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Evaluation of Vulcanized Rubber Consumption by Lignocellulolytic Fungi
Evaluación del Consumo de Caucho Vulcanizado por Hongos Ligninocelulolíticos

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SUMMARY

The biodegradation of vulcanized rubber is an environmental challenge due to its high resistance to decomposition. This study evaluated the ability of three lignocellulolytic fungal strains (*Phlebiopsis* sp., *Trametes sanguinea*, and *Trametes cingulata*) to degrade tire powder in a liquid medium, selecting these strains for their prior ability to degrade toxic compounds such as xylene.

The results highlighted *Phlebiopsis* sp. as the most effective strain, with a notable 43.163% reduction in rubber content in the treatment with 75% rubber and 25% PDB (T2). *T. sanguinea* also showed significant performance, with a 9.58% reduction in the same treatment. These results underscore the potential of these strains for rubber bioremediation, a material known for its environmental persistence.

FTIR spectroscopy confirmed that both *Phlebiopsis* sp. and *T. sanguinea* caused structural modifications in the rubber, showing a decrease in signals associated with the functional groups of rubber and carbon black. This demonstrates that these fungi can alter the chemical structure of rubber, facilitating its degradation.

This study highlights the importance of selecting specific strains for industrial bioremediation. *Phlebiopsis* sp. emerges as an up-and-coming option due to its high degradation capacity. *T. sanguinea*, although less effective, also presents considerable potential. These findings are crucial for developing effective strategies for managing rubber waste and suggest that future research should focus on optimizing cultivation conditions and exploring the enzymatic mechanisms of these strains.

Index words: *biodegradation, environmental, phlebiopsis sp., trametes, waste.*

RESUMEN

La biodegradación del caucho vulcanizado es un reto ambiental debido a su alta resistencia a la descomposición. E ste estudio evaluó la capacidad de tres cepas de hongos lignocelulolíticos (*Phlebiopsis* sp., *Trametes sanguinea* y *Trametes cingulata*) para degradar polvo de neumático en medio líquido, y seleccionó estas cepas por su habilidad previa para degradar compuestos tóxicos como el xileno.

Los resultados destacaron a *Phlebiopsis* sp. como la cepa más eficaz, con una reducción notable del 43.163% en el contenido de caucho en el tratamiento con 75% caucho y 25% PDB (T2). *T. sanguinea* también mostró un rendimiento significativo, con una reducción del 9.58% en el mismo tratamiento. Estos resultados subrayan el potencial de estas cepas para la biorremediación del caucho, un material conocido por su persistencia ambiental.

Las espectroscopías FTIR confirmaron que tanto *Phlebiopsis* sp. como *T. sanguinea* provocaron modificaciones estructurales en el caucho, evidenciando una disminución en las señales asociadas a los grupos funcionales del caucho y el negro de carbono. Esto demuestra que estos hongos pueden alterar la estructura química del caucho, facilitando su degradación.

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Este estudio destaca la importancia de seleccionar cepas específicas para la biorremediación industrial. *Phlebiopsis* sp. se revela como una opción particularmente prometedora debido a su alta capacidad de degradación. *T. sanguinea*, aunque menos eficaz, también presenta un potencial considerable. Estos hallazgos son cruciales para desarrollar estrategias efectivas para manejar residuos de caucho y sugieren que futuras investigaciones deberían centrarse en optimizar las condiciones de cultivo y explorar los mecanismos enzimáticos de estas cepas.

Palabras clave: *biodegradación, ambiental, phlebiopsis sp., trametes, residuos.*

INTRODUCTION

The management of waste derived from polymeric materials, such as vulcanized rubber, represents a significant challenge in both environmental and technological fields. The durability and resistance of this material hinder its natural decomposition (Cheng, Xia, and Yang, 2023), raising questions about the effectiveness of traditional degradation methods, which are mostly physical (Hu *et al.,* 2023), chemical (Wei, Yu, Du, and Sun, 2022), and recycling-based (Gawdzik, Matynia, and Błażejowski, 2020), as these often generate additional pollution during their execution (Zhao, Liu, Meng, Liu, and Duo, 2024). In light of this problem, there has been a growing interest in developing more sustainable and effective strategies to manage this type of pollutant.

In this context, lignocellulolytic fungi gain relevance due to their demonstrated ability to degrade complex compounds such as lignin (Del Cerro *et al.,* 2024). These organisms represent a potential solution to the challenges associated with rubber degradation and offer promising perspectives for waste management (Bennet, Wunch, and Faison, 2002).

Vulcanized rubber, found in numerous products and widely used in industry (Alarif, 2023), presents considerable challenges due to its robust structure (Fazli and Rodriguez, 2020), which hinders its natural decomposition (Shah, Hasan, Shah, Kanwal, and Zeb, 2013). However, recent research has explored the potential of certain fungi to degrade polymers such as polyurethane, polyethylene (Spina *et al.,* 2021), and even plastics (Bautista-Zamudio, Flórez, López, Monroy, and Segura, 2023), paving a promising way for the biodegradation of these materials.

To achieve these objectives, experiments were designed in which rubber samples were exposed to selected fungi. During these experiments, changes in the chemical structure of the rubber were studied, and weight loss was recorded as an indicator of degradation.

The study of rubber biodegradation by lignocellulolytic fungi is relevant in the context of seeking more effective solutions to address rubber waste and mitigate its environmental impact. The primary objective of this study is to evaluate the capacity of lignocellulolytic fungi to degrade used tire rubber. The purpose is to understand the biochemical mechanisms involved in this process, as well as its applicability in sustainable waste management.

MATERIALS AND METHODS

The present project was carried out in Laboratory 5 of the National Technological Pole for Research and Analytical Services in Biofuels at the Technological Institute of Tuxtla Gutiérrez in Chiapas, Mexico. To initiate the research, the first step involved selecting strains, which were chosen based on their history of producing specific enzymes such as Laccase and lignin peroxidase, based on a previous study where these same strains were used for hydrocarbon degradation (Sánchez-Corzo, *et al.,* 2021). The strains *T. cingulata*, *T. sanguinea*, and *Phlebiopsis sp*. were selected as suitable for this study. Subsequently, the strains were reactivated in a solid medium enriched with 5% coffee husk and mineral salts. Once the strains grew, they were transferred to a potato dextrose agar (PDA) medium for maintenance.

To carry out the vulcanized rubber consumption assay, the rubber sample, obtained from a discarded tire from a landfill in Villaflores, Chiapas, was treated by washing with distilled water until all visible impurities in the material were removed. Subsequently, the sample was pulverized using a high-pressure pulverizer. Once pulverized, it was sieved to a particle size of 0.25 mm, the desired size for further analysis. The material was sterilized with moist heat (15 psi for 15 minutes) to eliminate any microbial contamination.

Three treatments were established, each with a specific ratio of pulverized rubber to potato dextrose broth (PDB), expressed as a percentage of each solid component of the culture medium. These rubber-PDB solid ratios were set at C1 (50-50%), C2 (75-25%), and C3 (100-0%), respectively, based on the PDB technical sheet, which specifies that 24 grams of solids are required per liter of water for microbial growth. These concentrations were adjusted so rubber acted as the other proportion of total solids in the liquid culture medium. Each treatment was inoculated with each fungal strain and performed in triplicate to ensure reproducibility of the results. Additionally, the minimum mineral medium proposed by Koutny *et al*. 2006 was added to each treatment, as these mineral salts act as cofactors and are essential for the growth and development of the fungi in the solid culture medium.

To evaluate consumption, the samples underwent a sterilization process, followed by washing with 70% ethanol and then with 10% sodium hypochlorite for 24 hours to remove the biomass. Subsequently, they were filtered and dried at 55°C. To determine the consumption percentage, calculations were performed using the equation 1:

$$\text{consumption percentage} = \frac{(\text{initial weight} - \text{final weight})}{(\text{initial weight})} * 100 \tag{1}$$

The results were analyzed using Statgraphics Centurion version 19 software (Statgraphics Technologies, 2024) through a simple ANOVA. To evidence possible changes in the chemical structure of the material, FT-IR spectroscopy was performed, obtaining spectra in the range of 500-4000 cm^{-1} at a resolution of 4 cm^{-1} in treated and untreated samples.

RESULTS AND DISCUSSION

It has been demonstrated that various factors, such as temperature, humidity, pH, UV radiation, exposure to light, water, and air (Caparanga, Basilia, Dagbay, and Salvacion, 2009; Wang, Huang, Ji, Völker, and Wurm, 2020), can positively or negatively influence the process of polymer biodegradation by microorganisms. Even the type of culture medium can play a significant role. This is particularly the case with fungi, which are more commonly cultured on solid media, as this method facilitates handling by reducing the risk of contamination and is more cost-effective. However, liquid culture promotes fungal growth by presenting the substrate as suspended solids, which improves oxygen supply. This makes liquid media a more suitable option than solid media for optimizing enzyme production and recovery (Gandia, Brandhof, Appels, and Jones, 2021; Marchut-Mikołajczyk, Drożdżyński, Januszewicz, Domański, and Wrześniewska, 2019).

Another crucial factor is the degradation capacity of microorganisms. For example, certain bacteria such as *Rigidoporus microporus*, *Amycolaptosis*, and *Nocardia*, and fungi such as *Aspergillus*, *Penicillium*, *Fusarium solani*, and *Ceriporiopsis subvermispora* have demonstrated their ability to degrade natural rubber (Maiden, Atan, Syd-Ali, Ahmad, K., and Wong, 2024; Sato *et al.,* 2004; Andler, 2020; Shah, Hasan, Shah, Kanwal, and Zeb, 2013). Since 1928, the ability of certain fungi to degrade rubber has been documented, although most studies have focused on natural rubber and isoprene. Among the fungal genera known for their ability to degrade rubber are *Monascus*, *Aspergillus*, *Penicillium*, *Fusarium*, *Cladosporium*, *Paecilomyces*, *Phoma*, *Calotropis*, and *Phlebia* (Basik, Sanglier, Yeo, and Sudesh, 2021). However, few organisms have shown the ability to degrade vulcanized rubber, such as that derived from tires, due to its high resistance as a polymer.

Nevertheless, the latter are capable of degrading certain polymers, such as polypropylene. Some species, like *Coniochaeta hoffmannii* and *Pleurostoma richardsiae*, have been able to grow on this material (Porter *et al.,* 2023). Similarly, *Phanerochaete chrysosporium* PV1, *Lentinus tigrinus* PV2, *Aspergillus niger* PV3, and *Aspergillus sydowii* PV4 have demonstrated the ability to degrade polyvinyl chloride (Ali *et al.,* 2013). Likewise, *Penicillium oxalicum* NS4 (KU559906) and *Penicillium chrysogenum* NS10 (KU559907) managed to degrade both low- and high-density polyethylene (Ojha *et al.,* 2017), suggesting that these fungi could be capable of degrading more rigid substrates, such as vulcanized rubber.

In this context, Andler, D'Afonseca, Pino, Valdés, and Salazar-Viedma (2021) evaluated the degradation of vulcanized rubber using different fungal strains, analyzing radial growth in the presence of rubber particles in solid media. The results showed a biodegradation percentage of 7.5% with *Trametes versicolor* and 6.1% with *Pleurotus ostreatus*. Our study focused on the degradation of vulcanized rubber using white-rot fungi, selected for their biochemical capacities and their adaptation to various substrates. This analysis was conducted in liquid media, as the factors mentioned above favor the biodegradation process.

The results revealed that *T. cingulata* did not show consumption capacity in any of the treatments, while *T. sanguinea* exhibited significant degradation in the C2 treatment (9.58%). In contrast, *Phlebiopsis* sp. demonstrated consumption capacity in all treatments, reaching a maximum of 43.163% in C2. This highlights the variability in degradation capacity among the strains and treatments evaluated (Figure 1). *T. sanguinea* showed significant consumption in the C2 treatment (9.58%), consistent with the results of Andler *et al*.(2021), where *T. versicolor* also exhibited an approximate percentage of rubber degradation in solid media. This suggests that certain fungi of the genus *Trametes* have notable potential for rubber biodegradation, regardless of the type of medium used, although in our study, consumption in liquid media was 20% higher, reaffirming the influence of medium type and strain on the biodegradation process.

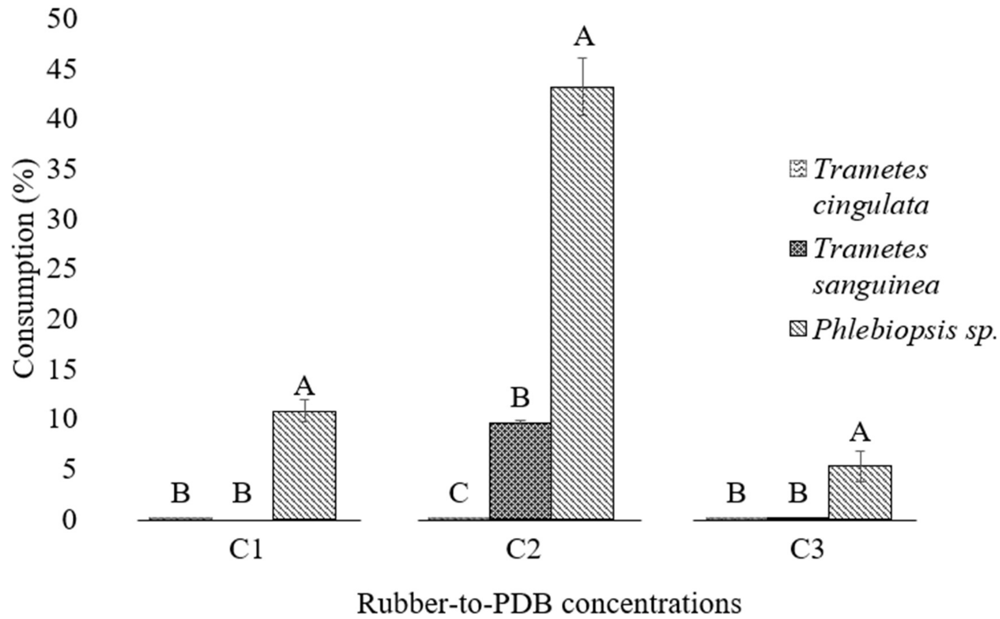

Figure 1. Percentage of vulcanized rubber consumption by different fungal strains under three substrate concentrations. Bars represent the mean values, and error bars indicate the standard deviation. Different letters above the bars denote statistically significant differences ($P < 0.05$) between treatments according to the applied statistical test.

On the other hand, *Phlebiopsis* sp. showed significantly higher capacity in all treatments compared to the strains evaluated in both this study and the work reported by Andler *et al*. (2021) In particular, *Phlebiopsis* sp. achieved a consumption of 43.163% in C2, far exceeding the levels observed for *T. sanguinea* (9.58%) and other strains evaluated by Andler *et al*. (2021), as it achieved a degradation rate four times higher than both organisms. This suggests that *Phlebiopsis* sp. is an exceptionally efficient strain for the biodegradation of vulcanized rubber in liquid media, likely due to its ability to produce degradative enzymes.

FTIR analyses revealed a reduction in signals associated with the vulcanized rubber structure after treatment with *T. sanguinea* and *Phlebiopsis* sp. In particular, a decrease was observed in the band corresponding to methylene stretching between 2920 and 2580 cm^{-1}, as well as in the methylene bending band around 1450 cm^{-1}. These signals are associated with the methylene-carbon bonds typical of natural rubber, which constitutes approximately 41% of the composition of tires (Palos *et al.,* 2021). Similarly, the signal around 870 cm^{-1}, corresponding to the carbon-methylene ($C-CH_2$) bond, also showed a significant reduction.

Aromatic ring vibrations and the signals corresponding to carboxylate and carbonyl groups (1520-1605 cm^{-1}), associated with carbon black, which represents 28% of the tire composition, also decreased after treatment with *T. sanguinea* and *Phlebiopsis* sp. (Palos *et al.,* 2021). This suggests that both fungi not only degrade natural and synthetic rubber but may also alter other components of the material, such as carbon black. Additionally, fungal growth could reduce the carbonyl groups of the original oxidation products, such as esters, lactones, and ketones (Sánchez, 2020) (Figure 2).

These observations suggest that *T. sanguinea* and *Phlebiopsis* sp. have a high capacity to modify the structure of vulcanized rubber. The reduction in the signals observed in the treatments indicates biodegradation, as evidenced by the decrease in the characteristic functional groups of the material. In contrast, *T. cingulata* did not show any significant reduction in the signals, presenting a spectrum similar to that of the control, indicating a lack of biodegradative activity. These results highlight the variability among fungi in their ability to degrade vulcanized rubber and confirm the superior effectiveness of *T. sanguinea* and *Phlebiopsis* sp. compared to *T. cingulata*.

When comparing our results with those of Andler *et al*. (2021), we observed a similar reduction in CH_2 bands and the loss of signals associated with functional groups after treatment with *T. versicolor* and *P. ostreatus*. However, in their study, the reduction was more drastic using EM media, while in our work, the most significant changes were observed in treatments with different strains in liquid media. The results suggest that liquid media may facilitate greater diffusion of extracellular enzymes and promote more effective biodegradation compared to the solid media used in previous studies. The difference in effectiveness observed in different media may be related to the fungi's ability to produce degradative enzymes that act on the rubber components.

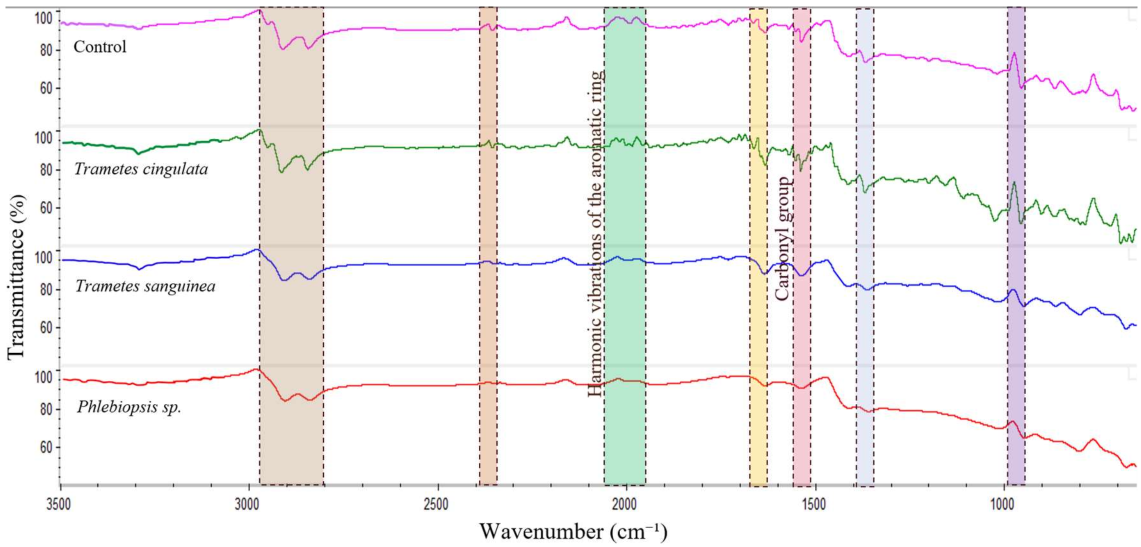

Figure 2. FTIR spectra of vulcanized rubber before and after fungal degradation using *T. cingulata*, *T. sanguinea*, and *Phlebiopsis* sp., compared to the control. The Y-axis represents transmittance (%), while the X-axis shows the wavenumber (cm^{-1}). The highlighted regions indicate key absorbance bands related to aromatic ring vibrations and other functional groups.

These results also suggest that *Phlebiopsis* sp. and *T. sanguinea* are capable of performing degradation mechanisms, as both fungal genera have the metabolic capabilities to break the chemical bonds of rubber polymers, making them promising candidates for the biodegradation of tire waste and other polymers.

CONCLUSIONS

This study demonstrates that certain lignocellulolytic fungi, specifically *Phlebiopsis* sp. and *T. sanguinea*, possess a remarkable capacity to degrade vulcanized rubber, with *Phlebiopsis* sp. showing the highest consumption potential under various treatment conditions. *T. sanguinea* also stood out, although with slightly lower efficacy, indicating that it remains a valuable strain for rubber degradation. The reduction in signals observed in the FTIR spectra after treatment confirms the structural modification of the rubber, underscoring the critical role these fungi play in the degradation of recalcitrant materials like vulcanized rubber. Moreover, the notable variability in consumption efficacy among different strains emphasizes the importance of selecting specific strains for industrial bioremediation applications. Given that factors such as the type of medium and environmental conditions significantly influence biodegradation efficiency, future research should prioritize the optimization of cultivation and treatment conditions to improve the consumption rates of these fungi. Additionally, further research into the enzymatic mechanisms involved in rubber degradation will be essential to fully understand and harness the potential of these fungi in addressing challenges related to rubber waste.

ETHICS STATEMENT

Not applicable.

CONSENT FOR PUBLICATION

Not applicable.

AVAILABILITY OF SUPPORTING DATA

The datasets used and analyzed during the current study are available from the corresponding author on reasonable request.

COMPETING INTERESTS

The authors declare that they have no competing interests.

FINANCING

The project funding comes from two significant sources: the National Council for Humanities Science and Technology (CONAHCYT) and the National Technological Institute of Mexico (TecNM). These institutions have actively supported the research and development of this project, providing the necessary resources to conduct meaningful studies and advance knowledge in this specific field. The financial support from CONAHCYT and the National Technological Institute of Mexico has been fundamental for the successful execution of this research and the achievement of the proposed objectives.

AUTHORS' CONTRIBUTIONS

The project conceptualization was carried out by: W.P.M.C., and S.E.S. While the methodology was developed by: W.P.M.C., S.E.S., and V.M.R.V. The software was provided by: J.H.C.G. and also used by W.P.M.C. Formal data analysis was conducted by: W.P.M.C., and J.J.V.M. The research was performed by W.P.M.C., and S.E.S. The original draft was written by: W.P.M.C. The review and editing of the document were done by S.E.S., V.M.R.V., J.J.V.M., and J.H.C.G. Project supervision was undertaken by S.E.S., V.M.R.V., J.J.V.M., and J.H.C.G. Project administration and funding acquisition were the responsibility of S.E.S.

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