Advance Sustainable Science, Engineering and Technology (ASSET)
Vol. 7, No.3, July 2025, pp. 02503020-01 ~ 02503020-09
ISSN: 2715-4211 DOI: https://doi.org/10.26877/asset.v7i3.2199

Biosorption of Chromiun in Batik Wastewater Using SCOBY Microbial Biomass: A Sustainable Bioremediation Approach

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Abstract. Batik wastewater poses an environmental threat due to hazardous heavy metals like lead, cadmium, and chromium (Cr). This study investigated the effectiveness of SCOBY (Symbiotic Culture of Bacteria and Yeast), a microbial consortium from kombucha production, in reducing Cr levels in batik wastewater. SCOBY is a promising biosorbent for heavy metals. The research aimed to assess SCOBY's ability to decrease Cr contamination in different types of batik wastewater (hand-drawn, stamped, and printed) over varying incubation times. Using a quasi-experimental approach, wastewater samples were collected from small and medium industries in Pekalongan City. Results showed that SCOBY effectively reduced Cr levels across all batik wastewater types and incubation periods. The most significant reduction occurred at 3 hours of incubation. Specifically, Cr levels decreased by 53% in hand-drawn batik wastewater, 44% in stamped batik wastewater, and an impressive 71% in printed batik wastewater. These findings suggest that SCOBY treatment is a viable and effective alternative for managing batik wastewater.

Keywords: batik wastewater, bioremediation, heavy metals, kombucha

(Received 2025-06-20, Revised 2025-06-22, Accepted 2025-06-22, Available Online by 2025-07-28)

1. Introduction

Batik, as an ancestral heritage recognized by UNESCO [1,2] , has become the identity of Pekalongan City, which is known as the world's batik city. In 2021, there were 261 batik SMEs in Jenggot Village with a workforce of 370 consisting of 250 male workers and 120 female workers. The high production of batik is directly proportional to the high liquid waste produced. Batik wastewater contains fatty oils, dyes, and several heavy metals such as *chromium* (Cr) [3–5], *cadmium* (Cd) [6,7], and other heavy metals such as *cobalt (*Co), *nickel* (Ni), *plumbum* (Pb), *copper* (Cu), and *arsenic* (As) [8,9] are also found in batik industry waste. According to the Pekalongan City Environmental Service (DLH), the batik business in Pekalongan City generates at least five million liters of wastewater every day. Since the IPAL can only hold 45% of it, the remainder is just thrown into the river [7].

The batik industry in its production process produces liquid waste, which amounts to 80% of the total amount of water used during the batik-making process. The content of liquid waste from the batik industry can be organic substances, suspended solids, phenols, heavy metals like chromium (Cr), fatty oils, and colors [10]. Data from the Pekalongan City Environmental Service in 2022 showed that the results of the analysis of the heavy metal content of Cr in batik wastewater samples in Jenggot Village exceeded the quality standard, reaching 3,565 mg/L. This high figure is because there are still several batik craftsmen who dump production waste directly into rivers or nearby waterways without going through a processing process.

Chromium (Cr) is a toxic heavy metal whose properties carried by this metal can result in acute poisoning and chronic poisioning [8,11]. Cr metal is persistent, bioaccumulative, and highly toxic and can decompose in the environment. The government has stipulated Government Regulation of the Republic of Indonesia Number 22 of 2021 concerning the Implementation of Life Management Protection that the maximum quality standard value for the heavy metal Cr in water is 1 mg/L [12].

Attempts to remove heavy metals using methods like precipitation, filtration, ion exchange, carbon adsorption, evaporation, membrane technology, reverse osmosis preconcentration, redox, electrowinning, chelation, wastewater coagulation, and electrochemistry have not worked [13]. In the last few decades, the biosorption method has received a lot of attention from researchers because it is considered environmentally friendly for removing particulates in water pollution [4,14]. Biosorption is a low-cost, easy-to-use, profitable, and efficient method of removing pollutants from water resources, and microorganisms are effective biosorbents [4,15,16]. The absorption of heavy metals by microbes can be carried out through absorption and adsorption processes. Absorption by absorption is also called a resistance mechanism. Resistance mechanisms can be carried out through the excretion or seepage of chelating compounds outside the cell, the binding of metal ions by intracellular molecules, or the accumulation of metals in certain organs such as vacuoles. Meanwhile, adsorption occurs when several molecules (compounds, ions, or atoms) are bound to the surface of a solid or liquid [12].

The biosorption method using microbes such as SCOBY is considered to be able to remove pollutants in wastewater [9]. Microbial biomass functions as an ion exchanger based on the reactive groups available on the cell surface, such as carboxyl, amine, phosphate, hydroxyl, and sulfate [17]. Symbiotic Culture of Bacteria and Yeast (SCOBY) is a consortium of microbes consisting of Acetobacter xylinoides, Komagataeibacter xylinus, Gluconacetobacter xylinus, Acetobacter aceti, and Acetobacter pasteurianus. Meanwhile, the yeast contained in SCOBY includes Schizosaccharomyces pombe, Saccharomycodes ludwigii, Kloeckera apiculata, Saccharomyces cerevisiae, Torulaspora, Zygosaccharomyces bailii, Brettanomyces bruxellensis, Brettanomyces lambicus, Brettanomyces custersii, Candida, and Pichia [18,19]. Acetobacter is a group of bacteria that can produce organic acids such as citric acid, maleic acid, and oxalic acid. These acid moieties when released to the medium join with metals by supplying both protons and metal complexing anions, thus leading to metal leaching [20]. Saccharomyces cerevisiae a dominant yeast in SCOBY also showed the ability to reduce chromium [21–24]. SCOBY as a mixed culture of various types of bacteria and yeast that has been proven to be able to eliminate heavy metals and is easy to culture and economical is an advantage as a biosorption agent.

The main of the present study is to describe the effectivities of SCOBY in reducing Cr from batik wastewater, especially the presentation of decreasing Cr in batik wastewater with different incubation periods. The batik wastewater used in this study is batik wastewater obtained from batik craftsmen in the Pekalongan City area from the types of hand-drawn batik, stamped batik, and printed batik.

2. Method

The type of research used in this study is a quasi-experimental design with a time series design with pretest-posttest. The sampling technique used was purposive sampling, starting with observations of the number of samples in the work area of Jenggot Village, Pekalongan City. Research on measuring Cr levels and incubation periods was carried out at the laboratory belonging to the Pekalongan City Environmental Service. The environmental laboratory of the Pekalongan City Service has been accredited by the National Accreditation Committee (KAN) with accreditation number LP-1757-IDN as a laboratory that consistently implements SNI ISO/IEC 17025:2017.

Scoby culture is obtained from the fermentation process of kombucha tea by combining 6 teaspoons and 21 tablespoons of granulated sugar with 3 liters of boiling water, which is inoculated with a 9 cm diameter SCOBY and 300 mL of water starter kombucha, and then incubated for 14 days in a glass jar at room temperature and protected from direct sunlight [25]. The characteristic SCOBY used in this research has a thickness of 0.6 cm and a diameter of 6 cm.

The sampling process is carried out at the first reservoir of the batik industry before being discharged into the river or soil infiltration. The obtained batik wastewater sample will be added by giving concentrated Nitric Acid (HN03) as much as 10 ml in 2 liters of sample. Then the sample is taken as much as 100 ml to carry out the destruction process until the metal sample dissolves 30 ml by heating at a temperature of 300 C for 3 hours. The sample then goes through a filtration process with a paper filter using a 0.45 cm membrane filter. Batik wastewater samples were measured for temperature and pH value both before and after treatment with SCOBY incubation.

The test of the ability of SCOBY to reduce Cr levels is carried out with different incubation periods of 1 hour, 2 hours, and 3 hours in different types of batik wastewater with the same temperature at room temperature which is replicated 3 times. The calculation of the reduction in Cr levels in batik wastewater is carried out using AAS. The sample is filtered with 100 ml of filter paper and then put into a PE bottle. Cr analysis in water samples is based on SNI 6989.17:2009. Cr extraction is carried out by the sample concentration method with concentrated nitric acid (HNO3). The water sample is shaken first, then 50 ml of sample is taken. The sample is poured into a measuring cup and covered with a watch glass. Concentrated HNO3 is added as much as 5 mL, then heated until ± 20 mL remains. Then rinse the watch glass, and the sample results are poured back into the measuring cup and diluted to 50 mL. The sample is filtered into a 50 mL measuring flask. The sample is transferred to a vial bottle and put into AAS to determine the Cr content [26].

Data analysis was conducted to determine the effectiveness of SCOBY in reducing Cr levels in batik waste. A data normality test was conducted using Shapiro-Wilk, and a dependent T-test was used to determine the difference in the average Cr reduction.

3. Result and Discussion

3.1. Result

The obtained batik wastewater samples were measured for temperature, pH, and chromium content. The results of temperature measurements on the samples showed the sample temperature in the range of 27˚C and a pH value of 6. The physical condition of this wastewater shows the same results in several studies where the temperature of batik wastewater is around 27˚C and the pH value is acidic [27–30]. The results of measuring Cr levels in batik waste samples showed that up to 50% had Cr levels exceeding the quality standard. The quality standard for chromium levels in the textile industry has a threshold value of 1 mg/L in accordance with SNI 6989 17 2009 and is regulated in the Regulation of the Minister of Environment Number P.16 of 2019 concerning Industrial Area Wastewater Quality Standards [28,31]

Table 1. Levels of Cr in batik wastewater samples

Sample	Level of Cr (mg/L)	Conclusion
BC1	1,0 mg/L	Maximum standar
BC2	1,0 mg/L	Maximum standar
BC3	0,5 mg/L	below standard quality
BP1	1,0 mg/L	Maximum standar
BP2	0,9 mg/L	below standard quality
BP3	0,4 mg/L	below standard quality
BT1	1,3 mg/L	above standard quality
BT2	1,1 mg/L	above standard quality
BT3	0,7 mg/L	below standard quality

Note: BC is stamped batik; BP is printed batik, and BT is hand-drawn batik

The results of the study showed that out of 5 samples of batik wastewater, only 4 samples had a Cr content below the industrial waste quality standard, that is, BC3, BP2, BP3, and BT3. The BC3 sample is batik wastewater from the stamped batik process, the BP2 and BP3 samples are batik printing, and the BT3 sample is from hand-drawn batik. Heavy metal waste from the textile industry mainly comes from dyes [32]. Total Cr is found in textile industrial wastewater because they are used as a catalysts during the dye manufacturing process; besides that, they are part of the dye molecules with other heavy metals such as arsenic (As), cadmium (Cd), lead (Pb), copper (Cu), and zinc (Zn) [33].

The decrease in Cr levels in batik liquid waste was seen with the treatment of giving scoby with an incubation of 60 minutes, 120 minutes, and 180 minutes. The results of Cr level measurements showed a decrease in Cr levels proportional to the length of incubation time. In the written batik sample, the decrease in Cr levels sequentially from 1.03 mg/L before treatment decreased by 0.8 mg/L, 0.63 mg/L, and 0.5 mg/L at 60 minutes, 120 minutes, and 180 minutes of incubation. Meanwhile, the decrease in Cr levels in batik stamp liquid waste did not show a significant number when compared to samples from the other two types of batik. The best decrease in Cr levels reached 0.22 mg/L in the printed batik sample at 180 minutes of incubation.

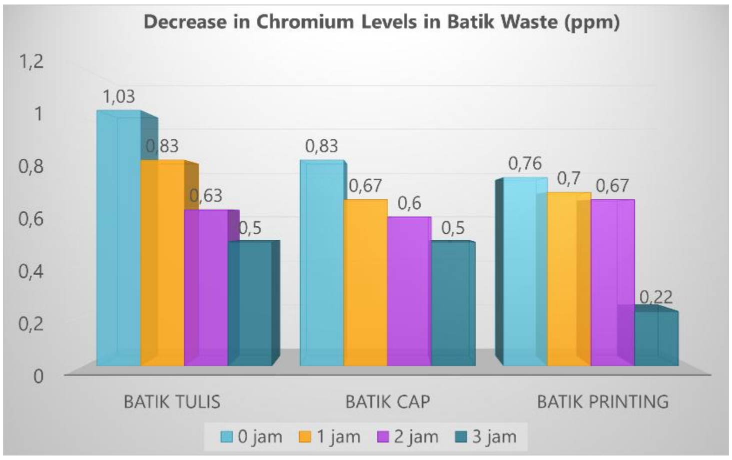

Figure 1. Decreasing Chromium Levels in Batik Wastewater

The effectiveness of SCB in reducing Cr levels is calculated by the difference in the reduction in levels of Cr compared to the initial Cr levels multiplied by 100%. Based on this calculation, the lowest effectiveness result of SCOBY in reducing Cr levels in batik printed wastewater was 8% at 60 minutes of incubation. The most significant effectiveness of Scoby in reducing Cr levels occurred in batik printed waste water at 180 minutes of incubation with an effectiveness of 72%.

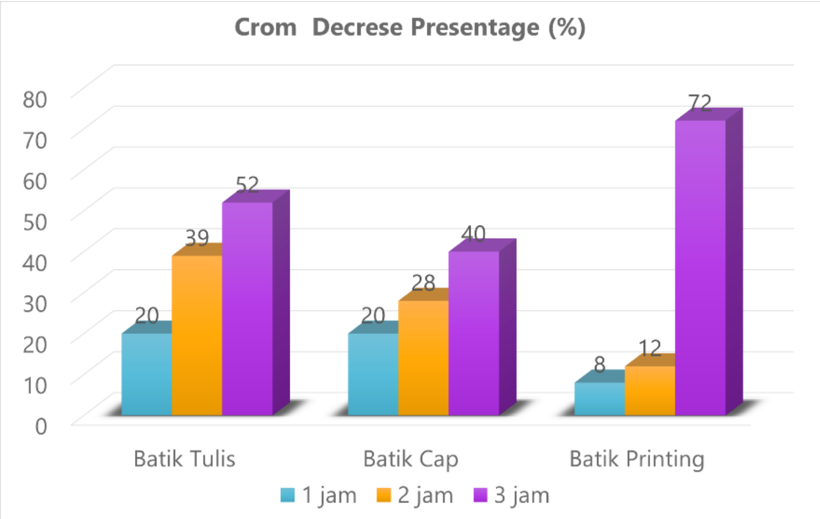

Figure 2. Effectiveness of Scoby to decreasing Cr Level

The results of the study showed that the chromium content in liquid batik waste experienced a significant decrease at 3 hours. The chromium content in hand-drawn batik waste at 1 hour decreased by 20%, at 2 hours decreased by 39%, and at 3 hours decreased by 52%. The chromium content in stamped batik waste at 1 hour decreased by 20%, at 2 hours decreased by 28%, and at 3 hours decreased by 40%.

3.2. Discussion

The chromium content in batik liquid waste comes from the use of synthetic dyes containing heavy metals. The more types of synthetic dyes used, the higher the chromium content in batik wastewater. Chromium heavy metals are found mainly in chromium yellow dyes containing iron chromium oxide ($FeCr_2O_4$) and chromium hexafluoride (CrF_6) [34,35]. Cr value in the Indigosol Red wastewater was the highest (1.01 mg/L) compared with Naphtol Soga Brown and Salt Naphtol Soga Brown that demonstrated values of 0.81, 0.75, and 0.41 mg/L, respectively [36]. Another factor that can influence the increase in chromium in batik liquid waste is the amount of air used in the dye solvent.

The results of the measurement of Cr levels in batik wastewater samples in this study were much higher when compared to the Cr levels found in batik wastewater in the Yogyakarta region, which only ranged from 9.87 to a maximum of 101 ppb [37]. Meanwhile, the measurement of Cr levels in Tanjung Bumi batik wastewater in the Madura regency showed higher results, reaching 31.07 - 45.47 mg/L, far exceeding the threshold set by the government regarding the maximum Cr levels in waste [38]

Scoby is a consortium of bacteria and yeast in the form of Acetobacter xylinum and Saccharomyces cerevisiae. In addition to being composed of microbial colonies, Scoby is also composed of cellulose particles that have unique results and performance in various fields. Scoby can be developed by making a reactor to remove heavy metals from wastewater because Scoby is a complex biological material. factors that affect the Cr absorption mechanism in Scoby are the surface and electrostatic interactions [9]. Cellulose from SCOBY has 5 main functional groups owned by cellulose. The O-H group (3345 cml) is a hydrogen bond that functions to bind the cellulose microfibrils to one another to keep them structured and compact. The CH_2 group (2898 cml/1314 cml) is a carboxyl group that can be used to estimate the crystallization rate of cellulose. H-O-H groups (1644–1650 cml) were used to determine the water adsorption rate. The C-O group (1107 cml) is a polyhydroxyl group which can state that SCOBY cellulose is formed from glucose or its derivatives. The C-O-C group (1050–1055 cml) is a glycosidic bond that plays a role in glucose polymer bonds so that it can form cellulose [39].

The results of the study showed that Scoby was able to reduce Cr levels up to 71% in batik wastewater within 180 minutes. Cellulose bacteria in scoby showed the ability to reduce Cr levels from batik wastewater effectively without pH intervention. These results indicate the similarity of the use of modified cellulose nanostructures (m-CNS) with thiols from wood pulp as a waste source to remove Cr(VI) ions from aqueous solutions with the highest Cr(VI) ion removal results of 95.95% at pH = 4.0 with a relatively short adsorption time (80 minutes) [40]. Other studies have shown the use of cellulose nanofibers (CNF) which can adsorb hexavalent chromium [41].

Absoprtion with Scoby are more effective when compared to the use of Pistia stratiotes plants in reducing Cr levels in batik liquid waste, which showed a percentage of 31.318% during a 6-day incubation [35] and reducing Cr with trembesi (Samanea saman) adsorbents and fitormediation of kiambang (Salvinia molesta) plants that need long time incubation [42]. The other research also showed that Scoby more effectively compared to Penicillium sp, that the range of values of Cr reduction varies from 83 to 86% in 5 day incubation time, with the largest biomass of 0.64 g [36]. The results of the study using Scoby were also more effective when compared to the original G4 indigenous bacteria consortium combination (Enterobacter mori, Enterobacter cloacae, Lysinibacillus fusiformis, and Bacillus thuringiensis) showed the fastest Cr (VI) removal, achieving an 81.93% Cr (VI) reduction within 48 h and a 99% reduction within 105 h [43].

These results are in line with Mousavi's research on the potential of Scoby in reducing heavy metal levels (Pb and Ni). The ability of the Scoby in heavy metal bioremediation has been studied, including the ability of the Scoby to reduce Ni (II) levels in liquid waste. The results of the study showed that the effectiveness of the Scoby in removing Ni reached 94.5% [9]. Other research shows that the effectiveness of the Scoby in removing Pb (II) from synthetic wastewater reaches 99.73%. This result is even better when compared with the use of magnetic GO [8].

The mechanism of heavy metal absorption can be done through passive and active absorption. The passive absorption mechanism known as biosorption can be done in two ways, namely by exchanging ions on the cell walls of microorganisms that are replaced by heavy metal ions and the formation of complex compounds between heavy metal ions with functional groups such as carbonyl, amino, thiol, hydroxy, phosphate, and hydroxy-carboxyl quickly and back and forth. While the active absorption mechanism is done by inserting heavy metals through the cell membrane with the process of entering essential metals through the membrane transport system, this is due to the similarity of properties between heavy metals and essential metals in the overall physicochemical characteristics of the waste [34].

The microbial biodegradation method of Cr is carried out by biotransformation of Cr6+ regulated by enzymes from a toxic to a non-toxic state (Cr3+) by bacteria and is considered a cheap and efficient method for removing Cr from contaminated waste soil and air [44]. According to Hilda Zulkufli, important factors that influence heavy metal biosorption are solution pH, solution ionic strength, initial pollutant concentration, the influence of other pollutants, biosorbent properties, temperature, and adsorbent dosage [12].

4. Conclusion

Based on the results and discussion, it can be concluded that SCOBY is effective in reducing Cr levels in batik wastewater at room temperature treatment and has potential to be used for batik wastewater management.

Acknowledgement

We would like to express our gratitude to the Pekalongan City Environmental Service, and Research and Community Service Institutions of Universitas Pekalongan.

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