

# Microstructural Mechanisms of Sugarcane Bagasse Biodegradation by Locally Isolated Tropical Cellulolytic Fungi for Environmentally Friendly Composting

Nurhaida Widiani<sup>1\*</sup>, Ovi Prasetya Winandari<sup>2\*\*</sup>

<sup>1</sup>Department of Biology, Faculty of Science and Technology; <sup>2</sup>Department of Biology Education, Faculty of Education and Teacher Training, UIN Raden Intan Lampung, Indonesia.

## Corresponding author

nurhaidawidiani@radenintan.ac.id\*, oviprasetyawinandari@radenintan.ac.id\*\*

Manuscript received: 27 November 2025. Revision accepted: 10 March 2026, Published: 19 April 2026.

## Abstract

Sugarcane bagasse is a highly recalcitrant lignocellulosic residue that decomposes slowly under natural conditions, requiring an effective biological agent to accelerate composting. This study evaluated the effectiveness of locally isolated tropical cellulolytic fungi in enhancing bagasse biodegradation based on macromorphological changes and microstructural evidence obtained through Scanning Electron Microscopy (SEM). Four treatments were tested: uninoculated control (P0), single inoculum A (P1), single inoculum B (P2), and consortium A+B (P3), with composting conducted for 56 days under a modified Takakura system. The consortium treatment (P3) exhibited the most advanced compost maturity, characterized by darker coloration, brittle texture, granular structure, and a distinct earthy odor. SEM micrographs further confirmed the greatest extent of lignocellulose breakdown in P3, showing deep longitudinal fissures, fibril delamination, lumen collapse, high porosity, and dense hyphal and spore colonization. These findings indicate that the consortium of cellulolytic fungi accelerates lignocellulose depolymerization through synergistic extracellular enzymatic activity, leading to faster decomposition than single isolates. Overall, the results highlight the bioconversion potential of indigenous tropical fungal consortia as environmentally friendly bioactivators for sustainable management of sugarcane bagasse and other lignocellulosic wastes.

**Keywords:** cellulolytic fungi; composting; fungal consortium; SEM; lignocellulose biodegradation; sugarcane bagasse.

## INTRODUCTION

Sugarcane bagasse is one of the most abundant lignocellulosic residues generated in tropical agro-industries. Its tightly compacted matrix composed of cellulose, hemicellulose, and lignin makes the material highly recalcitrant and difficult to degrade under natural conditions. This structural rigidity limits its conversion into value-added products such as organic compost, thereby presenting challenges to sustainable biomass management in sugarcane-producing regions (Khan et al., 2024). Accelerating the biodegradation of bagasse requires biological agents capable of depolymerizing lignocellulose efficiently under tropical environmental conditions.

Cellulolytic fungi are key biological degraders of lignocellulosic biomass due to their ability to secrete complementary enzyme complexes, including endoglucanases, exoglucanases,  $\beta$ -glucosidases, and lignin-modifying oxidoreductases. Recent studies have highlighted the advantages of microbial consortia over single strains in enhancing substrate colonization and enzymatic synergism during lignocellulose breakdown (S. Zhang et al., 2023). However, most investigations

rely on commercial or model fungal strains, while the biodegradation potential of locally isolated tropical fungi, which are naturally adapted to heat, humidity, and native lignocellulosic substrates, remains underexplored.

In many composting studies, the evaluation of lignocellulose degradation is limited to bulk metrics such as temperature dynamics, mass loss, pH changes, or C/N ratio reduction. These macroscopic parameters provide limited insight into how degradation actually proceeds at the microstructural level. Scanning Electron Microscopy (SEM) offers a powerful means of observing early and progressive changes in lignocellulosic cell walls, including fibril peeling, pore formation, hyphal penetration, and localized cracking features that serve as direct indicators of fungal enzymatic action (Qi et al., 2023; Zhao et al., 2023). Nevertheless, SEM-based mechanistic studies focusing specifically on tropical local fungal inoculants applied to sugarcane bagasse are still scarce.

This study aimed to elucidate the microstructural mechanisms of sugarcane bagasse biodegradation mediated by a consortium of locally isolated tropical cellulolytic fungi during composting. By integrating

SEM observations with morphological interpretation of fiber degradation events, this research provides new mechanistic evidence on how indigenous fungal consortia deconstruct lignocellulose under tropical composting conditions. The findings contribute to the development of efficient, environmentally friendly microbial inoculants for sustainable biomass conversion in tropical agro-ecosystems.

## METHODS

### Study Design and Preparation of Fungal Inoculum

The study was conducted from June to November 2025 at the Microbiology and Research Laboratories of UIN Raden Intan Lampung. A descriptive observational design with four treatments was applied: P0 (control), P1 (fungus A), P2 (fungus B), and P3 (consortium A+B).

Locally isolated cellulolytic fungi were prepared as inoculum by sterilizing culture bottles at 121 °C, filling them with 60 g substrate supplemented with CaCO<sub>3</sub> 2% and CaSO<sub>4</sub> 4% (7.5 mL each), and inoculating with one loopful of fungal culture. Bottles were incubated for 14 days until full mycelial growth (Gaiind et al., 2009).

### Composting Procedure

Sugarcane bagasse was chopped into 2 cm pieces and mixed with goat manure at a 2 : 1 (w/w) ratio, with moisture adjusted to 50 – 60%, a range considered optimal for aerobic microbial composting (Lombardi et al., 2022). Fungal inocula were added at 1% (v/w) for treatments P1 – P3 (Irawan et al., 2019). Composting followed a modified Takakura method using ventilated bins to maintain aerobic conditions, consistent with approaches commonly used for rapid household and lignocellulosic composting.

The composting process lasted 56 days, with material turned every 3 – 5 days during the first two weeks and every 7 – 10 days thereafter. Turning helps maintain

aeration and uniform microbial activity, preventing anaerobic pockets and supporting lignocellulose decomposition. Temperature, pH, and moisture were monitored periodically. Samples were collected on days 28 for morphological and microstructural evaluation.

### SEM Observation and Data Analysis

Bagasse samples were air-dried and prepared according to standard SEM protocols used in lignocellulose microstructure studies. SEM observations were performed at magnifications ranging from 500× to 5,000× to assess structural degradation, including fiber fragmentation, pore formation, surface erosion, and hyphal penetration key indicators of fungal enzymatic activity on lignocellulosic biomass (Mishra et al., 2017).

SEM images were analyzed descriptively, supported by macroscopic observations of color, texture, and physical consistency to compare the extent of biodegradation across treatments. Descriptive microstructural analysis is commonly used to evaluate the early and progressive stages of lignocellulose breakdown in fungal-mediated composting systems (Z. Zhang et al., 2021).

## RESULT AND DISCUSSION

### Results

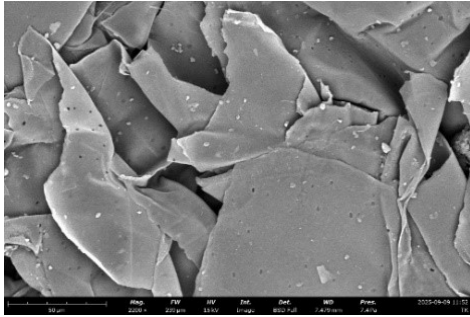
Physical and microstructural changes in sugarcane bagasse during composting varied across treatments. The control (P0) experienced very limited decomposition, indicated by unchanged coarse fiber texture, light brown color, and firm material structure throughout the 56-day observation. Treatments with single fungal inocula (P1 and P2) showed moderate transformation, including fiber softening, darker coloration, and partial fragmentation; however, the structural integrity of the material remained observable.



**Figure 1.** Physical transformation of sugarcane bagasse after composting with different cellulolytic fungal inoculum treatments. Panel **a** represents the uninoculated control, **b** and **c** represent single-fungal-inoculum treatments, and **d** represents the fungal consortium treatment.

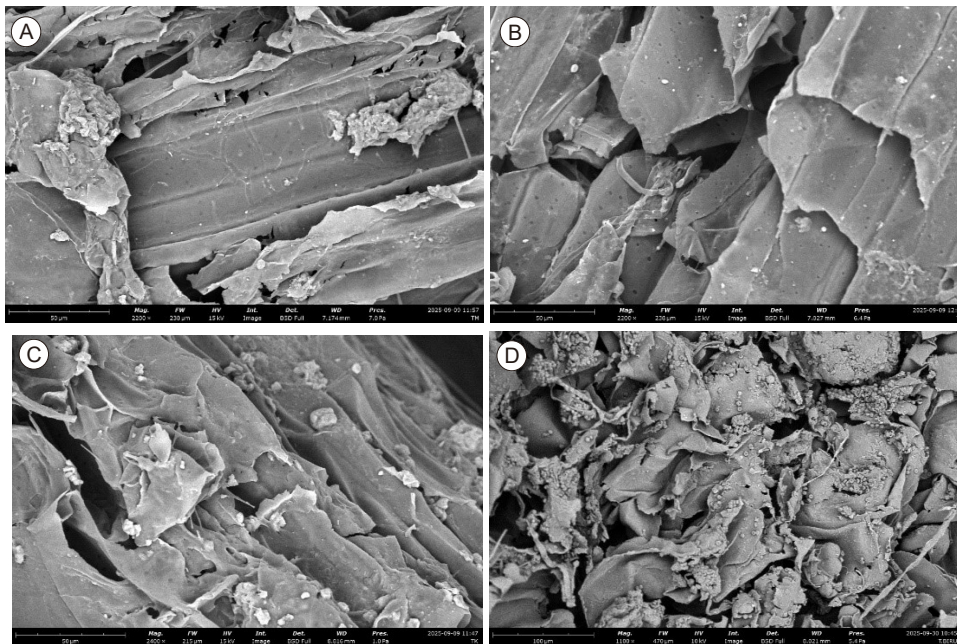
The consortium treatment (P3) produced the most advanced compost maturity indicators, including a dark brown to black appearance, brittle texture, granular

structure, and earthy odor. These parameters collectively signaled faster lignocellulosic degradation compared with single inocula and the uninoculated control.



**Figure 2.** Scanning Electron Microscope (SEM) micrograph of sugarcane bagasse prior to composting, showing intact and compact fiber structure at 2200× magnification.

SEM analysis revealed degradation patterns consistent with the visible physical changes. Before composting, bagasse fibers exhibited smooth and compact surfaces without cracks or pores (figure 2).

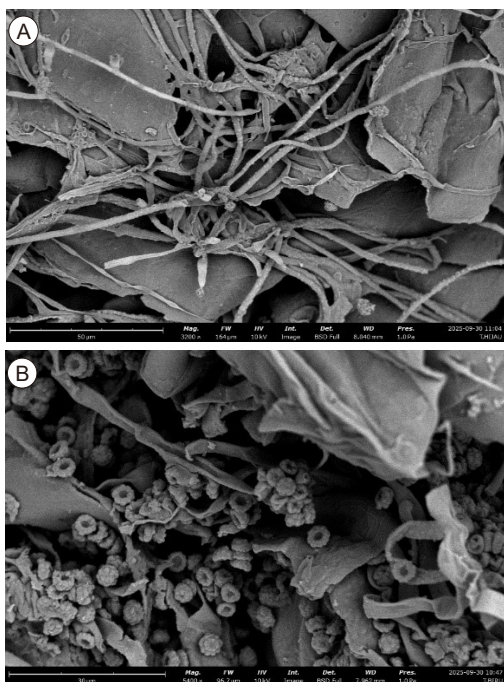


**Figure 3.** Scanning Electron Microscope (SEM) images of sugarcane bagasse after the composting process, showing different decomposition patterns under each treatment: (a) uninoculated control (P0), (b) single inoculum A (P1), (c) single inoculum B (P2), and (d) consortium of cellulolytic fungi (P3).

After composting, P0 demonstrated only shallow surface erosion, while P1 and P2 showed initial microfractures, early porosity, and partial surface peeling. P3 displayed the most intense microstructural disruption, consisting of deep longitudinal fissures, fibril delamination, lumen collapse, high porosity, and densely distributed hyphal penetration. These microstructural observations confirmed the progressive degradation of lignocellulosic fibers across treatments, with the strongest effect achieved in the consortium treatment (figure 3).

SEM images (Figure 5) reveal strong disintegration of the lignocellulosic fiber matrix, characterized by dense aggregates of fungal spores and compact hyphal networks firmly attached to the fiber surface, indicating active colonization and sporulation during composting. The close adhesion of spores and extensive hyphal penetration suggest that cellulolytic fungi exploited the lignocellulosic substrate as a carbon source through the secretion of extracellular enzymes such as cellulases and

hemicellulases, leading to the breakdown of complex plant cell wall polysaccharides. The resulting microstructural disruption manifested as surface cracking, peeling, and roughness reflects enzymatic degradation driven by cellulase and ligninase activities, consistent with previous reports of hyphal colonization and fiber disintegration in lignocellulosic residues inoculated with *Aspergillus* and *Trichoderma* species (Mohammad et al., 2011; W. Zhang et al., 2023). The coexistence of dense spore clusters and extensive hyphal growth indicates an active metabolic phase combining reproductive processes with substrate depolymerization, a colonization pattern commonly associated with high lignocellulose decomposition efficiency and accelerated organic matter turnover (Gong et al., 2023; Janusz et al., 2017).



**Figure 4.** SEM micrographs of sugarcane bagasse following the composting process with cellulolytic fungal inoculum. (a) Dense spore aggregation and severe lignocellulosic fiber disintegration at 5400× magnification. (b) Expanded hyphal network infiltrating and degrading bagasse fiber structures at 3200× magnification.

## Discussion

The compost treated with the fungal consortium (P3) exhibited the most advanced physical transformation, characterized by a darker color, crumb-like aggregation, and a brittle texture, indicating faster stabilization than the single-inoculum treatments (P1 and P2) and the control (P0). These macroscopic features are widely recognized as indicators of compost maturity in lignocellulosic waste systems, reflecting progressive humification and structural breakdown of plant residues as decomposition advances. SEM observations reinforced these trends by showing that P3 experienced the deepest microstructural disruption—including fibril fragmentation, pore formation, lumen collapse, and dense hyphal penetration—consistent with accelerated enzymatic attack on cellulose and hemicellulose as reported in fungal-mediated lignocellulose degradation studies. Taken together, the findings demonstrate that fungal consortia accelerate composting of lignocellulosic residues more effectively than single fungal strains, a trend widely reported in fungal-assisted biodegradation studies (Mohammad et al., 2011), and this work provides direct microstructural evidence that synergistic fungal interactions enhance substrate colonization and lignocellulose conversion efficiency.

SEM analysis provided mechanistic evidence supporting this conclusion. The intense cracking, lumen collapse, and fibril separation observed in P3 indicate extensive extracellular enzymatic depolymerization of cellulose and hemicellulose. Dense hyphal colonization across fiber surfaces and internal lumens confirms

sustained fungal activity throughout decomposition. These structural patterns correspond to known microstructural signatures of fungal-driven lignocellulose biodegradation reported in recent mechanistic studies (Qi et al., 2023; Zhao et al., 2023).

Taken together, the physical and SEM evidence confirms that the consortium of locally isolated tropical cellulolytic fungi promotes more rapid and deeper fiber decomposition than single isolates (Mohammad et al., 2011). The results support the strategic relevance of indigenous microbial resources as efficient biological agents for sustainable composting of sugarcane bagasse.

A key strength lies in integrating tropical local fungal isolates with SEM-based microstructural diagnostics, allowing direct observation of degradation pathways rather than relying solely on macroscopic compost maturity indicators. This elevates the mechanistic insight of the study relative to conventional compost research.

The study did not perform quantitative enzymatic or chemical evaluations (e.g., cellulase activity, FTIR, XRD, C/N ratio) and was conducted on a laboratory scale. Future research should include biochemical assays and field-scale trials to validate the performance of the fungal consortium for industrial composting applications.

## CONCLUSION

This study demonstrates that a consortium of locally isolated tropical cellulolytic fungi is substantially more effective in accelerating the biodegradation of sugarcane bagasse compared with single fungal isolates and the uninoculated control. The improvement in compost maturity indicators supported by microstructural evidence of fiber cracking, fibril delamination, and extensive hyphal penetration confirms that the enhanced decomposition in the consortium treatment is driven by synergistic extracellular enzymatic depolymerization of lignocellulose. These findings underscore the biodegradation potential of indigenous tropical fungal resources and affirm their relevance as environmentally friendly biological inoculants for lignocellulosic composting systems.

**Acknowledgements:** The authors gratefully acknowledge the financial support provided by the DIPA UIN Raden Intan Lampung for the 2025 fiscal year, which enabled the successful completion of this research. The authors also thank the Microbiology Laboratory and Research Laboratory of UIN Raden Intan Lampung for providing facilities and technical assistance throughout the study.

**Authors' Contributions:** N.W. conceived the research idea, designed the experimental framework, and supervised the overall study. O.P.W. conducted the laboratory experiments, performed sample preparation and SEM analysis, and collected observational data.

N.W. and O.P.W performed data interpretation and contributed to the development of the discussion. N.W. prepared the initial manuscript draft, while O.P.W edited, and improved the final version of the manuscript. All authors read and approved the final manuscript.

**Competing Interests:** The authors declare that there are no competing interests.

## REFERENCES

- Gaind, S., Nain, L., & Patel, V. B. (2009). Quality evaluation of co-composted wheat straw, poultry droppings and oil seed cakes. *Biodegradation*, 20(3), 307–317. <https://doi.org/10.1007/s10532-008-9223-1>
- Gong, Y., Chen, L., & Martin, F. (2023). *Comparative analysis of simulated in-situ colonization and degradation by Lentinula edodes on oak wafer and corn stalk*. November, 1–16. <https://doi.org/10.3389/fmicb.2023.1286064>
- Irawan, B., Septitasari, A. W., & Handayani, T. T. (2019). *Effect of Induced Compost by Cellulolytic (Aspergillus fumigatus) and Ligninolytic (Geotrichum sp.) Fungi Inoculum Application on Vegetative Growth of Red Chili (Capsicum annuum L.)*. 13(June), 815–821. <https://doi.org/10.22207/JPAM.13.2.16>
- Janusz, G., Pawlik, A., Sulej, J., Swiderska-burek, U., Jarosz-wilkolazka, A., & Paszczy, A. (2017). *Lignin degradation: microorganisms, enzymes involved, genomes analysis and evolution*. April, 941–962. <https://doi.org/10.1093/femsre/fux049>
- Khan, N. A., Khan, M., Sufyan, A., Saeed, A., Sun, L., Wang, S., Nazar, M., Tan, Z., Liu, Y., & Tang, S. (2024). *Biotechnological Processing of Sugarcane Bagasse through Solid-State Fermentation with White Rot Fungi into Nutritionally Rich and Digestible Ruminant Feed*. 1–16.
- Lombardi, F., Zingaretti, D., Verginelli, I., & Costa, G. (2022). Optimization of the Biostabilization Process of an Italian Mechanical – Biological Treatment Plant to Account for Changes in Waste Composition. *Waste and Biomass Valorization*, 0123456789. <https://doi.org/10.1007/s12649-022-01788-9>
- Mishra, V., Jana, A. K., Maiti, M., & Antriksh, J. (2017). Fungal pretreatment of sweet sorghum bagasse with supplements: improvement in lignin degradation, selectivity and enzymatic saccharification. *3 Biotech*, 7(2), 1–13. <https://doi.org/10.1007/s13205-017-0719-4>
- Mohammad, N., Alam, M. Z., Kabashi, N. A., & Adebayo, O. S. (2011). Development of compatible fungal mixed culture for composting process of oil palm industrial waste. *African Journal of Biotechnology*, 10(81), 18657–18665. <https://doi.org/10.5897/AJB11.2735>
- Qi, J., Li, F., Jia, L., Zhang, X., Deng, S., Luo, B., Zhou, Y., Fan, M., & Xia, Y. (2023). Fungal Selectivity and Biodegradation Effects by White and Brown Rot Fungi for Wood Biomass Pretreatment. *Polymers*, 15(8), 1–15. <https://doi.org/10.3390/polym15081957>
- Zhang, S., Duan, Y., Teng, C., Quan, H., Yang, X., Li, H., Li, X., & Yan, L. (2023). *Fast and Selective Degradation of Biomass for Xylose, Glucose and Lignin under Mild Conditions*. 1–16.
- Zhang, W., Diao, C., & Wang, L. (2023). Degradation of lignin in different lignocellulosic biomass by steam explosion combined with microbial consortium treatment. *Biotechnology for Biofuels and Bioproducts*, 1–15. <https://doi.org/10.1186/s13068-023-02306-2>
- Zhang, Z., Shah, A. M., Mohamed, H., Tsiklauri, N., & Song, Y. (2021). Isolation and Screening of Microorganisms for the Effective Pretreatment of Lignocellulosic Agricultural Wastes. *BioMed Research International*, 2021. <https://doi.org/10.1155/2021/5514745>
- Zhao, B., Liu, R., Guo, Q., Xu, G., Zhang, L., & Sun, P. (2023). *The use of newly isolated fungal cultures for the selective delignification of bamboo culms*. August, 1–9. <https://doi.org/10.3389/fbioe.2023.1265420>

**THIS PAGE INTENTIONALLY LEFT BLANK**