The decolorization effect by *Aspergillus* sp. 3 on Goldfish opercular beats

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Abstract

Batik effluent had high toxicity to aquatic organisms. Dye decolorization is a process used to reduce color density. Fungi which used for decolorization was *Aspergillus* sp 3. This study aimed to investigate the ability of fungi on decolorization of 3 kinds of batik effluents (Indigosol Green dye, Indigosol Purple dye, and Naphtol Black dye) and to investigate the ability of fungi on reducing TDS level. The study was also investigated the initial toxicity of batik effluents to Goldfish (*Cyprinus carpio*). Decolorization was measured by spectrophotometry, the pH value was measured by pH meter, and the TDS value was measured by TDS meter. In addition, the decolorized batik effluent was tested for toxic effect on *C. carpio* by total opercular beats. Decolorization assay showed that *Aspergillus* sp. 3 had the ability to decolorized only 2 kinds of batik effluents. The decolorization percentage of Indigosol Purple was 60.015% and Naphtol Black was 56.679%. The pH after treatment decreased from 8.5–9 to 5.3–6. The range of pH value of Indigosol Green, Indigosol Purple, and Naphtol Black 5.3–6. Besides that, *Aspergillus* sp. 3 also had the ability to reduce the TDS level in the effluent. The TDS level on Indigosol Green, Indigosol Purple, and Naphtol Black as 4,965%, 25,307%, and 15,129%, respectively. Initial toxicity assay of effluent to *C. carpio* showed that there was a difference of total opercular beats, which exposed by decolorized and before decolorized batik effluent. The total opercular beats of *C. carpio* on decolorized batik effluents showed high value than before decolorized batik effluents. It can be concluded that *Aspergillus* sp. 3 had the ability to decolorize and decreasing the initial toxicity of Indigosol Purple batik effluents on *C. carpio*.

Keywords: *Aspergillus* sp 3, *Cyprinus carpio*, decolorization, opercular beats, TDS

Introduction

Batik effluent is one of the environmental problems caused by their toxicity content on textile effluent. According to Gola et al. (2015), the textile industry is also one of the most significant contributors to this toxic effluent generation, which releases high concentrations of dyes, mordants, and other auxiliaries to the environment. The examples of textile dyes that produce toxic effluent are Indigosol Green, Indigosol Purple, and Naphtol Black. The batik effluent can be decolorized by biological decolorization. According to Ali et al. (2009), the domain of biological remediation has been gaining a great deal of attention during the last
couple of decades for its cost-effective and eco-friendly evolving nature. According to Karim et al. (2016), biological processes are advantageous as they convert organic compounds to non-toxic products. Biological methods as an eco-friendly alternative for remediation for heavy metal (Mishra & Malik 2013) and dyes pollutants (Kaushik & Malik 2009).

The example of a biological agent for decolorization is fungi. According to Kaushik & Malik (2009), fungi have been proven to be the most effective organism for textile effluent treatment and decolorization. Fungi have more advantages compared to single-cell organisms because their mycelia can dissolve insoluble substrates by producing extracellular enzymes. Fungi have greater physical and enzymatic contact with the environment because of their ratio to the cell surface. By far, the fungal decolorization degradation of dyes has been reported either through biosorption (Fu & Viraraghavan 2000) or enzymatic mineralization (lignin peroxidase, manganese peroxidase, manganese independent peroxidase, and laccases) (Ferreira et al. 2000; Wesenberg et al. 2003; Wong & Yu 1999; Zheng et al. 1999). Fungi offer an efficient system due to large surface area and easy solid-liquid separation (Mishra & Malik 2013). Fungi also possess multiple mechanisms for degradation of organic and inorganic contaminants (Awasthi et al. 2014). The example of fungi which commonly used is Aspergillus sp. Hefnawy et al. (2017) reported the fungi decolorization of dye could be achieved by treating with Aspergillus flavus and Aspergillus niger. Dewi et al. (2018a, 2019a) reported Aspergillus sp. 3 could decolorize batik effluent at 24 h, and its degradation products did not cause any toxicity. It was very effective in the removal of BOD, COD, TDS, TSS, EC, and reduce chromium, sulfide, ammonia, phenol, and fat from batik effluent (Dewi et al. 2018b, 2019b).

The problems of this research can be formulated how the ability of Aspergillus sp. 3 to decolorized the batik effluents and reducing the TDS level. The purposes of this research were consisted of investigating the ability of Aspergillus sp. 3 on batik effluents decolorization and reducing the TDS level.

Materials and Methods

Fungal isolate was used in this research was isolate of Aspergillus sp. 3, isolated from batik effluent from previous studies (Dewi et al. 2018b). Batik dye effluents used in this study were Indigosol Green, Indigosol Purple, and Naphtol Black. The batik effluents used in this study were diluted 10 times. The making of it was done by adding batik effluents as much as 40 mL into 360 mL of distilled water. So that the final volume of each dilution of 400 mL is obtained

Inoculant preparation

The Aspergillus sp. 3 was grown on Potato Dextrose Agar (PDA) medium (extract of 200 g potato, 15 g agar, 20 g dextrose in 1000 mL distilled water) in Petri dishes which were previously sterilized by autoclaving for 121 °C and 2 atm, at room temperature for 5 days. The five culture mycelium plugs (Ø 5 mm) were inoculated into 100 mL Potato Dextrose Broth (PDB) medium in 250 mL Erlenmeyer flasks aseptically then incubated for 3×24 hours on a 75 rpm shaker.

Decolorization assay

The fungal pellets isolate of Aspergillus sp. 3 formed after 3×24 h incubation time, then aseptically separated from its PDB medium. The separated pellets were added with all three types of batik waste of 100 mL aseptically, then incubated using shaker by 75 rpm at room temperature for 3×24 hours. Pellet mycelium Aspergillus sp. 3 formed then filtered using filter paper with a pressurized filter. The supernatants were analyzed for decolorization assay, pH measurement, TDS measurement, and toxicity assays. The decolorization was
analyzed by UV-vis spectrophotometry. The percentage of decolorization was analyzed by the formula:

\[
\text{% Decolorization} = \frac{\text{first absorbance} - \text{last absorbance}}{\text{first absorbance}} \times 100\%
\]

**pH measurement**
The pH of effluents was measured before and after decolorization using pHmeter.

**TDS measurement**
Batik effluents before and after decolorization were measured using a digital TDS meter. The percentage of TDS reduction is measured using the formula:

\[
\text{% TDS level} = \frac{\text{first TDS} - \text{last TDS}}{\text{first TDS}} \times 100\%
\]

**Toxicity assay**
The toxicity assay of effluent before and after treatment was conducted in aquarium volume 10 l. The *C. carpio* was first acclimated with water. The size of *C. carpio* was 3–4 cm. Then, the fish put into the aquarium containing the effluent before and after treatment with *Aspergillus* sp. 3 and counted the movement of the fish operculum in the first 5 minutes, second and third by repeating 3 times.

**Results**

**Batik effluents decolorization**
The decolorization was expressed by percentage decolorization (Fig. 1). The best decolorization of 3 kinds of batik effluents was evident with *Aspergillus* sp.3 on Indigosol Purple (60.015%) at 3x24 h incubation time followed by Naphtol Black (56.679%). Besides that, *Aspergillus* sp. 3 had a poor decolorization activity on Indigosol Green (-69.03%). This happens probably because Indigosol Green had a more complex structure than other dyes. Shaoxing Biying Textile Technology (2016) stated that the Molecular Formula of Indigosol Green is C_{36}H_{20}Na_{2}O_{10}S_{2} and C_{34}H_{14}Cl_{2}Na_{2}O_{8}S_{2} for Indigosol Purple.

![Figure 1](image-url)
Besides that, the color changed of concentrated batik effluent to less dense to clear yellow were indicated that *Aspergillus* sp. 3 had the ability to decolorize batik effluents. Color changed of batik effluent from concentrated to clear yellow is suspected because the complex compounds in batik dyes have broken down into simpler compounds. The color changed of batik effluent before and after the decolorization process is presented in Fig. 2.

The result of the TDS level showed that *Aspergillus* sp. 3 had the ability to reduce the TDS of batik effluents Indigosol Green, Indigosol Purple, and Naphthol Black at 10 times dilution, but it was not optimal. This is indicated by the small average value of the percentage reduction in TDS obtained (Fig. 3). This is presumably because the batik effluent used contained a lot of organic and inorganic compounds. Therefore it was difficult to degrade.

![Figure 2](image.png)

**Figure 2.** Decolorization of Batik effluent by *Aspergillus* sp. 3 at 3x24 hour incubation time. (A) Indigosol Green, (B) Indigosol Purple, (C) Naphthol Black.
(1) Control of Indigosol Green batik effluent by 10x dilution, (2) Indigosol Green batik effluent by 10x dilution after decolorization, (3) Control of Indigosol Purple batik effluent by 10x dilution, (4) Indigosol Purple batik effluent by 10x dilution after decolorization, (5) Control of Naphthol Black batik effluent by 10x dilution, (6) Naphthol Black batik effluent by 10x dilution after decolorization.

![Figure 3](image.png)

**Figure 3.** Histogram of decreasing of TDS value on batik effluent by *Aspergillus* sp at 3x24 hour incubation time

The result showed that the pH of Indigosol Green, Indigosol purple, and Naphthol Black batik effluent before the decolorization process were 9, 8.5, and 8.5. The pH of batik effluent before treatment tends to be alkaline. Then, the pH value of batik effluent from Indigosol Green, Indigosol Purple, and Naphthol Black after decolorization process were 7.8–8.2; 4.2–4.3; and 5.4–5.8 at 5 times dilution. The pH value of batik effluent from Indigosol Green, Indigosol Purple, and Naphthol Black after decolorization process were 5.8–6.1; 5–5.3; and 5.5–6 at 10 times dilution. These results indicate a decrease in pH due to the decomposition of organic and inorganic compounds that produce acidic compounds (Table 1).
Table 1. pH value of batik effluents decolorization by Aspergillus sp. 3.

| Treatments         | Indigosol Green | Indigosol Purple | Naphtol Black |
|--------------------|-----------------|------------------|---------------|
| Before decolorization | 9               | 8.5              | 8.5           |
| After decolorization     | 5.8 – 6.1       | 5 – 5.3          | 5.5 – 6      |

Besides that, the toxicity was assayed on C. carpio by operculum movement observation which showed in Table 2. Based on the table showed that the movement of the C. carpio operculum in degraded batik effluent is higher compared to batik effluent that has not been degraded. In fact, in the second five minutes the C. carpio is dead whereas the C. carpio can survive up to the third five minutes in the degraded batik effluent.

Table 2. Opercular beats of C. carpio as toxicity assay

| Treatment | Replication | Total of Operculum Motion |
|-----------|-------------|----------------------------|
|           |             | First 5 min | Second 5 min | Third 5 min |
| Water     | 1           | 700         | 490          | 762          |
|           | 2           | 650         | 756          | 800          |
|           | 3           | 500         | 930          | 910          |
| Effluent  | 1           | 81          | 0            | 0            |
|           | 2           | 38          | 0            | 0            |
|           | 3           | 59          | 0            | 0            |
| Neutral Effluent | 1   | 323         | 42           | 0            |
|           | 2           | 269         | 355          | 399          |
|           | 3           | 170         | 32           | 0            |

Discussion

Application of Aspergillus sp. 3 had been proved as a superior on decolorization of 2 kind batik effluent. Aspergillus sp. 3 had the ability to decolorized Indigosol Purple and Naphtol Black. Ali et al. (2009) reported that A. niger SA1, a brown-rot fungus has similar capabilities like white-rot in the efficient and comprehensive degradation of pollutants like dyes. Asses et al. (2018) reported that CR was successfully decolorized and biodegraded by A. niger. High decolorization efficiency (97%) was obtained after six days of culture.

The decolorization process by the fungus was analyzed by UV-spectrophotometry. Decolorization of dye was followed by changes in the absorbance value. There was a decreasing in absorbance value caused by reducing the color intensity. The decolorization process by fungus causes the clear less color of batik effluents. The color of batik effluents had been changed from concentrated color into clearness color, which means that the complex compound on batik effluent had been broken into simply compound. The reducing color intensity related to decolorization percentage. Dewi & Lestari (2010) reported that the lower concentration cause, the higher of decolorization percentage. Loss of color is an obvious indicator of dye removal from solutions but the assessment of products or metabolites; their chemical nature and further evaluation of toxicity become essential (Khandare & Govindwar 2015). Dewi et al. (2018a) reported that Aspergillus sp. 3 was the best isolate for decolorizing the effluent. Degradation by Aspergillus sp. 3 as the most effective fungi is seen more clearly than in the other species. The mycelium, which caused degradation using the Aspergillus sp. 3 fungus culture, was seen to be absorbing more dye than the other cultures.
Color removal from effluent containing dyes appeared to be due to mechanisms of the fungal biomass, primarily biosorption/bioabsorption. After decolorization of dyes by the biomass mechanism and then subsequent formation, dyes were metabolically degraded by fungal strains into their derivative products (Ali et al. 2009). The decrease in color intensity by the activity of fungi isolates was caused by adsorption and enzymatic activity (Schiegel & Schmit, 1994). Biosorption can occur in biomass; amino, carboxyl, thiol, lipid, and phosphate groups that exist in the fungal cell walls are responsible for binding the dye molecules in the biosorption process (Kyzas et al. 2013). Dye decolorization caused by a ligninolytic enzyme (laccase, manganese peroxidase, and lignin peroxidase). Lac enzyme activities of Pleurotus ostreatus is 200.43 U/l, MnP is 9.714 U/l, and LiP is 12938.60 (Dewi et al. 2019c).

Textile effluent is characterized by color; varies in hydraulic flow rate; and has high pH, temperature, biological oxygen demand (BOD), chemical oxygen demand (COD), total dissolved solids (TDS), and total suspended solids (TSS) (Banat et al. 1996; Ghoreishi & Haghighi 2003). Aspergillus sp. 3 was showed the reducing of the TDS level in the batik effluents after decolorization. This is indicated by the small average value of the percentage reduction in TDS obtained. According to Dewi et al. (2018b) reported Aspergillus sp. 3 was the best isolate for reducing TDS. Kanagaraj & Mandal (2011) reported that the reduction of TOC, TDS, and TSS of the dye had been initially treated with A. niger.

High TDS value reduces light penetration into the water and ultimately decreases the photosynthesis in aquatic flora. This causes a reduction in the dissolved oxygen level of water bodies, which results in extremely low purification of effluent by microorganisms (Namdhari et al. 2012). High TDS can disturb oxygen transfer and interfere with the biological metabolism of pollutants (Pophali et al. 2003).

Decolorization of batik effluent also affected by pH. The growth of fungi as a biological decolorization agent was affected by pH. Zafar et al. (2008) reported that solution pH is among the most significant parameters controlling the sorption. Ncibi et al. (2007) stated this occurs because of its impact on both the biosorbent surface binding site and the dye molecules that are ionized in the water. Aksu (2005) reported dye adsorption could change with the change of solution pH. Dye biosorption (positively charged cation) is influenced by the surface level of the adsorbent (negatively charged), which is determined by the pH of the solution. Sites with a negative charge on the adsorbent At low pH have low availability because the number of H+ ions compete with the cations of the dye for the adsorption site (Hameed et al. 2008) because, in the system of adsorption, there is a net positive charge due to the H2O+ presence. It can be interpreted that H+ ions in such a system compete with cations of the dye, produces a protonated active site by ruling out the dye cation that binds to the surface of the adsorbent (Wang et al. 2008). The amount of negative charged is low at a lower pH, which is not preferably a positively charged cation of dye for adsorption due to electrostatic repulsion (Nainasivayam et al. 2004). Negatively charged surfaces become more available when the pH of the dye solution increased due to biosorbents that have different functional groups undergo deprotonation (Saaed et al. 2009).

Besides that, the toxicity assays also testes on the opercular beats of C. carpio. As the degree of toxicity of water increases, it produces high-stress conditions on fishes, the degree of toxicity produced by the poisonous substance is dose-dependent upon environmental conditions such as temperature, pH of water, oxygen content and presence of residue molecules (Capkin et al., 2006; Singh & Mishra 2009). An increase in temperature, rise in metabolic rate of fish and hence its oxygen demand, but it decreases oxygen solubility in water. This counter tendency may result in depletion of oxygen to a lethal level (Tantarpale et al. 2012).
Conflict of Interest
The authors state no conflict of interest from this manuscript.

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Author Contributions
All authors have reviewed the final version of the manuscript and approved it for publication. RSD and KK designed the study, performed research, and collected the data; FAU for analysing the data and wrote the paper. RSD is the main contributor to this manuscript.

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