Decolorization of batik naphthol dye by local ligninolytic fungal isolates

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Abstract. Batik is very well-known in Indonesia. There are many centers of batik industries in Java. Meanwhile in Sumatera, mainly in North Sumatera we could find some home industries of batik, known as Sumatran batik. Those home industries often discharge the untreated liquid water into the streams. The main component of liquid waste containing dyes including naphthol is hardly decomposed and carcinogen. Four fungal isolates designated as TB01, TB04, TB06 and ZN01 were studied for their abilities on naphthol decolorization on agar medium containing 50 ppm of naphthol dye as the sole carbon source. Among four isolates, TB04 and TB06 showed the best growth and were selected for quantitative analysis of naphthol decolorization. Both isolates were grown in minimal salt medium with 50 ppm of naphthol as the carbon source, 25% of urea, 0.2% of glucose at pH 4.0. The best condition for naphthol decolorization was pH 4.0 with addition of 0.2% of glucose and 25% of urea which reduced the color up to 61.4% by TB04 and 79.01% by TB06 in shaking culture. Meanwhile in static culture, TB04 reduced the color of naphthol by 41.97% while TB06 was 53.45%. It seems that the decolorization process was initiated by naphthol absorption by the fungal mycelia followed by degradation of the dye.

1. Introduction

Batik is very famous cloth and it is considered as Indonesian traditional textile. It has been recognized as a World Cultural Heritage from the UNESCO since 2009. In 2006, the number of batik industries was 2,699 with the total asset was IDR 135.7 trillion. Those industries are located mostly in Java (57% in West Java, 14% in Central Java, 17% in Jakarta. The rest are distributed in East Java, Bali, Yogyakarta, and Sumatra [1].

In Sumatera, Batak batik is quite familiar even though not as well known as Java batik. No big batik industry is found in North Sumatera, there are some homemade batik industries instead. During the process of producing batik, many chemicals with different functions are used. Furthermore numerous types of dyes are applied during dyeing process. The dyes react with chemical agents to produce final color. Ludigol for example is used to get brighter and more vibrant color while potassium aluminum sulfate makes the design well applicated. Sodium alginate is used to thicken the dye for screening, painting, printing, or to control the spreading [2].

According to the solubility and chemical properties, dyes could be group into acid dyes, basic dyes, direct dyes, mordant dyes, vat dyes, reactive dyes, disperse dyes, sulfur dyes, and azo dye. The dye which is frequently used in batik dyeing process is naphthol dyes. Naphthol dyes are insoluble azo dyestuff that could produce very deep orange, red, and scarlet. These dyes have complex structure...
cause them to be very stable and decompose only at temperature higher than 200°C. Egli [3] synthetic dyes are more stable than that of natural dyes. Haron and Idris [4] wastewater from textile industry is one of the most important water pollution, if it is disposed to the environment it could exert great impact.

Many researchers reported some methods to reduce the effect which could be physically, chemically, and biologically. Numerous isolates have been reported to degrade and decolorize dyes and to accumulate some heavy metals from the waste. Singh et al. [5] reported that *Staphylococcus hominis* RMLRT03 decolorized the acid orange dye up to 94% in Bushnel Haas Medium amended with Acid Orange Dye and glucose at pH 7. The bacteria showed resist to high concentration of the dye which was 600 mg/L.  
Panerochaete chryssosporium, *Pleurotus sarjocaju* decolorized four dyes: Amaran, New Coccine, Orange G, and Tartrazine 20% to 60% in solid medium, while *Pleurotus sarjocaju* was not able to decolorize Tartrazine [6]. We have isolated four isolates that have been tested for their abilities to degrade lignin, to produce three enzymes: laccase, lignin peroxidase (LiP), and manganese peroxidase (MnP). In this report, we studied the abilities of those isolates in naphthol decolorization in both solid and liquid media under different nutrient concentration such as percentage of naphthol, glucose, and urea.

2. Materials and Methods

2.1. Fungal isolates

Four fungal isolates used in this study were of TB01, TB04, TB06, and ZN01. All were of the collection of Microbiology Laboratory, Faculty of Mathematics and Natural Sciences, University of Sumatera Utara.

2.2. Media composition and Culture condition

The fungal selection on their abilities to decolorize dyes was done according to Martani et al. [7] method. The fungal isolates were streaked on PDA and incubated until extensive mycelia achieved. Minimal Salt Medium (MSM) with composition in 1L is KH₂PO₄ 0.79g, K₂HPO₄ 0.79g, MSO₄·7H₂O 0.7g, NaCl 0.05g, FeSO₄·7H₂O 0.002g, ZnSO₄·7H₂O 0.002g, MnSO₄·7H₂O 0.001g, and NH₄NO₃ 0.1g. As for the screening ability of isolates to grow in naphthol containing media, four tested fungal isolates were inoculated on MSM agar supplemented with naphthol dye at concentration 10, 25, and 50 ppm. An agar plugs (Ø 5 mm) of fully growing colony was inoculated at the center of agar plate. The cultures were incubated for 6 days to observe the growth response and the changes the color of the media. An MSM agar without any fungal was served as a positive control to make sure whether the dyes decolorized spontaneously or not while MSM agar without addition of dyes but inoculated by fungal mycelia served as a negative control to observe the fungal ability to grow in the absence of naphthol dye. The isolates showed the best growth responses were used for further analyses.

2.3. Decolorization test of naphthol under various growth conditions

This step was designed to obtain the optimum growth condition of naphthol decolorization. Different growth conditions, such as initial pH media (4.0; 5.0; 6.0; and 7.0) and nitrogen content (0.25% and 0.5% of urea). The reduce color was determined on the day 2, 6, and 10 by measuring the absorbance at 418nm. Percentage of decolorization was determined according this formula [8]:

\[
\text{Percent decolorization} = \frac{\text{Initial absorbance} - \text{Final absorbance}}{\text{Initial absorbance}} \times 100\% 
\]
3. Results and Discussion

3.1. Growth of tested fungi in plate containing naphthol

All tested isolates grew in MSM agar both supplemented with 0.5% glucose and without glucose, as shown in Figure 1. The growth of isolates in media supplemented with naphthol relatively lower than that of in media containing naphthol. Macroscopic observation showed the growth of isolate in plates without naphthol were more intensive with a thicker mass of mycelia. Furthermore, increase naphthol concentration from 10 to 50 ppm seemingly did reduced the growth rate of isolate, except for isolate TB06, in which the growth reduced as concentration of naphthol increases.

![Figure 1](image1.png)

**Figure 1.** Colony diameter of isolates grown in agar plate containing different concentrations of naphthol

Based on colony diameter as shown in Figure 1, isolates TB04 and TB06 grew better than other two isolates both in media with no naphthol and in media containing naphthol. Then, the performance of mycelia was quite thin and rare in naphthol containing media indicating that naphthol inhibited the growth to some extends. After 10 days of incubation, the highest diameter of 50 ppm naphthol exhibited by TB04 followed by TB06. These two isolates were used for further analyses. The smallest diameter was found in isolate ZN04. The slow growth of the isolates in naphthol containing media indicated the isolates have difficulty to degrade and use naphthol for their growth. This was also suggests that naphthol is quite resistance to biodegradation. Figure 2 shows the growth of isolates on MSM plate containing naphthol.

![Figure 2](image2.png)

**Figure 2.** Growth of isolates on MSM agar containing 50 ppm of naphthol. A, negative control with no isolate; B, TB01; C, TB04; D, TB06; and E, Zn04
Previous study of the same fungi for their ligninolytic enzyme activities show that TB04 and TB06 showed the best growth and were able to produce related enzymes lignin peroxidase, manganese peroxidase, and laccase in medium pH range from 2.5 to 5.5. The fungi were able to grow in MSM plates containing any other carbon source, except wastewater of textile industry and they grew in liquid media containing 25% of liquid waste [9]. Different ability of fungi to grow in dyestuff media has been studied by some researchers. Although certain fungus, Lenzites elegans KSG32, grew well (8.2 cm) on naphthol green B agar on day 4 [10], other fungus, Pleurotus ostreatus (MTCC 142), grew much slowly on 50 ppm of crystal violet dye which was only 1.4 cm after 10 days of incubation [11].

3.2. Naphthol Decolorization
The ability of selected isolates to decolorize naphthol were studied under liquid culture conditions. WITH various pH, nitrogen source, and addition of glucose. Results as shown in Figure, 3, 4, 5 and 6 indicated that all isolates grew in all growth conditions that up to 15 days of incubation. The two isolates reduced the dye color in limited range. When glucose was not included to the culture as in Figure 3 and 4, the decolorization was relatively lower that that of culture in glucose containing media as in Figure 5 and 6.

![Figure 3](image_url)

**Figure 3.** The decolorization of naphthol by TB04 under various growth conditions (pH and nitrogen sources) and without glucose.

No significant difference between the two isolates, except the ability of TB06 isolate to reduce the dye color more varied. It was assumed that up to 15 days, the isolates degraded the dye very slowly even in the presence of nitrogen source. This caused the fungi to grow slowly as well. Researchers reported the slow degradation of numerous dyes due to their complex structures which in turn slow the growth of microorganism.
Microorganism under stress condition utilized synthetic organic matter as energy source. The slow growth rate was observed with *Bacillus subtilis* leading to a slow biodegradation rate. Initially the bacterial growth decreased up to day 6 of incubation, followed by an increase till 10 day [12].

In the presence of glucose at low concentration both isolates decreased the dye color significantly on the day 10 to 15 (Figure 5 and 6). Glucose function as an excellent carbon source to support the isolate growth, after 6 days of incubation the growth of isolates increased significantly, as the result, the rate of decolorization increased sharply. Although TB04 and TB06 isolates showed different patterns of decolorization, they share the optimum pH of medium. The best condition for both isolate to decolorize naphthol dye was at pH 4 and 0.25% urea concentration. Nitrogen concentration of 0.5% reduced the decolorization percentage. Figure 5 said that TB04 started to decolorize the dye on the day 6th and reached the highest activity on the day 15 which was 59%. Meanwhile Figure 6 told us that TB06 started to show decolorization on the day 10 with 62% and decrease slightly the activity on day 15.

**Figure 4.** The decolorization of naphthol by TB06 under various growth condition (pH and nitrogen sources) and without glucose

**Figure 5.** The decolorization of naphthol by TB04 under various growth conditions (pH and nitrogen sources) and with 0.2% glucose
A slightly different result was shown by Akdogan et al. [13]. He studied the effect of different nitrogen concentration on decolorization of reactive blue 19 dye by a fungal species, *Coprinus plicatilis*. He found that the best nitrogen (urea) concentration for the fungal growth was 5 g/L. At a higher concentration, 10 and 15 g/L of urea showed inhibitory effect. He said that the best pH for decolorization was 5.5, while in this study we found that the best pH for naphthol decolorization was at pH 4.0. The same result was obtained by Billal and Asgher [14] who studied the Manganese Peroxidase activity of *Ganoderma lucidum* IBL 05, in which the optimum pH was 4.0. In addition, the effect of aeration on decolorization was also evaluated in this study, results will be reported somewhere.

### 3.3. Absorption of naphthol dye by fungal mycelia

Decomposition and decolorization process is initiated by absorption of dye compounds by fungal mycelia. Electrostatic force between negatively charge of cell wall and positively charged of the dyes allows the absorption of dyes by fungal mycelia followed by the enzymatic break down of complex chemical bonds, and finally reducing the color intensity of the dyes [15].

**Figure 7.** Photomicrograph of naphthol dye absorption by TB06 mycelia

Based on this result it is assumed that decolorization of naphthol by these isolates occured mostly through binding or absorption of dyes to fungal mycelia. Absorption of dyes to fungal mycelia was
also documented in our previous work. Indigosol dyes contained in wastewater of textile were almost completely absorbed by fungal mycelia under static culture condition [16]. Then, from this study it could be concluded that TB06 and TB04 were able to utilize naphthol dye as the sole carbon source. The ability of both isolates increased significantly when 0.2% of glucose was added into the medium, and the best culture condition for decolorizing the dye was when medium contained 0.25% urea, and at pH 4.0.

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