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by Ratna Dewi

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Decolorization of indigosol blue batik effluent using *Lepiota* sp. isolated from Baturraden Botanical Garden

Ratna Stia Dewi^{1*}, Ajeng Arum Sari² and Rai Alvin Fazrian¹

¹ Faculty of Biology, Universitas Jenderal Soedirman

Jl. dr. Suparno 63 Grendeng, Purwokerto, Banyumas, Central Java, 53122, Indonesia

² Research Unit for Clean Technology, National Research and Innovation Agency,

Jl. Cisit, Sangkuriang, Bandung, West Java 40135 Indonesia

*E-mail: ratna.dewi0509@unsoed.ac.id

Abstract. Indigosol blue (IB) is a synthetic dye with good durability and is usually used in the batik industry. However, due to its toxicity, carcinogenicity, and mutagenicity, this compound negatively impacts the environment and humans. Herein, the local Basidiomycota, *Lepiota* sp., isolated from the Baturraden Botanical Garden on Mount Slamet, Central Java, Indonesia, was utilized to decolorize the IB. It is considered an alternative method because it can degrade dyes efficiently and environmentally friendly through adsorption and enzymatic mechanisms. The purpose of this study is to investigate the capability of *Lepiota* sp. to decolorize IB batik industry effluent with different incubation periods and glucose concentrations. In addition, the optimum condition for the biological treatment was also determined. The potential of *Lepiota* sp. was by calculating the level of decolorization of the IB batik industry effluent. It was grown in a liquid medium and then exposed to IB batik industry effluent. Decolorization was studied using fungal mycelium that had been incubated for 7 × 24 hours. The experimental results were then expressed as the percentage decolorization and the total dry weight of the mycelium. The *Lepiota* sp. exhibits its potential to decolorize IB with % degradation in the range of 45.29% to 85.78%. The highest percentage was shown at 72 hours incubation period and a 1% glucose concentration.

Keywords: Batik effluent; decolorization; indigosol blue; *Lepiota* sp.; optimization

1. Introduction

Batik is one of the traditional textile industries in Indonesia which has experienced rapid development. In reverse to positively impacting the community's economy, those industries produce increasing textile effluent containing dye, possibly resulting in ecosystem destruction. According to [1], the dominant synthetic dyes used in the batik industry are not readily biodegradable; therefore, the pollution of synthetic dyes in water bodies potentially becomes a severe environmental problem [2]. One of the synthetic dyes in the batik industry is Indigosol Blue (IB) [3]. IB effluent has a C-Br Association functional group, Aromatic and aliphatic groups (stretch C = C and C = N-), N-H cluster, and Hydroxyl group (O-H) [4]. IB has strong chemical bonds and is non-biodegradable. Therefore, this dye is wasted in the aquatic environment. Furthermore, it will last a long period to accumulate in a certain concentration level, which can negatively impact [5].



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In both physical and chemical treatments, efforts to reduce dyes and eliminate harmful organic compounds batik effluent have been carried out. However, considering the high cost and the secondary problems, an alternative treatment for dyes pollutants is still required [1]. Biological treatment by mushroom (Basidiomycota) is considered an alternative due to its advantages, which show the effectiveness of decolorizing dyes. More importantly, the mushroom can live in a toxic environment and various pH [6–8]. The characteristics of Baturaden Botanical Garden, which has cool air (18 to 25 °C) and high humidity (70 to 88%), are in agreement with the growth of mushrooms, in particular *Lepiota* sp. [9,10].

The macroscopic characteristics of *Lepiota* sp. are as follows: reddish-brown pileus color, has an annulus, stipe length 2 to 6 cm with a thickness of 0.2 to 0.7 cm [11]. Microscopically, *Lepiota* sp. has white hyphae with septate hyphae without spores. Although *Lepiota* sp. has been isolated; however, its potency for decolorizing IB batik industry effluent has not yet been reported.

The purpose of this study was to explore the potency of *Lepiota* sp. isolated from Baturaden Botanical Gardens for decolorizing IB batik industry effluent with various incubation periods and glucose concentrations. The incubation period reflects the total mass of dye absorbed by the mycelium [12]. According to previous studies, those two variations affect the decolorization of the dye.

1 2. Materials and Methods

2.1. Materials

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The media used for fungal growth were Potato Dextrose Agar (PDA: 200 g potato extract, 20 g dextrose, 20 g agar, 1000 ml aquadest) and Potato Dextrose Broth (PDB: 200 g potato extract, 20 g dextrose, 1000 ml aquadest). The dye used is IB dye, and the batik effluent used is IB effluent. Both were obtained from Batik craftsmen in the Sokaraja Batik industrial center, Banyumas, Central Java, Indonesia. The fungal isolate used was *Lepiota* sp., which was isolated from Baturaden Botanical Gardens.

2.2. Methods

2.2.1. Fungal isolate reculture

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The isolate of *Lepiota* sp. was recultured on sterile PDA media in a sterilized petri dish. The isolate was incubated for 7 × 24 hours at room temperature.

2.2.2. Fungal activation

Pure isolates were activated on PDA media by inoculation on sterile PDA media + sterile IB dye using a petri dish, then incubated for 7 × 24 hours at room temperature. Five plugs (1 × 1 cm) of the isolate were transferred to a 100 mL sterile PDB medium aseptically and incubated for 7 × 24 hours on a shaker (70 rpm).

2.2.3. IB effluent decolorization test

The activated fungal mycelium was then separated from the PDB medium aseptically. Subsequently, the activated mycelium was put into an Erlenmeyer flask (250 mL size) with various glucose concentrations (0, 0.5, 1, and 2 % w/v) in 100 mL real wastewater from batik effluent. And the incubation with shaking at 70 rpm was performed at 24, 48, 72, and 96 hours.

2.2.4. Decolorization percentage measurement

For determination of % decolorization, about 5 mL of the filtrate were taken, and the absorbance was measured at the maximum wavelength of 604.5 nm using a Spectrophotometer (721- Vis Spectrophotometer). The % decolorization was calculated using equation (1).

$$\text{Decolorization (\%)} = \frac{A_c - A_s}{A_c} \times 100\% \quad (1)$$

where A_c is the initial absorbance and A_s is the post-treatment absorbance.

2.2.5. Measurement of mycelium dry weight

The mycelium from the decolorization test was filtered using filter paper then put into an oven at 60 °C until it reached a constant weight. The dry weight of the mycelium was then measured by calculating the difference between the weight of the fungal mycelium and the weight of the filter paper used.

3. Results and Discussion

The following are the features of *Lepiota* sp.: a little creamy white fruiting body with a stipe length of to 6 cm and a pileus diameter of 1 to 5 cm with a brownish-red tint, a darker core, and lamellae. The macromorphology of Mycelium *Lepiota* sp. is white with a concentric spreading pattern. When seen under a microscope, the hyphae that compose have septates or partitions and no spores (Figure 1). This character is by the opinion of [13,14] which at least the lamellae are free and white to cream, the annulus or annular zone presents partial sheath remnants and white to cream spore prints, with characteristic length stipe and diameter of the pileus such as size.

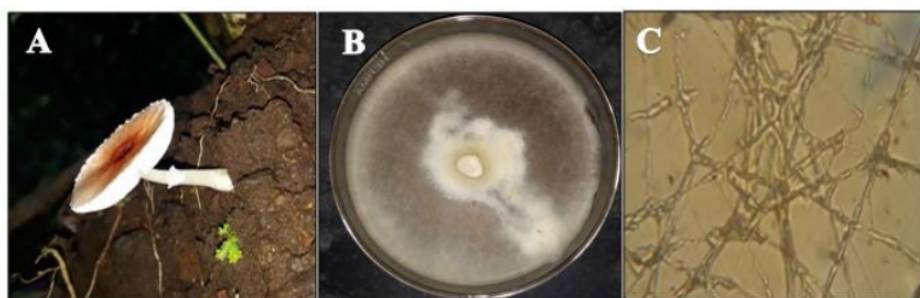
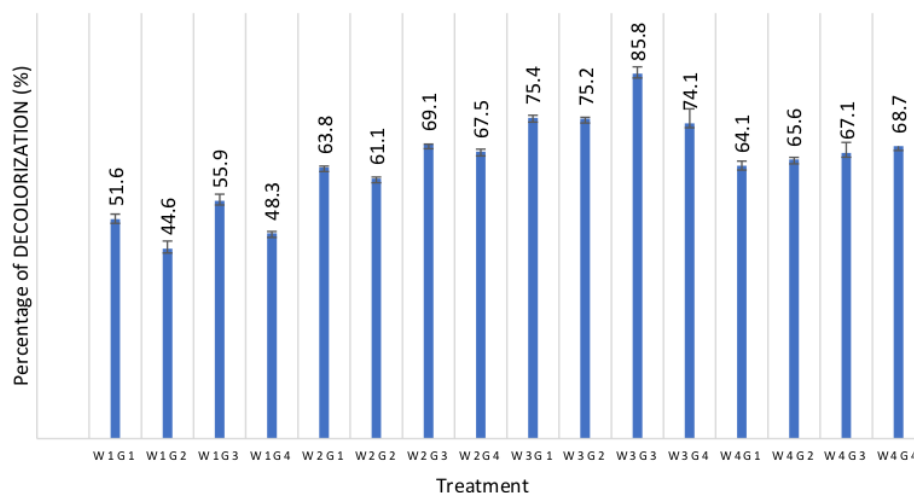


Figure 1. (A) The fruiting bodies of *Lepiota* sp. from Baturraden Botanical Gardens, (B) mycelium *Lepiota* sp. on PDA+indigosol blue medium, and (C) septate hyphae without *Lepiota* sp. under the microscope.

Figure 2 shows the results of an optimized treatment using *Lepiota* sp. from the Baturraden Botanical Gardens to decolorize IB batik industry effluent. The lowest value was 42.59% after 24 hours of incubation with 0.5% glucose concentration, while the highest value was 85.78% after 72 hours of incubation with 1% glucose concentration.

Figure 2 shows that *Lepiota* sp. may decolorize IB batik industry effluent at different incubation periods and glucose concentrations. According to Geethanjali [15], various major lignin-degrading fungi, such as *Clavaria*, *Clitocybecollybia*, *Flammula*, *Hypholoma*, *Lepiota*, *Mycena*, *Pleurotus*, *Agaricus*, *Polyporus*, *Fusarium*, *Arthrotrrys*, *Poria*, *Pholiota*, *Cephalosporium*, *Collybi*, and *Humicola* is capable of decomposing lignin and has been frequently used to remove synthetic dyes in the textile industry. Then there was [16], who indicated that the extracellular enzyme activity of *Lepiota* sp., i.e. laccase, MnP, and LiP, had been known in his research.

At 72 hours of incubation and 1 percent glucose concentration, the optimal decolorization treatment by *Lepiota* sp. had the highest average value of 85.78 percent. The best incubation period for *Lepiota* sp. for decolorizing IB batik industry effluent is 72 hours. These findings come from a study conducted by [17], which found that *Panerochaeta chrysosporium* decolorizes dye waste optimally within 72 hours. The more dyes absorbed by the fungal mycelium, the longer the contact period between the fungal mycelium and the dye. As a result, there is a greater percentage of decolorization. When the IB batik industry effluent was cultured for 96 hours, the percent decolorization of *Lepiota* sp. reduced. One of the most essential aspects in the decolorization of the dye is the incubation period, which is closely related to the pace of fungal development. Based on [18], the ability of fungi to manufacture ligninolytic enzymes optimally is affected by the incubation period. During the decolorization process, nutrient supplies are also crucial as a promoter of cell growth and an inductor of certain enzyme processes [19].



W1G1: 24 hours incubation period and 0% glucose concentration
W1G2: 24 hours incubation period and 0.5% glucose concentration
W1G3: 24 hours incubation period and 1% glucose concentration
W1G4: 24 hours incubation period and 2% glucose concentration
W2G1: 48 hours incubation period and 0% glucose concentration
W2G2: 48 hours incubation period and 0.5% glucose concentration
W2G3: 48 hours incubation period and 1% glucose concentration
W2G4: 48 hours incubation period and 2% glucose concentration
W3G1: 72 hours incubation period and 0% glucose concentration
W3G2: 72 hours incubation period and 0.5% glucose concentration
W3G3: 72 hours incubation period and 1% glucose concentration
W3G4: 72 hours incubation period and 2% glucose concentration
W4G1: 96 hours incubation period and 0% glucose concentration
W4G2: 96 hours incubation period and 0.5% glucose concentration
W4G3: 96 hours incubation period and 1% glucose concentration
W4G4: 96 hours incubation period and 2% glucose concentration

Figure 2. IB batik industry effluent decolorization by *Lepiota* sp. at different incubation periods and glucose concentration.

We investigate the potential decolorization of *Lepiota* sp. with various glucose concentrations of 0, 0.5, 1, and 2% to determine the optimum glucose concentration. The results revealed that glucose at 1% w/v is the best decolorizer for the IB batik business effluent. Glucose is essential for cell development and breakdown to occur. More crucially, glucose serves as a co-substrate, causing a specific enzyme reaction to occur. Our result aligns with [20], which showed that 1% glucose is required for an optimum biological treatment of dyes using *Schizophyllum comune*. [21] reported that the exponential or log phase of fungus occurred on the 3rd day. In addition, during the log phase, the number of cells is in the maximum and the enzyme production [22]. After 96 hours of incubation, it appears that *Lepiota* sp. has entered the stationary phase, and the number of mycelium growth has slowed. In addition, dye absorption gets saturated. As a result, the percentage of decolorization is reduced.

Glucose is a simple molecule that works as a primary nutrient for fungi. It will be consumed first before the dye's carbon source; however, the proportion of effluent decolorization decreased at a glucose concentration of 2%. It's thought that the fungus utilizes carbon sources in a diauxic way. Fungi tend to use only one carbon source [23]. The growth will take on a diauxic pattern if the molecular complexity

of the two carbons differs. This pattern causes the fungus to use the easier-to-digest carbon source until it is depleted, at which point the new fungus will switch to a more complex carbon source. The diauxic growth pattern is caused by catabolite suppression, which inhibits gene expression that encodes the manufacture of enzymes required to metabolize a carbon source since fungi more easily metabolize other carbon sources.

Every 24 hours, the dye intensity decreased (Figure 3), but then increased again at the 96th hour. The original dark blue IB batik industry effluent was optimally decolorized by *Lepiota* sp. at 72 hours, and the transformation of IB batik industry effluent to clear showed a 1 % glucose concentration. The dye's intensity declines initially due to absorption by the mycelium, then due to the breakage of complicated linkages and enzymatic degradation of the dye [24]. Other studies have also shown an adsorption mechanism that plays an important role in decolorizing synthetic dyes. Still, the absorption of dye by mycelium on the percentage of decolorization is deficient [25]. The absorption of dye by the fungal mycelium was due to the electrostatic attraction between the positively charged fungal cell wall and the negative charge on the dye [26]. Fungal cell walls are composed of chitin or chitosan, which absorb various substances [27]. In addition, the cell wall also secretes a gel that functions as an adhesive and can absorb dyes [6].

The absorption of dye by the mycelium was also proven, which was marked by a change in the color of the mycelium from cream white to dark blue (Figure 4). Munir *et al.* [28] earlier reported dye absorption in their research utilizing the Basidiomycetes fungus from Mount Barus, which absorbs all the dyes from batik waste, darkening the mycelium color.

Adsorption is considered one of the causes of the decolorization that occurs. The color of the mycelium surface, which was white before treatment and turned dark blue after treatment, confirms this (Figure 4). The process of dye adsorption by fungal cell walls is the mechanism of decolorization by fungus [29]. Suppose there is no change in mycelium color when compared to the control. In that case, there is no adsorption mechanism in the decolorization process, and vice versa if there is a change in mycelium color when compared to the control, there is an adsorption mechanism in the decolorization process [30].

Decolorization related to enzyme activity is another probable source of dye decolorization, in addition to absorption or adsorption. The azo dyes were degraded by *P. chrysosporium* through adsorption to the cell mass or the system of the lignin-degrading enzyme [31]. Other parameters such as the dry weight of mycelium were measured as supporting data in addition to the percentage of decolorization of Effluent IB. After completing the IB batik industry effluent decolorization test, the mycelium was dried in a 60°C oven until it attained a consistent weight. Figure 5 shows the results of the dry weight measurement of mycelium.

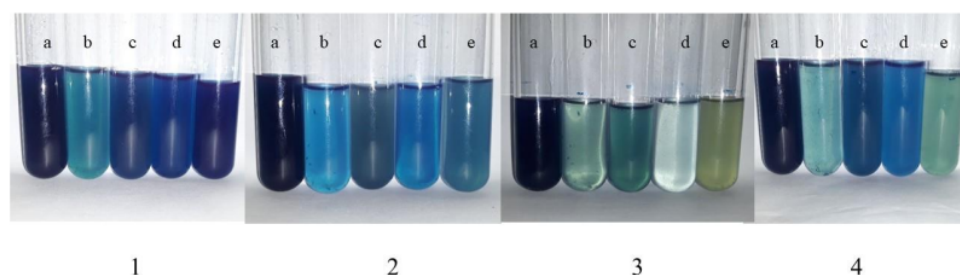


Figure 3. The result of decolorization of IB batik industry effluent by *Lepiota* sp. at incubation periods of (1) 24 hours, (2) 48 hours, (3) 72 hours, and (4) 96 hours; with glucose concentration (a) control, (b) 0%, (c) 0.5%, (d) 1 %, and (e) 2%.

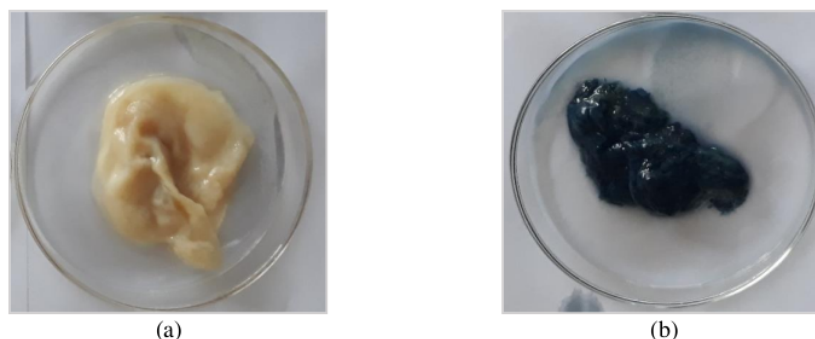


Figure 4. (a) Mycelium of *Lepiota* sp. without addition and (b) Mycelium of *Lepiota* sp. with the addition of IB batik industry effluent.

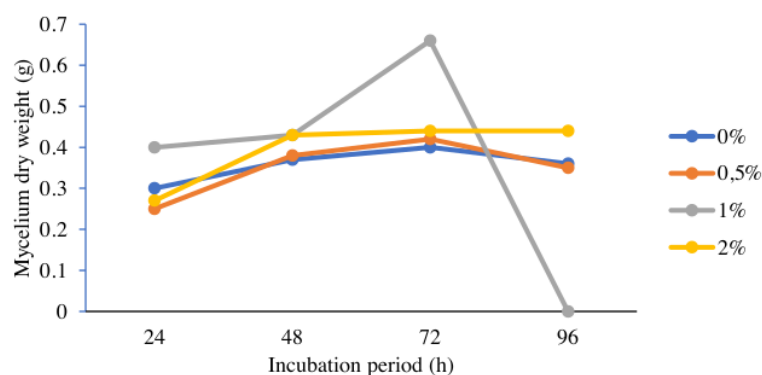


Figure 5. The dry weight of the mycelium of *Lepiota* sp. in different incubation periods and various glucose concentrations.

The W3G3 treatment had the highest dry weight of mycelium (0.66 g), while the W1G2 treatment had the lowest dry weight of mycelium (0.26 g). This is related to the proportion of IB batik industry effluent decolorization in W3G3 and W1G2 treatments, which are 85.78 and 44.62%, respectively. The fungus's consumption of carbon sources was closely related to the dry weight of the mycelium measured (Figure 5). Mushroom mycelium can develop because the nutrients in batik effluent are used by it. The wastewater from the IB batik industry includes functional groups that fungi can utilize as nutrition. Fungal mycelium may digest nutrients in batik wastewater to provide energy and promote mycelium growth in the formation of new cells. The fungus will break down the effluent into simple compounds that can be used as carbon and nitrogen sources if the nutrients in the solution run out. This is consistent with [32], that the dye's carbon and nitrogen sources will break down to create chemicals that make up the mycelium component.

The addition of up to 1% glucose to the mycelium can enhance the weight of the mycelium, resulting in a decolorization value of up to 85.78 %. Glucose supplementation also allows for an increase in biomass production, resulting in a higher proportion of decolorization [33]. The adsorption of dye by fungal mycelium is related to fungal biomass. Therefore, an increase in biomass can provide more surface area for the absorption of dye molecules [34].

4. Conclusion

Lepiota sp. from Beturraden Botanical Gardens can decolorize IB batik industry effluent, with the optimum incubation period of 72 hours, the glucose concentration of 1%, and the maximum decolorization percentage of 85.78%. Decolorization using fungus showed potential as a source of batik effluent processing system.

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