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RESEARCH ARTICLE

BIOREMEDIATION USING *Bacillus subtilis* AND *Saccharomyces cerevisiae* TO REDUCE CHROMIUM IN ELECTROPLATING LIQUID WASTE

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ABSTRACT

The electroplating industry produces liquid waste containing a small number of heavy metals but is toxic. Wastewater containing chromium (Cr) absorbed into the soil will affect soil fertility. Waste management is needed so that the abiotic and biotic environment is not poisoned by Cr. Bioremediation using bacterial and fungal microbes are applicable to reduce Cr levels in electroplating liquid waste. The purpose of this research was to investigate the reduction level of Cr in electroplating liquid waste through bioremediation using *Bacillus subtilis* and *Saccharomyces cerevisiae*. Laboratory experiments were conducted using variations in microbial concentrations ($10^{2.5}$ cells ml^{-1} and 10^{5} cells ml^{-1}), variations in microbial types (*Bacillus subtilis* bacteria, *Saccharomyces cerevisiae* fungi, and mixtures of both microbes), and variations in incubation time (6, 12, and 24 hours). The initial Cr concentration and the results of the bioremediation process were determined by measuring the absorbance and the Cr levels using Atomic Absorption Spectrophotometry (AAS). Based on experiments, the use of *Bacillus subtilis* $10^{2.5}$ cells ml^{-1} with a 24-hour incubation time reach the highest percentage reduction in Cr (88.96%), followed by 12-hours incubation time (84.73%), and 6-hours incubation time (79.21%). Furthermore, the use of a microbial mixture of *Bacillus subtilis* and *Saccharomyces cerevisiae* $10^{2.5}$ cells ml^{-1} with 6-hours, 12-hours and 24-hours incubation time was able to reduce the levels of Cr respectively by 77.46%; 80.18% and 83.04%. Next, *Saccharomyces cerevisiae* 10^{5} cells ml^{-1} with 6-hours, 12-hours, and 24-hours incubation time was able to reduce levels of Cr in a row by 50.17%; 52.35% and 55.63%. The results of this study indicate that the bioremediation process using the microbial *Bacillus subtilis* and *Saccharomyces cerevisiae* is proven to reduce the levels of Cr in the electroplating industry wastewater. The highest reduction results were achieved on the use of 24-hour incubation time and the use of *Bacillus subtilis* with a concentration of $10^{2.5}$ cells ml^{-1} at 88.96%.

Keywords: Bioremediation, *Bacillus subtilis*, Chromium heavy metal, Electroplating liquid waste, *Saccharomyces cerevisiae*

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INTRODUCTION

The electroplating industry is one of the

industries that produce liquid waste containing hazardous and toxic materials (Nurhasni, Salimin, & Nurfitriyani, 2013). Electroplating is an electrochemical way to coat the metal using a metal deposition principle. Metals commonly used for coatings are chromium, cadmium, copper, gold, nickel, silver, and other metals. The electroplating industry is one of the industrial activities that can produce hazardous and toxic liquid waste (Nurhasni et al., 2013). Electroplating industry (metal coating) is a type of industry that has the potential to produce waste, especially liquid waste, which can pollute the environment (Qin, Chai, Ju, & Hamamoto, 2018).

In the Periodic Table, Chrome (Cr) is a transition element in group VI-B, period 4. Due to the nature of the transition, Cr is included in the metal element. Chrome metal has an atomic number of 24 and has an atomic mass of 51.996; a density of 7.19 g mL^{-1}; boiling point 2,665°C, and melting point 1,875°C. Cr has various valences, including chrome (III) and chrome (VI) which are important in the ecological system (Focardi, Pepi, & Focardi, 2013; Sharaf, Gasmeleed, & Musa, 2013).

Cr is mostly found in the metal coating industry wastewater or paint industry in the form of dichromate anion ($Cr_2O_7^{2-}$). Cr is the seventh element with an abundant presence on earth (Mohanty & Patra, 2013). The use of Cr is in alloys, for example, iron metal alloys, steel coatings, paint, and pigment products, leather tanning, wood preservation, chemical production and pulp, and paper products. This industry plays a major role in the contamination of chrome metal with adverse effects on biological and ecological species (Ghani, 2011).

The presence of chrome (Cr) heavy metals in waters must be controlled because their negative impacts can pollute the environment and torment the health of humans, and other living things. The hazardous levels of Cr oxidation are +3 and +6, which being toxic to animals, humans, and plants (Focardi et al., 2013; Mohanty & Patra, 2013; Wolińska, Stępniewska, & Włosek, 2013). Cr(VI) ions can easily be absorbed by the human body and subsequently accumulate in the food chain then cause severe health problems including skin irritation, respiratory damage, intestinal corrosion, ulcers, even lung cancer (Qin et al., 2018), gene mutations, carcinogenic (Das et al., 2015; Hanifa, 2018), and teratogenic (Focardi, Pepi, & Focardi, 2013). The mutagenic nature of Cr(VI) ions is categorized as the number one carcinogen in humans according to the International Agency for Research on Cancer (Jaishankar, Tseten, Anbalagan, Mathew, & Beeregowda, 2014).

According to the Government Regulation of the Republic of Indonesia Number 101 Year 2014 (PP 101/2014) on the Management of Hazardous and Toxic Substances, any person who produces hazardous and toxic waste is obliged to manage the waste of hazardous and toxic substances produced (Peraturan Menteri, 2014a). The electroplating industry is obliged to manage waste before being discharged into the environment so that the heavy metal content does not exceed the maximum rate allowed by the government. The maximum rate of Cr in waste that is allowed is 0.5 mg l^{-1} (Peraturan Menteri, 2014b). Action is needed to reduce or even eliminate the Cr content, both in the form of total chrome and in the form of Cr(VI) ions by carrying out waste treatment before being discharged into free waters.

Wastewater treatment containing heavy metals has been carried out including precipitation (Mu, Wang, Bu, Liu, & Zhao, 2018), electrocoagulation (Sadyrbaeva, 2016; Makde & Hedaoo, 2018), electrodialysis, (Golbaz, Jonidi, Rafiee, & Rezaei, 2014; Hegazi, 2013), adsorption (Khan, Islam, Farooqi, Ayub, & Basheer, 2019; Permana, Haryati, & Bustan,

2017), and bioremediation. Bioremediation can be used to overcome environmental problems by utilizing microorganisms that aim to reduce levels of pollutants (Priadie, 2012). The bioremediation process is relatively economical, proven to be effective in reducing the adverse effects of pollution and making contaminated soils less polluted and free of toxic compounds (Verma & Kuila, 2019). *Pseudomonas aeruginosa* has been used to reduce nickel and chromium levels in metal coating industry wastewater (Mardiyono & Samsumaharto, 2016). The concentration of *Pseudomonas aeruginosa* and *Bacillus subtilis* 10^5 cells ml^{-1} and $10^{2.5}$ cells ml^{-1} with a variation of incubation time of 6 hours, 12 hours, and 24 hours was proven to reduce Cr(VI) levels (Kurniawan, 2014). The Concentration of $10^{2.5}$ cells ml^{-1} decreased Cr(VI) levels higher than the concentration of 10^5 cells ml^{-1}, while the 24-hour incubation time decreased the highest levels of Cr(VI).

Waste management is needed to reduce the level of Cr in waste before being discharged into the environment to overcome the negative impacts and dangers of Cr waste in the electroplating industry. The electroplating industry wastewater treatment is done using variations in microbial administration (*Bacillus subtilis* bacteria, *Saccharomyces cerevisiae* fungus, and a mixture of both), variations in microbial concentrations ($10^{2.5}$ cells ml^{-1} and 10^5 cells ml^{-1}), and variations in microbial incubation time (6 hours, 12 hours, and 24 hours). Before and after the bioremediation process, Cr was determined by the Atomic Absorption Spectrophotometry method. The purpose of this study is to reduce the levels of Cr in electroplating industrial wastewater through bioremediation using *Bacillus subtilis* and *Saccharomyces cerevisiae,* and variations in concentration and incubation time.

MATERIAL AND METHODS

This research was conducted in October 2018 to March 2019 in the central laboratory of Universitas Sebelas Maret and Microbiology Laboratory and Water Chemistry Laboratory of Setia Budi University, Surakarta. Tools used in this research include pH sticks, bottles, flasks, volume pipette, suction cup, centrifuge, filter paper, test tube, drop pipette, AAS. While the material used in this research was electroplating liquid waste, *Bacillus subtilis* bacteria, *Saccharomyces cerevisiae* fungi, H_2SO_4 0.2N, $K_2Cr_2O_7$ p.a., concentrated HNO_3, aquadest, aquabidestilata, label paper, and Whatman filter paper.

The procedure of this research was carried out using the bioremediation method through the following stages:

Microbial preparation procedures

The bacteria used are bacterial *Bacillus subtilis*, and the fungi *Saccharomyces cerevisiae*. Isolates of *Bacillus subtilis* are reproduced in a liquid medium LB (Luria Bertany) with a composition of isolates of 100 mL namely 1 G Tryptophan, 0.5 g Yeast extract, and 0.5 g NaCl. Furthermore, the isolates of *Saccharomyces cerevisiae* are reproduced in Sabouraud Glucose Agar (SGA) liquid media with a composition of isolates 100 ml of Mycological peptone 10 grams, Glucose 40 grams, and Agar 15 grams.

Carrier media preparation

The carrier media used in this study is rice bran. Rice bran weighs 30 grams and put in a petri dish. Petri dishes containing rice bran wrapped in paper and put in heat-resistant plastic. Then put in an autoclave to be sterilized with a temperature of 121°C at a pressure of 1 atm, for 15 minutes.

Planting bacteria into the media carrier

Planting bacteria into the carrier media is done by mixing 10 ml of bacteria, 15 ml aquadest, and 30 grams of rice bran for the colony's bacterial level $10^{2.5}$ cells ml^{-1}. While the

colony bacteria level 10^5 cells ml^{-1} is performed by mixing 10 ml of bacteria, 5 ml of aquadest, and 30 g of rice bran. The resulting mixture was then dried in the sun for one day in bright air conditions (Kurniawan, 2014).

Sampling

Samples were taken from the inlet at the Bina Chroom electroplating industry in Mojosongo, Surakarta, Central Java. Sampling was carried out three times a day such as in the morning, afternoon and evening, as much as 15 liters each. Initial characteristics of the wastewater were 35°C, smelling of sewage, pH 8.0 and yellowish color.

Preparation of a suspension of *Bacillus subtilis*, *Saccharomyces cerevisiae*, and the mixture of both microbes

The microbes were cultured on a suitable medium, taken 2-3 ose included in 100 ml of medium, then incubated at a temperature of 37°C for 6 hours, 12 hours, and 24 hours.

Variation types of microbe suspension given to electroplating waste samples

Samples treated with the addition of various types of microbe suspension with a concentration of $10^{2.5}$ cells ml^{-1} and 10^5 cells ml^{-1}, then incubated for 6 hours, 12 hours, and 24 hours with pH 7.4 and temperature of 37°C.

Samples were tested before and after processing with various types of microbes

Before and after incubation, the sample was taken 100 ml, added with 5 ml of concentrated HNO_3 and heated in electric heating until the test sample solution was almost dry, then added 50 ml of aquabidestilata, put into a 100 ml measuring flask through Whatman filter paper and matched with 100 ml solution H_2SO_4 0.2 N. The test solution is transferred into the cuvette then the absorbance was read using an (AAS) (SNI 06-6989.17-2004).

The research was conducted using Pretest Posttest Design, by determining the levels of Cr before and after the additional treatment of *Bacillus subtilis* and *Saccharomyces cerevisiae*. The independent variables (factors) in this research include variations in microbial administration, variations in microbial concentrations, and variations in microbial incubation time. The data that has been collected is further analyzed using the three-way Analysis of variance (ANOVA) to determine the difference in Cr concentrations reviewed from these three factors.

RESULTS

The concentration of Cr after bioremediation with variations in microbial administration, concentrations, and incubation time

Concentration of Cr after bioremediation with *Bacillus subtilis* (Bs), *Saccharomyces cerevisiae* (Sc) and mixtures of both microbes (Bs+Sc), variation of microbial concentrations ($10^{2.5}$ cells ml^{-1} and 10^5 cells ml^{-1}), and incubation times 6 hours, 12 hours, and 24 hours are presented in Table 1, Table 2, and Table 3. Figure 1, Figure 2, and Figure 3 give the visualization of Cr concentration after bioremediation.

Table 1 shows the concentration of Cr after the addition of various microbial administration, microbial concentration and 6 hours incubation time, which can reduce the Cr levels from the initial level of 2.5161 ppm. Levels of reduction depending on the type of microbe and concentration used. The highest reduction is in the addition of $10^{2.5}$ cells ml^{-1} *Bacillus subtilis* and the lowest reduction is in the addition of 10^5 cells ml^{-1} *Saccharomyces cerevisiae*.

Table 2 and Table 3 also show that in the incubation time of 12 hours and 24 hours, *Bacillus subtilis* reduce Cr levels higher than *Saccharomyces cerevisia*e. In the variation of $10^{2.5}$ cells ml^{-1} microbial concentrations can reduce the Cr levels higher than the concentration of 10^5 cells ml^{-1}. $10^{2.5}$ cells ml^{-1}

Bacillus subtilis was seen to reduce the highest levels of Cr levels, followed by $10^{2.5}$ cells ml^{-1} mixture of *Bacillus subtilis* and *Saccharomyces cerevisiae*. The addition of *Saccharomyces cerevisiae* 10^5 cells ml^{-1} reduced the lowest levels of Cr levels.

Average concentration percentage of Cr after bioremediation with variations in microbial administration, concentrations, and incubation time

The average Cr concentration percentage from the bioremediation process on the use of each microbial administration, concentration, and incubation time are presented in Table 4. The visualization of Cr concentration after bioremediation can be seen in Figure 3.

Statistical analysis

Based on the data in Table 5, the research hypothesis was tested using a three-way Analysis of Variance (ANOVA). The purpose of the three-way ANOVA is to determine the difference in Cr concentration by interaction of three factors, namely microbial administration (*Bacillus subtilis*, *Saccharomyces cerevisiae*, and a mixture of both), microbial concentration ($10^{2.5}$ cells ml^{-1} and 10^5 cells ml^{-1}), and incubation time (6 hours, 12 hours, and 24 hours). The requirements that must be fulfilled in the ANOVA test are the p-value in the interaction of three factors/variables was less than 0.05. The results of three-way ANOVA are presented in Table 5.

Based on three-way ANOVA, the F value of the interactions between three factors was 4.627 with a significance of 0.004 ($p < 0.05$). These results indicate that the interaction between variations in microbial administration, microbial concentration, and incubation time significantly influences the reduction in Cr concentration in electroplating wastewater. Furthermore, the F value on the interaction between variations in microbial administration and microbial concentration was 73.990 with a $p=0.0001$ ($p < 0.05$). Likewise, in the interaction between microbial administration and incubation time, the F value of 4.961 was obtained with $p=0.003$ ($p < 0.05$). Both of these results indicate that there are significant interactions between variations in microbial administration and microbial concentrations as well as between microbial administration and incubation time. But in the interaction between microbial concentration and incubation time, the F value was 1.599 with $p = 0.216$ ($p > 0.05$), so there was no significant interaction between microbial concentration and incubation time.

DISCUSSION

Based on Table 1 and Table 2, $10^{2.5}$ cells ml^{-1} *Bacillus subtilis* decreased Cr concentration greatest than all treatment during 6 hours incubation, with changes in Cr concentration from initial 2.5161 ppm to 0.5232 ppm (Cr concentration decrease was 1.9929 ppm). The lowest decrease is in the addition of 10^5 cells ml^{-1} *Saccharomyces cerevisiae* which is equal to 1.2623 ppm.

The reduction level of Cr concentration during 12 hours incubation from initial waste level of 2.5161 ppm from high to low, respectively in the use of $10^{2.5}$ cells ml^{-1} *Bacillus subtilis* (2.1320 ppm), $10^{2.5}$ cells ml^{-1} mixture of *Bacillus subtilis* and *Saccharomyces cerevisiae* (2.0174 ppm), 10^5 cells ml^{-1} mixture of *Bacillus subtilis* (1,9196 ppm), 10^5 cells ml^{-1} *Bacillus subtilis* and *Saccharomyces cerevisiae* (1.7955 ppm), $10^{2.5}$ cells ml^{-1} *Saccharomyces cerevisiae* (1.7194 ppm), and 10^5 cells ml^{-1} *Saccharomyces cerevisiae* (1.3172 ppm).

Table 1. Cr concentration after bioremediation with variation in microbial administration, concentration and incubation time (initial Cr concentration = 2.5161 ppm mg^{-1} $liter^{-1}$)

Treatment	Microbial concentration (cells ml^{-1})	Cr Concentration After Bioremediation		
		6 hours	12 hours	24 hours
Bs	$10^{2.5}$	0.5194 0.5229 0.5273 Average: 0.5232	0.3796 0.3861 0.3867 Average: 0.3841	0.3339 0.2053 0.2941 Average: 0.2778
Bs	10^{5}	0.6782 0.6712 0.6768 Average: 0.6754	0.5976 0.5973 0.5943 Average: 0.5965	0.6090 0.4968 0.5289 Average: 0.5449
Sc	$10^{2.5}$	0.8221 0.8212 0.8312 Average: 0.8248	0.7935 0.7952 0.8014 Average: 0.7967	0.6997 0.7485 0.8114 Average: 0.7532
Sc	10^{5}	1.2598 1.2481 1.2534 Average: 1.2538	1.1962 1.1948 1.2058 Average: 1.1989	1.1167 1.0844 1.1485 Average: 1.1165
Bs+Sc	$10^{2.5}$	0.5721 0.5663 0.5629 Average: 0.5671	0.4967 0.5021 0.4974 Average: 0.4987	0.4227 0.4294 0.4279 Average: 0.4267
Bs+Sc	10^{5}	0.7693 0.7655 0.7712 Average: 0.7687	0.7195 0.7188 0.7234 Average: 0.7206	0.6721 0.6741 0.6672 Average: 0.6711

Bs : Bacillus subtilis *Sc : Saccharomyces cereviciae* ppm : part per million (mg $liter^{-1}$)

Table 2. Average Cr concentration levels after bioremediation with variation in microbial administration, concentration and incubation time (initial Cr concentration = 2.5161 ppm)

Treatment	Microbial concentration (cells ml^{-1})	Cr concentration levels of changes (ppm)		
		6 hours	12 hours	24 hours
Bs	$10^{2.5}$	1.9929	2.1320	2.2383
Bs	10^{5}	1.8407	1.9196	1.9172
Sc	$10^{2.5}$	1.6913	1.7194	1.7629
Sc	10^{5}	1.2623	1.3172	1.3996
Bs+Sc	$10^{2.5}$	1.9490	2.0174	2.0894
Bs+Sc	10^{5}	1.7474	1.7955	1.8450

Table 3. Average Cr concentration percentage after bioremediation with variation in microbial administration, concentration and incubation time

Treatment	Microbial concentration (cells ml^{-1})	Average Cr concentration percentage (%)		
		6 hours	12 hours	24 hours
Bs	$10^{2.5}$	79.21	84.73	88.96
Bs	10^{5}	73.16	76.28	78.34
Sc	$10^{2.5}$	67.22	68.34	70.06
Sc	10^{5}	50.17	52.35	55.63
Bs+Sc	$10^{2.5}$	77.46	80.18	83.04
Bs+Sc	10^{5}	69.45	71.36	73.33

Tabel 4. Cr concentration in electroplating liquid waste after bioremediation using a mixture of both microbes (BS + SC)

Experiment number	Microbes	Microbial concentration	Cr concentration			Percentage of decrease level (%)
			Initial (ppm)	After bioremediation (ppm)	Changes (ppm)	
1	BS + SC	0	2.5909	2.5909	0	0
2	BS + SC	0	2.4894	2.4894	0	0
3	BS + SC	0	2.4680	2.4680	0	0
Average	BS + SC	0	2.5161	2.5161	0	0
1	BS + SC	$10^{2.5}$	2.5909	1.1111	1.4798	57.12
2	BS + SC	$10^{2.5}$	2.4894	1.0844	1.405	56.44
3	BS + SC	$10^{2.5}$	2.4680	1.1485	1.3195	53.46
Average	BS + SC	$10^{2.5}$	2.5161	1.1165	1.3996	55.63
1	BS + SC	10^{5}	2.5909	0.6945	1.8964	73.19
2	BS + SC	10^{5}	2.4894	0.7479	1.7415	69.96
3	BS + SC	10^{5}	2.4680	0.8120	1.656	67.10
Average	BS + SC	10^{5}	2.5161	0.7532	1.7629	70.06

Table 5. Analysis of variance

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	3.721[a]	17	.219	326.363	.000
Intercept	26.454	1	26.454	39441.825	.000
Microbes (A)	2.388	2	1.194	1780.358	.000
Concentration (B)	1.037	1	1.037	1545.580	.000
Incubation (C)	.169	2	.085	126.170	.000
A x B interaction	.099	2	.050	73.990	.000
A x C interaction	.013	4	.003	4.961	.003
B x C interaction	.002	2	.001	1.599	.216
A x B x C interaction	.012	4	.003	4.627	.004
Error	.024	36	.001		
Total	30.200	54			
Corrected Total	3.745	53			

Remark: a. R Squared = .994 (Adjusted R Squared = .991)

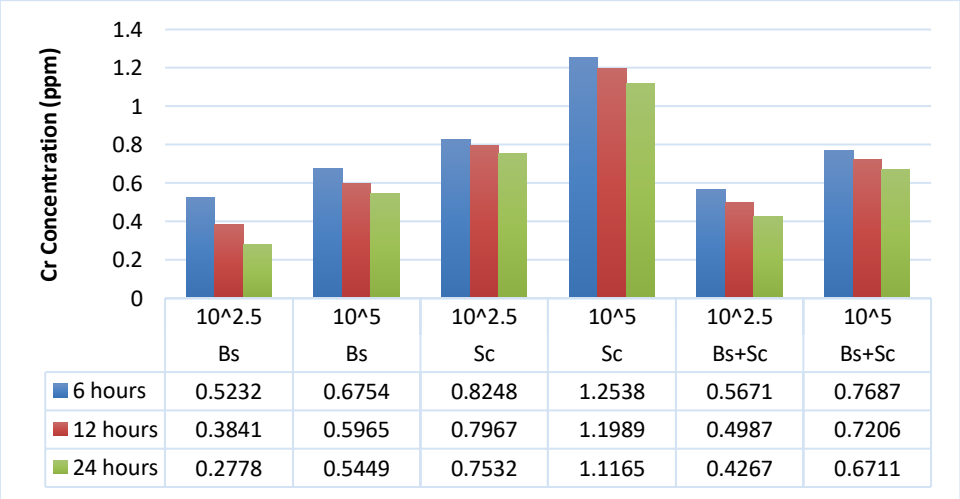
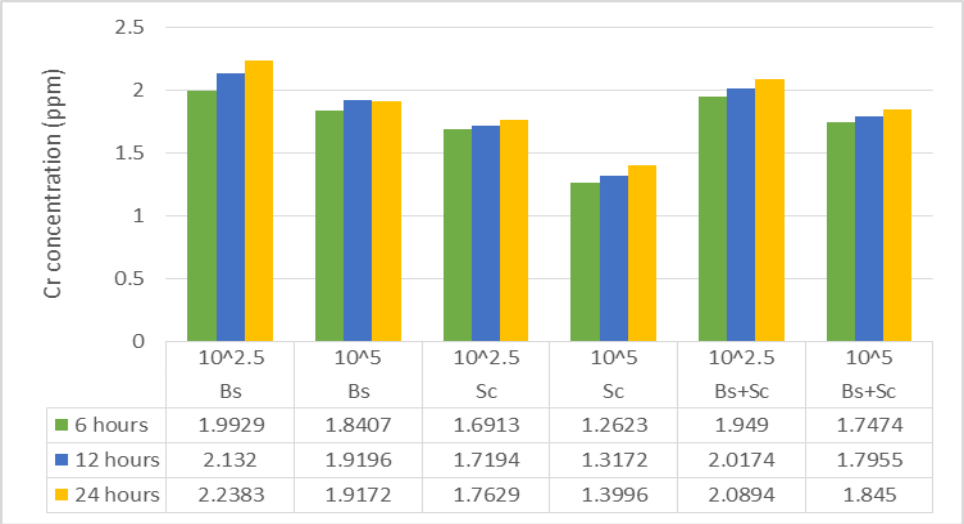
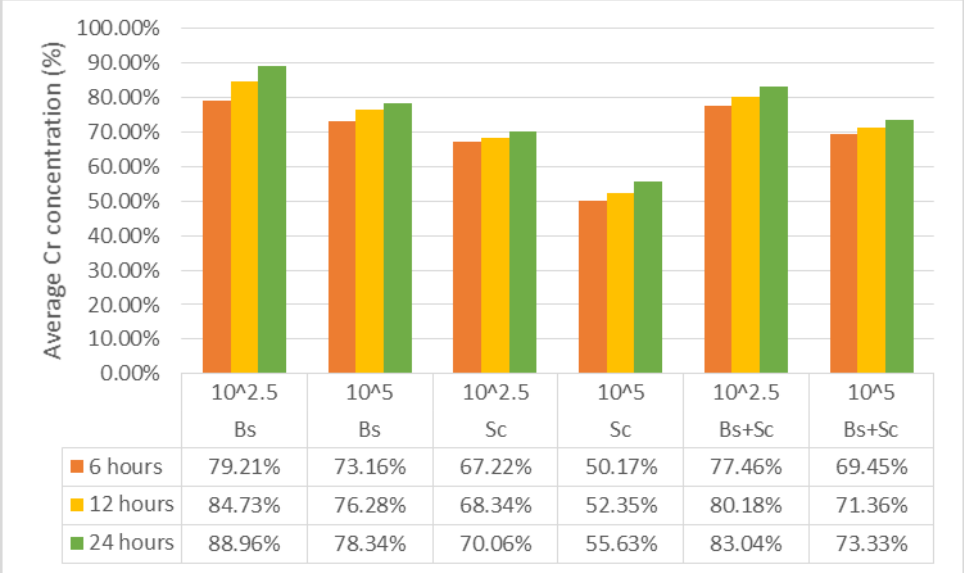


Figure 1. Cr Concentration after bioremediation with variation in microbial administration, concentration and incubation time (initial Cr concentration = 2.5161 ppm)



	10^2.5 Bs	10^5 Bs	10^2.5 Sc	10^5 Sc	10^2.5 Bs+Sc	10^5 Bs+Sc
6 hours	1.9929	1.8407	1.6913	1.2623	1.949	1.7474
12 hours	2.132	1.9196	1.7194	1.3172	2.0174	1.7955
24 hours	2.2383	1.9172	1.7629	1.3996	2.0894	1.845

Figure 2. Average concentration of change in Cr after Bioremediation with Variation in Microbial Administration, Concentration and Incubation Time (Initial Cr Concentration = 2.5161 ppm)



	10^2.5 Bs	10^5 Bs	10^2.5 Sc	10^5 Sc	10^2.5 Bs+Sc	10^5 Bs+Sc
6 hours	79.21%	73.16%	67.22%	50.17%	77.46%	69.45%
12 hours	84.73%	76.28%	68.34%	52.35%	80.18%	71.36%
24 hours	88.96%	78.34%	70.06%	55.63%	83.04%	73.33%

Figure 3. Average Cr Concentration Percentage after Bioremediation with Variation in Microbial Administration, Concentration and Incubation Time (Initial Cr Concentration = 2.5161 ppm)

The data from Table 1 dan Table 2 showed that the decrease in Cr concentration after bioremediation is influenced by the length of incubation time. The use of 6 hours incubation time has not shown a large number of Cr reductions, but the longer incubation time shows an increase in the reduction of Cr concentration (12 hours and 24 hours incubation time). Incubation time is related to the growth of microbes in the media, microbial growth factors are influenced by the time of interaction with the substrate on the microbial growth media. Microbial growth can be divided into 4 stages, including lag phase, log phase, stationary phase, and death phase (Tamam, 2016). At the 6-hour incubation stage, microbial growth in the lag period has a microorganism activity phase and prepares for the cleavage process, followed by 12-hour incubation, the activity of microorganisms increases and cell division occurs so that there is an increase in the number of cells in the

microorganism population. This phase occurs from the lag phase to the log phase and exponential growth occurs until finally at 24-hour incubation, experiencing a peak of microbial growth phase towards the next 24 hours the stationary phase is reached. So that at 24-hour incubation data obtained the highest reduction in chromium content in all microbial variations used. The microbial growth phase after 24 hours then experiences a death phase and it is possible that microbial activity will decrease (Tamam, 2016).

At the use of microbial concentrations of $10^{2.5}$ cells ml^{-1} showed a higher decrease compared to concentrations of 10^{5} cells ml^{-1}. The concentration of $10^{2.5}$ cells ml^{-1} microbes contains higher water, causing higher humidity. Bacterial and fungal growth is influenced by humidity and osmotic pressure (Tamam, 2016). The large concentration of bacteria will contain a large bacterial period, causing a greater ability to absorb Cr in the waste (Wazeck, 2013).

In this study, the concentration of *Bacillus subtilis* $10^{2.5}$ cells ml^{-1} showed higher results, made from 10 ml of bacteria plus 15 ml of aquadest, microbes with a concentration of 10^{5} cells ml^{-1} were made of 10 ml of bacteria plus 5 ml of aquadest, each using a rice bran carrier medium as much as 30 g (Kurniawan, 2014). Conditions of the addition of different aquadest in the volume of microbes and media carriers at the same magnitude will affect the moisture in the media grow microbes.

Another factor of microbial growth is influenced by the pressure of osmosis associated with the availability of water in the media. Water availability in microbial concentrations of 10^{5} cells ml^{-1} less, causing high concentrations in the media to grow microbes and the high concentration of the environment so as to exceed the concentration in cells and resulting in the occurrence of plasmolysis is the discharge of fluids in the cytoplasm of microbial cells. The occurrence of plasmolysis in this cytoplasm causes serious threats due to dehydration in the microbial cells so that the growth of microbes becomes obstructed (the number of microbes becomes fewer) at concentrations of 10^{5} cells ml^{-1} (Tamam, 2016).

The use of bacterial and fungal microbes at the same concentration shows that bacteria can reduce Cr levels higher. The mechanism of reduction is closely related to the ability of bacteria and fungi to survive in high metal concentrations (Akhmad, Yusran, Mariana, & Badruzsaufari, 2011). Bacteria in the process of detoxification can be by forming extra polymers that can bind metal as chelates, precipitate metals or transform metals into non-toxic forms (Gayathramma, Pavani, Singh, & Deepti, 2013).

Microorganisms use cells or metabolites in the form of enzymes to restore the polluted environment by means of absorption which can occur through a process of complexation, chelation, coordination, precipitation, ion exchange or oxidative-reductive processes (Ahemad, 2012; Juwarkar, Misra, & Sharma, 2014). The bioremediation process for the retrieval of chrome metal ions by Bacillus subtilis from a solution can be done in three ways i.e. (1) Metal adsorbed on the cell surface of microorganisms; (2) The absorption of metal ions enters into cell microorganisms and (3) the chemical transformation of metal ions by microorganisms (Pramono, Rosariastuti, Ngadiman, & Irfan, 2013).

Table 3 showed that the percentage reduction in Cr with $10^{2.5}$ cells ml^{-1} *Bacillus subtilis* and an incubation time of 6 hours, 12 hours, and 24 hours respectively from high to low were 79.21%, 84.73%, and 88.96%. Table 3 also showed that the percentage reduction in Cr with $10^{2.5}$ cells ml^{-1} mixture *Bacillus subtilis* and *Saccharomyces cerevisiae* with incubation times of 6, 12, and 24 hours, respectively

77.46%, 80.18%, and 83.04%. Table 4, showed that the lowest decrease of Cr is in the use of 10^5 cells ml^{-1} *Saccharomyces cerevisiae* with the incubation time of 6, 12, and 24 hours, respectively 50.17%, 52.35%, and 55.63%.

This research suggests that *Bacillus subtilis* manages to lower the chrome metal levels greater than that of *Saccharomyces cerevisiae*. A *Bacillus subtilis* ability in binding to chrome metal with extracellular fluid and the presence of *Cytochrome* as a manufacturer of chromate reductase enzymes can alter Cr(VI), then enter into the inner cell membrane and be converted into Cr(III) (Das et al., 2015). Saccharomyces cerevisiae has different abilities due to the structure of the cell wall consisting of a drum and mannan, so hen no incoming chrome ions will be placed in the Vacuola and partially secreted from the cell (Ribeiro et al., 2019). The presence of a portion of Chrome ions released again from the cell causes the absorption of chrome metal to be not maximal.

The use of mixed microbes can occur mutually affect (Waluyo, 2019), as seen in the mixture of *Bacillus subtilis* and *Saccharomyces cerevisiae*. The influence of one microbe with other microbes can be deadly or reduce life activities (antagonism, competition), microbes with one another there are also mutually reinforcing/beneficial activities of life (mutualism, synergism) and there is no influence (neutralism).

CONCLUSION

This research established that bioremediation using the microbial *Bacillus subtilis* and *Saccharomyces cerevisiae* has been proven to reduce levels of Cr in the electroplating industry wastewater, and the highest reduction results were achieved on the use of 24-hour incubation time and the use of *Bacillus subtilis* with a concentration of $10^{2.5}$ cells ml^{-1} which was 88.96%. Furthermore, the concentration of $10^{2.5}$ cells ml^{-1} *Bacillus subtilis* lowers the chrome metal levels most commonly compared to other microbial concentration variations.

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