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Chromium Removal of Batik Wastewater using *Aspergillus* sp. and *Penicillium* sp.

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Abstract. Indigosol red is one of batik dye used in Indonesia's batik industry. This industrial activity produces wastewater. Based on wastewater quality standards, it is explained that chromium (Cr) is one of the parameters in the standard of textile waste quality. Several selected fungi are potential to decolorize and remove heavy metal chromium from batik wastewater. Some fungal strains, such as *Aspergillus* sp. and *Penicillium* sp., isolated from batik wastewater, have been elaborated in this study, with *Phanerochaeta chrysoporium* as comparative isolate. This research reports the value of Cr in several batik wastewater in Banyumas Regency, and also discussed the role of these fungal removals of heavy metals from batik wastewater. The effect of the incubation period and the amount of biomass on the removal were also studied. Analysis of Cr content from wastewater sample ranged <0.12-1.01 mg/L. The results showed that the fungi were able to remove the Cr of batik wastewater. The *range* of values of Cr reduction varies from 83 to 86%. The most effective fungi to decolorize and remove chromium was *Penicillium* sp. in 5 day incubation time, with the largest biomass of 0.64 g. *Penicillium* sp. can be used as chromium removal.

1. Introduction

Batik is one of Indonesia's cultural richness that has high artistic value and as the identity of Indonesia that has been recognized by the world. Therefore, the development of the batik industry in Indonesia increases rapidly. West Sokaraja, Banyumas Regency has been potential batik production developed both in the industrial scale and home industry. However, the rapid growth of the batik industry in Indonesia raises a new problem of environmental pollution caused by batik wastewater from the dyeing process that is difficult to remove. According to previous research [1], batik wastewater contains organic materials, odor, and coloring. The source of pollution comes from the process of dying.

Chromium (Cr) is one of the parameters in the textile waste quality standard. The purpose of batik wastewater treatment is to remove the heavy metal Cr containing in batik wastewater. Thus, the waste is less harmfull to dispose of in the environment. Heavy metals such as Cr come from some batik dye, especially from the final washing process of batik [2]. If the waste material containing heavy metals is not treated correctly, it can pollute the environment. Chromium has accumulation characteristics, in the case of the Cr-untreated environment. It may accumulate and harm living beings.

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The dyes used in the batik industry are usually synthetic ones, such as red indigosol, which is included vat dyes. Vat dyes are one of the most popular dyes used on textiles, particularly on cotton textile materials, but it is naturally insoluble in water. In a conventional vat dyeing process, the dye is dissolved using sodium hydrosulfite (hydrose), a powerful reducing agent, followed by solubilizing with sodium hydroxide. This process creates high pollution problem [3]. Indigosol Red is chosen based on Cr contained in the batik dye wastewater, where, based on the standard quality, the permissible limit is 1 mg/L, while Indigosol Red dye wastewater has Cr content of 0.91 mg/L, higher than other dyes. However, Cr is quickly accumulated in an environment, and if it is not overcome, it affects the environment, especially the waters [2]. Chromium is needed as an essential nutrient and is needed in small amounts by some organisms, and high levels of chromium can be toxic and mutagenic [4]. Chromium is toxic and carcinogenic compounds. It causes health problems that are difficult to recover in a short time. Serious diseases include liver, kidney, dermatitis. Batik wastewater directly discharged into the environment without first processing will reduce the penetration of light that affects the activity of photosynthesis of aquatic organisms. The thin layer of dye in the waters also decreases the dissolved oxygen in the water [5].

Various ways, such as physical and chemical-based remediation, have been done to overcome the harmful effects of chromium [6]. The new method to remove harmful metals from the environment is a simple process known as bioabsorption. The selected microorganisms are introduced, grown, then contacted with heavy metal contaminated substrate [7]. A biological process is one of the favorable alternative methods in treating batik wastewater because it is less costly, works effectively, environmentally friendly, and generating non-toxic wastewater. Microorganisms are capable of converting or reducing pollutants into water, carbon dioxide, and various inorganic natural salts [8].

Fungi are selected as the color removing agents of the wastewater because they have a major component of cell wall constituents used for bioabsorption, the chitin. It is a highly effective N-acetyl-D-glucosamine polymer functioning as a bio-absorbant [9].
Bio-absorption can be interpreted as the ability of biological material to accumulate heavy metals from contaminated water through metabolic media or chemical pathways [10]. Based on previous research, the white-rot fungi *Phanerochaeta chrysoporium* is an ideal model for bioremediation to degrade toxic or insoluble materials. The fungus is very effective in degrading a wide range of organic molecules due to their release of extracellular lignin modifying enzymes [11].

In recent years, many studies have focused on some fungi, which can degrade and absorb dyes. Recently, there is a growing interest in studying other fungi, especially *Aspergillus* sp., for the decolorization and degradation of many different dyes. Its biomass can be used as an absorption agent and serve as a part of a technical solution in water pollution control [12]. *Penicillium* sp. also demonstrates its ability to degrade different xenobiotic compounds with low co-substrate requirements and could be potentially interesting for the development of economically feasible processes for pollutant transformation [13]. Likewise, there has been no published report of chromium removal in dye waste by *Aspergillus* sp. and *Penicillium* sp. Chromium removal is effective when using a priority scale (incubation period) to determine the optimum work on each fungus in removing chromium of batik dye [14]. The research objectives were to find out the value of Cr in several batik wastewater in Banyumas Regency, and to demonstrate the effectiveness of the fungi in Cr removal.

2. Methods

2.1. Chemicals

The culture media of fungi, including Potato Dextrose Agar (PDA) dan Potato Dextrose Broth (PDB), were made with a composition of potato extract, 15 g agar, 20 g dextrose, and 1000 mL distilled water. The potato extract was prepared as follows. Sliced potato (200 g) was half cooked. The difference between the type of media was that PDB did not use agar. The media were sterilized with autoclave 121°C, 2 atm for 15 minutes.

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2.2. Sample Collection

The wastewater was taken from the home industry in Sokaraja Kulon, Sokaraja, Banyumas Regency, Central Java. It was the wastewater from common dyes used in the batik industry.

2.3. Preparation of fungi

This research used three fungal isolates, i.e., *Aspergillus* sp. and *Penicillium* sp., recovered from batik waste contaminated effluent of Banyumas Batik Home Industry [15], and *Phanerochaeta chrysosporium* InaCc F206 (from Indonesia Culture Collection Research Center for LIPI, as positive control). The fungal isolates were rejuvenated into Petri dishes containing the PDA, then incubated for seven days at room temperature.

2.4. Analysis of batik wastewater Cr Content

Before the treatment was applied, the metal content of wastewater samples is measured. Samples were analyzed for chromium heavy metal content using Atomic Absorption Spectrophotometer (AAS).

AAS is a technique for measuring quantities of chemical elements present in environmental samples by measuring the absorbed radiation by the chemical element of interest. The data are obtained from reading the spectra produced when the sample is excited by radiation.

2.5. Cr removal assay in batik wastewater

The total of 27 Erlenmeyer flask contains PDB medium as much as 100 mL each. Fungal isolates (*Aspergillus* sp., *Penicillium* sp., *P. crysoporium*) containing five plugs (5 mm) in each treatment, then were incubated for three days at room temperature on a horizontal shaker. After three days, the PDB medium was drained, and 100 mL of Indigosol Red batik dye was pour. They were incubated at room temperature for three, five, and seven days.

Analysis of chromium content used AAS with a wavelength of 357.9 nm, a lamp current of 10 mA. The percentage decrease in Cr was expressed by:

$$\textbf{Decreased levels (\%)} = \frac{\textbf{initial concentration} - \textbf{final concentration}}{\textbf{initial concentration}} \textbf{ x 100\%}$$

2.6. Microscopic observation of mycelia before and after treatment

Microscopic observations were done to compare the mycelial surface before and after treatments. The mycelia were placed on an object-glass and observed to a magnification of 45x.

2.7. Measurement of pH

The Indigosol Red batik dye wastewater pH was measured using a universal pH by dipping the pH universal in the batik dye wastewater before and after treatments.

2.8. Growth assessment of fungi

Measurement of dry weight was done by measuring the fungal weight on the filter paper. The treated wastewater was filtered using filter paper; thus, the fungi were traped on the paper. They were dried in an oven (70^{o}C) and periodically weighted until they reach a constant value. The final result of mycelial dry weight was expressed as follows.

$$\text{dry weight of mycelia} = \text{final weight} - \text{weight of filter paper}$$

3. Results

3.1. Cr Content of batik wastewater

The results showed that heavy metal, especially Cr, in several samples of batik waste ranged from <0.12 to 0.101 mg/L. The detailed results are presented in Table 1. The content of Cr was more commonly found in Indigosol Red batik dye wastewater.

Table 1. The concentration of Cr from several wastewater samples.

Type of wastewater	Cr (mg/L)	
	Total	Average
Lorodan	0.43 0.39	0.41
Naphtol Soga Brown	0.78 0.83	0.81
Naphtol Red	<0.12 <0.12	<0.12
Naphtol Black	<0.12 <0.12	<0.12
Salt Naphtol Soga Brown	0.77 0.72	0.75
Salt Naphtol Red	<0.12 <0.12	<0.12
Indigosol Red	1.0 1.02	1.01
Indigosol Green	<0.12 <0.12	<0.12
Indigosol Yellow	<0.12 <0.12	<0.12

Indigosol Red wastewater had the highest Cr concentration (1.01 mg/L) followed by wastewater of Naphtol Soga Brown (0.81 mg / L), Salt Naphtol Soga Brown (0.75 mg / L), and Lorodan (0.41 mg / L). The lowest Cr content (<0.12 mg/L) was observed in Naphtol Red, Naphtol Black, Salt Naphtol Red, Indigosol Green Indigosol Yellow. Then Indigo Red wastewater was selected in the Cr removal treatment because the concentration was the highest compared to others.

3.2. Cr removal assay in batik wastewater

The decrease of chromium content in red indigosol batik dye wastewater (initial concentration of 1.01 mg/L) is presented in Figure 1.

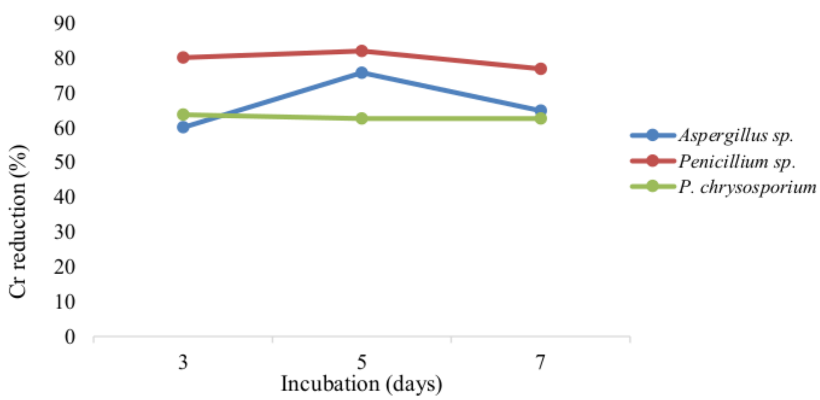

Figure 1. The percent decrease of Cr in Indigosol Red batik dye wastewater using *Aspergillus* sp., *Penicillium* sp., and *Phanerochaeta chrysoporium.*

The values of *Aspergillus* sp. in reducing the Cr content of the waste were 60.07% (day three), 75.82% (day five), and 64.83% (day seven). As for the *Penicillium* sp., it was 80.21% (day three), 82.05% (day five), and 76.92% (day seven). P. *chrysoporyum* reached 63.73% (day three), 62.63% (day five), and 62.63% (day seven).

3.3. Microscopic observation of mycelia

The visible mycelia of *Penicillium* sp before and after treatment was presented in Figure 1. Microscopic observations confirmed the adsorption and biosorption capacities in Indigo batik wastewater.

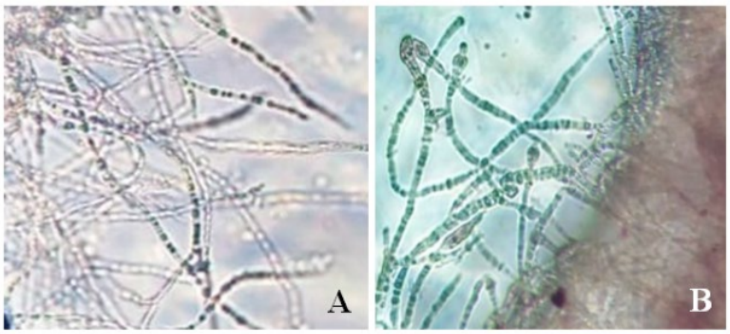

Figure 2. Microscopic observation of *Penicillium* sp. mycelia at day five incubation period (A) before and (B) after treatment

The microscopic observation (Figure 2) confirms the color presence on the fungus surface and inside the cell indicated Cr in these parts.

3.4. The pH Measurement

Another factor affecting biosorption was pH. Table 2 shows the effect of pH on decreasing Cr levels in red dye batik dye wastewater.

Table 2. pH Value of Red Indigosol Batik Dye Wastewater at Early and Last Treatments

Treatments	pH (Early)			pH (Last)		
	Replication					
	1	2	3	1	2	3
Aspergillus sp. within 3 days	8	8	8	7	7	7
Penicillium sp. within 3 days	8	8	8	7	7	7
P. chrysoporium within 3 days	8	8	8	7	7	7
Aspergillus sp. within 5 days	8	8	8	7	7	7
Penicillium sp.within 5 days	8	8	8	7	7	7
P. chrysoporium within 5 days	8	8	8	7	7	7
Aspergillus sp. within 7 days	8	8	8	6	6	6
Penicillium sp. within 7days	8	8	8	6	6	6
P. chrysosporium within 7 days	8	8	8	6	6	6

3.5. Growth assessment of fungi

Table 1 shows the effect of mycelial dry weight on decreasing Cr levels. Mycelium Dry Weight average of *Aspergillus* sp. in 3, 5, and 7 days reached 0.86, 0.79, 0.61 mg. For the *Penicillium* sp., it was 0.9, 0.99, 0.93, and *P. chrysosporium* was 0.46, 0.54, 0.48.

Table 3. Mycelium Dry Weight of *Aspergillus* sp., *Penicillium* sp. and *P. chrysoporium* within 3, 5, 7 days on decreasing Cr.

Treatments	Dry Weight of Mycelium (g)		
	Replication		
	1	2	3
Aspergillus sp. within 3 days	1.13	0.51	0.95
Penicillium sp. within 3 days	0.89	0.92	0.58
Phanerochaeta chrysoporium within 3 days	0.54	0.66	0.63
Aspergillus sp. within 5 days	1.11	0.77	0.82
Penicillium sp.within 5 days	0.82	0.86	1.28
Phanerochaeta chrysoporium within 5 days	0.65	1.05	1.08
Aspergillus sp. within 7 days	0.47	0.45	0.45
Penicillium sp. within 7days	0.53	0.65	0.44
Phanerochaeta chrysosporium within 7 days	0.48	0.52	0.44

4. Discussion

4.1. Cr Content of batik wastewater

The analysis of Cr values on several samples of wastewater from home batik industries in the Banyumas Regency is shown in Table 1. The Cr from these wastewater samples ranged from <0.12 to 1.01 mg/L. This result was higher than the values that have been reported [16], which reached 0.0546; 0.0488; 0.0464 mg/l Cr concentration of batik wastewater in groups 1, 2 and 3, but lower than in hexavalent chromium in batik liquid waste (4.6 mg/l) [17].

Cr value in the Indigosol Red wastewater was the highest (1.01 mg/L) compared to others. This value far exceeds the quality standard limits set by the Regulation of the Minister of Environment Number 03 of 2010 [18] regarding Wastewater Quality Standards for Industrial Estates at 0.5 mg/l. The next was Naphtol Soga Brown and Salt Naphtol Soga Brown that demonstrated values of 0.81, 0.75, and 0.41 mg/L, respectively.

Visually, Indigosol Red batik wastewater with the highest Cr value is red. This waste must be treated so that the concentration of chromium is below the stipulated quality standard. Furthermore, this wastewater will be used for the next research because its Cr concentration is considered the most dangerous.

4.2. Cr removal assay in batik wastewater

The value of *Aspergillus* sp. in reducing Cr was 60.07%; 75.82%; 64.83%, *Penicillium* sp. 80.21%; 82.05%; 76.92%; and *P. chrysosporyum* was 63.73%; 62.63%; 62.63% at day three, five, and seven, respectively (Figure 1). *P. chrysoporium* had lower values compared to *Aspergillus* sp. and *Penicillium* sp. The fungus is known as a white-rot fungus capable of degrading and remediating [19]. In our study, however, *Aspergillus* sp. and *Penicillium* sp. show a greater ability in decreasing chromium, as shown in a high percentage.

The percentage of chromium decrease in Indigosol Red batik dye was analyzed using HSD (Honestly Significant Difference). *Penicillium* sp. was the most effective fungus in reducing Cr content, which was not significantly different from *Aspergillus* sp. during day five incubation. Those results indicated that *Penicillium* sp. and *Aspergillus* sp. were equally useful for reducing Cr.
The best ability of *Penicillium* sp. and *Aspergillus* sp. to absorb Cr was on day 5. The results agree to published research reported that *Aspergillus* sp. N2 and *Penicillium* sp. N3 decreases chromium concentration with an initial concentration of 50 ppm to an almost undetectable level within 120 hours of incubation [20].

This ability is influenced by the maximum contact time for each isolate. Decreased chromium levels occur due to the mechanism of biosorption, which is influenced by growth stages (mycelia or spores) based on incubation time [21].

4.3. Microscopic observation of mycelia

Figure 2 represents the difference between before and after treatment using *Penicillium* sp. mycelia for five day incubation period. It indicates that Cr and dye absorption of batik wastewater occurs on the outer and inner mycelial surface. Our result was supported by a report claiming that heavy metals are absorbed along with the decolorization process [22]. The dye attachment outside and in the cell suggests decolorization and decreases the level of heavy metals. Fungal mycelium, enzymatically and non-enzymatically, is known to absorb dyes of batik waste. Fungal mycelia are hydrophobic, while dyes are hydrophilic, resulting in hydrophobic-hydrophilic interactions between mycelium fungi and dyes. The initial mechanism is absorption, then enzymatic and non-enzymatic processes take place.

4.4. Measurement of pH

Cr affects wastewater pH, as demonstrated in Table 2. The data showed the effect of decreasing Cr levels in red indigosol batik dye wastewater on pH. The treatment of *Aspergillus* sp. and *Penicillium* sp. of all incubation periods showed a decrease in pH before and after treatment at a pH of 8 to 7. The treatment of *P.chrysoporium* showed a decrease in each treatment, at a pH of 8 to 6. There is a positive correlation between Cr reduction and pH, where Cr reduction occurs at pH> 8, then completely reduce the Cr [23]. However, since Cr reduction is enzyme-mediated, pH changes may affect the enzyme ionization rate and the protein conformation, and consequently may affect enzyme activity [20].

4.5. Biosorption Growth assessment of fungi

Table 1 shows the measurements of the mycelium dry weight. The data described that decreasing Cr levels also addressed the dry weight of mycelia. The results showed no difference between *Penicillium* sp. and *Aspergillus* sp. However, the highest value was in *Penicillium* sp., supported by the decreased level of Cr.

It was revealed that the dry weight of mycelia decreased Cr level. The highest percentage of Cr reduction was in *Penicillium* sp. at day five (82.05%) with the highest dry mycelium weight of 0.99 g [Tabel 1]. One of the influencing factors in bioabsorption is contact time. The optimum contact time shows the time used by biosorbents to adsorb the maximum amount of metal ions that can be bonded, the optimum contact time of biosorption of metal ions Cr by fungi biomass. The effect of the magnitude of the Cr reduction is based on the ability of mycelium biomass in absorbing Cr, and the influence of contact time affected [24]. On day five, *Penicillium* sp. showed the maximum time for fungi to Cr absorption. The biomass obtained was comparable with absorbed Cr value.

Also, another suggested factor promoting a decrease in Cr levels is nutrients. Chromium is one of the nutrients used by the fungus to carried out metabolism. The ability of *Aspergillus* sp. and *Penicillium* sp. as a biosorption agent are very potential for bioremediation [20]. The chromium removal abilities of *Aspergillus* sp. and *Penicillium* sp. were better than *P. chrysoporium*. In particular, *Penicillium* sp. was superior to the other strains because it has the capacity for efficient chromium reduction under acidic or base conditions. Mostly, other Cr reduction studies were carried out at neutral pH [25].

5. Conclusion

The concentration of Cr in several batik wastewater in the Banyumas Regency ranged <0.12-1.01 mg/L. Indigosol Red was the highest one (1.01 mg/L). Fungi were able to remove Cr in Indigosol Red batik. The most effective fungi and incubation period to removed Cr level in red indigosol batik dye wastewater are *Penicillium* sp. in day five incubation period.

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