Removal of Chromium (VI) and Chromium (III) by using *Chlorella* sp Immobilized at Electroplating Wastewater

S Elystia¹, Edward H S¹, and A E Putri¹

¹ Departemen of Environmental Engineering, Faculty of Engineering, Riau University, 28293, Panam, Pekanbaru, Indonesia

Coresponding author’s email address:shintaelystia@yahoo.com

**Abstract.** Heavy metals which is mostly contained in electroplating wastewater is Cr, in the form of Cr (VI) and Cr (III). Cr (III) is relatively unstable but tends to be dangerous, Cr (VI) is very stable and has a high level of toxicity which is dangerous for the environment. This study aims to determine the ability of microalgae Chorella sp immobilized with calcium alginate in removing metals Cr (VI) and Cr (III) in electroplating wastewater with variations cell density in beads 0; 1.53x10⁸; 1.76x10⁷ and 1.54x10⁶ cells / bead, pH 3, 5, 7, and 9 with contact time 0; 12; 24; 36 and 48 hours. Allowance carried out by microalgae Chlorella sp immobilized in reducing Cr (VI) and Cr (III) metals in electroplating wastewater with initial concentrations of Cr (VI) 24.78 mg/l and Cr (III) 0.91 mg/l with highest efficiency value of 50.28% at pH 3, cell density 1.54x10⁶ cells/bead within 48 hours

**Keywords:** Biosorption, Chlorella sp, Contact Time, Cr, Electroplating Wastewater

1. Introduction

The activities in the electroplating industry produces the wastewater that containing heavy metal ions which are very toxic even in low concentration, and can be accumulated in water stream [1]. Heavy metal in electroplating wastewater came from rinsing activity and contained 5-50 mg/L [2]. The type of heavy metal ions that found in electroplating wastewater are Cr (III) in the form of Cr³⁺ and Cr (VI) cations in the form of Cr₂O₇²⁻ anion [3] where Cr³⁺ is relatively unstable but still dangerous, while Cr (VI) very stable and easily oxidized so it is very dangerous for the environment both low and high concentration.

One of alternative technology for treating electroplating wastewater is biosorption. Biosorption is a wastewater treatment technology that can eliminate heavy metals in wastewater by using live biomass or dead biomass [4]. Microalgae is a promising alternative for removing heavy metals because the populations of microalgae was really easy to be found like in the freshwater and in the sea, capable of providing fast, efficient removal of heavy metals and high metal binding capacity, producing the eco secondary product, algae can be regenerated, and metal ions adsorbed can be recovered [5]. One type of microalgae that commonly found in water is the type of microalgae *Chlorella* sp. *Chlorella* sp was able to reduce Pb metal concentration by 78% at a treatment of 20 ppm on a laboratory scale [6], *Chlorellasp* can also remove Cr by 90% and Cu 89% [7]. In the metal removal process *Chlorella* sp acts as a ligand, namely the R – COOH group, hydroxyl group, sulfate group and amine group. These functional groups play an important role in binding metals to the biosorption process [8].
Purnawati et al. [9] explained that Chlorella sp. has a high absorption rate but a weak structure, relatively small cells, and low mechanical strength that reduces the ability of cells to remove metals. To overcome this weakness, biomass immobilization was carried out. Biomass immobilization is a technique in which cells to be used are coated with a sufficiently porous polymer layer that allows the process of diffusion the substrate to the cell [10]. By immobilizing microalgae, the size of the biomass will be greater, minimizing clogging, more resistant to stress, not requiring maintenance and nutrition, even possibly reducing the concentration of pollutants more. Based on the description above, in this study electroplating wastewater treatment will be carried out by biosorption using microalgae Chlorella sp. which immobilized with calcium alginate in an airlift bioreactor.

2. Methods

2.1 Algae cultivation and immobilization

Stock suspension of Chlorella sp. was cultivated in Dahril Solution, aerated by filtered air at a rate of 2 L/min. After two weeks cultivation, algal cells were harvested by centrifugation at 3500 rpm for 10 min. The cell residues were washed by deionized water and resuspended in deionized water prior to use. The cell density in the suspension was $1 \times 10^9$ cell/ml. Cell number were counted under microscope with thomasithometer every 24 hours using thomasitometer and microscope. Different quantities of the algal cells were resuspended in deionized water to give different cell densities. Each diluted algal suspension was mixed with an equal volume of 4% sodium alginate to yield a mixture of 2% algae alginate suspension. Each mixture was dropped into a solution of 0.1 M CaCl$_2$ by using a peristaltic pump to form algal beads (immobilization). Three different types of beads with different cell densities were formed ($1.53 \times 10^8$, $1.76 \times 10^7$, $1.54 \times 10^6$ cells/bead). Blank beads were made without any algae as a control. Beads diameter is 4 mm the ratio of algae beads in wastewater is 1: 3.

2.2 Industrial wastewater

Industrial effluents were supplied by a chrome plating industry in city that generates a wastre stream containing primarily high Cr (VI) concentration. Samples of electroplating wastewaters from rinsing baths. Result of characterisation of wastewater were carried out at the laboratory of water analysis and presented in Table 1.

| Properties | Effluents (mg/L) | Quality standards (mg/L) |
|------------|-----------------|-------------------------|
| Cr (VI)    | 24.78           | 0.1                     |
| Cr (III)   | 0.91            | 0.5                     |
| Cd         | 0.02            | 0.05                    |
| Ni         | 1.0             | 1.0                     |
| Pb         | 0.1             | 0.1                     |
| Zn         | 0.2897          | 1.0                     |

2.3 Biosorption set-up

The biosorption set-up is shown in fig.1. an acrylic reactor of 5 L provided with a drain valve and diffuser plate was used in biosorption experiments. The dimension of a reactor is 15 cm x 30 cm.
2.4 Biosorption process
Biosorption experiments were carried out by putting into contact 0, 1.53x10^8, 1.76x10^7, 1.54x10^6 cells/bead with 3L of wastewater sample in a 5L airlift bioreactor at room temperature within contact time 0, 12, 24, 36 and 48 hours at pH 3. The result obtained there is the best density in the efficiency of metal removal, then proceed with the influence of pH on allowance. Each sample of electroplating wastewater (3L) is adjusted to pH 3, 5, 7 and 9 using NaOH solution. Then contacted with the best density of Chlorella sp beads within 0, 12, 24, 36 and 48 hours.

2.5 Analytical methods
The pH of the collected samples during the biosorption process was measured by a pH meter. Chromium and other metals concentration in the samples was analyzed by atomic absorption spectroscopy (AAS). Hexavalent chromium was analyzed by the SNI 6989.53-2010 by using atomic absorption spectroscopy (AAS). The concentration of trivalent chromium was calculated as the difference between total chromium and hexavalent chromium concentration. In addition to wastewater samples, cell density within the algae beads of the photobioreactor will be calculated daily during the allowance process. Ten algae beads will be removed from the photobioreactor, then dissolved in 1 mL of sterile sodium citrate 0.2 M for 30 minutes at room temperature. Algae that is soluble diluted with aquades. Next is the cell density calculation on the thomasitometer under a microscope.

3. Results and Discussions
3.1 Effect of difference of cell density in immobilized beads on its efficiency in chromium (VI) and (III) removal
The reduction of Cr (VI) and Cr (III) in the immobilized algal were significantly better than control beads (Figure 2a). The percentages of Cr (VI) removal after 24 h were 13.5, 38.05, 26.05, and 16.86 % for 0, 1.53x10^8, 1.76x10^7, 1.54x10^6 cells/bead and Cr (III) formed as a result of Cr (VI) reduction was the predominant chromium species in solution.
The $1 \times 10^6$ cells/bead achieved 50.24% removal of Cr (VI) after 48 hours. Lee [11], microalgae absorb Cr (VI) and reduce it to Cr (III) with the help of Chromate reductases. Microalgae can eliminate Cr (VI) by the mechanism of adsorption on cell walls, enzymatic reduction to Cr (III), complexation with metallothioneins, and detoxification of ROS [12]. Meanwhile, removal of Cr (VI) by cell density $1.53 \times 10^8, 1.76 \times 10^7$ is not as high as $1 \times 10^6$ because the high density of Chlorella sp will cause the access of metals to active sites terminated and as a result it makes absorption of metals reduced.

For the efficiency of Cr (III) cannot be calculated because the concentration of Cr (III) increases when there is a decrease of Cr (VI). The range of cell density that can be used in removing Cr (VI) is $1 \times 10^5 - 1 \times 10^8$ cells/ml. Based on the graph, it can be seen that the level of removal efficiency of Cr (VI) is inversely proportional to the density of microalgae cells [13]. Chlorella can do extracellular and intracellular detoxification. Extracellular detoxification occurs due to the interaction of Cr with the hydroxyl group on cellulose that lines the Chlorella cell wall [14]. Absorption of Cr by the cell wall can prevent Cr from entering the cell or reduce the amount of Cr entering the cell. The mechanism of intracellular detoxification is thought to occur in Cr absorption through the formation of phytochelatin. Cr which binds with phytochelatin will form complex compounds which are non-toxic.

Biosorption of heavy metals by microalgae consists of two phases [15]. The first phase is adsorption by the extracellular part of the cell and the material contained such as polysaccharides and components of cell wall constituents such as carboxyl, hydroxyl, sulfate and phosphate groups [16]. This phase is non-metabolic, takes place quickly and depends on several parameters such as pH, type of heavy metals, types of microalgae and biomass concentration. The second phase is absorption. This
process is metabolic, takes place slowly and there is active transport through the cell membrane and binds with proteins and other intracellular components.

The mechanism of reducing Cr (VI) to Cr (III) by microalgae can be seen in the following reactions:

\[
\begin{align*}
HCrO_4^- + R-COOH + H^+ &\leftrightarrow R-COOH_2^+ - HCrO_4^- \\
HCrO_4^- + R-NH_2 + H^+ &\leftrightarrow R-NH_3^- - HCrO_4^- \\
HCrO_4^- + R-SO_3H + H^+ &\leftrightarrow R-SO_3H_2^- - HCrO_4^- \\
HCrO_4^- + 3(-CH-) + 4H^+ &\leftrightarrow 3(-CH-) + 2Cr^{3+} + 4H_2O
\end{align*}
\]

The reduction of Cr (VI) to Cr (III) occurs due to complex formation by protonated functional groups on the surface of cell walls such as carboxyl groups, amine groups and sulfide groups. Based on the results of the absorption efficiency of Cr (VI) metal obtained from the biosorption process using immobilized Chlorella sp is still relatively low and above the quality standard PermenLH No. 05 of 2014 concerning quality standards for wastewater. This happens because electroplating liquid waste samples contain multicomponent ions, besides containing Cr metal ions, there are a number of other components (matrices), both cations and anions so that interactions occur more complicated and the possibility of interference during the biosorption process of each cation is very large [17].

3.2 Reduction of Cr (VI) and Cr (III) under different pH

One of the factors that give impact to biosorption process was pH. pH affect the type of chemical interaction that occur in the biosorption process between metals ion, functional groups on the surface of algae and the degree of ionization of the adsorbate during the reaction. In this study, pH variations were carried out at pH 3, 5, 7 and 9 with cell density of $1.54 \times 10^6$ cells/bead obtained from the results of experiments with variations in the cell density of Chlorella sp microalgae.

![Figure 3 (a)](image1.png)

**Figure 3 (a)** Relationship Between pH 3 on Cr (VI) and Cr (III) concentrations at 1.54x10^6 cell density

![Figure 3 (b)](image2.png)

**Figure 3 (b)** Relationship Between pH 5 on Cr (VI) and Cr (III) concentrations at 1.54x10^6 cell density
Removal of Cr (VI) and Cr (III) was inversely proportional when the removal of Cr (VI) is high, the concentration of Cr (III) will rise. The removal of Cr (VI) was strongly influenced by the pH where pH affects the value of metal solubility. Cr (VI) in the form of anions, namely $\text{Cr}_2\text{O}_7^{2-}$ so if the pH gets lower than the removal process will be better because of differences in the charge which causes the process of attaching or binding metal ions by the biosorbent surface. But in its application, it is not recommended to reduce the effluent pH to 1 because even though the optimum allowance value is risky in terms of safety [18].

While at low pH, Cr (III) has increased concentration due to the reduction of Cr (VI). Cr (III) contained in wastes has the form $\text{Cr}^{3+}$ or $\text{Cr(OH)}_2^+$ in the pH range 2-6 and in the pH range, the removal of Cr (III) is low due to the reject resistance between positive ions Cr (III) and positive ions from $\text{H}^+$ in waste on the surface of Chlorella sp. The highest absorption efficiency occurs at pH 3 while at pH 5-9 absorption efficiency is low. The highest absorption efficiency at pH 3, which is equal to 50.28%. At pH 5, the highest absorption efficiency is 39.06%. At pH 7 and 9 the highest efficiency of absorption at each pH is 35.27% for pH 7 and 29.94% for pH 9.

Based on the efficiency values obtained, the effective biosorption process is carried out at acidic pH, ie, pH 3. This is because the higher the pH, the metal precipitation will occur (metal precipitation). At low pH (below pH 3, the surface of the solid is positively charged because protonation occurs in anionic groups, such as carboxylic or amino, so that when the pH of the solution is lowered, there will be protonation of weak base groups on the cell surface of biomass so that the ability of biomass to absorb metals is weaker.

**4. Conclusion**

The present study demonstrates that Chlorella sp immobilized in alginate beads were more effective in removing Cr (VI) from wastewater than blank alginate beads. In the biosorption process Cr (VI) was partially eliminated and only a low amount of chromium in its trivalent form remained in solution. The optimum density cell is $1.54 \times 10^6$ cells/bead and the optimum pH is 3.

**References**

[1] De-Bashan L E and Bashan Y 2010 Immobilized Microalgae for Removing Pollutants: Reviews of Practical Aspects *Bioresource Technology* **101** 1611-1627

[2] Ahalya N, Ramachandra T V, and Kanamadi R D 2003 Biosorption of Heavy Metals *Research Journal Chemical Environment* **7**(4) 71-79

[3] Chen P W, Yen H W, Hsu C Y, and Lee L 2017 The Use of Autotrophic Chlorella vulgaris in Chromium (VI) Reduction Under Different Reduction Conditions *Journal of the Taiwan Institute of Chemical Engineers*
[4] Fanani A S 2017 Pemanfaatan Biomassa Alga Biru-Hijau Ananbaenacicadae sebagai Biosorben untuk Menurunkan Konsentrasi Logam Cr pada Limbah Cair Industri Elektroplating [Skripsi] Pekanbaru: Fakultas Teknik, Universitas Riau

[5] Gadd G M 2009 Biosorption: Critical Review of Scientific Rationale, Environmental Importance and Significance for Pollution Treatment Journal Chemical Technology Biotechnology 84 13–28

[6] Hameed A 2007 Effect of Algal Density in Bead, Bead Size and Bead Concentrations on Wastewater Nutrient Removal African Journal of Biotechnology 6

[7] Lee L, Hsu C Y, and Yen H W 2017 The Effect of Hydraulic Time Retention (HRT) on Chromium (VI) Reduction Using Autotrophic Cultivation of Chlorella vulgaris Bioprocess Biosystem Engineering

[8] Molazadeh P, Khatami N, Rahimi M R, and Nasiri A 2015 Adsorption of Lead by Microalgae Chaetoceros sp. and Chlorella sp. from Aquoeous Solution Journal of Community Health Research 4(2) 114-127

[9] Najiah A N 2016 Biosorpsi Logam Merkuri oleh Lactobacillus acidophilus pada Kolom Unggun Tetap: Eksperimen dan Prediksi Kurva Breakthrough Jurnal Teknik Universitas Hasanuddin

[10] SNI 6989.57.2008 Metode Pengambilan Contoh Air Permukaan Jakarta: Badan Standarisasi Nasional

[11] Sumada K 2006 Pengolahan Air Limbah Industri Elektroplating Secara Kimia dan Pertukaran Ion (Ion Exchange) Jurnal Teknik Kimia 26-36

[12] Susanti T 2009 Studi Biosorpsi Ion Logam Cr (VI) oleh Biomassa Alga Hijau yang Diimobilisasi pada Kalsium Alginat [Skripsi] Jakarta: Universitas Indonesia

[13] Syahputra B 2008 Pemanfaatan Alga Chlorella pyrenoidisa Untuk Menurunkan Tembaga (Cu) Pada Pelapisan Logam

[14] Yasril, Kasjono H S, and Ganefati S P 2009 Penurunan Kadar Krom (Cr) Dengan Menggunakan Biomasa Ampas Tebu Secara Bio-Adsorbs Jurnal Teknik Lingkungan 10(2) 145–151

[15] Wang J and Chen C 2009 Biosorbents for Heavy Metals Removal and Their Future

[16] Naja G and Volesky B 2011 The mechanism of metal cation and anion biosorption. In: Microbial Biosorption of Metals Springer: Dordrecht 19-58

[17] Chen P W, Yan H W, Hsu C Y, and Lee L 2017 The Use of Autotropic Chlorella vulgaris in Chromium (VI) Reduction Under Different Reduction Conditions Journal of the Taiwan Institute of Chemical Engineers

[18] Anita S D and Kardena E 2013 Biosorpsi Kromium Heksavalen Oleh Mikroalga Amobil Pada Limbah Industri Pelapisan Logam Jurnal Teknik Lingkungan FTSLITB 1-12