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 found to be potential organisms to decolorize and heavy metal chromium removal from batik wastewater. Some fungal strains which were isolated from batik wastewater and comprised of *Aspergillus* sp., and *Penicillium* sp. have been elaborated in this study, with *Phanerochaeta chrysoporium* as comparative isolate. This research report the value of Cr in several batik wastewater in Banyumas Regency, and also discussed the role of these fungal removal of heavy metals from batik wastewater. The effect of incubation period and amount of biomass on the removal was 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 chromium. The fungi were able to reduce the Cr concentration of batik wastewater. The range of values varies from 83 to 86%. The most effective fungi to decolorize and remove chromium was *Penicillium* sp. in 5 days incubation time, *with the largest Biomass* of 0.64 g. *Penicillium* sp. can be used as chromium removal.

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1. Introduction

Batik is one of Indonesia's cultural richness that has high artistic value and as the identity of the Indonesia that has been recognized by the world. Therefore, the development of batik industry in Indonesia increasingly rapidly. West Sokaraja, Banyumas Regency has potential in batik production development both in industrial scale and home industry. However, the rapid growth of 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, odour and coloring. The source of pollution comes from the process of dyeing.

Chromium (Cr) is one of parameter in the textile waste quality standard. The purpose of batik wastewater treatment is to remove the heavy metal of Cr that contained in batik wastewater to be safe when disposed of into 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 properly, they can pollute the environment. Chromium has accumulation characteristic, in case of Cr-untreated environment, it may accumulate and harm for living beings.

The dyes used in the batik industry are usually synthetic one, 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. However, vat dye is originally insoluble in water. In conventional vat dyeing process, the vat dye is dissolved using sodium hydrosulphite (hydrose) which is a powerful reducing agent followed by solubilizing with sodium hydroxide. This process creates high pollution problem [3]. Indigosol Red was chosen based on Cr contained in the batik dye wastewater, where based on the standard quality the permissible limit was 1 mg/L, while Indigosol Red dye wastewater had Cr content of 0.91 mg/L, higher than other dyes. However, the characteristic of Cr which is easily accumulated in an environment where if it is not overcome it will accumulate and affect the environment, especially the waters [2]. Chromium is needed as an essential nutrient and is needed in small amounts by some organisms, but at high levels of chromium, it can be toxic and mutagenic [4]. Chromium is toxic and carcinogenic compounds. Causing health problems that are difficult to recover in a short time. Dangerous diseases include liver, kidney, dermatitis and many more. 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 decrease the dissolved oxygen in the water [5].

There are many ways that have been done to overcome the dangers of chromium. Physical and chemical based remediation has been developed and applied to overcome pollution [6]. The search of a new method to remove harmful metals from the environment is by bioabsorption. The bioabsorption process is a very simple method. The selective microorganisms are introduced, grown and then contacted with heavy metal contaminated substrate [7]. Biological process is one of the more favorable alternative methods in treating batik wastewater, because it requires low cost, more effective, environmentally friendly and the end result is not toxic. Microorganisms are capable of converting or reducing pollutants into water, carbon dioxide, and various inorganic natural salts [8].

Fungi is chosen as the agents capable to removing the color of the wastewater because the fungi has a major component of cell wall constituents that are often used for bioabsorption, namely chitin which is a highly effective N-acetyl-D-glucosamine polymer as bioabsorbant. Part of the fungi that serves as a heavy metal absorption is a fungal cell wall. In this section there is chitin [9].

Bioabsorption 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. *P. chrysoporium* is so effective to degrade a wide range of organic molecules due to their release of extra-cellular lignin modifying enzymes [11].

In recent years, a number of studies have focused on some fungi, which are able to biodegrade, and bioabsorp dyes. Recently, there is a growing interest in studying of other fungi especially *Aspergillus* sp. for the decolorization and degradation of many different dyes, because their 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 demonstrated their 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]. But, it has not been reported that *Aspergillus* sp. and *Penicillium* sp. which can removal chromium in dye waste. To remove heavy metal chromium will be effective using priority scale (incubation period) to determine the optimum working on each fungus to remove chromium in batik dye [14]. Based on the description above, the objective of this research were to report the value of Cr in several batik wastewater in Banyumas Regency, and to illustrate 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 composition : extract of potatoes, 15 g of agar, 20 g dextrose, and aquades 1000 mL.

Some aquades was taken to make potatoes extract**.** 200 g of potatoes that have been slice were boiled until half cooked. The difference between both mediums was that PDB did not use agar. Perform sterilization with autoclave 121°C with 2 atm for 15 minutes.

2.2. Sample Collection

The wastewater were taken from home industry in Sokaraja Kulon, Sokaraja, Banyumas Regency, Central Java. Batik wastewater that used were wastewater from dyes that are often used in the batik industry.

2.3. Preparation of fungi

This research used three isolates of fungi,i.e *Aspergillus* sp. and *Penicilium* sp. which was isolated on batik waste contaminated effluent from Banyumas Batik Home Industry [15], and *Phanerochaeta chrysosporium* InaCc F206 (from Indonesia Culture Collection Research Center for LIPI, as positive control). The fungus isolates were used rejuvenated into petri dishes containing the medium of PDA, then incubated for 7 days at room temperature.

2.4. Analysis of batik wastewater Cr Content

Before the treatment is carried out, a metal content is measured in the wastewater samples. 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. This is done by reading the spectra produced when the sample is excited by radiation.

2.5. Cr removal assay in batik waste water

The total of 27 erlenmeyers flask contain PDB medium as much as 100 mL each flask. Fungi isolates (*Aspergillus* sp., *Penicilium* sp., *P. crysoporium*) that contain five plugs (5 mm) in each treatments, then incubated each treatment for 3 days at room temperature on horizontal shaker. After 3 days, drain the PDB medium and pour 100 mL of Indigosol Red batik dye. Incubate at room temperature for 3 days, 5 days, and 7 days.

Analysis of chromium content using AAS with wavelength of 357.9 nm, a lamp current of 10 mA. The percentage decrease in Cr can be 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

Observation of mycelia with a microscope to see clearly and to compare the surface of mycelia before and after treatment, by placing mycelia before and after the treatment above the object glass, and observed under a microscope to a magnification of 45x.

2.7. Measurement of pH

The Indigosol Red batik dye wastewater pH will be measured using an 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 is done by measuring the weight of filter paper which used. Then filter first the treatment results using filter paper and put the fungi of the incubation results on the filter paper. Drying using oven with temperature 70^{o}C, and measuring the weight gradually until the weight of the mycellium shows a constant number. Then measure the weight of fungi and filter paper to get final result. Measurement the dry weight of mycelia can be express by:

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

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 <0.12-0.101 mg/L. The full results can be seen in Table 1. The content of Cr was more commonly found in Indigosol Red batik dye wastewater.

Table 1. Concentration of Cr from several wastewater samples.

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

Indigosol Red wastewater had the highest concentration among others (1.01 mg / L). Then sequentially followed by wastewater of Naphtol Soga Brown, Salt Naphtol Soga Brown, Lorodan (0.81, 0.75, 0.41 mg / L, respectfully) and last which had a concentration of <0.12 namely 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 other wastewater.

3.2. Cr removal assay in batik wastewater

The results of the percentage decrease of chromium content in red indigosol batik dye wastewater with an initial concentration of 1.01 mg/L, can be seen in Figure 1.

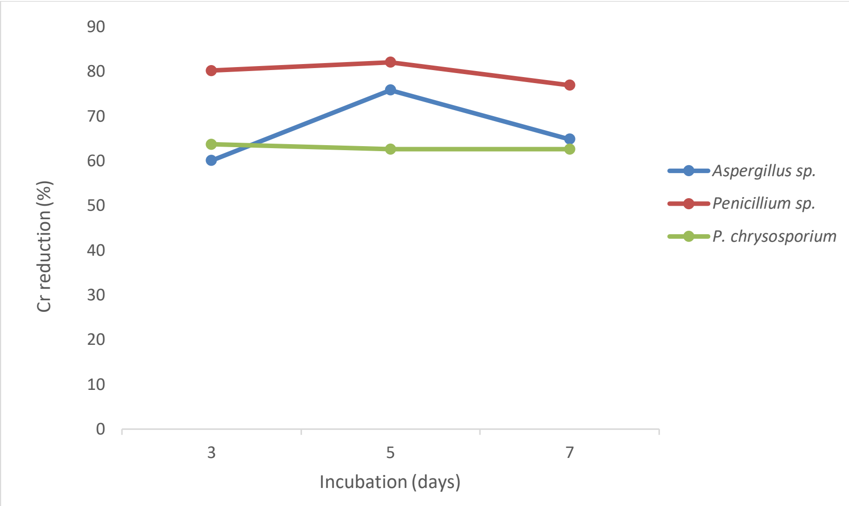

Figure 1. Percentage decreased of Cr in Indigosol Red batik dye wastewater using *Aspergillus* sp., *Penicillium* sp., and *Phanerochaeta chrysoporium.*

Based on Figure 1, respectively the value of *Aspergillus* sp. at 3^{rd}, 5^{th} and 7^{th} days were 60.07%; 75.82%; 64.83%. *Penicillium* sp. at 3^{rd}, 5^{th} and 7^{th} days were 80.21%; 82.05%; 76.92%. P. *chrysoporyum* at 3^{rd}, 5^{th} and 7^{th} days were 63.73%; 62.63%; 62.63%.

3.3. Microscopic observation of mycelia

Visible mycelia before and after treatment using the fungus *Penicillium* sp were presented in Figure 1. Microscopic observations confirmed the adsorption and biosorption capacities in Indigo batik wastewater.

Figure 2. Microscopic observation of *Penicillium* sp. mycellia at 5^{th} days incubation period (A) before treatment; (B) after treatment

Figure 2. confirms the presence of color on the surface and inside the cell. Cr contained in the dye. The appearance of color on the surface and inside the cell indicated Cr in these parts.

3.4. Measurement of pH

Other influences that affect biosorption was pH. Table 2 showed the effect of pH on decreasing Cr levels in red dye batik dye wastewater.

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

	pH (Early)			pH (Last)		
	Repeat					
Treatments	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 showed the effect of dry weight of mycelia on decreasing Cr levels. Mycelium Dry Weight average of *Aspergillus* sp. within 3,5,7 days were 0.86, 0.79, 0.61 mg, whereas in the *Penicillium* sp. were 0.9, 0.99, 0.93, and *P. chrysosporium* were 0.46, 0.54, 0.48, respectively.

Table 1. 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)		
	Repetition		
	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 results of the analysis of Cr values on several samples of wastewater from home batik industries in Banyumas Regency are shown in Table 1. Analysis data Cr from these wastewater sample ranged <0.12-1.01 mg / L. This result were higher than the research conducted by [16] that Cr concentration in batik wastewater in groups 1, 2 and 3 was 0.0546; 0.0488; 0.0464 mg / l, but lower than the [Natalia?] research that analyzing hexavalent chromium in batik liquid waste of 4.6 mg / l.

Cr value in the Indigosol Red wastewater was the highest concentration compared to other (1.01 mg/L). This value far exceeds the quality standard limits set by the Regulation of the Minister of Environment Number 03 of 2010 [17] concerning Wastewater Quality Standards for Industrial Estates at 0.5 mg/l. Following also waste Naphtol Soga Brown and Salt Naphtol Soga Brown that is equal to 0.81, 0.75 and 0.41 mg / L.

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

4.2. Cr removal assay in batik wastewater

Figure 1 showed that the value of *Aspergillus* sp. were 60.07%; 75.82%; 64.83%, *Penicillium* sp. 80.21%; 82.05%; 76.92%. *P. chrysosporyum* were 63.73%; 62.63%; 62.63% at 3rd, 5th and 7th days respctivelly. *P. chrysoporium* had a smaller yield compared to *Aspergillus* sp. and *Penicillium* sp. According to [18], *P. chrysosporium* is known as a white rot fungi that is capable of biodegradation and bioremediation, but in this case it can be seen the ability of *Aspergillus* sp. and *Penicillium* sp. shows a greater percentage decrease of chromium.

The percentage of chromium decrease in Indigosol Red batik dye analyzed using HSD (Honestly Significant Difference) (not mentioned). *Penicillium* sp. is the effective fungus to reducing the highest Cr content which was not significantly different from *Aspergillus* sp. 5th day of incubation period. This indicated that *Penicillium* sp. and *Aspergillus* sp. were equally good for reducing Cr.

The best ability of *Penicillium* sp. and *Aspergillus* sp. to absorb Cr is on day 5. This is in accordance with the research of [19], reported that *Aspergillus* sp. N2 and *Penicillium* sp. N3 decreases chromium concentration with an initial concentration of 50 ppm to an almost undetectable level or the within 120 hours of incubation.

This 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 [20].

4.3. Microscopic observation of mycelia

Figure 2 showed the difference between before and after treatment using *Penicillium* sp. mycelia within 5 days incubation period. After treatment indicated that Cr absorption occurs along with dye absorption of batik wastewater on the outer and inner surfaces of cells. This was supported by [21], heavy metals are absorbed along with the decolorization process. The sticking of the dye outside and in the cell proves the presence of decolorization and decreased levels of heavy metals. Fungal mycelium is known to absorb dyes found in batik waste, enzymatically and non-enzymatically. According to [22], mycellium fungi are hydrophobic while dyes are hydrophilic, resulting in hydrophobic-hydrophilic interactions between mycellium fungi and dyes. The initial mechanism is absorption and then is enzymatic.

4.4. Measurement of pH

Another thing that was affected by the of Cr is pH. Table 2 showed the effect of decreasing Cr level in wastewater batik dyes red dye on pH. Based on pH data showed the effect of decreasing Cr levels in red indigosol batik dye wastewater on pH. In the treatment of *Aspergillus* sp. and *Penicillium* sp. of all incubation periods showed a decrease in pH before and after treatment, at pH of 8 to 7. The treatment of *P.chrysoporium* showed a decrease in each treatment, at pH of 8 to 6. According to [23], there is a positive correlation between Cr reduction and pH, where Cr reduction occurs at pH> 8, then completely reduce the Cr. 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 [19].

4.5. Biosorption Growth assessment of fungi

Tabel 1 showed results of measurements of dry weight of mycelia. 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. But, the highest value possessed by *Penicillium* sp., supported level of Cr decrease.

Based on Tabel 1, it can be seen that the dry weight mycelia to decreased Cr level. The biggest percentage of Cr reduction was in *Penicillium* sp. 5th day of incubation of 82.05% with the highest dry mycelium weight of 0.99 g. According to [24], one of the influencing factors 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. Effect of the magnitude of the reduction in Cr level based on the ability of mycelia biomass in absorbing Cr, and the influence of contact time that affects. *Penicillium* sp. on day 5th is the maximum time for fungi to absorb Cr level. The biomass obtained was commensurate with absorbed Cr value.

In addition, other influences that support a decrease in Cr levels are nutrients. Chromium is one of the nutrients that can be used in the fungus to carried out metabolism. The ability of *Aspergillus* sp. and *Penicillium* sp. as a biosorption agent the very potential applicability especially as bioremediation [19]. 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. Most other Cr reduction studies were carried out at neutral pH [25].

5. Conclusion

Concentration of Cr in several batik wastewater in Banyumas Regency ranged <0.12-1.01 mg/L. Indigosol Red was the highest one (1.01 mg/L). Fungi were able to removed Cr in Indigosol Red batik.The most effective fungi and incubation period to removed Cr level in red indigosol batik dye wastewater is *Penicillium* sp. in 5th days incubation period.

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