

RESEARCH ARTICLE / REVIEW ARTICLE



EXTRACTION OF LACCASE ENZYME *PHANEROCHAETE CHRYSOSPORIUM* IN RICE HUSK MEDIA FOR TOTAL PETROLEUM HYDROCARBON REDUCTION

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Article history

Received

25 Mei 2026

Received in revised form

06 June 2026

Accepted

22 Okt 2026

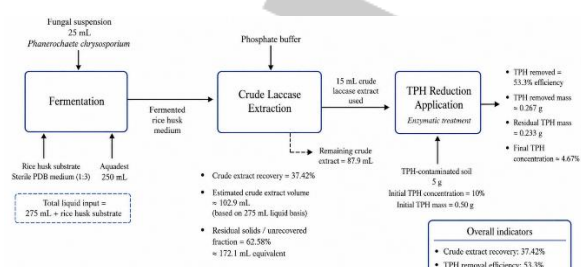
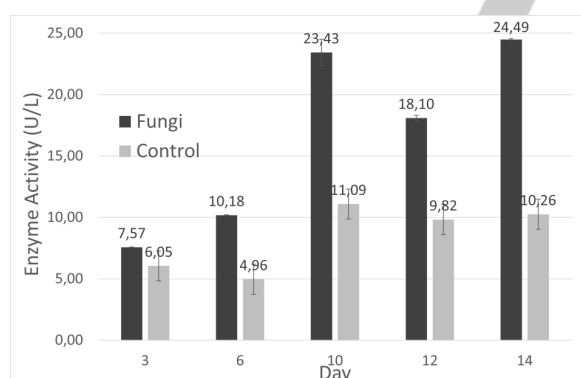
Published online

31 Des 2026

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Graphical abstract



ABSTRACT

Total Petroleum Hydrocarbon (TPH) is a persistent soil contaminant that requires effective and sustainable remediation strategies. This study investigates the production and application of crude laccase enzyme from *Phanerochaete chrysosporium* cultivated using rice husk as a low-cost lignocellulosic substrate for TPH reduction in contaminated soil. Fungal cultures were initially propagated on PDA medium and subsequently transferred to PDB supplemented with rice husk under controlled conditions of 30°C and pH 7. Crude laccase was extracted using phosphate buffer, and its enzymatic activity was measured spectrophotometrically using ABTS as a substrate. After 14 days of incubation, laccase activity reached 24.49 U/L, with a maximum crude enzyme yield of 37.42%. The application of 15 mL of crude laccase extract to 5 g of contaminated soil reduced TPH concentration from 10% to 4.67%, achieving a removal efficiency of 53.3%. Laccase facilitates hydrocarbon degradation through oxidation reactions, particularly targeting aromatic and phenolic compounds. These findings demonstrate the potential of rice husk as an economical substrate for fungal laccase production and support the application of crude laccase as a promising approach for sustainable soil bioremediation. Further optimization of enzyme production and treatment conditions is recommended to enhance remediation efficiency.

Keywords: Total Petroleum Hydrocarbon, Laccase, *Phanerochaete chrysosporium*, Rice Husk, Soil Bioremediation.

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1. INTRODUCTION

Total petroleum hydrocarbons (TPH) are complex mixtures of hydrocarbons derived from crude oil and petroleum-based products, consisting of aliphatic, alicyclic, aromatic, and high-molecular-weight hydrocarbon fractions. TPH contamination commonly occurs in soils, sediments, and aquatic environments. This type of contamination causes many fractions are hydrophobic, persistent, poorly biodegradable, and toxic to living organisms. Recent reviews emphasize that petroleum hydrocarbon-contaminated soils remain a major environmental concern and require remediation strategies not only effective but also environmentally sustainable and economically feasible (Mekonnen et al., 2024; Elshafei et al., 2024).

Mycoremediation has emerged as a promising biological approach for petroleum hydrocarbon contamination treatment. Unlike conventional physical and chemical remediation methods which more Unlike conventional physical and chemical remediation methods, which are often costly, energy-intensive, and may generate secondary pollutants. Fungal-based remediation relies on the ability of fungi to grow on complex organic substrates and produce extracellular enzymes capable of transforming recalcitrant pollutants. White-rot fungi possess ligninolytic enzyme systems, including laccase, lignin peroxidase, and manganese peroxidase, which are involved in the degradation of lignin and structurally related xenobiotic compounds. Recent studies have reported that mycoremediation can substantially reduce petroleum hydrocarbon concentrations in contaminated soils which is strongly influenced by substrate availability, incubation period, contaminant composition, pH, temperature, moisture, and oxygen availability (Antón-Herrero et al., 2023; Cipriani-Avila et al., 2025). Hence, potential fungal strain as inoculum bioagent is the most important factor for mycoremediation.

The huge diversity of fungi species alter further study to select preferred fungal strain. Among white-rot fungi, *Phanerochaete chrysosporium* has been widely studied as strong ligninolytic capability and its ability to degrade persistent organic pollutants, including petroleum-related aromatic compounds. The oxidative enzyme, especially ligninolytic enzyme, produced to breakdown of complex hydrocarbon structures. Recent experimental work on petroleum refinery wastewater also indicates that *P. chrysosporium* can contribute to pollutant removal through enzyme-mediated degradation mechanisms under controlled process conditions (Negi et al., 2025). Therefore, *P. chrysosporium* is a relevant fungal candidate for developing enzyme-based strategies for TPH reduction.

One of ligninolytic enzyme, laccase, is the key extracellular enzymes involved in fungal degradation of environmental pollutants. It is a multicopper oxidase that catalyses oxidation reactions using molecular oxygen as the final electron acceptor. Laccase can oxidize specific substrate such as phenolic compounds, aromatic hydrocarbons, dyes, endocrine-disrupting compounds, pharmaceuticals, and several petroleum-related pollutants. Recent reviews describe laccase as a versatile green biocatalyst for pollutant degradation, while studies on petroleum-contaminated matrices have shown that laccase-mediated treatment can significantly reduce persistent hydrocarbon fractions, including high-molecular-weight compounds that are usually resistant to degradation (Dong et al., 2023; Diefenbach et al., 2024).

Although direct incubation of fungi in contaminated media has been widely applied in mycoremediation, this approach may present several limitations. Direct fungal incubation requires the fungus to survive, colonize, and remain metabolically active under contaminated conditions,

which may be inhibited by petroleum toxicity, nutrient imbalance, limited oxygen transfer, unsuitable pH, low moisture, or competition with indigenous microorganisms. Reviews on petroleum hydrocarbon bioremediation emphasize that biological removal efficiency is highly dependent on environmental conditions and system design, suggesting that direct microbial or fungal treatment may not always provide consistent or rapid degradation performance (Mekonnen et al., 2024; Antón-Herrero et al., 2023).

For this reason, the use of extracted enzymes offers an alternative to pollutant removal. In an enzyme-based system, the active biocatalyst can be applied directly to the contaminated matrix without requiring fungal growth during the remediation stage. This approach may provide better control of enzyme dosage, contact time, reaction conditions, and treatment performance. The extraction of crude laccase also creates an important bridge toward future development of purified laccase or immobilized laccase systems. (Zhou et al., 2023; Adamian et al., 2021; Chen et al., 2025). Therefore, producing and extracting laccase from *P. chrysosporium* before application to TPH-contaminated samples is scientifically relevant because it separates enzyme production from pollutant degradation and provides a basis for future development of purified, kinetic, mediator-assisted, or immobilized enzyme systems with higher efficiency and better operational control (Hasan et al., 2023; Younus et al., 2025; Diefenbach et al., 2024)

Rice husk is a promising and low-cost lignocellulosic substrate for fungal laccase production because it is abundant in rice-producing countries and contains cellulose, hemicellulose, lignin, and silica, which can support fungal growth and stimulate ligninolytic enzyme production. Agricultural residues such as rice husk are suitable for solid-state fermentation because this method is simpler, cheaper, and more appropriate for fungal enzyme production than submerged fermentation (Wang et al., 2019). In addition, using rice husk provides an environmental benefit by converting agricultural waste into a value-added biocatalyst for pollution control. Previous studies have shown that rice husk and other agro-industrial residues can be used as substrates for laccase production, supporting their potential as fermentation media for *Phanerochaete chrysosporium* cultivation and future application in total petroleum hydrocarbon (Perdani et al., 2020).

Despite the growing body of research on mycoremediation, fungal laccase, and agricultural waste-based enzyme production, studies that directly combine rice husk-based laccase production by *P. chrysosporium* with the application of extracted enzyme for TPH reduction remain limited. Most previous studies have focused separately on whole-fungus mycoremediation, optimization of laccase production, or enzymatic degradation of selected pollutants. Therefore, this study addresses an important research gap by extracting laccase enzyme produced from *P. chrysosporium* cultivated on rice husk media and evaluating its potential for reducing TPH. This approach is expected to support the development of a low-cost, enzyme-based, and scalable bioremediation strategy for petroleum hydrocarbon-contaminated environments.

2. MATERIALS AND METHODS

A. Media Culture Preparation

The culture media used in this study consisted of Potato Dextrose Agar (PDA), Potato Dextrose Broth (PDB), and rice husk as the lignocellulosic substrate for laccase production. PDA medium was prepared for fungal maintenance and culture activation, while PDB was used as the liquid nutrient medium during enzyme production. Rice husk was used as the solid substrate to support fungal

growth and stimulate ligninolytic enzyme production. Prior to use, the rice husk was washed with distilled water to remove adhering impurities, then soaked with 1 M NaOH for 24 hours, and after that dried for 2 days. Rice husk cut or ground into smaller particles to increase the surface area for fungal colonization. The PDA, PDB, and rice husk media were sterilized using an autoclave at 121 °C and 15 psi for 15–20 minutes.

B. *Phanerochaete chrysosporium* Cultivation

Phanerochaete chrysosporium was cultivated on PDA medium under aseptic conditions in a laminar airflow cabinet. The fungal culture was transferred onto the surface of sterile PDA plates. The inoculated PDA plates were then incubated at 30°C for 7 days to allow sufficient mycelial growth. After incubation, actively growing fungal colonies were selected to ensure high metabolic activity and robust enzyme production, as only vigorous colonies can efficiently secrete extracellular laccases during subsequent fermentation. Active fungal colonies used as the inoculum for the subsequent laccase production stage.

C. Incubation in Rice Husk Media

After 7 days of cultivation on PDA, the *P. chrysosporium* culture was harvested and suspended in sterile Tween 80 (polysorbate)[R9.1][r9.2] solution to facilitate the release and dispersion of fungal spores or mycelial fragments. A total of 25 mL of fungal suspension was then inoculated into sterile PDB medium containing rice husk as the lignocellulosic substrate (1:3) with 250 ml of aquades. The mixture of PDB and rice husk served as the fermentation medium for laccase production. The inoculated media were incubated at 30 °C and pH 7 for 7 days under shaking conditions. During incubation, *P. chrysosporium* colonized the rice husk substrate and produced extracellular ligninolytic enzymes, including laccase, into the surrounding medium (Perdani et al., 2020; Wang et al., 2019; Wang et al., 2023).

D. Crude Enzyme of Laccase Extraction

After the incubation period, crude laccase enzyme was extracted from the fermented rice husk medium. The fermented medium was mixed with buffer phosphate at pH 7 to dissolve and recover extracellular enzymes produced during fungal incubation. The mixture was adjusted at pH 7, then gently shaken in water bath for 1 hour in 30 °C with 100 rpm. The filtrate was centrifuged at 7500 rpm for 15 minutes at 5 °C to remove remaining fungal biomass and suspended particles. The clear supernatant obtained after centrifugation was collected as the crude laccase enzyme extract and stored at 1 °C until further analysis and application (Singh et al., 2017; Santos et al., 2019; Zahoor et al., 2021).

E. Enzyme Activity Measurement

Laccase activity was measured to evaluate the enzymatic potential of the crude extract obtained from *P. chrysosporium* grown on rice husk media. The enzyme activity assay was conducted using ABTS as the oxidation substrate. The reaction mixture consisted of 1,5 mL of buffer solution, 0,45 mL of substrate ABTS, and 1,5 mL of crude enzyme extract. The reaction was incubated at 27 °C for 5 minutes, and the change in absorbance was measured using a UV–Vis spectrophotometer at 420 nm. Laccase activity was calculated based on the increase in absorbance due to substrate oxidation. One unit of laccase activity was defined as the amount of enzyme required to oxidize 1 µmol of substrate per minute under the assay conditions. The enzyme activity was expressed as U/mL.

A general formula for enzyme activity can be written as:

$$\text{Laccase Activity (U/L)} = \frac{\Delta A \times V_{\text{total}} \times 10^6}{\varepsilon \times l \times t \times V_{\text{enzyme}}} \quad (1)$$

Where ΔA is the change in absorbance, V_{total} is the total reaction volume, ε is the molar extinction coefficient of ABTS ($36000 \text{ M}^{-1} \text{ cm}^{-1}$), l is the cuvette path length, t is the reaction time, and V_{enzyme} is the volume of enzyme extract used in the assay (Jiang et al, 2019; Prawitasari&Kardena, 2026).

F. Total Petroleum Hydrocarbon Treatmeant

The crude laccase enzyme extract was applied to TPH-soil contaminated samples with concentration 10% to evaluate its potential for petroleum hydrocarbon reduction. The treatment system was prepared by adding 15 mL of crude laccase extract to 5 g of TPH-contaminated sample. The treatment was conducted in 50 ml flask under controlled conditions at 30°C for 6 hours. A control treatment without crude enzyme addition was prepared to compare the natural reduction of TPH with enzyme-assisted degradation. Additional controls, such as sterilized enzyme or buffer-only treatment, may also be included to confirm the role of active laccase in TPH reduction.

After the treatment period, residual TPH concentration was determined using a gravimetric method with n-hexane as the extraction solvent. Briefly, 10 ml of treated sample was transferred into a separatory funnel, followed by the addition of 10 mL of n-hexane. The mixture was shaken vigorously for 30 minutes to extract petroleum hydrocarbons into the organic phase. After phase separation, the n-hexane layer was collected. The combined n-hexane extract was then passed through anhydrous sodium sulphate to remove residual water and transferred into a pre-weighed evaporation dish or glass vial.

The n-hexane solvent was evaporated at 80°C until a constant weight was obtained. The remaining residue was considered as the extractable petroleum hydrocarbon fraction. The TPH concentration was calculated based on the difference between the final and initial weight of the evaporation. The TPH removal efficiency was calculated by comparing the initial and final TPH concentrations using the following equation:

$$\text{TPH Removal Efficiency (\%)} = \frac{C_0 - C_t}{C_0} \times 100 \quad (2)$$

where C_0 is the initial TPH concentration and C_t is the TPH concentration after treatment (Hopkinson & Smith, 2007; Ghazali et al., 2017).

3. RESULT AND DISCUSSION

A. Yield (%) Crude Extract Laccase Enzyme

The yield of crude laccase enzyme extracted from *Phanerochaete chrysosporium* cultivated on rice husk waste media showed a progressive increase during the incubation period. As shown in Table 1, the crude enzyme yield increased from $5.96 \pm 0.20\%$ on day 3 to $14.92 \pm 0.46\%$ on day 6, then continued to increase to $17.47 \pm 0.11\%$ on day 10, $21.60 \pm 0.40\%$ on day 12, and reached the highest value of $37.42 \pm 0.22\%$ on day 14 (Table 1).

Table 1. Yield of Crude Extract Laccase Enzyme

Day	%Yield
3	5,96 ± 0,2
6	14,92 ± 0,46
10	17,47 ± 0,11
12	21,60 ± 0,4
14	37,42 ± 0,22

This progressive increase indicates that laccase secretion is strongly dependent on fermentation time to colonize the rice husk substrate and produce extracellular ligninolytic enzymes. Similar temporal patterns have been reported for other white-rot fungi, for example, *Ganoderma applanatum* achieved peak laccase activity (220.14 U L^{-1}) only after 14 days of submerged cultivation on wheat straw (Ćilerdžić, Vukojević, & Stajić, 2017). The observed rise in yield up to day 14 suggests that *P. chrysosporium* continues to secrete laccase as it degrades the lignin-rich rice husk, and that the extraction process was able to recover the enzyme efficiently throughout this period without pronounced proteolytic degradation (Wang et al., 2019; Avanthi et al., 2016). This result is consistent with the general principle of solid-state fermentation, where lignocellulosic agricultural residues can function not only as physical support but also as nutrient sources and natural inducers for fungal laccase production (Wang et al., 2019).

The relatively low yield during the early incubation period may be related to the adaptation and initial growth phase of *P. chrysosporium*. During the first few days, fungal metabolism is mainly directed toward substrate colonization, mycelial development, and the utilization of easily available nutrients (Konan et al., 2024; Wang et al., 2019). As incubation proceeds, the fungus begins to interact more intensively with the lignocellulosic structure of rice husk, which contains cellulose, hemicellulose, lignin, and silica as carbon source. Lignin and lignin-derived compounds are important because they can stimulate the production of ligninolytic enzymes, including laccase. Therefore, the increasing yield from day 6 to day 14 suggests that rice husk was able to support fungal growth and induce enzyme production during the later fermentation stage. The present results demonstrate that rice husk is an effective inducer for *P. chrysosporium* laccase production under SSF conditions. Moreover, SSF itself is known to provide several advantages over submerged fermentation (SmF), including a higher volumetric productivity, lower risk of contamination, and better oxygenation of the fungal mycelium, all of which favour the accumulation of extracellular enzymes (Hasan et al, 2023). A comparative study of *P. chrysosporium* secretomes under SSF and SmF found that the fungus secreted more enzyme species and with higher activities when grown in SSF (Perdani et al, 2020). The rising yield from day 3 to day 14 therefore reflects both the inherent kinetics of enzyme production in this system and the favourable conditions provided by rice husk-based SSF.

The highest crude enzyme yield obtained on day 14 indicates that this incubation period may represent the most favourable extraction time. The increasing yield of crude laccase enzyme has important implications for the development of low-cost biocatalysts for environmental remediation. The use of rice husk waste as a fermentation medium can reduce the cost of enzyme production while also contributing to agricultural waste valorisation (Perdani et al., 2020; Wang et

al., 2019). This is relevant because one of the major limitations of enzyme-based treatment is the cost of enzyme production and downstream processing.

B. Enzyme Activity of Laccase

The laccase enzyme activity showed a dynamic pattern during the incubation period, with consistently higher activity in the fungal treatment than in the control. As shown in Figure 1, laccase activity in the fungal treatment increased from 7.57 U/L on day 3 to 10.18 U/L on day 6, followed by a sharp increase to 23.43 U/L on day 10. After that, the activity decreased to 18.10 U/L on day 12, before reaching the highest value of 24.49 U/L on day 14. In comparison, the control treatment showed lower enzyme activity throughout the incubation period, ranging from 4.96 to 11.09 U/L, and ending at 10.26 U/L on day 14. This result indicates that the presence of *Phanerochaete chrysosporium* in rice husk media substantially enhanced laccase activity compared with the control, confirming the role of fungal metabolism in producing extracellular oxidative enzymes.

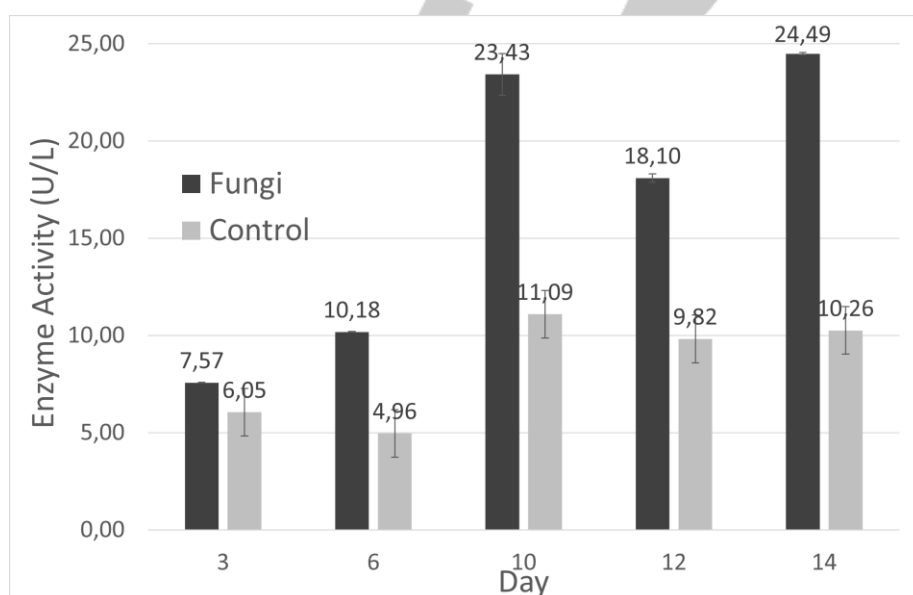


Figure 1. Enzyme Activity of Lacasse

The increase in laccase activity from day 3 to day 10 suggests that *P. chrysosporium* actively colonized the rice husk substrate and began producing ligninolytic enzymes as part of its metabolic adaptation, a pattern similarly observed in other white-rot fungi where enzymatic activity increased with prolonged incubation time (Reyes, Cajiao, & Ospina, 2021). Rice husk is a lignocellulosic material containing cellulose, hemicellulose, and lignin, and such materials can act as both nutrient sources and natural inducers for fungal laccase production. Lignocellulosic agricultural wastes are suitable substrates for fungal laccase production, especially under solid-state fermentation, because they can support fungal growth and stimulate ligninolytic enzyme synthesis (Wang et al. 2019). Therefore, the high activity observed on day 10 may reflect the phase when fungal biomass growth and enzyme secretion were actively occurring in response to the lignin-rich structure of rice husk.

The decrease in activity on day 12 may be caused by changes in nutrient availability, accumulation of metabolic by-products, enzyme instability, or partial inhibition during the incubation process. Enzyme production by fungi does not always increase linearly with incubation time because it is affected by pH, temperature, substrate composition, moisture, oxygen availability,

and fungal physiological stage. However, the subsequent increase to 24.49 U/L on day 14 indicates that laccase production was still active and may have entered another productive phase after further substrate degradation. This fluctuation is reasonable in fungal enzyme production because laccase is an extracellular enzyme whose activity depends not only on fungal growth but also on the availability of lignin-derived inducers and the stability of the enzyme in the fermentation medium (An et al, 2018). Fungal laccases are known as extracellular multicopper oxidases, and their structural and catalytic properties allow them to oxidize a wide range of phenolic and aromatic compounds (Mehra et al., 2018).

The higher laccase activity in the fungal treatment has important implications for pollutant degradation, particularly for Total Petroleum Hydrocarbon (TPH) reduction. Laccase catalyzes oxidation reactions using molecular oxygen as the final electron acceptor and can initiate the transformation of aromatic and phenolic pollutant structures (Diefenbach et al., 2024). Although TPH is a complex mixture containing aliphatic and aromatic hydrocarbon fractions, the oxidative activity of laccase may contribute to partial transformation of petroleum hydrocarbons, especially aromatic or high-molecular-weight fractions (Diefenbach et al., 2024; Younus et al., 2025). Dong et al. (2023) highlighted laccase as a versatile biocatalyst for pollutant degradation because of its broad substrate specificity and potential application under mild environmental conditions. This supports the interpretation that higher laccase activity may improve the ability of crude enzyme extract to transform or reduce petroleum-related contaminants.

The activity profile also suggests that incubation time is a critical parameter for determining the best harvesting period of crude laccase enzyme. Based on the data, day 14 produced the highest laccase activity, while day 10 also showed a strong activity peak. These two points may be considered as potential optimal extraction times, depending on whether the study prioritizes maximum enzyme activity, shorter production time, or process efficiency. For future application, the crude laccase extract can be further optimized through pH adjustment, enzyme purification, mediator-assisted oxidation, or immobilization to improve stability and reusability. This is particularly relevant for TPH remediation because recent evidence showed that laccase-mediated treatment significantly reduced persistent petroleum hydrocarbons in contaminated soil, including heavier hydrocarbon fractions (Diefenbach et al., 2024). Therefore, the laccase activity obtained from rice husk-based fungal cultivation provides a promising basis for developing low-cost enzyme-based treatment systems for petroleum hydrocarbon reduction.

C. Laccase Enzyme Application for Total Hydrocarbon Petroleum (TPH) Removal

The application of crude laccase enzyme showed a clear reduction in Total Petroleum Hydrocarbon (TPH) concentration. As presented in Figure 2[R10.1][r10.2], the initial TPH concentration before enzymatic treatment was 15%, while the residual TPH concentration after treatment decreased to 7%. This result indicates an absolute decrease of 8 percentage points, corresponding to approximately 53.3% TPH removal efficiency. The decrease suggests that the crude laccase extract produced from *Phanerochaete chrysosporium* cultivated on rice husk media was able to contribute to the transformation or degradation of petroleum hydrocarbon compounds. This finding showing that laccase-mediated treatment can enhance the degradation of petroleum hydrocarbons, including more persistent hydrocarbon fractions in contaminated matrices (Diefenbach et al., 2024).

The observed TPH reduction supports the potential role of laccase as an oxidative biocatalyst for petroleum hydrocarbon remediation. Laccase is a multicopper oxidase enzyme that catalyze one-electron oxidation reactions using molecular oxygen as the final electron acceptor. Because of its broad substrate specificity, laccase can oxidize several aromatic and phenolic pollutants, and its reactivity may be extended to more complex non-phenolic compounds through the presence of natural or added redox mediators (Mate et al., 2016; Younus et al., 2025). Laccase can operate under relatively mild conditions and participate in oxidative transformation of recalcitrant organic pollutants (Dong et al. 2023). Therefore, the reduction of TPH in this study may be attributed to the oxidative activity of crude laccase toward petroleum hydrocarbon components, especially aromatic or partially oxidizable fractions.

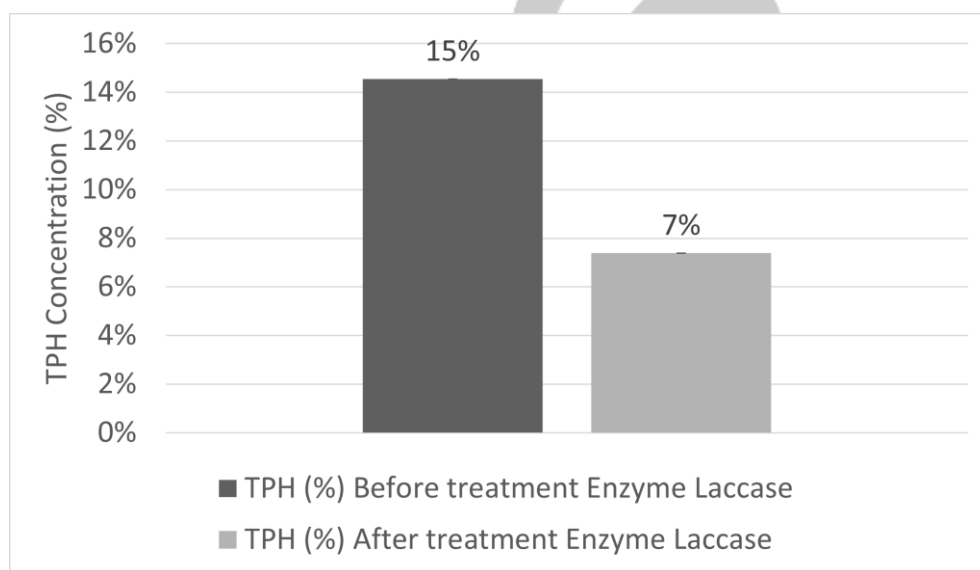


Figure 2. TPH Reduction with Crude Enzyme Laccase Treatment

Mechanistically, laccase does not necessarily mineralize all petroleum hydrocarbon compounds directly into carbon dioxide and water in a single step. Instead, laccase may initiate oxidation reactions that produce hydrocarbon radicals, quinone-like structures, alcohols, aldehydes, ketones, carboxylic acids, or other oxygenated intermediates (Diefenbach et al., 2024; Dai et al., 2021). These transformed products are generally more polar than the parent hydrocarbons, which may increase their susceptibility to further biodegradation or reduce their extractability by non-polar solvents such as n-hexane during gravimetric TPH analysis. In this context, the decrease in measured TPH may reflect a combination of enzymatic oxidation, partial degradation, structural transformation, and reduced recovery of extractable hydrocarbon residues. Diefenbach et al. (2024) also reported that laccase treatment was particularly effective in reducing heavier petroleum hydrocarbon fractions, indicating that laccase-mediated oxidation can contribute to the transformation of persistent hydrocarbons.

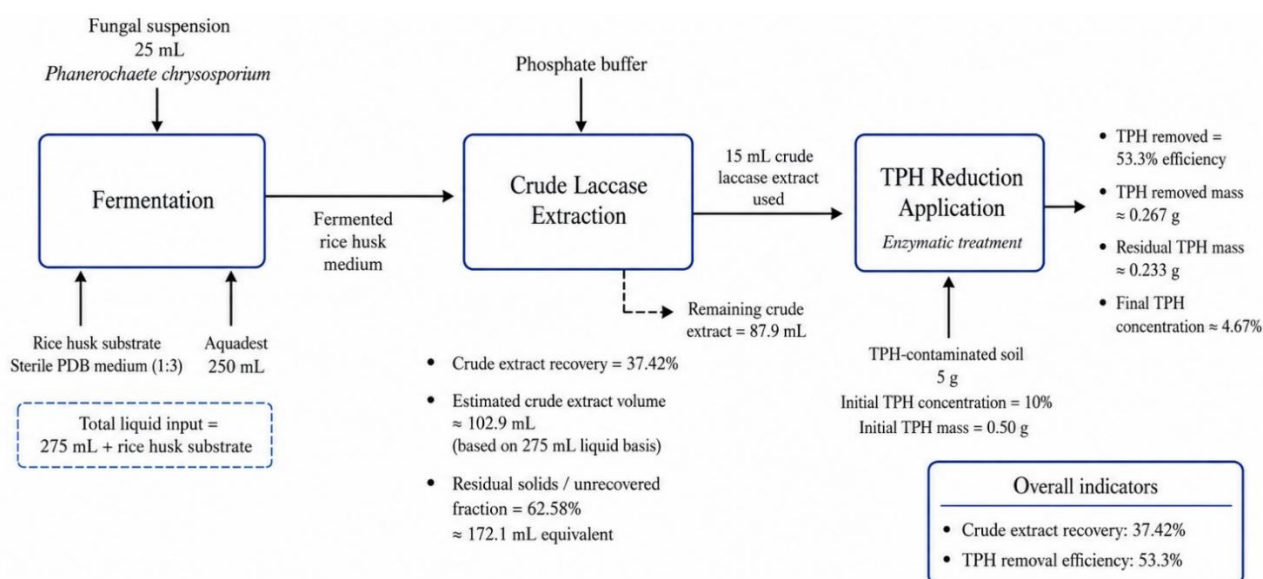


Figure 3. Mass Balance Crude Enzyme Extraction and TPH Reduction Application

The use of crude laccase enzyme also provides an important implication compared with direct fungal incubation. In direct mycoremediation, pollutant removal depends on fungal survival, adaptation, growth, and continuous enzyme secretion in contaminated media (Cahuich-Flores et al., 2026). These processes can be affected by petroleum toxicity, nutrient limitation, oxygen availability, pH, and competition with indigenous microorganisms. By contrast, crude enzyme application separates the enzyme production stage from the pollutant degradation stage, allowing the active enzyme extract to be applied directly to the contaminated sample. This approach provides better control over enzyme dosage, contact time, and treatment conditions. It also creates a pathway for future development of purified laccase, mediator-assisted laccase systems, or immobilized laccase to improve stability, reusability, and degradation efficiency. Recent reviews highlight that immobilization can help overcome several limitations of free laccase, especially low operational stability and limited reusability in environmental applications (Dong et al., 2023).

Overall, the reduction of TPH from 15% to 7% demonstrates that crude laccase enzyme extracted from *P. chrysosporium* cultivated on rice husk media has promising potential as a low-cost biocatalyst for petroleum hydrocarbon reduction to strengthens the environmental value. Lignocellulosic agricultural residues have been widely reported as suitable substrates for fungal laccase production under solid-state fermentation due to their low cost, availability, and ability to stimulate ligninolytic enzyme production (Wang et al., 2019). Therefore, this study offers a preliminary basis for integrating agricultural waste valorization with enzyme-based TPH remediation. However, further analysis using GC-FID or GC-MS is recommended to identify which hydrocarbon fractions are degraded, transformed, or merely reduced in extractability after enzymatic treatment.

D. Mass Balance Crude Enzyme Extraction and TPH Reduction Application

process highlights the efficiency and potential of rice husk-based fungal cultivation as a sustainable enzyme source (Figure 3). From a total liquid input of 250 mL PDB medium plus 25 mL fungal suspension containing rice husk as substrate, 37.42% of the medium was recovered as crude

laccase extract, while the remaining 62.58% constituted residual solids, unextracted medium, and biomass. The extraction yielded an enzymatically active solution with a laccase activity of 24.49 U/L, providing sufficient catalytic potential for downstream application to TPH-contaminated soil.

This demonstrates that lignocellulosic agricultural residues can serve both as low-cost nutrient sources and inducers for laccase production, aligning with recent studies that emphasize the valorization of agro-wastes for fungal enzyme generation into a value-added enzyme product and bioremediation applications. When applied to 5 g of TPH-contaminated soil with an initial hydrocarbon concentration of 10%, 15 mL of the crude laccase extract removed approximately 53.3% of the TPH, leaving a residual hydrocarbon fraction of 4.67%. This indicates that the enzyme not only catalysed oxidation reactions on aromatic and aliphatic hydrocarbons but also enhanced the solubilization and bioavailability of TPH for microbial or chemical transformation. The mass balance analysis confirms that controlled enzyme dosing can optimize pollutant reduction while minimizing residual unreacted material, a strategy supported by the observed effectiveness of filamentous fungal laccases in industrial wastewater and soil remediation contexts.

The implications of this mass balance extend to sustainable process design and industrial application. By separating enzyme production from pollutant treatment, process control can be improved in terms of dosage, incubation time, and operational stability, while enabling the possibility of enzyme immobilization or purification for repeated use. Immobilized enzyme systems are particularly promising because they can enhance operational stability and enable repeated use of enzymes in wastewater or contaminated soil treatment applications (Rana et al., 2022). This approach also reduces dependency on in situ fungal growth, mitigates the risk of inhibitory effects from pollutants, and aligns with circular bioeconomy principles by converting rice husk into a high-value enzyme product.

4. CONCLUSION

Crude laccase enzyme produced by *Phanerochaete chrysosporium* using rice husk as a substrate exhibits substantial potential for petroleum hydrocarbon remediation. The progressive increase in enzyme yield and activity demonstrates effective fungal growth and ligninolytic enzyme production under solid-state fermentation conditions. Application of the crude enzyme to TPH-contaminated soil can be achieved 53.3% reduction, highlighting its catalytic capacity in oxidizing aromatic and high-molecular-weight hydrocarbon fractions. Utilizing rice husk not only provides a low-cost medium for enzyme production but also adds value to agricultural residues. This enzyme-based approach separates production from pollutant degradation, allowing controlled application and paving the way for future optimization through purification, mediator-assisted oxidation, or immobilization. Overall, the study confirms the feasibility of integrating agro-waste valorization with sustainable bioremediation strategies for petroleum-contaminated environments.

Although this study demonstrates the potential of crude laccase enzyme from *Phanerochaete chrysosporium* for TPH reduction, it has several limitations. The experiments were conducted at laboratory scale, and the enzyme extraction and application conditions may not fully reflect environmental variability in field soils. Additionally, only crude enzyme activity was measured, without purification or immobilization, which could affect stability and efficiency. Future research should explore enzyme purification, immobilization, mediator-assisted oxidation, and optimization of fermentation conditions to improve TPH removal efficiency. Investigations on scale-up, soil type

variability, and long-term enzyme stability in situ are also recommended to advance the practical application of this bioremediation strategy.

Acknowledgment

The authors would like to express their sincere gratitude to the Department of Environmental Engineering, Universitas Islam Indonesia, for providing the laboratory facilities and technical support necessary for conducting this research. This study was funded and supported by the Department of Environmental Engineering, Universitas Islam Indonesia, which made the experimental work and analysis possible. The authors also thank all colleagues and staff who contributed to the successful completion of this project.

REFERENCES

- An, Q., Ma, H., Han, M., Si, J., & Dai, Y. (2018). Effects of different induction media as inducers on laccase activities of *Pleurotus ostreatus* strains in submerged fermentation. *BioResources*, 13(1), 1143–1156. <https://bioresources.cnr.ncsu.edu/resources/effects-of-different-induction-media-as-inducers-on-laccase-activities-of-pleurotus-ostreatus-strains-in-submerged-fermentation/>
- Aziz, G. M., Hussein, S. I., M-Ridha, M. J., Mohammed, S. J., Abed, K. M., Muhamad, M. H., & Abu Hasan, H. (2023). Activity of laccase enzyme extracted from *Malva parviflora* and its potential for degradation of reactive dyes in aqueous solution. *Biocatalysis and Agricultural Biotechnology*, 50, 102671. <https://doi.org/10.1016/j.bcab.2023.102671>
- Ćilerdžić, J. L., Vukojević, J. B., & Stajić, M. M. (2017). Dynamics of ligninolytic enzyme production in *Ganoderma applanatum* depending on cultivation type. *Zbornik Matice Srpske za Prirodne Nauke*. <https://doaj.org/article/fe612820425d4d998724b0d22c30abf4>
- Cahuich Flores, S. G., López Martínez, S., Cappello García, S., Morales Bautista, C. M., Pampillón González, L., Ramírez Álvarez, N., & Zurita Macías Valadez, M. (2026). Mycoremediation of a hydrocarbon contaminated soil using white rot fungi. *Bioremediation Journal*. Advance online publication. <https://doi.org/10.1080/10889868.2026.2641536>
- Dai, X., Chen, W., Zhang, T., & Liu, L. (2021). Effects of adding laccase to bacterial consortia degrading heavy oil. *Processes*, 9(11), 2025. <https://doi.org/10.3390/pr9112025>
- Diefenbach, T., Sumetzberger-Hasinger, M., Braunschmid, V., Konegger, H., Heipieper, H. J., Guebitz, G. M., Lackner, M., Ribitsch, D., & Loibner, A. P. (2024). Laccase-mediated degradation of petroleum hydrocarbons in historically contaminated soil. *Chemosphere*, 348, 140733. <https://doi.org/10.1016/j.chemosphere.2023.140733>
- Dong, C.-D., Tiwari, A., Anisha, G. S., Chen, C.-W., Singh, A., Haldar, D., Patel, A. K., & Singhania, R. R. (2023). Laccase: A potential biocatalyst for pollutant degradation. *Environmental Pollution*, 319, 120999. <https://doi.org/10.1016/j.envpol.2023.120999>
- Ghazali, F. M., Rahman, R. N. Z. A., Salleh, A. B., Basri, M., & Yassin, M. M. (2017). *Biodegradation of petroleum hydrocarbons by indigenous microbial consortium in contaminated soil*. International Biodeterioration & Biodegradation, 117, 36–47. <https://doi.org/10.1016/j.ibiod.2016.11.009>
- Hasan, S., et al. (2023). Laccase production from local biomass using solid state fermentation: Insights into enzyme stability and productivity advantages. *Fermentation*, 9(2), 179. <https://doi.org/10.3390/fermentation9020179>

- Hopkinson, P., & Smith, P. (2007). *Comparison between gas chromatographic and gravimetric techniques for the determination of total petroleum hydrocarbons in soil samples*. *Environmental Chemistry Letters*, 5(4), 271–274. <https://doi.org/10.1007/s10311-007-0096-1>
- Jiang, F., Zeng, Q., & Xu, X. (2019). *Optimization of laccase production and ABTS enzyme assay for medicinal mushroom Ganoderma species*. *Journal of Applied Microbiology*, 126(2), 448–459. <https://doi.org/10.1111/jam.14255>.
- Konan, D., Ndao, A., Koffi, E., Elkoun, S., Robert, M., Rodrigue, D., & Adjallé, K. (2024). *Biodecomposition with Phanerochaete chrysosporium: A review*. *AIMS Microbiology*, 10(4), 1068–1101. <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC11609428/>
- Louhasakul, Y., Treu, L., Kougias, P. G., Campanaro, S., Cheirsilp, B., & Angelidaki, I. (2021). *Valorization of palm oil mill wastewater for integrated production of microbial oil and biogas in a biorefinery approach*. *Journal of Cleaner Production*, 296, 126606. <https://doi.org/10.1016/j.jclepro.2021.126606>
- Mate, D. M., & Alcalde, M. (2016). *Laccase: A multi-purpose biocatalyst at the forefront of biotechnology*. *Microbial Biotechnology*, 9(5), 666–677. <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5658592/>
- Perdani, M. S., Margaretha, G., Sahlan, M., & Hermansyah, H. (2020). *Solid state fermentation method for production of laccase enzyme with bagasse, cornstalk and rice husk as substrates for adrenaline biosensor*. *Energy Reports*, 6, 336–340. <https://doi.org/10.1016/j.egy.2019.08.065>
- Pundir, A., Thakur, M. S., Prakash, S., Kumari, N., Sharma, N., Parameswari, E., He, Z., Nam, S., Thakur, M., Puri, S., Puranik, S., Kumar, S., Madhu, & Kumar, M. (2024). *Fungi as versatile biocatalytic tool for treatment of textile wastewater effluents*. *Environmental Sciences Europe*, 36, 185. <https://doi.org/10.1186/s12302-024-01007-3>
- Prawitasari, D. A., & Kardenia, E. (2025). *Biotransformation of the Textile Dye Reactive Black 5 with Aspergillus niger and Analysis of Ligninolytic Enzyme Activity*. *HAYATI Journal of Biosciences*, 33(2), 297–309. <https://doi.org/10.4308/hjb.33.2.297-309>
- Rana, H., Sharma, A., Dutta, S., & Goswami, S. (2022). *Recent approaches on the application of agro waste derived biocomposites as green support matrix for enzyme immobilization*. *Journal of Polymers and the Environment*. <https://doi.org/10.1007/s10924-022-02574-3>
- Reyes, C., Cajiao, F., & Ospina, D. J. (2021). *Enzyme activities of five white rot fungi in the presence of TEMPO and malt extract*. *Journal of Fungi*, 7(3), 222. <https://doi.org/10.3390/jof7030222>
- Rodrigues, E. N. J., da Rosa, R. A., Peralta, R. M., & Freitas, F. (2018). *Evaluation of purified and crude laccase from Trametes versicolor in the decolorization of textile effluent*. *Environmental Technology & Innovation*, 9, 246–257. <https://doi.org/10.1016/j.eti.2017.12.011>
- Santos, L., Silva, D., Squina, F., & de Pádua, G. S. (2019). *Enzyme extraction of ligninolytic enzymes from solid state cultures with different buffer solutions: effect on enzyme activity*. *Enzyme and Microbial Technology*, 125, 12–20. <https://doi.org/10.1016/j.enzmictec.2019.03.001>
- Sar, T., Marchlewicz, A., Harirchi, S., Mantzouridou, F. Th., Hosoglu, M. I., Akbas, M. Y., Hellwig, C., & Taherzadeh, M. J. (2024). *Resource recovery and treatment of wastewaters using filamentous fungi*. *Science of the Total Environment*, 951, 175752. <https://doi.org/10.1016/j.scitotenv.2024.175752>

- Singh, R., Tiwari, M. K., Singh, S., & Sarma, S. (2017). Extracellular laccase production and extraction from *Aspergillus flavus* using solid state fermentation and its characterization. *Biocatalysis and Agricultural Biotechnology*, 11, 236–243. <https://doi.org/10.1016/j.bcab.2017.02.017>
- Wang, F., Xu, L., Zhao, L., Ding, Z., Ma, H., & Terry, N. (2019). Fungal laccase production from lignocellulosic agricultural wastes by solid-state fermentation: A review. *Microorganisms*, 7(12), 665. <https://doi.org/10.3390/microorganisms7120665>
- Younus, H., Khan, A., Iqbal, S., & Ali, F. (2025). Eco-friendly biocatalysts: Laccase applications, mechanisms, and environmental roles. *Catalysts*, 15(10), 921. <https://www.mdpi.com/2073-4344/15/10/921>
- Zahoor, M., Khalil, S., & Rawat, S. (2021). Optimization of laccase extraction from *Pleurotus ostreatus* under solid state fermentation for phenolic pollutant degradation. *Biocatalysis and Agricultural Biotechnology*, 31, 101869. <https://doi.org/10.1016/j.bcab.2020.101869>