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Efficiency of *Aspergillus* sp. 3 to reduce chromium, sulfide, ammonia, phenol, and fat from batik wastewater

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Abstract. Batik coloring waste contains heavy metal chromium (Cr), and other components such as, Sulfide (S^{2-}), Ammonia (NH_3), phenol and oil-fat. The Batik industries are generally classified as small and medium enterprises, which usually do not process their waste. The aim of this study was to observe the ability of *Aspergillus* sp. 3 to reduce the concentration of Cr, sulfide, ammonia, phenol, and oil-fat component from batik wastewater. The selected fungus, *Aspergillus* sp. 3 was isolated from batik waste. Based on previous study, selected fungus, *Aspergillus* sp. 3 was able to decolorize and remediate Indigosol Blue batik wastewater. Potato dextrose broth medium was used for growing the mycelium. Reduction process was occurred with omitted of medium (formed mycelium-supplemented the batik wastewater). Based on experiments, *Aspergillus* sp. 3 was able to reduce 89.09%, 83.05%, 56.37%, 48.48%, 95.09%, 32.56, 39.28 and 38.15% of Cr sulfide, NH_3 , phenol and total oil-fat concentration, respectively. *Aspergillus* sp. 3 had potential application in bioremediation of water polluted by batik wastewater.

1. Introduction

One type of waste produced from batik business activities is wastewater. The waste comes from the dyeing or coloring process. Wastewater is usually accommodated in a pond. Before being discharged into the environment, it must be processed first so that the waste component that can pollute the environment can be reduced or eliminated so that negative impacts can be minimized.

The production process of the batik industry produces liquid waste which amounts to 80% of the total amount of water used. The wastewater of the batik industry contains organic substances, suspended solids, colors and also chromium (Cr), sulfide, ammonia phenols, fatty and oils [1, 2].

Cr is one of the heavy metals that can pollute water. The presence of chromium in the waters can cause a decrease in water quality and endanger the environment and aquatic organisms. The negative impact in aquatic organisms is the disruption of metabolism due to obstruction of the workings of enzymes in physiological processes. Chromium can accumulate in the body and is chronic which ultimately results in the death of aquatic organisms. Cr metal is a toxic heavy metal. Its toxic



properties can lead to acute poisoning and chronic poisoning. Cr enters the environment, either in the water, or in the soil. Most of Cr released into waters in two ways, naturally and non naturally. The natural entrance of Cr can be caused by some physics factors, for example erosion that happened to mineral rocks. Non natural Cr entrance is the impact of human activities in the form of industry wastewater [3].

Sulfide is sulfuric acid gas. Sulfide within wastewater is the result of the decay of organic matter in the form of hydrogen sulfide (H_2S). Hydrogen sulfide is produced by decomposing microorganisms from organic substances that is toxic to algae and other microorganisms. Otherwise, hydrogen sulfide can be used by photosynthetic bacteria as electron/hydrogen donors to reduce carbon dioxide (CO_2). The results of the decay of organic substances cause unpleasant odors in the surrounding environment. Determination of sulfide aims to analyze sulfuric acid gas in wastewater that occurs from the decomposition process of organic substances (sulfur compounds). It causes the occurrence of foul odors in the waters [4].

Inorganic nitrogen consists of ammonia (NH_3), ammonium (NH_4^+), nitrite (NO_2^-), and nitrogen (N_2) [5]. Free ammonia is also called ammonia nitrogen, produced from the decay of bacterial organic substances. New wastewater is relatively low of free ammonia and has high organic nitrogen content. Ammonia (NH_3) is a nitrogen compound that becomes NH_4^+ at a low pH. It is called ammonium. Ammonia itself is in a reduced state (-3) [4]. Ammonia (NH_3) concentration in the wastewater can come from aerobic and anaerobic degradation containing nitrogen.

The presence of ammonia contained in water can cause odor. The odor of ammonia is unpleasant, so its concentration should be low. The maximum allowable limit in wastewater is 8 mg/l (Central Java Provincial Regulation No. 5/2012). About 0.037 mg/l of ammonia concentration can cause slight ammonia.

Phenol in liquid waste is derived from aromatic hydrocarbons containing OH groups. One of the properties of this compound is very toxic so that if it breaks down into the environment it can endanger the living biota in the environment [6]. Aromatic hydrocarbons often cause environmental problems and are often encountered, especially phenols in waters areas are phenols. One of can come from the batik industry [7].

Oil and fat are type of pollutants that come from liquid waste because it uses a layer of wax called "Malam" in making batik motifs. Batik industrial pollutants will cause the threatened life of biota in the environment. These pollutants contain aromatic compounds that have low to high molecular weight [8]. This pollutant is produced quite large in relation to the coloring process.

The concentration of Cr, S^{2-} , NH_3 , phenol, ammonia and oil-fat components from wastewater before being discharged into the environment should be reduced to the permissible quality standard with the aim that the waste does not pollute the surrounding environment..

The effect over capacity of those components in environment will lead to negative impact especially in aquatic biota and plants around it. The technique of reducing it in the wastewater can be done by increasing the quality of the environment by decreasing the concentration of these components. Several studies have been carried out as an effort to reduce the component of wastewater by adding chemicals and physical separation methods. These methods are effective, but they can cause other environmental pollution. Besides, it needs high cost to overcome the problems. The biological methods are important approaches because of its cost-effectiveness, high efficiency, and minimal toxicity to the environment [9].

An alternative method of processing batik wastewater, especially in reducing the concentration of Cr, sulfide, phenol, ammonia, and oil-fat component is mycoremediation. Mycoremediation is one of the methods that use biological agents that is fungi, to reduce waste from the environment. The fungi have the enzymatic activity for degradation of waste/pollutants [10]. The toxic effects of tannery wastewater are reduced by *Aspergillus niger* after treatment. The physicochemical parameters wastewater (Colour, COD, TS, TDS, TSS, chlorides) are reduced. Moreover, the chromium and sulfides concentration reduction is 57.8%, and 65.7%, respectively [11]. *Aspergillus* sp. is considered

effective in waste remediation. This genus is promising to be used as an agent for remediation of wastewater.

This research used *Aspergillus* sp. 3 obtained from previous studies [12,13]. Isolated *Aspergillus* sp. 3 has highest ability to decolorize Indigosol Blue batik wastewater and to reduce the physicochemical parameters. *Aspergillus* sp. 3 is the best isolate for decolorizing the level of Biochemical Oxygen Demand (BOD), Chemical Oxygen Demand (COD), total dissolved solids (TDS), total suspended solids (TSS), and electrical conductance (EC) pH and temperature compared to other *Aspergillus* spp. [12,13]. The aim of this study was to observe the ability of *Aspergillus* sp. 3 to reduce the concentration of Cr, sulfide, ammonia, phenol, and oil-fat component from batik wastewater.

2. Materials and Methods

2.1. Wastewater

The Indigosol Blue batik wastewater was generated from batik home industries of Sokaraja, Banyumas, Central Java, Indonesia.

2.2. Microbial culture

Aspergillus sp. 3 used in the study was obtained from previous studies [12,13]. It was isolated from effluent that was taken from the environment of batik wastewater ponds. This the isolate was screened for its ability to decolorize and remediate Indigosol Blue batik wastewater. The isolate maintained in Potato Dextrose Agar (PDA) medium slant and stored at 4°C. PDA made from Potato infusion that can be made by boiling 200 g of potatoes in 1 L distilled water and added 20 g dextrose and 20 g agar powder. Whereas the Potato dextrose broth (PDB) formulation was identical to PDA, omitting the agar.

2.3. Reduction study

Aspergillus sp. 3 was cultured on PDB medium. The 100 ml of PDB medium was placed in Erlenmeyer flasks (250 ml) and autoclaved. The flasks were inoculated with five plugs from 7 d old isolate culture (10 mm diameter) grown in PDA plates and incubated at shaker reciprocal 70 rpm for 3 days. Then the wastewater was added after pellet mycelium formed, omitting the solution medium and incubated at shaker reciprocal 70 rpm for 3 days. The experiments were executed in triplicates. After incubation, the treated wastewater was separated from fungal biomass. The supernatants were used for determinations of Cr, sulfide, phenol, ammonia and oil-fat component. The fungal biomass of isolate was determined after drying at 70°C to constant weight.

The concentration of Cr, sulfide, phenol, ammonia and oil-fat were measured before and after treatment. The determination of each component obtained by the procedure outlined in APHA (American Public Health Association) [14]. The reduction of concentration each component was calculated by a formula as follows:

$$\text{Reduction (\%)} = \frac{\text{Initial concentration} - \text{final concentration}}{\text{final concentration}} \times 100 \quad (1)$$

3. Results and Discussion

The results of measuring the concentration of Cr, sulfide, ammonia, phenol, and oil-fat in batik wastewater before and after treatment using *Aspergillus* sp. 3 isolate (Table 1) showed that the concentration of Cr metal decreased from 1.1 mg/l to <0.12 mg/l (undetectable). It was indicated that there was a large decrease in Cr concentration even though the initial concentration was slightly above the quality standard. The lower the number or dose (% b/v) of the adsorbent used, the higher the absorption capacity [15].

The sulfide and ammonia concentrations decreased from 2.95 mg/l and 127.2 mg/l to 0.5 mg/l and 55.5 mg/l, respectively. Although the initial concentration of phenol was below the quality standard,

this isolate can reduce the concentration from 0.33 mg/l to 0.17 mg/l. the concentration was very high in the oil and fat measurements before treatment, which was 10,992.75 mg / l but could decrease to 539.75 mg/l. The data showed that the decrease in concentration after treatment even though sulfide, ammonia and fat oil parameters were still above the quality standard by the government regulation. The amount of decreasing concentration was determined using percentage reduction.

The percentage reduction of wastewater characteristics (Figure 1) showed that the isolate of *Aspergillus* sp. 3 reduced the total concentration of Cr, S²⁻, NH₃, phenol and total oil-fat with a percentage reduction of concentration 89.09%, 83.05%, 56.37%, 48.48%, 95.09%, respectively.

Table 1. Characteristics of wastewater before and after a treatment using *Aspergillus* sp. 3.

| No | Characteristics | Unit | Concentration | | Quality standards* |
|----|------------------|------|---------------|--------|--------------------|
| | | | Before | After | |
| 1 | Total Chrom (Cr) | mg/L | 1.1 | <0.12 | 1.0 |
| 2 | Sulfide | mg/L | 2.95 | 0.5 | 0.3 |
| 3 | Total Ammonia | mg/L | 127.2 | 55.5 | 8.0 |
| 4 | Phenol | mg/L | 0.33 | 0.17 | 0.5 |
| 5 | Total oil-fat | mg/L | 10.992,75 | 539.75 | 3.0 |

Source*: Central Java Provincial Regulation No. 5/2012

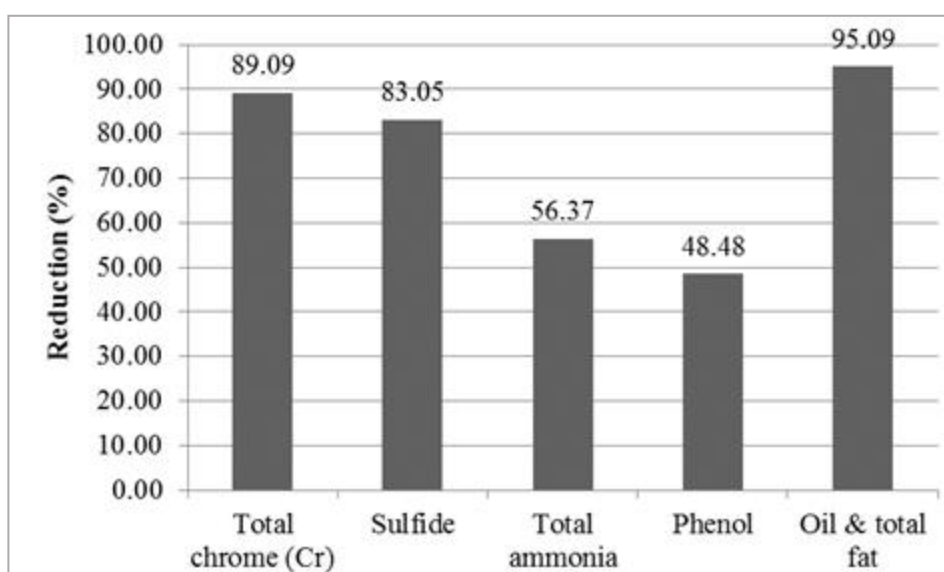


Figure 1. Percentage reduction of wastewater characteristics using *Aspergillus* sp. 3.

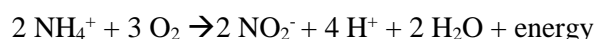
Figure 1 showed that *Aspergillus* sp. 3 isolate succeeded in reducing Cr concentration. This was the presence of the isolate response to the Cr of dyes containing batik wastewater. It is known that Cr content in the batik industry comes from the coloring process. The color of batik used textile dyes containing Cr. Cr content in wastewater occurs during the washing process after coloring batik fabric material. Cr content is determined by the concentration of Cr in the dye used. Cr concentration is higher if the dye used was using high Cr. Another factor that affected Cr content is the amount of dye solution, where the more solution the Cr concentration was increasingly limited and otherwise, the dissolution of Cr with a certain amount of solution greatly affected Cr concentration in wastewater. The frequency of washing also affects the content of Cr dissolved in water. The use of the dye type

and the amount of dye had an effect on the Cr content in wastewater. The use of more diverse dyes, the potential for Cr content in wastewater is also high [3].

Decreasing the concentration value can occur due to degradation so that the resulting concentration becomes smaller. Especially in the reduction of sulfide concentrations can be explained because of oxidation of sulfides. According to [16], sulfide degradation of dye wastewater was due to oxidation of sulfides and producing salt.

It is known that there are 26 types of compounds that are sources of odor emitted from industrial activities. Two of the types of compounds are used as odor parameters including hydrogen sulfide (H₂S), and ammonia (NH₃) [17]. Reduction efficiency of sulfide and total ammonia were recorded for *Aspergillus* sp. 3 as 83.05% and 56.37% respectively (Figure 1). This indicated that the treatment using *Aspergillus* sp. 3 isolate was very effective for the degradation of strong odors in Indigosol Blue batik wastewater, especially in this case ammonia and sulfide.

Total ammonia present in high concentration in batik wastewater (127.2 mg/l) were far above the quality standard allowed by the regulation (8 mg/l) (Table 1). High ammonia concentration can indicate contamination. The presence of NH₃ caused unpleasant odors, so NH₃ concentration must be lowered. NH₃ in wastewater can be treated microbiologically through the process of nitrification to become nitrite and nitrate. In nitrification, ammonia will interact with oxygen and produce nitrite, then interact with microbes and turn into nitrate, a safer compound [18]. The reaction was as follows:



In this study, isolate *Aspergillus* sp. 3 was incubated in aerobic conditions. Fulfillment of oxygen requirements for growth was given by agitation to the medium using a shaker. This condition was used for the degradation of ammonia in wastewater. This was according to [19], aerobic conditions that are equipped with an abundance of nitrifying microorganisms (microorganisms that help process ammonia reform to nitrite and nitrate) are sufficient to produce a conducive aquatic environment and were relatively safe from pollutants. Nitrification is an oxidation reaction, which was the process of forming nitrite or nitrate from ammonia. The process of nitrification, oxidizing ammonia to nitrite in the nitrogen cycle by microorganisms, also in the process of nitrogen decomposition in the wastewater treatment system.

Aspergillus sp. 3 isolate that used in this study play important roles as a source of nitrifying microorganism in ammonia reduction. Some heterotrophic microorganisms are also reported to be able to oxidize and nitrify [20]. Fungi belonging to the genera *Aspergillus* is heterotrophic microorganisms. The *Arthrobacter* and *Aspergillus flavus* are able to produce nitrate in media containing ammonia as a nitrogen source. The difference between them is the carbon source used. Autotrophic bacteria used CO₂ as a carbon source while heterotrophic microorganisms used organic compounds as carbon sources [21].

The concentration of ammonia reduction also coincides with the concentration of sulfide reduction with the presence of *Aspergillus* that conditioned on aerobic incubation. Oxygen can prevent most toxic substances from being formed in water, they were oxidized to non-toxic forms through by biological activity in aerobic conditions. Nitrite would be oxidized to nitrate, Ferro is converted to ferric and to hydrogen sulfide (H₂S) is converted to sulfate [19].

Table 1 and Figure 1 showed that there was a decrease in phenol concentration after treatment. This indicates that *Aspergillus* sp. 3 can degrade phenol. Phenol is a toxic compound, difficult to degrade and causes taste and odor in water even with a concentration of 0.002 mg/l [22]. Fungi can be used for phenol degradation because active fungi produce various enzymes [23], which can reduce cyclic ring compounds. Biodegradation enzymes of lignin cyclic ring breakers are lignin peroxidase, manganese peroxidase, laccase. In biodegradation of phenols, they were key enzymes, which are responsible for oxidizing phenols through electron oxidation by forming cation intermediates radicals [24].

The ability of *Aspergillus* sp. 3 in degradation of oil and fat within Indigosol Blue batik wastewater was examined (95.09%). *Aspergillus oryzae* biomass can be used for application of fat degradation

by the release of glycerol simultaneously. Fungi can reduce the concentration of fat by using lipase [25].

Aspergillus sp. 3 in batik wastewater had proven to reduce the concentration of Cr metal and other components (Figure 2). This can be shown by the color changes after treatment that indicating the reduction of component and degradation of toxic solid components in the batik wastewater by the isolate. Reduction of Cr, S^{2-} , NH_3 , phenol and oil-fat concentration from wastewater after treatment makes it less toxic.

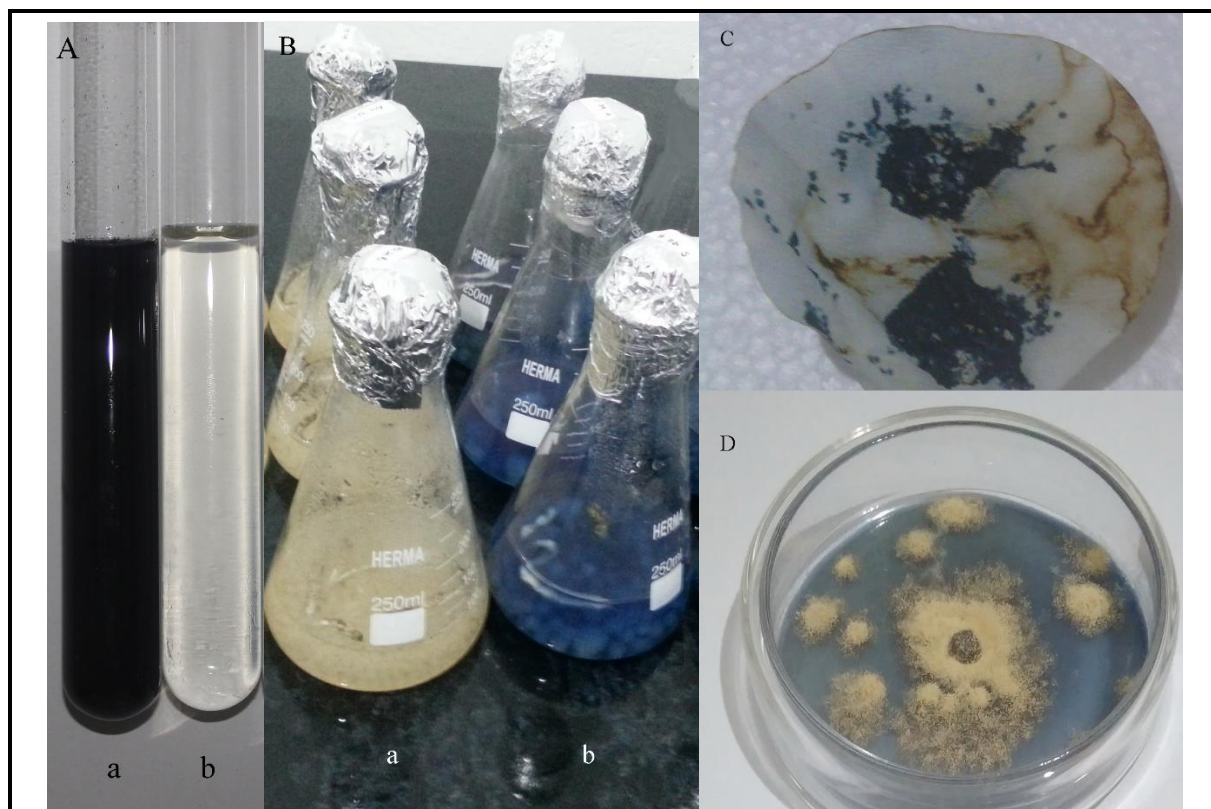


Figure 2. Effects of *Aspergillus* sp. 3 the growth on batik Indigosol Blue wastewater. Supernatant (A), mycelium (B) [before (a), after (b) treatment], Biomass after treatment (C) and Pure culture of *Aspergillus* sp. 3 (D).

The decrease in all parameters was evidenced by the growth of *Aspergillus* sp. 3 isolate (Figure 2B). The mycelium appeared white before the treatment, and blue after the treatment. Biomass appeared blue like the color of wastewater (Figure 2C), this indicates the absorption of toxic components by mycelium.

Aspergillus sp. 3 may had potential application in bioremediation of batik wastewater because of its toxic components reduction ability. Concentrations of S^{2-} , phenol, and NH_3 , nitrogen in contaminated soil, are reduced to a permissible quality standard with the microbial consortium (*Penicillium chrysogenum* and *Aspergillus niger*) after treatment [26]. Filamentous fungi are known to be able of immobilizing Cr by binding of a cell wall, metal-binding metabolites secretion, and intracellular uptake [27,28]. *Aspergillus* and *Penicillium* are associated with reduction of phenolic compounds in soils [29,30].

4. Conclusion

Concentration of Chromium, sulfide, total ammonia, phenols and oil-fat from Indigosol Blue batik wastewater were reduced by treatment of *Aspergillus* sp. 3. Potato dextrose broth medium was used

for growing the mycelium. Reduction process was occurred with omitted of medium (formed mycelium-supplemented the batik wastewater). Based on experiments, *Aspergillus* sp. 3 efficiently was able to reduce 89.09%, 83.05%, 56.37%, 48.48%, 95.09%, 32.56, 39.28 and 38.15% of Cr, S²⁻, NH₃, phenol and total oil-fat concentration, respectively.

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