Hexavalent Chromium Removal from Electroplating Waste Using Biochitin from *L. Vanamme* Shrimp Shell Waste

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Received 7 November 2019; Accepted 12 May 2020

**ABSTRACT**

The increasing number of electroplating industries in this globalization era has a negative impact on the environment because of its liquid waste containing high concentrations of hexavalent chromium, Cr (VI). To overcome this problem, biochitin extracted from white shrimp shell waste (*L. vanamme*) has been used to adsorb Cr (VI) from electroplating waste. Biochitins were extracted through single and sequential fermentation method using *L. plantarum* and *B. thuringiensis* bacteria. The optimization process of Cr (VI) removal was done by using biochitin from different methods, at various mass of biochitin, the time of adsorption, and Cr (VI) concentration. Results showed that biochitin produced from the sequential fermentation method could optimally adsorb Cr (VI). By using 2 g of biochitin, Cr (VI) could be removed as much as 51.99 ± 0.41% during 6 hours. At the optimum condition, the selected adsorbent could remove Cr (VI) in the concentration range of 9.38 to 92.39 mg/L, which showed the adsorption capacity of 0.915 mg/g. The capability of biochitin for Cr (VI) removal was reinforced by the SEM EDS images, which show the presence of chromium compounds in the biochitin.

Key words: biochitin, hexavalent chromium, electroplating waste, shrimp shell waste, adsorption

**INTRODUCTION**

The modern society lifestyle in this globalization era has provided an increasing number of industries, especially in the electroplating industry. The increasing number of electroplating industries certainly provides positive benefits for the economic sector in Indonesia. However, electroplating industries using high concentration of hexavalent chromium, Cr (VI), for prevent metal rusting that can be dangerous for the environment. Beside that, high concentrations of hexavalent chromium can cause various diseases including skin rashes, respiratory problems, kidney and liver damage. Cr (VI) is also carcinogenic, mutagenic, and even at high concentrations can cause death [1].

Because of the negative effects of hexavalent chromium, it is necessary to treat the electroplating wastewater so it is not harmful for health and the environment. There are several methods that can be used for wastewater treatment include reverse osmosis, sedimentation, filtration, ion exchange, and adsorption. In the reverse osmosis method, precipitation, filtration, and ion exchange require high operational costs and a large amount of chemicals. In addition, the reverse osmosis method produce sludge as side products, so high cost was required for its
sludge removal. Therefore the efficient and environmentally friendly method of wastewater treatment can be obtained via adsorption method. Through adsorption, in addition to being more efficient, the waste treatment process is also more profitable compared to other methods because it has a high level of recovery and the adsorbent can be reused [2].

Using Indonesia's potential as the 4th largest maritime shrimp-producing country in the world, the abundant amount of shrimp shell waste can be used for chitin production. Chitin is biopolymer that can be used for removing Cr (VI) in electroplating waste. The chemical methods has been generally used for isolating chitin from shrimp shell waste. In the chemical process of removing minerals and protein from shrimp shells, strong acid and bases were used. Sodium hypochlorite was also used at high temperatures for the decolorization process [3]. Chemical process is not only cause high electricity consuming, but also can endanger both the human health and environment. To overcome this problem, the environmentally friendly biological extraction method was conducted. The biological extraction method is advantageous as reported by Rao, 2016 [4], because it has ability to generate good quality chitin which extracted from shrimp shell waste with lower extraction costs compared to chemical process. The use of various bacteria types in fermentation method will generate biochitins with different characteristics. These different natures of biochitins can affect the adsorption efficiency of Cr (VI). Therefore, in this study, biochitins were isolated by single and sequential fermentation method by using Lactobacillus plantarum and or Bacillus thuringiensis bacteria. Through the different nature of biochitin, the removal of Cr (VI) in electroplating waste could be optimized.

There are several factors affecting the adsorption efficiency of hexavalent chromium adsorption, including the adsorbent mass, the adsorption time, and the adsorbate concentration [5]. Hence, the aims of this presented research were to investigate the effect of biochitins nature, adsorption time, adsorbent mass, and the initial concentration of Cr (VI) to the removal efficiency of Cr (VI) in electroplating waste.

EXPERIMENT
Chemicals and instrumentation
Biochitins were extracted from shrimp shell waste collected from a shrimp processing industry in Sidoarjo, Indonesia. The microorganisms used were Lactobacillus plantarum, obtained from Biology Department, Faculty of Mathematics and Natural Sciences, Brawijaya University (isolated from the small intestine Solen spp) and Bacillus thuringiensis, obtained from the Soil Department, Faculty of Agriculture, Brawijaya University (isolated from a silkworm cocoon). Chemicals used were: potassium dichromate (Merck), MRSB (de Man, Rogosa and Sharpe Broth) as growth media for Lactobacillus plantarum dan NB (Nutrient Broth) as growth media for Bacillus thuringiensis, sodium hydroxide (Merck), sodium hypochlorite (Merck), sulfuric acid (Merck), 1,5-diphenylcarbazide (Merck), acetone (Merck), and distilled water. Hydrobate was used in bacterial culture and shrimp shell fermentation processes.

The apparatus and instrumentations used in this study were: a set of glassware brands of pyrex and iwaki, digital pen type pH meter (PH-009(II)A), autoclave for apparatus and material sterilization, Heraeus incubator for culturing L.plantarum and B.thuringiensis bacteria, Biobase shaker incubator for shrimp shell fermentation, Genesys 2.0 Spectronic-20 for measuring absorbance of hexavalent chromium standard solution and electroplating waste, FTIR spectrophotometer (Shimadzu FTIR QP89500 ) for functional group identification, SEM-EDS (FEI Quanta FEG 650, using AZteOne software) for surface morphology and
compound content identification. Bacterial culture was carried out in Laminar Air Flow (LAF).

**Procedure**

**Isolation of biochitin**

Biochitin was produced from 80 mesh shrimp shell powder. Three types of biochitin used in this study were produced based on the previous procedure [6]. Biochitin A was produced by single fermentation using *L. plantarum* bacteria which was inoculated in de Man Rogose Sharpe Broth (MRSB); biochitin B was produced by single fermentation using *B. thuringiensis* bacteria which was inoculated in Nutrient Broth (NB); and biochitin C was produced by sequential fermentation using *L. plantarum* and then continued with *B. thuringiensis* bacteria.

**The effect of biochitin nature to the efficiency of hexavalent chromium removal**

Biochitins produced by fermentation process using different bacteria had different characteristics. This experiments were carried out to determine the biochitin that have high performance as an adsorbent to remove Cr (VI). About 0.2 grams of biochitin A, B, and C was mixed with 25 mL of Cr (VI) 50 mg/L and were stirred at 100 rpm in room temperature for 6 hours. Then the mixture was filtered using fine filter paper. The initial and final concentrations of Cr (VI) in the solution were determined using US-EPA procedure [7]. This experiment was carried out in triplo. The removal efficiency of Cr (VI) was determined based on the percentage of Cr (VI) adsorbed on the surface of biochitin.

**Determination of the adsorption time equilibrium**

At least there are two factors that affected the amount of Cr (VI) adsorbed on the biochitin, i.e. the adsorption time and the adsorbent mass. To determine the equilibrium time for Cr (VI) adsorption, the adsorption was conducted using batch system by mixing. About 0.2 grams of biochitin C was mixed with 25 mL of Cr (VI) 50 mg/L. The mixture was then stirred at room temperature for 0; 2; 4; 6; 8; and 10 hours. The amount of Cr (VI) adsorbed was determined based on the differentiate concentrations of Cr (VI) between in the initial and final solutions. The optimum adsorption time was determined based on the optimum amount of Cr (VI) adsorbed on the biochitin.

**Determination of the optimum adsorbent mass**

The adsorbent mass determines the number of active sites which is responsible for the interaction with the adsorbates. In this experiment, biochitin C at various mass (0.5; 1.0; 1.5; 2.0; and 2.5 grams) was mixed with 25 mL of Cr (VI) 50 mg/L. The mixture was stirred (at 100 rpm) for 6 hours. These experiments were carried out in triplo. The optimum of adsorbent mass is the mass of adsorbent with optimum percentage of Cr (VI) removal.

**Removal of hexavalent chromium from electroplating waste**

Cr (VI) removal test was carried out using electroplating waste which was diluted 10x, 100x, and 1000x. The waste was mixed with 2 g of biochitin C and stirred for 6 hours at 100 rpm. The removal efficiency of Cr (VI) then was calculated as the amount of chromium hexavalent adsorbed. The adsorption isotherm was examined using the Langmuir and Freundlich adsorption isotherm. The isotherm that fit to the experimental data was assessed based on the correlation coefficient for the regression ($R^2$ value closest to unity) and the value of separation factor (RL) in the range from 0 to 1.
Determination of hexavalent chromium concentration
Concentration of Cr (VI) in the solution was determined based on the formation of the diphenylcarbazone complex using spectrophotometry method (US-EPA procedure [7]). The Cr (VI) solution as much as 1 mL was put into a 50 mL volumetric flask, then was added by 45 mL of distilled water. The solution pH was adjusted to 2.0 ± 0.5 using 10% H2SO4. Next, about 1 mL of diphenylcarbazide was added, and the mixture was diluted up to 50 ml with distilled water. The solution was allowed to stand for 8 minutes so that the resulting color is stable. Absorbance of the complex compound then was measured at 540 nm.

RESULT AND DISCUSSION
The effect of biochitin nature to the efficiency of hexavalent chromium removal
Chitin can be isolated from the shrimp shell waste after deproteination and demineralization process. In this presented study, biochitins were produced after deproteination and demineralization using L. plantarum or/and B. turingiensis. The B. turingensis as proteolytic bacteria produces protease enzyme which responsible for deproteination process. Meanwhile L. plantarum produces lactic acid enzymes, which react with calcium in the shrimp shell during demineralization process. Hence, single or sequential fermentation produces biochitin with different nature that affect the capacity of the adsorbents in the Cr (VI) removal.

![Figure 1](image_url)

**Figure 1.** The effect of biochitin nature on the removal efficiency of Cr (VI) using A: L.plantarum fermentation; B: B.thuringiensis fermentation; and C: sequential fermentation by L.plantarum and B.thuringiensis. Adsorption was conducted by mixing 25 mL of 50 mg/L hexavalent chromium solution with 0.2 g adsorbent and stirred at 100 rpm for 6 hours.

Based on Figure 1, biochitin C has the highest capacity to remove Cr (VI), i.e. 23.93 ± 0.51% with the amount of Cr (VI) adsorbed was 1.436 ± 0.04 mg/g. Biochitin B produced by single fermentation using B.thuringiensis fermentation could adsorp Cr (VI) 1.083 ± 0.06 mg/g with the removal efficiency of 17.99 ± 0.82%. This shows that biochitin produced from the sequential fermentation method can increase the adsorption capacity up to 32.58% compared to single fermentation method. This is because biochitin that produced from sequential fermentation has a highest demineralization efficiency (34.41 ± 1.84%) compared to a single fermentation method (16.22 ± 1.64% in average) [6].
The higher the percentage of demineralization, the less impurities minerals are left in the biochitin, consequently the Cr (VI) adsorption is optimal. Higher demineralization efficiency in sequential fermentation compared to single fermentation is due to two steps fermentation by gram-positive bacteria so it can optimize the enzymes production.

In addition, biochitin produced from different fermentation method has different particle size, i.e: 158.61; 142.96; 125.27 μm (based on particle size measurement using PSA) respectively for biochitin A, B, and C. This character affects the capability of the biochitin as adsorbent used for Cr (VI) removal. The smaller the particle size of the adsorbent, the greater the biochitin surface area.

Based on the functional group identification using FTIR spectrophotometry as studied in the previous experiment [5], it can be obtained that sequential fermented biochitin extracted from L. vanamme shrimp shell waste has a similar spectrum pattern compared to the reference [8]. The biochitin has functional groups at wave numbers 3389 - 3451; 3104 and 3121; 1630-1655; and 1026-1030 cm\(^{-1}\) that indicating O-H group; N-H acetamide (-NHCOCH\(_3\)); carbonyl (-C = O) from acetamide; and the -C-O-C group from the glucosamine chitin ring respectively. The present of –NH and C=O functional groups are important factor for controlling Cr (VI) adsorption on biochitin. The intensity ratio between C=O groups of acetamides and O-H groups in biochitin shows the acetylation degree [6]. The greater the degree of acetylation, the greater the number of acetamides groups in chitin, so that the ability to bind Cr (VI) is higher. The acetylation degree of biochitin C was 53.81 ± 7.39%, so the biochitin has high capacity to adsorb Cr (VI), as shown in Figure 2.

![Figure 2](image_url)

**Figure 2.** The adsorption time for Cr (VI) adsorption. 25 mL of Cr (VI) 50 mg/L was adsorbed by 0.2 g biochitin C. The solution was stirred at room temperature.

**Determination of adsorption equilibrium time**

Determination of the optimum conditions for the Cr (VI) removal were carried out using biochitin C. Time of adsorption determines the adsorbed amount of analyte. The rate of adsorption is usually fast at the first minute of contact and slightly increases for longer adsorption. The adsorption will reach equilibrium at the certain time where the rate of adsorption equals to the desorption rate.

To determine the adsorption equilibrium time, about 0.2 grams of biochitin C was mixed with 25 mL of Cr (VI) 50 mg/L and stirred for several hours. Based on Figure 2, it can be seen that the adsorption rate of Cr (VI) was fast at the first 2 hours and then increased slowly for up
to 6 hours. The equilibrium adsorption time was observed at 6 hours of adsorption. At the optimum time, biochitin C could adsorb Cr (VI) of $1.24 \pm 0.04 \text{ mg/g}$ ($21.93 \pm 0.71\%$).

**The effect of adsorbent mass to the efficiency of hexavalent chromium removal**

In order to determine the biochitin quantity for an optimal removal of Cr (VI), the effect of biochitin mass to the Cr (VI) removal efficiency has been studied. According to Figure 3, it can be seen that Cr (VI) removal by biochitin C was increased with the increasing mass of biochitin (0.5-2 g). This can be explained by the fact that increasing the mass of adsorbent will increasing its contact surface and its followed by the increasing the amount of active groups which can contact with Cr (VI), so its offered to the sorption of Cr (VI). At this optimal mass of biochitin C (2 g), the efficiency of Cr (VI) removal by the biochitin C was $51.99 \pm 0.41\%$.

![Figure 3](image)

**Figure 3.** The effect of biochitin mass on the Cr (VI) removal efficiency using biochitin C from sequential fermentation by *L. plantarum* and *B. thuringiensis*. The adsorption was conducted by mixing 25 mL of Cr (VI) 50 mg/L with agitation speed of 100 rpm for 6 hours at room temperature.

**Removal of hexavalent chromium from electroplating waste**

Electroplating waste is one of anthropogenic source of Cr (VI) pollutant. To determine the capacity of Cr (VI) adsorbed at biochitin, the electroplating waste was diluted. Based on Figure 4, it can be concluded that the biochitin could be used to remove Cr (VI) at high concentration. The adsorbent could adsorb Cr (VI) of 13198.57 mg/L with the removal efficiency was more than 60%. The greater the concentration of Cr (VI), the lower the pH of its solution. The lower the pH, the more active sites (–NH groups from acetamide) in biochitin would be protonated, while at the lower pH (<7), Cr (VI) is in the form of HCrO$_4^-$ dan Cr$_2$O$_7^{2-}$ [9], so that its protonated biochitin can interact with hexavalent chromium solution easily.

In this study, the heavy metal removal efficiency in the freshwater that contain trace amount of Cr (VI) was also investigated. Two grams of biochitin C was adsorb freshwater (pH 7) that contain 0.92 mg/L of Cr (VI) at the same adsorption condition with previous experiment (triplo condition with 100 rpm agitation speed at room temperature). At this condition, biochitin C can adsorb 0.92 mg/L of Cr (VI) until it remained 0.168 mg/L in the freshwater. The decreasing of Cr (VI) concentration in the water could be higher if the pH of the solution was lowered, so that the concentration of Cr (VI) in the freshwater within the threshold would be expected.
**Figure 4.** The effect of initial concentration on the hexavalent chromium removal efficiency using 2 g of biochitin C. 25 mL of electroplating waste with various concentration of Cr (VI) was adsorbed by biochitin C at 100 rpm in room temperature.

**Table 1.** Summary of Langmuir and Freundlich Parameter for hexavalent chromium removal by sequential fermented biochitin

| Parameter of Langmuir | Qm (mg/g) | K_L (L/g) | R^2  |
|-----------------------|-----------|-----------|------|
|                       | 0.915     | 0.117     | 0.994|

| Parameter of Freundlich | n | K_F | R^2  |
|-------------------------|---|-----|------|
|                         | 1.078 | 0.057 | 0.986 |

**Table 2.** The value of separation factor (R_L)

| K_L (L/mg) | C_0 (mg/L) | R_L  |
|------------|------------|------|
| 0.117      | 9.38       | 0.477 |
| 0.117      | 92.39      | 0.085 |
| 0.117      | 986.42     | 0.009 |
| 0.117      | 13198.57   | 0.001 |

To understand the adsorption mechanism, the fit linear of the equilibrium concentration to the amount of Cr (VI) adsorbed was constructed using the Langmuir and Freundlich isotherm equation. According to the Table 1, Langmuir model has a better correlation coefficients compared to Freundlich model. This is reinforced with the information from Table 2, which shows the separation factor (R_L) value in the Langmuir isotherm were within range 0.001 – 0.477 (1/(1+K_L*C_0)) [10] that indicates more favorable heavy metal adsorption than Freundlich isoterm. A separation factor (R_L) in a range value from 0 to 1 indicates a favorable adsorption, while R_L > 1 indicates an unfavorable adsorption [10].
Table 3. The composition of sequential fermented biochitin which had absorbed 986.42 mg/L Cr (VI) from electroplating wastewater, analyzed by SEM-EDS.

| Element | Atomic (%) | Weight (%) |
|---------|------------|------------|
| C       | 62.79      | 32.19      |
| Cr      | 3.21       | 7.12       |
| Fe      | 18.2       | 43.39      |
| O       | 8.31       | 5.67       |
| Ca      | 1.01       | 1.74       |
| Al      | 3.27       | 3.77       |
| Mn      | 1.84       | 4.31       |
| K       | 0.41       | 0.68       |
| Mg      | 0.42       | 0.44       |
| P       | 0.36       | 0.48       |
| Si      | 0.18       | 0.21       |

In the Freundlich isotherm, the n value is similar with R_L value. According to the Table 1, n value of the Freundlich isotherm was > 1 which indicates unfavorable adsorption. By using the Langmuir adsorption isotherm, it can be obtained that the optimum concentration of adsorbate is in the range of 9.38 to 92.39 mg/L with the adsorption capacity was 0.915 mg/g.

Figure 5. SEM of biochitin C which adsorbed 986.42 mg/L Cr (VI) in electroplating wastewater, with 250 x magnification (left side) and 4,500 x magnification (right side).

Adsorption occurs on the biochitin surface due to the electrostatic interaction between the positive charge of protonated –NH groups and the negative charge of Cr (VI) species on the surface of adsorbent. The potential energy of the molecule surface decreased as the molecule approached the surface. The adsorbed molecule can be considered to form a two-dimensional phase and concentrated on the surface or interface as shown in SEM images (Figure 5).
According to the SEM images in Figure 5, the presence of white small flake indicates the Cr (VI) adsorbed. This is reinforced by the EDS data in Table 3, which show the presence of Cr (VI) in the adsorbent for 7.12%. The presence of Fe and Mn, i.e. 43.39% and 4.31%, respectively, as shown in Table 3, indicate that the biochitin has also a good ability to adsorb another metal, which contained in electroplating waste.

CONCLUSION
Biochitin that produced by sequential fermentation method using *L. plantaraum* in conjunction with *B. thuringiensis* has a good performance as an adsorbent for Cr (VI). By using 2.0 g of this adsorbed, Cr (VI) could be adsorbed optimally during for 6 hours. The adsorbent could adsorb Cr (VI) from electroplating waste with the concentration in the range of 9.38 to 92.39 mg/L with optimum adsorption capacity of 0.915 mg/g. At this condition, Cr (VI) in the waste could be removed by 50.05 ± 1.65%. Adsorption of Cr (VI) on the surface of the biochitin followed the Langmuir isotherm where the adsorption took place homogenously at the first layer of adsorbent. Adsorption of Cr (VI) by biochitin is occurred due to electrostatic interaction between the negative charge of Cr (VI) species and the positive charge of protonated –NH groups in the glucosamine ring.

CONFLICT OF INTEREST
Authors declare that no competing interest from published the manuscript.

ACKNOWLEDGMENT
Authors would like to thank to the Ministry of Research, Technology and Higher Education, for the funding research under the “Penelitian Tesis Magister” funding scheme with the Research contract number of 055/SP2H/LT/DRPM/2019, 11 March 2019.

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