Candidate Selection for Fungi Growing Media in Dye Waste Degradation

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Abstract. Exploration of microorganisms such as bacteria and fungi this decade increases significantly to studied and developed because it is potentiality. However, fungi have more potential than bacteria because fungi produce ligninolytic extracellular enzymes such as lignin peroxidase, manganese peroxidase and laccase which play an important role in the degradation process of organic compounds such as dyestuff waste. This study will examine the potential of Pleurotus cystidiosus fungi in degrading textile dyes direct congo red (DCR) using different growing media. The stages include the ratio of fungi to growing media and the concentration of dyes that can be degraded at variations in dye concentrations of 150 mg L^{-1}, 200 mg L^{-1}, 250 mg L^{-1}, and 300 mg L^{-1}. The results showed a ratio of 3% (b/b) in rice husk media with a degraded DCR concentration of 269 mg L^{-1} and 9% (b/b) in sawdust media with a degraded DCR concentration of 298 mg L^{-1}. The result shows that sawdust media is better in degrading DCR with degradation efficiency of 94%.

Keywords: Pleurotus cystidiosus, direct congo red, degradation, rice hull, sawdust

1. Introduction
The development of the textile industries in Indonesia are dominated by the production of garments, fabrics, yarns, and fibers [1]. One of the developing textile industries in South Sulawesi is the silk textile industry, one of which is located in Wajo Regency. Sutera is a promising textile industry in terms of quality; it is very beneficial for the country, it’s managers as well as consumers in particular. Synthetic dyes are generally used because they have a complex structure and are stable compared to natural color dyes, consequently, the dye does not fade easily but the waste generated is difficult to degrade [2]. Based on the waste treatment system, several large industries have been equipped with Wastewater Treatment Installation (WWTP) facilities, but there are still some industries throw away their waste directly into the river without management process first, especially if the river is one of the main sources of water supply for the community around the industry [3].

Waste dyes are generally disposed of without prior processing, as a result, is the contamination of water biota in the water body due to reduced oxygen demand during photosynthesis. At the bottom of the waters will produce aromatic amine compounds more toxic than azo dyes themselves [4]-[7]. Therefore, an effective dye treatment technology is needed. The treatment of dye by using chemical and physical wastewater technique is quite effective to remove color, but it is not efficient in terms of cost, chemicals use, and it is also causing a lot of sludge [8]. The use of microorganisms has the
potential to be developed because textile waste with high organic matter content can be used directly or indirectly by microorganisms as nutrients for growth like fungi.

Fungi are able to degrade color components that are toxic because they have the ability to transform waste into a form that is less or not harmful [9][10]. Phanerochaete chrysosporium and Pleurotus ostreatus able to degrade textile dyestuffs are the most active types of wood rot fungi because they produce lignin peroxidase (Li-P) enzyme, manganese peroxidase (Mn-P) and lacase (Lac) play a role in the degradation process of organic compounds such as dyes textiles [10]-[13]. Biological waste processing has the potential and efficiency that is promising [14]. Therefore, in this study uses Pleurotus cystidiosus mushroom using sawdust media, rice hull, and Potatoes Dextrose Agar (PDA) to understand the growing media candidates that provide good degradation results.

2. Research Method

2.1 Chemicals and instruments

The chemicals and instruments using are Spectrophotometer UV-Vis (Shimadzu UV-1800), pH-meter (GEMMY), centrifuge (TOMY LC-200), oven (MEMMERT), autoclave (TOMY SX-500), analytical balance (Cheetahfa2204B), incubator (Stuart SI500), oases, glassware, Bunsen. P.cystidiosus IPB (InaCCF122) fungi, P. flabellatus fungi, alcohol 70%, rice hull, polybag, color substance DCR (Merck, catalog number 101340), dextrose, sodium nitrate(s), potassium dihydrogen phosphate(s), magnesium sulfate heptahydrate(s), sodium acetate(s), acetic acid(0.5 M, rice bran CaCO₃, cotton, distilled water, aluminum foil, plastic food wrap, sawdust.

2.2 Research procedure

2.2.1 The growth of P. cystidiosus fungi

The sawdust pH is adjusted until it reaches pH 8 (the pH setting using CaCO₃) then dried and added rice bran (20% of the weight of sawdust) and stirred. Homogeneous sawdust is covered with airtight plastic and left for three days for weathering, and the pH is measured after weathering. Weathering sawdust media is put into a bottle container compacted. Weighed 20 g of media and seeds of P. cystidiosus fungi as much as 3%, 6%, 9%, 12%, and 15% (b/b) of media weight and inoculated into a bottle until the mycelium filled the bottle. The same treatment for rice hull media.

2.2.2 Determination concentration of degradable DCR dyes substance

Determination of the maximum concentration of degradable DCR dyes by fungi using four variations of concentration, respectively 150 mg L⁻¹, 200 mg L⁻¹, 250 mg L⁻¹, and 300 mg L⁻¹. A total of 0.02 kg of media containing fungi (optimum ratio results) were put into a sterile container and then added as much as 0.05 L of DCR dyestuff. Then covered with cotton and then incubated for 7 days at room temperature. The liquid was centrifuged for 15 minutes. The supernatant obtained is then absorbed in maximum DCR wavelength, the same treatment for control.

2.2.3 Color quality reduction test

The decrease in CDR dye quality can be observed by looking at color changes, variations of pH values, and decreases of dye concentration by comparing initial and final degradation. Color degradation can be observed visually by comparing it to dyestuff solutions before degradation, declining pH using a pH-meter, declining the concentration used UV-Vis spectrophotometer at the maximum DCR dye wavelength optimization results. The degradation efficiency was determined by comparing the concentration of dyes degraded to the initial DCR dye concentration multiplied by 100%. Mathematically the efficiency percent (% E) can be calculated using the formula below:

\[ \%E = \frac{C_a - C_s}{C_a} \times 100\% \] (1)
Description: $C_a$ is the initial concentration; $C_s$ is the residual concentration, and $\%E$ is the percentage of dye degradation efficiency.

### 3. Results and Discussion

The maximum wavelength of DCR dye stuff was obtained at 490 nm. The diagram of wavelength optimization result DCR dyestuff that used can be seen in Figure 1. The optimization was conducted to determine the wavelength for the initial measurement.

![Figure 1. The wavelength optimization of DCR dyestuff](image)

Fungal ratio to rice hull media and sawdust as the optimum fungus growth medium is used to degrade DCR synthetic dyes. Besides the media growing fungi also supports the degradation process. Measurement of the pH of the media grows before and after the incubation of the fungus which is rice husk at pH 6.7 and sawdust pH 6.4.

![Figure 2. Fungal ratio to rice hull and sawdust media (%)](image)

Growing media without mushrooms showed that the reduction in DCR dye concentration was greater than media which containing fungi. It was obtained the final concentration of dyes is 16.273 mg L$^{-1}$ smaller than the growth media containing fungi 3% (b/b) on rice hull media of 43.445 mg L$^{-1}$, while the media control of sawdust, the final concentration of dyestuff is 10.732 mg L$^{-1}$ smaller than
The final concentration of media sawdust containing fungi 9% (b/b) was 14.426 mg L\(^{-1}\). This is due to non-enzymatic processes, namely the adsorption process. Therefore it can be said that the addition of fungi, decreases the adsorption power of rice husk media. This is due to the growing fungal mycelium enveloping the growing media as a result the adsorption ability of the growing media was decreased. In the treatment of addition of fungi an enzymatic process occurs, known as the degradation process using ligninolytic enzymes produced by fungi. Revealed by the color changes on dyes. Degradation is supported through the measurement of the wavelength of DCR dyes resulting from the degradation, to prove that degradation has been shown by a shift in wavelength to DCR dyes accompanied by a decrease in absorption intensity. This is in accordance with the research of Elisangela et al. [15] and N. Enayatizamir et al. [16] that azo type dyes which are severed on azo groups will disappear after experiencing degradation and new absorption in the ultraviolet (UV) region.

Rice husk media was obtained from the ratio of optimum fungus amount of 3% (b/b) with pH 5.6. In sawdust growing media, the ratio of optimum fungus amount is 9% (w/w) pH 5.9. The pH deposition is caused by the formation of organic acids. In accordance with Stular et al. [17] that changes in pH occur due to the degradation process of lignocellulose and other organic compounds that produce organic acids. Changes in pH in the dyestuff resulting from degradation did not meet the required criteria for quality standards for textile wastewater reviewed by the Kepmen LHN. 51/ MENLH/10/1995 [18].

Figure 3 shows the efficiency of DCR dye degradation. Greater efficiency is seen in the DCR dye concentration of 200 mg L\(^{-1}\) with degradation efficiency of 94%. This evidence that \textit{P. cystidiosus} fungi in degrading are up to 200 mg L\(^{-1}\), pH is 3.69, and the color is lighter than the concentration of other dyes.

![Graph](image_url)  
**Figure 3. Efficiency curve of DCR various concentration**

Based on this result, it provides information that the use of rice husks without fungi is more potential in adsorbing dyes. But it also needs to be taken into account in terms of degradation that occurs that the data obtained in the adsorption process and the degradation process involving fungi do not have significant differences. Therefore, textile waste treatment using \textit{P. cystidiosus} fungi needs to be considered.

4. Conclusion

Sawdust growing media provides greater degradation than rice husks. Because of the high cellulose chemical content so that the growth of fungi is good as a result of the increased production of enzymes from fungi. The optimum fungal ratio of 9% (b/b) with degradation efficiency of 94%. It is necessary to isolate the fungal enzyme \textit{P. cystidiosus} and test the characteristics of enzymes to determine the type of enzyme that plays a role.
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