Potential of Basidiomycetous Fungi Isolated from Gunung Barus Forest North Sumatera in Decolorization of Wastewater of Textile Industry

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Abstract. A study of basidiomycetous fungi in decolorization of wastewater of textile industry has been started in our laboratory. The objective of this study was to obtain potential isolates and to examine their decolorization acitity. The fungi were isolated from local forest, Gunung Barus Forest, in North Sumatera and screened their ligninolytic activity qualitatively by bavendam method and the waste was obtained from local textile industry in Medan. Nineteen fungal isolates grew on plate agar medium containing 100% of waste supplemented with 2% glucose, and 6 of those exhibited good growth when glucose in the media was reduced to 1%. Surprisingly, these six potential isolates grew, although relatively at lower rate, when glucose was not included in the media. Meanwhile, there was no substantial decolorization of media could be observed on all plates cultures. Analyses of decolorization on liquid condition containing 25% of wastewater and no glucose showed that fungal grew at the bottom culture flask. All 6 isolates exhibited decolorization activity. Interestingly, mass of mycelia growth at the bottom absorbed dyes and dissolved suspended solid which was seemingly separated from very clean solution medium surrounding. These results indicated that the cultures utilized carbon source from waste and the extracellular matrixes produced by fungal isolates might involve in decolorization of textile wastewater.

1. Introduction
Textile industry uses various chemical and dyes and produces large amount of wastewater. The amount has been predicted to increases the growing demand for textile products every year. Thus, the release of wastewater to the environment can be the reason for serious health and environmental problems. Egli [1] many organic compounds are used in dyeing and finishing process of textile industry. Base on the dyeing process, textile dyes are classified as reactive, direct, disperse, acid, basic and vat dyes. Chemical reaction occurring during manufacturing of textile can cause formation of toxic chemical. Dyes used in textile industry are group as natural and synthetic dyes. Natural dyes are obtained naturally and mostly extracted from plant materials. The synthetic dyes are derivative of aromatic hydrocarbon such as benzene, toluene, naphthalene and anthrasene. Synthetic dyes are more stable than that of natural dyes. Marmion [2] synthetic dyes are widely used in several industries such as textile, paper, printing, cosmetics, pharmaceuticals, photography color and petroleum. Campos et al. [3] dyes are classified into acidic, basic, disperse, azo, diazo, anthraquinone and metal complex based on their structures.
Large proportion of chemicals and dyes are lost during manufacturing of textile and exists in wastewater. Somasiri et al. [4], Haroun and Idris [5] colored wastewater from the textile industries is one of the most obvious indicators of water pollution. Colored dye wastewater causes severe effects on aquatic environment even in small amounts. In addition to color, dye wastewater also contains other pollutants like degradable organics, nutrients, pH altering agent, salts, sulphur, toxicants and refractory organics. Furthermore, Adyel et al. [6] has reported that untreated sludge from textile industries contain the heavy metals. Thirteen heavy metals, viz., Mn, Ti, Cu, Zn, Ni, Sr, V, Cr, Zr, Hg, Cd, Nb and Ga, were detected and some exceeded the permissible limit to apply in agricultural land.

Many works on degradation and decolorization of textile dyes or wastewater of textile industry have been reported to reduce the risk of pollutant to the environment. At least there are three ways to minimize pollution effect of textile wastewater, first applying new technology to reduce the amount of waste, second practicing effective wastewater management and third recycling system [7]. Furthermore, three types of treatment techniques of decolorization of wastewaters of textile industry have been intensively studied; chemical, physical and biological approaches. Chemical treatment techniques include ozonation, fenton oxidation and photo catalytic oxidation and reduction reactions. They convert hazardous pollutant to nonhazardous or less toxic compounds. The physical techniques are adsorption, ion exchange, irradiation, membrane filtration, electrolysis, coagulation/flocculation and ultrasonic mineralization methods. The last treatment is through biological technique, which includes bacterial and fungal biodegradation and biosorption in aerobic, anaerobic or combined treatment processes [8, 9]. The latter has been obtaining much attention from environmental safety viewpoint compared to others. Recently, researchers have focused on biological techniques as the best alternative.

As far as literature has been survey, white rot fungi seem to be a good choice due to its ligninolytic activity and their decolorization activity. Phanerachaete chrysosporium, Ganoderma sp., Trametes trogii, Irpex lacteus, Dichomitus squalens, and many others have been used in decolorization studies [10,11,12,13,14]. Dayaram [15] reports laccase enzyme from Polyporus rubidus immobilized on alginate degrades tested dyes more than 80% within 5 days under stationary incubation conditions. Moreira-Neto [16], twelve basidiomycetes strains from the genera Pleurotus, Trametes, Lentinus, Peniophora, Pycnoporus, Rigidoporus, Hygrocybeand, Psilocybe decolorized the reactive dyes Cibacron Brilliant Blue H-GR and Cibacron Red FN-2BL in solid and liquid media. Some have great ability to decolorize the synthetic textile effluent more than 70% and laccase was the main ligninolytic enzyme involved on dyes decolorization.

This study has been designed to obtain potential basidiomycetous fungi isolated from local forest of North Sumatera and to examine their decolorization activity on textile wastewater. The isolates were screened in agar plate containing waste water and the decolorization activity was evaluated on liquid condition. This paper describes that basidiomycetous fungi isolated from local forest in North Sumatera have an ability to decolorize textile wastewater.

2. Material and Methods

2.1. Chemicals and organisms

All chemicals used were of reagent grade obtained from Fluka and other chemical companies. Basidiomycetous fungi used in this study were isolated from Gunung Barus forest of North Sumatera and always maintained in media containing wastewater through the study. Wastewater containing chemical dyes was obtained from local batik industry in Medan. The study was done in two steeps, first was the screening potential isolates to grow in wastewater and the second decolorization analyses of potential selected isolates. All tested media were supplemented with minimal salt nutrient.

2.2. Screening potential isolates
All fungal isolates used in the study were previously screened for their ligninolytic activity qualitatively by bavendam method. This step was done to obtain isolates with high ligninolytic potential, and many works on degradation of hydrocarbon compounds including chemical dyes were done on the basis of ligninolytic activity. Screening was done by culturing isolates in plate media containing wastewater (100%) in which waste was used as a solvent of nutrient salt. The medium was first supplemented with 2% glucose as carbon source in addition to carbon of chemical dyes. The isolates exhibiting good growth were subcultured to media with a lower level of glucose content (1%). Visual observation was done for the growth response and decolorization activity of each isolate. Then, isolates growing better in the latter media were used for decolorization under liquid condition.

2.3. Decolorization test of potential isolates
A volume of 100 ml liquid media was prepared in 250 ml Erlenmeyer flask containing 25% wastewater and minimal salt nutrient with no glucose. The media were sterilized for 15 minutes. Ten agar plagues (ø5 mm) of active growing colony were inoculated separately into each sterilized media, and the culture were incubated at stationery condition at room temperature for one month. Growth response and decolorization activity of each culture was observed during cultivation.

2.4. Analyses of decolorization activity
Analyses of decolorization activity were done spectrophotometrically at the end of cultivation period. The maximal absorbance of dyes in wastewater was read from a wavelength of 100 to 700 nm, and maximal wavelength was used to measured decolorization activity. The liquid of culture media was carefully pipetted and transferred to spectrophotometer cuvette, and the reading was done at room temperature. Media containing 25% wastewater was used as control and distilled water was used for comparison. Percentage of decolorization was calculated following formula proposed by Mahmoodi et al. [17].

3. Results and Discussion
3.1. Growth of basidiomycetous fungi in media containing wastewater of textile industry
The screening step was done to obtain potential isolates which will be utilized in decolorization study. This study used adaptation method that is so far has not been reported in the screening study of decolorization by microbe. Nineteen of 28 isolates cultured in 100% media supplemented with 1% glucose showed good growth response, and six of those exhibited best growth performance. However there was no significant decolorization was observed visually in each culture plate, as shown in the following figure.
Results as shown above indicated that isolates did not utilize dyes as carbon source, the color of plate did not substantially change. The isolates preferred to use glucose instead of carbon existed in wastewater. Furthermore growth of isolates in media containing textile waste assumed to contain various chemicals including dyes and heavy metals indicated that the isolates could grow under stress environment.

Importantly, all six isolates grew on plate media containing 100% waste although glucose was not added to media. Even growth of isolates was noticed much lower than the growth response when they were cultivated in media supplemented with 1% glucose, this result has encouraged us very much. However, there was no significant reduction of color intensity of plate could be detected visually after one week cultivation as it was expected at the beginning of this study. To this result, for temporary conclusion, it was assumed that the fungi not at this growth condition. It is believed that the fungus utilized carbon from textile waste. Figure 2 below shows one week growth of selected isolate on agar plate containing wastewater (100%) and no glucose.

3.2. Decolorization activity of selected isolates
Decolorization study was done in liquid media. Although isolates exhibited good growth on agar plate containing 100% waste, it was considered to toxic and stressful under liquid condition, and importantly glucose was also not provided in the media. Thus a concentration of 25% liquid was used for preliminary test. Results showed all isolates exhibited the growth and it was detected from the second day of cultivation. However, unlike common type of fungal growth performance, the isolates grew concentrated more to the bottom of culture flaks, as shown in Figure 3. Interestingly, mass of fungal
mycelia absorbed all dyes and suspended solid content in the media, creating clean solution media surrounding dark green mycelial mass. In addition to absorbing dyes, dark color of mycelial mass could have been forming as a consequence of suspended solid absorbed at mycelia. This assumption was further proved microscopically. Preliminary observation showed that dyes and suspended solid existed in wastewater absorbed to fungal mycelia, as shown in Figure 3.

![Figure 3](image)

**Figure 3.** Growth Of Selected Isolate in Liquid Media Containing 25% Wastewater and No Glucose, Left Before Inoculation, Center After Incubation For One Month, and Right Photo Micrograph of Fungal Mycelial Mass

Result as shown in Figure 3 has attracted more attention to a different decolorization viewpoint during the course of our study. At the beginning design of this study, the decolorization in this work has been focused on the degradation of chemical dyes containing on the wastewater of textile industry. Nonetheless, we have a strong believe that the isolate have utilized dyes carbon sources even under low rate. This was probably due to the analyses of optimal growth condition has not been performed. Mycelial fungi as shown in photo micrograph (Figure 3) clearly indicated that the fungi grew. Measurement of fungal growth (fungal dry weight) in liquid culture is currently under investigation. Thus, it has shifted our main interest, at least temporarily, from degradation to other possible decolorization process. It has been assumed that extracellular matrix such as polysaccharide produced by fungal hyphae might have an important compound or molecule in decolorization processes, the result will be presented somewhere else.

![Figure 4](image)

**Figure 4.** Clean Portion of Culture Media (wastewater) After Treated with Selected Isolates for One Month
Preliminary analyses of absorbance of the clean part of culture media of selected isolates resulted in significant reduction of absorbance value compared to control. Decolorization rate reached 90% or above, results as shown at Figure 4. This part is now under investigation, and a comprehensive result will be published in short. These results are comparable with previously reported study by Moreira-Neto [16], basidiomycetes fungi from the genera of Trametes, Lentinus, Peniophora, Pycnoporus, Rigidoporus, Hygrocybeand, Psilocybe have an ability to decolorize CBB H-GR and Cibacron Red FN-2BL by more than 70%. Leidig et al, [18], Trametes versicolour grown in a 1.0-L aerated stirred tank bioreactor under non-sterile conditions was capable of decolourizing polyvinylamine sulphonate anthrapyridone (Poly R-478) with the average dye elimination of 80% and it was achieved after 19 days cultivation. In conclusion, basidiomycetous fungi isolated from Gunung Barus Forest are potential agents in decolorization of wastewater of textile industry.

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