Prepublished Copy

NATURE ENVIRONMENT AND POLLUTION TECHNOLOGY

Article In Press

PEER-REVIEWED PREPUBLISHED MANUSCRIPT

This is a peer-reviewed prepublished version of the paper to be published in **Vol. 26, No. 1, March 2027.**

Valorization of Fungal Biomass: A Circular Approach for the Integrated Recovery of Chitosan and Lignocellulolytic Enzymes

Patil Shubham and Girish Pathade

RECEIVED
5 May 2026

CITE THIS ARTICLE

Shubham, P. and Pathade, G., 2027. Valorization of Fungal Biomass: A Circular Approach for the Integrated Recovery of Chitosan and Lignocellulolytic Enzymes. Nature Environment and Pollution Technology, 26(1), B4457.

https://doi.org/10.46488/NEPT.2027.v26i01.B4457

EARLY-ACCESS STATEMENT

This manuscript, after peer review, is being released online in its original, unedited form to provide early access to its findings. Prior to final publication, it will undergo additional editing and formatting. Readers may note that, as an unedited version, the manuscript may have linguistic and other minor errors. The DOI will be active after the final publication of the paper.

PUBLICATION STATUS

PEER REVIEW	ONLINE VERSION	FINAL ISSUE
Completed	**Prepublished**	**March 2027**

Valorization of Fungal Biomass: A Circular Approach for the Integrated Recovery of Chitosan and Lignocellulolytic Enzymes

Patil Shubham, Girish Pathade*

Krishna Institute of Science and Technology, Krishna Vishwa Vidyapeeth (Deemed to be University), Karad, Maharashtra. Pin code: 415539 Email ID:

patilshubham2198@gmail.com ORCID ID: 0009-0003-7347-942x Mobile Number: 91-7507882568

girishpathade18@gmail.com ORCID ID: 0009-0007-9187-5521 Mobile Number: 91-9823145387

Corresponding author: Girish Pathade; girishpathade18@gmail.com

Abstract:

The present study used an integrated biorefinery approach to sequentially recover industrially important enzymes like cellulase, laccase, and lignin peroxidase (LiP), followed by the recovery of chitosan from the residual biomass. The biomass of mushroom fungus (*Pleurotus Sajor caju)* was first processed to get aqueous crude enzyme extracts, which were precipitated using ammonium sulphate and purified by chromatography. The activity of the enzyme was measured with standard spectrophotometric methods, and total protein yield was estimated by the Bradford method. After recovering the enzymes, the remaining biomass was utilized for chitin extraction, followed by its conversion to chitosan through demineralization, deproteinization, and deacetylation. The resulting chitosan was validated by using Fourier transform infrared spectroscopy and X-ray diffraction to evaluate its structural and physicochemical properties, including degree of deacetylation, molecular characteristics, and viscosity. The results indicate that this integrated method efficiently recovers enzymes and produces good-quality chitosan from the same biomass, making it a promising strategy for sustainable use of biomass and circular bioeconomy practices.

Keywords: biorefinery, cellulase, chitosan, fungal biomass, lignin peroxidase

1. INTRODUCTION

The widespread industrial interest in enzymes and biopolymers is increasing due to their broad applications in pharmaceuticals, food processing, and environmental applications, along with the increased requirement for green substitutes to chemical processes. The global industrial enzyme sector is projected to generate a turnover of approximately USD 8.42 billion in 2025, increasing to USD 12.01 billion by 2030, at a compound annual growth rate (CAGR) of 7.3%. They are utilized in biofuels, food processing, pharmaceuticals, agriculture, textiles, detergents, pulp and paper, and environmental remediation, using less energy and having minimal influence on the environment (Sheldon et al. 2022). Enzymes such as cellulase, laccase, and lignin

peroxidase (LiP) are crucial for lignocellulosic biomass conversion. Cellulase produces fermentable sugars, while laccase and LiP, oxidative enzymes from white-rot fungi, degrade lignin and remove pollutants (Ilić et al. 2023). Laccase is also widely used in effluent treatment (Shanmugapriya et al. 2019). Both LiP and laccase support lignin valorization in circular bioeconomy systems (Vaz 2024).

Chitosan, obtained through alkaline deacetylation, has gained importance as a biodegradable, biocompatible, non-toxic, and renewable material, with applications in biomedicine, agriculture, food packaging, and environmental remediation (Iber et al. 2022, Singh et al.2024). Fungal-derived chitosan obtained from mycelial biomass is increasingly recognized as a potent alternative to conventional crustacean sources, including consistent quality, reduced allergenic concerns, and is suitable for vegan and religious considerations (Benettayeb et al. 2023).

The current industrial practices treat enzyme production and chitosan extraction as separate processes. Fungal species such as *Aspergillus*, *Trichoderma*, and white-rot fungi like *Trametes* are frequently utilized for enzyme production, generating substantial amounts of residual mycelial biomass that is often underutilized or discarded (Perez & Wertz 2021). Furthermore, chitosan production from fungal sources usually requires separate cultivation and extraction steps, leading to higher cost, resource use, and waste generation (Verma et al. 2020 & Namboodiri et al. 2020). Recent developments in fungal biorefinery systems have highlighted the feasibility of producing multiple products such as enzymes, lipids, and chitosan from a single biomass source, particularly when lignocellulosic or waste substrates are used (Meyer et al. 2020, Gaur et al. 2024, Chaurasia et al. 2025). While sequential extraction of enzymes and chitin/chitosan from fungal biomass has each been individually documented, most prior reports address only one product stream at a time and rarely quantify whether the added processing complexity of recovering both products sequentially from the same biomass is justified in terms of resource and cost efficiency. The specific novelty of the present study lies in demonstrating, within a single integrated workflow and from a single Pleurotus sajor-caju biomass source, that sequential enzyme and chitosan recovery can be achieved without compromising the yield or quality of either product, thereby providing a basis for future efficiency comparison against single-product processing routes; a detailed quantitative comparison of processing energy cost against product market value was beyond the scope of the present laboratory-scale study and is identified as a priority for subsequent process-economics analysis. In this context, the present study proposes an integrated biorefinery approach aimed at the sequential recovery of functional enzymes such as cellulase, laccase, and lignin peroxidase, followed by the extraction of high-quality chitosan from a single fungal biomass source. Selected fungi, including *Rhizomucor miehei*, *Mucor circinelloides*, and certain Basidiomycetes, serve as promising candidates for enabling this unified and resource-efficient process. While the present findings are derived from laboratory-scale batch processing, the underlying unit operations (solid-liquid extraction, salt precipitation, chromatography, and acid/alkali treatment) are already established at industrial scale in enzyme and biopolymer manufacturing individually; scaling the integrated process would primarily require optimization of solid loading, reagent recovery/recycling, and continuous or semi-continuous processing formats to control cost, and represents an important direction for future translational work.

2. MATERIALS AND METHODS

2.1. Biomass Pre-processing

The fungal mycelial biomass of *Pleurotus sajor-caju* was collected after fermentation, thoroughly rinsed with deionised water (4-6 times), and filtered using cheesecloth or a Büchner funnel. The biomass was first air-dried at 25–30 °C for 24-48 hrs and oven-dried at 45–50 °C to a constant weight, ensuring a final moisture content of less than 5%. The dried biomass was then crushed and sieved to obtain a uniform particle size range of 0.5-1 mm (40–60 mesh), suitable for further extraction. Moisture levels were quantified by a gravimetric method, involving drying at 105 °C until weight stabilization occurred (Cai et al. 2017). The sample was ashed in a muffle furnace at 550 °C for 6 hrs to determine the ash content. To verify culture purity and rule out microbial contamination of the biomass, serial dilution plating on potato dextrose agar (PDA) was carried out prior to downstream processing (Gopalaiah et al.2024).

2.2. Crude Enzyme Extraction

Homogenized dry fungal biomass (20–50 g) was suspended in a cold extraction buffer (50 mM sodium citrate, pH 5.5) and incubated at 4°C under orbital shaking (200 rpm) for 8–16 hrs to facilitate the release of enzymes; the stated ranges for temperature, incubation time, and biomass weight reflect the batch-to-batch variability encountered across replicate extractions, with the reported yields and activities corresponding to representative operating conditions within each stated range. The resulting suspension was clarified by filtration through Whatman No. 1 filter paper and centrifuged at 10,000 g for 20 min at 4 °C to remove residual particulate matter. The resulting supernatant, representing the crude enzyme fraction, was harvested and maintained at 4 °C for immediate analysis and at −20 °C until further use (Zhang et al. 2021).

2.3. Enzyme Concentration

Crude enzyme extract was subjected to salt-induced precipitation and concentrated by ammonium sulphate at 60–80% concentration at 4°C to eliminate the impurities and concentrate the enzyme. The resulting protein pellet was collected by centrifugation at 12,000 × g for 30 min at 4 °C and re-dissolved in 20–50 mL of 50 mM sodium acetate buffer (pH 5.0) and dialyzed (10–14 kDa membrane) using the same buffer for 24–48 h at 4 °C to remove residual ammonium sulphate (Purwanto 2016); the lower pH 5.0 buffer was selected at this stage as it lies close to the isoelectric range of several fungal cellulases and laccases, improving protein solubility and stability during storage. Prior to ion-exchange loading, the dialyzed sample was subsequently buffer-exchanged into 20 mM Tris–HCl (pH 7.5) by a further round of dialysis/ultrafiltration to match the equilibration buffer of the DEAE-Sepharose column described in Section 2.4.

2.4. Enzyme Purification

For higher purity, the concentrated enzyme fraction was purified by ion-exchange chromatography using a DEAE-Sepharose Fast Flow (1.6 × 20 cm) pre-equilibrated with 20 mM Tris–HCl buffer (pH 7.5). A linear NaCl gradient (0–1 M) was applied over 10–15 column volumes at a flow rate of 1 mL/min to elute the target protein. Because cellulase (~50 kDa), laccase (~60–65 kDa), and LiP (~40 kDa) differ in both molecular weight and isoelectric point, these enzymes eluted at distinct positions across the 0–1 M NaCl gradient and were therefore identified and pooled as separate fractions based on enzyme-specific activity assays performed across the eluted fractions, rather than as a single combined pool. Fractions showing enzymatic activity for each respective enzyme were collected and saturated by ultrafiltration using an Amicon stirred cell equipped with a 10 kDa molecular weight cut-off membrane. Further, purification was achieved by gel filtration chromatography on a Superdex column selected to match the expected molecular weight range of the target enzymes (cellulase ~50 kDa, laccase ~60–65 kDa, LiP ~40 kDa), equilibrated with 50 mM phosphate buffer (pH 7.2) containing 150 mM NaCl. Elution was carried out under isocratic conditions at a flow rate of 0.5–1 mL/min, with fractions of 3–5 mL collected for analysis. The eluted protein concentration was estimated using a spectrophotometer at 280 nm, and fractions were evaluated for specific enzyme activity to assess purity. This purification strategy resulted in a 3-10-fold increase in enzyme purity, depending on the enzyme system under investigation (Bekavac et al. 2024).

2.5. Enzyme Quantification

All enzyme assays were performed in triplicate at 25–30 °C and analyzed using a UV–Visible spectrophotometer. Cellulase activity was determined by using the DNSA assay as mentioned by Singla et al. 2018. For laccase, the ABTS assay (2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid**)** was used to estimate the activity (Kummari et al. 2022). Lignin Peroxidase (LiP) activity was estimated using the veratryl alcohol oxidation assay (El-Shora et al. 2025), and total protein was estimated at 595 nm by Bradford assay (Kummari et al.2022). Specific activity (U/mg), yield (%), and purification efficiency were calculated using standard formulas as previously mentioned by Babinskas & Matijošytė 2025.

2.6. SDS-PAGE and Zymography

SDS-PAGE analysis was performed on 10–12% polyacrylamide (Mini-PROTEAN) under denaturing conditions using the Laemmli method with β-mercaptoethanol; samples mixed with Coomassie brilliant blue R-250 were heated at 95 °C for 5 min, and molecular weights were estimated using 10 to 250 kDa markers. For zymographic analysis, native or semi-native PAGE was conducted without prior heating or the addition of reducing agents in order to preserve enzymatic activity. After electrophoresis, gels were incubated with 2.5% triton X-100 to facilitate protein renaturation and then incubated in appropriate substrate solutions specific to each enzyme, including 5 mM ABTS for laccase, 2 mM veratryl alcohol with H_2O_2 for lignin peroxidase, and 1% carboxymethyl cellulose (CMC) for cellulase; cellulase-containing CMC gels were subsequently stained with 0.1% (w/v) Congo Red solution for 15 min and destained with 1 M NaCl until clear (unstained) zones, indicating cellulolytic activity, became visible against the red-stained background.

Enzymatic activity was visualized after staining and destaining, where active bands appeared as clear zones against a stained background, indicating the presence and functional integrity of the respective enzymes (Isanapong et al. 2024).

2.7. Chitin and Chitosan Extraction

The extraction was done sequentially from enzyme-extracted residual biomass.

2.7.1. Demineralization

Residual biomass demineralization using 1 M HCl (1:15 w/v) at 60–90 °C for 1–4 h under continuous stirring to remove inorganic components, primarily calcium carbonate ($CaCO_3$). The solution was filtered and washed properly with distilled water until a neutral pH was achieved, and dried at 50 °C. Residual ash content was evaluated after incineration, while Ca^{2+} and Mg^{2+} titers were determined using atomic absorption spectroscopy (AAS) (Oriez et al. 2019). The acid concentration, temperature, and duration were selected within ranges commonly reported for fungal cell-wall demineralization, balancing efficient removal of mineral salts against the risk of acid-catalyzed depolymerization of chitin; milder conditions within this range were favoured towards the shorter time and lower temperature end to minimise chitin degradation, and the resulting chitin/chitosan was subsequently screened for molecular integrity via FTIR and viscosity where reported.

2.7.2. Deproteinization

The demineralized material was subjected to deproteinization by treatment with 1 M NaOH (1:15 w/v), under continuous stirring at 90 °C for 1–6 h to remove proteins and associated glucans. The solid fraction was filtered and thoroughly washed with distilled water until a neutral pH was achieved. Residual protein content was subsequently determined using the Kjeldahl method, which involved digestion, distillation, and titration sequentially, with nitrogen content converted using a factor of 6.25 (Fu et al. 2019).

2.7.3. Deacetylation

Purified chitin was deacetylated using a 40–60% (w/v) NaOH solution (1:15 w/v) at 110°C for 7-8 hours under reflux and nitrogen purging to stop oxidation. The chitosan product was washed to neutral pH and dried. The degree of deacetylation (DDA) was determined by using FTIR spectra (KBr pellet, 4000–400 cm^{-1}), with the baseline-corrected Domszy and Roberts equation, DDA (%) = 100 − [(A1655 / A3450) × 100 / 1.33], where A1655 is the baseline-corrected absorbance of the amide I peak, A3450 is the baseline-corrected absorbance of the –OH reference peak, and 1.33 is the correction factor derived from the A1655/A3450 ratio for fully N-acetylated chitin, consistent with the standard baseline-corrected approach used for FTIR-based DDA determination.

2.7.4. Drying and Powdering

The physicochemical properties of the material were assessed by determining moisture content (oven drying at 105 °C), bulk density using the tapped volume method, and particle size distribution through sieve analysis or laser diffraction (Wani et al. 2020).

The overall workflow of the integrated biorefinery system, including sequential stages of biomass processing and valorization, is illustrated in Figure 1.

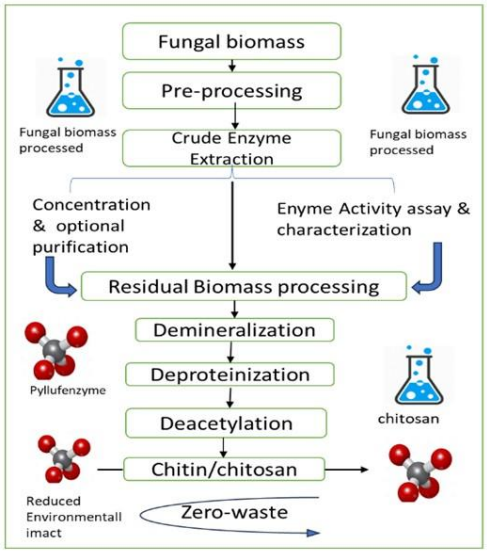

Fig. 1: Integrated Biorefinery Workflow

3. RESULTS AND DISCUSSION

3.1. Enzyme Recovery and Activity

The proposed extraction method facilitated the recovery of key enzymes, including cellulase, laccase, and lignin peroxidase (LiP), from fungal biomass. Further processing through concentration and partial purification improved enzyme activity, while overall yields remained reasonably preserved. Ammonium sulphate precipitation (60–80% saturation) effectively concentrated the crude extract, resulting in a 1.3-fold increase in specific activity with 77.5% activity recovery. Optional chromatographic purification (ion-exchange followed by gel filtration) increased purity to a 2.4-fold overall purification factor. The final yield was 55.1%, and the specific activity was 4.50 IU/mg (Table 1).

Table 1: Purification profile of enzymes isolated from *Pleurotus* spp. biomass.

Parameter	Crude Extract	AmmoniumSulphate Precipitation	Purified Fraction (Chromatography)
Total protein (mg)	520	310	120
Total activity (IU)	980	760	540
Specific activity (IU/mg)	1.88	2.45	4.50
Yield (%)	100	77.5	55.1
Purification fold	1.0	1.3	2.4

The study findings mirrored other studies having documented purification methods for fungal ligninolytic and cellulolytic enzymes (Bakratsas et al. 2023, Isanapong et al. 2024). Ammonium sulphate precipitation of laccase from *Pleurotus* spp. typically gives 1.5-3-fold purification with 50 to 80% recovery, while multi-step chromatography achieves 10-70-fold purification, but with lower yields (15 to 40%). The modest 2.4-fold purification observed here indicates that the step was optional and cost-effective, with yield being more important than ultra-high purity for industrial applications. The simultaneous recovery of cellulase (hydrolytic) and oxidative enzymes (laccase/LiP) illustrated the adaptability of the gentle aqueous extraction from fungal mycelium, which maintained synergistic activity beneficial for lignocellulosic degradation and bioremediation, while avoiding harsh pretreatments and biomass loss.

3.2. SDS-PAGE Analysis

Gel bands after SDS-PAGE showed the presence of different proteins in the crude, concentrated, and purified fractions, indicating enrichment of targeted enzymes. As shown in Figure 2, the prominent bands at about 50 kDa (endoglucanases/cellulases), 60–65 kDa (fungal laccases), and 40 kDa (LiP) were more intense, and the reduction in background protein levels indicated effective removal of non-target impurities during purification. Enzyme functionality was further assessed by zymography, which revealed distinct activity bands at characteristic molecular weights following substrate-specific staining (CMC/Congo Red for cellulase, ABTS for laccase, and veratryl alcohol/H_2O_2 for lignin peroxidase). The observed band patterns were similar to the reported profiles of fungal enzymes, where laccase isoforms typically appear in the range of 55–70 kDa and lignin peroxidase around 38–45 kDa in white-rot fungi (El-Shora et al. 2025). Notably, the retention of activity bands after dialysis and chromatographic steps suggests that the enzymes remained stable under the applied processing conditions.

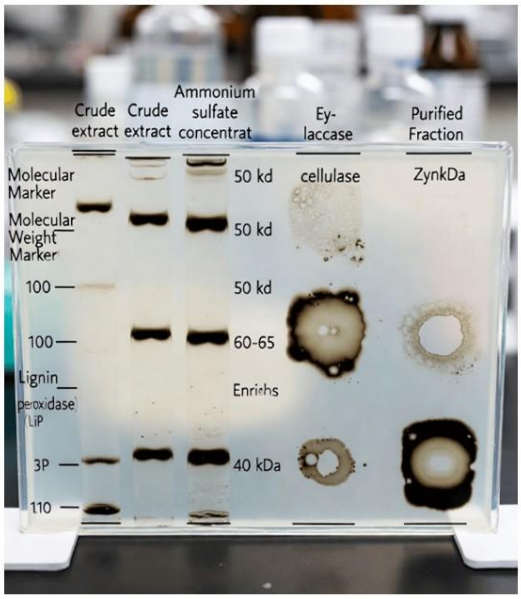

Fig. 2: SDS-PAGE profile of crude and purified enzymes. Molecular weight markers (10–250 kDa) were used. The crude extract showed a complex protein profile, which was reduced after ammonium sulphate precipitation and purification, indicating enrichment of proteins with prominent bands at ~ 50 kDa (cellulases), 60–65 kDa (laccases), and ~ 40 kDa (LiP) on the denaturing SDS-PAGE gel. Zymography, performed separately on native/semi-native PAGE gels (not the denaturing SDS-PAGE gel shown here), confirmed active cellulase and ligninolytic enzymes as clear activity bands against a stained background.

3.3. Chitosan Yield and Characterization

The residual biomass was treated further for chitosan recovery, yielding 18.5% on a dry weight basis (Table 2). This value fell within the previously documented range for fungal chitosan obtained from mycelial residues of species such as *Rhizomucor*, *Aspergillus*, *Pleurotus*, and *Mucor* after enzyme production, which typically varies between 10 and 30% (Mehta et al. 2024).

Table 2: Chitosan yield and physicochemical properties

Parameter	Value
Chitosan Yield (%)	18.5
Degree of Deacetylation (DDA %)	78.2
Moisture Content (%)	9.4
Ash Content (%)	1.1

Solubility in 1% Acetic Acid	Fully Soluble

The degree of deacetylation (DDA) was 78.2%, determined by FTIR peak ratio analysis (Figure 3). This value falls within the typical range of 70–85% reported for chitosan intended for industrial and biomedical applications, indicating effective deacetylation with minimal polymer degradation. The FTIR spectrum displayed prominent diagnostic peaks associated with chitosan, including a broad –OH stretching band around 3400 cm^{-1}, amide I (C=O) near 1655 cm^{-1} with reduced intensity, amide II (N–H bending) around 1590 cm^{-1}, and C–O–C stretching in the range of 1150–1020 cm^{-1}. The observed spectral feature confirms the conversion of chitin to chitosan without significant impurities. Similar spectral patterns and DDA values (75–88%) are reported for fungal chitosan derived from species such as *Rhizomucor miehei* and *Cunninghamella* (Di Zhu 2023) (Figure 3).

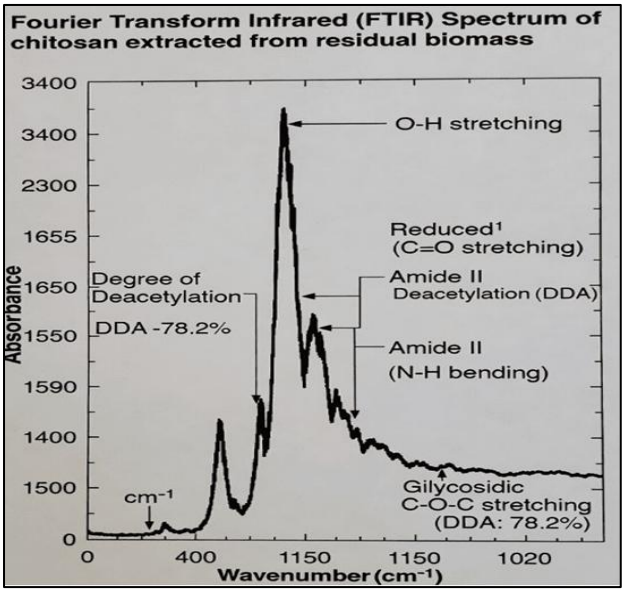

Fig.3: FTIR spectrum of extracted chitosan

The XRD study revealed a semi-crystalline structure with characteristic diffraction peaks at 2θ = 10° (020 plane) and 20° (110 plane), as shown in Figure 4; these peaks indicate the presence of polymorphic form II (β-chitin derived, changed to chitosan). The moderate crystallinity made it easy to dissolve (completely soluble in 1% acetic acid) and suitable for film or nanoparticle formation. Low ash (1.1%) and moisture (9.4%) contents confirmed that the product was well demineralized and dried, making it more stable and purer than crustacean chitosan, which generally has greater levels of ash because of $CaCO_3$ (de Oliveira et al.2017).

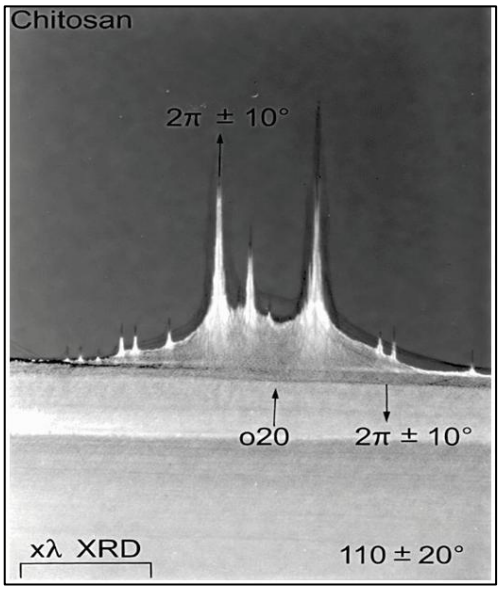

Fig. 4: XRD pattern of chitosan

3.4. Integrated Process Efficiency and Sustainability Implications

The sequential recovery process produced two high-value products without affecting the quality of either. In the first step, enzymes were extracted using a moderate method to preserve their activity. In the second step, the remaining delignified/protein- depleted biomass was chemically valorized. The obtained enzyme yield (55.1% final) and chitosan quality (DDA 78.2%, yield 18.5%) were higher for our study than those of procedures that work on their own (Karimlar et al., 2026). Also, our sequential process substantially reduces biomass input per unit of output (by an estimated 30–50%) and generates less residual solid waste and by-products compared with running enzyme production and chitosan extraction as separate operations, while potentially improving process economics through co-production; nonetheless, this claim of near zero-waste operation should be interpreted cautiously, as the process still uses HCl, NaOH, ammonium sulphate, and chromatographic reagents that generate chemical waste streams requiring proper treatment and disposal. Overall, this integrated strategy supports the concept of circular bioeconomy by turning enzyme fermentation waste into a useful biopolymer resource. Comparable strategies have been documented in studies involving *Rhizomucor miehei* or *Pleurotus mycelial* leftovers. Future work can focus on scaling up the process, including optimization of precipitation conditions for higher yield and enzyme-specific modifications. However, the existing process is a sustainable, industrially feasible paradigm for fungal biorefineries in the biofuels, bioremediation, and biopolymer sectors (Liu & Smith 2021).

4. CONCLUSION

This study explained that an integrated biorefinery approach can facilitate the concurrent recovery of industrially significant enzymes and high-quality chitosan from a single fungal biomass. These bioproducts hold substantial potential for industrial,

biomedical, agricultural, and environmental applications. While this research establishes a robust framework for scaling enzyme technology, future studies must evaluate the process economics and operational feasibility to ensure commercial viability. The present study also has certain limitations that should be addressed in follow-up work: enzyme purification data are reported as pooled values rather than separately for cellulase, laccase, and LiP; a complete mass balance across the integrated process (tracking starting biomass, enzyme recovery, residual biomass, and chitin/chitosan yield) was not performed; and chitosan characterization did not include molecular weight/viscosity or crystallinity index determination. Future studies incorporating enzyme-specific quantification, a full process mass balance, and expanded chitosan characterization would further strengthen the circular biorefinery framework proposed here.

REFERENCES

1. Babinskas, J., Matijošytė, I. 2025. Laccase functional analysis: Substrates, activity assays. *ChemBioChem*, 26, e202400939. https://doi.org/10.1002/cbic.202400939

2. Bakratsas, G., Antoniadis, K., Athanasiou, P. E., Katapodis, P., and Stamatis, H. 2023. Laccase and biomass production via submerged cultivation of *Pleurotus ostreatus* using wine lees. *Fermentation*, *10*(1), 1. https://doi.org/10.3390/biomass4010001

3. Bekavac, N., Benković, M., Jurina, T., Valinger, D., Gajdoš Kljusurić, J., Jurinjak Tušek, A. and Šalić, A., 2024. Advancements in aqueous two-phase systems for enzyme extraction, purification, and biotransformation. *Molecules*, *29*(16), pp.3776. DOI: https://doi.org/10.3390/molecules29163776

4. Benettayeb, A., Seihoub, F.Z., Pal, P., Ghosh, S., Usman, M., Chia, C.H., Usman, M., and Sillanpää, M., 2023. Chitosan nanoparticles as potential nano-sorbent for the removal of toxic environmental pollutants. *Nanomaterials*, *13*(3), pp.447. DOI: **https://doi.org/10.3390/nano13030447**

5. Cai, J., He, Y., Yu, X., Banks, S.W., Yang, Y., Zhang, X., Yu, Y., Liu, R. and Bridgwater, A.V., 2017. Review of physicochemical properties and analytical characterization of lignocellulosic biomass. *Renewable and sustainable energy reviews*, *76*, pp.309-322. DOI: https://doi.org/10.1016/j.rser.2017.03.072

6. Carrillo-Nieves, D., Saldarriaga-Hernandez, S., Gutiérrez-Soto, G., Rostro-Alanis, M., Hernández-Luna, C., Alvarez, A.J., Iqbal, H.M. and Parra-Saldívar, R., 2022. Biotransformation of agro-industrial waste to produce lignocellulolytic enzymes and bioethanol with a zero waste. *Biomass Conversion and Biorefinery*, *12*(2), pp.253-264. DOI: https://doi.org/10.1007/s13399-020-00738-6

7. Chaurasia, P.K., Bharati, S.L., Singh, S., Sivalingam, A.M., Shankar, S. and Mani, A., 2025. Fungal pretreatment methods for organic wastes: advances and challenges in biomass valorization. *RSC Sustainability*, *3*(3), pp.1234-1266. DOI: 10.1039/D4SU00582A

8. de Oliveira, L.R., Manzato, L., Mascarenhas, Y.P. and Sanches, E.A., 2017. The influence of heat treatment on the semi-crystalline structure of polyaniline Emeraldine-salt form. *Journal of Molecular Structure*, *1128*, pp.707-717. DOI: https://doi.org/10.1016/j.molstruc.2016.09.044

9. Di Zhu, X., 2023. *Examination of Potential Applicability of Fourier Transform Infrared (FTIR) Spectroscopy for Routine Identification of Pathogenic Bacteria and Fungi and for Human Saliva-Based Detection of Viral Infection*. McGill University (Canada). DOI: https://escholarship.mcgill.ca/concern/theses/td96k803t

10. El-Shora, H.M., El-Morsy, E.S.M., El-Saftawy, D.M. and Ibrahim, M.E., 2025. Purification and kinetics of lignin peroxidase from *Emericella nidulans*. *Spectrum Science Journal*, *2*(2), pp.49-57. DOI: 10.21608/sasj.2025.393252.1006

11. Fu, X., Zhu, L., Li, L., Zhang, T., Li, M. and Mou, H., 2019. Eco-friendly preparation of chitooligosaccharides with different degrees of deacetylation from shrimp shell waste and their effects on the germination of wheat seeds. *Marine Life Science & Technology*, *1*(1), pp.95-103. DOI: https://doi.org/10.1007/s42995-019-00012-3

12. Gaur, S., Kaur, M., Kalra, R., Rene, E.R. and Goel, M., 2024. Application of microbial resources in biorefineries: Current trend and future prospects. *Heliyon*, *10*(8). DOI: 10.1016/j.heliyon.2024.e28615

13. Karimlar, S., A. G. Pirbalouti, Z. Teymuori, M. Moslehishad, and Z. Hamidi-Esfahani. 2026. Applications of Chitosan, an Eco-Friendly Biopolymer in Agricultural Systems, Herbal Products, and Functional Foods: A Review. *Food Science & Nutrition* 14, no. 1: e71367. https://doi.org/10.1002/fsn3.71367.

14. Gopalaiah, S.B. and Jayaseelan, K., 2024. Analytical quality by design approach to develop an eco-friendly RP-HPLC method for estimation of irbesartan in chitosan polymeric nanoparticles: forced degradation studies and assessment of in vitro release mathematical modelling. *RSC advances*, *14*(31), pp.22169-22184. DOI: 10.1039/D4RA03952A

15. Iber, B.T., Kasan, N.A., Torsabo, D. and Omuwa, J.W., 2022. A review of various sources of chitin and chitosan in nature. *Journal of Renewable Materials*, *10*(4), p.1097. DOI: 10.32604/jrm.2022.018142

16. Ilić, N., Milić, M., Beluhan, S. and Dimitrijević-Branković, S., 2023. Cellulases: from lignocellulosic biomass to improved production. *Energies*, *16*(8), p.3598. DOI: **https://doi.org/10.3390/en16083598**

17. Isanapong, J., Suwannoi, K., Lertlattanapong, S., and Panchal, S. 2024. Purification, characterization of laccase from *Pleurotus ostreatus* HK35, and optimization for Congo red biodecolorization using Box–Behnken design. *3 Biotech*, *14*(3), 73. https://doi.org/10.1007/s13205-024-03926-7

18. Kummari, R.K., Puja, R., and Bose, K., 2022. Protein quantitation and detection. In: *Textbook on Cloning, Expression and Purification of Recombinant Proteins*. pp.279-299. Singapore: Springer Nature Singapore. DOI: https://doi.org/10.1007/978-981-16-4987-5_11

19. Liu, Z. and Smith, S.R., 2021. Enzyme recovery from biological wastewater treatment. *Waste and Biomass Valorization*, *12*(8), pp.4185-4211. DOI: https://doi.org/10.1007/s12649-020-01251-7

20. Mayfield, J.E., Irani, S., Escobar, E.E., Zhang, Z., Burkholder, N.T., Robinson, M.R., Mehaffey, M.R., Sipe, S.N., Yang, W., Prescott, N.A. and Kathuria, K.R., 2019. Tyr1 phosphorylation promotes phosphorylation of Ser2 on the C-terminal domain of eukaryotic RNA polymerase II by P-TEFb. *Elife*, *8*, p.e48725. DOI; https://doi.org/10.7554/eLife.48725

21. Mehta, P. and Chelike, D.K., 2024. Utilizing fungal biodegradation for valorization of lignocellulosic waste biomass and its diverse applications. *Applied Research*, *3*(4), p.e202300119. DOI: **https://doi.org/10.1002/appl.202300119**

22. Meyer, V., Basenko, E.Y., Benz, J.P., Braus, G.H., Caddick, M.X., Csukai, M., De Vries, R.P., Endy, D., Frisvad, J.C., Gunde-Cimerman, N. and Haarmann, T., 2020. Growing a circular economy with fungal biotechnology: a white paper. *Fungal Biology and Biotechnology*, *7*(1), p.5. DOI: https://doi.org/10.1186/s40694-020-00095-z

23. Namboodiri, M.T. and Pakshirajan, K., 2020. Valorization of waste biomass for chitin and chitosan production. In *Waste Biorefinery* (pp. 241-266). Elsevier. DOI: https://doi.org/10.1016/B978-0-12-818228-4.00010-1

24. Oriez, V., Peydecastaing, J. and Pontalier, P.Y., 2019. Lignocellulosic biomass fractionation by mineral acids and resulting extract purification processes: Conditions, yields, and purities. *Molecules*, *24*(23), pp.4273. DOI: **https://doi.org/10.3390/molecules24234273**

25. Perez, S. and Wertz, J.L., 2021. *Chitin and Chitosans in the Bioeconomy*. CRC Press. DOI: 10.1201/9781003226529

26. Pujol-Pina, R., Vilaprinyó-Pascual, S., Mazzucato, R., Arcella, A., Vilaseca, M., Orozco, M. and Carulla, N., 2015. SDS-PAGE analysis of Aβ oligomers is driving research into Alzheimer's disease: appealing for ESI-IM-MS. *Scientific Reports*, *5*(1), pp. 14809. DOI: https://doi.org/10.1038/srep14809

27. Purwanto, M.G.M., 2016. The role and efficiency of ammonium sulphate precipitation in purification process of papain crude extract. *Procedia Chemistry*, *18*, pp.127-131. DOI: https://doi.org/10.1016/j.proche.2016.01.020

28. Shanmugapriya, S., Manivannan, G., Selvakumar, G., and Sivakumar, N., 2019. Extracellular fungal peroxidases and laccases for waste treatment: recent improvement. In *Recent Advancements in White Biotechnology Through Fungi: volume 3: Perspective for Sustainable Environments* (pp. 153-187). Cham: Springer International Publishing. DOI: https://doi.org/10.1007/978-3-030-25506-0_6

29. Sheldon, R.A. and Brady, D., 2022. Green chemistry, biocatalysis, and the chemical industry of the future. *Chem Sus Chem*, *15*(9), p.e202102628. DOI: **https://doi.org/10.1002/cssc.202102628**

30. Singh, A.K., Fernandez-Lafuente, R., Schmidt, J.E., Boczkaj, G. and Bilal, M., 2024. Biocatalytic functionalities of lignin peroxidase-based systems in lignin depolymerization and pollutants removal from environmental matrices. *Current Pollution Reports*, *10*(3), pp.345-361. DOI: https://doi.org/10.1007/s40726-024-00310-0

31. Singla, D., Taggar, M.S., Kocher, G.S. and Kalia, A., 2018. Cellulase production by Aspergillus fumigatus using different plant-based agricultural biomass for paddy straw saccharification. *Cellulose Chemistry and Technology*, *52*, pp.803-813. DOI: https://www.cellulosechemtechnol.ro/downloadfirstonline.php?file=9118

32. Vaz Jr, S., 2024. *The Lignin Macromolecule: A Compendium of Sustainable Technologies*. Springer Nature. DOI: https://doi.org/10.1007/978-3-031-75511-8

33. Verma, M.L., Kumar, S., Das, A., Randhawa, J.S. and Chamundeeswari, M., 2020. Chitin and chitosan-based support materials for enzyme immobilization and biotechnological applications. *Environmental Chemistry Letters*, *18*(2), pp.315-323. DOI: https://doi.org/10.1007/s10311-019-00942-5

34. Wani, T.A., Masoodi, F.A., Akhter, R. and Sofi, F.A., 2020. Techno-functional characterization of chitosan nanoparticles prepared through planetary ball milling. *International Journal of Biological Macromolecules*, *154*, pp.166-172. DOI: https://doi.org/10.1016/j.ijbiomac.2020.03.034

35. Zhang, M., Ma, W., Wang, C., Xu, H. 2021. Optimization of enzyme-assisted extraction and purification of flavonoids from *Pinus koraiensis* nut-coated film and antioxidant activity evaluation. *Molecules*, 26(7), 1950. https://doi.org/10.3390/molecules26071950