Removal of Cr(VI) Using Biochitin from White Shrimp (Litopenaeus vannamei) Shell Modified by Dithizone

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Abstract. Biochitin is a chitin produced from the sequential fermentation of white shrimp shells using Lactobacillus plantarum followed by Bacillus thuringiensis. Biochitin was then modified by adding 0.015 g dithizone/ g adsorbent in toluene followed by reflux for 4 hours at 70°C. The purpose of this study was to determine the optimum conditions for removal of Cr(VI) using the dithizone modified biochitin (DiBch). This research was carried out using 25 mL Cr(VI) 50 mg/L mixed with DiBch adsorbents in various masses (0.1; 0.2; 0.3; 0.4 and 0.5 gram). The mixture was stirred for various time (0, 2, 4, 6 and 8 hours). Furthermore, the adsorption of electroplating waste with known concentrations was also carried out (0.92; 9.38; 92.39; 986.42 and 13215.94 mg/L). The results of this study indicate that Cr(VI) can be removed optimally using 0.4 g DiBch with stirring time of 2 hours. The removal efficiency of Cr(VI) from electroplating waste reached 83.45% ± 0.78.

1. Introduction

Chromium is one of the metals that has a higher concentration in waste from electroplating, paint, coloring, the paper industry, and others. This metal is very toxic and exposure in high concentrations can cause epigastric pain, nausea, vomiting, severe diarrhea and bleeding. The maximum level of chromium contaminants for drinking water is 0.05 mg/L [1]. Thus, to reduce the concentration of heavy metal ions to environmentally acceptable levels, effective and efficient separation methods must be developed. Of all the various water treatment techniques, adsorption is generally more desirable to remove heavy metal ions because it has high efficiency, using a simple method, low cost, and can be used widely. Adsorption can be done on heavy metals using various types of adsorbents such as chitin extracted from shrimp shells.

Chitin can be obtained from the white shrimp (Litopenaeus vannamei) shell obtained from the waste of fish processing plants. Shrimp shell waste consists of three main components namely protein (25% -44%), calcium carbonate (45% -50%), and chitin (15% -20%) [2]. Therefore the waste needs to be handled more seriously, especially because the waste contains chemical compounds that can be used for the basic ingredients of adsorbents. Shrimp shell can be used as an adsorbent because it has an active group that is the acetamide group (NH₃COCH₃) and has a reactive amine group and stable chelation [3]. In this study, the process of chitin extraction was carried out using fermentation method because this method uses only a few chemicals that are harmful to the environment and shrimp shell waste can be utilized more efficiently at a low cost. Chitin obtained can be modified with dithizone.

Previous research by Mudasir showed that immobilization of dithizone in chitin resulted in a more selective and higher adsorption capacity for Cd (II) ions compared to unmodified chitin[4]. Dithizon is one of the ligands that can be used to modify the surface of chitin, because it contains many N, -NH and -SH donor atoms which are sensitive to metal ions. The use of dithizone in making adsorbents has aimed to increase the selectivity and adsorption capacity of chitin for removal of heavy metal. Hence, this study aimed to determine the optimum condition for the removal of Cr(VI) using biochitin modified by dithizone (DiBch).
2. Experiment

2.1. Chemicals and Instrumentation

Equipment used in this study included: Laminar Air Flow (LAF), cool box, oven, blender, 80 mesh sieves, magnetic stirrers, glass apparatus, shaker, cuvette, pH meter, UV-Vis 1601 Shimadzu spectrophotometer, Fourier Transform Infrared (FTIR) Spectrophotometer - 8400S Shimadzu brand, Scanning Electron Microscopy (SEM), Particle Size Distribution 1090 Dry Rinse.

Materials and chemicals used were: white shrimp (L. vannamei) shell, L. plantarum in MRSA (deMann Rogosa Sharpe Agar) modified SHES (Shrimp head extract solution), 2% glucose, 10% glucose, 1% salt, B. Thuringiensis SA (sialic acid) on NA (Nutrient Agar) modified SHES, NaOH 4% (Merck 106498.1000), distilled water, 1 M HCl, 4% NaOCl (Merck 105614), diphenylcarbazide crystal (Merck 103091), acetone (Merck 100012), K2Cr2O7 crystal (Merck 104865), 10% H2SO4 (Merck 109073), electroplating wastewater samples.

2.2. Biochitin Extraction using Fermentation Method

Biochitin was extracted from the white shrimp shell using sequential fermentation method which was published elsewhere [5]. In principle, the culture of 15 hours L. plantarum was used in SHSA-modified MRSA with 2% glucose. About 500 mL starter was then added with 500 g of shrimp shell flakes (1: 1) [2]. Next 10% glucose and 1% salt were added to the Erlenmeyer. Then 30 hours fermentation was carried out at 37 °C. Chitin was then washed with water and dried in the oven overnight with a temperature of 80 °C. A total of 3% (w/v) dried chitin was then added to 10% (v/v) of the 24-hours culture of B. thuringiensis SA on the SHES-modified NA medium with 10% glucose. The mixture was incubated at 37 °C with agitation speed at 200 rpm for 81 hours. Chitin was then washed with water and dried in the oven overnight at 80 °C.

2.3. Modification of Biochitin with Dithizone

To prepare the modified biochitin, the following procedure was applied: 2.0 g of biochitin was added to 40 mL of toluene and mixed with 0.015 grams of dithizone in a 500 mL flask. The mixture was refluxed and stirred for 4 hours at 70 °C. The product was filtered and washed sequentially with toluene, ethanol, and water several times until the filtrate did not show the characteristics of dithizone color. The immobilized Dithizone biochitin (DiBch) was then dried in an oven at 60 °C for 12 hours and filtered through an 80-mesh filter.

2.4. Adsorption Study of Cr(VI)

Adsorption of Cr(VI) solution was carried out using batch adsorption methods. Cr(VI) solution with a concentration of 50 mg/L was added to DiBch 0.0; 0.1; 0.2; 0.3; 0.4 and 0.5 g. The mixture was stirred for 0, 2, 4, 6 and 8 hours at 100 rpm. Afterwards, the mixture was filtered using filter paper and the concentration of Cr(VI) in the filtrate was analyzed by a spectrophotometer visible at a wavelength of 540 nm. The number of metal ions adsorbed can be calculated by the following equation,

\[ Q = \frac{[(C_0 - C_A)V]}{m} \]  \hspace{1cm} (1)

where Q is the amount of Cr(VI) adsorbed onto unit amount of the adsorbents (mg/g), C0 and C_A are the initial and final concentrations of Cr(VI) (mg/L), respectively, V is the volume of the aqueous phase (mL), and m is the weight of DiBch adsorbent.

Furthermore, the percentage of adsorption can be calculated by the following formula,

\[ \% \text{ Adsorption} = \frac{m_0 - m_t}{m_0} \times 100\% \]  \hspace{1cm} (2)

where \( m_0 \) and \( m_t \) are the initial and the final mass of Cr(VI) in the solution, respectively.
3. Result and Discussion

3.1. The Equilibrium Time for Cr(VI) Adsorption using Dithizone Modified Biochitin

The equilibrium time of adsorption is the time where the rate of adsorption equals to the desorption rate. At this condition, the surface of adsorbent is filled by the adsorbate. It can be seen from figure 1, the adsorption of 50 mg/L Cr(VI) with 0.2 g of DiBch adsorbent at the initial hours experienced a high increase because at the beginning of adsorption the entire pore surface remained empty and the Cr(VI) ions would be attached to the surface of the adsorbent so that the rate of adsorption will take place quickly. The rate of adsorption was increased slowly up to 2 hours. As shown in Figure 1, the equilibrium time for Cr(VI) adsorption by DiBch was 2 hours with the Cr(VI) adsorbed was 3.71 mg/g.

![Figure 1](image1.jpg)

Figure 1. The influence of adsorption time to the amount of Cr(VI) adsorbed (mg/g) on the surface of DiBch adsorbent.

After 2 hours, the rate of adsorption was slightly decreased in equilibrium with the desorption rate. During the adsorption, the Cr(VI) ions were either attached to the active sides available on the surface of the adsorbent or filled the pore of adsorbent.

3.2. The Influence of DiBch Mass

The influence of the DiBch mass to the adsorption of Cr(VI) was carried out by using 25 mL of Cr(VI) solution of 50 mg/L mixed with the DiBch adsorbent at the various mass of 0.1; 0.2; 0.3; 0.4 and 0.5 g. Adsorption was conducted by stirring the mixture for 2 hours. Figure 2 shows that increasing the mass of adsorbent affected the efficiency of Cr(VI) adsorption. The number of active sites on the surface of adsorbent will increase with the increasing of the adsorbent mass. The percentage of Cr(VI) adsorbed reached its optimum value at 62.83% when using 0.4 grams of DiBch as adsorbent. Further increase of the adsorbent mass decreased the efficiency of Cr(VI) adsorbed due to the saturation of the active site of the adsorbent which forms lumps in the adsorbent thereby reducing the surface area of the adsorbent [8].

![Figure 2](image2.jpg)

Figure 2. The Influence of DiBch Adsorbent Mass to the removal efficiency of Cr(VI).
3.3. The Influence of Cr(VI) Initial Concentrations

Adsorption on Cr(VI) in electroplating waste was carried out by means of 0.4 g of DiBch adsorbent mixed with 25 mL of electroplating waste at various concentrations of 0.92; 9.38; 92.39; 986.42 and 13215.94 mg/L. Figure 3 shows that the optimum adsorption occurred at a concentration of 986.42 mg/L with the percentage of Cr(VI) removal was 83.45%. With increasing Cr(VI) concentration, the adsorption efficiency decreases, because the ability of DiBch to adsorb Cr(VI) is already optimum. In other words, the maximum capacity of DiBch adsorbents has been reached at a concentration of 986.42 mg/L.

![Figure 3](image-url)

**Figure 3.** The influence of initial concentration of Cr(VI) in the electroplating waste to the Cr(VI) removal efficiency.

The decrease in adsorption efficiency is caused by the unproportioned number of DiBch active sites for interaction with the Cr(VI) at high concentration [9]. This is consistent with Langmuir's theory which says that the active sites on the surface of the adsorbent has an amount proportional to the surface area of the adsorbent, so that if the active site on the surface of the cell wall of the adsorbent is saturated with adsorbates, then the addition of adsorbates concentration can no longer increase the adsorption capacity of the adsorbent. Therefore, the adsorption efficiency of Cr(VI) was decreased at concentrations more than 986.42 mg/L. It means the optimum removal efficiency of Cr(VI) could be reached by means of initial concentration of Cr(VI) was 986.42 mg/L.

Adsorption mechanism of Cr(VI) on the DiBch can be studied from the adsorption isotherms. We use the Langmuir and Freundlich adsorption isotherms to figure out the mechanism of Cr(VI) adsorption with the linear fit showed in Figure 4. For the Langmuir line graph, the linearization model was obtained from a plot between 1/Ce and 1/Qe. It can be seen that the graph obtained is linear with equal \( y = 67.193x - 1.8916 \), with a correlation value \( R^2 \) of 0.993. The Freundlich Isotherm graph was obtained from the linearization line from the plot between \( \log Ce \) and \( \log Qe \) which shows a linear equation \( y = -2.055x + 2.556 \) with a value of \( R^2 = 0.8374 \).

![Figure 4](image-url)

**Figure 4.** Graph of Langmuir Isotherm (left) and Freundlich (right) of Cr(VI).
Table 1. Parameters of Langmuir and Freundlich Adsorption Isotherm.

| Langmuir Isotherm | Freundlich Isotherm |
|-------------------|---------------------|
| Q_{max} (mg/g) = 0.53 | K_f = 359.75 |
| b (L/mg) = 0.39 | n = 0.49 |
| R^2 = 0.993 | R^2 = 0.8374 |

It can be seen from the Figure 4 that the Langmuir isotherm has a correlation value (R^2) which is close to 1. Hence, it can be suggested that the adsorption of Cr(VI) on the DiBch follows the Langmuir isotherm. The adsorption of Cr(VI) on the DiBch surface occurs chemically in one layer with the assumption that the active site on the adsorbent surface has the same energy and the adsorption occurred reversible [10]. From this model, the maximum capacity of the DiBch adsorbent for Cr(VI) was 0.53 mg/g. The surface of DiBch consists of active sites which responsible to interact with Cr(VI) oxyanions, including hydroxyl groups of chitin, primary amine groups of chitin and dithizone, and –SH groups of dithizone. At lower pH than the pzc of chitin (6.7) and pKa of dithizone (pKa 1 = 4.5), the active groups undergo protonation resulting in partially positive charge of the sites. At this study, the pH of electroplating waste was as low as 3.0, so the oxyanions of Cr(VI) could be attached to the surface of DiBch adsorbent due to the electrostatic force between the negative charge of Cr(VI) oxyanions and the positive charge of adsorbent sites.

4. Conclusion
The results showed that the dithizone modified biochitin (DiBch) can be used to adsorb Cr(VI) up to 986.42 mg/L by using 0.4-gram adsorbent for 2 hours with the percentage of Cr(VI) removal was 83.45% ± 0.78. The Cr(VI) adsorption mechanism using DiBch follows the Langmuir isotherm equation model which shows the adsorption capacity of DiBcH was 0.53 mg/g.

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