Assessing the impact of ligninolytic enzyme production by galactomyces geotrichum in solid-state fermentation utilizing oil palm empty fruit bunch

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Abstract: Oil palm empty fruit bunch (OPEFB) is a prominent agricultural waste in Malaysia, with an annual production exceeding 18 million tons. Despite its vast abundance, the utilization of OPEFB and oil palm waste, in general, remains limited. This study therefore evaluates the significant impact of OPEFB as avital bioresource within the Biorefinery sector, particularly for biofuel and biochemical production. The study focused on assessing the enzymatic activity in evolved lignin and cellulose degradation, which are critical processes in biomass conversion. To achieve this, solid-state fermentation (SSF) was conducted in flasks using OPEFB fibre's (with an average length of 250mm) at a moisture content of 70% and a temperature of 30°C, for a time span of for 30-days. The result revealed that under solid-state fermentation, the highest recorded activity was observed for manganese peroxidase (11.44U/g), followed by lignin peroxidase (8.05U/g), and laccase (3.15U/g). These findings in dictate the substantial potential for cellulose degradation and subsequent biofuel production. Furthermore, the results of this study revealed that solid-state fermentation partially degraded the lignocellulosic compounds presenting OPEFB. Overall, the monitored enzyme activities in ligninolytic categorised demonstrated moderate levels of performance, emphasizing the promising prospects of OPEFB as a feedstock for biorefinery applications.

Keywords: Lignin lytic, enzyme, galactomyces geotrichum, solidstatefragmentation

1. INTRODUCTION

The utilization of OPEFB and oil palm waste, in general, are pretty limited. Up to now, OPEFB wastes have been used for soil replenishment and as animal feed, while a more significant portion is burned to save up plantation space and avoid

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contamination [1]. *Galactomyces geotrichum* is a fungus that was isolated from sample of oil palm trunk and oil palm empty fruit bunch that shows Basidiomycetes growth. Most publications related to *Glactomyces geotrichum* are reporting the application of this fungus for biodegradation of dye. It shows high production of ligninolytic enzymes that are beneficial for the removal of lignin in lignocelluloses biodegradation [1].

2. MATERIALS AND METHODS

2.1 Optimizing Solid Medium for Galactomyces geotrichum Cultivation and Media Preparation

Cultivating *Galactomyces geotrichum* on solid medium is essential for numerous biotechnological applications. This study presents a precise protocol for fungal growth and medium preparation to ensure the viability and longevity of cultures. For Galactomyces *geotrichum cultivation*, we employed potato dextrose agar (PDA) as the solid medium. PDA was prepared by accurately combining 19.5 g of Difco PDA with 500mL of distilled water, following established procedures (Longeetal.,2018). Subsequently, the PDA solution was meticulously dissolved and subjected to sterilization via autoclaving at 121°C for a duration of 20 minutes, maintaining strict as optic conditions throughout. Up on completion of sterilization, the molten agar was cautiously cooled to at temperature range of 50-60°C. Under rigorous as optic conditions. (Pourkhanalietal.,2021).

2.2 Ligninolytic enzymes

2.2.1 Laccase

Laccase is an oxidative enzyme involved in the degradation of phenolic component of lignin. In a test tube, 1.7mL of sodium acetate buffer (0.05M, pH 5.0) was added, followed by 0.2mL of ABTS. A 0.1mL of sample was then added and immediately capped with an aluminum foil. The mixture was vortex and incubated at 30°C for 60 minutes. Absorbance was measured using a UV-VIS spectrophotometer at wavelength of 420nm against the reagent blank (Greenberg, 2019). Laccase activity is calculated as follow.

$$\text{Laccase activity}\left(\frac{\text{U}}{\text{L}}\right)=\frac{\text{OD x Total volume}(\mu\text{L})}{\text{Volume of sample }(\mu\text{L})\text{x }\epsilon(\text{mM}^{-1}\text{cm}^{-1})\text{x1 cm}}$$

2.3 Lignin Peroxidase

Lignin peroxidase (Lip) is a peroxidase enzyme involved in the degradation of both phenolic and non-phenolic component of lignin. In a test tube, 0.4 mL of sodium acetate buffer (0.05M, pH 5.0) was added, followed by 0.1 mL of veratryl alcohol and 0.5mL sample [2]. Then, 40.0 µL of hydrogen peroxide was added and immediately capped with an aluminum foil. The mixture was then vortex and incubated at 37°C for 3 minutes. Absorbance was measured using a UV-VIS

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spectrophotometer at wavelength of 310 nm against the reagent blank. LiP activity is calculated as follow.

Lignin peroxidase activity (U/g)=Lignin peroxidase activity(U/L)x(Extraction buffer(mL)/Amount of extracted solid (g))x solid ratio

2.4 Manganese Peroxidase

Manganese peroxidase (MnP) is an oxidative enzyme involved in the degradation of the phenolic component of lignin. In a test tube, 0.1 mL of phenol red was added, followed by 0.1mL of manganese (II) sulfate monohydrate and 0.5mL sample (Varnaitė and Raudonienė, 2005) Then, 1.2mL of sodium acetate buffer (0.05M, pH 5.0) was added and followed by 0.1mL of hydrogen peroxide before immediately capping with an aluminum foil. The mixture was vortex and incubated at 30°C for 60 minutes. Absorbance was measured using a UV-VIS spectrophotometer at wavelength of 610nm against the reagent blank. MnP activity is calculated as follow.

2.4 Manganese Peroxidase

Manganese peroxidase (MnP) is an oxidative enzyme involved in the degradation of the phenolic component of lignin. In a test tube, 0.1 mL of phenol red was added, followed by 0.1mL of manganese (II) sulfate monohydrate and 0.5mL sample (Varnaitė and Raudonienė, 2005) Then, 1.2mL of sodium acetate buffer (0.05M, pH 5.0) was added and followed by 0.1mL of hydrogen peroxide before immediately capping with an aluminum foil. The mixture was vortex and incubated at 30°C for 60 minutes. Absorbance was measured using a UV-VIS spectrophotometer at wavelength of 610nm against the reagent blank. MnP activity is calculated as follow.

Manganese peroxidase activity (U/g) = Manganese peroxidase activity(U/L) x (Extraction buffer(mL)/Amount of extracted solid (g))x solid ratio.

3. RESULTS AND DISCUSSION

This article is aimed at investigating and providing more information on the ability of *Galactomyces geotrichum* to produce ligninolytic enzymes from the fermentation of oil palm empty fruit bunch. Lignin- and cellulose-degrading enzymes are amongst two most important enzymes that play important role in the degradation of complex structure of lignocellulose biomass. Ligninolytic enzymes particularly lignin peroxidase, manganese peroxidase and laccase are the prime enzymes to initiate and enhance degradation of lignocellulose [2].

3.1 Ligninolytic Enzymes Activity

Based on the result obtained showed all enzymes activity from day 1 until day 30. On day 1 all ligninolytic enzymes showed activity .It might be due to the complex structure of ligninolytic enzymes because microbe is not able to degrade the ligninolytic. However, lignin peroxidase at the beginning showed highest activity

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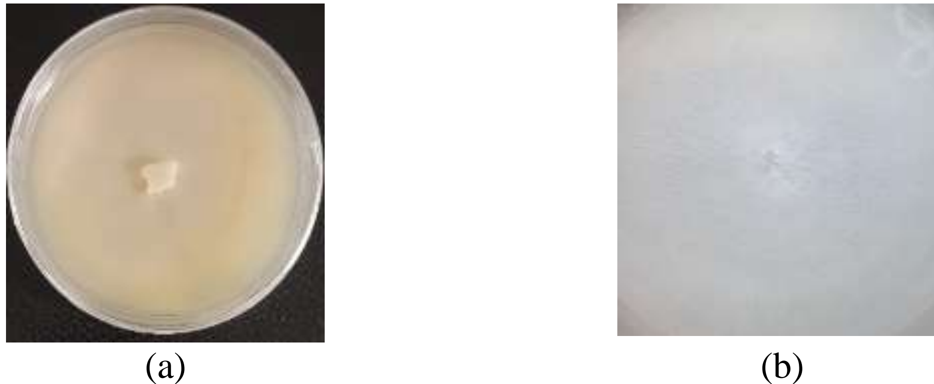
(8.054 U/ g). This could be due to the broad substrates and high redox potential it can directly attack on the compounds on the other hand do not need to a mediator to do degradation (Datta et al., 2017). Therefore, lignin peroxidase has high redox as compared with other enzymes (laccase and MnP). Therefore, this indicated that lignin peroxidase is very common for its high redox potential and it can oxidize phenolic and non-phenolic compounds of lignin directly without any mediator (Datta et al., 2017). The activity of manganese peroxidase was increased as compared to laccase. Based on the results manganese peroxidase was at the high amount (11.44 U/ g) but it would not directly attack the lignin unit because it has required (Mn^{2+}) act as a mediator at during degradation process. During fermentation, the complex reaction of fungi in the SSF may maximize to trigger the amount of lignin and manganese peroxidase (More et al., 2011). Fig. 2 show that laccase was the poorest ligninolytic enzyme produced by *Galactomyces geotrichum*.



(a) (b)

Fig. 1. Growth of *Galactomyces geotrichum* on PDA plate: (a) front view of the culture and (b) rear view of the culture

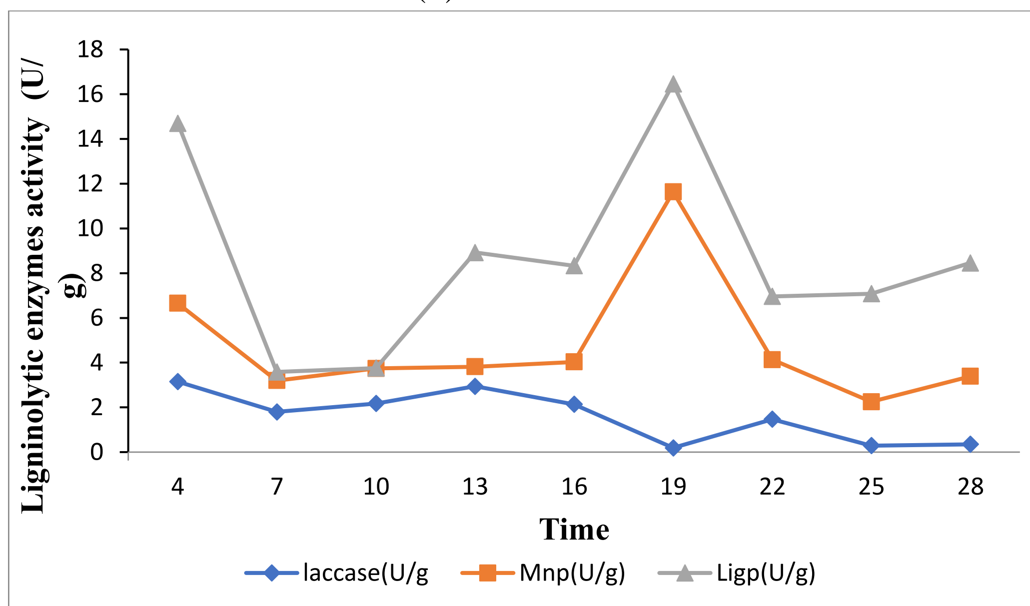


Fig. 2. Profile of ligninolytic enzymes (a) laccase (b) manganese peroxidase and (c) lignin peroxidase produced in solid state fermentation (SSF) period of 30 days

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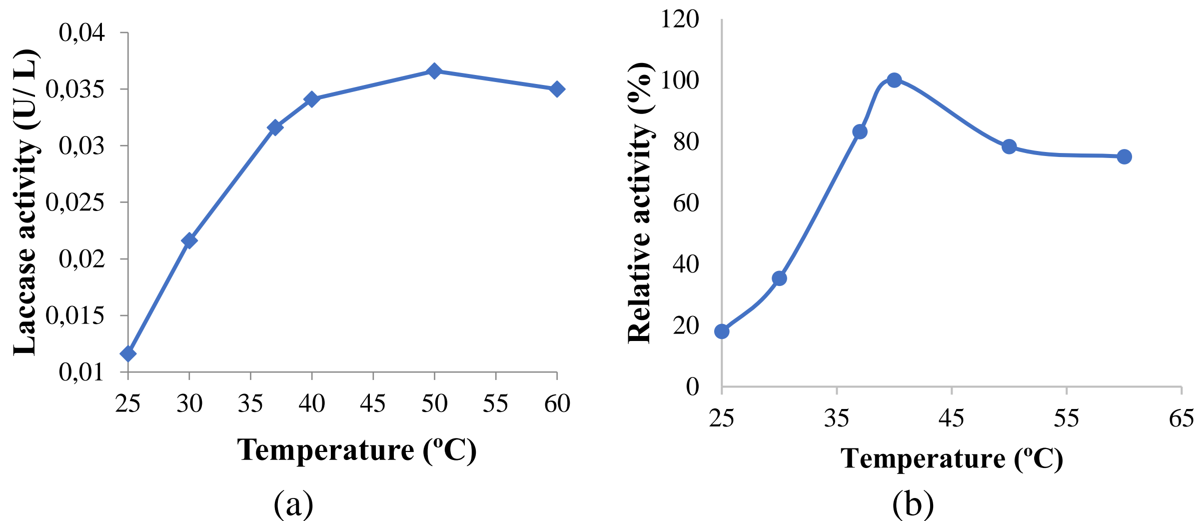
The maximum laccase produced was 3.15 U/g at day 1 of SSF. Low laccase production was highly related to the low redox nature of the enzyme. Under SSF condition, it was not possible to control mass transfer thus poor mass transfer developed in the SSF. This would possibly create low redox condition that is preferable for the laccase catalytic activity. Therefore, under SSF condition more laccase was produced by *Galactomyces geotrichum*) The presence of higher laccase in SSF condition would be advantageous for the extensive degradation of lignin as it would result in depolymerisation of lignin for both phenolic and non-phenolic substrate (Jiao et al., 2018).

3.2 Biochemical Characterization of Lignin lytic Enzymes

The biochemical parameters of all five enzymes, such as the optimum pH, and optimum incubation temperature, as well as the pH and temperature stability of ligninolytic enzymes were determined.



(a) (b)

Fig. 3. Effect of temperature on the a) activity and b) stability of laccase at different temperature condition.

This is because the laccase mostly loss its activity in high temperature and fungal laccase often cease functioning quickly at high temperatures. For the optimum pH of laccase, the higher activity was observed at pH3 with 0.8012 U/L total activities (Fig. 4.a). The high activity observed could be due to the oxidation of substrate because laccase loss its activity in high pH. Therefore, the activity of laccase depends on rate of ionization. While, the pH stability for laccase was observed at pH8 with maximum activity (1.0012U/L) as shown in Fig. 4.(b) therefore laccase could be quite stable in this pH the trend outcome of this study was similar to those reported in Afreen et al., [3].

The optimum temperature was detected at 40 ºC with optimum activity (0.0572). The high activity was due to the adaption nature of fungi. However a study suggested MnP production was influenced by the temperature of the growing medium, with low activity (Urek & Pazarlioglu, 2007). Therefore, (0.0316U/L) found at 25 ºC. When

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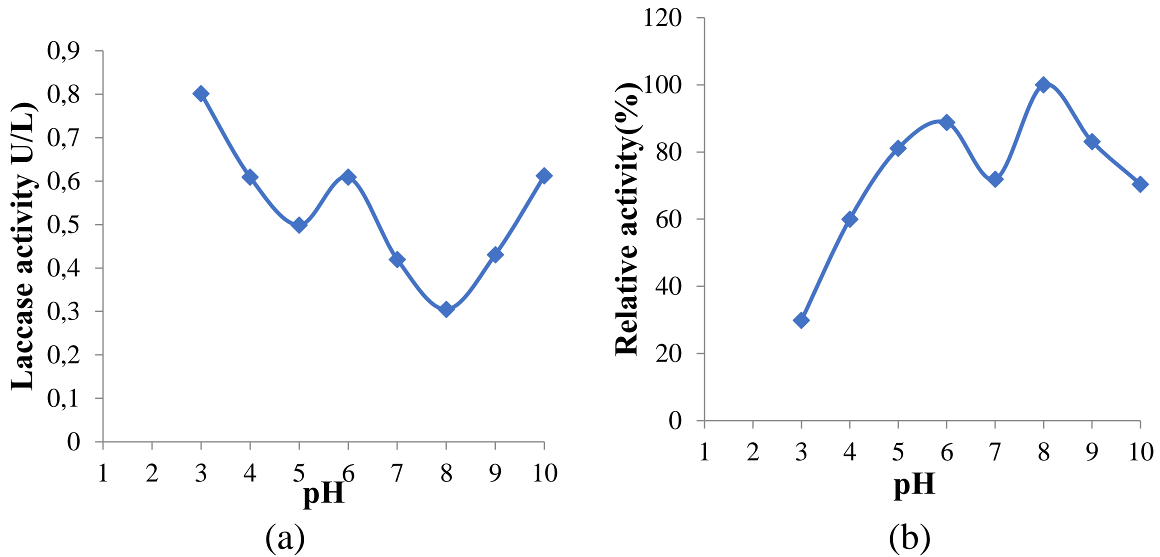
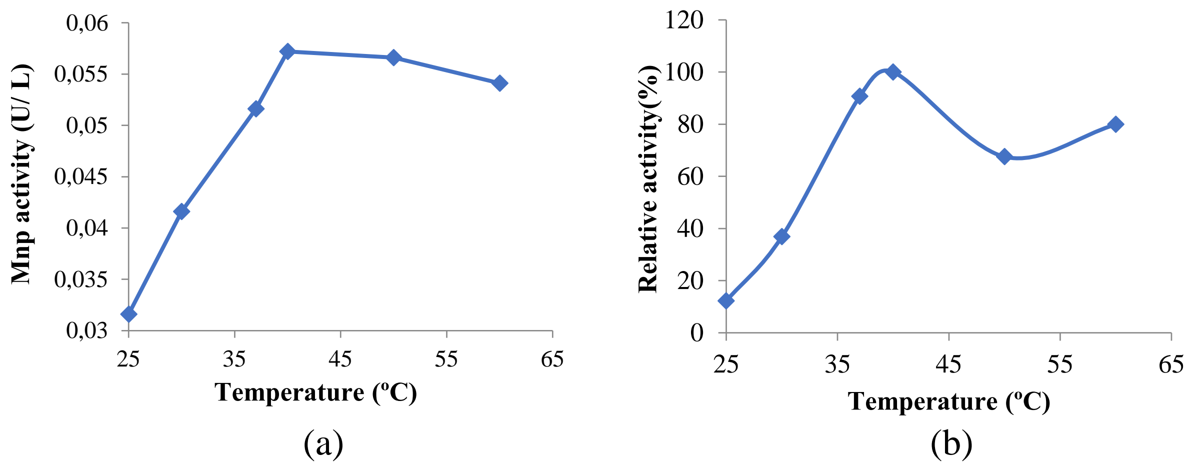
the temperature was raised to 40ºC, small amounts of MnP were produced at 25 to 30ºC.



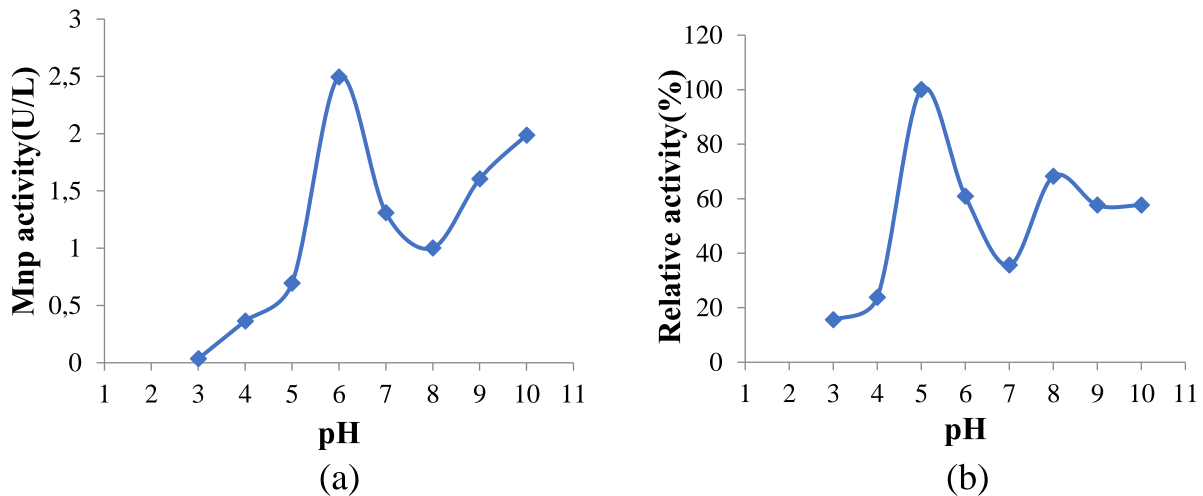
(a) (b)

Fig. **Error! No text of specified style in document.**. Effect of pH on the; (a) activity and (b) stability of laccase at different pH condition



(a) (b)

Fig. 5. Effects of temperature on the a) activity and b) stability of manganese peroxidase (MnP) at different temperature conditions



(a) (b)

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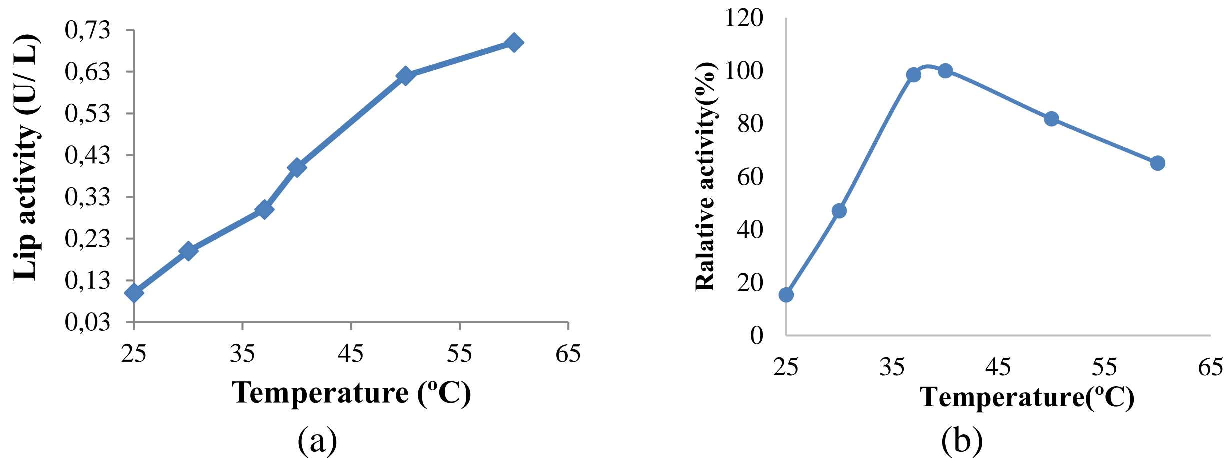

Fig. 7. Graphical presentation showing Lip activity and Relative activity against temperature

The fungal manganese peroxidase showed an optimal activity at the temperature of 40ºC (Fig. 5 (a). For temperature stability, the result revealed the temperature stability for MnP at 40ºC as shown in Fig. 5 (b). The outcome of this research was closed to findings reported by (Urek & Pazarlioglu, 2007). The optimum pH for MnP was detected at pH 6 with showed the maximum activity of 2.4936U/L (Fig. 6 (a). This is because the MnP has a good potential and adapting in slightly acidic environment therefore the optimum pH caused exhibiting good resistance in acidic environment (Kong et al., 2016). Thus, the outcome of this study was similar to findings reported by (Jiang et al., 2008). However, the MnP produced by *Galactomyces geotrichum* was most stable at pH 5 (2.7936U/L) as shown in Fig. 6(b) this is because of MnP well known in active produce at this pH while it showed declining at pH 6 until the pH 10 the findings of this analysing was closed to findings (Jiang et al., 2008).

As the result is presented in Fig. 7 (a), an increase in activity at 30 ºC (0.21U/L) and at 40 ºC (0.412U/L) and finally showed its optimum up to 60 ºC (0.711U/L) within the reaction time interval. As the drop in absorbance at 30 ºC was comparable to that seen at higher temperatures this is due the high redox potential of lip. The optimum pH for lignin peroxidase was observed at pH 6 (0.7101U/L). The high activity observed might be due to the effect of buffering on the surface carrier. Therefore, this enzyme shifted at acidic range. The findings of this study similar to findings reported by]. At too acidic condition, (pH < 3) LiP would gradually started to lose its activity [4].

4. CONCLUSION

This study aimed to assess the impact of *Galactomyces Geotrichum* on various aspects of fermentation. The findings suggest that *Galactomyces Geotrichum* holds promise a as suitable candidate for solid fermentation, particularly due to its moderate enzyme production capabilities within the fungal domain. The investigation also

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revealed partial degradation of lignocellulosic compounds present in Oil Palm Empty Fruit Bunches (OPEFB). However, the complex structural composition of ligninolytic enzymes led to fluctuations in their activity, especially in solid conditions, which posed challenges for effective fungal degradation. Notably, among all the ligninolytic enzymes, Lignin Peroxidase (Lip) exhibited superior activity and production levels compared to Manganese Peroxidase (MnP) and Laccase .Overall, enzyme activity was deemed satisfactory.

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