

Degradation of Low-Density Polyethylene Using Laccase Produced by *Trametes versicolor* (Turkey Tail) Cultured on *Oryza sativa* (Rice) Stems

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Abstract. Plastic pollution remains an environmental crisis both locally and globally, and the Philippines' insufficient waste management and significant dependence on single-use plastics generate 2.7 million tons of plastic waste annually. LDPE is commonly used in bottles, garbage bags, plastic gloves, and single-use containers that decompose for up to 1,000 years. Rice (*Oryza sativa*) stems are discarded as agricultural waste, containing cellulose and hemicellulose, which are essential to act as substrates for solid-state fermentation. Turkey tail (*Trametes versicolor*) is a fungus capable of producing laccase that can break down chemical structures due to mono-electronic oxidation. This study tests the degrading ability of laccase produced using turkey tail in rice stems as a fermentation medium. Researchers buried LDPE strips (1 x 0.5) inches with initial weights of 0.63 grams and in quintuplicates per set-up, incubating them at 5, 10, and 15 days, respectively. Groups A, B, and C were laccase-treated in each incubation period, while Groups D, E, and F received no treatment. After incubation, LDPE strips were cleaned, weighed, and had a corresponding percentage weight loss of 12.30%, 18.26%, and 30.56% for experimental; 11.11%, 12.30%, and 14.68% were computed for negative control. One-way ANOVA, paired, and independent samples t-tests were performed, setting the alpha value to 0.05 to determine significant differences between set-ups. The ANOVA result showed that 15 days in the experimental set-up had the highest degradation compared to 5 and 10 days of groups, indicating significant differences with a p-value of 3.84E-06. T-tests revealed that Group C significantly differs from all groups, resulting in a p-value of 3.38E-05. Findings suggest that laccase produced by turkey tail cultured on rice stems degraded LDPE. All set-ups degraded LDPE, but the experimental groups demonstrated a higher degradation than the negative control groups. The degradation process is more efficient the longer it is incubated.

Keywords: Plastic pollution; Low-density polyethylene; Rice stems; Turkey tail; Laccase; Plastic degradation.

1.0 Introduction

Approximately 400 million tons of plastic waste is produced annually worldwide. The amount of plastic produced will be tripled if the trend continues by 2060. Around 60% of all generated plastic waste causes environmental damage and is seen in surroundings or landfills (Lai, 2024). In 2020, 367 million metric tons of plastic waste were produced, and only 9% was successfully recycled. It has indicated that plastic production has increased two-fold since 2000, reaching 460 million tons in 2019 (OECD, 2022). In the same year, the Philippines contributed the most significant amount of plastic to the ocean, adversely impacting marine ecosystems (Ramos, 2024). It is marked by

inadequate waste management and a heavy reliance on single-use plastics, generating 2.7 million tons of plastic waste annually in the Philippines (Moaje, 2024). A study revealed that 80% of plastic waste originated from the polluted Pasig River, making it the most contaminated river globally (Meijer et al., 2021).

Low-density polyethylene (LDPE), derived from the monomer ethylene, is commonly used in products like squeezable bottles, garbage bags, plastic gloves, and single-use containers (Jambeck et al., 2015). It is marked on plastic products by the number "4" within an arrow triangle. LDPE is a thermoplastic polymer that is flexible, odorless, transparent, and fully recyclable. The molecular structure of LDPE resembles that of a tree branch. The tree-branch-like structure gives LDPE lower crystallinity, resulting in flexibility (De Naoum & Schadeegg, 2024). Currently, there are 28.09 million tons 2024 of LDPE produced worldwide, and there is an expected growth in its demand between 2019 and 2024 (Mordor Intelligence, 2024). Unfortunately, LDPE products can take up to 1000 years to break down, highlighting the urgency for environmentally friendly alternatives (Promotherapy, 2019).

Microorganisms can break down plastic and offer potential solutions to plastic waste. Using microbial biodegradation techniques provides an economical and environmentally reliable approach to addressing the plastic waste crisis (Shilpa et al., 2022). Enzymes can be sourced from microorganisms such as Turkey tail (*Trametes versicolor*), which are fungi capable of producing laccase. Plastic degradation through microbial or enzymatic means is a promising strategy to break down petro-plastics into recyclable monomers or mineralizing them into carbon dioxide, water, and biomass, generating higher-value bioproducts (Montazer et al., 2019).

Laccases are monomeric glycoproteins with multifaceted enzymatic properties belonging to blue copper oxidases (Temporiti et al., 2022). They are used in breaking down chemical contaminants due to their broad substrate specificity and a wide spectrum of complexes through mono-electronic oxidation (Dong et al., 2023). According to Guerberoff and Camusso (2019), laccase acts as a catalyst that speeds up degradation, with the oxidation of lignin concentration influencing their production. A study by Mishra and Upadhyay (2020) stated that enzymes exhibit two fundamental properties: one increases reaction rates without affecting itself, and the other affects reaction rates without altering the chemical equilibrium between reactants and products.

Rice (*Oryza sativa*) is an abundant crop in the Philippines, with its stems consisting of 13.5% lignin, 24.0% cellulose, and 27.8% hemicellulose by weight (Rosado et al., 2021). Liu et al. (2017) studied the presence of laccase within the genome of 30 genes, which showed that this enzyme can degrade various organic compounds. Swetha and Shivaprasad (2019) extracted and isolated laccase from rice stems, which is essential in this study that makes an ideal fermentation medium where it uses the chemical composition of rice stems as a food source for Turkey tail for growth and the ability to degrade LDPE.

Perdani et al. (2020) performed solid-state fermentation using Turkey tail that produced laccase, where they used steam-exploded rice husks, bagasse, and cornstalk as substrates, which was a candidate for enzyme production as an application in biosensor. It exhibited that the enzyme is oxidized with decreasing substrate. Similarly, Chandrasekaran et al. (2021) performed serial dilution of 1 g of collected soil samples where laccases were isolated. A plate assay was performed, dropping 0.01% of guaiacol, and the appearance of a brown zone around the bacterial growth suggested laccase production. 1 g samples were buried in soil treated with bacterial culture and evaluated its degradation for three months. The percentage weight loss was calculated to demonstrate the extent of laccase as a plastic degrading ability.

This study uses rice stems as a fermentation medium and food source for growing turkey tails to produce laccase. The application aims to degrade low-density polyethylene as it is a predominant material for packaging goods and contributes to plastic waste. This research assessed the degrading capability of laccase produced through solid-state fermentation, isolated, and transferred them to soil pits embedded in LDPE. The researchers examined only three incubation periods (5, 10, and 15 days) with 5-day intervals. The researchers observed its percentage weight loss as a parameter determining the extent of LDPE degradation. Furthermore, the study's findings can help understand how laccase can break down organic compounds, contributing to a more efficient and less polluting way to reduce plastic waste. Further research can help establish the concentration and threshold of laccase produced using turkey tail cultured on rice stems from degrading LDPE and other plastics.

2.0 Methodology

2.1 Research Design

A quantitative research design was used as a systematic approach to examine and resolve a specific problem. This approach tested hypotheses and produced results using numerical data and statistical analysis. Furthermore, statistical analyses, like one-way ANOVA, Paired, and Independent Samples t-tests, were used to evaluate if there were significant differences. The researchers employed experimental quantitative data to determine the current issue, using a scientific method that involves altering one or more variables to see how they influence another variable. It offers numerical facts that can be verified and quantified in nature. In line with the quantitative approach and experimental design, the study consists of six groups, divided into three set-ups for experimental (laccase treated in LDPE) and three for negative control (no treatment in LDPE). Both groups underwent five trials to establish validity and incubated at 5, 10, and 15 days. The study's design aimed to determine the highest degradation among incubation periods.

2.2 Research Locale

The study was conducted at San Beda University-Rizal, the Chemistry laboratory on the 4th floor, and the Physics Stockroom on the 3rd floor of the JHS building.

2.3 Research Participants

No research participants were used in this study. The researchers used low-density polyethylene and divided into six groups: three for experimental (Group A, B, and C) and three for negative control groups (Group D, E, and F), with incubation periods of 5, 10, and 15 days each, respectively.

2.4 Research Instrument

This study used laboratory equipment, such as a spectrophotometer, to determine laccase activity obtained from solid-state fermentation of turkey tail in rice stems. While an analytical balance measured the initial and final mass of LDPE per group and calculated the percentage weight loss, the formula is expressed as:

$$\% \text{ Weight Loss} = \frac{W_0 - W}{W_0} \times 100$$

W_0 is the initial weight before plastic degradation (mg), and W is the residual weight after plastic degradation (mg).

2.5 Data Gathering Procedure

Materials

Four hundred and thirty grams of rice stems were obtained from Purok 1 Bakir, Nueva Vizcaya, and Duran Farm, Bulacan. A plant sample was authenticated at the University of the Philippines-Diliman. At the same time, five turkey tail cultures and ten potato dextrose agar plates were purchased from Mycosphere, Nueva Ecija, and were certified for authenticity. All other materials, including low-density polyethylene, guaiacol, containers, sodium acetate, and soil pH buffer, were purchased from their suppliers.

Substrate Preparation

The leaves and roots were separated and discarded after harvesting rice stems at their respective locations. It was washed with distilled water and air-dried for a day. 430 g rice stems were obtained after being weighed, cut into (0.25 in), and autoclaved at 121°C at 15 psi for 15 minutes and set aside to cool at room temperature (Najafpour, 2013).

Turkey Tail Sub-cultures

Five turkey tail cultures were refrigerated for one day before being subculturing to potato dextrose agar (PDA) plates (Birhanli & Yesilada, 2010). The researchers used a scalpel to cut a portion (22 x 22 cm) of turkey tail culture containing mycelia. The culture was transferred to ten PDA plates and incubated for two weeks at 25°C for the maturation of fungi (Bains & Chawla, 2020).

Laccase Plate Assay

A plate assay was performed to confirm if turkey tail subcultures excreted laccase. 4 mM of guaiacol was dropped to the ten PDA plates and incubated for 24-48 hours at 25°C. The reddish-brown result indicated an oxidation reaction that confirmed laccase was present (Chandrasekaran et al., 2021).

Solid-State Fermentation

The prepared rice stems were placed in a sterilized sizeable plastic tray and in a fume hood to prevent contamination. Researchers used a scalpel to cut a (22 x 22 cm) turkey tail subculture, which was placed evenly on rice stems. It was incubated for 12 days at room temperature for cultivation (Perdani et al., 2020). Solid-state fermentation was achieved to stimulate the growth of the turkey tail on rice stems for laccase production (Adekunle et al., 2016).

Laccase Extraction

After fermentation, each batch contains 8.27 g of fermented rice stems with 10 mL of sodium acetate buffer (50 mM, pH 5.0), was mixed for 4 minutes, and was ice bath at 4°C for 5 minutes. The solution was centrifuged at 3,000 rpm for 30 minutes, filtered by Whatman no. 1 filter paper, and mixed with 80 mL of sodium acetate buffer (Perdani et al., 2020). 50 µL of guaiacol was dropped on the resulting solution as screening for laccase production. An enzyme screening was performed with two solutions, both with standard buffer, but one was dropped with guaiacol and incubated for 24 hours before spectrophotometric analysis.

Spectrophotometric Analysis of Laccase

The spectrophotometer was used to determine the wavelength of laccase activity at its peak absorbance. 2 samples were used: both with standard sodium acetate buffer but 1 containing guaiacol for oxidation. The reading was set to (200-1000 nm) with increments of (25 nm) where all graphical data was presented, and samples were scanned in triplicates and averaged (Avian Technologies LLC, 2020). Pant (2022) stated that the fluctuation of the absorbency of the samples was observed, and the sample's absorbency indicates enzyme activity.

Soil Pit Preparation

The soil was sieved to remove rocks, and the pH level was controlled using an acidifier mixture and monitored using a pH meter daily. LDPE sheets were cut into strips with a measurement of (1 x 0.5 in) and weighed with an initial weight of 0.63 g per group (Chandrasekaran et al., 2021). Six large containers were prepared: 3 for experimental and 3 for negative control. LDPE sheets were embedded in five soil pits containing 75 grams each and placed inside a larger container for each group to ensure environmental control.

Incubation of LDPE

After soil pit preparation, 160 mL of the laccase solution was poured into the containers embedded with LDPE. The researchers incubated the soil pits with laccase for the experimental groups for 5, 10, and 15 days. In contrast, soil pits had no treatment for the negative control groups with the same incubation periods as the experimental (Helen et al., 2017). After each incubation, the LDPE strips were collected and washed with distilled water, air-dried, weighed, and recorded.

Percentage Weight Loss Measurement

After collecting LDPE from soil pits embedded in laccase solution and with no treatment, the strips were weighed before and after treatment using an analytical balance to assess the extent of percentage weight loss. The equation $\% \text{ Weight Loss} = \frac{\text{initial weight} - \text{final weight}}{\text{initial weight}} \times 100$ calculated the percentage weight loss of the LDPE.

Statistical Analysis and Data Interpretation

The researchers analyzed and interpreted the weight loss data collected, calculating whether there were significant differences between the weight loss in the incubation periods of the experimental group (laccase-treated) and the negative control (no treatment) groups. A one-way analysis of variance (ANOVA) and t-tests (paired and independent samples) were used.

2.6 Safety Considerations

Risks

The study involves subculturing turkey tails to increase hyphae development during solid-state fermentation. This method increases the risk of breathing fungal spores; researchers can cause allergic reactions without personal protective equipment. All procedures are carried out under the supervision of laboratory professionals to ensure safety and mitigate risk while protocols are being followed.

Fungi handling

The fungi used in the study, specifically the turkey tail, is known to cause allergic reactions in humans once its spores are inhaled. Therefore, the researchers should properly store the fungi cultures in a place where no one usually goes to avoid inhalation. Furthermore, practicing the wearing of surgical masks can also prevent the inhalation of spores.

Proper waste disposal

Researchers collected rice stems from solid-state fermentation and turkey tail subcultures and transferred them to large containers, where chemical disinfection was employed while PPE was equipped. A 10% household bleach solution was soaked in the waste for 30 minutes. After disinfection, the stems were transferred to leakproof plastic containers labeled as biohazards.

3.0 Results and Discussion

Enzyme screening for laccase was done before the soil treatment with the isolated product. The extracted solution was separated and transferred into two separate beakers. Both solutions were treated with standard sodium acetate buffer (50 mM, pH 5.0), one as is, while the other was dropped with guaiacol. A change in the laccase-treated solution's color was observed in Figures 1 and 2 after 24 hours.



Figure 1. Presence of laccase in standard buffer solution without guaiacol

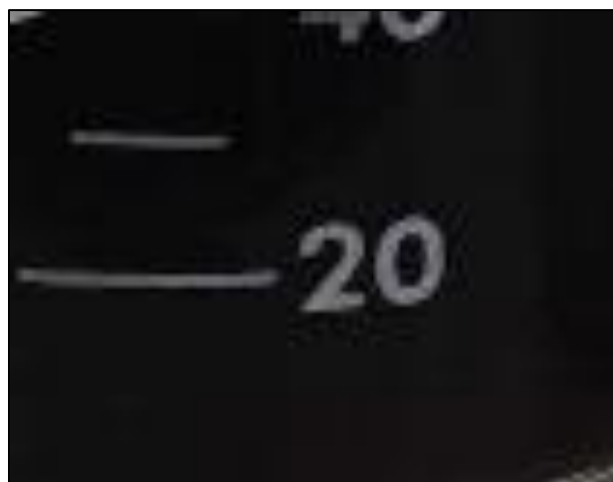


Figure 2. Presence of laccase in standard buffer solution with guaiacol

Perdani et al. (2020) combined the fermented product with fifty milliliters of sodium acetate buffer (50 mM, pH 5.0). For 60 minutes, the mixture was agitated using a rotary shaker set to 120 rpm. Afterward, a muslin cloth was used and filtered; mixed results were centrifuged for 10 minutes at 4°C at 9000 rpm. A crude laccase extract is the resultant supernatant. In this study, laccase was observed in Figure 1 after 24-hour incubation. At the same time, Paraschiv et al. (2022) isolated laccase-producing microorganisms from natural substrates using a spread-plate or streak-plate method. Screening methods using chromogenic substrates, like guaiacol, are carried out to determine the most productive colonies. Umar and Ahmed (2022) stated that the color change after dropping guaiacol into the solution indicates the presence of laccase. Oxidation reaction occurs when guaiacol interacts with laccase. Figure 2 shows that the solution's color changed to reddish-brown due to the guaiacol's reaction (Shrestha et al., 2016).

Figure 3 shows the laccase solution's average absorbency with and without guaiacol after enzyme screening. Observations reveal that at lower wavelengths (200-450 nm), both solutions exhibit high absorbance, where guaiacol solution is maintained at a constant up to 500 nm. In comparison, the sodium acetate buffer solution gradually declines at 450 nm. As the wavelength decreases at 500 nm, the absorbance of the guaiacol solution decreases steadily, and the sodium acetate buffer solution demonstrates a more significant decrease. It suggests that laccase has a higher activity with guaiacol due to oxidation reaction, while the decreasing value of standard solution implies minimal light absorption by the solution itself.

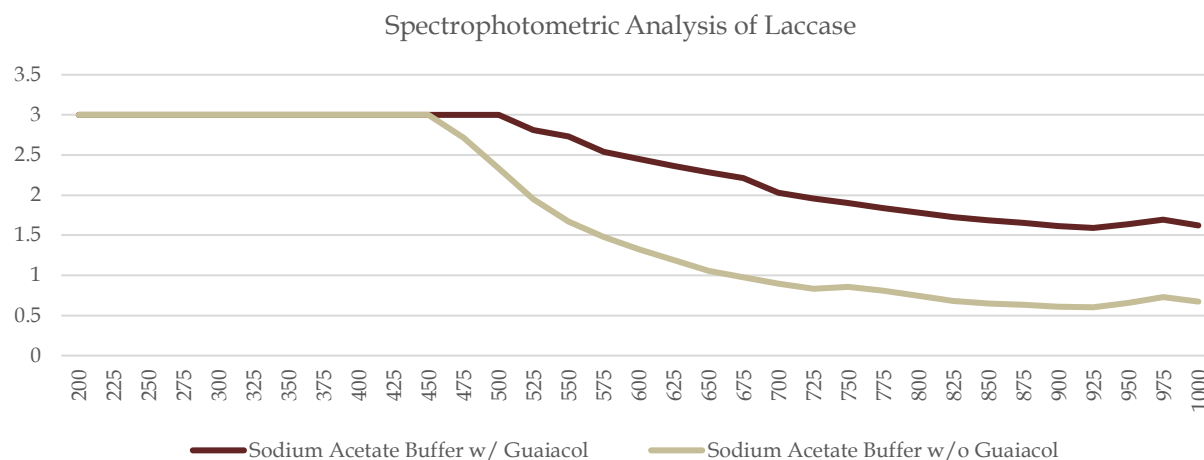


Figure 3. Spectrophotometric analysis and comparison of sodium acetate buffer solution of laccase w/ and w/o guaiacol

According to Senthivelan et al. (2019), a spectrophotometer observes the sample's color change through absorbency. This phenomenon is explained by Beer's Law, which states that absorbance (A) is directly proportional to the concentration (c) of the absorbing sample in a solution and the path length (ℓ) of the light passing through the sample, expressed mathematically as $A = \epsilon \ell c$ (Mayerhöfer & Popp, 2018). In this study, the absorbing sample is laccase oxidation using guaiacol. The change in absorbance is directly proportional to the concentration of the color produced, which determines the laccase activity. According to Hoekstra and Smith (2023), enzymes like Proteinase K and PLA depolymerase effectively degrade bioplastics, achieving a 20-30% breakdown. By measuring absorbance changes at specific wavelengths, researchers can quantify enzyme activity and optimize conditions for enhanced degradation. It provides a strong framework for advancing research in bioplastic biodegradation and developing more efficient strategies for mitigating plastic pollution.

Table 1 summarizes the degradation analysis of experimental and negative controlled groups' average pre- and post-weight, expressed as grams and percentage weight loss in percent, with the raw data in quintuplicates. At the same time, soil pH changes were noted and maintained using a pH meter. With 15 incubation days, the experimental group, Group C, exhibited the highest weight loss, highlighting the impact of the laccase present, which shortens the time for plastic degradation. In contrast, Group F, with 15 incubation days, indicated a slower degradation due to the absence of laccase in the soil pits but had the highest degradation among negative control groups.

Table 1. Comparison of average pre-, post-weight, and percentage weight loss of experimental and negative control groups

Set-up	Group	Incubation Period (days)	Pre-weight (g)	Post-weight (g)	Percentage weight loss (%)
Laccase-treated	A	5	0.63	0.55	12.30
	B	10	0.63	0.52	18.26
	C	15	0.63	0.44	30.56
	D	5	0.63	0.56	11.11
No treatment	E	10	0.63	0.55	12.30
	F	15	0.63	0.54	14.68

These results correlate with the study of Helen et al. (2017), where they performed an in-vitro biodegradation assay of the bacteria isolates for 40 days with 5 days as intervals. It was shown that one isolate peaked at 15 days

while the other at 35 days. It can depend on the microorganism capable of producing laccase, which is directly proportional to the incubation days of its production and threshold. Another study by Makut et al. (2023) indicated the effect of pH at (5, 6, 7, 8, and 9) during incubation periods as it supports regulating the soil pH at 5.0 during incubation.

Table 2 presents the one-way ANOVA test performed on the experimental and negative control groups with an F value of 16.34. With the alpha value set at 0.05, the $F_{0.05}$ was 5,18 = 2.77. The p-value of 2.77 is 3.84E-06, indicating that the test statistic is larger than the critical value deemed significant. Therefore, there are significant differences in the weight loss difference of LDPE.

Table 2. ANOVA analysis on weight loss difference between groups

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	0.042	5	0.0084	16.34	3.84E-06	2.772
Within Groups	0.009	18	0.0005			
Total	0.051	23				

Moreover, Table 3 presents the paired samples t-test analysis regarding the weight loss difference between the experimental and negative control groups. Significant differences are observed between the pre- and post-weight results of Groups A to F with p-values of 0.01831, 0.00982, 3.38E-05, 0.00121, 5.13E-04, and 0.00168, respectively.

Table 3. Summary of paired samples t-test analysis of pre- and post-weight per group

Comparison		t Statistic	P-value	Significance
Pre-weight	Post-weight			
A	After 5 days	4.69	0.01	p < .05
B	After 10 days	5.88	0.00	p < .05
C	After 15 days	40.2	0.00	p < .05
D	After 5 days	12.1	0.00	p < .05
E	After 10 days	16.1	0.00	p < .05
F	After 15 days	10.8	0.00	p < .05

In a study by Kim and Kim (2019), enzymes function as biological catalysts, which hastens molecular-level chemical processes in organisms. They significantly regulate several biological processes, including metabolism, signaling, transcription, replication, and protein synthesis. In addition, Xia et al. (2023) stated that laccase is a highly effective biocatalyst for environmentally harmful substances. These enzymes work similarly to inorganic catalysts, increasing the rate of chemical reactions by lowering the activation energy of those reactions. In this study, Groups A, B, and C containing laccase broke down LDPE strips faster than untreated Groups D, E, and F.

Table 4 provides an analysis using independent samples t-test between experimental groups to determine significant differences in weight loss per group. There is no significant difference in Group A vs. B with a p-value = 0.24061. Compared to Group A vs. C and B vs. C, significant differences result in p-values of 5.43E-04 and 0.00525.

Table 4. Summary of independent samples t-test analysis of between experimental groups

Pairwise comparison	t Statistic	P-value	Significance
A (after 5 days) vs B (after 10 days)	1.30	0.24	insignificant
A (after 5 days) vs C (after 15 days)	6.69	0.00	p < .05
B (after 10 days) vs C (after 15 days)	4.27	0.00	p < .05

It supports a study by Roohi et al. (2017), who explored the use of enzymes to control the degradation of biodegradable plastics and emphasized their potential role in managing plastic waste. In addition, a study by Santo et al. (2012) specifically focused on using bacterial laccase enzymes for plastic degradation. Similarly, Banerjee et al. (2014) demonstrated the ability of enzymes to degrade polymers, opening doors for more sustainable solutions in various industries.

Table 5 provides an analysis using an independent samples t-test between negative control groups to determine significant differences in weight loss per group. There are no significant differences between Groups D, E, and F, resulting in a p-value = 0.35592, 0.07178, and 0.17634, respectively.

Table 5. Summary of independent samples t-test analysis between negative control groups

Pairwise comparison	t Statistic	p-value	Significance
D (after 5 days) vs E (after 10 days)	1.00	0.35	insignificant
D (after 5 days) vs F (after 15 days)	2.18	0.07	insignificant
E (after 10 days) vs F (after 15 days)	1.53	0.17	insignificant

In contrast, the degradation of the negative control groups is supported by Bläsing and Amelung (2017), who reported that minimal degradation of synthetic polymers was tested in soil. In addition, Scalenghe (2018) cited a study conducted by Albertsson and Karlsson (1988) where 10 years of medium-term studies on 0.02-0.16 mm of LDPE films shows that without oxidation additives, only evolved 0.2% weight of CO₂, while UV sensitizer was irradiated of the percentage of weight loss in a few weeks. Similar results were obtained by Selke et al. (2015), who concluded that 3 years is a very short degradation time for films.

Furthermore, Table 6 provides an analysis using independent samples t-test between groups to determine significant differences in weight loss per set-up. No significant differences are observed between Groups A, B, D, and F when compared, resulting in a p-value = 0.68319, 1.00000, 0.45068, 0.08708, 0.14337, and 0.42744, respectively. Compared to Group C and grouped with D, E, and F, there are significant differences with p-values of 3.35E-06, 2.66E-06, and 5.13E-05.

Table 6. Summary of independent samples t-test analysis between groups

Comparison		t Statistic	p-value	Significance
Treated	Untreated			
A (after 5 days)	D (after 5 days)	-0.43	0.68	insignificant
A (after 5 days)	E (after 10 days)	0.00	1.00	insignificant
A (after 5 days)	F (after 15 days)	0.81	0.45	insignificant
B (after 10 days)	D (after 5 days)	-2.04	0.08	insignificant
B (after 10 days)	E (after 10 days)	-1.68	0.14	insignificant
B (after 10 days)	F (after 15 days)	-0.85	0.42	insignificant
C (after 15 days)	D (after 5 days)	-16.3	0.00	p < .05
C (after 15 days)	E (after 10 days)	-16.9	0.00	p < .05
C (after 15 days)	F (after 15 days)	-10.2	0.00	p < .05

According to Pandey (2003) and Shraddha et al. (2011), solid-state fermentation is the most suitable way to produce enzymes using fungi. Using agricultural residues that contain lignin, cellulose, and hemicellulose. It aims to grow microorganisms in an environment with little to no complimentary water or free-flowing aqueous phase. Furthermore, in a study by Perdani et al. (2020), this method was used to produce laccase enzymes. Their study subjected turkey tail cultures with bagasse, cornstalk, and rice husks serving as the substrate. It significantly increased laccase production using SSF through steam-exploded pre-treated corn stalk as a substrate (Adekunle et al., 2016). As a result, Group C, which is laccase-treated, had the highest degradation.

4.0 Conclusion

The researchers concluded the following based on the findings concerning the effect of incubation periods of low-density polyethylene strips buried in soil pits treated with laccase extracted from the solid-state fermentation of cultured turkey tail on rice stems and without treatment. The extraction and isolation of laccase from turkey tail cultured on rice stems using a standard buffer solution was successful. Based on the spectrophotometric analysis, the interaction of laccase with guaiacol caused an oxidation reaction, which led to fluctuations in the sample's absorbency compared to the sodium acetate buffer solution with fewer fluctuations. The data indicates that Group C (laccase-treated) had the highest percentage of weight loss degradation at 30.56% among experimental and negative control groups. In contrast, Group F (no treatment) had the highest percentage of weight loss degradation at 14.68% among negative control groups. The researchers noted that as the incubation period is longer, the percentage of weight loss degradation is higher. The data analysis shows that one-way ANOVA had significant differences between groups in terms of their weight loss difference at varying incubation periods. At the same time, paired t-tests revealed that comparing pre- and post-weights of Groups A, B, C, D, E, and F was significant, while independent t-tests showed that only Group C was significant compared to all groups. The researchers' findings indicate that more extended incubation periods in the experimental groups can enhance laccase enzyme activation, thereby determining its threshold in the degradation process where industrial applications seeking

efficient plastic waste management can be adopted. Implementing this approach could result in more effective and sustainable plastic degradation processes while reducing the environmental impact of plastic waste.

5.0 Contributions of Authors

All authors contributed equally to each section, reviewed, and approved the final work.

6.0 Funding

No research grant was given to this study.

7.0 Conflict of Interests

The authors declare no conflict of interest in this study.

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