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Biological valorization of coconut coir by *Trametes hirsuta* Bm-2: delignification, laccase production, phenolic release, and saccharification response

Valorización biológica del bonote de coco mediante *Trametes hirsuta* Bm-2: deslignificación, producción de lacasa, liberación de compuestos fenólicos y respuesta de sacarificación

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Abstract

Coconut coir is an abundant lignocellulosic residue whose valorization is limited by the high recalcitrance of its lignin-rich matrix. This study evaluated the biological modification of milled coconut coir using *Trametes hirsuta* Bm-2 under solid-state fermentation (SSF) and submerged fermentation (SmF) for 28 days, and compared it with short ultrasound-assisted enzymatic pretreatments (1 h) using either a *T. hirsuta* Bm-2 crude extracellular enzyme extract or a commercial laccase. The raw material showed a lignocellulosic profile with high lignin content (36.97%), alongside cellulose (33.31%) and hemicellulose (11.36%). Biological pretreatment achieved the highest lignin removal, reaching 37.77 ± 0.07% delignification in SSF and 43.44 ± 0.10% in SmF after 28 days, demonstrating the ability of *T. hirsuta* Bm-2 to modify a highly recalcitrant substrate under mild biological conditions. Coconut coir also supported high extracellular laccase production, particularly under SmF, where activity peaked at day 7, highlighting its potential as a low-cost substrate for enzymes of industrial interest. However, saccharification did not increase proportionally with lignin removal. SmF-pretreated solids showed a time-dependent optimum in sugar release at day 7 (9.57 ± 0.15 g L^{-1}), followed by a sharp decline at longer incubation times despite further lignin reduction. This decoupling suggests that lignin removal alone was insufficient to ensure efficient hydrolysis and may be associated with partial deconstruction of the lignocellulosic matrix, fungal biomass accumulation, possible loss or consumption of accessible carbohydrate fractions, and residual inhibitory compounds. Ultrasound alone produced 15.25 ± 0.12% delignification, while ultrasound combined with crude extract or commercial laccase increased delignification to 20.48 ± 0.05% and 20.77 ± 0.05%, respectively, but did not improve sugar release under the evaluated conditions. Overall, *T. hirsuta* Bm-2 enabled biological valorization of coconut coir, with stronger potential for lignin modification and laccase production than for saccharification.

Keywords: coconut coir, *Trametes hirsuta* Bm-2, delignification, ultrasound-assisted enzymatic pretreatment, laccase, crude extracellular enzyme extract.

Resumen

La fibra de cáscara de coco (bonote) es un residuo lignocelulósico abundante cuya valorización está limitada por la alta recalcitrancia de su matriz rica en lignina. En este estudio se evaluó la modificación biológica de fibra de cáscara de coco molida utilizando *Trametes hirsuta* Bm-2 bajo fermentación en estado sólido (SSF) y fermentación sumergida (SmF) durante 28 días, y se comparó con pretratamientos enzimáticos asistidos por ultrasonido de corta duración (1 h), empleando ya sea un extracto enzimático extracelular crudo de *T. hirsuta* Bm-2 o una lacasa comercial. La materia prima mostró un perfil lignocelulósico con alto contenido de lignina (36.97%), junto con celulosa (33.31%) y hemicelulosa (11.36%). El pretratamiento biológico logró la mayor remoción de lignina, alcanzando 37.77 ± 0.07% de deslignificación en SSF y 43.44 ± 0.10% en SmF después de 28 días, lo que demuestra la capacidad de *T. hirsuta* Bm-2 para modificar un sustrato altamente recalcitrante bajo condiciones biológicas suaves. La fibra de coco también favoreció una alta producción extracelular de lacasa, particularmente bajo SmF, donde la actividad alcanzó su máximo al día 7, resaltando su potencial como sustrato de bajo costo para la obtención de enzimas de interés industrial. Sin embargo, la sacarificación no aumentó proporcionalmente con la remoción de lignina. Los sólidos pretratados por SmF mostraron un óptimo temporal en la liberación de azúcares al día 7 (9.57 ± 0.15 g L^{-1}), seguido de una disminución marcada a tiempos de incubación más largos, a pesar de una mayor reducción de lignina. Este desacoplamiento sugiere que la remoción de lignina por sí sola fue insuficiente para asegurar una hidrólisis eficiente y puede estar asociada con una deconstrucción parcial de la matriz lignocelulósica, acumulación de biomasa fúngica, posible consumo de fracciones carbohidratadas accesibles y compuestos inhibidores residuales. El ultrasonido por sí solo produjo 15.25 ± 0.12% de deslignificación, mientras que el ultrasonido combinado con extracto crudo o con lacasa comercial incrementó la deslignificación a 20.48 ± 0.05% y 20.77 ± 0.05%, respectivamente, pero no mejoró la liberación de azúcares bajo las condiciones evaluadas. En conjunto, *T. hirsuta* Bm-2

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permitió la valorización tecnológica de la fibra de coco, con miras a su potencial uso en la modificación de lignina y la producción de lacasa que para la sacarificación.

Palabras clave: fibra de cáscara de coco (bonote), *Trametes hirsuta* Bm-2, deslignificación, pretratamiento asistido por ultrasonido, lacasa, extracto crudo de enzimas extracelulares.

1 Introduction

Coconut (*Cocos nucifera* L.) is a major agricultural crop mainly used by the coconut water and oil industries, with a global products market projected to grow from USD 11.5 billion in 2018 to USD 31.1 billion by 2026 (Johnson *et al*., 2024; Tripathi *et al*., 2024). In addition to food uses, coconut serves as a versatile source of raw materials for industries ranging from automotive and beverages to biofuels, confectionery, cosmetics, housing, oleochemicals, and pharmaceuticals (Johnson *et al*., 2024). Large volumes of coconut husk are produced annually in coastal regions, where inadequate disposal can cause persistent solid-waste accumulation because their high lignin content slows natural biodegradation (de Almeida & de Albuquerque, 2025; Kaur *et al*., 2025; Nissar *et al*., 2025). This accumulation can affect coastal communities by reducing environmental quality in tourist areas, increasing sanitation and public-health burdens, and impacting soil and groundwater resources (Koliotasi *et al*., 2023). When coconut husk-derived residues, including coir, are stockpiled and exposed to rainfall, tannins and other phenolic compounds can leach into soil and water bodies; this is particularly relevant in vulnerable settings such as the Yucatan Peninsula, where a highly connected karst aquifer can facilitate rapid contaminant transport from the surface to groundwater and coastal zones (Narendar & Priya Dasan, 2014; Neena *et al*., 2007; Arcega-Cabrera *et al*., 2021). In addition, open burning of coconut residues can release fine particulate matter (PM2.5) and other air pollutants, degrading air quality and increasing respiratory and cardiovascular health risks (Obeng *et al*., 2020; Pinakana *et al*., 2024; Sangkham *et al*., 2024; Weichenthal *et al*., 2017). This creates an urgent need to implement appropriate disposal practices for coconut residues and to develop processes that facilitate their degradation and valorization, thereby mitigating the environmental and public-health challenges associated with their accumulation.

In Mexico, the world's eighth-largest coconut producer (~1.14 million tonnes in 2023), coconut production contributes to a substantial lignocellulosic residue stream; globally, coconut husk represents approximately 12–15% of fruit weight, with coir fiber accounting for about 75% of the husk fraction (de Almeida et al., 2026; de Almeida & de Albuquerque, 2025). Yucatán contributes approximately 5% of national fresh coconut production and ranks sixth in the country; in 2019, state production reached 10,172.3 t, with nearly 86% concentrated in Litoral Centro and Sur, while production increased by 25.32% between 2018 and 2024, from 10,042.17 to 12,585.29 t (Ramos Clamont Montfort *et al*., 2021; SIAP, 2026). Despite this productive base, the local coconut value chain remains weakly integrated. Ramos Clamont Montfort *et al*. (2021) reported that only 11.5% of surveyed producers were involved in coconut-derived products, mainly coconut water and traditional sweets, whereas 74.2% did not track the final destination of fruits sold through intermediaries. Residue management is also poorly documented: only 16.9% of producers reported removing coir from the fruit, frequently accumulating it in storage sheds, while 60% were unaware of current uses of coir, such as substrates, pots, or ropes. Moreover, there are no local records on the amount of peeled coconut sold or coir generated in the region.

In the Yucatan Peninsula, coconut-derived products such as coconut water, virgin coconut oil, and coconut sap-based beverages have shown market potential; however, local processing and value addition remain limited, reinforcing the relevance of exploring feasible uses for underutilized fractions such as coir (Uzcanga Pérez *et al*., 2015; Ramos Clamont Montfort *et al*., 2021). Although coconut fiber has been identified in Yucatan as a locally available material for agricultural substrates, its direct use may require prior conditioning due to physicochemical constraints such as soluble salts (Gayosso Rodríguez et al., 2016; Gayosso-Rodríguez *et al*., 2018). Thus, coconut coir is not only an underdocumented residue stream, but also a locally available lignocellulosic material whose effective use depends on appropriate processing. Beyond agricultural conditioning, biological modification with regional ligninolytic fungi such as *Trametes hirsuta* Bm-2 represents a complementary valorization route for lignin-rich substrates (Tapia-Tussell *et al*., 2011; Tapia-Tussell *et al*., 2015; Hernández-Calderón *et al*., 2023).

The valorization of coconut residues can enable not only the production of fermentable sugars for second-generation biofuels but also the recovery of lignin-derived streams and phenolic compounds for higher-value applications, as well as enzymes with commercial and industrial applications, such as laccases, supporting a more circular use of all lignocellulosic fractions (de Almeida & Albuquerque, 2025; Vieira *et al*., 2024; Vasina *et al*., 2016). However, coconut husk is intrinsically recalcitrant because the lignin–carbohydrate matrix restricts enzyme access to cellulose, making pretreatment a prerequisite for efficient saccharification or methanization (de Araujo *et al*., 2025; Gómora-Hernández *et al*., 2025; Vieira *et al*., 2024). For this reason, pretreatment technologies are commonly reported to modify or partially remove lignin/hemicellulose and increase cellulose accessibility (de Almeida & Albuquerque, 2025; de Araujo *et al*., 2025). While chemical and

physicochemical routes (e.g., alkaline delignification, oxidative bleaching, steam explosion, and sequential combinations) can achieve strong structural disruption and substantial fractionation, they often entail higher chemical/energy inputs and may require downstream conditioning or washing depending on process severity (de Almeida & Albuquerque, 2025; Din *et al*., 2020; de Araujo *et al*., 2025). Conversely, biological pretreatments based on ligninolytic fungi operate under mild conditions and reduced chemical demand, but typically require longer residence times to achieve measurable delignification (Murtius et al., 2025; de Almeida & Albuquerque, 2025). Importantly, integrating complementary pretreatments can also be leveraged to co-produce phenolic fractions while improving matrix accessibility (González-Muñoz *et al*., 2026), reinforcing the value of hybrid strategies in circular production schemes. Within this context, white-rot fungi, particularly species of the genus *Trametes*, have shown strong potential for selective lignin degradation due to their production of oxidative enzymes such as laccases, manganese peroxidases, and lignin peroxidases (Vasina *et al*., 2016). *Trametes hirsuta* Bm-2 has been recognized as a high laccase producer capable of acting on a wide variety of phenolic substrates, making it an attractive candidate for lignin removal from recalcitrant biomass and as a potential biofactory for enzymes and phenolic compounds (González-Muñoz *et al*., 2026; Hernández-Calderón et al., 2023).

The present study addresses the limited information on *Trametes hirsuta* Bm-2-mediated biological modification of coconut coir and its trade-offs among delignification, laccase production, phenolic release, and saccharification. Specifically, this work evaluates the performance of *T. hirsuta* Bm-2 under solid-state and submerged fermentation systems for lignin removal and extracellular laccase production, and compares these biological pretreatments with short ultrasound-assisted enzymatic treatments. Rather than focusing solely on sugar release, the results integrate lignin removal, soluble phenolic release, laccase activity, and reducing sugar production to identify process-specific responses and support realistic valorization pathways for coconut coir as a tropical lignocellulosic residue.

2 Materials and methods

2.1 Raw material and characterization

Coconut coir (*Cocos nucifera* L.), corresponding to the fibrous fraction obtained from the coconut husk, was collected as an agroindustrial residue from a coconut oil processing facility in Bacalar, Quintana Roo, Mexico. Coconut coir was milled using a Pagani DYCOMET mill (Mexico) equipped with a 1 mm screen, sieved to obtain particles of 300–500 μm, washed with distilled water to remove soluble impurities, and oven-dried at 80 °C for five days. The chemical composition (cellulose, hemicellulose, and lignin contents) was determined according to the National Renewable Energy Laboratory (NREL) protocol for structural carbohydrates and lignin in biomass (Sluiter *et al*., 2008).

2.2 Microorganism and inoculum preparation

The white-rot fungus *Trametes hirsuta* Bm-2 (GenBank accession GQ280373), isolated from decaying wood in Yucatan, Mexico (Tapia-Tussell *et al*., 2011), was employed for biological pretreatments. The strain was maintained on malt extract agar (2 % w/v) at 35 °C. For inoculum preparation, 1 cm^2 mycelial plugs from 4-day-old cultures were transferred into 250 mL Erlenmeyer flasks containing 50 mL of liquid YMPG medium (10 g L^{-1} glucose, 10 g L^{-1} malt extract, 2 g L^{-1} peptone, 2 g L^{-1} yeast extract, 2 g L^{-1} KH_2PO_4, 1 g L^{-1} $MgSO_4{\cdot}7H_2O$, and 1 mg L^{-1} thiamine-HCl; pH 4.5) as described by Ancona-Escalante *et al*., (2018). Cultures were incubated at 35 °C and 150 rpm for 4 days, and homogenized using an Ultra-Turrax homogenizer (IKA T18 Digital, Germany).

2.3 Biological pretreatments

Two biological pretreatments were evaluated: solid-state fermentation (SSF) and submerged fermentation (SmF), adapted from Sun *et al*. (2011). For SSF, 5 g of coconut coir were placed in 250 mL Erlenmeyer flasks containing 27 mL of Rodríguez-Couto medium (2 g L^{-1} glucose, 15 g L^{-1} malt extract, 0.9 g L^{-1} $(NH_4)_2SO_4$, 2 g L^{-1} KH_2PO_4, 0.5 g L^{-1} $MgSO_4{\cdot}7H_2O$, 0.1 g L^{-1} $CaCl_2{\cdot}2H_2O$, and 0.5 mL L^{-1} thiamine-HCl; pH 5). Flasks were sterilized at 121 °C and 1.2 atm for 20 minutes to reduce the microbial load, inoculated with 5 mL of homogenized *T. hirsuta* Bm-2 inoculum, and incubated statically at 35 °C for 7, 14, 21, and 28 days. For SmF, 5 g of fiber were mixed with 83 mL of Rodríguez-Couto medium, sterilized under the same conditions, inoculated with 5 mL of *T. hirsuta* Bm-2 inoculum, and incubated under agitation (150 rpm, 35 °C) for the same periods. After incubation, samples were filtered to separate solid and liquid fractions. The liquid phase was centrifuged and stored at 4 °C, while the solid fraction was dried at 80 °C for 72 h for subsequent analysis.

2.4 Combined physical and biological pretreatments

An ultrasound pretreatment was performed by suspending 5 % (w/v) of fiber in 0.1 M sodium acetate buffer (pH 4.8). The suspension was sealed in double hermetic plastic bags (Ziploc, Mexico) and placed in a one-gallon stainless-steel ultrasonic bath (Cole-Parmer, model 08895-14, USA). Sonication was carried out at 42 kHz, 100 W, and 45 °C for 1 h without mechanical agitation, according to Nagula and Pandit (2016). After treatment, the suspension was filtered to separate the liquid and solid fractions. Additionally, ultrasound-assisted enzymatic pretreatments were performed by combining ultrasound with either (i) a crude enzyme extract of *T. hirsuta* Bm-2 or (ii) a commercial laccase from *Aspergillus* sp. (Sigma-Aldrich, USA), both at an equivalent activity of 1,470 U g^{-1} of substrate.

The crude enzyme extract was produced by cultivating *T. hirsuta* Bm-2 in 60 mM phosphate buffer (pH 6.0) supplemented with 3 % (w/v) wheat bran, as described by Ancona-Escalante *et al*. (2018). After 7 days of incubation at 35 °C, the culture broth was filtered and centrifuged (3,500 rpm, 15 min, 10 °C), and the supernatant was used as the crude extracellular enzyme extract.

2.5 Laccase activity assay

Laccase activity was measured using ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)) as substrate, defining one unit (U) as the amount of enzyme that oxidizes 1 μmol min^{-1} of ABTS (ε_{420} = 36,000 L moL^{-1} cm^{-1}) (Tapia-Tussell *et al*., 2015).

2.6 Lignin and total phenolic content

Phenolics were quantified according to Box (1983). Briefly, 50 μL of extract were mixed with 1.55 mL distilled water and 100 μL Folin–Ciocalteu reagent. After 5 min in the dark, 300 μL of 7.5% (w/v) Na_2CO_3 were added. The mixture was incubated at 40 °C for 30 min, and absorbance was measured at 760 nm (Varian Cary 3 UV–Vis). Results were expressed as mg gallic acid equivalents (GAE) g^{-1} of substrate. The acid-insoluble lignin (Klason lignin) content was determined following the NREL protocol (Sluiter *et al*., 2008), and the percentage of delignification was calculated relative to untreated fiber.

2.7 Enzymatic hydrolysis

Enzymatic saccharification was performed using Cellic CTec3 (Novozymes, Denmark) at an enzyme loading of 10 FPU g^{-1} substrate in 50 mM sodium citrate buffer (pH 4.8). Reactions containing 1 g of pretreated or untreated fiber in 25 mL buffer were incubated at 50 °C and 150 rpm for up to 72 h. Samples were withdrawn at defined intervals (0–72 h) and analyzed for reducing sugars by the DNS method using a glucose standard (Miller, 1959).

2.8 Statistical analysis

All experiments were performed in triplicate. Statistical analyses were carried out using one-way analysis of variance (ANOVA) to evaluate the effect of pretreatments. When differences were significant, means were compared using Tukey's honestly significant difference post hoc test. Statistical significance was set at $p < 0.05$.

In addition, an exploratory correlation analysis was performed to examine the relationships among biological pretreatment responses and saccharification performance. Pearson correlation coefficients (r) were calculated using treatment mean values for SSF and SmF (n = 8), considering reducing sugar release as the main hydrolysis response and delignification, Klason lignin, solid recovery, mycelial biomass, phenolic release, laccase activity, and incubation time as explanatory process variables. Day 0 was excluded from this analysis to focus on the active biological pretreatment period.

3 Results and discussion

3.1 Raw material characterization

The chemical composition of the coconut coir was first determined to establish the baseline characteristics of the raw material. On a dry-weight basis, the fiber contained 36.97% lignin, 33.31% cellulose, and 11.36% hemicellulose. These values fall within the ranges reported for coconut coir in recent studies, with lignin, cellulose, and hemicellulose spanning about 27.4–43.31%, 25.6–40.0%, and 10.5–23.5%, respectively (Nogueira *et al*., 2019; Din *et al*., 2020; Nogueira *et al*., 2021; Rezeki *et al*., 2023; de Brito *et al*., 2025; Ribeiro *et al*., 2024; de Araujo *et al*., 2025). The lignin content was toward the upper range, which is consistent with the durability and recalcitrant behavior commonly associated with coconut coir and reinforces the need for pretreatment prior to efficient saccharification (Carrijo *et al*., 2002; Albuquerque *et al*., 2016). Variability in reported coconut compositions may be related to differences in fruit maturity, end-use, and geographic and climatic conditions that influence the raw material (Brígida *et al*., 2010). The Klason lignin content observed here (~37%) is comparatively high relative to several sustainable SSF substrates reported in the literature, which often show lignin contents in the ~7.5–25.1% range (e.g., wheat/rice straw, brewers' spent grains, tea

residues, and sorghum bagasse), supporting the view of coconut coir as a more lignin-rich and potentially more recalcitrant feedstock (Loi *et al*., 2021).

3.2 *Delignification of coconut coir by Trametes hirsuta Bm-2*

Given the high lignin content of the raw material (~37% Klason lignin), pretreatment is required to reduce matrix recalcitrance and to enable downstream valorization routes. Therefore, delignification was evaluated using *T. hirsuta* Bm-2 under SSF and SmF and compared with short ultrasound-assisted enzymatic pretreatments.

3.2.1 *Delignification under SSF and SmF (0–28 d)*

Biological pretreatment with *Trametes hirsuta* Bm-2 induced measurable changes in the lignin fraction of coconut coir under both solid-state fermentation (SSF) and submerged fermentation (SmF). The effect of each system was examined through the time-course of delignification and solid recovery throughout the 28-day period (Figure 1). Both SSF and SmF showed a rapid early decrease in Klason lignin, which dropped from 36.87 ± 0.1%^{a} at day 0 to 25.46 ± 0.2%^{b} and 25.86 ± 0.06%^{b} at day 7, corresponding to 30.96 ± 0.2%^{b} and 29.86 ± 0.06%^{b} delignification in SSF and SmF, respectively. The highest delignification percentage was observed at day 28, reaching 37.77 ± 0.07%^{d} in SSF (Klason lignin 22.95 ± 0.07%^{f}) and 43.44 ± 0.1%^{d} in SmF (Klason lignin 20.85 ± 0.1%^{g}). Mycelial growth increased throughout biological pretreatment, confirming active colonization of coconut coir by *T. hirsuta* Bm-2. Mycelial biomass in SSF increased to 0.45 ± 0.02 g^{b}, 0.91 ± 0.03 g^{c}, and 1.36 ± 0.05 g^{e} at days 7, 14, and 21, respectively; in SmF, it increased to 0.39 ± 0.02 g^{b}, 0.74 ± 0.03 g^{d}, and 1.12 ± 0.04 g^{f} over the same period. This increase paralleled the decrease in solid recovery and suggests that extended incubation promoted not only lignin modification but also fungal biomass accumulation and possible consumption of accessible organic fractions, including carbohydrate-rich fractions that could be degraded by enzymes involved in cellulose and hemicellulose turnover, such as cellulases and xylanases (Vasina *et al*., 2016; Andriani *et al*., 2020). Andriani *et al*. (2020) demonstrated that *T. hirsuta* AA-017 produces laccase, manganese peroxidase, and lignin peroxidase from 13 varieties of sorghum straw, with maximum activity around day 8. In this work, the largest decrease in Klason lignin occurred during the first 7 days of incubation. At day 28, mycelial biomass and solid recovery were not determined because separation of fungal biomass from the residual substrate was no longer technically feasible.

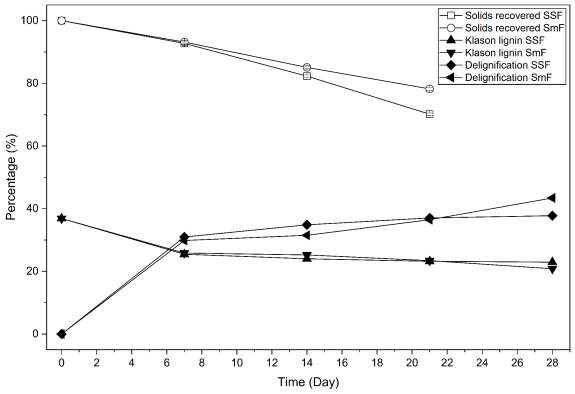

Figure 1. Solid recovery (%), Klason lignin (%), and delignification (%) of coconut coir after 28 days of biological pretreatment with *Trametes hirsuta* Bm-2 under solid-state fermentation (SSF) and submerged fermentation (SmF). Values are reported as mean ± SD (n = 3).

To contextualize the magnitude of lignin removal achieved here, the resulting Klason lignin values and delignification levels were compared with those reported for coconut coir under physicochemical and biological pretreatment strategies, showing that values before and after biological pretreatment were within the range reported for other pretreatment approaches.

For instance, Nogueira *et al*. (2019) reduced lignin from 32.22 ± 3.39% to 20.33 ± 2.84% and 24.02 ± 0.74% using an alkaline NaOH pretreatment (140 atm, 70 °C, 2.5 h) applied alone or followed by ethanol extraction, achieving a maximum delignification of 54%, which is slightly higher than the values obtained here. In that study, solids recovery was 72%, whereas in the present work it reached 70.16 ± 0.41% and 78.24 ± 0.49% under SSF and SmF, respectively. Similar trends were reported by de Brito *et al*. (2025) and de Araujo *et al*. (2025), who decreased lignin from 36.05% to 30.33 ± 0.14% after steam explosion (20 atm, 210 °C, 10 min) and to 27.07 ± 0.70% after sequential steam explosion and alkaline pretreatment (NaOH 4%, 2 atm, 121 °C, 30 min), corresponding to delignification levels of approximately 16% and 25%, respectively, with 44% solids recovery (de Araujo *et al*., 2025). Ribeiro *et al*. (2024) reported comparable lignin reductions, from 30.64 ± 1.05% to 21.21 ± 0.53% after steam explosion combined with NaOH 2% (13 atm, 197 °C, 10 min), although with solids recoveries below 30%. Other studies have reported greater lignin reductions and delignification levels, such as Ebrahimi *et al*. (2018), who decreased lignin to 21.36% and 19.48% and reported removals of 58.35% and 64.14% after pretreatment with 20% and 25% ammonium carbonate (1 atm, 80 °C, 12 h), respectively (solids recoveries of 70% and 66%). Overall, the performance of *T. hirsuta* Bm-2 in reducing lignin content was comparable to that achieved by pressure-assisted

alkaline treatments; however, the residence time required in the biological approach (7–28 d) was substantially longer than the minutes-to-hours range typically reported for alkaline pretreatments (de Araujo *et al*., 2025; de Brito *et al*., 2025; Ribeiro *et al*., 2024; Nogueira *et al*., 2019). Nevertheless, biological delignification offers potential advantages in terms of lower energy requirements and reduced generation of spent chemical/solvent streams compared with physicochemical and chemical routes, supporting its potential as a more environmentally friendly alternative (Loi *et al*., 2021).

On the other hand, the maximum delignification percentage obtained here under SmF was similar to those reported using *Stenotrophomonas* sp. CFB-09 on coconut husk (42.95% and 41.18%) and higher than those reported for both configurations evaluated here when compared with *Paenibacillus* sp., *Aneurinibacillus aneurinilyticus*, and *Bacillus* sp., which yielded 37%, 33%, and 30% delignification, respectively (Olajuyigbe *et al*., 2018).

3.2.2 *Delignification under ultrasound-assisted enzymatic pretreatments (1 h)*

While SSF/SmF achieved competitive delignification performances, their residence times are inherently longer than those required for physicochemical pretreatments. To explore a rapid alternative and assess whether short physical intensification could partially replicate lignin removal, ultrasound-assisted pretreatments (1 h) were evaluated with and without laccase-containing systems. The addition of laccase-containing systems to ultrasound further reduced Klason lignin relative to ultrasound alone and modified the concentration of soluble phenolics in the liquid fraction (Figure 2). The untreated fiber contained 36.87 ± 0.1%^{a} Klason lignin, whereas ultrasound alone decreased lignin to 31.25 ± 0.12%^{b}, corresponding to 15.25 ± 0.12%^{b} delignification. When ultrasound was combined with laccase-based systems, the lowest lignin contents were obtained, with 29.21 ± 0.05%^{c} for the ultrasound plus commercial laccase treatment and 29.32±0.05%^{c} for the ultrasound and *T. hirsuta* Bm-2 crude extract treatment, yielding delignification values of 20.77 ± 0.05%^{c} and 20.48 ± 0.05%^{d}, respectively. This indicates that ultrasound alone produced measurable structural disruption, while the addition of oxidative enzyme systems provided a modest but consistent gain in lignin removal, which is consistent with the role of laccase in promoting lignin oxidative modification beyond purely physical effects (González-Muñoz *et al*., 2026). Although delignification was lower than that achieved in the fermentations described in the previous section, it is noteworthy that the delignification levels obtained here were comparable to those reported for coconut coir pretreated with

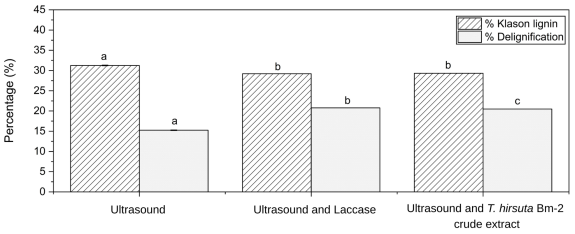

Figure 2. Effect of ultrasound-assisted pretreatments on coconut coir lignin modification. Klason lignin content and delignification percentage after ultrasound alone, ultrasound combined with commercial laccase, and ultrasound combined with *T. hirsuta* Bm-2 crude extract. Values represent mean ± standard deviation. Different lowercase letters indicate significant differences among treatments according to Tukey's test ($p < 0.05$).

pressure-assisted alkaline treatments (as described in section 3.2), within 1 h, which is substantially shorter than the 7 days required to observe measurable delignification under SSF and SmF. Delignification values found here were superior to those achieved by SSF with *T. hirsuta* Bm-2 (17.48 ± 0.45% at 27 °C and 15.51 ± 0.15 % at 35 °C) using pineapple leaf waste as substrate (Chablé-Villacis *et al*., 2023).

3.2.3 *Structural evidence by SEM and FTIR*

SEM micrographs were used to qualitatively assess surface modifications of coconut coir following pretreatment with *T. hirsuta* Bm-2. The untreated fiber exhibited a comparatively smooth and compact outer surface (Figure 3). After 7 d of submerged fermentation, the surface appeared rougher and showed clearer signs of erosion than the solid-state condition at the same incubation time, which was consistent with the higher laccase activity measured in SmF at day 7 (79,417.18 ± 1,727.26^{c} U g^{-1}) relative to SSF (7,577.46 ± 64.94^{b} U g^{-1}). Pore-like features were also observed on the pretreated fibers, similar to those previously reported after aqueous glycerol treatment (Ebrahimi *et al*., 2017) and H_2O_2-based chemical treatments (Brígida *et al*., 2010). These observations confirm that fermentation with *T. hirsuta* Bm-2 alters the structure of the lignocellulosic substrate, with the effects being more evident under SmF.

Consistent with these surface-level alterations, FTIR spectroscopy was used to probe whether lignin and hemicellulose associated functional groups showed attenuation or redistribution relative to the untreated control (Figure 4). A broad band around 3340 cm^{-1} was observed which is mainly attributed to O–H stretching associated with hydrogen bonding in polymeric structures (Ding *et al*., 2020). Bands at 2942 and 2896 cm^{-1} correspond to asymmetric and symmetric C–H stretching of

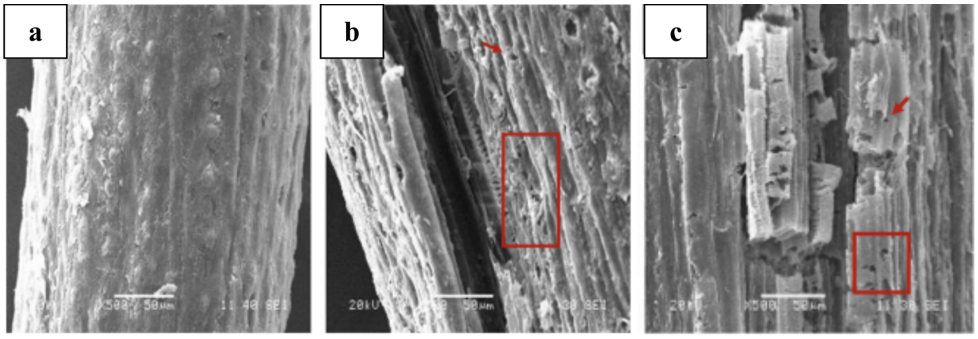

Figure 3. SEM micrographs of coconut coir: (a) untreated fiber, (b) fiber after 7 days of solid-state fermentation (SSF), and (c) fiber after 7 days of submerged fermentation (SmF) with *Trametes hirsuta* Bm-2.

aliphatic chains commonly present in lignocellulosic components (cellulose, hemicellulose, and lignin). A peak at 1728 cm^{-1} was assigned to C=O stretching (esters/carboxylic acids), which is typically linked to acetyl groups in hemicellulose and carbonyl functionalities in lignin (Maceda-Rodriguez *et al*., 2020). Signals at 1600 and 1509 cm^{-1} were associated with aromatic skeletal vibrations (C=C) of lignin (Apadyn-Varol and Mutlu, 2023), while the band at 1331 cm^{-1} has been related to C–H deformation and/or C–O stretching in syringyl units and/or cellulose (Gonçalves *et al*., 2016). Bands at 1238, 1165, and 995 cm^{-1} correspond to C–O–C and C–O stretching vibrations typical of polysaccharides and ether linkages in hemicellulose/lignin (Contreras, 2010), and the band at 895 cm^{-1} is attributed to the β-(1→4) glycosidic linkage characteristic of cellulose (Kunusa *et al*., 2020). As shown in Figure 4, both solid-state and submerged fermentation induce changes in the intensity of several FTIR bands, although differences between these two biological pretreatments were subtle. Overall, the spectra indicate partial deconstruction of the lignocellulosic matrix, evidenced by reduced signals associated with carbonyl groups (1728 cm^{-1}) and aromatic lignin structures (1509–1600 cm^{-1}), along with attenuation of the O–H band (~3340 cm^{-1}). These shifts are consistent with partial hemicellulose removal, partial lignin modification, and alterations in the hydrogen-bond network relative to the untreated control.

After documenting the structural effects of fungal pretreatment, the same microscopic approach was applied to ultrasound-treated fibers to determine whether cavitation-driven disruption produced comparable surface features. SEM micrographs confirmed that ultrasound pretreatments disrupted the coconut fiber surface relative to the untreated fiber (Figure 5), producing fractures and surface tearing consistent with cavitation. In addition to roughening comparable to that observed after fungal pretreatment, ultrasound yielded distinctive ring-like detachments and rolling/coil-like deformations that have been reported during cavitation-driven sonication

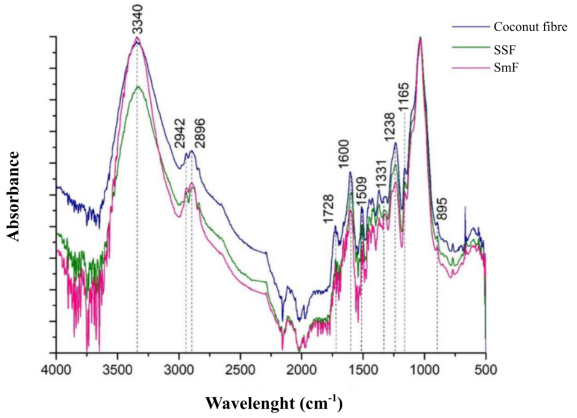

Figure 4. FTIR spectra of coconut coir. Untreated fiber and fiber after 7 days of biological pretreatment with *Trametes hirsuta* Bm-2 under solid-state fermentation (SSF) and submerged fermentation (SmF).

of cellulose fibers, where fibers can undergo bending and localized breakage detectable by SEM (Redlinger-Pohn *et al*., 2022). Pore-like features were less evident than those observed in the biologically pretreated fibers, and localized brightened areas, often associated with laccase-driven oxidative bleaching of lignin (Piscitelli *et al*., 2011), were not clearly distinguished under the evaluated conditions.

FTIR analysis confirmed the presence of the main functional groups typical of lignocellulosic materials and enabled evaluation of structural changes induced by the applied physical and enzymatic pretreatments (Figure 6). All spectra showed bands associated with cellulose, hemicellulose, and lignin, with variations in band intensity attributable to the treatments. The broad band at ~3340 cm^{-1} (O–H stretching) reflects hydroxyl groups from polysaccharides and phenolic structures as well as hydrogen-bonded water. Samples treated with ultrasound showed a change in the intensity of this band relative to the untreated fiber, suggesting alterations in the hydrogen-bond network, likely due to partial matrix disruption by cavitation and increased exposure of hydroxyl groups (Fernandes *et al*., 2023).

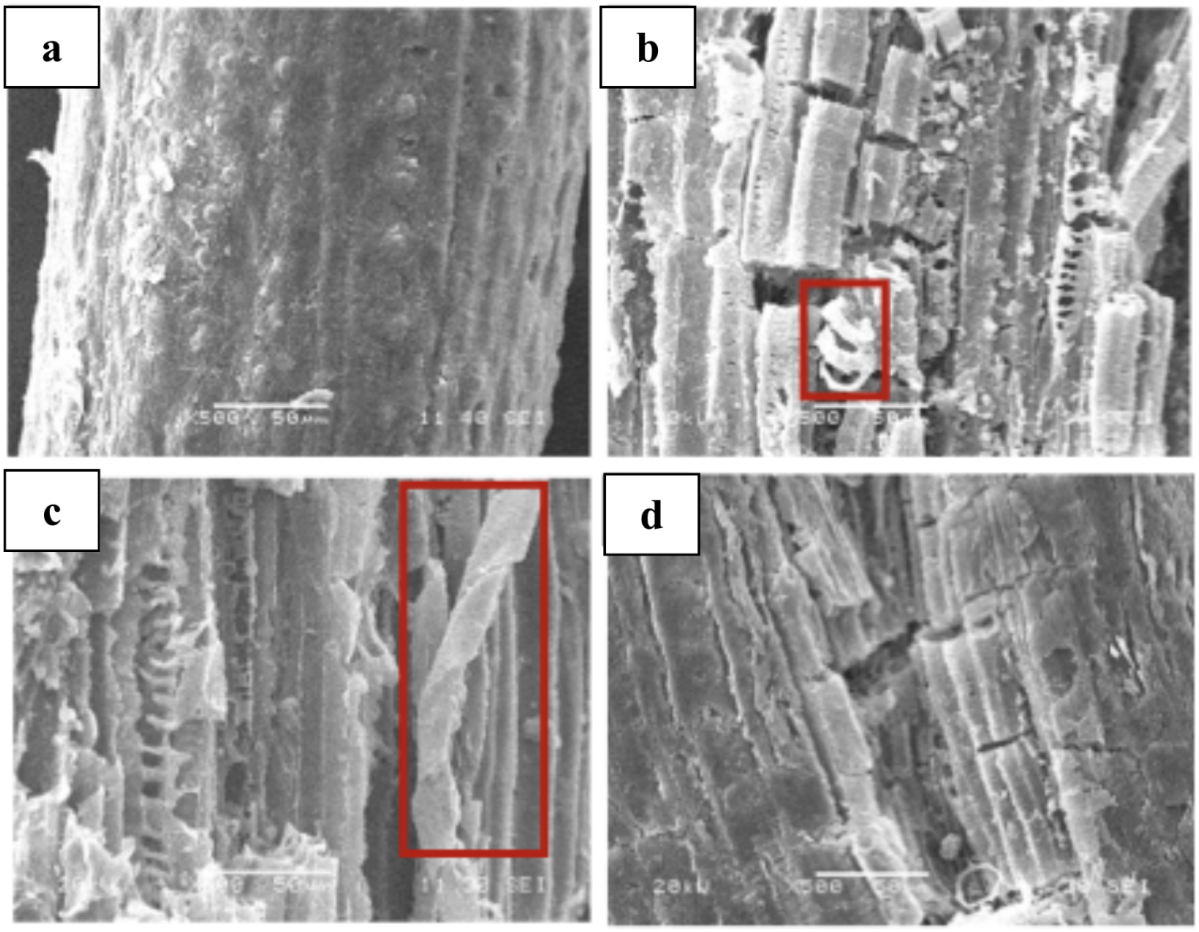

Figure 5. SEM micrographs of coconut coir: (a) untreated fiber, (b) ultrasound-treated fiber, (c) ultrasound treatment with *Trametes hirsuta* Bm-2 enzyme extract, and (d) ultrasound treatment with commercial laccase (*Aspergillus spp*.).

This behavior was not observed when ultrasound was combined with enzyme preparations (crude extract or commercial laccase). Bands at 2942–2900 cm^{-1} (C–H stretching of methyl/methylene groups) displayed intensity changes in treated samples, indicating modifications in the chemical environment of aliphatic chains within the structural polymers (Gonçalves *et al*., 2016). A decrease in the 1730–1728 cm^{-1} band (C=O stretching of ester/acetyl groups characteristic of hemicellulose) was observed after pretreatment, most clearly for ultrasound alone, suggesting partial hemicellulose removal and/or deacetylation (Gonçalves *et al*., 2014). Likewise, reduced intensity at 1600 and 1509 cm^{-1} (aromatic skeletal vibrations) indicated partial modification or redistribution of lignin in treated samples (Gonçalves *et al*., 2016). Changes in the 1331–1165 cm^{-1} region (C–O and C–O–C vibrations) and at ~895 cm^{-1} (β-glycosidic linkages associated with amorphous cellulose) further supported treatment-induced rearrangements in polysaccharide organization. Overall, the FTIR results indicate that the pretreatments caused no drastic changes in chemical identity but produced subtle yet consistent shifts in key bands, consistent with partial deconstruction of the lignocellulosic matrix and possible improvement in carbohydrate accessibility.

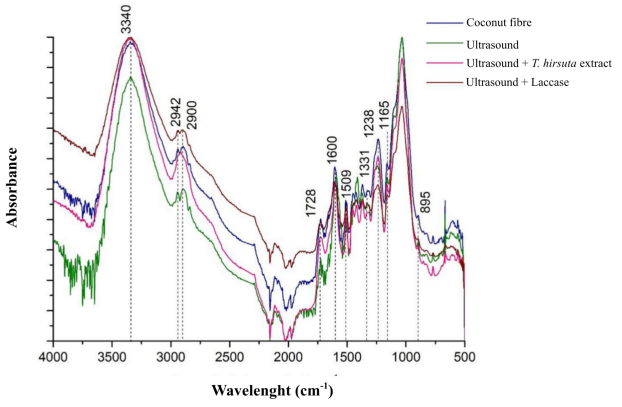

Figure 6. FTIR spectra of coconut coir: untreated fiber and fibers after ultrasound pretreatment alone, ultrasound plus *Trametes hirsuta* Bm-2 enzyme extract, and ultrasound plus commercial laccase.

3.3 Laccase activity, phenolic release, and saccharification response

Beyond lignin removal, *T. hirsuta* pretreatments can generate value streams such as extracellular laccase and soluble phenolics, which may also influence downstream hydrolysis through inhibition or non-productive interactions. Thus, laccase activity and phenolic release were analyzed together with saccharification performance.

3.3.1 Laccase and phenolic profiles in SSF and SmF

Phenolic release and laccase activity followed distinct profiles for SSF and SmF (Figure 7). Laccase activity peaked at day 7, with 7,577±64.94^{b} U g^{-1} (1.18 $\times 10^{6}$ U L^{-1}) for SSF, and 79,417.18±1727.26^{c} U g^{-1} (4.51 $\times 10^{6}$ U L^{-1}) for SmF, which was more than ten-fold higher than in SSF with no addition of inducers. The laccase activities obtained using coconut coir place our system toward the upper end of values reported for sustainable substrates under SSF, which span from ~31 to 579 U g^{-1} for several residues (e.g., tea residues, olive leaves/wheat straw, and cereal/grass by-products), include intermediate cases around ~2,600 U g^{-1}, and only occasionally reach much higher levels such as ~13,506 U g^{-1} (with phenols as inducers) or ~89,800 U g^{-1} reported for *Tricholoma giganteum*, which was similar to the activity found for SmF at day 7 (Loi *et al*., 2021); this is noteworthy because coconut coir, despite its high lignin content, appears to be a suitable substrate for SSF and SmF laccase production with *T. hirsuta* for different applications while achieving competitive delignification performances (Figure 1). ĆilerdžiĆ *et al*. (2011) reported that *T. hirsuta* BEOFB 30 produced ligninolytic enzymes under SSF using agricultural residues, with maximum activities of 3,827 ± 219 U L^{-1} for laccase on mandarin orange peels, 1,971.5 ± 23.0 U L^{-1} for MnP on wheat straw, and 1,173 ± 100 U L^{-1} for versatile peroxidase on glucose-enriched mandarin orange peels. Andriani *et al*. (2020) reported that SmF by *T. hirsuta* AA-017 grown on Indonesian sorghum biomass produced higher laccase and manganese peroxidase activities in accessions with high (19.7–22.9%) and moderate (17.9–18.4%) lignin contents, with total lignin showing a positive correlation with Lac and MnP production. In their optimized system (addition of mineral inducers), maximum activities reached 25,700 U L^{-1} for Lac, 46,700 U L^{-1} for MnP, and 91 U L^{-1} for LiP, with peak production at approximately days 8, 5, and 8, respectively. Interestingly, Hernández-Calderón *et al*. (2023) found a linear increase in laccase activity from day 3, with a maximum activity at day 8, reaching 9,000 U mL^{-1} when *T. hirsuta* Bm-2 was cultured using wheat bran 3% w/v (35 °C, 150 rpm). Notably, laccase peak activity in this work was achieved within

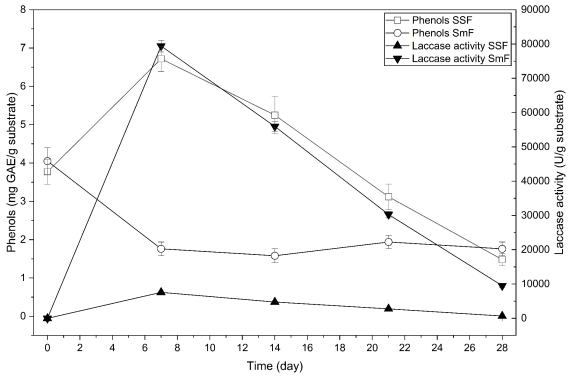

Figure 7. Total phenolic content (mg gallic acid equivalents, GAE) and laccase activity (U g^{-1} substrate) during 28 days of biological pretreatment of coconut coir with Trametes hirsuta Bm-2 under solid-state fermentation (SSF) and submerged fermentation (SmF). Values are reported as mean ± SD (n = 3).

7 days, which is similar to the results reported by Andriani *et al*. (2020) and Hernández-Calderón *et al*. (2023), but shorter than the incubation times commonly reported for different ligninolytic fungi (typically 14–35 days) (Loi *et al*., 2021). This is relevant because it highlights the potential of coconut coir as a low-cost and underutilized lignin-rich substrate for producing industrially relevant enzymes such as laccases, particularly through a mild biological route based on *T. hirsuta* Bm-2. Laccase production showed a better performance in SmF configuration, suggesting that those culture conditions were more suitable for laccase expression and activity in the presence of coconut coir. Consistently, the exploratory combined correlation analysis showed a positive association between laccase activity and reducing sugar release ($r = 0.884$, $p = 0.0036$), suggesting that the early high-laccase window coincided with better saccharification rather than with the later stage of maximum cumulative delignification.

In SSF, the early increase in phenols and the simultaneous peak in laccase activity at day 7 (Figure 7) suggested an initial oxidative modification of lignin that promotes the release of soluble phenolic compounds, as commonly observed across ligninolytic fungi/enzymes and diverse substrates. For example, *T. hirsuta* pretreatment of rice straw yielded a phenol concentration of 1.7 mg g^{-1} substrate (Saritha *et al*., 2012), and *Sargassum* spp. treated with a crude extract from the same fungus released 6.31 mg GAE eq g^{-1} substrate (González-Muñoz *et al*., 2026). The subsequent decline in phenols toward day 28 could reflect a shift in the balance between release and the downstream fate of these compounds, including fungal uptake/metabolism and oxidative transformation not only by laccases (Adelpour *et al*., 2025) but also by other enzymes present in *Trametes* spp. secretomes, such as manganese peroxidases and versatile peroxidases (Vasina *et al*., 2016). In

addition, laccase-mediated repolymerization reactions (Zhang *et al*., 2023; Mattinen *et al*., 2009) and biosorption onto the solid matrix and fungal biomass (Legorreta-Castañeda *et al*., 2020) could contribute to the observed decrease in phenolic concentration. Although mycelial biomass was lower in SmF than in SSF, this is not necessarily contradictory, because, under agitated SmF, the biomass formed pellets, which can increase effective surface area and potentially influence sorption processes (Legorreta-Castañeda *et al*., 2020).

In SmF, laccase activity was substantially higher than in SSF, while phenolic concentrations remained significantly low across the experiments in comparison with SSF (day 7 to 21), except at day 28, when no significant differences were found. This pattern could indicate that, under liquid cultivation, soluble phenolics did not accumulate because they are further oxidized or transformed as they were generated, approaching a quasi steady-state between production and consumption in early stages (Adelpour *et al*., 2025; Hernández-Calderón *et al*., 2023; Vasina *et al*., 2016; Mattinen *et al*., 2009). Consistent with this interpretation, the maximum enzymatic activity coincided with the highest delignification rate (between day 0 and day 7) without significant accumulation of phenolic compounds in SmF in comparison with SSF, suggesting that early-stage phenol oxidation/repolimerization was closely linked to the observed peak in laccase activity in SmF.

Differences between SSF and SmF configurations are also consistent with mode-dependent mass-transfer effects. In SSF, the lower water availability and smaller liquid volume per unit of solid may favor higher apparent accumulation of soluble phenolics in the aqueous phase, while also limiting diffusion and slowing their oxidative transformation by the extracellular oxidoreductase system of *T. hirsuta* Bm-2. In contrast, the higher water availability and larger liquid volume per unit of solids in SmF can dilute locally accumulated inhibitory compounds and facilitate diffusion of extracellular enzymes and reaction products (Chablé-Villacis *et al*., 2023; Si *et al*., 2021; Hernández-Calderón *et al*., 2023), thereby altering both enzyme expression/activity and the apparent phenolic profile present in the liquid fraction (Piscitelli *et al*., 2011; Hernández-Calderón *et al*., 2023). Agitation may also enhance oxygen diffusion and consequently laccase oxidative activity in SmF. Umar *et al*. (2023) showed that agitation strongly affected laccase production, dissolved oxygen, and mycelial morphology in SmF using *Ganoderma multistipitatum* and wheat bran as substrate; optimum production was found at 150 rpm, where laccase activity reached 19.44 $\times 10^5$ U L^{-1} and dissolved oxygen reached 65%, whereas lower agitation at 50 rpm produced 12.82 $\times 10^5$ U L^{-1} and higher

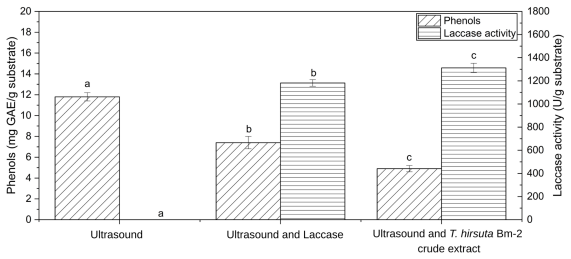

Figure 8. Effect of ultrasound-assisted pretreatments on coconut coir phenol release and laccase activity. Soluble phenolic content and residual laccase activity in the liquid fraction after ultrasound alone, ultrasound combined with commercial laccase, and ultrasound combined with *T. hirsuta* Bm-2 crude extract. Values represent mean ± standard deviation. Different lowercase letters indicate significant differences among treatments according to Tukey's test ($p < 0.05$).

agitation around 200 rpm damaged the mycelium, promoted autolysis, and reduced laccase production. Therefore, SmF likely favored extracellular laccase activity because agitation and higher water availability improved oxygen transfer, enzyme diffusion, and contact between soluble lignin-derived inducers and fungal biomass. From a valorization perspective, these contrasting dynamics suggest that the timing of liquid-phase recovery and the selected process configuration may be critical when phenolic streams or laccase production are targeted as coproducts alongside delignification.

3.3.2 Phenolics and laccase behavior under ultrasound-assisted enzymatic pretreatments

Whereas SSF/SmF profiles reflect time-dependent production and transformation of phenolics under fungal growth, the ultrasound-assisted assays represent a short, intensified event intended to rapidly solubilize phenolics and test the immediate effect of laccase-containing preparations on the soluble pool. Soluble phenolics increased markedly after ultrasound (11.8 ± 0.4^b mg GAE eq g^{-1}) compared with a non-sonicated sample (4.6 ± 0.2^a mg GAE eq g^{-1}), while lower phenolic levels were observed when ultrasound was combined with enzymes (7.4 ± 0.6^c and 4.90 ± 0.3^a mg GAE eq g^{-1}) (Figure 8). A plausible explanation is that sonication promotes rapid solubilization of low-molecular-weight phenolic fragments, whereas in the presence of laccase-containing preparations part of this soluble pool may be further oxidized, transformed, or re-associated with the solid phase, resulting in lower accumulation in the liquid fraction at the sampling point (Nagula & Pandit, 2016; Piscitelli *et al*., 2011; Mattinen *et al*., 2009; Legorreta-Castañeda *et al*., 2020).

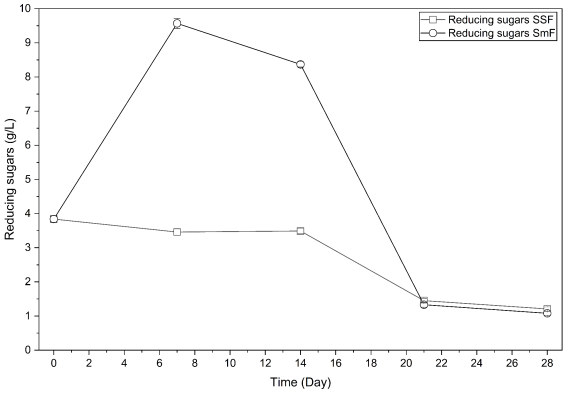

Figure 9. Total reducing sugars released during 28 days of biological pretreatment of coconut coir with *Trametes hirsuta* Bm-2 under solid-state fermentation (SSF) and submerged fermentation (SmF). Values are reported as mean ± SD (n = 3).

Laccase activity was quantified only for the enzyme-containing treatments (Figure 8). The initial reference activity prior to sonication was 1,379.6±51.6^{c} U g^{-1} for the enzyme-containing systems, decreasing to 1,311.8 ± 40^{c} U g^{-1} after ultrasound (4.91% reduction) for the *T. hirsuta* Bm-2 crude extract. Meanwhile, mean activity for the commercial laccase decreased 14.32% (1,182.00 ± 28.2^{b} U g^{-1}) after sonication, showing statistical significance and indicating greater sensitivity of the commercial preparation to ultrasound under the evaluated conditions. This behavior is consistent with the fact that cavitation can partially inactivate enzymes through shear, localized heating, and radical-driven structural changes and aggregation, with the magnitude of the effect being highly dependent on the surrounding medium and formulation (Delgado-Povedano & Luque de Castro, 2015; Rathnakumar *et al*., 2023). In this context, crude extracts may display higher apparent robustness under sonication because coexisting proteins and other macromolecular components can act as protective or stabilizing agents, whereas more purified/commercial enzyme preparations can be more susceptible under the same ultrasound regime (Delgado-Povedano & Luque de Castro, 2015; Martins *et al*., 2015), suggesting that a crude enzymatic extract from *T. hirsuta* Bm-2 could be a practical alternative to purified enzymes for application in complex substrates or media, given its greater tolerance to sonication and its potentially lower processing cost.

3.3.3 Enzymatic hydrolysis: reducing sugars and cellulose conversion

Hydrolysis was evaluated based on reducing sugar release from biomass biologically pretreated under SSF and SmF for up to 28 days (Figure 9). In SSF, reducing sugars remained close to the baseline value at day 0 (3.84 ± 0.1 g L^{-1}) through day 14 (3.49 ± 0.08 g L^{-1}), followed by a sharp decrease at later times to 1.21 ± 0.06 g L^{-1} by day 28. Accordingly, the estimated cellulose conversion in SSF decreased from 2.88% at day 0 to 0.91% at day 28. In contrast, SmF pretreatment enhanced sugar release relative to SSF at all incubation times after day 0. Reducing sugars increased sharply to a maximum at day 7 (9.57 ± 0.15 g L^{-1}) and remained high at day 14 (8.37 ± 0.08 g L^{-1}), before dropping abruptly to 1.08 ± 0.06 g L^{-1} by day 28. The corresponding cellulose conversion values in SmF peaked at 7.17% (day 7) and declined thereafter, reaching 0.81% at day 28.

Interestingly, the amounts of reducing sugars per gram of dry substrate in this work (87 ± 2 for SSF at day 14 and 239 ± 2 mg g^{-1} substrate for SmF at day 7) were higher than those reported by Saritha *et al*. (2012), who reported about 10 mg g^{-1} substrate for paddy straw with a 1 to 6 day *T. hirsuta* MTCC136 culture. In addition, the results observed here were similar to those obtained with conventional pretreatments on coconut fiber, such as those reported by Nogueira *et al*. (2019), where sugar yields were between 50 and 230 mg g^{-1} substrate (~13 to 40% glucose conversion) using hydrothermal, acid, alkaline, and alkaline–organosolv treatments; on the other hand, Ebrahimi *et al*. (2018) obtained values well above the maximum of 9.57 ± 0.15^{c} g L^{-1} (239 ± 2 mg g^{-1} substrate) reached in this work, reporting close to 77 g L^{-1}. However, it is worth noting that most treatments were applied under a pressure of around 148 atm and a temperature of 70 °C (Nogueira *et al*., 2021; Nogueira *et al*., 2019; Ebrahimi *et al*., 2018), which, although below the temperature of autohydrolysis, the combination of pressure, temperature, and chemical agents may be the factor behind the differences observed in the release of fermentable sugars, as they promote greater disruption of the lignocellulosic matrix compared with the ambient temperature and atmospheric pressure used in this study.

It is noteworthy that saccharification improves when washing steps are applied after pretreatments (Nogueira *et al*., 2019), possibly because some phenolic compounds such as vanillin, syringaldehyde, and ferulic acid can inhibit sugar release (TPC ~80 to 175 mg GAE g^{-1} substrate). In addition, products derived from the oxidation of phenolic compounds by laccases during delignification can inhibit cellulase activity (Qin *et al*., 2016; Oliva-Taravilla *et al*., 2015), highlighting the importance of washing and detoxification processes (Nogueira *et al*., 2021; Nogueira *et al*., 2019) before carrying out fermentation when T. hirsuta is used as a pretreatment for delignification aiming at generating fermentable sugars for conversion to ethanol, even when the phenolic concentration of the liquid fraction

is moderate (Nogueira *et al*., 2021).

The phenolic profile indicates that Folin–Ciocalteu-reactive soluble phenolics alone did not explain the loss of saccharification at longer incubation times, since phenolic concentrations were not highest at days 21–28, when sugar release was poorest. However, this does not exclude the contribution of lignin-derived inhibitory species, because the Folin–Ciocalteu assay reflects reducing capacity but does not identify specific oxidized, coupled, or matrix-associated products (Box, 1983). Laccase-mediated oxidation can transform phenolic compounds into oligomeric or polymeric products through radical coupling reactions (Mattinen *et al*., 2009; Zhang *et al*., 2023), while manganese peroxidase and related peroxidative systems can generate Mn^{3+}-mediated phenoxy radicals, quinones, aryl cation radicals, peroxyl radicals, and low-molecular-weight oxidation products during lignin conversion (Hofrichter, 2002). These products may be poorly represented as soluble Folin-reactive phenolics but may still impair hydrolysis by modifying residual lignin properties, promoting non-productive cellulase adsorption, or directly inhibiting cellulolytic enzymes (Oliva-Taravilla *et al*., 2015; Qin *et al*., 2016).

On the other hand, the negative association between incubation time and sugar release (r = -0.730, p = 0.0399) further supports that prolonged residence time did not favor saccharification under these conditions. Mao *et al*. (2013) reported that biological pretreatment of *Carex meyeriana* with *T. hirsuta* C7784 for 6 weeks removed approximately 12% of lignin, but also decreased cellulose and hemicellulose by approximately 22% and 13%, respectively, supporting that prolonged fungal pretreatment can modify lignin while simultaneously consuming structural carbohydrates. This behavior suggests a potential trade-off commonly reported for fungal pretreatments, where extended residence times can increase lignin modification but also promote biomass losses and/or consumption of accessible carbohydrates associated with fungal growth, ultimately reducing the substrate fraction available for enzymatic hydrolysis (Mao *et al*., 2013; Vasina *et al*., 2016; Metreveli *et al*., 2017; Andriani *et al*., 2020). The decoupling between delignification and reducing sugar release was supported by the correlation analysis, which showed a negative association between sugar release and delignification (r = -0.781, p = 0.0221) and a positive association between sugar release and residual Klason lignin (r = 0.781, p = 0.0223). This is consistent with Andriani *et al*. (2020), who reported sequential production of ligninolytic enzymes followed by xylanase and cellulase in submerged cultures of *T. hirsuta* AA-017, with xylanase and cellulase detected from days 7–8 and peaking at days 10–12, after the main laccase/MnP/LiP activity

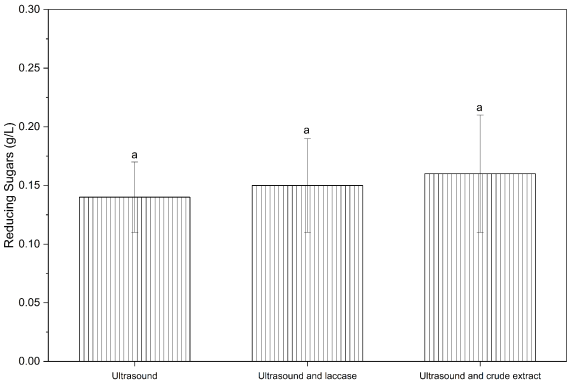

Figure 10. Reducing sugar release from coconut coir after ultrasound-assisted pretreatments. Bars show reducing sugars (g L^{-1}) measured in the liquid fraction for ultrasound alone, ultrasound with commercial laccase, and ultrasound with a Trametes hirsuta Bm-2 crude enzyme extract. Values are reported as mean ± SD (n = 3); different letters indicate significant differences among treatments within the same response variable ($p < 0.05$).

window. Thus, the progressive increase in mycelial biomass observed up to day 21 suggests active fungal growth that may have consumed part of the most accessible carbohydrate fraction before hydrolysis, helping to explain why SmF at 7–14 d represented the most favorable saccharification window, whereas prolonged incubation to 21–28 d favored cumulative delignification but resulted in poor sugar release. Although Andriani *et al*. (2020) observed glucose-dependent repression of cellulase and xylanase, depletion of readily assimilable carbon sources during the longer batch incubation used here could have favored a later shift toward lignocellulosic polysaccharide utilization.

Mechanistically, this decoupling may arise from the combined effect of partial matrix deconstruction, fungal consumption of accessible carbohydrates, and changes in the chemical nature of residual lignin. SEM and FTIR results indicated surface erosion, pore-like features, attenuation of lignin-associated aromatic bands, and changes in hemicellulose/carbohydrate-related regions; however, these modifications were subtle and consistent with partial rather than extensive deconstruction of the coconut coir matrix. Therefore, although *T. hirsuta* Bm-2 modified and removed part of the lignin fraction, the remaining lignin–carbohydrate architecture may have continued to restrict cellulase access or promote non-productive enzyme adsorption (Saini *et al*., 2016).

Unlike SSF and SmF, where residence time, fungal growth, lignin modification, and carbohydrate preservation were simultaneously affected, ultrasound-assisted pretreatments provided a short-term conditioning system to evaluate whether rapid physical/enzymatic modification of the fiber

was sufficient to enhance saccharification. Overall, reducing sugars were low and similar across treatments, ranging from 0.14 ± 0.03 to 0.16 ± 0.05 g L^{-1} (equivalent to 3.5 ± 0.75 to 4 ± 1.25 mg g^{-1}) (Figure 10). As discussed above, phenolic concentrations, however, differed among pretreatments, with the highest value observed for ultrasound alone (11.8 ± 0.4 mg GAE g^{-1}) and lower levels when ultrasound was combined with enzymes (7.40 ± 0.60 with commercial laccase and 4.9 ± 0.3 mg GAE g^{-1} with the *T. hirsuta* Bm-2 crude extract).

Despite measurable effects on phenolic solubilization and detectable laccase activity in the enzyme-containing treatments, ultrasound-assisted pretreatments did not improve saccharification under the evaluated conditions, as reducing sugar release remained low and statistically similar across treatments (Figure 10). This suggests that ultrasound primarily promoted rapid solubilization and chemical modification of lignin-derived phenolics, evidenced by the higher phenolic concentration after ultrasound alone and the lower values when laccase-containing systems were applied, without producing sufficient matrix deconstruction to enhance hydrolysis (Nagula & Pandit, 2016; Delgado-Povedano & Luque de Castro, 2015). A similar functional mismatch has been observed for sweet sorghum bagasse treated with a crude enzymatic extract from *T. hirsuta* Bm-2, where SEM and FTIR showed evidence of structural modification of the lignocellulosic matrix, but glucose release after enzymatic saccharification remained low compared with values reported in the literature. This was attributed, at least in part, to incomplete cellulose exposure to the cellulolytic cocktail, supporting the view that lignin modification alone does not ensure efficient saccharification (Hernández-Calderón, 2018). In addition, residual phenolics in the solid matrix can contribute to non-productive interactions or inhibition during enzymatic hydrolysis, which may further limit sugar formation (Qin *et al*., 2016; Yao *et al*., 2022).

In contrast to the higher reducing sugar release achieved after biological pretreatment under SmF at 7–14 d, these results indicate that the ultrasound–enzyme configurations tested here are more effective at modulating phenolic streams than at increasing saccharification, unless further process optimization or complementary steps are implemented, such as extending sonication time beyond 1 h, incorporating washing or detoxification prior to hydrolysis, and optimizing enzymatic hydrolysis conditions (e.g., using sequestrant additives to mitigate inhibitory interactions) (Gundupalli *et al*., 2022; Contesini *et al*., 2021; Nogueira *et al*., 2019; Nogueira *et al*., 2021; Gómora-Hernández *et al*., 2025). These findings reinforce the versatility of *T. hirsuta* Bm-2 for valorizing lignin-rich substrates such as coconut coir and highlight the need to tailor operational configurations (SSF vs SmF, solid-to-liquid ratio, agitation/oxygen transfer) and downstream conditioning steps to selectively maximize the desired product portfolio (phenolics, laccase, or sugars). Overall, the results indicate that delignification, phenolic release, and hydrolysis response were not linearly coupled; instead, they depended on configuration (SSF vs SmF) and residence time, thereby defining the most suitable route for a given coproduct portfolio.

Conclusions

Biological pretreatment with *Trametes hirsuta* Bm-2 was the most effective strategy for lignin reduction in coconut coir, reaching 37.77 ± 0.07% delignification under SSF and 43.44 ± 0.10% under SmF after 28 days. These values demonstrate that *T. hirsuta* Bm-2 can achieve competitive delignification of a highly recalcitrant lignocellulosic residue under mild biological conditions, without the high pressures, elevated temperatures, or solvent-intensive conditions commonly required by physicochemical pretreatments. In addition, coconut coir supported high extracellular laccase production, particularly under SmF during the early stages of cultivation, highlighting its potential as a low-cost and underutilized substrate for producing enzymes of industrial interest.

However, the highest lignin removal did not coincide with the highest saccharification response. SmF provided the best sugar release at early incubation times, particularly at day 7, whereas prolonged fermentation to 21–28 days increased cumulative delignification but resulted in poor reducing sugar release and low cellulose conversion. This decoupling indicates that lignin removal alone was not sufficient to ensure efficient hydrolysis under the evaluated conditions. The low saccharification response was likely associated with partial rather than extensive matrix deconstruction, fungal biomass accumulation, possible loss or consumption of accessible carbohydrate fractions, and the presence of residual inhibitory compounds. Therefore, when biological pretreatment with *T. hirsuta* Bm-2 is intended to support fermentable sugar production, washing or detoxification steps should be considered, together with strategies that ensure effective opening of the lignocellulosic matrix and adequate cellulase access.

Overall, coconut coir valorization with *T. hirsuta* Bm-2 should be approached as a process with response-specific operational windows. Short SmF treatments may favor laccase production and moderate saccharification, SSF may be useful for phenolic-rich streams, and longer biological

treatments may favor delignification but compromise hydrolysable carbohydrate availability. Future work should optimize residence time, solid conditioning, washing/detoxification, and intensified pretreatment–hydrolysis strategies to improve sugar recovery while preserving the enzyme-producing potential of this fungal system.

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Nomenclature

GAE	Gallic acid equivalent(s)
SmF	Submerged fermentation
SSF	Solid-state fermentation
TPC	Total polyphenol content

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