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Effect of hydrogen peroxide as an inducing agent of peroxidase enzymes for the polyethylene biodegradation by *Pleurotus ostreatus* in liquid fermentation

Efecto del peróxido de hidrógeno como agente inductor de enzimas peroxidasas en la biodegradación de polietileno por *Pleurotus ostreatus* en fermentación líquida

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ABSTRACT

Widely used petroleum-derived polymers, such as polyethylene (PE), are a significant source of environmental pollution due to their accumulation in the ecosystem. In the present study, *P. ostreatus* was grown in a PE-supplemented medium added with H_2O_2 as a peroxidase enzyme-inducing agent for 5 weeks in liquid fermentation to evaluate the biodegradation of this polymer. A medium lacking H_2O_2 was used as a control. Enzyme production by *P. ostreatus* and the contact angle of the PE surface were evaluated. Chemical structural changes of the PE surface were determined by FTIR spectroscopy. The activities of laccase, unspecific peroxigenase, and

lignin peroxidase were found to be higher in the control medium (4072, 3692 and 4224 U/L, respectively) than in the medium supplemented with H_2O_2 (3531, 2910 and 2878 U/L, respectively). Changes in the chemical structure and the decrease in the contact angle of the PE surface showed biodegradation of the polymer. The PE surface of control cultures had a lower contact angle (60.9°) than that observed in PE of cultures added with H_2O_2 (66.8°). The addition of 5 mM of H_2O_2 to the medium increased the production of manganese peroxidase, however, it did not have a significant impact on the biodegradation of PE.

Keywords: biodegradation, hydrogen peroxide, peroxidases, *Pleurotus ostreatus*, polyethylene.

RESUMEN

Los polímeros derivados del petróleo de uso común, como el polietileno (PE), son una fuente significativa de contaminación ambiental debido a su acumulación en los ecosistemas. En el presente estudio, *P. ostreatus* se cultivó en un medio suplementado con PE y adicionado con H_2O_2 como agente inductor de la enzima peroxidasa durante 5 semanas en fermentación líquida para evaluar la biodegradación de este polímero. Un medio sin H_2O_2 se utilizó como control. Se evaluó la producción de enzimas por *P. ostreatus* y el ángulo de contacto de la superficie del PE. Los cambios en la estructura química de la superficie del PE se determinaron mediante espectroscopía FTIR. Las actividades de lacasa, peroxigenasa inespecífica y lignina peroxidasa fueron mayores en el medio de control (4072, 3692 y 4224 U/L, respectivamente) que en el medio suplementado con H_2O_2 (3531, 2910 y 2878 U/L, respectivamente). Los cambios en la estructura química y la disminución del ángulo de contacto de la superficie del PE evidenciaron la biodegradación del polímero. La superficie de PE en los cultivos control presentó un ángulo de contacto menor (60.9°) en comparación con la observada en los cultivos con H_2O_2 (66.8°). La adición de 5 mM de H_2O_2 al medio aumentó la producción de manganeso peroxidasa; sin embargo, no tuvo un impacto significativo en la biodegradación del PE.

Palabras clave: biodegradación, peroxidasas, peróxido de hidrógeno, polietileno, *Pleurotus ostreatus.*

1. INTRODUCTION

Petroleum-derived polymers, such as polyethylene (PE), account for more than 60% of the plastic production and are considered non-biodegradable polymers due to their highly inert C-C structures, which lack functional groups (Han *et al.,* 2024). However, PE possesses properties that make it one of the most widely produced polymers globally, including low cost, durability, and versatility (Zhang *et al.,* 2018; Jin *et al.,* 2023). The most extensively studied and well-known types of PE are low-density polyethylene (LDPE) and high-density polyethylene (Bardají *et al.,* 2020; Patel *et al.,* 2022). PE degradation is highly complex due to its long, stable linear chains of carbon and hydrogen.

The accumulation of plastic waste has become a serious environmental pollution issue, affecting biodiversity due to its high molecular stability and low natural degradability. Depending on the type of plastic and environmental conditions, it can persist for decades or even centuries (Taghavi *et al.,* 2021; He *et al.,* 2024). For this reason, scientists have continuously conducted research to develop strategies for plastic waste management, focusing on more efficient and

environmentally sustainable treatments, such as plastic biodegradation (Sánchez, 2020; Tian *et al.*, 2021; Delangiz *et al.,* 2022; Han *et al.*, 2024). Biodegradation is a process in which polymers are broken down by microorganisms, including bacteria, fungi, and algae, which utilize the polymeric chain as a carbon source (Eliaz *et al.,* 2018; Delangiz *et al.,* 2022).

In recent years, microorganisms capable of degrading synthetic plastics have been identified and studied. Fungi play a significant role in polymeric material degradation, as their mycelium can penetrate the polymer surface, facilitating its breakdown, while their extracellular enzymatic system fragments the polymeric chain. Additionally, their intracellular enzymatic system assimilates and mineralizes these fragments (Sánchez, 2020; Ali *et al.,* 2021). Currently, more than 14,000 species of white-rot fungi have been reported to fragment polymers and secrete lignolytic enzymes, including *Trametes versicolor, Agrocybe aegerita*, *Pleurotus ostreatus*, and *Ganoderma lucidum* (Kumar and Chandra, 2020; Bertolacci *et al.,* 2022; González-Márquez and Sánchez, 2024; Okal *et al.,* 2023; Ocaña-Romo *et al.,* 2024). These species utilize PE as a carbon source through enzyme secretion (Srikanth *et al.,* 2022).

PE degradation occurs in two stages. The first stage, depolymerization, involves reducing the molecular weight of high-molecular-weight compounds through fungal enzymatic action, thereby improving PE surface hydrophobicity (Thakur *et al.,* 2023). The second stage, mineralization, occurs when hyphal receptors internalize PE fragments, assimilating them within cells through their intracellular enzymatic system and converting them into biomass, energy, CO_2, CO_4, and H_2O (Ali *et al.,* 2021; Mat Yasin *et al.,* 2022; Bacha *et al.,* 2023). During the degradation process, PE undergoes modifications in both its surface structure, increasing hydrophilicity and promoting microbial attack, and in its chemical structure due to C-H bond cleavage and the formation of oxygen-containing functional groups, such as hydroxyls and carboxyls (Zhang *et al.,* 2024).

Enzymes have the ability to significantly shorten the lifespan of plastics from hundreds of years to just a few weeks (Han *et al.,* 2024). However, only a limited number of enzymes have been identified that can effectively oxidize complex structures such as PE (Bacha *et al.,* 2023). The enzyme laccase (Lac) primarily functions by oxidizing phenolic compounds, reducing O_2 to H_2O through the removal of an electron from the aromatic substrate, generating phenoxyl radicals (Bettin *et al.,* 2011; Agrawal *et al.,* 2018). Peroxidase enzymes utilize H_2O_2 for break down the polymeric chain by oxidizing side chains of phenols, benzene, methylene, aldehydes, and ketones (Okal *et al.,* 2023). Manganese peroxidase (MnP), a member of the oxidoreductase family, is considered one of the most powerful ligninolytic enzymes due to its ability to oxidize Mn^{2+} to Mn^{3+} in the presence of H_2O_2 (Liers *et al.,* 2011; Kumar and Chandra, 2020). Studies have demonstrated that MnP expression is regulated by the presence of Mn^{2+} and H_2O_2 in the culture medium (Santacruz-Juárez *et al.* 2021). Lignin peroxidase (LiP), also belonging to the oxidoreductase family, is responsible for oxidizing phenolic aromatic substrates and non-phenolic lignin-like compounds in the presence of H_2O_2 (Santacruz-Juárez *et al.* 2021). The unspecific peroxygenase enzyme (UnP) is an extracellular enzyme that exhibits peroxidase activity by oxidizing an electron, as well as peroxygenase activity by transferring an oxygen atom from H_2O_2 (Santacruz-Juárez *et al.*, 2021). Additionally, microbial cells have been stimulated by the addition of non-lethal concentrations of H_2O_2 to enhance peroxidase enzyme production (Taghavi *et al.,* 2021).

In this study, the biodegradation of PE by *P. ostreatus* was evaluated by assessing enzyme production and contact angle of the PE surface, as well as chemical structural changes of the PE surface using FTIR spectroscopy.

2. MATERIALS AND METHODS

2.1. Fungal strain and PE samples

The fungal strain *P. ostreatus* was obtained from the culture collection from the Biotechnology Laboratory of the Research Center for Biological Sciences at the Universidad Autónoma de Tlaxcala (CICB-UATx, Mexico). The strain was grown on malt extract agar (EMA) and incubated at 27°C for 5 d, then stored at 2°C until used as inoculum. LDPE films measuring 1 cm x 1 cm were used. The PE was washed and stored as described by González-Márquez *et al.* (2024).

2.2. Liquid fermentation conditions and supernatant collection

125 mL flasks with 50 mL culture medium were used. A culture medium enriched with 25 g malt extract (ME), 5 g yeast extract, 5mM of H_2O_2, straw extract (100 g of straw per liter of distilled water) and mineral salts were prepared: 0.1872 g K_2HP_4, 0.2032 g $MgNO_3$, 0.5 g $Ca(H_2PO_4)_2$, 1 g $MgSO_4$, 0.005 g $FeSO_4$, 0.007 g $ZnSO_4$, 0.009 g $MnSO_4$, and 0.011 g $CuSO_4$ (per liter of water). The previously washed PE (1 cm x 1 cm) was added to flasks containing culture medium. Then, 10 mycelium fragments (8 mm of diameter) of *P. ostreatus* taken from the periphery of a fresh colony were used as inoculum. The flasks were incubated in an orbital incubator (Prendo, Mexico) at 27 °C and 120 rpm for 5 weeks. Samples were taken in triplicate every 7 d. The supernatant from the flasks was separated from the culture medium through filtration using filter paper (Whatman No. 4) and a vacuum pump (Millipore Merck, Germany). The supernatant was transferred to Eppendorf tubes and stored at -40°C for later use in enzyme activity analyses.

2.3. Enzyme production and enzymatic parameters

The enzymatic activity of Lac was determined using a Jenway 7305 UV-Vis spectrophotometer at 468 nm. The substrate used was 2,6-dimethoxyphenol (DMP) (ε 469 = 49,600 1/M cm), as previously reported by González-Márquez *et al.* (2024). The reaction mixture consisted of 900 µL of DMP dissolved in 0.1 M acetate buffer at pH 4.5 and 100 µL of the supernatant. The mixture was then incubated at 40 °C for 1 minute.

The enzymatic activity of MnP was determined using a Jenway 7305 UV-Vis spectrophotometer at 334 nm, as previously reported by González-Márquez *et al.* (2024). The substrate used was 10 mM guaicol (ε 334 = 18,300 1/M cm). The reaction mixture consisted of 75 µL of 1 mM manganese(II) sulfate tetrahydrate ($MnSO_4$), 790 µL of 0.1 M tartrate buffer at pH 4.2, 50 µL of 40 mM hydrogen peroxide, 75 µL of 10 mM guaicol, and 10 µL of the supernatant. The mixture was then incubated at room temperature for 10 minutes.

The activity of UnP was determined using a Jenway 7305 UV-Vis spectrophotometer at 310 nm, employing 40 mM veratryl alcohol as the substrate (ε 310 = 9300 1/M cm). The reaction mixture consisted of 100 µL of 40 mM veratryl alcohol, 50 µL of 40 mM hydrogen peroxide, 840 µL of 0.1 M citrate buffer at pH 4.5, and 10 µL of the supernatant. The mixture was incubated at room temperature for 10 minutes, as previously reported by González-Márquez *et al.* (2024).

The activity of LiP was determined using a Jenway 7305 UV-Vis spectrophotometer at 310 nm, as previously reported by González-Márquez *et al.* (2024).The substrate used was 40 mM veratryl alcohol (ε 310 = 9300 1/M cm). The reaction mixture consisted of 200 µL of 40 mM

veratryl alcohol, 200 µL of 40 mM hydrogen peroxide, 580 µL of 0.1 M tartrate buffer at pH 4.2, and 20 µL of the supernatant. The mixture was incubated at room temperature for 10 minutes. For all cases, one enzyme unit (U) is defined as the amount of enzyme required to convert 1 µmol of substrate per minute. The enzymatic parameters calculated included maximum enzyme production (E_{max}) (U/L) and enzymatic productivity (P_{RO}), defined as the ratio of E_{max} (U/L) to the fermentation time required to reach E_{max}, as previously reported by Andrade-Alvarado *et al.* (2024).

2.4. Evaluation of surface hydrophobicity and FTIR analysis after biodegradation

The surface hydrophobicity of PE was assessed by measuring the contact angle using a 5 µL drop of distilled water placed on the PE surface (Lamour *et al.,* 2010). The images of the contact angle were captured with an iPhone 15 Pro camera (ISO 250, f 1.8, 1/60 s) and analyzed with ImageJ software (version 1.53).
The chemical structure changes of PE were analyzed with a Thermo Nicolet iS10 infrared spectrometer (Massachusetts, USA), using a Ge mirror, provided by the Instrumental Analysis Laboratory at the National Technological Institute of Mexico (Technological Institute of Aguascalientes, Mexico), and employing the attenuated total reflectance (ATR) technique. The absorption spectra of PE were scanned 64 times, producing an optimized final spectrum with a resolution of 4 cm^{-1}, within the range of 4000-500 cm^{-1}. The FTIR spectra were displayed in baseline mode.

2.5 Statistical Analysis

The results were validated under the assumptions of normality (Shapiro-Wilk test) and homoscedasticity (Levene's test). Statistical evaluations were conducted using analysis of variance (ANOVA), followed by Tukey's post-hoc test. Data were performed using the Statistical Analysis System (SAS, free software trial), with a significance level of $p<0.05$, and presented as the mean ± standard deviation from experiments conducted in triplicate.

3. RESULTS

The E_{max} of Lac was achieved at week 1 for the control medium and week 3 for the H_2O_2 medium of liquid fermentation (4072.58 U/L and 3531.58 U/L, respectively) (Fig. 1). In Table 1, *P. ostreatus* demonstrated higher P_{RO} in the control medium (24.24 U/L/h) compared to the culture medium with H_2O_2 (7.00 U/L/h). Manganese peroxidase had higher production in the H_2O_2 medium than in the control medium (Fig. 2). *P. ostreatus* showed a E_{max} of 1273.22 U/L at week 3 of fermentation in the H_2O_2 medium (Table 1). *P. ostreatus* showed higher P_{RO} in the H_2O_2 medium (4.36 U/L/h) compared to the control medium (2.52 U/L/h).

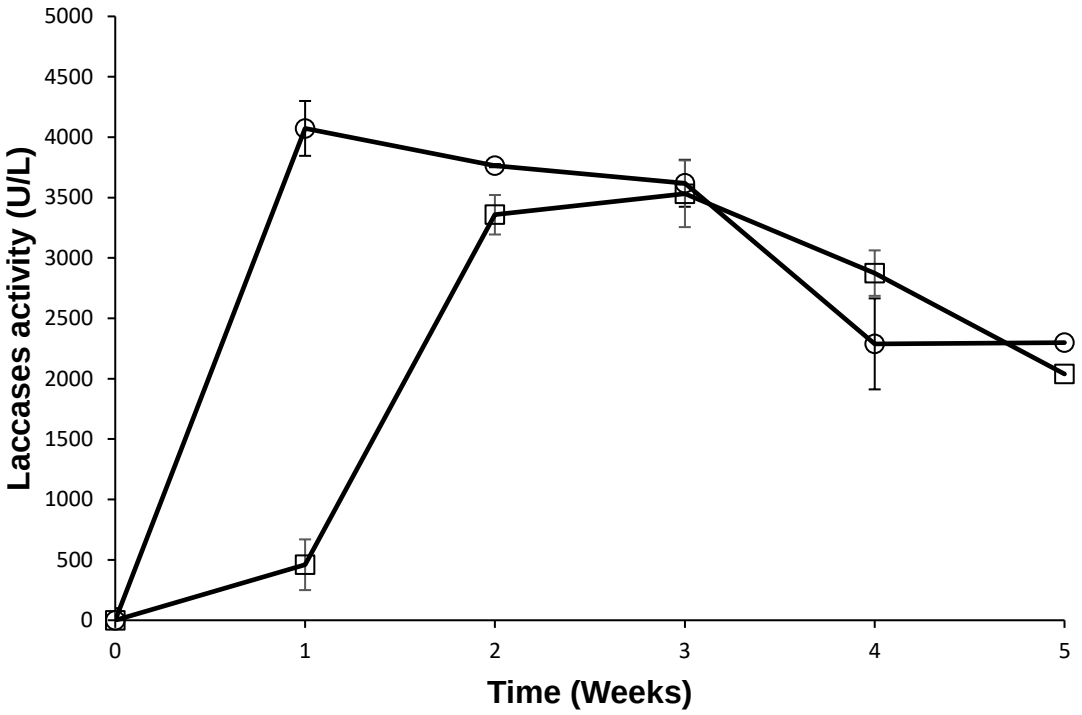


Fig. 1. Laccases activity of *P. ostreatus* grown in control medium (circle) and in H_2O_2 medium (square) in liquid fermentation.

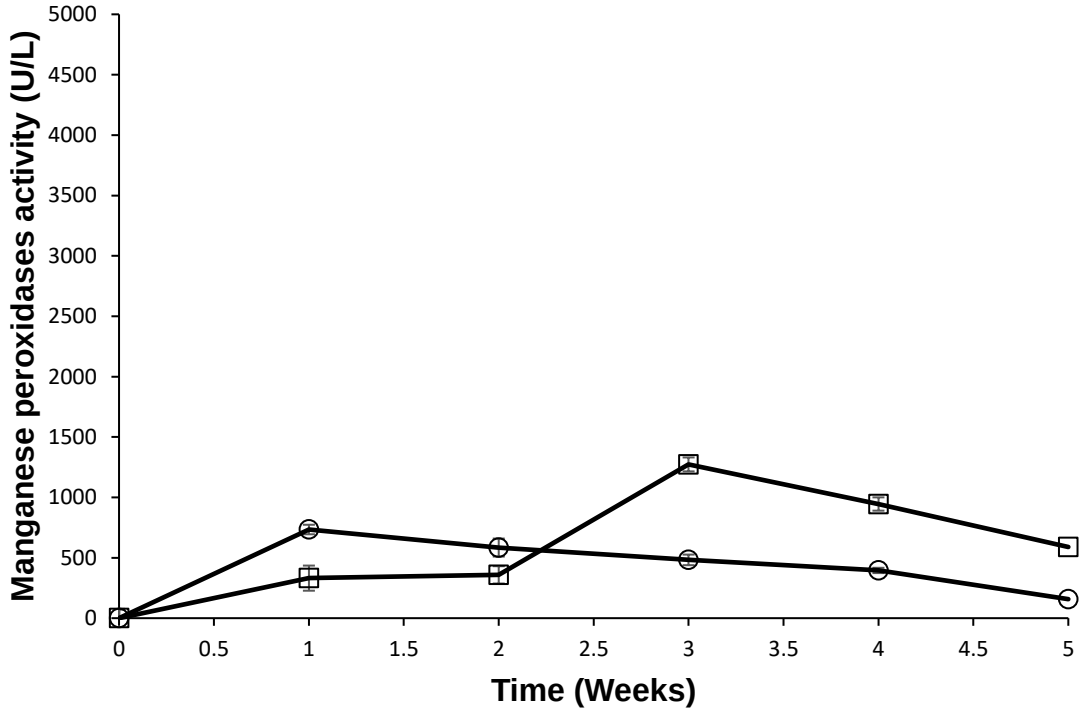


Fig. 2. Manganese peroxidases activity of *P. ostreatus* grown in control medium (circle) and in H_2O_2 medium (square) in liquid fermentation.

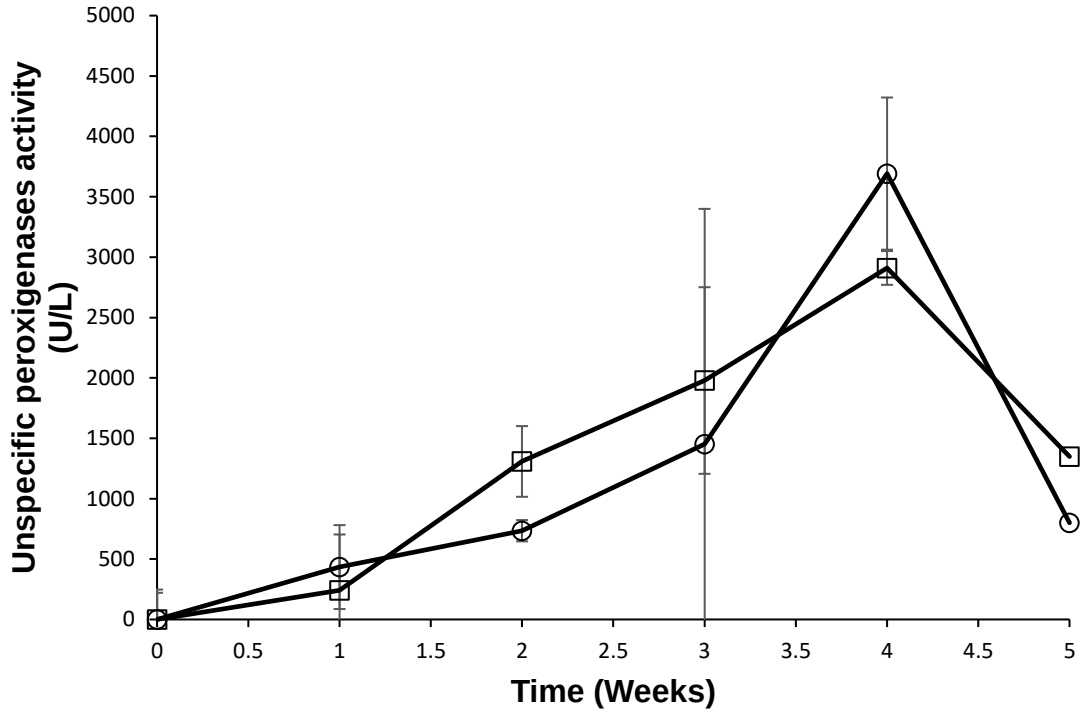


Fig. 3. Unspecific peroxigenases activity of *P. ostreatus* grown in control medium (circle) and in H_2O_2 medium (square) in liquid fermentation.

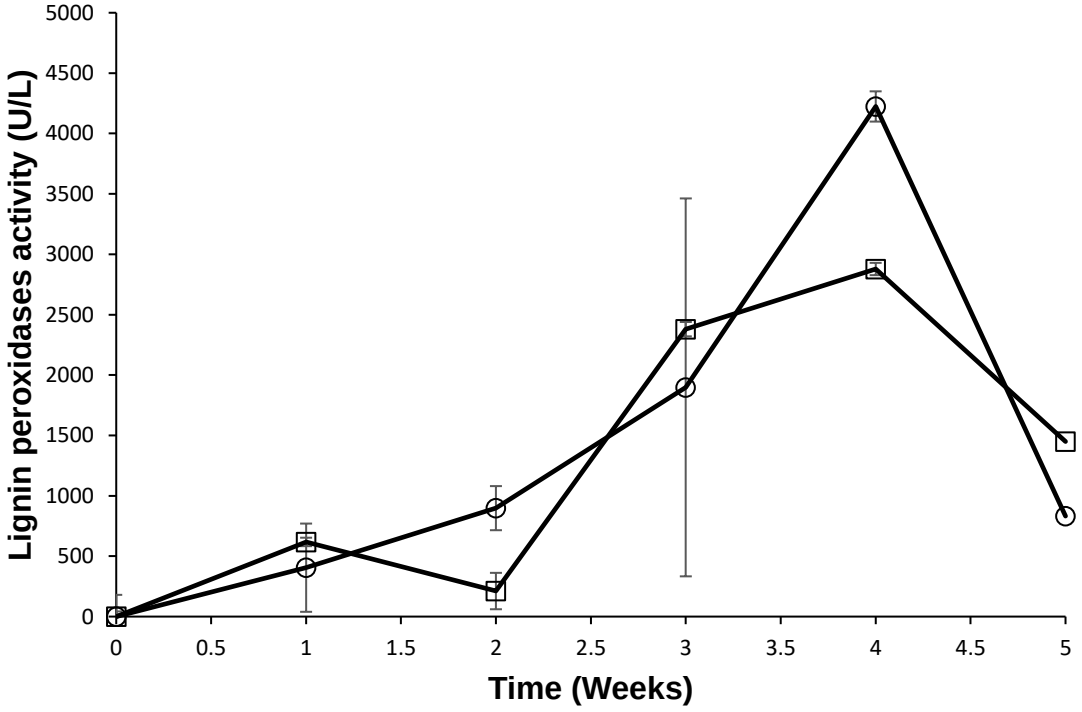


Fig. 4. Lignin peroxidases activity of *P. ostreatus* grown in control medium (circle) and in H_2O_2 medium (square) in liquid fermentation.

Figure 3 shows the activity of UnP, *P. ostreatus* showed higher activity of UnP in the control medium (3691.75 U/L) compared to the H_2O_2 medium (2910.39 U/L) at week 4 of fermentation. In Table 1, *P. ostreatus* exhibited higher P_{RO} in the control medium (5.49 U/L/h) compared to the medium added with H_2O_2 (4.33 U/L/h). Figure 4 shows that *P. ostreatus* had higher lignin peroxidase activity in the control medium compared to the H_2O_2 medium. *P. ostreatus* exhibited a E_{max} of 4224.01 U/L at 4 weeks of fermentation. *P. ostreatus* demonstrated higher enzymatic productivity in the control medium (6.28 U/L/h) compared to the H_2O_2 medium (4.28 U/L/h) (Table 1). *P. ostreatus* showed an E_{max} of 2878.13 U/L in the H_2O_2 medium (Table 1).

Table. 1. Enzymatic parameters of *P. ostreatus* grown in control medium and in H_2O_2 medium under liquid fermentation conditions.

	Culture media	
Enzymatic parameters	**Control**	**H_2O_2**
Laccases		
E_{max} (U/L)	4072.58[a] ± 60.52	3531.58[b] ± 185.24
P_{RO} (U/L/h)	24.24[a] ± 0.18	7.00[b] ± 1.10
Manganese peroxidases		
E_{max} (U/L)	734.06[b] ± 49.18	1273.22[a] ± 37.19
P_{RO} (U/L/h)	4.36[a] ± 0.15	2.52[b] ± 0.07
Unspecific peroxigenases		
E_{max} (U/L)	3691.75[a] ± 117.44	2910.39[b] ± 107.70
P_{RO} (U/L/h)	5.49[a] ± 0.11	4.33[b] ± 0.11
Lignin peroxidases		
E_{max} (U/L)	4224.01[a] ± 200.10	2878.13[b] ± 56.89
P_{RO} (U/L/h)	6.28[a] ± 0.40	4.28[b] ± 0.11

The mean and standard deviation (±) of triplicate experiments are reported. Measurements within the same row with different letters are significantly different. The statistical significance level for the ANOVA is $p < 0.05$ according to the Tukey test. E_{max}, maximum enzyme activity and P_{RO}, enzymatic productivity (see Sect. 2.3).

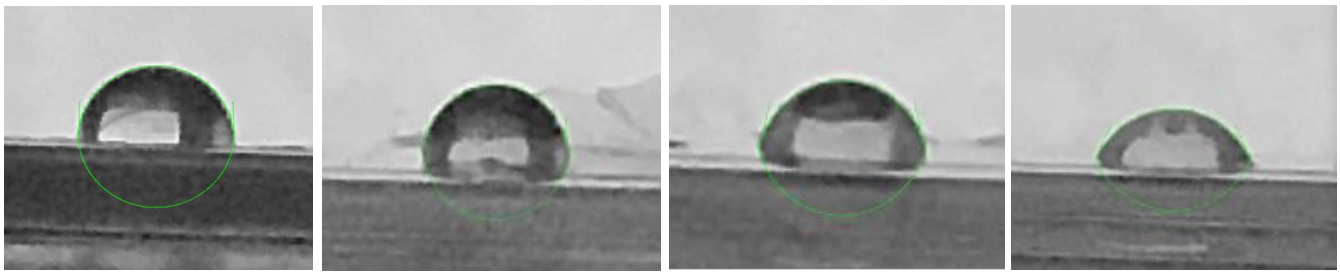
The surface hydrophobicity of PE samples decreased after degradation by *P. ostreatus* in liquid fermentation (Table 2). At week 5, the contact angle decreased from 90.33° to 60.9° in the control medium, and from 90.33° to 66.83° in H_2O_2 medium. The decreased surface hydrophobicity was greater in the control medium after week 4 of degradation (Fig. 5).

Table. 2. Contact angle (°) of PE surface after biodegradation by *P. ostreatus* in control medium and with H_2O_2 in liquid fermentation.

Treatment	Fermentation time (weeks)					
	0	1	2	3	4	5
Control	90.33[a] ± 0.57	79.16[b] ± 1.52	74.76[c] ± 0.57	73.2[c] ± 1.15	61.3[d] ± 0.57	60.9[d] ± 1.15
H_2O_2	90.33[a] ± 0.57	75.63[b] ± 0.57	71.86[c] ± 1.00	72.4[c] ± 0.57	71.26[c] ± 0.57	66.83[d] ± 1.00

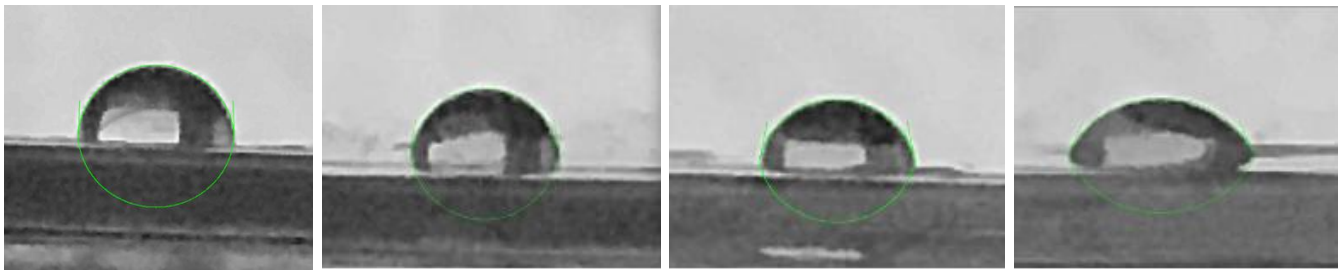
The mean and standard deviation (±) of triplicate experiments are reported. Measurements within the same row with different letters are significantly different. The statistical significance level for the ANOVA is $p < 0.05$ according to the Tukey test (see Sect. 2.4).

Control medium



0 Wk **1 Wk** **3 Wk** **5 Wk**

H_2O_2 medium



0 Wk **1 Wk** **3 Wk** **5 Wk**

Fig. 5. Digital images showing a drop of water on the PE sample surface. The angle formed between the water drop and sample was measured (i.e. contact angle) to determine the hydrophobicity of the surface of PE after *P. ostreatus* growth in control medium (a) and in H_2O_2 (b) at week 0, 1, 3 and 5 of fermentation.

The FTIR spectra of PE was analyzed after biodegradation by *P. ostreatus* in both control and H_2O_2 media. A band corresponding to the rocking vibration of methylene (CH_2) was observed in the range of 700–750 cm^{-1} (Fig. 6). This band formed a displaced doublet after the

biodegradation process, with the values increasing and decreasing during fermentation. The FTIR band in the 1750–1600 cm^{-1} range, associated with the carbonyl group, increased following biodegradation by *P. ostreatus* (Fig. 7). Additionally, the FTIR band at 1440–1490 cm^{-1}, which corresponds to the asymmetric rocking vibration of terminal methyl (CH_3), exhibited a splitting pattern during fermentation (Fig. 8). No significant differences were observed between the control medium and the H_2O_2 medium during fermentation.

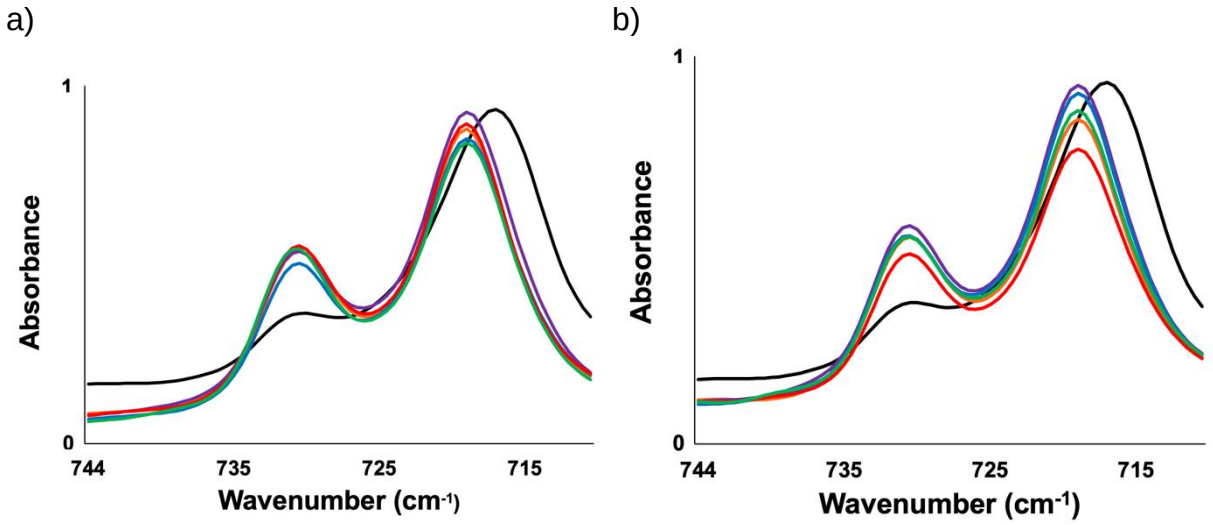


Fig. 6. FTIR spectrum of the surface of PE, showing the rocking vibrations of the methylene group in samples degraded by *P. ostreatus* grown in control medium (a) and in H_2O_2 medium (b) in liquid fermentation. Control (black line), week 1 (orange line), week 2 (purple line), week 3 (blue line), week 4 (red line), and week 5 (green line).

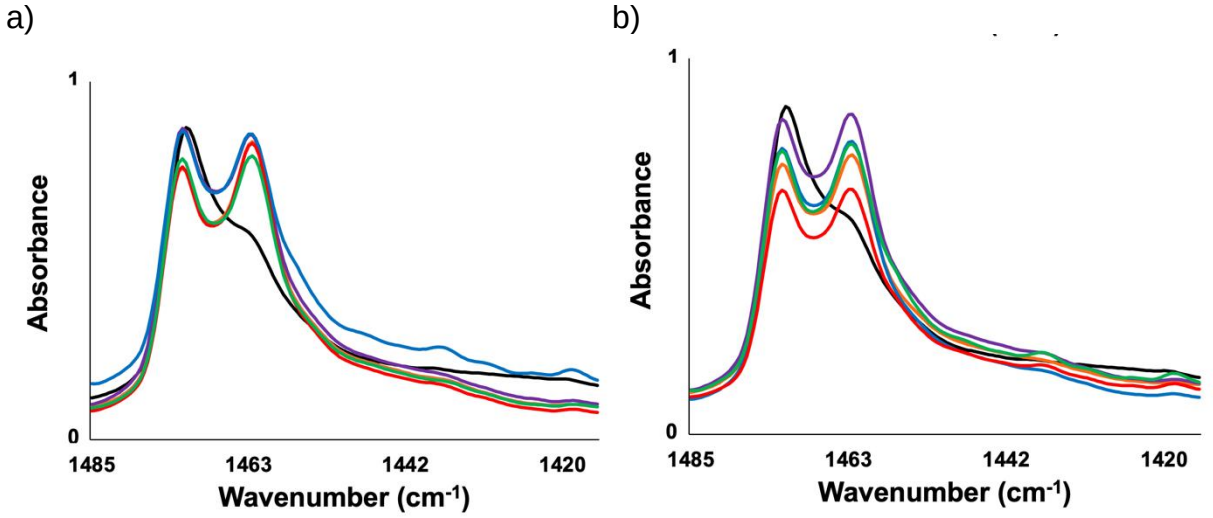


Fig. 7. FTIR spectrum of the surface of PE, showing the asymmetric rocking vibration of terminal methyl group in samples degraded by *P. ostreatus* grown in control medium (a) and in H_2O_2 medium (b) in liquid fermentation. Control (black line), week 1 (orange line), week 2 (purple line), week 3 (blue line), week 4 (red line), and week 5 (green line).

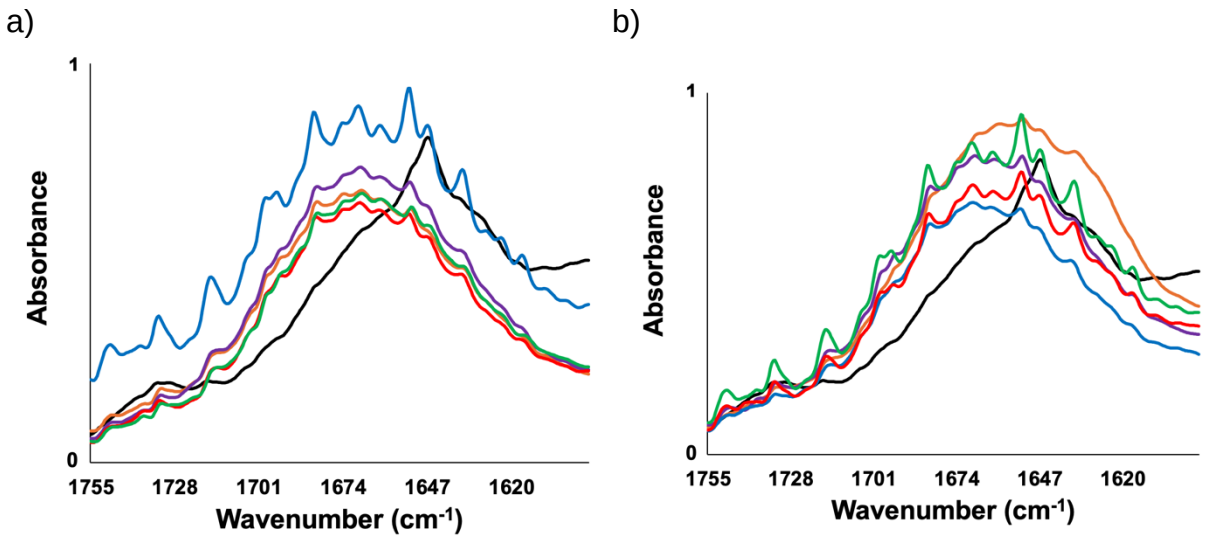


Fig. 8. FTIR spectrum of the surface of PE, showing the carbonyl group (1600–1750 cm^{-1}) in samples degraded by *P. ostreatus* grown in control medium (a) and in H_2O_2 medium (b) in liquid fermentation. Control (black line), week 1 (orange line), week 2 (purple line), week 3 (blue line), week 4 (red line), and week 5 (green line).

4. DISCUSSION

The fungal enzymatic oxidation mechanisms involved in plastic degradation are strongly linked to lignin catabolism (Ellouze and Sayadi, 2016). Laccase and peroxidase, through redox reactions, break down the plastics materials in a similar manner to the hydrolysis of lignin. Various fungal species, such as *Penicillium chrysogenum, Trichoderma harzianum, Phanerochaete chrysosporium*, and *P. ostreatus*, secrete oxidase enzymes capable of decomposing synthetic polymers (Okal *et al.,* 2023).

P. ostreatus has been recognized for its ability to degrade lignocellulosic materials and various agro-industrial substrates. This ability is attributed to its enzymatic system, which includes oxidase enzymes such as Lac and peroxidases. Lac are involved in breaking down lignin by targeting phenolic bonds, while peroxidases like LiP and MnP are involved in the cleavage of non-phenolic linkages in the lignin structure. In the present research, Lac activity increased during the first week of fermentation but decreased in the second week, which coincided with the production of MnP, LiP, and UnP. This pattern is consistent with findings reported by Andrade-Alvarado *et al.* (2024).

In the present study, *P. ostreatus* reached its maximum Lac activity (4072.58 U/L) on 7 d of fermentation in the control medium. The Lac activity started to decrease by the second week. González-Rodríguez *et al.* (2023) reported an E_{max} of 1336 U/L for *A. aegerita* in liquid fermentation using a soybean flour medium. Bánfi *et al.* (2015) studied that *P. ostreatus* had the highest Lac activity (6000 Um/g) when grown on wheat straw at 8 d. González-Márquez and Sánchez (2024) reported a Lac activity of 12,200 U/L for *P. ostreatus* in a malt extract medium under liquid fermentation.

Research previously reported suggests that the LDPE degradation process occurs in two phases. In the first phase, Andrade-Alvarado *et al.* (2024) reported that *P. ostreatus* secretes Lac enzymes during its growth. These enzymes degrade the phenolic compounds present in

bioavailable substrates and release H_2O_2 into the medium. In the second phase, H_2O_2-dependent enzymes, including MnP, LiP, and UnP, contribute to the degradation of non-phenolic compounds (Ambatkar *et al.,* 2022).

The lignin degradation process requires peroxidase enzymes (LiP and MnP) to initiate catalysis, which depend on H_2O_2, naturally produced by fungi through their enzymatic system (Yu *et al.,* 2019). Ambatkar *et al.* (2022) reported that the fungus *P. ostreatus* produces peroxidase enzymes (MnP and LiP) for lignin degradation.

In the present study, *P. ostreatus* exhibited maximum MnP activity (E_{max} of 734.06 U/L) at week 3 of fermentation and maximum LiP activity (E_{max} of 4224.01 U/L) at week 4 in the control medium. However, in the H_2O_2 medium, *P. ostreatus* reached maximum MnP activity (E_{max} of 1273.22 U/L) at week 3 and maximum LiP activity (E_{max} of 2878.38 U/L) at week 4. Perera *et al.* (2023) reported MnP activity of 6.10 ± 0.11 mU/mL for *S. commune* and 39.81 ± 2.3 mU/mL for *F. pseudensiforme* in a lignin-rich medium. In the present research, *P. ostreatus* produced a higher amount of MnP in a medium supplemented with H_2O_2 as a co-substrate. It is suggested that LiP activity was higher in the control medium due to H_2O_2 induction, resulting from substrate oxidation carried out by Lac. Meanwhile, MnP utilized the H_2O_2 added to the culture medium to enhance its production.

In the current study, the first enzymes to be produced were Lac enzymes (week 1), followed by H_2O_2-dependent enzymes: MnP (week 3), and LiP and UnP (week 4). These latter enzymes play a role in the final degradation of PE, as they have a larger enzymatic cavity that allows them to accept larger molecules (Santacruz-Juárez *et al.* 2021). Santacruz-Juárez *et al.* (2021) reported that PE degradation can occur through the synergistic activity of Lac, MnP, LiP, and UnP. Lac acts first by producing the H_2O_2 necessary for peroxidases. Then, LiP is activated, generating an oxidation of the heme iron center to form free radicals from the polymer chain of PE. Lastly, MnP are responsible for degrading the molecules produced in PE decomposition into organic acids, which enter the Krebs cycle for mineralization. On the other hand, UnP enzymes are also activated by the presence of H_2O_2 and form an oxo-ferryl complex, which reacts with PE to initiate catalysis by generating ethyl and alkane free radicals. These are successively oxidized to acetic acid, which enters the Krebs cycle for mineralization into CO_2 and H_2O.

In the present study, H_2O_2 was added as an inducing agent to enhance peroxidase enzyme production. However, the enzymatic activity shown in H_2O_2 medium did not show significant differences to that activity obtained in the control medium. Ambatkar *et al.* (2022) also studied those wild fungi increased the peroxygenase production in the presence of H_2O_2 as a co-substrate.

In the present study, PE exhibited a significant reduction in surface hydrophobicity. The gradual decrease in the contact angle may be due to modifications in the hydrophobic surface due to the action of oxidoreductases secreted by *P. ostreatus*. Ray *et al.* (2023) reported similar findings, observing a decrease in the contact angle of PE (from 107.4° to 99.4°) after enzymatic treatment and biofilm formation using commercial laccase enzyme from *P. chrysosporium* in sodium acetate buffer solution. These results highlight the crucial role of these enzymes in increasing the susceptibility of the material to fungal attack, facilitating its degradation (Bai *et al.,* 2025). The reduction in surface hydrophobicity resulted from the formation of fungal biofilms on the surface during liquid fermentation, which altered the physicochemical properties of PE by changing the concentration of functional groups and modifying surface morphology (Miri *et al.,* 2022). Similar findings were also reported by González-Márquez *et al.* (2024).

In the present research, the principal functional groups of PE were modified after degradation by *P. ostreatus*. The doublet observed in the 700–750 cm^{-1} range suggested an increase in

crystallinity, as this IR band appears as a doublet when the material is in a crystalline phase. The findings of this study confirm those of a previous study (Martínez-Romo *et al.*, 2018). The formation of a shoulder in the CH_3 band (1440–1490 cm^{-1}) indicated that the PE backbone underwent the cleavage of branched chains, leading to the formation of low-molecular-weight polymer chains (Martínez-Romo *et al.*, 2018). In the current study, the decrease in the intensity of the C=O band (1750–1600 cm^{-1} range) during biodegradation was primarily attributed to the fungal consumption of carbonyl groups as part of the PE degradation process, which enhanced the polymer surface hydrophobicity, consistent with previous findings (Zahra *et al.,* 2010).

5. CONCLUSION

These results show that the addition of 5mM of H_2O_2 to the culture medium increased the production of MnP, however, it did not induce the production of Lac, LiP and UnP. *P. ostreatus* can produce enzymes for PE degradation, since the changes in the chemical structure and the decrease in the contact angle of the PE surface showed biodegradation of the polymer.
To better understand the effect of H_2O_2 on the biodegradation of PE by *P. ostreatus*, future studies are required on testing different concentrations of H_2O_2 to know its effect on the biodegradation process of this polymer.

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AUTHOR CONTRIBUTION

Angel González-Márquez and Ariadna Denisse Andrade-Alvarado performed the experimental work, analyzed data, and wrote the first draff of the manuscript. Rosario González-Mota analyzed data and supervised the research. Carmen Sánchez planned the experiments, conceived the initial idea, supervised the research, analyzed data, and wrote the final version of the manuscript. All authors read and approved the final version of the manuscript.

CONFLICT OF INTERESTS

The authors declare that there is no conflict of interest.

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