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Microbial reduction of Cr (VI) in to Cr (III) by locally isolated Pseudomonas aeruginosa

H S H Munawaroh*, G G Gumilar2, A B D Nandiyanto3, S Kartikasari 1, D Kusumawaty5, L Hasanah6

1-4Program Studi Kimia, FPMIPA Universitas Pendidikan Indonesia
3Program Studi Biologi, FPMIPA Universitas Pendidikan Indonesia
6Program Studi Fisika, FPMIPA Universitas Pendidikan Indonesia

Jl. Dr. Setiabudhi No. 229, Bandung 40154, Jawa Barat-Indonesia

*heli@upi.edu

Abstract. This study aims at investigating the influence of Cr (VI) on growth of pseudomonas aeruginosa and Cr (VI) reduction in to Cr (III) on leather tannery effluent model by indigenous pseudomonas aeruginosa. The effect of Cr (VI) on pseudomonas aeruginosa growth was checked by growing the isolated in the medium containing certain concentration of sodium dichromat (K_2Cr_2O_7) as source of hexavalent chromium. Spectrophotometer analysis showed that the rate of pseudomonas aeruginosa growth decreased at the concentration of 100 ppm of hexavalent chromium (Cr (VI)). In addition, the reduction of Cr (VI) was monitored by growing the isolated in the medium with K_2Cr_2O_7. The result of AAS and spectrophotometer show the decreased of Cr (VI) concentration in the medium from 100 to 5.86 ppm with the conversion efficiency reach to 94.73% during 48 hours of the treatment. High efficiency conversion of Cr (VI) in to Cr (III) indicates the possibility of pseudomonas aeruginosa as promising bioremediation agent to reduce Cr (VI) in the environment.

1. Introduction

Leather industries employ chromium-tanning processes to boost their production and leather quality [1][2]. Unfortunately the most of the residual chromium thus is discharge in solid or liquid effluent. Indeed, the waste problem gained from chromium cannot be avoided. Naturally, chromium may exist in different oxidation states with the most stable and common forms are the hexavalent chromium [Cr(VI)] and trivalent chromium [Cr(III)] [1]. The existance of Cr(VI) in the environment mostly comes from the industrial activities, while Cr(III), naturally predominates in the environment [1] especially in the acidic conditions. Due to the Cr(VI) strong oxidation properties, significant quantities of Cr(VI) in the environment may constitute toxicological risk to living organisms [2]. Cr(VI) also causes sometimes life-threatening illness including irreversible damage to vital body system [3].

Many physical and chemical removal-based methods were studied to solve the Cr(VI) waste. Currently, the effluents are treated with ferrous sulphate, chemical reduction, followed by either alkaline precipitation or removal by ion exchange; however, the adsorption that suffers from precipitation and additional treatment methods to remove those is to be sorted [3]. However, these methods require high cost and high energy to conduct the process. Instead of it, the process also
sometimes produce non eco-friendly waste product. In response to this challenge, bioremediation of Cr (VI) using indigenous bacteria emerged as sustainable and an environmentally compatible technology.

Some microbial species have been reported to reduce Cr (VI) under either aerobic or anaerobic condition. These microorganisms have developed the capabilities to protect themselves by various mechanisms, such as adsorption, uptake, methylation, oxidation, and reduction [4]. Illustration model of microabial-based bisorption was shown on Fig. 1. Some bacteria can remove Cr(VI) by uptake it as a nutrient for their metabolism, or convert Cr(VI) into Cr(III), which is less toxic and less mobile compared to Cr(VI). For example, *Acinetobacter haemolyticus* show an ability to remove Cr(VI) by adsorb it into their cell membrane [5], while *Pseudochrobactrum* sp., *Proteus* sp., *Bacillus* sp., and *Bacillus methylotrophicus* are found to be Cr(VI) resistant and reduce it existence in the environment by reduction it into Cr(III) [6][7][8]. Another enzymatic transformation of Cr(VI) into Cr(III) was performed by *Ochrobactrum* sp.[9]. Other mechanism which is conducting by bacteria is detoxifying and immobilizing Cr(VI) by converting it into the insoluble chromium hydroxides (Wang (1990); see reference [1]).

The unique ability of each strain bacteria in reducing Cr(VI) may be of use as promising means of bioremediation of Cr(VI). In the present study, an indigenous Cr(VI) resistant bacteria, *Pseudomonas Aeruginosa*, was characterized and the effects of Cr(VI) on its growth and Cr(VI) reducing ability were determined by spectrophotometer and AAS analysis. The result found the decreased of *Pseudomonas aeruginosa* growth rate at the medium with 100 ppm of Cr (VI). It was monitored the decreased of Cr (VI) from 100 ppm to 5.86 ppm with the efficiency reach to 94.73% during 48 hours of the treatment, indicate locally bacterial *Pseudomonas aeruginosa* is potential to be used as a bioremediation agent chromium hexavalent [Cr (VI)] reduction to chromium trivalent [Cr (III)] on leather tannery wastewater. These findings indicate the potential of local Pseudomonas aeruginosa to be further applied as bioremediation agent in Cr(VI) contaminated environment.

![Figure 1. Model of Cr(VI) reduction into Cr(III) by *Pseudomonas aeruginosa*](image)

**2. Experimental Method**

**2.1. Raw Material**
Isolat *Pseudomonas aeruginosa* (from Microbiology laboratory, Institut Teknologi Bandung), ethanol 70%, King’s B medium, Potassium dichromate (K₂Cr₂O₇), 1,5-difenilkarbazida and de-ionized water. All purchased raw material were used without further purification.

**2.2. Bacterial growth conditions**
Isolated Pseudomonas aeruginosa was inoculated on to nutrient agar amended with 100 mg/L of Cr (VI). A filter-sterilized solution of K₂Cr₂O₇ was used as the source of Cr (VI), which was added to the
sterile molten nutrient agar to prevent problems associated with autoclaving chromate-containing solutions [10]. The inoculated plates were incubated at 37°C for 24 h.

2.3. Determination of minimum inhibitory concentration (MIC) of Cr(VI)
The minimal inhibitory concentration (MIC) of chromium at which no colony growth occurred was determined by broth agar dilution method. The isolates were inoculated individually into 10 ml nutrient agar medium of King’s B and incubated at 37°C for 24 h. Nutrient agar plates containing different concentrations of Cr(VI) (20–200 mg/l) were inoculated aseptically from the exponential growing cultures of each bacterial strain. These plates were incubated at 37°C for 24 h. The MIC was considered to be the lowest concentration of Cr(VI) at which no growth occurred.

2.4. Growth Kinetics Measurement
Growth of Cr(VI) resistance potential bacterial (Pseudomonas aeruginosa) was studied in 250 ml flasks containing 100 ml of King’s B medium supplemented with 100 mg/l potassium dichromate as a source of Cr(VI). Flasks were inoculated with 0.1 ml of freshly prepared inoculum and incubated at 37°C with 150 rpm shaking for 24 h. Samples were drawn at regular 2h time intervals. The Optical density changes of the culture during growth were recorded at 600 nm using a spectrophotometer UV [11]. Culture media without chromium treatment served as a control for the growth experiment.

2.5. Reduction of Cr(VI) by isolated Pseudomonas aeruginosa
Nutrient broth amended with concentrations of chromate of 100 mg/l was inoculated with selected bacterial isolate V3 culture so as to get an OD of 0.05 from overnight grown culture; it was then incubated at 30°C in a shaker at 150 rpm. 10 ml aliquots were withdrawn at regular 5-h intervals and analyzed for chromium reduction. Cr(VI) uptake and reduction during growth was followed by color alteration of potassium dichromate solution and by the quantitative decrease in Cr(VI) concentration in culture [12][13][14], the increase concentration of trivalent chromium [Cr(III)], and total chromium, CrT. The total concentration Cr(VI) in the media was determined spectrophotometrically using 1,5-diphenilcarbazide as complexing agent [14]. CrT were determined using atomic absorption spectrophotometer, while Cr(III) was calculated from CrT and Cr(VI), [CrT-Cr(VI)].

3. Results and Discussion

3.1. Resistant analysis of Indigenous Pseudomonas aeruginosa on Cr(VI)
The resistant analysis of Pseudomonas aeruginosa was determined by colony counting method. Plates contain nutrient agar medium supplemented with different concentration (20,50, 70, 80, 90, 100, 150, and 200 ppm) of potassium dichromate (K2Cr2O7), as Cr(VI) sources, were inoculated by indigenous Pseudomonas aeruginosa. The resistancy was shown in the Table 1.

| Parameters          | Cr (VI) (ppm) |
|---------------------|---------------|
|                     | 0  | 20 | 50 | 100| 150| 200|
| Medium color        | Yellow greenish transparent | Yellow transparent | Yellow transparent | Yellow transparent (+++) | Yellow transparent (+++) | Yellow transparent (++++) |
| Colony density      | countless | Countless | Countless | None | None | None |
| Colony morphology   | Round shape | Round shape | Round shape | NI | NI | NI |
| Colony color        | White | White | White | NI | NI | NI |
| Colony distribution | Random | Random | Random | NI | NI | NI |
The resistance of the isolated *Pseudomonas aeruginosa* towards the bactericidal action of Cr(VI) was studied at varying concentration of Cr(VI) (Table 1). The *Pseudomonas aeruginosa* was monitored to be resisted up to 50 ppm of Cr(VI), and totally killed at 100 ppm of Cr(VI) and higher concentrations in the solid medium of King’s supplemented by Cr(VI). Since the range 50-100 ppm of Cr(VI) concentration too broaden, the resistance analysis was elaborated to see the effect of Cr(VI) on cell viability at 50-90 ppm (Table 2). It was observed that the *Pseudomonas aeruginosa* shows their resistance up to 70 ppm of Cr(VI).

### Table 2. Resistant analysis of *Pseudomonas aeruginosa* to Cr(VI) at range of 0-90 ppm

| Parameters                  | Cr (VI) (ppm) |
|-----------------------------|---------------|
|                             | 0  | 50 | 60 | 70 | 80 | 90 |
| Medium color                | Yellow greenish transparent | Yellow transparent | Yellow transparent | Yellow transparent | Yellow transparent | Yellow transparent |
| Colony density              | countless | Countless | Countless | low | None | None |
| Colony morphology           | Round shape | Round shape | Round shape | shape | NI   | NI   |
| Colony color                | White | White | White | White | NI   | NI   |
| Colony distribution         | Random | uniform | uniform | Random | NI   | NI   |
| Number of colony            | >300 | >300 | >300 | >30 | None | None |

NI: not identified

3.2. Effect of Cr(VI) on *Pseudomonas aeruginosa* Growth Rate

The effect of Cr (VI) on growth of *Pseudomonas aeruginosa* was determined by comparing the growth profile of *Pseudomonas aeruginosa* at temperature of 37°C and pH 6.8 in the medium with and without supplementation of Cr(VI) as can be seen in the Fig. 2.

![Figure 2. Growth Profile of *Pseudomonas aeruginosa* in the absence and in the presence of 100 ppm of Cr(VI).](image)
It was found that the presence of 100 ppm of Cr(VI) slightly delay the bacterial growth (Fig. 2). This indicates that the growth of *Pseudomonas aeruginosa* was considerably inhibited due to the toxicity of Cr(VI). This observations are in agreement with the previous work by Singh et al. (2012) who reported that the growth rate of Bacillus cereus decreased as the concentration of Cr(VI) concentration in the culture medium increased [15].

3.3. Bioreduction of Cr(VI) into Cr(III) by *Pseudomonas aeruginosa*

Biotransformation of Cr(VI) was determined by growing the *Pseudomonas aeruginosa* in the medium with the presence of Cr(VI) at the concentration of 75 ppm for 24 h of observation. The reduction of Cr(VI) was evaluated for each 8 h of observations as can be seen in the Fig. 3.

![Figure 3. Bioreduction of Cr(VI) by *Pseudomonas aeruginosa*](image)

Bioreduction evaluation of Cr(VI) by *Pseudomonas aeruginosa* shows the decrease of Cr(VI) concentration with the time. This results are in agreement with pervious work reported by Ozturk et al.(2012) [4]. At an initial Cr(VI) concentration of 103.94 ppm, nearly 60% of Cr(VI) was reduced after 24 h of reaction, while the value further decreased to 94% after 48 h of reaction time (Fig. 4). This research shows the ability of indigenous *Pseudomonas aeruginosa* to reduce the higher concentration of Cr(VI) in the medium.

![Figure 4. Efficiency of Cr(VI) reduction by *Pseudomonas aeruginosa*. The initial concentration of Cr(VI) in the medium is 103.94 ppm.](image)
4. Conclusion
The Cr(VI) bioreduction of locally Pseudomonas aeruginosa, was successfully demonstrated. Further understanding of the proper mechanism of Cr(VI) biotransformation and factors affecting Cr(VI) reduction should assist toward its application in chromium bioremediation processes.

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