http://periodicos.uem.br/ojs
ISSN on-line: 1807-8664
https://doi.org/10.4025/actascitechnol.v48i1.74839

Biodegradation of a textile dye by *Ganoderma lucidum*: scale-up into packed-bed bioreactors

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ABSTRACT. This study presents the scale-up of the discoloration process of the dye Remazol Brilliant Blue R (RBBR) by the white-rot fungus *Ganoderma lucidum*. Experiments were initially conducted in a 2-L lab-scale bioreactor and subsequently scaled up to a 45-L packed-bed bioreactor operated in batch mode under nonsterile conditions. The dye solution (150 mg L^{-1}) was recirculated through the packed bed, which supported the growth of *G. lucidum* on an inert and biodegradable carrier — peach palm waste. Discoloration, enzyme activity, pH, and microbial community composition were analyzed. In the lab-scale system, discoloration reached 80%, with laccase activity measured at 847.2 IU mL^{-1}. Upon scale-up, discoloration reached 91%, with laccase identified as the main factor contributing to biodecoloration. The bacterial community was initially dominated by the phylum *Proteobacteria* (93.4%), followed by an increase in *Acidobacteria*, *Actinobacteria*, and *Bacteroidetes*. Among eukaryotes, *Opisthokonta* represented over 96% of the community. These results suggest that *G. lucidum* is a promising agent for the industrial-scale biodegradation of RBBR dye.

Keywords: *Bactris gasipaes*; discolorization; *ganoderma lucidum*; ligninolytic enzymes; peach palm waste; solid-state fermentation.

Received on Dezember 03, 2024.
Accepted on July 04, 2025.

Introduction

The textile industry generates large volumes of wastewater worldwide. Due to the presence of stable and complex dye structures, most of the effluent discharged from textile plants is heavily polluted (Ghaffar et al., 2023; Javaid et al., 2016; Solaiman et al., 2024), posing a significant environmental challenge. The impact of this sector — including water and soil contamination and toxic effects on human health — has been recently documented by several researchers (Bueno et al., 2022; Solaiman et al., 2024).

The limitations of conventional treatments include the incomplete removal of dyes, high chemical costs, generation of hazardous secondary waste, and disposal issues (Hermann et al., 2020; van Brenk et al., 2024). Bioremediation has emerged as a promising alternative, offering advantages such as cost-effectiveness, the absence of chemical by-products, and environmentally friendly technology (van Brenk et al., 2024; Yadav et al., 2021).

Most studies on the biodegradation of these hazardous compounds are performed at the laboratory scale and often involve ligninolytic enzymes (such as laccase, lignin peroxidase, and manganese peroxidase) produced by white-rot fungi (WRF) (Hermann et al., 2020; Serbent et al., 2023; van Brenk et al., 2024; Yadav et al., 2021). These oxidative enzymes contribute to fungal metabolism and enable the degradation of synthetic dyes commonly (Li et al., 2015; van Brenk et al., 2024; Yadav et al., 2021). Therefore, the development of new studies involving WRF in scaled-up bioreactors for textile wastewater treatment is of great scientific and practical interest (Hermann et al., 2020).

However, enzyme production depends on several parameters (van Brenk et al., 2024). Laccase activity in fungi is enhanced not only by the presence of dyes but also by lignocellulosic materials during solid-state fermentation (SSF) (Hermann et al., 2020). Agro-industrial residues such as peach palm (*Bactris gasipaes*) waste are rich in lignocellulose and can serve as substrates for SSF by WRF. Peach palm shells, generated as byproducts during heart-of-palm extraction, account for 85 to 95 g kg^{-1} of the total palm weight and can cause negative environmental, social, and economic impacts if improperly disposed of (Pasko et al., 2022; Timm et al., 2024a). The cellulose, hemicellulose, and lignin in these shells can be degraded by WRF, stimulating laccase production and subsequent dye discoloration (Hermann et al., 2020), while also mitigating

environmental damage. The reuse of agro-industrial byproducts supports progress toward the UN Sustainable Development Goals (SDGs) (Timm et al., 2024a). Furthermore, fungal-based technologies align with the principles of a bio-based circular economy, which is increasingly emphasized (Serbent et al., 2023).

Among the various dyes used in the textile industry, Remazol Brilliant Blue R (RBBR) is one of the most widely used. It is commonly used as a starting material in the synthesis of polymeric dyes. RBBR has an anthraquinone structure and belongs to a class of toxic and recalcitrant organopollutants (Yadav et al., 2021). Although the discoloration of RBBR has been extensively studied (Yadav et al., 2021), and the use of WRF in bioremediation processes is increasingly explored (Serbent et al., 2023; Timm et al., 2024b; Timm et al., 2024c), the role of laccase in RBBR degradation remains poorly understood (Yadav et al., 2021). Moreover, the development of new technologies for pollutant degradation using microorganisms in packed-bed bioreactors (PBB) has shown promise. Still, a major challenge in applying WRF technology lies in designing and implementing suitable reactors (Hermann et al., 2020). Scaling up is particularly difficult due to the complexity of controlling the physicochemical processes involved and the limited understanding of the microbiota that develops within such bioreactors.

The present study aimed to design and scale up a PBB operated in batch mode under non-sterile conditions for the discoloration of RBBR. Notably, this is the first scale-up study to use a fixed-bed reactor with peach palm waste (shells) colonized by the fungus *Ganoderma lucidum* to decolorize a textile dye — RBBR. The study also included microbial DNA isolation and high-throughput sequencing for the rapid and accurate identification of bacterial contaminants potentially affecting the discoloration process.

Materials and methods

To design and scale up a PBB, *Ganoderma lucidum* was cultivated on peach palm waste under solid-state conditions. WRF and lignocellulosic substrate used in this study were selected based on findings from a previous investigation (Chicatto et al., 2018a). Biodegradation assays were first conducted at the laboratory scale and subsequently scaled up. The processes were evaluated in terms of pH, discoloration efficiency, laccase and manganese peroxidase activity, and microbial community composition. Additionally, the contribution of dye adsorption to the overall discoloration was assessed.

Fungal strains and culture conditions

The WRF *G. lucidum* EF31 used in this study was kindly provided by the National Research Center for Forestry/Embrapa Forests (Colombo, PR, Brazil). The fungal isolate was cultivated on Petri dishes containing Potato Dextrose Agar (PDA) medium for 10 days at 25 ± 1°C in the absence of light. Cultures were stored at 4°C until further use.

Dye solution and peach palm waste

The dye RBBR (molecular formula $C_{22}H_{16}N_2Na_2O_{11}S_3$; molar mass: 626.5 g mol^{-1}; λ_{max}: 592 nm) was provided by a local textile company (Santa Catarina, Brazil). The dye solution used in all experiments was prepared at a concentration of 150 mg L^{-1}.

Peach palm (*Bactris gasipaes*) waste, referred to as 'shells,' was used as the solid substrate in all discoloration tests. The material was supplied by a local agribusiness in Brazil, milled in a knife mill to a particle size of 2 mm, and dried at 60°C for 24 hours prior to use.

Discoloration experiments in a lab-scale PBB

Initially, RBBR degradation was evaluated in a 2-L PBB, following the method described by Chicatto et al. (2018b). For this, 20 g of peach palm waste was placed into each of ten 500-mL Erlenmeyer flasks, along with 20 mL of a mineral medium (Leung & Pointing, 2002), and autoclaved at 121°C for 15 minutes. After inoculation with *G. lucidum*, the flasks were incubated at 28 ± 2°C in the dark for 21 days (Hermann et al., 2020).

Following incubation, the contents of the flasks (200 g of colonized biomass) were transferred to a 2-L graduated cylinder functioning as the PBB. The bioreactor was maintained under non-sterile conditions at a controlled temperature of 25°C for a 5-day adaptation period. After this period, the dye solution was manually recirculated through the system once per day for seven days, maintaining adequate moisture levels to support fungal growth. Daily samples were collected to assess discoloration efficiency and the enzymatic activities of laccase and manganese peroxidase.

Discoloration experiments in an up-scale PBB

The scale-up of the PBB system was performed in a 45-L batch bioreactor with recirculation, operated under non-sterile conditions. The scale-up procedure was based on the study by Chicatto et al. (2018b), who conducted experiments using 1-L flasks containing industrial effluents and the fungus *Ganoderma lucidum*.

Figure 1 illustrates the schematic setup used for the scale-up of RBBR biodegradation. Three tanks were connected in series, each with a nominal capacity of 15 L (totaling 45 L): (i) the first tank contained the RBBR solution (150 mg $L^{-}1$); (ii) the second tank served as the bioreactor, filled with *G. lucidum* previously cultivated on peach palm waste; (iii) the third tank was used as the effluent reservoir, where the dye solution was collected during circulation and stored overnight. All tanks were made of acrylic and had the same dimensions: 30 cm in diameter and 20 cm in height.

Prior to the biodegradation assays, *G. lucidum* was cultivated on peach palm waste as described in Section 2.3, but the number of flasks increased to 40. A total of 1,200 g of colonized biomass was transferred to the bioreactor. After a 5-day adaptation period, the discoloration assays were initiated. In total, six consecutive batches were carried out, each lasting 7 days. For each batch, a fresh RBBR solution was prepared and pumped through the packed bed at a flow rate of 36 L h^{-1} for 9 hours per day. Upon completion of one batch, the next was immediately started, totaling 42 days of operation.

To evaluate the extent of RBBR adsorption onto the peach palm waste, control experiments were performed under the same conditions but without fungal inoculation. In this case, three 7-day batches were conducted. To maintain enzyme production, the bioreactor was supplemented daily with mineral medium, which was recirculated for 1 hour per day.

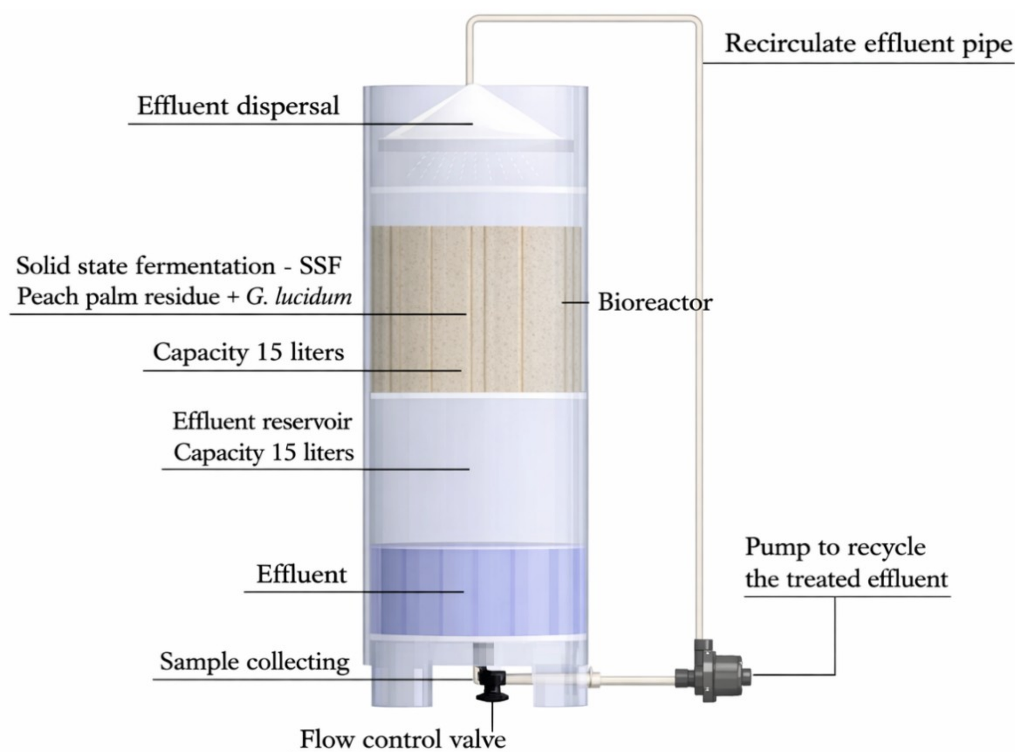

Figure 1 - Schematic overview of the up-scaled PBB (45 L).

Analytical assays

The efficacy of dye removal, laccase and manganese peroxidase activities, and pH were monitored throughout the discoloration assays. Enzymatic activity measurements were performed based on the methods described by (Chicatto et al., 2018a; Chicatto et al., 2018b).

To evaluate the discoloration process, the efficiency of color removal was determined by comparing the initial absorbance of the dye solution with its absorbance at the end of fermentation. Measurements were conducted using a UV–VIS spectrophotometer (Shimadzu UV–Vis-1650 PC) at 590 nm.

Laccase activity was determined following the protocol described by (Hou et al., 2004). Briefly, 0.1 mL of a liquid extract obtained from the bioreactor was added to test tubes containing 0.8 mL of 2,2'-Azino-bis (3-

ethylbenzthiazoline-6-sulphonic acid) (ABTS) solution, 0.1 mL of acetate buffer (pH 4.0), and 0.1 mL of distilled water. Control samples consisted of 0.8 mL ABTS and 0.1 mL acetate buffer. The tubes were incubated at 30°C for 20 minutes. ABTS oxidation was monitored by measuring absorbance at 420 nm (ε = 36 mM^{-1} cm^{-1}) every 15 seconds for 2 minutes using a UV–VIS spectrophotometer (Shimadzu UV–Vis-1650 PC).

Manganese peroxidase activity was determined according to (Wariishi et al., 1992). In brief, 0.4 mL of liquid extract was mixed with 0.4 mL of hydrogen peroxide (0.5 mM), 2.8 mL of sodium malonate buffer (50 mM, pH 4.5), and 0.4 mL of manganese sulfate (10 mM). The oxidation of $MnSO_4$ in sodium malonate buffer in the presence of H_2O_2 forms a complex with manganic ions (Mn^{3+}) and malonate. This reaction was monitored by measuring absorbance at 270 nm (ε = 11.59 mM^{-1} cm^{-1}) using a UV–VIS spectrophotometer (Shimadzu UV–Vis-1650 PC). Enzyme activities were expressed in U mL^{-1}, where one unit (U) is defined as the amount of enzyme required to oxidize 1 µmol of substrate per minute under the assay conditions.

The pH of samples was measured daily, from time zero until the end of fermentation, using a benchtop pH meter (Tecnal). All experiments were conducted in triplicate.

Microbial DNA isolation, high-throughput sequencing, and microbial rRNA gene taxonomic analyses

To identify the microbial communities present in the up-scaled PBB system, samples of solid biomass from the fixed bed were collected from the bioreactor on the first and last experimental days, both for the fungal cultivation and the control systems.

Total DNA was extracted from these samples using the DNeasy PowerSoil Kit (QIAGEN), according to the manufacturer’s protocol. To characterize the prokaryotic population, fragments of the 16S rRNA gene were amplified using universal primers 515F and 806R (Bates et al., 2011). For eukaryotic population analysis, fragments of the 18S rRNA gene were amplified using the primer pair Fw and Rv (Nolte et al., 2010).

The initial PCR amplification was carried out in a 50-µL reaction mixture containing 1.5 mM $MgCl_2$, 0.2 µM of each primer, 0.2 mM of each dNTP, 1 U of Platinum Taq DNA polymerase, 1× PCR reaction buffer, and 10 ng of genomic DNA. For prokaryotic amplification, the PCR protocol consisted of an initial denaturation step at 95°C for 3 minutes, followed by 25 cycles of denaturation at 95°C for 30 seconds, annealing at 52°C for 1 minute, and extension at 72°C for 1 minute, with a final extension at 72°C for 7 minutes. For eukaryotic amplification, the PCR conditions were initial denaturation at 94°C for 4 minutes; 30 cycles of denaturation at 94°C for 45 seconds, annealing at 50°C for 30 seconds, and extension at 72°C for 1 minute; followed by a final extension step at 72°C for 7 minutes.

After amplification, PCR products were purified using Agencourt AMPure XP Beads (Beckman Coulter). Libraries were then constructed according to the Ion Plus Fragment Library Kit protocol, using 100 ng of DNA as input. As all samples were sequenced in a multiplexed run, barcode sequences were incorporated to allow individual identification of each sample in the final sequencing dataset. Sequencing was performed using an Ion PGM System (Thermo Fisher Scientific) with an Ion 316 chip, following the manufacturer’s instructions.

The rRNA reads generated were trimmed to a minimum length of 100 bp and a minimum Phred quality score of 20 using PRINSEQ (Schmieder & Edwards, 2011). The remaining sequences were dereplicated, sorted by decreasing read abundance, and filtered to exclude singletons using USEARCH v7.0.1090 (Edgar, 2010) according to the algorithm UPARSE (Edgar, 2013). Clusters were assembled using a minimum sequence identity threshold of 99%, and chimeric sequences were removed using the RDP reference database for 16S rRNA (Cole et al., 2014).

For 16S rRNA fragments, taxonomic assignment was performed using UCLUST, while 18S rRNA fragments were classified using the RDP Classifier within the QIIME v1.8 environment (Caporaso et al., 2010). Operational taxonomic units (OTUs) were defined at 97% sequence similarity. Taxonomic classification for 16S rRNA sequences was conducted using the Greengenes database, version 13.8 (DeSantis et al., 2006), and the SILVA database, version 111 (Quast et al., 2012) for 16S and 18S rRNA, respectively.

Rarefaction of the OTU table was performed with a maximum subsampling depth of 2,100 reads for prokaryotic datasets and 5,100 reads for eukaryotic datasets. All sequencing data were deposited in the National Center for Biotechnology Information (NCBI) under BioProject accession number PRJNA473226.

Statistical analysis

Experimental data were analyzed using ANOVA followed by Tukey’s test for multiple comparisons at a significance level of $p \leq 0.05$. All statistical analyses were performed using Statistica software, version 7.0 (StatSoft).

Results and discussion

Discoloration experiments in a lab-scale PBB

Figure 2 illustrates the RBBR discoloration percentage and the laccase activity produced by *G. lucidum* over the experimental period in the 2-L PBB (Figure 2a), as well as the linear relationship between these two variables (Figure 2b).

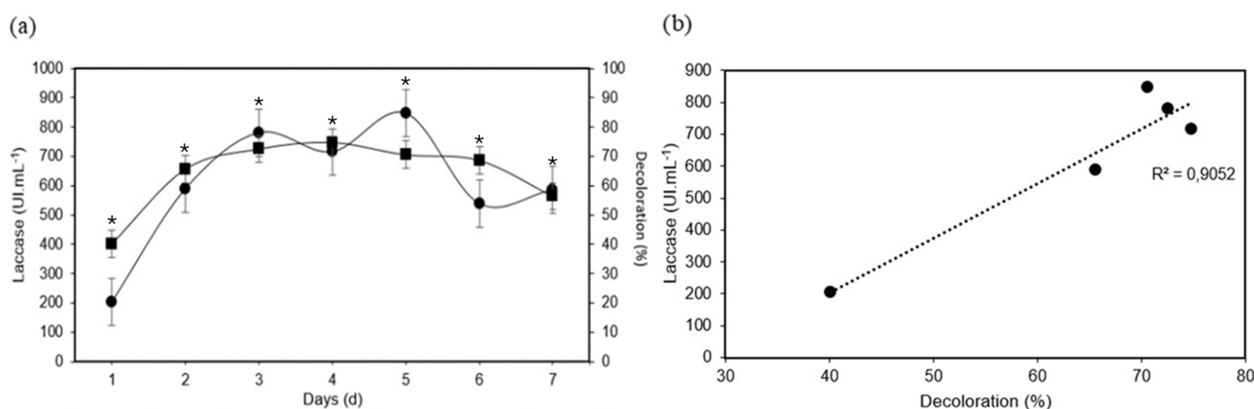

Figure 2 - RBBR discoloration percentage (■) and laccase activity produced by G. lucidum (●) throughout the experimental period in the lab-scale PBB (2 L) (a), and the correlation between both parameters (b). Asterisks (*) above error bars indicate statistically significant differences among sampling days ($p \leq 0.05$). Points without asterisks and error bars indicate no statistically significant differences between means.

Laccase activity reached up to 847.2 IU mL^{-1}, and RBBR degradation/discoloration began on the first day of operation, progressively increasing over time and showing statistically significant differences among the collected data (Figure 2a). A strong positive linear correlation was observed between laccase activity and RBBR discoloration (R^2 = 0.9042; Figure 2b). Although significant laccase activity was detected, manganese peroxidase was not observed, consistent with the findings of Chicatto et al. (2018b) in a similar laboratory-scale study. This absence suggests that manganese peroxidase activity may have occurred primarily during the initial fungal growth phase in the flasks (Serbent et al., 2023), which aligns with known WRF behavior. The production of ligninolytic enzymes by WRF is known to be influenced by several factors, including microbial interactions, medium composition, pH, temperature, water activity, moisture availability, aeration, and agitation (Hermann et al., 2020).

Therefore, the observed color removal can be primarily attributed to laccase activity. Similar findings have been reported in other solid-state fermentation (SSF) systems using basidiomycete fungi (Das et al., 2016; Hermann et al., 2020; Serbent et al., 2023; Zeng et al., 2015). Several studies have highlighted the role of WRF-derived laccase in the degradation and decolorization of azo, anthraquinone, and xanthene dyes (Bilal et al., 2017; Cantele et al., 2017; Hermann et al., 2020; Suwannawong et al., 2010).

The maximum laccase activity achieved in this study (847.2 IU mL^{-1}) is considerably higher than values typically reported for similar systems. For example, *Marasmiellus palmivorus* produced approximately 315 IU mL^{-1} under optimized SSF conditions in a packed-bed pilot-scale reactor treating textile dyes (Cantele et al., 2017), while *Pleurotus ostreatus* reached around 120 IU mL^{-1} in a co-substrate SSF system for dye decolorization (Das et al., 2016). Additionally, immobilized laccases commonly used in industrial dye degradation processes usually exhibit activities ranging from 50 to 500 IU mL^{-1} (Bilal et al., 2017). These comparisons highlight the biotechnological potential of *G. lucidum* for dye bioremediation, particularly in PBBs using low-cost lignocellulosic substrates. The high enzymatic activity observed positions *G. lucidum* as a promising biocatalyst not only for dye degradation but also for broader industrial applications requiring oxidative enzymes.

Increased laccase activity leads to faster decolorization (Chicatto et al., 2018b). *G. lucidum* is recognized as an efficient laccase producer, with the ability to act as a biocatalyst in the oxidative cleavage of synthetic dyes (Chicatto et al., 2018b). Laccase can oxidize aromatic rings of both phenolic and non-phenolic substrates, enabling the biotransformation of various pollutants. This characteristic makes the enzyme particularly suitable for use in textile wastewater treatment (Yadav et al., 2021).

Discoloration experiments in an up-scale PBB

Figure 3 presents the discoloration percentage, laccase activity, and pH values obtained in the pilot-scale bioreactor operated under nonsterile conditions. No manganese peroxidase activity was detected in any of the batches, corroborating the results observed at the laboratory scale (Chicatto et al., 2018b).

Among the different batches, the highest discoloration efficiencies were observed in the first (91%) and fourth (85%) batches, while the peak laccase activity was recorded in the third batch. The pH values showed slight variation over time, ranging approximately from 4.5 to 6.7, indicating limited bacterial proliferation within the bioreactor. Although statistically significant differences were observed between the first and second batches, no significant differences were found among the third to sixth batches.

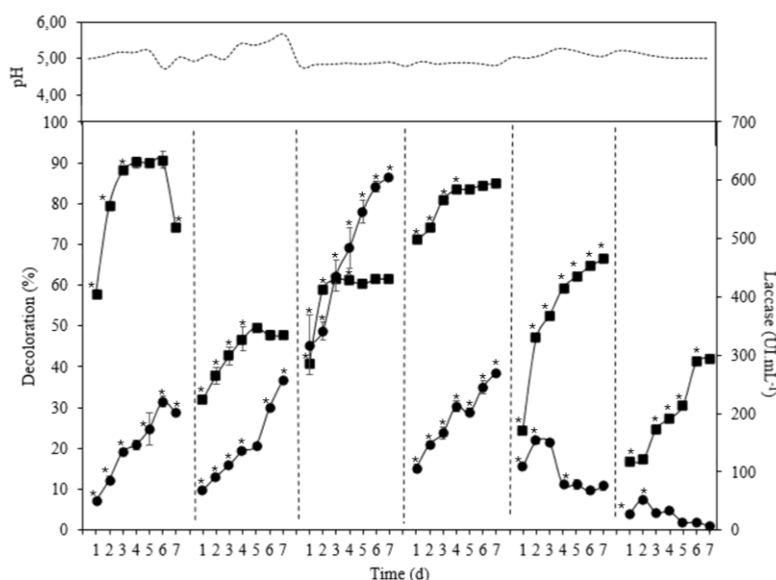

Figure 3 - RBBR discoloration percentage (■), laccase activity produced by *G. lucidum* (●), and pH values (---) over the experimental period in the up-scaled PBB (45 L). Asterisks (*) above the error bars indicate statistically significant differences across sampling days ($p \leqslant 0.05$). Points without asterisks or error bars indicate no statistically significant differences between means.

Overall, two distinct behavioral patterns were observed across the experimental batches: (i) in batches 1 to 4, discoloration efficiency increased until the fourth day, showing statistically significant differences between sampling days. Afterward, discoloration stabilized with no further significant variation until the seventh day. In parallel, laccase activity increased almost linearly over time and remained statistically distinct between days; (ii) from batch 4 onward, discoloration efficiency no longer reached a plateau, and laccase activity progressively declined over time.

These findings indicate that the duration of bioreactor operation influences process performance, with the most effective results observed during the initial stages. Additionally, a positive correlation between discoloration efficiency and laccase activity was evident across the different batches (Chicatto et al., 2018b).

A particularly notable case occurred in batch 2, where the maximum discoloration reached only 50%, yet laccase activity was higher than in batch 1. The correlation between discoloration and laccase activity in this batch was very strong ($R^2 = 0.9956$). It is likely that in batch 1, both biodegradation and adsorption of the dye onto peach palm fibers contributed significantly to dye removal, as previously observed by Hermann et al. (Hermann et al., 2020).

Interestingly, although a significant increase in laccase production was recorded in batch 3, the corresponding impact on discoloration was only observed in batch 4, where dye removal reached up to 85%. A subsequent decline in laccase activity was observed in batch 5. Finally, in batch 6, both laccase activity and discoloration efficiency declined proportionally ($R^2 = 0.9413$), indicating overall system exhaustion and performance deterioration. The data suggest a linear relationship between laccase activity and discoloration efficiency, supporting the suitability of a linear model to describe the observed trends. Variations in laccase activity appear to explain a substantial portion of the variation in discoloration efficiency (Chicatto et al., 2018b).

In the absence of manganese peroxidase, the results emphasize the potential of WRF-derived laccase as a promising agent for bioremediation applications (Serbent et al., 2023). A similar pattern in RBBR dye degradation using laccase from *Arthrographis kalrae* was also reported by Yadav et al. (2021).

To better understand the discoloration process, the contribution of dye adsorption in the absence of fungal activity was also evaluated. Figure 4 presents the results of control assays conducted under nonsterile conditions, showing discoloration efficiency and pH values throughout the first three batches. In the first batch, the adsorption process of the dye could be divided into three distinct phases: (i) phase 1 (0-24 h): Color removal reached nearly 50%, while the pH remained above 3. This phase is characterized by rapid adsorption and saturation of the active sites on the peach palm waste fibers; (ii) phase 2 (2 4-96 h): Discoloration efficiency dropped to nearly zero, indicating complete saturation of the fibers. During this period, the pH declined sharply to 1.36, likely due to the proliferation of acid-producing microorganisms in the bioreactor; and (ii) phase 3 (96-168 h): The pH continued to decrease, while discoloration unexpectedly rose to approximately 30%.

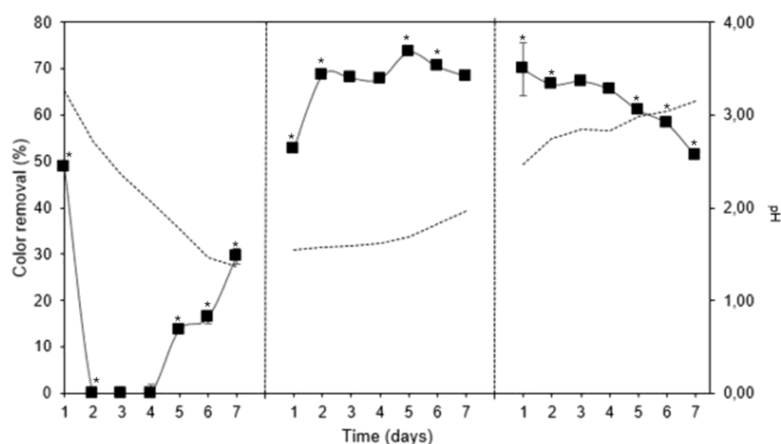

Figure 4 - RBBR discoloration percentage (■) and pH values (---) over the experimental period in the up-scaled PBB (45 L). Asterisks (*) above the error bars indicate statistically significant differences across sampling days ($p \leq 0.05$). Points without asterisks or error bars indicate no statistically significant differences between means.

In batches 2 and 3, increases in both pH and discoloration efficiency were observed. These changes are likely related to the growth of opportunistic microorganisms, which may have benefited from the absence of fungal competition. By the end of the 21-day period, discoloration efficiency returned to approximately 50%.

These control assay results are consistent with the findings of Chicatto et al. (2018a), who reported maximum dye adsorption within the first 8 hours of biomass contact (Chicatto et al., 2018b). The behavior observed during Phase 2 suggests bacterial proliferation in a favorable environment without fungal competition, resulting in a significant drop in pH. In Phase 3, the likely death of acid-sensitive bacteria and the possible colonization by WRF could explain the increase in discoloration. A similar pattern of phase-dependent discoloration was observed by Hermann et al. (Hermann et al., 2020) during the scale-up of rhodamine B removal under non-sterile conditions using *Agaricus brasiliensis*.

When compared with other fungal-based systems reported in the literature, the performance of the PBB used in this study is notably high. For instance, Cantele et al. (2017) reported discoloration efficiencies of approximately 75% for textile dyes using *Marasmiellus palmivorus* under solid-state fermentation (SSF), with laccase activities near 315 IU mL^{-1}. Similarly, Das et al. (2016) achieved approximately 80% discoloration of Congo red using *Pleurotus ostreatus*, with laccase activity around 120 IU mL^{-1}. Furthermore, the 91% RBBR removal achieved in the present study, with laccase activity reaching 847.2 IU mL^{-1}, surpasses the 75% discoloration reported in pilot-scale reactors using *Coriolopsis* sp. (Chen & Yien, 2015) and approaches the efficiency observed in continuous stirred tank reactors operated with *Phanerochaete chrysosporium*, which achieved 85-90% dye removal — but under sterile conditions and requiring strict aeration control (Li et al., 2015).

These results demonstrate the potential of *G. lucidum* as a promising biocatalyst for the discoloration of RBBR in PBBs operated under solid-state conditions at pilot scale. This study provides new insights into scaling up dye bioremediation processes using WRF and peach palm waste as a low-cost, lignocellulosic substrate. The findings reinforce the dual functionality of agro-industrial waste: acting both as a dye adsorbent and as a support for fungal colonization and enzyme production for xenobiotic degradation (Hermann et al., 2020; Serbent et al., 2023; Timm et al., 2024c).

Research into the biotechnological and bioremediation potential of basidiomycete fungi offers promising alternatives for addressing environmental challenges (Serbent et al., 2023; Timm et al., 2024c). The reuse of agro-industrial residues contributes to environmental protection and supports the achievement of the SDGs

and the zero-waste agenda (Timm et al., 2024a). Therefore, the approach adopted in this study may serve as an effective mycoremediation strategy for dye-contaminated effluents while simultaneously addressing the environmental burden of peach palm waste disposal — ultimately contributing to the advancement of SDGs and the circular bioeconomy.

Microbial rRNA gene taxonomic analyses

The analysis of amplified and sequenced rRNA genes has become a widely adopted method for identifying and classifying prokaryotic and eukaryotic communities. Metabarcoding through deep sequencing enables the generation of thousands of short rRNA reads per sample. Unlike traditional Sanger sequencing, it eliminates the need for labor-intensive clone library construction (Hamady et al., 2008; Liu et al., 2007). These techniques produce short reads (averaging approximately 200 bp), which have been effectively used to characterize microbial communities in both natural and artificial environments (Goffredi et al., 2011).

In the present study, prokaryotic and eukaryotic communities were analyzed via high-throughput sequencing of amplified fragments of the 16S and 18S rRNA genes. A total of 381,451 sequences for 16S rRNA and 220,325 sequences for 18S rRNA were obtained and used in downstream analyses. Prokaryotic microorganisms were classified into five phyla: *Acidobacteria*, *Actinobacteria*, *Bacteroidetes*, *Firmicutes*, and *Proteobacteria*. These were further distributed across 9 classes, 13 orders, 25 families (or respective OTUs), and 33 genera (or respective OTUs). No archaeal sequences were detected in the samples. The eukaryotic community was composed of two major groups: *Amoebozoa*, represented by the genus *Acanthamoeba*, and *Opisthokonta*, which included members from the phyla *Ascomycota*, *Basidiomycota*, and *Glomeromycota*.

Figure 5 presents the dominant microbial groups identified in biomass samples collected from the bioreactor at different time points: the initial time of the fungal inoculation process (A1 – after 21 days of inoculation plus 5 days of adaptation), the initial time of the control system (A3), and the final time point of the experimental process (A1 – 42 days after dye circulation). The results are shown at the phylum level for prokaryotes (a) and eukaryotes (b), and at the genus level for prokaryotes (c) and eukaryotes (d).

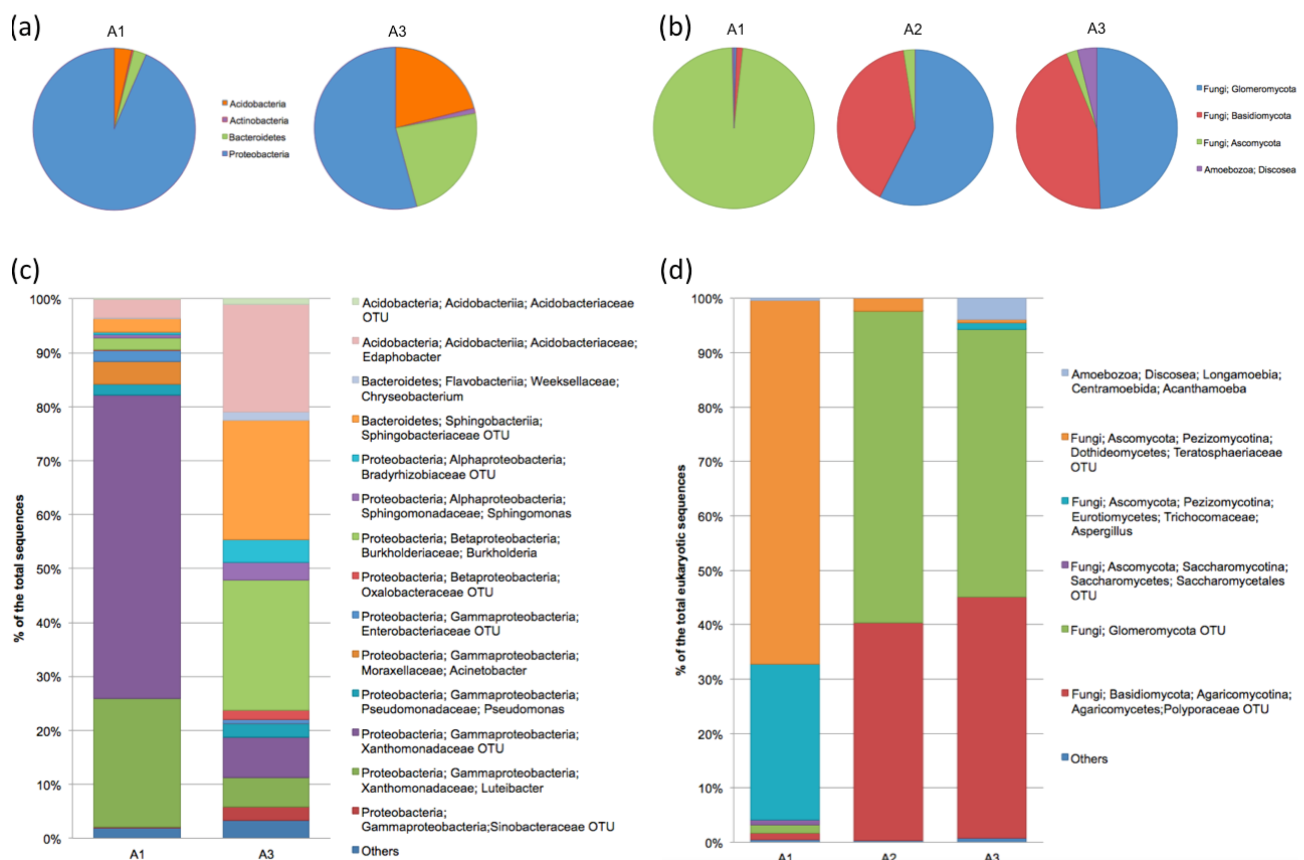

Figure 5 - Dominant microbial groups in biomass samples from the up-scaled PBB at different time points: initial stage of the fungal process (A1 – 21 days after inoculation and 5 days of adaptation), initial stage of the control system (A3), and final stage of the experimental process (A1 – 42 days after dye circulation). Data are presented at the phylum level for prokaryotic (a) and eukaryotic (b) microorganisms, and at the genus level for prokaryotic (c) and eukaryotic (d) microorganisms.

The bacterial community in the bioreactor was predominantly composed of the phylum *Proteobacteria* (Figure 5a). Similar results were reported by Chen et al. (2015), in a PBB used for the discoloration of Victoria Blue R. The consistent presence of *Proteobacteria* in PBBs has been observed in several other studies, highlighting their adaptability to various reactor configurations and environmental conditions (Fang et al., 2024; Hermann et al., 2020; Qiu et al., 2013; Rejish Kumar et al., 2009; Wery et al., 2003).

Over the course of the experiment, the relative abundance of *Proteobacteria* decreased from 93.4% in sample A1 to 54.2% in sample A3. Concurrently, there was a noticeable increase in the phyla *Acidobacteria*, *Actinobacteria*, and *Bacteroidetes* (Figure 5a). At the genus level (Figure 5c), two members of the family *Xanthomonadaceae* dominated the prokaryotic community in sample A1: an unclassified OTU (56.4%) and the genus *Luteibacter* (23.8%). Other relevant genera included *Acinetobacter* (4.2%) and *Edaphobacter* (3.5%). In sample A3, the most abundant genera were *Burkholderia* (24%), *Edaphobacter* (22.1%), and an OTU belonging to the family *Sphingobacteriaceae* (20%), together accounting for more than 66% of the total sequences.

Regarding the eukaryotic community (Figure 5b), members of *Opisthokonta* represented more than 99.6% of the total sequences in samples A1 and A2, and 96.1% in sample A3. The remaining 3.9% in A3 were classified as *Amoebozoa*. At the genus level (Figure 5d), a *Teratosphaeriaceae* OTU and the genus *Aspergillus* comprised 96% of the total sequences in sample A1 (28.6% and 66.9%, respectively). In samples A2 and A3, a *Polyporaceae* OTU and a *Glomeromycota* OTU accounted for more than 95% of the sequences (with average abundances of 42.2% and 53.2%, respectively).

The absence of sterilization led to modifications in the bioreactor microbiota, which were closely associated with pH fluctuations. This phenomenon has also been observed by Hermann et al. (Hermann et al., 2020). Notably, the predominance of basidiomycete fungi — according to rRNA gene sequencing — may have contributed to the relative pH stability, as this group is known for its ability to regulate environmental pH (Timm et al., 2022). In contrast, acidification of the system was observed when new microorganisms colonized the bioreactor during extended operation under non-sterile conditions (Hermann et al., 2020).

Amoebozoa, for example, is a diverse group of microorganisms capable of inhabiting a wide range of environments, including soil, air, freshwater, and wastewater (Duarte et al., 2013; Hermann et al., 2020). Amoebae feed on bacteria through predation, thereby influencing bacterial populations, nutrient recycling, and energy flow within microbial communities. Free-living amoebae have previously been identified in textile wastewater, despite the presence of toxic compounds such as dyes (Hermann et al., 2020; Ramírez-Cavazos et al., 2014). Despite the presence of diverse microbial populations, the discoloration process remained effective under the adopted experimental conditions, indicating the robustness of the system.

To the best of our knowledge, this is the first study to investigate the mycoremediation of RBBR by *Ganoderma lucidum* cultivated on peach palm shells. Further research is required to assess the techno-economic feasibility of scaling up this technology for industrial applications.

Although various techniques have been developed for the treatment of textile wastewater containing hazardous dyes (Ghaffar et al., 2023; Solaiman et al., 2024; Surana et al., 2024), it remains essential to not only advance in dye remediation but also to promote the development of safer, greener, and more sustainable technologies. Such efforts should aim to reduce chemical complexity and support the transition toward a circular, bio-based economy.

Conclusion

Recent advances in environmental decontamination research have stimulated the search for new alternatives for treating textile effluents. The use of basidiomycete fungi for dye decolorization represents a promising complementary treatment following conventional physicochemical processes, as demonstrated by the results of this study. *Ganoderma lucidum*, grown on peach palm waste under solid-state cultivation, produced extracellular laccase that played a key role in the discoloration process. In the lab-scale PBB (2 L), the discoloration efficiency reached 80%, while in the up-scaled system (45 L), it reached 91%. These findings underscore the potential of WRF and low-cost agro-industrial waste as a viable strategy for dye removal. This study provides an important first step toward the cyclic use of lignocellulosic biomass in up-scaled solid-state fermentation systems — an approach not previously reported in literature.

Data availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Acknowledgments

The authors would like to thank the Graduate Program in Environmental Engineering at the Universidade Regional de Blumenau (FURB), Blumenau, Santa Catarina, Brazil; the Forestry Unit of the Brazilian Agricultural Research Corporation (Embrapa Florestas), Colombo, Paraná, Brazil; and the High-Performance Computing Laboratory (LAD/PUCRS) for providing access to resources used in the high-throughput sequencing analyses. Special thanks are extended to Dr. Adriana Giongo and Dr. Leticia Marconatto from the Institute of Petroleum and Natural Resources, PUCRS, Rio Grande do Sul, Brazil, for their support with DNA sequencing and data analysis. This work was supported by the Brazilian Ministry of Science, Technology and Innovation (MCTI) (Grant 402593/2013-8), the Forestry unit of Brazilian Agricultural Research Corporation (Embrapa Florestas, Brazil), and the National Council for Scientific and Technological Development (CNPq, Brazil) [Grant number MCTI/CNPq Nº 20/2017]. The project was funded through scholarships provided by the Coordination for the Improvement of Higher Education Personnel (CAPES), Brazil [Finance Code 001] and [DS 88887.490173/2020-00]) and the National Council for Scientific and Technological Development (CNPq). The author LBB Tavares is a fellowship holder of CNPq (Grant number [305880/2020-9-*CNPq No 02/2020]).

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