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The Use of Agricultural Waste to Increase the Production Ligninolytic Enzyme by Fungus *Polyporus* sp.

I Nyoman Sukarta[1], I Dewa Ketut Sastrawidana[2]

[1]Department of Analysis Chemistry, Ganesha University of Education, Bali, Indonesia
[2]Department of Chemistry Education, Ganesha University of Education, Bali, Indonesia
Email: inyomansukarta@yahoo.co.id, idewasastra@yahoo.com

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Abstract

This research was aimed at enhancing the production of ligninolytic enzymes produced by fungus *Polyporus* sp. isolated from Buleleng as a means to treat the textile waste water. The optimum enzyme production was carried out by adding agricultural waste such as banana skin, straw, and sawdust. The research was completed in several stages including i) growing the fungus *Polyporus* sp. in PDA media, ii) enzymes production, iii) enzyme activity tests, and iv) decomposition analysis of textile waste water in several enzyme concentrations and in various incubation time lengths. The results showed that the optimum activities of laccase, MnP, and LiP enzymes that were produced without the addition of any agricultural waste were 20.5, 25.7, and 75.4 µmol/mL·minute. On the other hand, the enzymes activities on the addition of banana skins were 139.0, 116.0, and 654.0 µmol/mL·minute, on the addition of sawdust were 194.4, 41.0, and 259.0, and on the addition of straw were 148.2, 131.0, and 392.0 µmol/mL·minute, respectively. The efficiency of the degradation of 25 mL of remazol black B and remazol red RB (100 mg/L) by using 6 mL ligninolytic enzyme during the six-hour incubation was 53% - 58% and 55.4% - 69.3%, respectively.

Keywords

Ligininolytic Enzyme, Sawdust, Banana Skin, Straw

Subject Areas: **Biological Chemistry, Environmental Chemistry**

1. Introduction

Most textile industries produced a large amount of wastewater with the excessive colour. A portion of 1 mg/L of

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dye gave the significant coloration. Most of textile waste water which contained approximately 20 - 200 mg/L of dye that could degrade the water quality [1]. Untreated waste water discarded to the environment could also harm the aquatic ecosystem.

Recently, few small-scale textile industries could afford to build their own wastewater installation. There were also limited wastewater technologies available for them. Instead, they tend to use physical and chemical methods to manage their wastewater including the addition of sodium hypochlorite, alum and limestone. These materials are quite cheap but often producing a large amount of sludge that would further shallow the wastewater tanks. The disadvantages of the physical and chemical methods can be overcome by the application of biological method including the use of fungus and bacteria. The advantages of using microbial in wastewater treatment are: a) quite straight forward to operate, b) relatively low cost, c) immobilized microbes are recyclable and d) environmentally friendly [2].

Fungus is one of the potential microbes employed in the future to degrade xenobiotic compounds including *Bjerkandera adusta* [3], *Coriolus versicolor* [4], and *Trametes versicolor* [5]. Sastrawidana *et al.* [6] have explored Balinese fungi strains that could be used to degrade textile dye. Three out of fourteen Balinese fungus strains (*Polyporus* sp., *Ganoderma* sp., and *Microporus* sp.) were found to decolorized azo dyes in 72% - 82% within 7-day incubation [6]. These fungi strain were suspected to produce extracellular enzymes including ligninolytic (manganese peroxidase, lignin peroxidase, to degrade the azo dyes [7]. The use of ligninolytic enzymes could turn the dyes into non-toxic, unspecific with broader spectrum when the enzymes immobilized on a solid support [8]. The higher the ligninolytic enzyme produced by the fungi, the more efficient the enzyme in degrading the dyes. Aslam and Asgher [9] reported that some agricultural waste exemplified by wheat, straw, banana skins, cane sugar and corn produced *Pleurotus ostreatus* fungi that could enhance the production of ligninolytic enzyme.

In this article, the use of agricultural waste, sawdust, straw, and banana skin to increase the production ligninolytic enzyme by *Polyporus* sp. is reported. These wastes are easy to obtain and rich source of cellulose, hemicellulose as well as lignin.

2. Material and Methods

2.1. Subject and Object of the Research

The subject of this research is the fungi *Polyporus* sp. while the objects are the enzyme activity on the addition of agricultural wastes as well as the efficiency of textile dyes decoloration. The fungi were obtained from a plantation in Gitgit Village, Sukasada-Buleleng Bali as shown in **Figure 1**.

2.2. Data Collections

Culturing the *Polyporus* sp.

The fungi were chopped and put into a test tube filled with water. A 1 mL of this mixture was transferred into a petri disc contained sterilized potato dextrose agar (PDA) media and incubated over seven days until the fungi started to grow. In a 1 L of PDA media consisted of 200 g potatoes, 20 g dextrose, 20 g agar, and a chloroamphenicol tablet. The myceliums of the fungi were put into a 500 mL Erlenmeyer flask contained 250 mL Czapec

Figure 1. The *Polyporus* sp. fungi.

media, and incubated over seven days in rotary shaker. In 1 L of Czapex consisted of 15 g sucrose, 3 g $NaNO_3$, 0.5 g KCl, 0.5 g $MgSO_4 \cdot 7H_2O$, 0.01 g $FeSO_4 \cdot 7H_2O$ and 1 g KH_2PO_4.

2.3. Production of Ligninolytic Enzymes

The enzyme was produced by using solid state fermentation (SSF) method in which agricultural wastes (sawdust, banana skin and straw) were employed as solid support. A 50 mL fungal suspension was transferred into a 500 mL container filled with 175 g banana skin and 50 mL Czapex media. The pH of this mixture was adjusted to 5 and water content around 60% - 70%. The mixture was incubated over 7 days before being added 150 mL water and blended. The filtrate was then centrifuged (4000 rpm) in 15 minutes to give ligninnolytic enzyme. The same procedure was repeated for sawdust and straw, at the same time the control was prepared by using submerged fermentation (SmF) without adding any agriculture wastes as solid supports.

2.4. Determination of the Ligninolytic Activity

2.4.1. Manganese Peroxidase Activity (MnP)

An aliquot of enzyme ligninolytic (3 mL) was put into a test tube and successively added phenol red (1 mL, 100 mM), $MnSO_4$ (1 mL, 500 mM), H_2O_2 (1 m, 3%) and phosphate buffer (4 mL, pH 5.5). The mixture was left at room temperature for 15 minutes before being measured at 465 nm. The enzyme activity was determined based on the amount of substrate being oxidized by 1 mL enzyme per minute.

2.4.2. Lignin Peroxidase Activity (LiP)

An aliquot of enzyme (3 mL) was transfered into a test tube and the following substances were added: n-propanol (1 mL, 100 mM), tartaric acid (1 mL, 50 mM), H_2O_2 (1 mL, 3%), and phosphate buffer (4 mL, pH 5.5). The mixture stayed at room temperature for 15 minutes before the absorbance being measured at 310 nm.

2.4.3. Laccase Activity

A test tube filled with an aliquot of enzyme (3 mL) was subsequently added with buffer citrate-phosphate (0.3 mL, pH 5) and 2,2'-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid) (ABTS) (0.1 mL, 50 mmol/L). The absorbance of the mixture was recorded at 420 nm. One unit of laccase in equal amount of enzyme was required to oxidize 1 μmol of ABTS per minute for each mL of the enzyme.

2.5. Decomposisition of Textile Dyes

2.5.1. Decomposition of Textile Dyes on Different Length of Incubation Time

A 150 mL Erlenmeyer filled with textile dyes (50 mL, 100 mg/L) was added Czapek (3 mL) and ligninolytic (5 mL) before being incubated at 1 - 7 days at pH 5 in constant shaker. The mixture subsequently was centrifuged at 4000 rpm for 30 minutes and filtered before the absorbance being measured at 597 nm for remazol black B and at 526 nm for remazol red RB.

2.5.2. Decomposition of Textile Dyes on Different Amount of Ligninolytic Enzyme

A 150 mL Erlenmeyer filled with textile dyes (50 mL, 100 mg/L) was added Czapek (3 mL) and enzyme (1 mL). The mixture was incubated at pH 5 at the optimum time determined from previous experiment. The mixture was then centrifuged at 4000 rpm for 30 minutes and filtered. The absorbance of the supernatant was recorded at its maximum wavelength. The decomposition of the textile dyes on the addition of various concentration of enzyme (2 - 7 mL) was carried out by following exactly the same procedure.

3. Results and Discussions

3.1. Results

The photographs of culturing *Polyporus* sp. in PDA media over seven-day incubation is displayed in **Figure 2**.

The yellow colored formed during incubation was a signal that ligninolytic enzym was already produced. The enzyme was produced without and with solid support using agricultural waste, sawdust, straw, and banana skins.

The ligninolytic enzyme produced during incubation were exemplified in **Figure 3**.

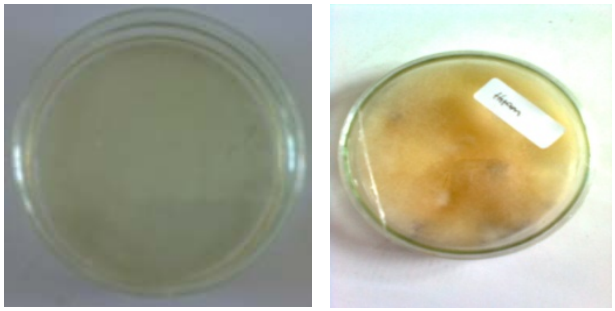

Figure 2. Growing of the *Polyporus* sp. in PDA media.

(a) (b) (c)

Figure 3. Ligninolytic enzyme produced without solid support (a), using straw (b), banana skins, and sawdust (c).

Ligninolytic enzyme activity data were tabulated in **Table 1**.

As indicated in **Table 1** the activity of ligninolytic enzyme produced by using the addition of agricultural waste were reasonably higher compared to those without solid support.

The efficiency of degradation of dye textile remazol black B and remazol red RB against ligninolytic enzyme (5 mL) in varied time was displayed in **Figure 4**. While the efficiency of degradation of dye textile on six-day incubation was depicted in **Figure 5**.

3.2. Discussion

The production of ligninolytic enzyme by *Polyporus* sp. in PDA media was qualitatively seen from the color change of the media. The role of the agricultural waste was to provide rough and solid support as well as source of carbon for the fungi. In addition, the solid support also served as inducer since they contained lignin, cellulose, and hemicelluloses that could boost the optimum production of the enzyme. The preference of agriculture solid support were mainly based on their availability, price, and particle size. The smaller the particle size the larger the surface area was where the microorganism life. Which was too small size was also affected oxygen transfer required by the fungi and further reduce the production of enzyme. This is essential to keep the fungi growing in the slightly humid condition. Most commonly used solid support are wheat bran, rice bran, wood pulp, sugar beet and corn flour. Moreover, agriculture waste are also considered such as banana skins, straw, and sawdust.

The production of ligninolytic enzyme by *Polyporus* sp. using banana skins, straw, and sawdust solid support during seven-day incubation were 116, 131, and 21 µmol/minute·mL respectively that corresponding to MnP; 654, 392, 259 µmol/minute·mL associated to LiP; and 139, 148, 194 µmol/minute·mL related to laccase enzyme. A similar research was also carried out using pulp cane by Hossain & Anantharman [10]. They found that the LiP enzyme produced without the addition of pulp cane during varied incubation time (3, 6, 9, 12, 15, and 18

Table 1. The activity of ligninolytic enzyme.

Inducers	Enzyme activity (μmol/mL minute)		
	Laccase	MnP	LiP
Banana skins	139	116	654
Sawdust	194	41	259
Straw	148	13	392
Without media	21	26	75

MnP = mangan peroxidase; LiP = lignin peroxidase.

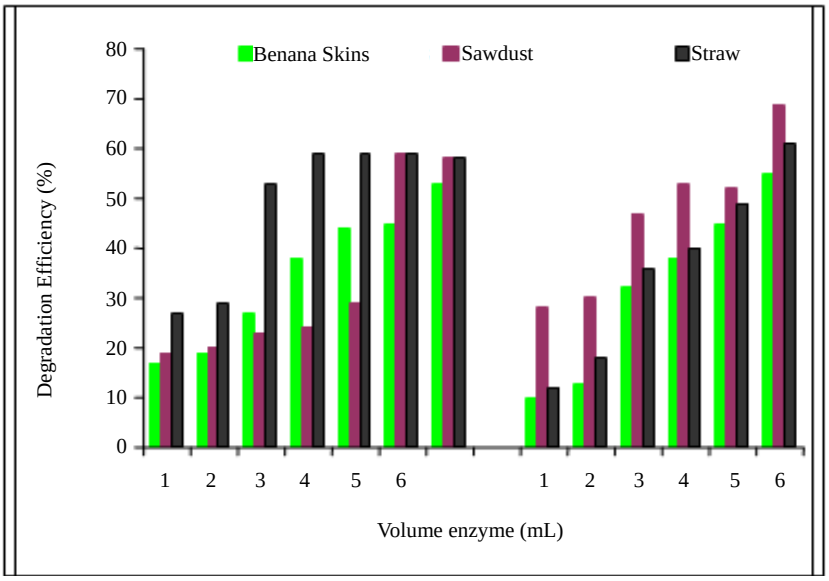

Figure 4. The degradation profile of dye textile by ligninolytic enzyme in varied incubation time.

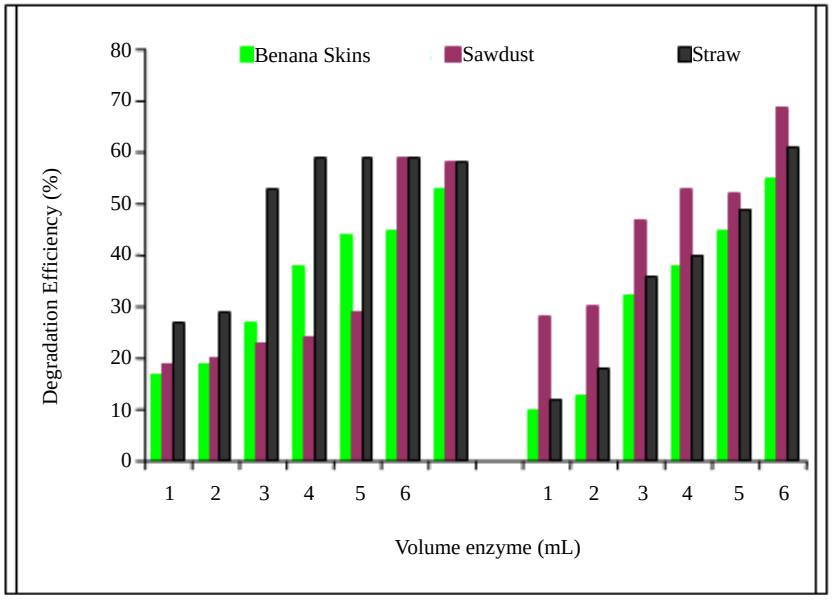

Figure 5. The degradation profile of dye textile by ligninolytic enzyme over six-day incubation.

days) were 20, 80, 105, 125, 125, and 295 µmol/minute·mL, respectively. Meanwhile, the addition of pulp cane (2%) gave 85, 175, 285, 335, 335, and 295 µmol/minute·mL. The MnP enzyme activity with no solid support were 15, 60, 85, 105, 105, and 80 µmol/minute·mL. The activity with solid support increased to 65, 145, 265, 305, 305, and 350 µmol/minute·mL. The laccase enzyme activity produced without pulp cane were 15, 45, 75, 115, 115, and 80 µmol/minute·mL. While the activity were found to increase with the addition of pulp cane, 65, 125, 220, 290, 290, 250 µmol/minute·mL.

A recent update (2008) by the same researchers showed that the MnP and LiP enzyme activity produced by *Phanerochate chrysosporium* during twelve-day incubation without the addition of straw were 127 and 154 µmol/minute·mL. However, the activity increased significantly to 318 and 339 µmol/minute·mL with the addition of the solid support.

The optimum condition found on the degradation of remazol black B and remazol RB (25 mL, 100 mg/L) was on the addition of ligninolytic enzyme (6 mL) during six-day incubation. The efficiency were 45% - 59% and 55.4% - 69.3% for remazole black B and remazole red RB respectively.

It was also found that sawdust was the best agricultural waste used as solid support during the degradation of textile dye. In addition, laccase enzyme was best produced on addition of sawdust, while MnP LiP and prefer straw and banana skins respectively.

4. Conclusions

Agriculture waste including banana skins, straw and sawdust could be used to boost the activity of ligninolytic enzyme produced by *Polyporus* sp.

The activity of laccase, MnP, and LiP enzymes produced without the addition of solid support were 20.5, 25.7, and 75.4 µmol/minute·mL. While the addition of banana skins gave 139, 116.0, and 654.0 µmol/minute·mL, respectively, sawdust produced 194, 41.0, 259.0 µmol/minute·mL, and straw resulted 148.0, 131.0, 392.0 µmol/minute·mL.

The degradation efficiency of remazole black B and remazole red RB (25 mL, 100 mg/L) using ligninolytic (6 mL) during six-day incubation were 53% - 58% and 55.4% - 69.3%.

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