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Review effectiveness of indigenous local microorganisms in degrading hexavalent chromium (Cr(VI)) in Batik liquid waste

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ABSTRACT

The batik industry in Indonesia has an IKM (Small and Medium Industry) scale so that it does not yet have adequate waste treatment. In the long term, waste is disposed of directly into the environment which can damage aquatic ecosystems and harm human health. Textile wastewater has a complementary picture and has a deep color. One of the most dangerous heavy metals contained in textile waste is hexavalent chromium (Cr(VI)). Several ways can be done to reduce hexavalent chromium (Cr(VI)) by bioremediation. Based on the results of the literature review, it shows that the bioremediation agents from single isolate microorganisms that are most effective in degrading chromium with high efficiency are Bacillus subtilis and *Pseudomonas aeruginosa*. The most effective consortium servers with constant reduction rates are the consortium of bacteria genus Mesophilobacter, Methylococcus, Agrobacterium, Neisseria, Xanthobacter, Deinococcus, Sporosarcina, and Bacillus by reducing BOD levels by 85.71%. The hexavalent chromium-degrading microorganisms are characterized by the presence of chromate reductase enzymes, mostly gram-negative bacteria, and a high growth rate.

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Introduction

Batik is a typical Indonesian cloth that has a variety of patterns that are printed with the special wax called "*malam*". Based on the manufacturing technique, the types of batik fabrics consist of stamped batik, written batik, and printed batik. The process of making batik generally consists of fabric processing, pattern making, waxing/coating wax, coloring, cooking or waxing, washing or rinsing, and drying[1–3]. The manufacture of batik certainly produces waste resulting from the processing of cloth, dyeing or coloring, cooking or pelorodan (remove the wax that is still attached to the cloth), and rinsing[4]. In general, the waste generated from batik making can be in the form of solid, gas, liquid, and particulate[1]. Batik liquid waste has the highest percentage compared to other types of waste, which is 80 – 95% of the total water use[4,5]. Physical characteristics of liquid waste are in the form of unpleasant odor, dark color, and irritation to the skin[6]. Therefore, batik liquid waste that is discharged into the environment in the long term can reduce environmental quality and cause health problems for the community around the batik industry.

Batik liquid waste contains organic matter, suspended solids, and dyes consisting of heavy metals such as chromium (Cr), sulfide (S2-), ammonia (NH_3), cadmium (Cd), lead (Pb), phenol, and fat. and pathogenic microorganism[2,5,6]. Among the heavy metals found in batik wastewater, chromium is one of the most toxic metals according to the Agency for Toxic Substances and Diseases Registry (ATSDR) and is listed as a level 'A' human carcinogen by the United States Environmental Protection Agency (US EPA)[7]. It exists in nature in the form of trivalent (Cr(III)) and hexavalent (Cr(VI)). Hexavalent chromium is the second most stable oxidation state of chromium produced from anthropogenic sources such as plating or electroplating, petroleum refining, leather tanning, pickling, textile manufacturing and pulp processing) and rarely occurs naturally, except for crocoite ($PbCrO_4$)[8]. Hexavalent chromium is very dangerous and mutagenic because it has strong oxidation potential, higher solubility in water, and rapid permeability through biological cell membranes[9,10]. Thus, it is necessary to treat batik liquid waste effectively before being discharged into the environment to reduce the content of heavy metals, especially hexavalent chromium.

Handling of batik liquid waste that has been carried out currently uses abiotic methods such as deposition technology, adsorption, filtration, ion exchange, oxidation or reduction, reverse osmosis, and electrochemistry[11,12]. However, abiotic treatment is considered less effective because it is expensive, not environmentally friendly, less effective, produce secondary pollutants, and depends on the concentration of waste[7]. Therefore, a more effective and efficient method is needed to handle batik liquid waste, namely the biotic method (bioremediation) because the cost is relatively cheap, environmentally friendly, does not cause secondary pollutants, and the effectiveness and safety level are more potential to be developed further in the future[12,13]. Therefore, the handling of batik liquid waste using biotic methods is more effective and efficient.

The bioremediation method is assisted by microorganisms capable of reducing batik wastewater, especially heavy metals such as hexavalent chromium (Cr (VI)). Microorganisms that can effectively reduce chromium in batik wastewater include *Bacillus* sp[14] *Bacillus subtilis* NAP1[15], *Pseudomonas* sp.[8,16,17], *Geobacillus* sp.[14], and *Lactobacillus delbrueckii*[18]. In addition, several consortiums of microorganisms have also been tested to degrade wastewater such as the consortium of *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Klebsiella pneumoniae* and *Escherichia coli* in azo dyes in the textile industry[19], consortium of bacteria Mesophilobacter, Methylococcus, Agrobaacterium, Neisseria, Xantobacter, Deinococcus, Sporosarcina, and Bacillus in batik waste [4], the consortium of *Sargassum* sp. and *Saccharomyces cerevisiae* in batik waste[20], *Aspergillus* sp.[5], *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Escherichia coli*[21]. Therefore, bioremediation can be carried out by a consortium of microorganisms that can reduce batik wastewater, especially heavy metals such as hexavalent chromium (Cr (VI)) derived from batik dyes. The purpose of this study was to find the most effective microorganism in degrading hexavalent chromium (Cr (VI)) in batik wastewater based on previous literature.

Method

This study uses a systematic literature review approach adopted from Durach[22], which consists of 5 steps, namely 1) developing a framework for formulating review questions, 2) identifying relevant research, 3) assessing the quality of the research to be reviewed, 4) recapitulating the results of the study, and 5) analyze and interpret the results of the study.

The first step is to determine the topic of questions to be investigated in a structured manner, such as 1) the most dangerous types of heavy metals found in batik wastewater (hexavalent chromium (Cr(VI)), 2) Heavy metal degradation methods in batik wastewater, 3) Bioremediation method, 4) Bacterial consortium to degrade batik liquid waste. Searches to support the questions that have been set using keywords include: "batik liquid waste", "hexavalent chromium (Cr(VI))", "bioremediation", and "bacteria consortium".

In the second step, before searching for articles, inclusion and exclusion criteria are set which are used to limit the search for articles and select the initial articles. Inclusion and exclusion criteria are described in Table 1.

Table 1. Inclusion and exclusion criteria

Inclusion Criteria	**Exclusion Criteria**
Articles published from 2010-2021 from accredited journals	Non-journal articles
The article focuses on the effect of the bacterial consortium on the degradation of hexavalent chromium (Cr(VI)) waste	Articles that do not contain clear information about the effect of the battery consortium on the degradation of hexavalent chromium (Cr(VI)) waste
The article contains qualitative and quantitative research	Articles that have weaknesses in methods and lack of references

The third step is to search for articles using databases from Google Scholar, Scopus, Emerald, Dimensions, Eric Database, and Web of Science.

The fourth step is to review and synthesize each article that is used as a reference in compiling review articles related to the effectiveness of the effect of the bacterial consortium on the degradation of hexavalent chromium (Cr(VI)) waste.

Results and Discussion

Chromium content in batik wastewater

Characteristics of turbid batik liquid waste depending on the type of dye used, foamy, high pH (alkaline), high BOD concentration, and alkaline fat content[1,23,24]. The dyes used in making batik are synthetic dyes containing heavy metals such as cadmium (Cd), chromium (Cr), lead (Pb), cobalt (Co), copper (Cu), mercury (Hg), nickel (Ni), magnesium (Mg), iron (Fe) and manganese (Mn)[2]. The more types of synthetic dyes used, it will increase the more chromium content in batik waste, especially chromium yellow dye which contains iron chromium oxide (FeCr2O4) and chromium hexafluoride CrF_6[24]. In addition to the many types of dyes, another factor that can affect the increase in chromium in batik wastewater is the amount of water that functions as a dye solvent [25]. The type of dye that contains a lot of chromium content is usually the dye that gives the yellow color.

The provision of quality standards for chromium levels in the textile industry has a threshold value of 0.5 mg/L following SNI 6989 17 2009 and is regulated in the Minister of Environment Regulation Number 03 of 2010 concerning Wastewater Quality Standards for Industrial Estates[4,26]. Batik liquid waste is taken before processing, after processing, and after being discharged into the waters (discharged into the environment) have varying levels of chromium, and all three still exceed the threshold for the quality standard of textile industry wastewater. This is evidenced by the results of measurements of chromium levels in several batik industries in Indonesia such as the batik industry in Solo before processing has an average chromium content of 16.6747 mg/L, after processing the concentration becomes 10.1181 mg/L, and after being disposed of in The river water flow which is 2 km from the factory has a concentration of 7.6277 mg/L[6], while the batik industry in Solo specifically, such as in Laweyan, which before processing has a chromium content of 74.298 mg/L[20], batik industry wastewater in Kauman Tulungagung before processing has a chromium content of 2.3 mg/L[15], batik liquid waste in Lampung before processing had a chromium content of 4.6 mg/L[27], batik liquid waste in Sokaraja, Banyumas before processing was 1.1 mg/L[5] and Pekalongan batik wastewater before processing 0.645 mg/L[24], so that the levels of hexavalent chromium (Cr(VI)) contained in batik wastewater have levels that exceed the standard limits for waste quality provisions in industrial activities and the Long-term, this water pollution can damage the ecosystem of aquatic bodies, because the presence of B3 waste in the environment can cause toxicity such as damage to aquatic ecosystems, death of aquatic organisms, to toxicity to the human body such as mutagenic, teratogenic and carcinogenic[28]. Trivalent chromium causes respiratory and nervous system disorders, and hexavalent chromium (Cr(VI)) can be carcinogenic and is categorized as the most toxic heavy metal according to the *Agency for Toxic Substances and Diseases Registry* (ATSDR)[7]. Another impact of the presence of batik liquid waste is that the photosynthesis process that occurs in plants will be hindered and the dissolved oxygen level in the water will exceed the threshold[21].

Microorganisms in batik wastewater

Microorganisms in batik wastewater are very diverse and can be obtained through certain stages such as sample dilution, isolation, identification of microorganisms by gram staining and biochemical tests, pure culture (multiplication of indigenous microorganisms), and measurement of reducing activity by isolates[15]. Some of the bacteria found in batik wastewater are *Bacillus* sp., *Pseudomonas* sp., *Geobacillus* sp.[14], *Lactobacillus delbrueckii*[18], *Pseudomonas putida*, *Pseudomonas aeruginosa*, *Klebsiella pneumonia*, and *Pantoea* sp.[23], *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Citrobacter freundii*, serta *Alcaligenes*[21], and *Bacillus subtilis* NAP1[15] under both aerobic and anaerobic conditions. The microorganisms other than bacteria were also found, such as the consortium *Sargassum sp.* and *Saccharomyces cerevisiae*[20], serta isolat tunggal berupa *Aspergillus* sp.[5], *Saccharomyces cerevisiae*[23], dan *Pleoratus ostreatus*[29]. Some of the microorganisms that can reduce the heavy metal hexavalent chromium (Cr(VI)) are *Bacillus* sp., *Geobacillus* sp., *Pseudomonas* sp., *Saccharomyces cerevisiae, Pseudomonas aeruginosa*[16], *Alcaligenese*[30], *Klebsiella pneumoniae*, *Pantoea* sp., and *Pseudomonas putida*[23], *Bacillus subtilis* NAP1[15], also *Aspergillus* sp[5]. Microorganisms that reduce BOD and COD levels are *Pleoratus ostreatus*, and the decolorization of synthetic dyes is *Lactobacillus delbrueckii*. The presence of gram-negative bacteria dominates in batik wastewater and its ability to mutate more quickly to develop resistance leads to the absorption of heavy metals such as hexavalent chromium (Cr(VI)) through sulfate transport channels in cell membranes and bacterial cytoplasm[9]. Several microorganisms contained in batik wastewater interact with each other enzymatically and work together in reducing heavy metal levels. With the presence of various microorganisms in batik liquid waste, of course, it can degrade heavy metals effectively and efficiently.

A Consortium of Hexavalent Chromium-Degrading Microorganisms

Bioremediation agents on heavy metals are usually carried out by a consortium of several microorganisms such as bacteria, archaebacteria, actinomycetes, microalgae, and fungi including molds and yeasts, even macroalgae can consort with other microorganisms[4]. However, the use of bacteria and fungi is more used as a chromium degrader than other types of microorganisms.

A consortium of microorganisms in the form of bacteria that can degrade hexavalent chromium include a consortium of bacteria genus Mesophilobacter, Methylococcus, Agrobacterium, Neisseria, Xantobacter, Deinococcus, Sporosarcina, and Bacillus which are mutualism in batik wastewater and are effective in reducing BOD levels by 85.71%[4], This is due to the decomposition of organic matter in batik wastewater caused by the action of the azo reductase enzyme, which also applies to the consortium of bacteria *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Klebsiella pneumoniae* and *Escherichia coli* in azo dyes in the textile industry, which is effective in reducing the level of decolorization of azo dyes of dyes Dylon' Navy Blue variant with the composition of *Acid Blue*[19,21]. Therefore, hexavalent chromium (Cr(VI)) can be degraded by microorganisms that have certain characteristics such as most types of gram-negative bacteria, the presence of azoreductase or chromate reductase enzymes, and have high growth and reproduction rates. The consortium of microorganisms in the form of macroalgae and yeast, namely *Sargassum* sp. and *Saccharomyces cerevisiae*, are also effective in reducing hexavalent chromium (Cr(VI)) in batik wastewater reaching 73.03% with an initial concentration of chromium 74.298 ppm to 20.04 ppm, this is due to the exchange of ions in the cell membrane wall with heavy metal[20].

The consortium of microorganisms was carried out by combining several isolated microorganisms that were previously propagated by pure culture on a laboratory scale, then combined to test the potential for degradation of the environment contaminated with heavy metals. The interaction of microorganisms in the environment cannot be predicted unless observed in the laboratory and can increase the final result of heavy metal degradation when compared to the use of single isolates[13]. This happens because the work of enzymes from each type of microorganism can complement each other to survive using the available nutrient sources in the growth medium[31]. So that the consortium of microorganisms is considered to be more effective as a bioremediation agent than a single isolate.

Hexavalent Chromium Reduction Mechanism

Various types of heavy metal bioremediation such as biosorption, metal-microbial interactions, bioaccumulation, biomineralization, biotransformation, and bioleaching have been carried out on local indigenous microorganisms[11]. The reduction of hexavalent chromium is carried out through several stages such as heavy metal absorption, extracellular reduction, and intracellular reduction[13,14,20]. The mechanism of heavy metal absorption can be done through passive and active uptake. The passive uptake mechanism known as biosorption can be carried out in two ways, namely by exchanging ions in the cell walls of microorganisms replaced by heavy metal ions and the formation of complex compounds between heavy metal ions and functional groups such as carbonyl, amino, thiol, hydroxy, phosphate, and hydroxy- carboxyl rapidly and back and forth. While the active uptake mechanism is carried out by entering 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 character of the waste. The process of active uptake in microorganisms can occur in line with the consumption of metal ions for growth and intracellular accumulation of metal ions[20]. Both of these metal absorption processes occur in microorganisms by involving the transport of heavy metal ions on the walls and cell membranes of microorganisms. The hexavalent chromium reduction mechanism is presented in Figure 1.

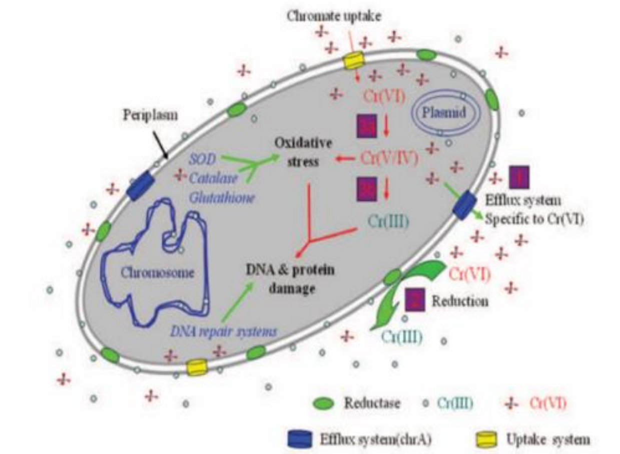


Fig 1. Hexavalent Chromium Reduction Mechanism[13]

The mechanism of degradation of hexavalent chromium generally occurs through enzymatic reduction pathways (direct reduction) or non-enzymatic reduction pathways (indirect reduction)[13]. The enzymatic reduction pathway is carried out by taking chromate by microorganisms and then reducing it enzymatically using chromate reductase enzymes located in the membrane, cytoplasm (intracellular), or periplasm (extracellular). When chromate reductase genes such as ChrR, YieF, NemA and LpDH are expressed in bioremediation, they can increase the rate of Cr(VI) reduction and cell viability by minimizing oxidative stress[14]. Among chromate reductases that form fewer ROS (Reactive Oxygen Species) can be used in bioremediation because quinone reductase activity increases tolerance to peroxides so that it does not affect detoxification of cell viability[9], in the decomposition of organic matter in batik wastewater, bacteria release enzymes to decompose organic compounds and produce by-products in the form of carbon dioxide (CO_2), methane (CH_4), hydrogen (H_2) and water (H_2O), trivalent chromium hydroxide or oxide precipitates, as well as supporting energy[4,13]. The non-enzymatic reduction pathway is carried out by utilizing the concentration of microbial biomass in the form of microbial metabolism products or decomposition in the form of other metal ion pollutants such as Fe2+ or H2S, and organic molecules such as intracellular thiol compounds or extracellular polymeric substances that can reduce Cr(VI) to Cr(III). Dissimilated iron-reducing bacteria reduce Fe3+ to Fe2+. Both sulfide and Fe2+ are effective reducing agents for Cr(VI) under certain conditions[20]. The mechanism of degradation by enzymatic and non-enzymatic pathways occurs continuously in reducing hexavalent chromium in wastewater.

Effectiveness of Chromium Reduction in Wastewater

Several chromium-degrading microorganisms isolated from wastewater before or after treatment have different effectiveness. This is influenced by several factors such as pH, temperature, light intensity, type and amount of metal, nature of growth medium, and species of microorganisms[11,20,32]. The chromium-degrading microorganisms based on environmental characteristics can be seen in Table 2. The pH level is very influential, if the pH level is too low then chromium reduction is inhibited because the chromate reductase enzyme works optimally at pH 6.5 - 8.5[32]. The single isolate microorganism that was most effective in degrading chromium with high efficiency was *Bacillus sp*, which can be seen in Table 2, 3.

Table 2. Chromium-degrading microorganisms based on environmental characteristic

Type of microorganism	pH	Temperature	Medium character/ waste sample
Bacillus sp., *Pseudomonas* sp. & *Geobacillus* sp[14,33]	7	50°C	Batik waste
Bacillus subtilis NAP1[15]	7	30°C	Batik waste
Lactobacillus deelbrueckii[18]	7	37°C	Batik waste
Saccharomyces cerevisiae[20]	5	30°C	Batik waste

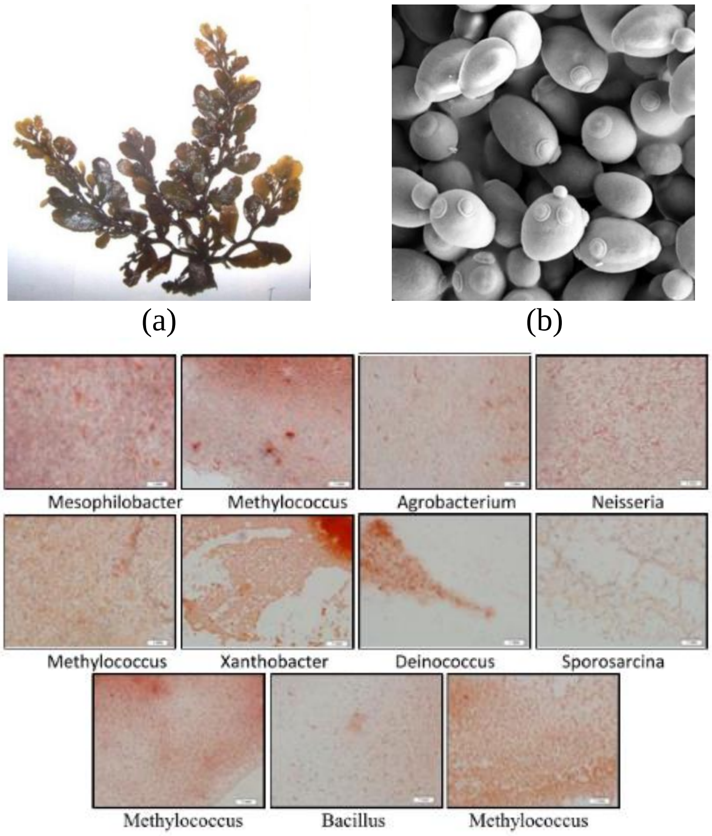
Table 3. Effectiveness of single isolate of chromium-degrading microorganism

Type of microorganism	Percentage	Time needed	Initial chromium concentration	Final chromium concentration
Bacillus sp.[14]	97%	24 hours	50 and 125 ppm	1.5 and 3.75 ppm
Pseudomonas aeruginosa[17]	94%	48 hours	100 ppm	5.86 ppm
Aspergillus sp.[5]	89.09%	72 hours	1.1 mg/L	0.12 mg/L

In an environment contaminated with waste, each microorganism reduces certain heavy metals specifically in a relatively long time. Usually these microorganisms will be isolated to measure the effectiveness of heavy metal reduction. Several single isolate microorganisms were combined to increase the rate of reduction of hexavalent chromium as a consortium of microorganisms. After the incorporation of various microorganisms, usually the surface water or mud becomes flocculated and becomes more foamy day by day which indicates the presence of remediation activity[33]. The use of a consortium of microorganisms in industrial wastewater, especially textile factories such as batik and leather tanning has different effectiveness due to the long period of colonization of microorganisms[12]. The use of a consortium of microorganisms in industrial wastewater, especially textile factories such as batik and leather tanning has different effectiveness due to the long period of colonization of microorganisms[12]. The effectiveness of the consortium of microorganisms degrading chromium and BOD is presented in Table 4.

Table 4. Effectiveness of consortium of chromium degrading microorganisms and BOD

Microorganism consortium	**Percentage**	**Time required**	**Concentration of initial contamination levels**	**Concentration of Final Contaminant Level**
Sargassum sp. dan Saccharomyces cerevisiae[20]	73.03%	24 hours	chromium (74.298 ppm)	chromium (20.04 ppm)
Genus Mesophilobacter, Methylococcus, Agrobacterium, Neisseria, Xantobacter, Deinococcus, Sporosarcina, dan Bacillus[4]	85.71%	144 hours	BOD (14 mg/L)	BOD (2 mg/L)



(c)

Fig 2. Morphology of hexavalent chromium (Cr(VI))(a) degrading species *Sargassum* sp.[34](b) *Saccharomyces cerevisiae* shown on SEM[35], and (c) bacteria of the genera Mesophilobacter, Methylococcus, Agrobacterium, Neisseria, Xanthobacter, Deinococcus, Sporosarcina, and Bacillus[4]

The decrease in BOD levels also affects the decrease in the levels of other heavy metals such as hexavalent chromium. Thus, the most effective use of the consortium of microorganisms is the consortium of the genera Mesophilobacter, Methylococcus, Agrobacterium, Neisseria, Xantobacter, Deinococcus, Sporosarcina, and Bacillus.

Bioremediation using bacteria is considered more effective than other microorganisms because of the large population of bacteria in nature. Especially its presence in wastewater can show resistance to 100 mg/L chromium and has a higher bioaccumulation ability during the growth process only which is involved with metabolic activity[33,36]. Success in selecting a consortium depends on the similarity of the waste properties (reduced heavy metal ions) to the structure of the cell wall membrane of the type of microorganisms[6,20]. The use of monospecies microorganisms is considered more effective than multispecies because the consortium development needs to pay attention to the study of the interaction of the types of microorganisms and the enzymes they secrete whether they can synergize in the degradation of heavy metals or not, because the interaction of microorganisms results in the degradation of heavy metals which is higher and stable to fluctuating environmental conditions[7]. The use of a consortium of microorganisms as a bioremediation agent depends on various factors that can affect its superiority in degrading heavy metals such as the type of heavy metal, the level of physicochemical parameters of the waste, and microorganisms.

Conclusion

The most effective single isolate microorganism in degrading chromium with high efficiency was *Bacillus sp.* The most effective consortium and constant reduction rate was the consortium of bacteria genus *Mesophilobacter, Methylococcus, Agrobacterium, Neisseria, Xantobacter, Deinococcus, Sporosarcina, and Bacillus* which reduced BOD levels by 85.71%. Several factors that affect the effectiveness of chromium reduction are the availability of metals, types of microorganisms, pH, temperature, and the nature of the medium or sample of batik liquid waste.

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Author contributions

All the authors have equal contribution in preparation of the manuscript. The first, second, and third author have original idea, conceptualization, methodology, analysis and validation. The fourth author contributed in revision, editing, review and improvement of the first draft of the manuscript. First and fourth authors did organization of the manuscript including language corrections and formal analysis.