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Decolorization of Indigosol Blue Dye Using *Trametes versicolor* F200 and *Aspergillus* sp.

Sunu Pertiwi[1*], Ratna Stia Dewi[1], Ajeng Arum Sari[2]

[1]Fakultas Biologi, Universitas Jenderal Soedirman
[2]Pusat Penelitian Kimia, Lembaga Ilmu Pengetahuan Indonesia (LIPI)
*Email: sunupertiwi68@gmail.com

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Abstract
The dyeing process of batik eventually produces much of wastewater. The difficult degradation and the dangers posed within the synthetic dyes are the main concerns in finding efficient wastewater treatment. Biological treatment has been known to be an effective technique of reducing or eliminating color intensity in wastewater. Fungi is one organism that can decompose many environmental pollutants. The aims of this research were to determine the ability of fungal isolates in decolorizing the synthetic dyes and analyzed which treatment has the highest decolorization percentage. Fungal isolates of *Trametes versicolor* F200 and *Aspergillus* sp. were used as a biological agent to decolorize of Indigosol Blue dye. The decolorization percentage was analyzed by spectrophotometer method. The result showed that *T. versicolor* F200 and *Aspergillus* sp. able to decolorize Indigosol Blue dye. The decolorization treatment of Indigosol Blue dyes using *T. versicolor* F200 showed the highest decolorization percentage reaching 97.21%.
Keyword: *Aspergillus* sp., Decolorization, Synthetic dyes, *Trametes versicolor* F200

INTRODUCTION

Batik is a traditional art that has wisdom value and has been a part of Indonesian culture for a long time. Nowadays, batik industries are dominantly used the synthetic dye due to has various types of color, easier to be found, used in large scale production, and more simply practical use. Indigoid dye is one of the synthetic dyes that are often used in industrial batik.

The dyeing process eventually produces much wastewater, which contains a lot of organic compounds, smelly, and stained. The direct disposal of wastewater into the waters without any treatment will cause many environmental problems, such as generates lethal conditions for the aquatic organism and potentially affected to human health. Various methods of wastewater treatment to remove dye have been done, for example, chemical, physical and biological treatment. However, the physical and chemical treatments have a potential high cost and produce another toxic molecule (Namboodri *et al.*, 1994; Slokar & Le Marechal, 1998; Tadda *et al.*, 2016). Therefore, biological treatment more recommended and effective for decolorizing dyes because it takes place naturally, low cost, and there is no residual reaction that requires further processing (Sorta, 2013; Dewi *et al.*, 2016).

Trametes versicolor and *Aspergillus* sp. have been known able to decolorize several of synthetic dyes. A recent study by Yang *et al.* (2017) showed that *T. versicolor* CBR43 able to decolorize of acid (Acid Red 114 and Acid Black 172), disperse (Disperse Black 1) and reactive dyes (Reactive Red 120, Reactive Blue 4, Reactive Orange 16, and Reactive Black 5). The decolorization of Acid Red 114 up to 86.5% over 2-4 days. Moreover, Acid Black 172 were decolorized attain 91.2% by six days. The Disperse Black 1 decolorized up to 80.2% in 9 days. Furthermore, the reduction of each reactive dyes reaches 96-97% dye removal at six days. Furthermore, the study by Hemalatha *et al.* (2016) reported that *Aspergillus* sp. collected from marine soil in Bay of Bengal Kakinada can decolorize several synthetic dyes. *Aspergillus* sp. showed 75% dye removal in Congo Red, 60% in Malachite Green, 45% in Brilliant Green, and 45% in Methylene Blue after five days incubation time. The change and disappearance of color contained in synthetic dyes indicate the degradation process carried out by fungal isolate.

The decolorization mechanism consists of adsorption, enzymatic degradation, and utilization as a carbon source. Adsorption is the primary step that occurs in the separation of dye molecular substance from the wastewater. Furthermore, the degradation process is the breakdown of complex molecules into simple compounds by ligninolytic enzymes (MnP, LiP, and laccase) produced by mycelial fungi (Setiadi, 2002). These enzymes have a role in degrading synthetic dye molecule through redox reaction, which completely oxidized become water, carbon dioxide, and or any other inorganic compound (Bennet & Fasion, 1994). Finally, the cleaved molecules of dye are utilized by fungi as a carbon source (Singh, 2006). This research aimed to

determine the ability of *T. versicolor* F200 and *Aspergillus* sp. to decolorize of Indigosol Blue dyes and analyzed which treatment that has the highest decolorization percentage.

MATERIALS AND METHOD

Chemical

Chemical compounds used in this research were obtained from The Research Center for Chemistry – Indonesian Institute of Science (LIPI) Puspiptek area, Serpong Tangerang. The Indigosol Blue dye was obtained from the batik industry in Banyumas, Central Java.

Fungal isolate

The fungal isolates used in this research are *Trametes versicolor* F200 obtained from Indonesia Culture Collection Research Center (InaCC) – LIPI, and *Aspergillus* sp. which obtained from the isolation process of Indigosol Blue effluent (Dewi *et al*., 2018).

Rejuvenation of Fungal Isolate

T. versicolor F200 and *Aspergillus* sp. were maintained in potato dextrose agar (PDA) medium in the Petri dish at 4°C until use. Fungal isolates are rejuvenated in PDA medium on the Petri dishes for seven days at room temperature.

Decolorization assay (Copete-Pertuz *et al.* 2019 modified)

Decolorization assay was performed in 250 mL Erlenmeyer flasks with 100 mL of culture medium broth (pH 5.6) containing 10 g L−1 glucose, 2 g L−1 ammonium L-(+)-tartrate, 5 g L^{-1} peptone, 1 g L^{-1} KH_2PO_4, 1 g L^{-1} yeast extract, 0.5 g L^{-1} $MgSO_4$. $7H_2O$ and 0.5 g L^{-1} KCl, 1 mL mineral solution [100 mg L^{-1} $B_4O_7Na_2$. $10H_2O$, 70 mg L^{-1}, $ZnSO_4$. $7H_2O$, 50 mg L^{-1} $FeSO_4$. $7H_2O$, 10 mg L^{-1} $MnSO_4.7H_2O$, and 10 mg L^{-1} $(NH4)_6Mo_7O_{24}$. $4H_2O$]. The isolate of *T. versicolor* F200 and *Aspergillus* sp. as much as 5 plugs (5 mm) were inoculated and growth until seven days at 28ºC 70 rpm for 24 hours. Afterward, separately supplemented with Indigosol Blue (100 mg L^{-1}) dyes. As a comparison, the culture medium without inoculated fungi were used as a control. The treatment and the control were incubated at 28ºC 70 rpm for 24 hours.

Spectrophotometrically UV-vis analyzed the treatments and control at 0 hours and 24 hours. The change in absorbance value of Indigosol Blue was determined at 604.5 nm. The percentage of decolorization (D%) was calculated as follows:

$$D\% = \frac{A0-At}{A0} \times 100$$

Detail:
D% : Decolorization percentage
A_0 : The initial dye absorbance
A_t : The absorbance at the t incubation period

RESULT AND DISCUSSION

The *Trametes versicolor* F200 and *Aspergillus* sp. were able to decolorize Indigosol Blue dyes. The average of decolorization percentage on Indigosol Blue dyes using *T. versicolor* F200 showed 97.21% dye removal whereas using *Aspergillus* sp. 60.63% and also control 1.15%. It is accordance with the study by Young & Yu (1997), *T. versicolor* able to decolorize indigo dye. 40-50 mg/L concentration of Indigosol Carmine dye was removed up to 92.8% at nine days incubation period. Furthermore, six different types of *Aspergillus* spp. in the study by Dewi *et al*. (2018) show decolorization activity against the batik dye effluent containing Indigosol Blue 04B. The highest decolorization percentages reach 99.05, 98.50, and 99.90%.

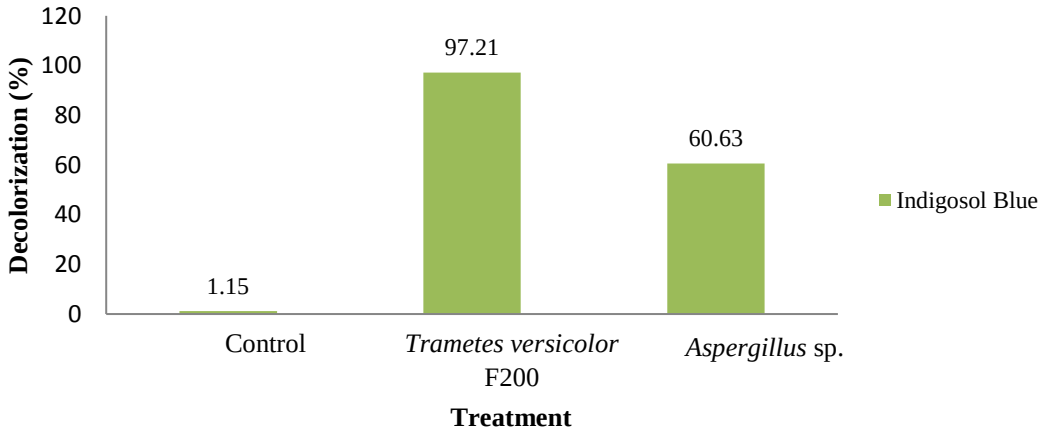

Figure 1. Histogram of the average decolorization percentage of RBBR and Indigosol Blue dyes using *T.versicolor* F200 and *Aspergillus* sp. at 24 hours incubation.

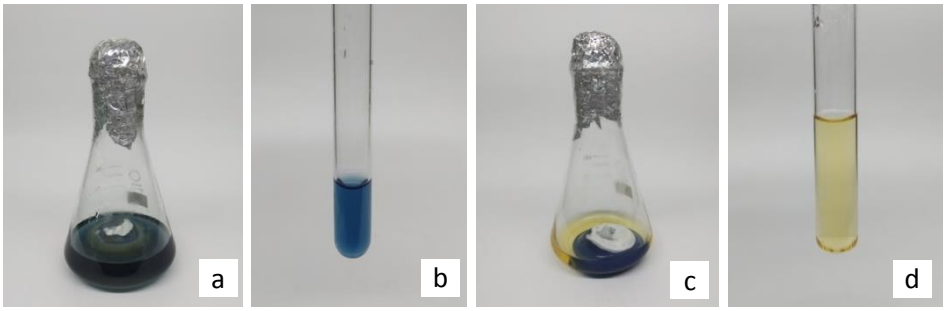

Figure 2. Decolorization of Indigosol Blue dye by *Trametes versicolor* F200 at (a)(b) 0 hour incubation period and (c)(d) 24 hours incubation period

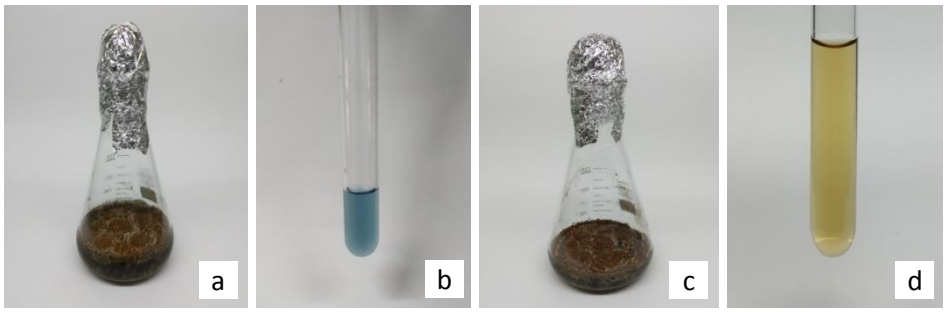

Figure 3. Decolorization of Indigosol Blue dye by *Aspergillus* sp. at (a)(b) 0 hour incubation period and (c)(d) 24 hours incubation period

T. versicolor F200 showed higher ability than Aspergillus sp. in decolorizing of Indigosol Blue dyes. It is indicated that fungal strain affects the decolorization rate. The research by Park *et al.* (2004) reported that the decolorization rate of synthetic dyes was depended on fungal specificity towards synthetic dyes. *T. versicolor* ATCC 200801, *T. versicolor* KCTC and *Panerochaete chrysosporium* KCCM 60256 show different decolorization rates. *T. versicolor* KCTC showed the highest decolorization activity. About 100% of Acid yellow 99, Acid Blue 350 and Acid red 114 decolorized by *T. versicolor* KCTC on 48 hours incubation time. Even though, *T. versicolor* ATCC 200801 showed a higher capability for dye degradation than *P. chrysosporium* KCCM 60256.

Based on the previous description, it also indicates *T. versicolor* F200 from Basidiomycota has a greater ability than Aspergillus sp. (Ascomycota). According to the study by Claus *et al*. (2002), Basidiomycota has higher oxidative capacities and more efficient to decolorize of high concentration of synthetic dyes rather than Ascomycota. Decolorization rate by *Polyporus pinistus* and *T. versicolor* from Basidiomycota were significantly higher than *Myceliophthora thermophila* (Ascomycota). At 0-100 ppm of dye concentration, *P. pinisitus* and *T. versicolor* are able decolorize indigoid Acid Blue 24 reach 65-80%, and anthraquinone Reactive Blue 19 up to 70-85% in 4 hours incubation period while *M. thermophila* exhibit slow decolorization rate and progressively decrease as long as the increasing of dye concentration.

The mechanism of decolorization can be occurred by non-enzymatically and enzymatically (Singh *et al.* 2006). Non-enzymatic mechanism occurs through the adsorption process, which is defined as the accumulation of toxins through the cells (Ngo *et al*. 2016). The color changes of fungal mycelium evidence this. The isolate of *T. versicolor* F200 showed the color change of mycelium from initial white to bluish. Similarly, *Aspergillus* sp. was turned into dark. It is in accordance with the statement of Yulisna (2000), the color changes of fungal mycelium indicates the decolorization process caused by the mechanism of dye adsorption by fungi. The color changes of *T. versicolor* F200 and *Aspergillus* sp. can be seen in Figure 4.

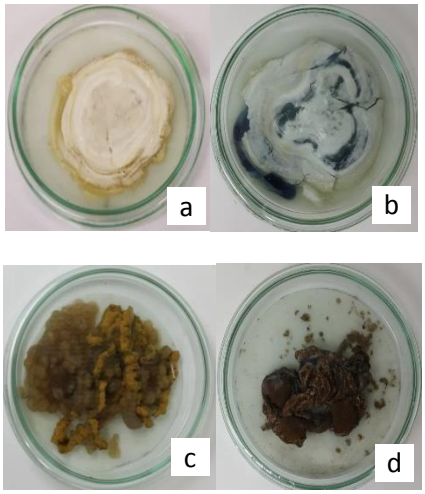

Figure 4. The color changes of fungal isolate
(a) *T. versicolor* F200 without synthetic dyes,
(b) *T. versicolor* F200 with Indigosol Blue dye,
(c) *Aspergillus* sp. without synthetic dyes,
(d) *Aspergillus* sp. with Indigosol Blue dye.

The enzymatic mechanism involves the secretion of the ligninolytic enzyme. Ligninolytic enzymes are an extracellular oxidative enzyme which responsible for the degradation of the aromatics compound. Ligninolytic enzymes consist of Manganese Peroxidase (MnP), Lignin Peroxidase (LiP) and laccase (Mester & Tien, 2000). These enzymes can degrade substrate nonspecifically. Therefore, fungi can decolorize various synthetic dyes. Ligninolytic enzymes are responsible for the breakdown of aromatic compounds into water, CO_2, and other inorganic product (Hammel, 1997; Bennett & Faison, 1997).

The decolorization activity of *T. versicolor* F200 towards Indigosol Blue dye showed the highest decolorization percentage, reach 97.21% dye removal. Benito *et al.* (1997), *T. versicolor* can decolorize of synthetic dyes. As much as 10% of total color removed by adsorption and 90% removed by enzymatically of fungal mycellium. Study by Floudas *et al.* (2012), *T. versicolor* genetically showed the presence of 26 class II peroxidases (peroxidase) and ten multicopper oxidase (laccase) genes. It is indicated that *T. versicolor* able to produce MnP, LiP, and laccase.

CONCLUSION

Trametes versicolor F200 and *Aspergillus* sp. can decolorize of Indigosol Blue dye. The decolorization treatment of Indigosol Blue dyes using *T. versicolor* F200 show the highest decolorization percentage, reach 97.21%.

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