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Protein expression on Cr resistant microorganism using electrophoresis method

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Abstract. *Fatmawati U, Suranto, Sajidan. 2009. Protein expression on Cr resistant microorganism using electrophoresis method. Nusantara Bioscience 1: 31-37.* Hexavalent chromium (Cr(VI)) is known as toxic heavy metals, so the need is reduced to Cr(III) is much less toxicity. *Pseudomonas aeruginosa, Pseudomonas putida, Klebsiella pneumoniae, Pantoea* sp., and *Saccharomyces cerevisiae* are resistant Cr(VI) microorganisms and can reduce Cr(VI). The aim of this research is to know ability of the microorganism to reduce Cr(VI) and to know the protein band pattern between Cr(VI) resistant microorganisms and non-resistant microorganisms inoculated on LB broth. SDS-PAGE was used to identify protein expression. At the same time, Cr(VI) concentration was identified by the 1.5 diphenyl carbazide method. The quantitative data were analyzed by two factorial ANOVA that continued with DMRT at a 1% level test. The qualitative data, i.e., protein expression analyzed by relative mobility (Rf). The results showed that the ability of microorganisms to reduce Cr(VI) at an initial concentration of 0.5 ppm, one ppm, five ppm, and 10 ppm might vary; the average percentage of the ability of each microorganism in reducing Cr(VI) is *P. putida* (65%) > *S. cerevisiae* (64.45%) >. *P. aeruginosa* (60.73%) > *Pantoea* sp. (50.22%) > *K. pneumoniae* (47.82%) > without microorganisms (34.25%). The adding microorganisms have significantly influenced toward reduction of Cr(VI). The SDS-PAGE shows that protein expression between resistant and not resistant microorganisms are no different, but resistant microorganisms have more protein (protein band is thicker).

Keywords: Cr heavy metal, microorganism, protein, electrophoresis.

Abstrak. *Fatmawati U, Suranto, Sajidan. 2009. Ekspresi protein pada mikroorganisme resisten Cr dengan metode elektroforesis*. *Nusantara Bioscience 1: 31-37.* Krom heksavalen (Cr(VI)) dikenal sebagai logam berat beracun, sehingga perlu direduksi menjadi Cr(III) yang lebih rendah toksisitasnya. *Pseudomonas aeruginosa, Pseudomonas putida, Klebsiella pneumoniae, Pantoea* sp. dan *Saccharomyces cerevisiae* adalah mikroorganisme resisten dan mampu mereduksi Cr(VI). Tujuan penelitian ini adalah mengetahui kemampuan mikroorganisme dalam mengurangi Cr(VI) dan mengetahui pola pita protein antara mikroorganisme resisten Cr(VI) dan mikroorganisme tidak resisten yang diinokulasi pada medium kaldu LB. SDS-PAGE digunakan untuk mengetahui ekspresi protein, sementara konsentrasi Cr(VI) diidentifikasi dengan metode 1,5 difenilkarbazid. Data kuantitatif dianalisis dengan ANAVA dua faktorial dilanjutkan dengan uji jarak berganda Duncan pada taraf 1%. Data kualitatif yaitu ekspresi protein dianalisis dengan mobilitas relatif (*Rf*). Hasil penelitian menunjukkan bahwa kemampuan mikroorganisme dalam mereduksi Cr(VI) pada konsentrasi awal 0.5 ppm, 1 ppm, 5 ppm dan 10 ppm berbeda-beda, persentase rata-rata kemampuan masing-masing mikroorganisme dalam mereduksi Cr(VI) adalah: *P. putida* (65%) > *S. cerevisiae* (64,45%) > *P. aeruginosa* (60,73%) > *Pantoea* sp. (50,22%) > *K. pneumoniae* (47,82%) > tanpa mikroorganisme (34,25%). Penambahan mikroorganisme secara nyata mempengaruhi reduksi Cr(VI). SDS-PAGE menunjukkan bahwa ekspresi protein antara mikroorganisme resisten dan tidak resisten tidak berbeda, tetapi mikroorganisme resisten memiliki lebih banyak protein (pita protein lebih tebal).

Kata kunci: logam berat Cr, mikroorganisme, protein, elektroforesis.

INTRODUCTION

Chromium (Cr), as one of the heavy metal contaminants, can potentially become a pollutant as a result of coloring fabrics in the textile industry, paints, leather tanning, metal plating, batteries, or industrial chromium (Ackerley et al. 2004). Through the food chains, chromium can be deposited in a living body part, which can cause toxicity at a certain size (Mulyani 2004). Generally, chromium in open nature is in the valence of 3 (Cr^{3+}) and valence 6 (Cr 6 +). Cr^{6+} is more toxic than Cr^{3+}. The toxicity of Cr^{6+} is due to its high solubility and mobility in the environment (Palar 1994; Lowe et al. 2002; Uprati et al. 2003; Rahman et al. 2007). It can cause DNA structural damage or generate mutations (Larashati 2004).

Several microorganisms species such as *Pseudomonas putida, Pseudomonas aeruginosa, Klebsiella pneumoniae, Pantoea* sp., and *Saccharomyces cerevisiae* are resistant to heavy metal contamination to reduce Cr(VI) to Cr(III). Research conducted by Krauter and Krauter (2002) concludes that *S. cerevisiae* can reduce Cr(VI) at 100% of the initial 1.89 ppm concentration at an optimum pH of 6.5-7. While in acidic conditions (pH = 2.1), the corps of *S. cerevisiae* can still reduce the Cr(VI) level by 70%.

Jianlong *et al.* (2003) tested the tolerance level of *S. cerevisiae* at a concentration of Cr(VI) 5 uM, and it was found that it did not affect microbial growth, while at the concentration of Cr(VI) 15 uM it inhibited microbial growth by 30%.

Ganguli and Tripathi (2002) report that the bacteria *P. aruginosa* can reduce Cr(VI) by 96% of the initial concentration of Cr(VI) at 10 ppm. But the bacteria *P aeruginosa* also has a limited ability to reduce the concentration of 50 ppm, which is only reduced by 16%. *P. putida* is a bacterium resistant to Cr and Cd to be used to reduce Cr in a certain medium (Timothy et al. 1989; Lowe et al. 2002; Ackerley et al. 2004; Rahman et al. 2007). Bacteria *P. putida* can reduce Cr(VI) with a speed of 6 ppb min-1, which was previously tested on gelatin-containing Cr (IV) (Lowe et al. 2002). *K. pneumoniae* which was inoculated in BHI medium, can reduce Cr(VI) by 27% (Mardiyono 2005). Obraztsova *et al.* (2002) suggested that the reduction of Cr(VI) 150 ppm by *Pantoea sp* was optimum with the addition of sulfate (SO4-2), which took 20 hours.

Knowing the potential for some microorganisms such as *P aeruginosa, P. putida, K. pneumoniae, Pantoea* sp., and *S. cerevisiae* in reducing heavy metal Cr, then research was conducted on the ability of these microorganisms to reduce Cr(VI) in liquid media containing heavy metal Cr, to know the genetic changes in microorganisms through the expression of protein bands by polyacrylamide gel electrophoresis detection in SDS-PAGE. Electrophoresis is the ideal analysis to purify the protein component of the mixture sample by adding the medium that can bind proteins during the electrophoresis process. Polyacrylamide gel electrophoresis is the best method to purify proteins by electrophoresis (PAGE). Polyacrylamide gel is a solution of acrylamide and bis-acrylamide (Davis and Heywood 1963; Hames and Rickwood 1990; Matsudaira 1993).

This research aimed to know the microorganism's knowability to reduce Cr(VI) and to know the protein band pattern between Cr(VI) resistant microorganisms and non-resistant microorganisms inoculated on LB broth.

MATERIALS AND METHODS

The microorganism used. Microorganisms such as *K. pneumoniae, P. aeruginosa, P. putida, Pantoea* sp., and *S. cerevisiae* are used to test the reduction of Cr(VI). The expression pattern of protein bands is obtained from the University of Gadjah Mada University, Yogyakarta. Bacterial isolates are multiplied in LB liquid medium. The composition of each isolate was 100 mL of 1 g Tryptone, 0.5 g yeast extract, and 0.5 g NaCl, and for the proliferation of molds liquid PDA (potato dextrose agar) medium was used.

Preparation of liquid media which contains heavy metal Cr. A total of 0.1414 g $K_2Cr_2O_7$ was reconstituted with distilled water in a 1-liter measuring flask and diluted until it reached mark boundaries to obtain solution concentrations of 0.05 mg/mL to prepare a supply of standard Cr. Then standard chromium solution was made by diluting 1 mL of Cr stock solution into 100 mL liquid medium until we obtained liquid media containing concentrations of heavy metals Cr 0.5 ppm. For concentrations of 1 ppm, five ppm, and 10 ppm, Cr preparations were done by diluting standard solution into a liquid medium (LB or PDA) as much as 100 mL.

Counting the number of microorganism cells. To determine the ability to live microorganisms to survive in media containing heavy metals, the number of cells inoculated in microorganisms was calculated in liquid media with Cr(VI) 0 ppm and 10 ppm for 16 hours. The growing culture was diluted several times by 10^{-5} and 10^{-6}. The result of dilution was grown on 100 µL of solid LB media, and then it was incubated again for 16 hours at 37ºC. Colonies formed then were calculated by *colony counter*, and the number of microorganisms cells was also counted in units of cells/mL (Hadioetomo 1993). Cultures that grew on both Cr(VI) were resistant microorganisms, while the cultures that only grew on the control were not resistant.

Inoculation of microorganisms in liquid LB media. To determine the ability to reduce Cr(VI), each microorganism was taken with an ose needle and was grown in Erlenmeyer containing liquid LB media (Luria-Bertani media) with Cr(VI) 0 ppm, 0.5 ppm, one ppm, five ppm, and ten ppm concentration. Each species of microorganism was grown in 100 mL of LB liquid medium on five different initial concentrations of Cr(VI) above. They were then incubated in an incubator with a temperature of 30-36ºC for 16 hours.

Hexavalent chromium test. A total of 50 mL liquid bacterial culture medium containing chromium was put into Eppendorf tubes and centrifuged at 3000 rpm for 30 minutes; the supernatant was collected and filtered with Whatman filter paper of 0.2 µm and then the heavy metal content was analyzed (Lowe et al. 2002). The solution was then neutralized by adding H_2SO_4 (1+1) or NH_4OH, and then it was added with 1 mL of H_2SO_4 (1+1) and 0.3 mL of 85% H_3PO_4. The solution was then quantitatively transferred into a 100 mL measuring flask, and a 2 mL diphenyl carbazide solution was added, diluted to mark boundaries, and whipped until it was well mixed. After 5-10 minutes, it was measured with a UV-VIS spectrophotometer with a wavelength of 540 nm.

Preparation of standard solution of chromium. Standard supply chromium solvent as much as 20-20 mL was taken with a pipette (2, 4, 6, 8, and 10, and so on in stages) into several pieces of measuring 100 mL flask. Put 25 mL of distilled water into the other measuring flask, a blank. To add 1 mL of H2SO4 (1 +1) into each measuring flask, 0.3 mL 85% H_3PO_4 and 2 mL of solution diphenylcarbazide, then diluted until it gets the boundary mark and whip until blended, allowed to stand for 5-10 minutes. Define the absorption in the wavelength of 540 nm, and a calibration curve is made. Then, calculate the chromium content in mg/L for the calibration curve.

SDS-PAGE electrophoresis. A total of 5 mL bacterial culture was poured into 1.5 mL an Eppendorf tube, and the cells were sedimented by centrifuging for 5 minutes at a

speed of 13,000 rpm. The cell sediment was then cleaned from a liquid LB medium by removing its supernatant. The pellet cell, which settled then, was suspended with Phosphate Buffered Saline Solution (PBS) twice, then it was again centrifuged back, and the PBS supernatant was discarded. One mL of PBS was added to the pellets. To break the cells, sonication was used for 30 seconds 4 times. Redisentrifuse it and take the supernatant for running by adding sample buffer (4:1/v: v). Before being placed in wells, the mixture of sample and sample buffer was boiled in boiling water for 2 minutes, then put in ice for + 5 minutes, after which the samples were ready for running. The gel formed (*discontinuous gel* 10% and 3% *stacking gel*) was transferred into the electrophoresis tank (Hames and Rickwood 1990).

Furthermore, the electrophoresis tank was filled with *running buffer* (0.19 M Glycine, 10 mL SDS 10%, and 0.0248 M Tris in 1 L) until full. As much as 10 µL of micropipette of a mixture of the sample and the sample buffer was carefully poured into the electrophoresis wells. Then close the tank lid and set the voltage (100V, 90 minutes). To identify the molecular weight of proteins, use protein markers with a molecular weight range of 212-11.3 kDa.

Coomassie Blue staining. The solution was made with the composition of 1 g Coomassie Blue coloring that was dissolved in 1 L of destaining solution (100 mL acetic acid, 400 mL of methanol, then diluted with the addition of distilled water until it reached the volume of 1 L) (Hames and Rickwood 1990). After running, the gel was soaked in dye solution Coomassie blue for 12 hours; then, it was washed with destaining 3-4 times for 2 hours until the protein band pattern was formed.

Data analysis. Differences in the ability of each microorganism to reduce the heavy metals Cr(VI) at each concentration were analyzed by analysis of variance (ANOVA) followed by a further test of Duncan Multiple Range Test (DMRT). The differences in protein expression between the microorganisms were described descriptively.

RESULTS AND DISCUSSION

The growth of microorganisms in media containing Cr

Preliminary test results in the form of counting the number of colonies, or *Colony Form Unit (CFU)* of five species of microorganisms which were grown in a gel medium containing 10 ppm Cr(VI) indicated that the five species of microorganisms were able to live in a media containing Cr(VI) (Table 1). The highest number of cells contained in *S. cerevisiae* was 460x106 cells/mL at a concentration of 0 ppm Cr(VI), while the concentration of 10 ppm Cr(VI) *S. cerevisiae* cells also produce most of the 317x106 cells/mL. The number was obtained by calculating the average number of colonies of two types of dilution: 10-5 and 10-6. The gel medium used to grow *S. cerevisiae* was Potato Dextrose Agar (PDA) because *S. cerevisiae* is a yeast that can ferment. It needs a lot of substrate fermentation of glucose that will be converted into ethanol. The ability of *S. cerevisiae* to survive in a gel medium containing Cr(VI) indicates that these microorganisms are resistant or tolerant to Cr heavy metals (Jianlong et al. 2003; Mulyani 2004; Gao *et al*. 2006). *P. aeruginosa* also live in a media containing heavy metal Cr(VI) 10 ppm. It is proven by the growth of colonies on LB agar media containing Cr(VI) 10 ppm with an average cell amount of 287x106 cells/mL. Other microorganisms also can live in a media containing heavy metal Cr(VI) 10 ppm. The fewest number of cells observed on *Pantoea* sp. with the number of cells of 177x106 cells/mL.

Five species of microorganisms, in general, have a decrease in the number of cells in media containing heavy metal Cr(VI). The lowest reduction in the number of cells is present in *K. pneumoniae* by 20.7%, while the highest decrease is in *P. putida* at 34.5%. The decrease in the number of cells of inoculated microorganisms in media containing heavy metal Cr(VI) shows that these microorganisms select the tolerant variant toward the heavy metals. Based on Table 1, the decrease in the number of cells of microorganisms after being inoculated into the gel medium with the addition 10 ppm of Cr(VI), generally as much as 20-30%, a decline that is not too extreme and indicates that the five species of microorganisms are resistant to the environment containing the metal weight of Cr(VI).

The microorganisms that are capable of living in media containing Cr(VI) can also serve as a reduction of heavy metal Cr(VI) to Cr(III). Several studies have proven that the presence of Cr(VI) at levels of 0-50 ppm in the cells of the microorganisms does not interfere with cell growth microorganisms (Jianlong et al. 2003; Gao et al. 2006; Rahman et al. 2007) because, besides growth, the microorganisms will make side product of H2S. The increase in the number of cells of microorganisms will increase the speed of H2S production that will accelerate the reduction of Cr(VI). H_2S, produced by the bacteria, will react with chromium to form chromium sulfides that are not stable in solution and will more quickly be deposited to form Cr $(OH)_3$, namely Cr with a valence of three who has lower toxicity of Cr valence six.

Table 1. The number of cells inoculated microorganisms on solid LB medium with the addition of Cr(VI) 0 ppm and 10 ppm.

Species	Average number of cells (cell/mL)		Percentage decrease in the number of cells
	0 ppm	10 ppm	
P. aeruginosa	$370x10^6$	$287x10^6$	22.4%
P. putida	$352x10^6$	$230x10^6$	34.6%
K. pneumoniae	$303x10^6$	$240x10^6$	20.7%
Pantoea sp.	$254x10^6$	$177x10^6$	30.3%
S. cerevisiae	$460x10^6$	$317x10^6$	31%

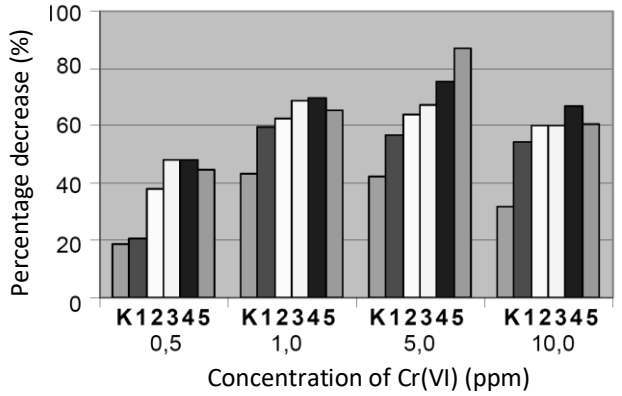

Figure 1. Percentage decrease in Cr(VI) by five species of microorganisms. Note: K = without microorganisms or control, 1 = *K. pneumoniae*, 2 = *Pantoea* sp., 3 = *P. aeruginosa*, 4 = *P. putida*, 5 = *S. cerevisiae*.

Reduction of Cr(VI) in liquid media

Based on the result of the preliminary research, it is proven that the five species of microorganisms are capable of living in a gel medium containing heavy metal Cr(VI). Then the test was conducted to determine the changes in levels of Cr(VI) before and after treatment. The treatment was done with five species of microorganisms, namely: *K. pneumoniae, Pantoea* sp., *P. aeruginosa, P. putida,* and *S. cerevisiae* which were inoculated in LB liquid media containing Cr(VI) 0.5 ppm, one ppm, five ppm, and ten ppm with the initial inoculum concentration of 1% (Mardiyono 2005) and was incubated for 16 hours at room temperature on a shaker. The decreased percentage in Cr(VI) by five species of microorganisms at different concentrations is shown in Figure 1.

The reduction ability of Cr(VI) by the five species of microorganisms is tested on LB liquid medium with a solution of 1 ppm Cr(VI). On visual observation, there is a difference between liquid LB media that are not inoculated microorganisms and LB liquid medium, which is inoculated with five species of microorganisms. The liquid media that are inoculated by microorganisms look more cloudy. This indicates that there are growth and proliferation of cells in the media. In addition to the growing activity, some microorganisms also can reduce Cr(VI) (Suzuki et al. 1992; Ganguli and Tripathi 2002; Krauter and Krauter 2002; Jianlong et al. 2003; Mulyani 2004; Upreti et al. 2004; Mardiyono 2005). *Saccharomyces cerevisiae* has the highest ability to reduce among other microorganisms. The decreased percentage in *S. cerevisiae* is as much as 87% at the initial concentration of 5 ppm. At the initial concentration of this five ppm, the lowest percentage decline occurred in K. pneumoniae, as much as 56.7%. In the treatment without bacteria, a decrease also happens by 42.5%. Several other studies state that Saccharomyces is a microorganism with the highest effectiveness in reducing Cr(VI). Krauter and Krauter (2002) state that *S. cerevisiae* can reduce Cr(VI) 100% at pH 6.5-7, while its bio removal capabilities are less effective at acidic pH.

One of the benefits for *Saccharomyces* as an agent of biosorption of Cr(VI) is that its character is not pathogenic compared to other bacterial pathogens such as *P. aeruginosa* and *K. pneumoniae. S. cerevisiae* can also reduce other heavy metals such as Mo, Co, Ca, Zn, Sr, Hg, and Cu in water (Krauter and Krauter 2002; Mulyani 2004).

Pseudomonas putida has the second-largest percentage in reducing Cr(VI), 66.8% in the initial concentration of Cr(VI) initial five ppm. The lowest ability is bacteria *K. pneumoniae* at 20.4% on the initial concentration of 0.5 ppm. In the treatment without adding microorganisms, there was also a decrease of 31%. Several other studies also use bacterium *P. putida* to reduce Cr(VI) into Cr(III); among others are Ackerley et al. (2004), Lowe et al. (2002), Timothy et al. (1989), and Rahman et al. (2007). The results show that *P. putida* can reduce Cr(VI).

The highest initial concentration of Cr(VI) used in this study is ten ppm. At this concentration, the microorganisms still can grow and reproduce; it is seen from the turbidity of liquid LB medium inoculated with five species of microorganisms. Logically, with higher survival ability, indeed, there must be more microorganisms that live to increase the ability to reduce Cr(VI) into Cr(III) in the environment where Cr(VI) is also greater. The highest percentage in decreasing Cr(VI) occurs at concentrations of one ppm and five ppm.

Cr(VI) on five species of microorganisms

Based on Figure 2, we can see the order of microorganisms' ability to reduce Cr(VI). The highest ability is in bacteria *P. putida* with an average percentage of reduction as much as 65%, while the lowest is bacteria *K. pneumoniae* with an average percentage reduction of 47.8%. The sequence of comparison of the ability to reduce Cr(VI) among the microorganisms are as follows: *P. putida* (65%)> *S. cerevisiae* (64.45%)> *P. aeruginosa* (60.73%)> *Pantoea* sp. (50.22%)> *K. pneumoniae* (47.82%)> without microorganisms (34.25%).

The analysis results of variance calculations of the influence of initial concentration of heavy metals Cr(VI) and species of microorganisms toward the decrease in Cr(VI) is shown in Table 2. Based on the calculation of two-factorial ANOVA where the main factor is the species of bacteria and the subplot factor is the concentration, it can be concluded that the species of microorganism have real effects on decreasing the concentration of Cr(VI) at level 1%. The initial concentration of Cr(VI) has a real impact on reducing the concentration of Cr(VI) at level 1%, and the interaction of species of microorganisms with an initial concentration of Cr(VI) has a significant effect on decreasing the concentration of Cr(VI) at level 1%.

From the fact above, there are significant variations of effect in the species of microorganisms and the initial concentration of heavy metals Cr(VI) to the decline of heavy metal concentrations of Cr(VI). Further tests, namely the DMRT, to the decline of heavy metal concentrations of Cr(VI) are shown in Table 3.

The presence of Cr(VI) in the environment can interfere with the organism and result in the selection of resistant

Table 2. Results of analysis of variance the variation of initial concentration of Cr(VI) and species of microorganisms to the decrease of Cr(VI).

Source of diversity	Degrees of freedom	Sum of squares	Middle Squares	F Calculate	F table 5%	F table 1%
Replication	2	108.258	54.129			
Microorganisms (A)	5	8626.752	1752.350	50.858	3.330*	5.640
Error (a)	10	339.249	33.925			
Initial conc. of Cr (VI) (B)	3	8988.810	2996.270	101.644	2.860*	4.380
AxB	15	1350.517	90.034	3.054	1.960*	2.580
Error (b)	36	1061.207	29.478			
General	71	20474.792				

Note: kk (a) = 10.631%; kk (b) = 9.910%; * = significant difference at level 1%

Table 3. Further test results significantly different from Duncan's to the decline of Cr (VI) concentrations.

Species of microorganisms	Initial concentrations of Cr (VI)			
	0.5 ppm	1 ppm	5 ppm	10 ppm
Without microorganisms	18.8^{e}	43.06^{e}	42.52^{e}	31.83^{e}
K. pneumoniae	20.46^{e}	59.82^{abcd}	56.72^{cd}	54.42^{abcd}
Pantoea sp	38.16^{abcd}	62.59^{abcd}	64.00^{bcd}	59.94^{abcd}
P. aeruginosa	48.12^{ab}	68.95^{ab}	67.32^{bc}	60.14^{abc}
P. putida	48.16^{a}	69.50^{a}	75.63^{ab}	60.65^{a}
S. cerevisiae	44.81^{abc}	65.4^{abc}	87.00^{a}	66.87^{ab}

Note: numbers followed the same letter showed no significant difference in the level of DMRT 1%.

bacteria. Compounds of Cr(VI) are more dangerous than Cr(III) due to their high solubility in water, rapid permeability, and subsequent interaction with intracellular proteins and nucleic acids (Upreti et al. 2004). Microorganisms can develop resistance mechanisms to select the next resistant variants.

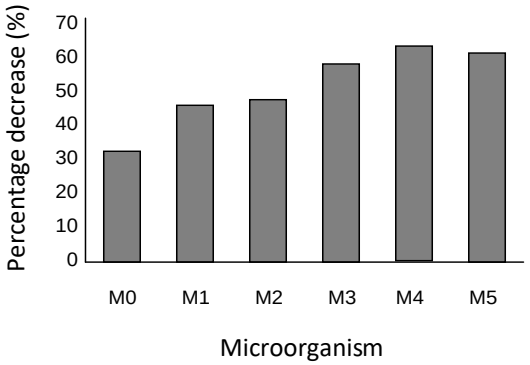

Figure 2. The average reduction capability. Note: M0 = without microorganisms (control), M1 = *K. pneumoniae*, M2 = *Pantoea* sp., M3 = *P. aeruginosa*, M4 = *P. putida*, M5 = *S. cerevisiae*

As noted previously, according to Rahman et al. (2007), reduction of Cr(VI) happens because not only of growth but also the fact that microorganism produces byproducts in the form of H2S. The increasing number of cells of microorganisms will increase the speed of H2S production that will accelerate the reduction of Cr(VI). H2S produced by the bacteria will react with chromium to form chromium sulfides that are not stable in solution and will more quickly be deposited to form Cr $(OH)_{3,}$ which is Cr with a valence of three who has lower toxicity of Cr with a valence of six. Meanwhile, according to Suhendrayatna (2001), reduction of Cr(VI) to Cr(III) by microorganisms called bio removal has two kinds of mechanisms, namely passive and active. Passive absorption is known as biosorption. This process occurs when heavy metal ions bind to the cell wall in two different ways, namely (i) ion exchange of monovalent and divalent ions which like Na, Mg, and Ca on the cell wall was replaced by heavy metal ions, and (ii) complex formation between heavy metal ions with functional groups such as carbonyl, amino, thiol, hydroxyl, phosphate, hydroxyl, carboxyl located on the cell wall.

The biosorption process can occur back and forth and quickly. The process of alternating bonds of heavy metal ions on the surface of these cells can occur in both dead cells and living cells from biomass. The biosorption process can also be more effective at certain pH and other ions in the medium in which heavy metals can be deposited as salt, which is not dissolved. Heavy metal absorption can also occur actively, which occurs in various types of living cells. This mechanism simultaneously occurs in line with the consumption of metal ions for the growth of microorganisms or the accumulation of intracellular heavy metal ions. Heavy metals can also be deposited in the metabolism and excretion process. This process depends on its energy and the sensitivity of different parameters such as pH, temperature, ionic strength, and light. This process can also be inhibited by low temperatures, lack of energy sources, and inhibition of cell metabolism (Suhendrayatna 2001).

Protein expression in microorganisms resistant Cr(VI) in Figure 3 shows the results of running the pattern of protein bands on SDS-PAGE of the five species of microorganisms, namely *K. pneumoniae*, *Pantoea* sp., *P. aeruginosa*, *P. putida*, and *S. cerevisiae*, each sample of which is extracted from the cells of microorganisms that are grown in LB liquid culture with concentrations of Cr(VI) 0 ppm and ten ppm. These two kinds of concentration are chosen to distinguish between populations of microorganisms that are resistant and that are not resistant.

Protein markers can be used to identify the molecular weight of a mixture of polypeptides (Hames and Rickwood 1990). In this study, the protein characteristics used to have a molecular weight range of 212-11.3 kDa. From the electrophoresis results, there are several protein bands with different thicknesses. The protein with more thickness and greater color intensity than any other protein and is always there in every variety is called major protein (Wijaya and Rahman 2005).

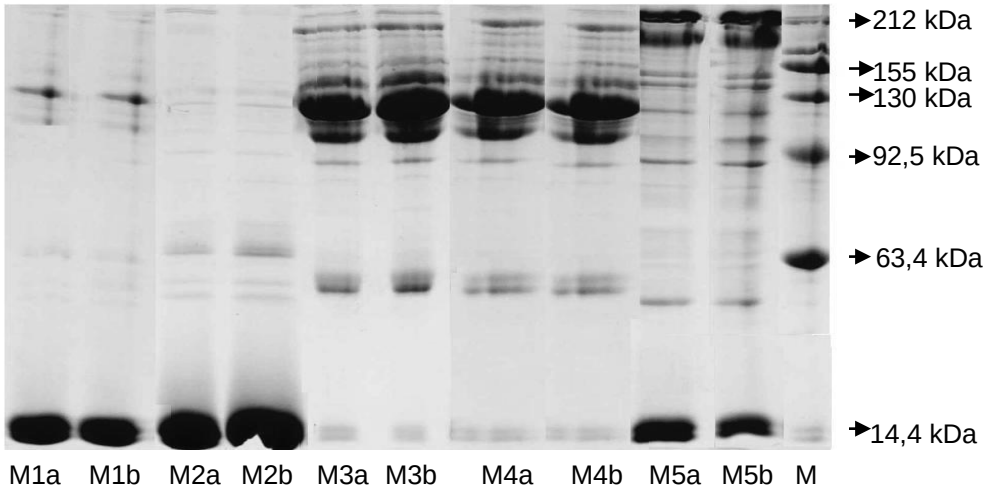

Figure 3. Protein expression in five species of microorganisms are grown on LB liquid medium with Cr(VI) 0 ppm and 10 ppm. Note: M = marker proteins, M1a, M1b = *K. pneumoniae*, M2A, M2b = *Pantoea* sp., M3a, M3b = *P. aeruginosa*, M4A, M4b = *P. putida*, M5a, M5b = *S. cerevisiae*. a = 0 b = 10 ppm ppm

In the electrophoresis results, there are several major proteins on the species of *P. aeruginosa* (M3) and *P. putida* (M4). This major protein molecular weight ranges from 148.7 kDa, 121.6 kDa, and 105 kDa. The two major proteins of this type have the same color intensity and thickness because these two microorganisms belong to the same genus, Pseudomonas. Bacteria *K. pneumoniae* (M1) has several protein bands. Still, because proteins from the running sample are only a few, the formation of bands on polyacrylamide gel is less than optimal. From Figure 3, we can note that there are three major protein bands of resistant microorganisms and not resistant microorganisms with the molecular weight of 121.6 kDa, 14.4 and 14 kDa, three kDa. Generally, the pattern of protein bands from two running samples does not look dramatically different because they are actually of one type.

Protein running of *S. cerevisiae* (M5) shows the same banding pattern among the microorganisms that are resistant and which are not resistant. These microorganisms have three pairs of major proteins with a molecular weight of 212 kDa, 188.5 kDa, 14.5 kDa, and 14.4 kDa. In addition, there are some bands with less color intensity due to less protein concentration. The lower the location of protein bands is, the smaller the molecular weight. This happens because the low molecular weight has a greater speed of migrating in the matrix medium polyacrylamide.

The reason why electrophoresis was used in this study is that it has a vital role in separating biological molecules, especially proteins. This method does not affect the structure of biopolymers and is very sensitive to the difference in charge and small molecular weight (Bachrudin 1999). Proteins that run in a medium that contains an electric field cause the charged compounds to move in the solution due to the nature of the opposite polarity so that the mobility of a molecule is a function of shape, molecule size, and large content type.

The use of SDS and mercaptoethanol accompanied by heating will break the three-dimensional structure of proteins, particularly the disulfide bonds into polypeptides subunits individually. SDS also wraps the chain of proteins not bound by the same negative charge to form an SDS-protein complex. SDS-protein complexes have an identical charge density and move on the gel based on the size of the protein (Wijaya and Rohman 2005). Therefore, the greater SDS-protein complexes have slower mobility than the smaller SDS-protein complexes.

Methods for extracting proteins in microorganisms are done with *Phosphate Buffer Saline Solution* (PBS) solution followed by breaking the cell using sonification (cell-breaking equipment using sound waves that produce high frequency). The purpose of cell breaking is to provide opportunities to extract the protein that will be purified. The cells that have been broken must be preserved from the influence of oxygen because oxygen can cause the protein to be inactive, denatured, and compact (Bachrudin 1999).

CONCLUSION

The species of microorganisms *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Pseudomonas putida*, *Pantoea* sp., and *Saccharomyces cerevisiae* have a significant effect on the percentage reduction of Cr(VI). The variation of the initial concentration of Cr(VI) also has a very substantial impact on the percentage reduction of Cr(VI) and interaction species of microorganism with an initial concentration of Cr(VI), which have a significant effect on decreasing the concentration of Cr(VI). Microorganisms' ability in reducing Cr(VI) to Cr(III) can be sorted as follows: *P. putida* (65%)> *S. cerevisiae* (64.45%)> *P. aeruginosa* (60.73%)> *Pantoea* sp. (50.22%)> *K. pneumoniae* (47.82%)> without microorganisms (34.25%).

Expression of proteins formed on each of the microorganisms that are resistant and are not resistant have almost the same pattern of protein bands.

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