Decolorization and Degradation of Batik Dye Effluent using
*Ganoderma lucidum*

Diah Pratiwi, A.W. Indrianingsih, Cici Darsih, Hernawan

Research Unit for Natural Product Technology, Indonesian Institute of Science, Yogyakarta, Indonesia 55861

E-mail: dee.diahpratiwi@gmail.com

**Abstract**. Batik is product of traditional Indonesia culture that developed into a large textile industry. Synthetic dyes which widely used in textile industries including batik. Colour can be removed from wastewater effluent by chemical, physical, and biology methods. Bioremediation is one of the methods that used for processing colored effluent. Isolated White-rot fungi *Ganoderma lucidum* was used for bioremediation process for batik effluent. *G. lucidum* were growth at pH 5-6 and temperature 25°C at various Naphtol Black dye with concentration 20 ppm, 50 ppm, and 100 ppm for 30 day incubation time. The result from this study increased decolorization in line with the increasing of COD degradation. Increasing percentage of decolorization and COD degradation gradually increased with incubation time and dye concentration. The maximum decolorization and COD reduction were found to be 60.53% and 81.03%. *G. lucidum* had potential to decolorized and degraded COD for NB dye effluent on higher concentration.

1. **Introduction**

Batik is product of traditional Indonesia culture that developed into a large textile industry. In the past, dyes and supporting materials used in the process of batik use natural ingredients, but over its the development into a industry then many batik using materials synthesis. Of course this affects the quality and quantity of effluent generated from the process batik production.

Nowadays, water pollution is a major problem faced by the world. It is one of the main cause of death and diseases all over the world [1]. Removal of various pollutants such as heavy metals and dyes from industrial effluents has become a critical issue due to strict environmental regulations [2]. In particular, the dyes wastewater such as azo dyes are the potential polluting synthetic dyes with an extensive range of structures as well as colors as they are used widely in batik industry. Wastewater containing dyes from batik industries affects the aesthetic value of surface water and reduces light penetration, endangering aquatic lives and hindering photosynthesis [3]. Dyes can also cause skin irritation, allergic dermatitis and some of them have been reported to be carcinogenic and mutagenic [4].

Azo dyes are synthetic aromatic compounds [5]. The aromatic group around the azo bond helps to stabilize the azo group. Aromatic azo compounds are usually stable and produce strong colors [6]. Naphthol Black (NB) [Disodium;(6Z)-4-amino-3-[(4-nitropheryl)diazenyl]-5-oxo-6-(phenyl-hydrayzinylidene)naphthalene-2,7-disulfonate] is the most commonly used benzidine-based azo dye. It
is usually found in the effluents discharged from batik industries. It has a complex structure and high solubility in water. Due to all these nature, NB was selected as a model anionic dye in this study.

Some methods can be applied to remove dyes from the waste water, such as membrane filtration, electrocoagulation, electrochemical process, reverse osmosis, irradiation, oxidation, precipitation, and adsorption [7]. Each method has its advantages and disadvantages. The application of traditional physicochemical methods has been restricted due to high cost and low efficiency. In recent years, biological decolorization methods have been considered as a useful, specific, less energy, and environmentally benign, since it results in bioconversion of pollutants to stable and non-toxic end products [8,9]. White-rot fungi (WRF) have received much attention due to their ability to degrade synthetic dyes, giving rise to non-toxic compounds. Some WRF has been reported to degrade rather azo dyes, such as _Phanerochaete chrysosporium_, _Pycnoporus cinnabarinus_, _Pleurotus ostreatus_, and _Basidiomycetous Fungi_ [8]. But there not much progress has been made in decolorization of textile effluents such as batik industries. Batik effluents constitute high chemical and biological oxygen demands, suspended solids, and toxic compounds. Thus, there is need to search other microorganisms for treating batik effluents, which are capable of growing at alkaline pH and having sturdy ligninolytic activities.

_G. lucidum_ is a representative of white-rot fungi. _G. lucidum_ is highly liable to grow on and degrade agro-industrial lignocellulosic biomass. _G. lucidum_ can grow on alkali lignin and expresses high laccase activity [10]. This work was aimed at evaluating _G. lucidum_ for their ability to decolorize NB dyes in liquid cultures.

2. Material and Methods

2.1. Batik Dye Effluent

The batik effluent was collected from a private small and medium Batik enterprises located at Petir, Rongkop, Gunungkidul Regency. Synthetic dyes used in Batik enterprises is NB that included azo dyes.

2.2. Organism and Inoculum

_G. lucidum_ was developed by _G. lucidum_ cultivation on centers of mushroom farmer Media Agro Merapi Kaliurang, Yogyakarta. Taxonomy this mushroom has been verified at Pharmaceutical Biology Divison, Faculty of Pharmacy, Universitas Gadjah Mada. The strain is maintained on PDA (Potato Dextrose Agar) Sigma Aldrich at 30°C. The mycelium was transferred to PDA plates and allowed grow for 7th days. Liquid culture experiments were conducted in 500 ml Erlenmeyer flasks containing 230 ml of liquid medium PDB (Potato Dextrose Broth) and Batik dye effluent at different concentration. Liquid medium PDB containing 20 g/L dextrose and 4 g/L potato extract. Each flask was inoculated with 3 number of agar plug cut from PDA plates and incubated for 30 days.

2.3. Enzyme Assays

The extraction using the centrifugation enzyme method [11]. One gram sample is mixed with 50 mm phosphate buffer PH 6.5 with a comparison of 10/1 (v/w), then shake on speed 150 rpm for 1 hour. After that, samples centrifuged at 7000 x g for 20 minutes. Supernatant liquid separated and used to analyze the activity of the enzyme.

Laccase activity was measured at 30 °C using 1 mM ABTS as substrate. The enzyme analysis mixture contained 0.5 ml of 0.5 M sodium acetate buffer (pH 5), 0.1 ml of 0.1 mM ABTS (Roche Diagnostics), and 0.4 ml enzyme filtrate. The increase in absorbance of the solution was monitored at 420 nm (E_{420} = 36 mM⁻¹ cm⁻¹) using Halo UV-VIS Spectrophotometer RB-10.

2.4. Decolorization

Decolorization was monitored by a spectrophotometer and expressed as the percentage of removed dye. Percentage of decolorization was calculated as follows:
Decolorization (%) = \frac{(Abs_0 - Abs_t)}{Abs_0} \times 100

Where the absorbance at time 0 is Abs\(_0\) and Abs\(_t\) is the absorbance at time t, and \(\lambda_{\text{max}}\) is measured at the maximum visible wavelength of dye.

2.5. COD Degradation

Chemical Oxygen Demand is an amount of oxygen consumed by the organic compound and inorganic matter which was oxidized to the water. COD reflect the pollution degree of water and comprehensive index of relative content of organics [12]. Degradation of batik dye effluent in term of COD removal was also investigated. This COD analysis was done at Universitas Gadjah Mada using the volumetric method.

3. Results and discussions

3.1. Mycelial Growth

The Growth of the mycelial already filled all surface of the media on the 7 day, reaching 10.18 cm in diameter. The thickness of mycelia also increased in line with the incubation time. Mycelium was white, but after 20 day the color changed to yellowish or yellow and till 30 day mycelial color began to change to brownish. There factors that affecting growth mycelial biomass are medium, pH and strain. Medium play an important role, followed by pH and type of strain [13]. The growth of \(G.\ lucidum\) mycelial to fully covered medium depends on diameter surface, amount and nutrients that contained at medium. This parameter growth has been reported by Kapoor and Sharma, that the mycelial growth depends on such factor as culture media, pH, temperature, and nutrient component. These factors greatly influenced the expansion of fungi in each field and laboratory condition [14].

Figure 1. The Growth of Mycelial at (a) day 7; (b) day 20; and (c) day 30
3.2. Effect of Dye Concentration and Incubation Time on Decolorization

The percentage of decolorization increased with time incubation. These results are accordance with research conducted by Sharma et.al (2015) [15], where an increasing percentage of decolorization gradually increased with incubation time up to a point till it became constant. In this study, the incubation time where decolorization became constant is not reached during the 30 day incubation time or reached after 30 day. The optimum decolorization of batik effluent in this study reaching 58.79% at 20 day on 50 ppm concentration. The maximum color removal of textile dye using *G. Lucidum* found to be 81.4% at 5 day operation [16]. Textile effluent Arzoo textile (ART) and Crescent textile (CRT) was maximally decolorized 49.5 ± 0.71% and 36.2 ± 0.56% on 10th day of incubation. The ability of white rot fungi to degrade textile industrial effluent is correlated directly to its ability to degrade different dyes present in effluent [17]. Decolorization of Reactive Blue 19 by Ganoderma Sp. achieved 95% after 5 day at pH 7 and 25°C [18]. Decolorization of synthetic dyes by *G. lucidum* taken time 7 to 15 days [19]. There are two factors that influenced decolorization process by fungi, one is related to fungal growth conditions and other is characteristics of dye solution or effluent [20].

The amount usage of dyes, affect the concentration of effluent textiles and dyeing industries. The effects of dye concentration on decolorization of batik effluent by *G. lucidum* was studied at different concentrations from 20 mg/L to 100 mg/L till 30 day. The result of this study revealed that the decolorization was increased with the increasing dye concentration. This is contrast with the results of previous study stated that dye concentration affects the efficiency of decolorization, high dye concentration decreased the decolorization rates [20]. Decolorization was decrease with increasing dye concentration [21]. The removal of dyes increases with decrease in initial dye concentration. At high concentration, the chemical and other pollutants present in the dye effluent inhibit the growth of microorganism [16]. But, this result of study are in line with Revankar and Lele which states that time required to complete decolorization increased over the increased of concentration. That could be possible to sequentially adapt the biomass to higher concentrations and thus improve the performance [22]. Decolorization ability of white rot fungi can be substantially increased by carefully optimizing the operational conditions such as nutrient content of media culture, age of fungus and environmental condition [18]. *G. lucidum* had potential to decolorized and degraded COD for NB dye effluent on higher concentration.
3.3. Effect of Dye Concentration and Incubation Time on COD Degradation

![Figure 3. The Effect of Dye Concentration and Incubation Time on COD Degradation of Batik Effluent by G. lucidum](image)

Complete decolorization of effluent dyes does not mean that the effluent is completely degraded [12], so degradation of batik dye effluent regarding of COD removal were also investigated. COD removal increased with incubation time. Long incubation times giving *G. lucidum* more time to degrade the COD contained in batik effluent. The maximum percentage of COD degradation achieving 81.03% at 30 day incubation time. Higher concentration of batik dye effluent increased COD removal. This result different with a general study which stated that the higher concentration of dye in the effluent decrease the ability of COD removal. It possible that this caused the presence of elements contained in batik effluent which is a nutrient for *G. lucidum* to growth. An increased of dye concentration increase the elements that be a nutrient for *G. lucidum*. The medium that fungi are mostly grown with dyes or dye effluent to develop a biosorbent containing living fungi mainly composed of carbon source, nitrogen source, and other nutrients [20]. From this study, the result shows that increased decolorization in line with the increasing of COD degradation.

3.4. Enzyme Activity

A method has been patented for decolorization dye effluent using WRF to absorb, degrade, and remove the color of dye effluent[23]. Biodegradation mechanism of textile dyes by fungi because they can produce the lignin modifying enzymes, laccase, manganese peroxidase (MnP) and Lignin peroxidase (LiP) [24]. *G. lucidum* has potential to produce laccase as main ligninolytic enzyme at optimized parameters were incubation period of 16 days, pH 5, and temperature of 30°C [25].

The concentration of dye affect the enzyme activity, increased of dye concentration decreased the enzyme activity. An increasing of enzyme activity gradually increased with incubation time up to optimum condition. The result of the study showed that when enzyme activity higher then the percentage of decolorization and COD removal lower, neither the opposite when the percentage of decolorization and COD removal higher then the enzyme activity lower. There is possible mechanisms of fungal decolorization and degradation of dyes, i.e. adsorption, biodegradation, mineralization, enzymatic degradation, and utilization as a carbon source [26]. Decolorization was carried out by a variety of WRF and in most cases laccase was responsible for color removal [17]. In this study, COD removal and
Decolorization may not be affected by laccase activity but other extracellular enzymes such as LiP and MnP. Extracellular enzymes such as LiP and MnP are involved in biodegradation of dyes as well as in color removal. The extent of dye removal depends on the degree of dye complexity, nitrogen availability, and enzymatic in the medium [26]. Other mechanisms of fungal decolorization such as adsorption may due happen on this study. Adsorption as mechanisms of decolorization a wide range dye wastewater by *Ganoderma sp.*[27]. Revankar and lele stated that to ensure that the dye decolorization was not as a result of adsorption of dye on fungal mycelium, the mycelial cultures were filtered, re-suspended in methanol, extracted and centrifuged. If there was no color detected within the extract supernatant, this indicated that decolorization of effluent was achieved because dye degradation by ligninolytic enzyme systems of *Ganoderma sp.*[22].

![Figure 4](image-url)

**Figure 4.** Decolorization, COD degradation, and Enzyme Activity of Batik Dyes Effluent by *G. lucidum*

### 4. Conclusion

*G. lucidum* as one of WRF that tremendous potential for biodegradation of a variety textile effluent. In these study *G. lucidum* were growth at pH 5-6 and temperature 25°C at various NB dyes concentration: 20 ppm, 50 ppm, and 100 ppm for 30 day incubation time. Increased decolorization in line with the increasing of COD degradation. Increasing percentage of decolorization and COD degradation gradually increased with incubation time and dye concentration. The maximum decolorization and COD reduction were found to be 60.53% and 81.03%. *G. lucidum* had potential to decolorized and degrade COD for NB dye effluent on higher concentration. Laccase activity may not affected the decolorization and COD removal of batik NB dyes effluent by *G. lucidum*. Need to do more study to find out the optimum concentration of NB as well as to know really mechanisms of decolorization and COD degradation.
References

[1] Shadeera Rouf and M. Nagapadma 2015 *Int. J. Sci. Eng. Researc* 6 538–44
[2] Li C, Cui J, Wang F, Peng W and He Y 2016 *Desalination Water Treat.* 57 14060–6
[3] Farzana M H and Meenakshi S 2015 *Int. J. Biol. Macromol.* 72 900–10
[4] Gong R, Li M, Yang C, Sun Y and Chen J 2005 *J. Hazard. Mater.* 121 247–50
[5] Raval N P, Shah P U, Ladha D G, Wadhwani P M and Shah N K 2016 *Desalination Water Treat.* 57 9247–62
[6] Anon Modeling of Fixed Bed Column Studies for Adsorption of Azo Dye on Chitosan Impregnated with a Cationic Surfactant - Modeling-of-Fixed-Bed-Column-Studies-for-Adsorption-of-Azo-Dye.pdf
[7] Jain R and Sikarwar S 2008 *J. Hazard. Mater.* 152 942–8
[8] Kuhad R C, Sood N, Tripathi K K, Singh A and Ward O P 2004 *Advances in Applied Microbiology* vol 56 (Elsevier) pp 185–213
[9] Diwaniyan S, Kharb D, Raghukumar C and Kuhad R C 2010 *Water. Air. Soil Pollut.* 210 409–19
[10] Sitarz A K, Mikkelsen J D, Højrup P and Meyer A S 2013 *Enzyme Microb. Technol.* 53 378–85
[11] Gaspara F, Brar S K, Tyagi R D, Verma M and Surampalli R Y 2010 *Biochem. Eng. J.* 49 388–94
[12] Yang Q, Liu Z and Yang J 2009 *J. Water Resour. Prot.* 01 286–9
[13] Qianling Zhou, Wei Yang, Jun Fang Lin and Li Qiong Guo 2015 *Eur. J. Med. Plants* 6 17–25
[14] Pooja Kapoor and B.M. Sharma 2014 *Int. J. Sci. Environ. Technol.* 3 621–31
[15] Selvakumar S, Manivasagan R and Chinnappan K 2013 *Biotech* 3 71–9
[16] Ashger M, Nooreen S and Bhatti H N 2010 *Water Environ. Res. Res. Publ. Water Environ. Fed.* 82 357–61
[17] Fazli M M, A.R. Mesdaghinia, K. Naddafi, S. Nasseri, M. Yusnesian, M. Mazaheri Assadi, S. Rezaie and M. Yusnesian 2010 *Iran J Env. Health Sci Eng* 7 35–42
[18] Lingan Rajendran, Ramjegathesh Rajendran, Ramu Senthil, Venkatesan Shanthiyaa, Thiruvengadam Raguchander and Ramasamy Samiyappan 2014 *Wudpecker J. Agric. Res.* 3 130–5
[19] Fu Y and Viraraghavan T 2001 *Bioresour. Technol.* 79 251–62
[20] Murugesan K, Nam I-H, Kim Y M and Chang Y-S 2007 *Enzyme Microb. Technol.* 40 1662–72
[21] Revankar M and Lele S 2007 *Bioresour. Technol.* 98 775–80
[22] Shen, H.-P., D.-G. Mou, K.-K. Lim, P. Feng and C.-H. Chen Microbial decolorization of wastewater
[23] Ertugay N and Acar F N 2017 *Arab. J. Chem.* 10 S1158–63
[24] Zill-e-Huma Aftab and Shakil Ahmad 2015 *Pak J Phytopathol* 27 95103
[25] Singh H 2006 *Mycoremediation* (Hoboken, NJ, USA: John Wiley & Sons, Inc.)
[26] Mou D-G, Lim K K and Shen H P 1991 *Biotechnol. Adv.* 9 613–22