Potency of dye absorption of exopolysaccharide producing fungi isolated from Gunung Barus North Sumatera, Indonesia

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Abstract. The ability of fungi to decolorize wastewater containing dyes has been obtaining much attention from different aspects. The purpose of this study was to obtain exopolysaccharide producing fungi and to know the ability exopolysaccharide produced in decolorization of wastewater containing dyes. The fungi were isolated from local forest Gunung Barus North Sumatera. Six isolates of decolorizing fungi cultivated on basal medium and basal medium enriched with wastewater containing dyes for the production of exopolysaccharide. The isolates were then cultivated on basal medium with and without glucose content. Results showed that only one isolate ZN03 of six tested isolates produced the exopolysaccharide both in basal medium and basal medium enriched with wastewater containing dyes. Isolate ZN03 showed the highest decolorization activity (58.5%) among other fungi. Then the production of exopolysaccharide was found only in culture supplemented with glucose. The exopolysaccharide produced by ZN03 absorbed dyes containing in wastewater and changed its color from white to dark brown.

1. Introduction
The development of industrial textile has caused the accumulation of dyestuff waste in aquatic environment. Dyestuff waste from synthetic dyes, even in low concentration, will affect aesthetics, and turbidity of water. Sometimes chemical ingredients of dyes are toxic for aquatic organism, plants and even humans [1]. Efforts to control dyestuff waste have been done both chemically and physically, but the processes are considered less environmentally friendly, since they are expensive and difficult to apply [2]. An alternative treatment of dyestuff is urgent to use and apply in management of waste containing dyestuff. Some researchers have used biological agent such as bacteria [3] or fungi [4] to decolorize the dye. Several decolorizing fungi are able to degrade on of dyes such as Phanerochaete chrysosporium, Pleurotus ostreatus, Stachybotrys, Cladosporium, Penicillium, and Aspergillus [5]. The ability of Basidiomycota and Ascomycota fungi to decolorizing dye stuff is reported to be higher than that of bacteria. This decolorizing activity correlates to the activity of fungi to degrade lignin [6]. The structure of dye chromophore such as azo dye is similit to the structure of lignin. Azo dyes is the most widely used dyes due to their strong adherence to textile material, and it is not easily degraded [7]. The decolorization activity of dyestuff by fungus caused by the activity of hydrolytic enzyme to degrade the chemical active side of azo dye, and polysaccharides glycanin fungal mycelium can absorb the dyestuffs. Beside, filamentous and yeast of Ascomycota and Basidiomycota could produce exopolysaccharide (EPS) into their growth culture medium [8]. The exopolysaccharide produced by phylum Basidiomycota (genera of Ganoderma, Agaricus, Cordyceps, Trametes, Pleurotus, and Lentinus) and phylum Ascomycota (genera Lasiodiplodia, Phoma, and Diaporthe) have various biological activities and functions [5].
Exopolysaccharide is a long-chain polymer of secondary metabolites produced by some microorganisms and secreted to the environment or growth medium. Exopolysaccharides are composed of polysaccharides, proteins, nucleic acids and fats [9] and some non-carbohydrate constituents such as acetate, pyruvate, succinate, and phosphate [5]. These polymers have bioactivation power that can be used in the use of drugs as antioxidants, anti-tumors and anti-inflammation [10]. In food industry, exopolysaccharide is used as thickeners and emulsifier. Exopolysaccharide is also used as bioabsorption of heavy metals and constituent of biofilms [11]. From the previous research it was obtain several fungi from soil of Gunung Barus in North Sumatera Indonesia, that potential as decolorizing of dye [4]. The objective of this research was to find the potency of the decolorizing fungi to produce exopolysaccharide as their activity to decolorize wastewater.

2. Materials and Methods

2.1. Fungal isolates and dyestuff waste

The six isolates of decolorizing fungi (TB02, TB2K, TB03, TB11, ZN03, and ZN06) were obtained from previous research. The fungi were originally isolated from soil of local forest Gunung Barus, the Province of North Sumatera, Indonesia. The isolates were cultured at potato dextrose agar (PDA oxoid®) slant and stored at ambient temperature. The isolates were then sub-cultured in the PDA plate before using for analyses. Dyestuff waste used was wastewater containing naphthol form local batik industry in Medan.

2.2. Production of exopolysaccharide

The exopolysaccharide was produced in two types of media; the basal medium without dyestuff waste, and the basal medium enriched with dyestuff waste. The basal medium contains (g/l): 1.0 peptone; 2.0 yeast extract; 1.0 K₂HPO₄; 0.2MgSO₄·7H₂O; 5.0NH₄NO₃; 40.0 glucose; pH 6.0 [12]. Basal medium (100 ml) in 250 ml Erlenmeyer was inoculated with 10 agar plugs (Ø 5 mm) of active growing fungal mycelium. The cultures were incubated in room temperature for 7 days in shaking condition (100 rpm). Dyestuff waste at a concentration of 25 % was used in culture medium. At the end of cultivated time, the absorbance of medium was measured by spectrophotometer at λ 440 nm. Percentage of decolorization was determined in equation [13]. Decolorization (%) = \[\frac{(A₀ - At)A₀}{A₀}\] × 100, where A₀ is the initial absorbance in control (untreated dye) and Aₜ is the absorbance in fungal treatment. The fungi were also cultivated on modified medium with no glucose and 1 % glucose. Culture condition was the same with previous condition. The exopolysaccharide produced was collected and purified as described below.

2.3. Purification of exopolysaccharide

The fungal culture medium was filtered with Whatman no.1. The mycelium was dried at 70° C for 24 hours for measuring the fungal growth. The exopolysaccharide was harvested by precipitation of the culture filtrate with cold ethanol (1:2, v/v) at 4ºC for 24 hours. The precipitate was collected and dried at 70° C. The reducing sugar was calculated by the Dubois method [14].

2.4. Dye absorbtion test of exopolysaccharide

As much as 0.2 g wet weight of extracted exopolysaccharide was soaked on 20 ml of 25% dyestuff waste at agitated condition. The absorbance of solution was measured every 30 minutes using spectrophotometer at λ 440 nm.

3. Results and Discussion

3.1. Production of exopolysaccharide

The production of exopolysaccharide was measured under two media, the basal medium and the media containing dyestuffs. The basal media used contained glucose as carbon and NH₄NO₃ as nitrogen sources. Results as depicted in Table 1 showed that all fungi grew well on media, indicated by relatively the same dry weight of fungal mycelia (Table 1). The exopolysaccharide analyzed in this study was the soluble exopolysaccharide. From six decolorizing fungi tested, only ZN03 did produce
exopolysaccharide. The production of exopolysaccharide has been reported by several researchers. Growth culture condition and composition of medium production were important parameter for production of exopolysaccharide [10].

### Table 1. Production of exopolysaccharide by six isolates of decolorizing fungi on basal medium

| No. | Code of isolates | EPS (g/l) | Dry weight of mycelium (g) |
|-----|------------------|----------|---------------------------|
| 1.  | TB02             | Nd*      | 0.78                      |
| 2.  | TB2K             | Nd       | 0.68                      |
| 3.  | TB03             | Nd       | 0.82                      |
| 4.  | TB11             | Nd       | 0.64                      |
| 5.  | ZNO3             | 0.053    | 0.73                      |
| 6.  | ZNO6             | Nd       | 0.67                      |

*nd, not detected.

When wastewater containing dye stuff at a concentration of 25% was added to the culture, all culture also grew at almost the same rate as indicated in Table 2. Then, exopolysaccharide was only produced by isolate ZN03. Surprisingly the production of exopolysaccharide increased almost double in culture supplemented with wastewater containing dye stuffs.

### Table 2. Production of exopolysaccharide by six isolates of decolorizing fungi in basal media containing dyestuff waste

| No. | Code of isolates | EPS (g/l) | Dry weight of mycelium (g) | Decolorization activity (%) |
|-----|------------------|----------|---------------------------|-----------------------------|
| 1.  | Control          | -        | 0                         | 0.0                         |
| 2.  | TB02             | -        | 0.70 g                    | 9.6                         |
| 3.  | TB2K             | -        | 0.50 g                    | 3.2                         |
| 4.  | TB03             | -        | 0.65 g                    | 16.8                        |
| 5.  | TB11             | -        | 0.67 g                    | 39.6                        |
| 6.  | ZN03             | 0.094    | 0.78 g                    | 58.5                        |
| 7.  | ZN06             | -        | 0.55 g                    | 6.0                         |

These results indicated that wastewater containing dyestuff triggered the isolate to synthesize more exopolysaccharide. Then it is suggested that when fungi are grown under environmental stress in this case was dye stuff, the fungi tend to synthesize more exopolysaccharide as for their defense mechanism. However, under stationary, it was clearly noticed that fungal mycelia of culture with no glucose absorbed more color or higher decolorization activity compared with the culture containing glucose, and the dyestuffs were clearly bound to fungal mycelia under microscopic analyses [4]. Therefore decolorization activity of isolate TB02, TB02K, TB03, TB11, and ZN06 occurred through binding of dyestuff to fungal mycelia.

#### 3.2. Production of exopolysaccharide under low glucose content

Production of exopolysaccharide was also determined under low glucose content of the media. One percent of glucose was used, and media containing glucose for control of exopolysaccharide formation. The culture was incubated for one week under shaking condition. Result as shown in Table 3 indicated that exopolysaccharide was produced in culture containing 1% glucose, and no exopolysaccharide produced when glucose was not included in the media.
Table 3. Production of exopolysaccharide by isolate ZN03

| No. | Treatment | No glucose Decolorization activity (%) | EPS (g/l) | 1% glucose Decolorization activity (%) | EPS (g/l) |
|-----|-----------|----------------------------------------|----------|----------------------------------------|----------|
| 1.  | Control*  | 0                                      | 0        | 0                                      | 0        |
| 2.  | ZN03      | 44.8                                   | 0        | 70.4                                   | 0.046    |

* Media with no isolate.

Results as shown in Table 3, importantly, indicated that decolorization was only detected in culture supplemented with glucose with decolorization reached 70.4%, and no decolorization was observed when glucose was not included in the culture. This result clearly confirmed the role of exopolysaccharide in decolorization of dyestuff or color containing in the waste, and in line with our previous works. We noticed clear binding of dyestuff on the fungal mycelia both macroscopic and microscopic observation and reduced intensity of color up to 90% under stationary culture condition [4]. Analyses of reducing sugar showed total sugar of the extracted exopolysaccharide was 62.5 ppm.

3.3. Dye absorption of extracted exopolysaccharide

The potency of extracted exopolysaccharide in decolorization was measured. Zero point twenty mg of exopolysaccharide was soaked in to 20 ml of solution containing 25% of dyestuff waste. Result in Figure 1 shows the absorbance value of solution decreased from 30 minutes and continued to maximal reduction at 90 minutes with the percentage of decolorization of 0.23%. No more decolorization was observed at 120 minutes. From the results it is indicated that once saturation was achieved, the exopolysaccharide was not able to absorb more color. 15] previously noticed the same model in which decolorization increased in parallel with incubation time, and the absorption of color reduced and stop when saturation was achieved.

![Figure 1. Reduction absorbance value after extracted exopolysaccharide was soaked into solution containing dyestuff waste](image)

Furthermore, Figure 2 clearly confirmed decolorization ability of extracted exopolysaccharide. The color of exopolysaccharide changed from clear white on the left into dark brown before and after soaked into solution containing dyestuff waste. The same result was previously reported by [16] exopolysaccharide of *Rhodotorula mucilaginosa* turned its color from white to brownish when it was soaked to wastewater. The exopolysaccharide produced by *R. mucilaginosa* absorbed blue methylene maximally. Comparing to the rate of decolorization under culture condition as described above, this value was considered quite low. This was probably due to the amount of extracted exopolysaccharide used was very little (only 0.20 g). On the other hand, under culture condition, more processes might involve in decolorization, such as through enzymatic process and by mycelial absorption.
Figure 2. Exopolysaccharide produced by ZN03. Left before and right after soaked into solution containing dyestuff waste

In conclusion isolate ZN03 produced soluble exopolysaccharide only in glucose containing media, and the production of exopolysaccharide increased when wastewater containing dyestuff was added to the culture. Culture ZN03 decolorized dyestuff by 58.5% and its extracted exopolysaccharide absorbed the color.

Acknowledgement
We gratefully acknowledge the funding from University of Sumatera Utara through TALENTA Research Grant (Nomber5338/UN5.1.R/PPM/2017).

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