering approaches governing enzyme production and biomass valorization. A structured literature review was conducted using peer-reviewed publication 2019 to 2025.

The analysis highlights synergistic interactions among cellulases, hemicellulases, and oxidative enzymes in overcoming biomass recalcitrance. Key process parameters—including aeration, moisture content, temperature regulation, and bioreactor design—are identified as a primary determinants of productivity and efficiency. Solid-state fermentation is recognized as an efficient strategy for valorizing agricultural residues such as rice straw, although challenges in heat and mass transfer and scale-up remain.

Advances in strain engineering, regulatory pathway optimization, and enzyme cocktail design improve hydrolytic performance, but trade-offs between productivity, stability, and process robustness persist. Integration within circular biorefinery frameworks is essential for scalable and sustainable biomass conversion.

Keywords: *Hydrolytic enzymes, Lignocellulosic valorization, Solid state fermentation, Strain engineering, Trichoderma spp*

ABSTRAK

Enzim hidrolitik *Trichoderma* spp. berperan penting dalam pengkonversian biomassa lignoselulosa karena berkapasitas sekresi tinggi dan fleksibilitas katalitik luas. Artikel review ini menyajikan analisis kritis-terintegrasi terhadap sistem enzim, strategi fermentasi, serta pendekatan rekayasa bioproses yang mengendalikan produksi enzim dan valorisasi biomassa. Kajian literatur terstruktur ini menggunakan publikasi ilmiah bereputasi periode 2019-2025.

Hasil analisis menyoroti sinergi antara selulase, hemiselulase, dan enzim oksidatif dalam mengatasi sifat rekalsitran biomassa. Parameter proses utama—meliputi aerasi, kadar air, suhu, dan desain bioreaktor diidentifikasi sebagai faktor penentu produktivitas dan efisiensi proses. Fermentasi padat (solid-state fermentation) menjadi strategi efisien untuk pemanfaatan residu pertanian seperti jerami pad, meskipun masih menghadapi persoalan perpindahan panas dan massa untuk dijadikan berskala industri.

Kemajuan dalam rekayasa strain, regulasi lintasan metabolik, dan optimasi enzyme cocktail meningkatkan kinerja enzim hidrolitik namun masih disertai trade-off antara produktivitas, stabilitas, dan ketangguhan proses. Untuk itu, pengintegrasian rekayasa molekuler dengan

teknologi fermentasi dalam kerangka biorefinery sirkular menjadi kunci pengembangan sistem konversi biomassa yang berkelanjutan dan terukur.

Kata kunci: *Enzim hidrolitik, Rekayasa Strain, Solid state fermentation, Trichoderma spp, Valorisasi lignoselulosik*

INTRODUCTION

The increasing demand for sustainable and environmentally responsible biotechnological processes has accelerated the development of enzyme-based industrial applications. Among microbial producers, filamentous fungi are particularly valued for their ability to secrete large quantities of extracellular enzymes directly into the cultivation medium, making them highly suitable for large-scale bioconversion processes (Sala et al. 2024). Within this group, *Trichoderma* spp. have emerged as one of the most promising genera due to their high enzymatic productivity, non-pathogenic nature, and adaptability to diverse substrates and environmental conditions (Abbas et al. 2022).

The enzymatic system of *Trichoderma* is dominated by hydrolytic enzymes, particularly cellulases and hemicellulases (including xylanases), which play an important role in lignocellulosic biomass conversion. In addition, certain species produce oxidative enzymes such as laccases, albeit at lower levels, contributing to partial lignin modification. These enzyme systems have been widely utilized across multiple industries, including biofuel production, pulp and paper processing, textiles, food and feed, and environmental bioremediation (Arnau et al. 2020; Wallenius et al. 2021). Among them, *Trichoderma reesei* is widely recognized as an industrial benchmark for cellulase production due to its well-characterized genome and high secretion capacity, while other species such as *T. harzianum* and *T. asperellum* provide complementary enzymatic functionalities (Zhang et al. 2022).

Despite extensive research on enzyme systems and strain development, the integration of these biological aspects with bioprocess engineering remains relatively underexplored. Many existing studies focus primarily on molecular regulation or industrial applications, with limited attention to the

interaction between enzyme systems and process conditions. This gap becomes particularly important in the context of solid-state fermentation (SSF), which is widely regarded as a promising platform due to its low water requirement, compatibility with agro-industrial residues, and potential for enhanced enzyme production.

However, the practical implementation of SSF is constrained by several engineering challenges, including heat accumulation, oxygen transfer limitations, moisture heterogeneity, and difficulties in process scale-up (Leite et al. 2021; Perwez and Al Asheh 2025). These limitations indicate that improving enzyme performance alone is insufficient; instead, a more integrated approach that considers both enzyme system optimization and bioprocess design is required to achieve efficient and scalable lignocellulosic valorization.

METHODOLOGY OF LITERATURE REVIEW

This review was conducted using a structured literature search and selection approach to ensure transparency and reproducibility. Three major scientific databases—Scopus, Web of Science, and Google Scholar—were utilized to capture a comprehensive range of peer-reviewed publications. Scopus and Web of Science were prioritized due to their high indexing standards and coverage of reputable journals, while Google Scholar was included to ensure broader retrieval of relevant studies, including recent and interdisciplinary publications.

The literature search was performed for articles published between 2019 and 2025 using a combination of predefined keywords and Boolean operators. The primary search string included: ("*Trichoderma*" AND "hydrolytic enzymes") OR ("cellulase production" AND "*Trichoderma*") OR ("solid-

state fermentation” OR “submerged fermentation”) AND (“lignocellulosic biomass” OR “biomass conversion”). The search was further refined by limiting results to English-language publications and peer-reviewed journal articles.

The selection process followed a stepwise screening procedure adapted from systematic review practices. Initially, titles and abstracts were screened to exclude irrelevant studies. Subsequently, full-text articles were assessed based on predefined inclusion and exclusion criteria. Studies were included if they: (i) focused on *Trichoderma* spp.; (ii) addressed hydrolytic enzyme systems, fermentation strategies, strain improvement, or lignocellulosic biomass conversion; and (iii) provided experimental, mechanistic, or applied insights relevant to bioprocess optimization. Studies were excluded if they: (i) focused on unrelated fungal general (ii) addressed non-biotechnological applications; or (iii) lacked sufficient methodological or experimental detail.

It is acknowledged that restricting the review to English-language publications may introduce language bias, potentially excluding relevant studies published in other languages. However, this limitation was considered necessary to ensure consistency in analysis and interpretation.

The selected articles were systematically analyzed using a comparative and thematic synthesis approach. Key variables, including enzyme types, fermentation conditions, substrate characteristics, and reported performance outcomes, were extracted and categorized to identify current trends, technological advances, and research gaps. This approach enabled the integration of biological and bioprocess perspectives within a coherent analytical framework.

HYDROLYTIC ENZYME SYSTEMS OF *TRICHODERMA* spp.

Filamentous fungi of the genus *Trichoderma* are widely recognized as prolific producers of extracellular hydrolytic enzymes, positioning them as key biological platforms for lignocellulosic biomass conversion. Their ecological roles as saprophytes and mycoparasites have driven the evolution of

enzymatic systems capable of efficiently degrading complex plant polymers, particularly cellulose and hemicellulose, while exhibiting limited but functionally relevant lignin-modifying capacity (Zhu et al. 2025). This ecological adaptation underpins their industrial significance, where efficient depolymerization of recalcitrant biomass remains a critical bottleneck.

The enzymatic machinery of *Trichoderma* spp. operates as a coordinated system composed of cellulases, hemicellulases, and accessory enzymes. Rather than acting independently, these enzymes function in a highly synergistic manner, where the effectiveness of biomass degradation depends on both enzyme diversity and balanced expression. In addition to hydrolytic enzymes, certain species produce oxidative enzymes such as laccases and peroxidases, which contribute to lignin modification and detoxification of phenolic compounds (Tonin et al. 2020; Civzele et al. 2023). Although their contribution is comparatively limited, the integration of hydrolytic and oxidative activities can enhance substrate accessibility, particularly in partially pretreated biomass systems.

Among the studied species, *Trichoderma reesei* remains the most extensively characterized and commercially exploited due to its high protein secretion capacity and well-established genetic system (Martinez et al. 2023). However, increasing attention has been directed toward *T. asperellum* and *T. harzianum*, which exhibit broader enzyme repertoires and improved adaptability under solid-state fermentation (SSF) conditions. This shift reflects a growing recognition that no single strain is universally optimal; instead, enzyme system performance must be aligned with substrate characteristics and process conditions (Hinterdobler et al. 2021).

Cellulases

Cellulases constitute the most industrially significant enzyme group produced by *Trichoderma* spp.. The cellulase complex comprises three principal enzyme classes: endoglucanases (EC 3.2.1.4), cellobiohydrolases (EC 3.2.1.91), and β -glucosidases (EC 3.2.1.21), which function synergistically

to convert cellulose into glucose (Castrillo et al., 2021; Organo et al., 2022).

Endoglucanases initiate hydrolysis by cleaving internal β -1,4-glycosidic bonds within amorphous cellulose regions, generating new chain ends. Cellobiohydrolases then act processively on these chain termini to release cellobiose, which is subsequently hydrolyzed into glucose by β -glucosidases (P. Zhang et al., 2022). This sequential mechanism highlights that overall hydrolysis efficiency is governed not only by individual enzyme activities but also by their relative proportions and interaction dynamics.

A critical limitation in *T. reesei* is its relatively low β -glucosidase activity, which often leads to cellobiose accumulation and feedback inhibition. This bottleneck illustrates a broader challenge in enzyme system design, where imbalanced enzyme expression can significantly reduce process efficiency. To address this issue, strategies such as co-cultivation, enzyme supplementation, and genetic engineering have been employed to rebalance enzyme ratios and improve catalytic performance (N. Wang et al., 2025a).

These findings emphasize that optimizing enzyme synergy is as important as increasing total enzyme production in industrial applications. Alat dan bahan utama yang digunakan dalam penelitian harus ditulis dengan cara ilmiah, yaitu rasional, empiris dan sistematis. Sampel yang diambil wajib mencantumkan asalnya, termasuk koordinat GPS yang dapat dilakukan dengan menggunakan aplikasi google map.

Xylanases and Other Hemicellulases

Hemicellulases represent a complementary enzyme group that facilitates cellulose accessibility by degrading hemicellulosic components. Among them, xylanases (EC 3.2.1.8) play an important role by hydrolyzing β -1,4-xylosidic linkages in xylan, a major structural polysaccharide in agricultural residues (Castrillo et al. 2021). Rather than acting as primary depolymerizing agents, hemicellulases function by disrupting the hemicellulosic matrix that surrounds cellulose microfibrils. This structural modification significantly enhances the accessibility of cellulases to their substrate, thereby improving overall saccharification efficiency.

In this context, the contribution of hemicellulases is indirect but essential, highlighting the importance of coordinated enzyme systems.

Trichoderma spp. produce multiple xylanase isoforms with varying molecular weights, pH optima, and substrate specificities, enabling adaptation to structurally heterogeneous biomass. In addition, accessory enzymes such as β -xylosidases, arabinofuranosidases, and acetylxylan esterases are co-secreted to ensure more complete hemicellulose degradation (Alananbeh et al. 2024). However, the efficiency of hemicellulose removal is highly dependent on substrate composition and pretreatment history, indicating that enzyme performance cannot be evaluated independently of process conditions.

From an application perspective, hemicellulases have been utilized in pulp bleaching, textile processing, and the production of value-added oligosaccharides. Their effectiveness under mildly acidic conditions further supports their integration with fungal cellulase systems in biomass conversion processes.

Laccases and Other Oxidative Enzymes

Although *Trichoderma* spp. are primarily recognized for hydrolytic enzyme production, certain strains are capable of producing oxidative enzymes such as laccases (EC 1.10.3.2) and peroxidases. Laccases, as multicopper oxidases, catalyze the oxidation of phenolic substrates using molecular oxygen as the terminal electron acceptor (Wallenius et al. 2021).

In comparison with lignin-degrading fungi, the oxidative capacity of *Trichoderma* is relatively limited. Nevertheless, even low levels of laccase activity can play a strategic role in integrated biomass processing by modifying lignin structures and reducing phenolic inhibitors generated during pretreatment. These effects can indirectly enhance the accessibility of polysaccharides to hydrolytic enzymes. Laccase production in *Trichoderma* is often inducible and influenced by environmental factors such as copper ions and aromatic compounds, particularly under SSF conditions (Abd El-latif et al. 2024). However, challenges remain in achieving sufficient enzyme yield, catalytic

stability, and process scalability (Civzele et al. 2023). Therefore, the contribution of oxidative enzymes should be viewed as supportive rather than primary, requiring careful integration with hydrolytic systems.

Overall, the efficiency of *Trichoderma*-based biomass conversion is governed by the composition, balance, and interaction of multiple enzyme systems rather than the performance of individual enzymes alone. This highlights the importance of integrating enzymatic functionality with process design considerations. A deeper understanding of these interactions provides a foundation for developing optimized fermentation strategies and bioprocess systems, which are discussed in the following section.

FERMENTATION STRATEGIES AND BIOPROCESS ENGINEERING

The productivity and industrial feasibility of hydrolytic enzymes derived from *Trichoderma spp.* are strongly influenced by both fermentation strategy and bioprocess design. The selection between submerged fermentation (SmF) and solid-state fermentation (SSF) extends beyond operational preference, as it reflects fundamental differences in fungal physiology, oxygen transfer mechanisms, substrate accessibility, and scale-up complexity. Consequently, optimizing enzyme production requires a system-level understanding that integrates biological performance with engineering constraints.

Submerged Fermentation (SmF)

Submerged fermentation remains the dominant platform for large-scale enzyme production due to its high level of process control, reproducibility, and compatibility with automated operations. In SmF systems, fungal growth and enzyme secretion occur in liquid media under controlled conditions of temperature, pH, aeration, and agitation (Sharma et al. 2025). Oxygen availability is a key factor influencing enzyme production in *Trichoderma* cultures, as aerobic metabolism directly supports protein synthesis and secretion. In this context, oxygen transfer is commonly characterized by the volumetric mass transfer coefficient (k_{La}), which serves as a critical parameter for reactor performance. Higher k_{La} values are

generally associated with increased enzyme productivity; however, excessive agitation may induce shear stress, alter fungal morphology, and disrupt pellet formation, ultimately reducing secretion efficiency (Michelin et al. 2019). This trade-off highlights the need for careful optimization of hydrodynamic conditions rather than simple maximization of oxygen transfer.

Despite its advantages, SmF exhibits limitations when processing lignocellulosic substrates. Solid residues typically require pretreatment prior to fermentation, increasing process complexity and cost. In addition, high substrate loading can elevate broth viscosity, impair oxygen transfer, and complicate downstream processing (Wang et al. 2025). These constraints reduce the overall economic efficiency of SmF for biomass-based applications and motivate the exploration of alternative systems.

Solid-State Fermentation (SSF)

Solid-state fermentation has emerged as a promising alternative for enzyme production using filamentous fungi such as *Trichoderma*, whose natural growth environment resembles solid substrates with limited free water. In SSF, microorganisms grow on moist solid matrices without free-flowing liquid, often resulting in enhanced enzyme secretion (Mitchell et al. 2023).

One of the main advantages of SSF is its ability to directly utilize low-cost lignocellulosic residues as both carbon source and physical support. This dual functionality improves resource efficiency and reduces water and energy requirements. In many cases, SSF systems yield higher enzyme titers than SmF, which has been attributed to reduced catabolite repression and more favorable fungal morphology (Bamidele et al. 2025).

However, these advantages are accompanied by significant engineering challenges. The low thermal conductivity of solid substrates limits heat dissipation, leading to temperature gradients that can inhibit fungal growth. Similarly, restricted mass transfer results in heterogeneous oxygen distribution within the substrate bed. These limitations become more pronounced during scale-up, where maintaining uniform environmental conditions is particularly difficult (Finkler et

al. 2021; Vauris et al. 2022; Perwez and Al Asheh 2025).

Importantly, while SSF is often reported to outperform SmF in enzyme yield, its scalability and process control remain less mature. Therefore, the selection between SmF and SSF should be based on a balance between productivity, process controllability, and economic feasibility rather than enzyme yield alone.

Aeration and Oxygen Transfer

Aeration is a critical parameter in both SmF and SSF systems, as oxygen availability directly influences fungal metabolism, growth, and enzyme synthesis. In SmF, oxygen transfer is relatively well-controlled through agitation and air sparging, enabling homogeneous distribution throughout the reactor. Numerous studies have demonstrated a strong correlation between oxygen transfer efficiency and enzyme productivity in *Trichoderma* cultures (Zhang et al. 2022).

In contrast, oxygen transfer in SSF is inherently heterogeneous due to the absence of bulk liquid mixing and limited substrate porosity. To address this limitation, forced aeration using humidified air has been applied to improve oxygen penetration and temperature regulation (Vauris et al. 2022). However, the effect of aeration is highly system-dependent. While some studies report improved enzyme production, others observe minimal or even negative effects, indicating that aeration must be carefully optimized based on reactor configuration, substrate properties, and fungal strain (Rayhane et al. 2019; Méndez-González et al. 2020).

Temperature and Moisture Control

Temperature regulation differs significantly between SmF and SSF systems. In SmF, heat removal is efficiently managed using cooling jackets or internal heat exchangers. In contrast, SSF systems are prone to metabolic heat accumulation, which can result in localized overheating and reduced enzyme productivity (Vauris et al. 2022).

Moisture content and water activity (a_w) are critical parameters governing microbial activity in SSF. Optimal moisture levels typically range between 60% and 80%,

ensuring adequate nutrient diffusion while maintaining substrate porosity. Excess moisture reduces oxygen transfer, whereas insufficient moisture limits microbial metabolism and enzyme secretion (He et al. 2019).

Water distribution in SSF is dynamic and influenced by evaporation, respiration, and internal migration. These changes affect substrate structure, enzyme accessibility, and overall hydrolysis efficiency. Therefore, precise control of moisture content is essential for maintaining stable and reproducible enzyme production (Kalaiselvan et al. 2025).

Bioreactor Design and Scale-Up

The scale-up of enzyme production processes requires reactor designs that accommodate the morphological characteristics of filamentous fungi while maintaining efficient heat and mass transfer. In SmF, stirred-tank reactors remain the industrial standard due to their scalability and operational flexibility. Alternative configurations such as airlift and bubble column reactors have also been explored to reduce shear stress and energy consumption (Cerrone and O'Connor 2025).

In SSF, several reactor designs have been developed, including tray, packed-bed, and rotating drum systems. Packed-bed and rotating drum reactors are particularly promising for *Trichoderma* cultivation, as they improve aeration and temperature control while maintaining structural simplicity (Rayhane et al. 2019).

Despite these developments, SSF scale-up remains challenging. Achieving uniform temperature, oxygen, and moisture distribution across large substrate volumes is technically demanding. Emerging approaches, including real-time sensors, computational modeling, and advanced monitoring systems, offer potential solutions for improving process control and scalability.

Given the strong dependence of enzyme productivity on substrate characteristics, the selection and valorization of lignocellulosic feedstocks—particularly rice straw—represent a critical component of integrated bioprocess design, as discussed in the following section.

RICE STRAW AS A LIGNOCELLULOSIC SUBSTRATE FOR ENZYME PRODUCTION

Rice straw represents one of the most abundant agricultural residues globally and has attracted considerable attention as a low-cost feedstock for enzyme production and biomass valorization. In major rice-producing regions, annual straw generation often equals or exceeds grain yield, creating significant management challenges. Conventional disposal practices, particularly open-field burning, contribute substantially to greenhouse gas emissions and air pollution. Consequently, the utilization of rice straw through biotechnological processes is increasingly regarded not only as a resource recovery strategy but also as an environmental necessity (Khantibongse and Ratanatamskul 2023).

From a bioprocess perspective, rice straw presents both opportunities and constraints. Its relatively high cellulose and hemicellulose content supports the induction of hydrolytic enzymes, particularly under solid-state fermentation (SSF) conditions where its fibrous structure facilitates fungal colonization. However, its physicochemical complexity imposes significant limitations that must be addressed through appropriate pretreatment and process optimization (Mitchell et al. 2023). These contrasting characteristics highlight the need to evaluate rice straw not only in terms of composition, but also in relation to its structural and process-dependent behavior.

Structural and Compositional Constraints

Rice straw typically contains 32–47% cellulose, 19–27% hemicellulose, and 5–15% lignin, although these values vary depending on cultivar, soil conditions, and harvesting practices. In addition, rice straw is characterized by significant silica deposition within its epidermal layers, which distinguishes it from many other agricultural residues (Khantibongse and Ratanatamskul 2023).

The hierarchical organization of cellulose microfibrils within a hemicellulose–lignin matrix creates structural resistance to enzymatic hydrolysis. High cellulose crystallinity and lignin crosslinking reduce enzyme

accessibility and limit hydrolysis efficiency (Chen et al. 2021). As a result, although rice straw is chemically rich in fermentable polysaccharides, its effective conversion is often constrained by its recalcitrant structure.

From an engineering perspective, this recalcitrance directly influences enzyme loading requirements, pretreatment intensity, and overall process cost. Therefore, rice straw should be considered a structurally complex substrate that requires integrated upstream processing rather than a uniformly low-cost feedstock.

Variability and Process Standardization

A critical challenge in rice straw utilization is its inherent variability across geographic regions, cultivars, and post-harvest conditions. Differences among rice varieties, such as indica and japonica, influence lignin composition, cellulose crystallinity, and ash content (Chen Chen et al., 2021; Mayolo-Deloisa et al., 2020). In addition, storage conditions and seasonal fluctuations in moisture content further affect substrate quality (Del Río et al. 2012; Chen et al. 2021). In addition, storage conditions and seasonal fluctuations in moisture content further affect substrate quality.

This variability has direct implications for process standardization. Fermentation systems optimized for a specific batch of rice straw may not perform consistently when applied to substrates with different physicochemical characteristics. Variations in substrate properties influence fungal growth kinetics, oxygen transfer behavior in SSF systems, and enzyme induction patterns (Ningthoujam et al. 2023). Therefore, reliable industrial application requires not only substrate characterization but also adaptive process control strategies capable of accommodating feedstock variability.

Pretreatment as a Process Bottleneck

Pretreatment is widely recognized as a necessary step to enhance the enzymatic accessibility of rice straw. Various approaches, including physical, chemical, physicochemical, and biological methods, have been investigated, each involving trade-offs between effectiveness, cost, and environmental impact (Kumari and Singh 2022; Ningthoujam et al. 2023).

Chemical and physicochemical methods—such as alkaline treatment, acid hydrolysis, and steam explosion—are effective in disrupting lignin structures and increasing surface area. However, these approaches may generate inhibitory by-products, including phenolic compounds and organic acids, which negatively affect fungal growth and enzyme activity. In addition, intensive pretreatment increases energy demand and operational costs, potentially offsetting the economic benefits of low-cost biomass (Kumari and Singh 2022; Igbojionu et al. 2025).

Alternative physicochemical techniques, including ammonia fiber explosion (AFEX) and CO₂ explosion, have been explored to reduce chemical usage while maintaining effectiveness (Ningthoujam et al. 2023). Biological pretreatment offers a milder and more environmentally friendly option but is generally limited by slower processing rates and incomplete lignin removal. From a systems perspective, pretreatment should not be optimized solely for maximum lignin removal, but rather for achieving a balance between improved digestibility, inhibitor formation, and overall process economics.

Rice Straw in Solid-State Fermentation Systems

Rice straw is particularly suitable for SSF due to its fibrous structure, which supports fungal attachment and growth. As both a carbon source and a physical matrix, it enables integrated enzyme production without extensive medium supplementation. Several studies have reported higher cellulase and xylanase titers in SSF compared with submerged systems when rice straw is used as the substrate (Chen et al. 2021). Enhanced enzymatic activity in SSF is often associated with improved fungal colonization and favorable morphological development. However, these advantages are strongly dependent on substrate preparation, including particle size, moisture content, and nutrient supplementation. For example, blending rice straw with materials such as wheat bran can improve porosity, nutrient availability, and overall fermentation performance (Organo et al. 2022). Therefore, the effectiveness of rice straw in SSF

is not intrinsic but depends on appropriate process optimization and substrate conditioning.

Opportunities within a Circular Bioeconomy Framework

Despite its technical challenges, rice straw remains a strategically important feedstock within circular bioeconomy systems. Its valorization for enzyme production offers dual benefits: reducing environmental pollution associated with agricultural waste and generating value-added bioproducts.

Integrated processes in which enzymes are produced in situ and directly applied for biomass hydrolysis can reduce transportation costs and dependence on commercial enzyme formulations. However, while SSF is often described as economically favorable, its scalability and reproducibility remain highly dependent on strain selection, reactor configuration, and process control. Future research should therefore prioritize the development of: cost-effective and low-impact pretreatment methods, adaptive process strategies to manage substrate variability, strain engineering tailored to rice straw characteristics, and comprehensive techno-economic and life cycle assessments

A multidisciplinary approach integrating microbial physiology, materials science, and bioprocess engineering will be essential to transform rice straw from an abundant agricultural residue into a reliable and scalable industrial substrate.

STRAIN IMPROVEMENT AND GENETIC ENGINEERING OF *TRICHODERMA* SPP.

Although native *Trichoderma* spp. are inherently efficient producers of hydrolytic enzymes, industrial applications require strains with enhanced secretion capacity, regulatory robustness, and adaptability to heterogeneous substrates such as rice straw. Consequently, strain improvement strategies have evolved from empirical mutagenesis to targeted genome engineering approaches, reflecting a shift from phenotype-driven selection to mechanism-based design (Guzmán-guzmán, Etesami, & Santoyo, 2025; Kim, Ha, Lee, Lee, & Yun, 2025). Importantly, no single strategy is universally

optimal. Each approach—ranging from classical mutagenesis to advanced genome editing—presents distinct advantages and limitations in terms of efficiency, genetic stability, scalability, and regulatory acceptance. Therefore, selecting an appropriate strain improvement strategy requires balancing technological capability with industrial feasibility.

Classical Mutagenesis and Industrial Strain Development

Classical mutagenesis, based on random genetic alterations followed by phenotypic screening, represents the earliest approach to strain improvement. Techniques such as ultraviolet irradiation and chemical mutagenesis (e.g., ethyl methanesulfonate) have been widely used to generate mutants with enhanced enzyme production (Guzmán-guzmán et al., 2025; Kim et al., 2025). A notable example is the development of hypercellulolytic *Trichoderma reesei* strains derived from the QM6a lineage, including RUT-C30, which exhibits significantly improved cellulase secretion (Bolognesi et al., 2025; Luu et al., 2023). These improvements have been associated with mutations affecting carbon catabolite repression and global regulatory pathways.

However, the efficiency of classical mutagenesis is inherently limited by its stochastic nature. While it can generate high-performing strains, the underlying genetic changes are often poorly defined, making reproducibility and rational optimization difficult. In addition, pleiotropic effects may negatively impact growth, morphology, and long-term stability (Rosolen, Aono, & Almeida, 2022). Despite these limitations, classical mutagenesis remains relevant in industrial settings due to its non-GMO status, which simplifies regulatory approval. This creates a practical trade-off: lower precision but higher regulatory acceptance.

Regulation of Enzyme Expression and Carbon Catabolite Repression

Advances in molecular understanding have highlighted that enzyme productivity is not solely determined by gene presence but by complex regulatory networks. In *Trichoderma* spp., transcription factors such as XYR1, CRE1, and ACE proteins coordinate

the expression of cellulolytic and hemicellulolytic enzymes in response to environmental signals (Sveholm et al., 2024). Carbon catabolite repression (CCR), primarily mediated by CRE1, represents a key regulatory bottleneck. The presence of easily metabolizable carbon sources suppresses enzyme gene expression, limiting productivity under certain fermentation conditions (Sveholm et al., 2024).

Targeted modification of regulatory pathways has demonstrated significant improvements in enzyme yields. However, complete disruption of CCR can destabilize cellular metabolism and reduce strain fitness. This highlights a central challenge in strain engineering: maximizing enzyme production without compromising cellular homeostasis. Thus, current strategies increasingly focus on partial deregulation or dynamic control of regulatory networks rather than complete gene knockout. Nevertheless, the optimal level of CCR modulation remains unclear and may vary depending on substrate type and fermentation system, representing an important area for further investigation (Rantasalo, Vitikainen, Paasikallio, Jäntti, & Landowski, 2019).

Recombinant DNA Technology and Genome Editing

Recombinant DNA technology has enabled precise manipulation of genes involved in enzyme production, allowing targeted overexpression, deletion, and pathway modification. These approaches have been used to enhance cellulase and hemicellulase production, as well as to introduce heterologous enzymes that complement native systems (Y. Wang et al., 2022; Xia & Lin, 2022).

Compared with classical mutagenesis, recombinant approaches offer higher specificity and reproducibility. Techniques such as promoter engineering, gene copy number optimization, and codon optimization have significantly improved transcriptional efficiency and protein secretion (J. Sugitha, Kavitha, R, & Merrylin, 2021; Y. Wang et al., 2022). More recently, CRISPR/Cas9-based genome editing has emerged as a powerful tool for rapid and precise genetic modification (J. Zhang et al., 2024). This technology enables targeted

manipulation of regulatory networks and metabolic pathways with unprecedented accuracy.

However, these advantages are accompanied by several challenges. Genome-edited strains may face regulatory barriers in certain jurisdictions, limiting industrial deployment. In addition, concerns related to off-target effects and long-term genetic stability remain unresolved. (J. Zhang et al., 2024). Thus, while CRISPR-based approaches offer superior precision and design flexibility, their industrial adoption is influenced not only by technical performance but also by regulatory and socio-economic considerations.

Protein Secretion Engineering and Cellular Machinery Optimization

Beyond gene expression, the efficiency of the cellular secretion system plays a critical role in determining extracellular enzyme yield. Processes such as protein folding, endoplasmic reticulum stress response, vesicle trafficking, and post-translational modification directly influence enzyme stability and activity (Bulgari et al., 2023).

Engineering strategies targeting chaperone systems, unfolded protein response pathways, and vesicular transport have demonstrated measurable improvements in secretion efficiency. However, increasing protein synthesis beyond cellular capacity can impose metabolic burden, leading to stress responses that ultimately reduce productivity.

This trade-off between production rate and cellular stability represents a key limitation in secretion engineering. Therefore, balanced optimization—rather than maximal overexpression—is essential to achieve sustainable enzyme production.

Co-cultivation Strategies and Enzyme Cocktail Optimization

In addition to genetic modification of single strains, co-cultivation strategies have been explored to overcome intrinsic enzymatic limitations. Co-culturing *Trichoderma* spp. with complementary microorganisms can enhance specific enzyme activities, such as β -glucosidase or oxidative enzymes, thereby improving overall hydrolysis efficiency (Y. Wang et al., 2022). Similarly,

the design of optimized enzyme cocktails has become a central strategy in industrial biomass conversion. Adjusting the relative proportions of cellulases, hemicellulases, and accessory enzymes can significantly reduce enzyme loading and improve process economics.

However, co-cultivation systems introduce additional complexity in process control and reproducibility. Maintaining stable microbial interactions under industrial conditions remains a challenge, particularly in large-scale SSF systems. These considerations indicate that enzyme system optimization should focus not only on maximizing individual enzyme activities but also on achieving stable and predictable synergistic interactions.

Challenges and Future Perspectives

Despite substantial progress, several challenges remain in the development of improved *Trichoderma* strains. These include maintaining genetic stability during prolonged cultivation, ensuring consistent performance under industrial conditions, and addressing regulatory constraints associated with genetically modified organisms. A key gap in current research is the limited integration between molecular engineering and bioprocess conditions. Strains optimized under laboratory conditions often exhibit reduced performance at scale, particularly in SSF systems where morphological characteristics influence mass transfer and substrate colonization. Future research should therefore emphasize the integration of systems biology tools—such as transcriptomics, proteomics, and metabolic modeling—with advanced bioprocess engineering. Bridging this gap will be essential for developing robust, high-performance strains capable of consistent and scalable lignocellulosic biomass conversion.

APPLICATIONS OF TRICHODERMA-DERIVED HYDROLYTIC ENZYMES

Hydrolytic enzymes produced by *Trichoderma* spp., including cellulases, hemicellulases, and oxidative enzymes such as laccases, play an important role in multiple industrial sectors. Their industrial

relevance is largely attributed to high secretion capacity and compatibility with lignocellulosic substrates. However, their practical implementation is governed not only by catalytic performance but also by cost efficiency, operational stability, and integration within existing industrial systems (Bulgari et al., 2023).

A critical consideration is that enzyme-based processes often compete with established physicochemical technologies. While enzymatic approaches offer environmental advantages, their adoption depends on achieving competitive performance under industrial conditions.

Lignocellulosic Biomass Conversion and Bioenergy

The primary application of *Trichoderma*-derived enzymes is in the saccharification of lignocellulosic biomass for biofuel and biochemical production. Although *T. reesei* widely used, their industrial implementation remains constrained by residues into fermentable sugars (Xiao, Zhao, Li, Gao, & Liu, 2023; Zhu et al., 2025). Efficient hydrolysis requires a synergistic enzyme system, including endoglucanases, cellobiohydrolases, β -glucosidases, and hemicellulases. The addition of auxiliary enzymes can enhance lignin modification and improve sugar yields from recalcitrant biomass (Augusta, Horta, Alves, Filho, & Murad, 2018; Wallenius et al., 2021).

However, compared with thermochemical conversion methods, enzymatic hydrolysis operates under milder conditions but typically requires longer processing times and higher enzyme costs. Enzyme loading remains a key economic constraint, often contributing significantly to overall production costs in second-generation bioethanol processes. As a result, industrial feasibility depends on reducing enzyme dosage while maintaining conversion efficiency. Strategies such as on-site enzyme production, strain engineering, and optimized enzyme cocktails are increasingly explored to address this limitation. Nevertheless, achieving consistent performance across diverse biomass types remains an unresolved challenge.

Pulp, Paper, and Textile Industries

In the pulp and paper industry, *Trichoderma*-derived cellulases and xylanases are applied to improve fiber processing through biobleaching and deinking. Compared with conventional chemical treatments, enzymatic approaches reduce the use of chlorine-based reagents and lower environmental impact (Rajput et al., 2024). Despite these advantages, enzyme-based processes must compete with highly optimized chemical systems that offer faster reaction rates and established scalability. As a result, enzymatic treatments are often used as complementary steps rather than complete replacements. (Bolognesi et al., 2025; Triwahyuni et al., 2024). In textile applications, cellulases are used for fabric finishing processes such as biopolishing and biostoning. These treatments improve fabric quality while reducing mechanical damage. However, enzyme performance is sensitive to process conditions, including temperature, pH, and the presence of surfactants. This sensitivity can limit robustness under industrial operating conditions.

Thus, future development in these sectors depends on improving enzyme stability and compatibility with existing industrial workflows rather than solely enhancing catalytic activity.

Food, Feed, and Agricultural Applications

In food and feed industries, hydrolytic enzymes derived from *Trichoderma* are used to enhance nutrient availability and processing efficiency. In animal feed, cellulases and xylanases degrade non-starch polysaccharides, improving digestibility and nutrient absorption (Edoardo et al., 2024; Perim et al., 2024). Compared with chemical feed additives, enzyme-based solutions offer improved specificity and reduced environmental impact. However, their effectiveness is influenced by feed composition, processing conditions, and enzyme stability during storage and digestion.

In agriculture, *Trichoderma* spp. are also applied as biocontrol agents due to their production of cell wall-degrading enzymes such as chitinases and

β -glucanases. This dual role as enzyme producer and microbial inoculant creates opportunities for integrated agricultural systems (NS, MP, GA, & ML, 2024). Nevertheless, commercialization is influenced by regulatory frameworks governing microbial products and enzyme additives, which vary across regions and may limit large-scale adoption.

Bioremediation and Environmental Biotechnology

Oxidative enzymes such as laccases have demonstrated potential in the degradation of environmental pollutants, including dyes, pesticides, and aromatic compounds (Alabdall et al., 2022; Hussein, Dufossé, Wu, & Lu, 2024). Compared with conventional physicochemical treatment methods, enzymatic processes operate under milder conditions and require fewer chemical inputs. However, their practical application is often constrained by enzyme instability, limited operational lifetime, and challenges in recovery and reuse.

In industrial wastewater treatment, these limitations can offset the environmental advantages of enzyme-based systems. As a result, strategies such as enzyme immobilization and reactor integration are necessary to improve process viability (Yamaguchi & Miyazaki, 2024).

Emerging Applications and Integrated Biorefineries

Recent developments emphasize the integration of *Trichoderma*-derived enzymes into biorefinery systems, where biomass is converted into multiple value-added products. In this context, enzyme production, biomass hydrolysis, and downstream processing are interconnected within a single process framework (Bulgari et al., 2023; Xiao et al., 2023). On-site enzyme production using low-cost substrates such as rice straw offers a potential pathway to reduce operational costs. However, the success of such systems depends on maintaining consistent enzyme performance under variable process conditions.

A key challenge in integrated biorefineries is balancing process complexity with economic feasibility. While enzyme integration can improve resource efficiency, it also

introduces additional variables in process control and optimization (Y. Wang et al., 2022; J. Zhang et al., 2024). Future research should therefore focus on: quantitative evaluation of enzyme cost contribution in industrial systems, development of robust enzyme formulations with improved stability, process integration strategies that minimize operational complexity, and comprehensive techno-economic and life cycle assessments

Ultimately, the industrial impact of *Trichoderma*-derived enzymes depends not only on their catalytic efficiency but on their ability to compete with established technologies in terms of cost, scalability, and reliability.

CHALLENGES, LIMITATIONS, AND FUTURE PERSPECTIVES

Despite the well-established industrial relevance of hydrolytic enzymes derived from *Trichoderma* spp., their large-scale implementation remains constrained by interconnected challenges spanning biological performance, process engineering, and economic feasibility. Importantly, these limitations are not independent; rather, they interact across multiple system levels, from molecular regulation to industrial-scale operation. Therefore, addressing these challenges requires a hierarchical and integrated approach.

Key Bottlenecks Across System Levels

Among the various constraints, three interrelated bottlenecks can be identified as the most critical: (i) imbalance in enzyme systems at the molecular level, (ii) mass and heat transfer limitations at the process level, and (iii) cost-intensive substrate pretreatment at the system level. These factors collectively determine the overall efficiency and economic viability of *Trichoderma*-based bioprocesses. Improvements in one domain often introduce new limitations in another, highlighting the need for coordinated optimization rather than isolated solutions.

Biological Constraint and Enzyme System Limitations.

At the molecular level, enzyme system imbalance remains a primary constraint. In

Trichoderma reesei, the relatively low β -glucosidase activity compared with cellulase production leads to cellobiose accumulation and feedback inhibition, reducing hydrolysis efficiency (N. Wang et al., 2025c). Although genetic engineering strategies have partially addressed this issue, optimizing enzyme ratios without compromising cellular fitness remains challenging. This reflects a broader trade-off between enzyme overproduction and metabolic stability.

In addition, the limited ligninolytic capability of *Trichoderma* spp. restricts its ability to independently degrade complex lignocellulosic structures. This limitation necessitates either pretreatment or integration with auxiliary enzymes, increasing process complexity. Thus, future research should focus not only on increasing enzyme titers but also on improving enzyme robustness and synergistic functionality under realistic process conditions (Zhu et al., 2025).

Similarly, the comparatively low ligninolytic capacity of *Trichoderma* spp., especially when contrasted with white-rot fungi, restricts their standalone application in complete lignocellulose deconstruction. Enzyme inhibition by phenolic compounds and other by-products generated during biomass pretreatment further compromises process robustness (Zhu et al., 2025). Future efforts must therefore focus not only on increasing enzyme titers but also on improving enzyme tolerance to inhibitors and enhancing synergistic enzyme interactions.

Process Engineering and Scale-Up Constraints

At the process level, the transition from laboratory-scale optimization to industrial-scale operation introduces significant challenges. In SSF systems, limitations in heat dissipation, moisture control, and oxygen transfer create heterogeneous conditions that reduce process reproducibility (del Río et al., 2012; Mitchell et al., 2023). In contrast, SmF systems offer better controllability but require high energy input for aeration and agitation. Furthermore, excessive shear stress can negatively affect fungal morphology and enzyme secretion. These contrasting limitations illustrate that no single fermentation strategy is universally optimal. Instead, hybrid or adaptive systems that

combine the advantages of SmF and SSF may offer a more balanced solution. However, such systems introduce additional complexity in reactor design and process control (Rohr et al., 2024).

Substrate and Pretreatment Constraints

At the system level, substrate variability and pretreatment complexity represent major economic bottlenecks. Agricultural residues such as rice straw exhibit significant variability in composition and structural properties, which directly affect enzyme performance and process reproducibility (Xiao et al., 2023).

Pretreatment is required to improve substrate accessibility but remains one of the most energy-intensive and cost-dominant steps in biomass conversion. In addition, the formation of inhibitory compounds during pretreatment can reduce enzyme activity and microbial performance (Liu et al., 2020). Therefore, the challenge is not simply to maximize pretreatment efficiency but to achieve an optimal balance between substrate digestibility, inhibitor formation, and process cost.

Regulatory and Genetic Engineering Challenges

Advances in genome editing technologies have significantly improved the ability to engineer *Trichoderma* strains. However, the industrial implementation of genetically modified strains is influenced by regulatory constraints and public acceptance, particularly in food and agricultural applications (Jin, 2025; Y. Wang et al., 2022). In addition, the regulatory networks governing enzyme production, secretion, and stress response remain incompletely understood. This limits the predictability of engineered strains, especially under industrial conditions. Bridging this gap requires integration of systems biology approaches with process-level validation to ensure that engineered traits remain stable and functional during large-scale operation (Zheng et al., 2022).

Toward Integrated and Sustainable Bioprocess Systems

The future of *Trichoderma*-based enzyme systems lies in the integration of molecular engineering, process optimization,

and system-level design. Rather than focusing solely on maximizing enzyme production, future strategies should aim to optimize the entire bioprocess ecosystem (Novy, Nielsen, Seiboth, & Nidetzky, 2019).

Key directions include: development of substrate-specific enzyme systems with improved synergy, integration of fermentation strategies to balance productivity and controllability, implementation of real-time monitoring and data-driven process optimization and comprehensive techno-economic and life cycle assessments

Ultimately, the scalability and industrial competitiveness of *Trichoderma*-derived enzyme systems will depend on the ability to coordinate improvements across molecular, process, and economic dimensions within a unified framework.

CONCLUSIONS

Hydrolytic enzymes derived from *Trichoderma* spp. represent a well-established platform for lignocellulosic biomass conversion, driven by the synergistic action of cellulases, hemicellulases, and oxidative enzymes. Among these, *Trichoderma reesei* remains the primary industrial producer, while emerging species and engineered strains expand functional diversity and process adaptability.

Despite significant advances, industrial implementation remains constrained by interconnected challenges, including enzyme system imbalance, substrate variability, pretreatment complexity, and scale-up limitations in both submerged and solid-state fermentation systems. These constraints operate across multiple system levels, linking molecular regulation with process performance and industrial scalability.

Future development should therefore prioritize integrated optimization strategies. Tailored enzyme cocktails, hybrid fermentation systems, and real-time process monitoring offer promising pathways to improve efficiency and robustness. In addition, integration within circular biorefinery frameworks—particularly through on-site utilization of agricultural residues—can enhance economic and environmental sustainability.

Ultimately, the competitiveness of *Trichoderma*-based enzyme systems will depend on the convergence of molecular

engineering and advanced bioprocess design within scalable and economically viable production platforms.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES

- Abbas A, Mubeen M, Zheng H, Sohail MA, Shakeel Q, Solanki MK, Iftikhar Y, Sharma S, Kashyap BK, Hussain S, Zuñiga Romano MC, Moya-Elizondo EA, Zhou L (2022) *Trichoderma* spp. genes involved in the biocontrol activity against *Rhizoctonia solani*. *Front Microbiol* 13. doi: 10.3389/fmicb.2022.884469
- Abd EI-latif AS, Zohri ANA, EI-Aref HM, Mahmoud GAE (2024) Kinetic studies on optimized extracellular laccase from *Trichoderma harzianum* PP389612 and its capabilities for azo dye removal. *Microb Cell Fact* 23:1–19. doi: 10.1186/s12934-024-02412-2
- Alananbeh KM, Alkfoof R, Muhaidat R, Massadeh M (2024) Production of xylanase by *Trichoderma* species growing on olive mill pomace and barley bran in a packed-bed bioreactor. *J Fungi* 10:49. doi: 10.3390/jof10010049
- Arnau J, Yaver D, Hjort CM (2020) Strategies and challenges for the development of industrial enzymes using fungal cell factories. In: *Grand Challenges in Biology and Biotechnology*, pp 179–210
- Bamidele MO, Bamikale MB, Cárdenas-Hernández E, Bamidele MA, Castillo-

- Olvera G, Sandoval-Cortes J, Aguilar CN (2025) Bioengineering in solid-state fermentation for next sustainable food bioprocessing. *Next Sustain* 6:100105. doi: 10.1016/j.nxsust.2025.100105
- Castrillo ML, Bich GÁ, Amerio NS, Rodríguez MD, Zapata PD, Villalba LL (2021) Assessment of cellulase complex secretory capacity of *Trichoderma* strains and morphological and molecular identification of the isolate with the highest enzymatic secretion capacity. *J Microbiol Biotechnol Food Sci* 10:1–8. doi: 10.15414/jmbfs.1357
- Cerrone F, O'Connor KE (2025) Cultivation of filamentous fungi in airlift bioreactors: advantages and disadvantages. *Appl Microbiol Biotechnol* 109. doi: 10.1007/s00253-025-13422-4
- Chen C, Deng X, Kong W, Qaseem MF, Zhao S, Li Y, Wu AM (2021) Rice straws with different cell wall components differ on abilities of saccharification. *Front Bioeng Biotechnol* 8. doi: 10.3389/fbioe.2020.624314
- Civzele A, Stipniece-Jekimova AA, Mezule L (2023) Fungal ligninolytic enzymes and their application in biomass lignin pretreatment. *J Fungi* 9. doi: 10.3390/jof9070780
- Del Río JC, Rencoret J, Prinsen P, Martínez ÁT, Ralph J, Gutiérrez A (2012) Structural characterization of wheat straw lignin as revealed by analytical pyrolysis, 2D-NMR, and reductive cleavage methods. *J Agric Food Chem* 60:5922–5935. doi: 10.1021/jf301002n
- Finkler ATJ, Weber MZ, Fuchs GA, Scholz LA, de Lima Luz LF, Krieger N, Mitchell DA, Jorge LM de M (2021) Estimation of heat and mass transfer coefficients in a pilot packed-bed solid-state fermentation bioreactor. *Chem Eng J* 408:127246. doi: 10.1016/j.cej.2020.127246
- He Q, Peng H, Sheng M, Hu S, Qiu J, Gu J (2019) Humidity control strategies for solid-state fermentation: capillary water supply by water-retention materials and negative-pressure auto-controlled irrigation. *Front Bioeng Biotechnol* 7:1–13. doi: 10.3389/fbioe.2019.00263
- Hinterdobler W, Li G, Spiegel K, Basyouni-Khamis S, Gorfer M, Schmoll M (2021) *Trichoderma reesei* isolated from Austrian soil with high potential for biotechnological application. *Front Microbiol* 12:1–14. doi: 10.3389/fmicb.2021.552301
- Igbojionu LI, Mao Y, Binner E, Fernandez-Castane A (2025) A comparative study of effects of microwave-assisted deep eutectic solvents pretreatment of rice straw on cellulose enrichment and enzymatic digestibility. *Cellulose* 32:10513–10528. doi: 10.1007/s10570-025-06834-y
- Kalaiselvan P, Devi NC, Deepti M, Devi AA, Akamad K, Dheeran P, Debbarma S, Vadivel D, Rajesh D (2025) Solid-state fermentation—a sustainable future technology in aquafeeds? *Front Mar Sci* 12:1–21. doi: 10.3389/fmars.2025.1669719
- Khantibongse P, Ratanatamskul C (2023) Insight into pathway of monosaccharide production from integrated enzymatic hydrolysis of rice straw waste as feedstock for anaerobic digestion. *Sci Rep* 13:1–14. doi: 10.1038/s41598-023-27398-6
- Kumari D, Singh R (2022) Rice straw structure changes following green pretreatment with petha wastewater for economically viable bioethanol production. *Sci Rep* 12:10443. doi: 10.1038/s41598-022-14627-7
- Leite P, Sousa D, Fernandes H, Ferreira M, Costa AR, Filipe D, Gonçalves M, Peres H, Belo I, Salgado JM (2021) Recent advances in production of lignocellulolytic enzymes by solid-state fermentation of agro-industrial wastes. *Curr Opin Green Sustain Chem* 27:100407. doi: 10.1016/j.cogsc.2020.100407
- Martinez Y, Ribera J, Schwarze FW, De France K (2023) Biotechnological development of *Trichoderma*-based formulations for biological control. *Appl Microbiol Biotechnol* 107:5595–5612. doi: 10.1007/s00253-023-12687-x

- Méndez-González F, Loera O, Saucedo-Castañeda G, Favela-Torres E (2020) Forced aeration promotes high production and productivity of infective conidia from *Metarhizium robertsii* in solid-state fermentation. *Biochem Eng J* 156. doi: 10.1016/j.bej.2020.107492
- Michelin M, Mota AMO, Silva DP, Ruzene DS, Vicente AA, Teixeira JA (2019) Production of biomass-degrading enzymes by *Trichoderma reesei* using liquid hot water-pretreated corncob in different conditions of oxygen transfer. *Bioenergy Res* 12:583–592. doi: 10.1007/s12155-019-09991-8
- Mitchell DA, Ruiz HA, Krieger N (2023) A critical evaluation of recent studies on packed-bed bioreactors for solid-state fermentation. *Processes* 11:1–32. doi: 10.3390/pr11030872
- Ningthoujam R, Jangid P, Yadav VK, Sahoo DK, Patel A, Dhingra HK (2023) Bioethanol production from alkali-pretreated rice straw: effects on fermentation yield, structural characterization, and ethanol analysis. *Front Bioeng Biotechnol* 11:1–9. doi: 10.3389/fbioe.2023.1243856
- Organo ND, Granada SMJM, Pineda HGS, Sandro JM, Nguyen VH, Gummert M (2022) Assessing the potential of a *Trichoderma*-based compost activator to hasten the decomposition of incorporated rice straw. *Sci Rep* 12:448. doi: 10.1038/s41598-021-03828-1
- Perwez M, Al Asheh S (2025) Valorization of agro-industrial waste through solid-state fermentation: mini review. *Biotechnol Rep* 45:e00873. doi: 10.1016/j.btre.2024.e00873
- Rayhane H, Josiane M, Gregoria M, Yiannis K, Nathalie D, Ahmed M, Sevastianos R (2019) From flasks to single used bioreactor: scale-up of solid state fermentation process for metabolites and conidia production by *Trichoderma asperellum*. *J Environ Manage* 252:109496. doi: 10.1016/j.jenvman.2019.109496
- Sala A, Artola A, Barrena R, Sánchez A (2024) Harnessing packed-bed bioreactors' potential in solid-state fermentation: the case of *Beauveria bassiana* conidia production. *Fermentation* 10:481. doi: 10.3390/fermentation10090481
- Sharma N, Ahlawat YK, Stalin N, Mehmood S, Morya S, Malik A, Malathi H, Nellore J, Bhanot D (2025) Microbial enzymes in industrial biotechnology: sources, production, and significant applications of lipases. *J Ind Microbiol Biotechnol* 52. doi: 10.1093/jimb/kuaf010
- Tonin F, Marti E, Arends IWCE, Hanefeld U (2020) Laccase did it again: a scalable and clean regeneration system for NAD⁺ and its application in the synthesis of 12-oxo-hydroxysteroids. *Catalysts* 10:1–14. doi: 10.3390/catal10060677
- Vauris A, Valcauda S, Husson F, De Coninck J (2022) A novel method to assess heat transfer and impact of relevant physicochemical parameters for the scaling up of solid-state fermentation systems. *Biotechnol Rep* 36. doi: 10.1016/j.btre.2022.e00764
- Wallenius J, Kontro J, Lyra C, Kuuskeri J, Wan X, Kähkönen MA, Baig I, Kamer PCJ, Sipilä J, Mäkelä MR, Nousiainen P, Hildén K (2021) Depolymerization of biorefinery lignin by improved laccases of the white-rot fungus *Obba rivulosa*. *Microb Biotechnol* 14:2140–2151. doi: 10.1111/1751-7915.13896
- Wang N, Lin Q, Wang Z, Shi H, Gao Y, Zen J, Lou K, Huo X (2025) Multi-omics analysis of *Trichoderma reesei* mutant with high glucanase activity. *Sci Rep* 15:25184. doi: 10.1038/s41598-025-07954-y
- Zhang P, Li Q, Chen Y, Peng N, Liu W, Wang X, Li Y (2022) Induction of cellulase production in *Trichoderma reesei* by a glucose–sophorose mixture as an inducer prepared using stevioside. *RSC Adv* 12:17392–17400. doi: 10.1039/d2ra01192a
- Zhu Q, Liu W, Song L, Guo Z, Bian Z, Han Y, Cai H, Yang P, Meng K (2025) The potential of *Trichoderma asperellum* for degrading wheat straw and its key genes in lignocellulose degradation. *Front Microbiol* 16:1–16. doi: 10.3389/fmicb.2025.1550495