Modification of Biochitin Immobilized Dithizone as Adsorbent for Cr (VI) Removal

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Modification of Biochitin Immobilized Dithizone as Adsorbent for Cr (VI) Removal

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Abstract: Biochitin is chitin obtained from a biological fermentation that could be used as adsorbent. Biochitin used in this study was produced by sequential fermentation method from white shrimp shell using *Lactobacillus plantarum (L.plantarum)* followed by using *Bacillus thuringiensis (B.thuringiensis)*. Modification of biochitin was investigated by immobilization of dithizone onto the biochitin. Immobilization parameters were optimized including the mass of dithizone and reflux time. The optimum conditions were determined based on the optimum adsorption efficiency of Cr(VI) on the modified biochitin. Adsorption was conducted triplo using 50 mg/L Cr (VI) at pH 5.0 for 6 hours The modified biochitin produced by optimum parameters was then characterized including the functional groups, surface morphology, particle size, and adsorption efficiency for Cr(VI) from electroplating waste. Results of this study showed that the optimum modification conditions were achieved by immobilization of 0.015 g dithizone/ g of biochitin and the mixture was refluxed for 4 hours in toluene medium at 70^{o} C. This modified biochitin had the FTIR spectra at wavelength numbers of 2376.13 cm^{-1} (S-H group), 1382.87 cm^{-1} (S=C group), and at 1558.38 cm^{-1} (N-H group) which shows that dithizone had been successfully immobilized onto biochitin. It was also showed from SEM images that the modified biochitin had more uniform pores than unmodified biochitin. In addition, the modified biochitin was micro particle with the size was in the range of 90 - 200 µm. In addition, the modified biochitin could be used to remove the diluted Cr(VI) from electroplating waste at pH 4.6 with the adsorption efficiency was 83.45% ± 0.40, higher than the one using the unmodified biochitin.

Keywords: biochitin, immobilization, dithizone, adsorption, Cr(VI)

1. Introduction

Heavy metals are one of contaminant causing pollution in soil and water. The extent of the distribution of pollution is related to a large number of industries that produce toxic wastes, such as mercury (Hg), lead (Pb), arsenic (As), cadmium (Cd), chromium (Cr), and nickel (Ni)[1)]. Chrome is a metal that has high toxicity and can cause acute poisoning[2–4)]. In the aqueous solution, chrome is available in two oxide states, *i.e.* Cr(III) and Cr(VI) in the form of oxyanions. Cr(VI) is more toxic than Cr(III). It causes dermatitis and allergic skin reactions, respiratory symptoms including coughing, shortness of breath, and itchy nose. In addition, Cr(VI) is mutagenic and carcinogenic contaminant for all organisms.

In general, the industries discharge the wastes into the water system without removing the toxic compounds in the wastes. Such removal methods have been taken into consideration to treat the wastes including precipitation, adsorption on solids, ion exchange, and separation with foam[1)]. Adsorption is a method that more widely used in industries to remove metals in the waste. Advantages of adsorption method are more economic, not causing toxic side effects, and are capable of removing metals.[5)]. Chitin was one of adsorbents that is widely used to remove heavy metals. Chitin is the poly(β-(1-4)-*N*-acetyl-D-glucosamine biopolymer. Some researchers consider that chitin has 0-50% degree of deacetylation (DD)[6)]. It means, chitin has about 0-50% degree of acetylation (DA). It makes chitin has higher crystallinity and lower solubility in acid condition respect to that of chitosan. The amine groups in chitin are responsible sites for heavy metals adsorption[7,8)]. In some cases, adsorption of heavy metals on chitin is not specific and selective.

Modification of chitin by using dithizone had been investigated to increase selectivity of the chitin for adsorption of cationic heavy metals species, especially in the wastewater sample with high concentration of alkali and earth alkali metal ions[9)]. As specific and sensitive ligand, immobilization of dithizone could add the adsorption sites in the chitin because of the availability of N donor atoms, as well as -NH and -SH as groups which

are respossible for chelation with cationic heavy metals, such as Cd(II) ion[9].

Chitin could be isolated from shrimp shells, crab shell, and squid bones[10,11]. Extraction of chitin could be performed chemically through protein hydrolysis (deproteination) using high concentration of basic solution and followed by removal of inorganic material (demineralization) using concentrated acid solution[12]. The use of these chemicals causes product depolymerization, thereby affecting properties such as molecular weight, viscosity, and acetylation rates [13–15]. The disposal of waste from chemically chitin extraction is very dangerous, consuming energy and threatening the environment[7,10]. To overcome the problem of handling chemically, the biotechnology process can be used as an alternative method that is more environmentally friendly. Microorganisms (microbiological fermentation) and proteolytic enzymes (enzymatic extracts or isolated enzymes) have been used for deproteination and demineralization process in chitin extraction.

Lactic acid fermentation is one of the biological methods that can be used to extract chitin from shrimp shells. *Lactobacillus plantarum (*L. *plantarum*) can extract chitin from shrimp heads with a demineralization value (DM) of 71%[12]. Lactic acid bacteria in the form of *L. paracasei* has been used to extract chitin from Nephrops norvegicus with deproteination value (DP) of 71% [16]. In addition, by using the *L. plantarum* that was added with 10% inoculum and 5% glucose, the chitin had DM value of 81.4%[17]. Wahyuntari *et al* investigated the effect of the sequence of deproteination and demineralization process by microbes[18]. She used *Bacillus licheniformis* to conduct deproteination process and *Lactobacillus acidophilus* FNCC116 for demineralization process. The result showed that deproteination-demineralization process produced chitin with lower percent yield than the result from demineralization-deproteination process. Biochitin is then used to refer chitin extracted biologically through fermentation process.

Rosmawati *et al*[11] has produced biochitin through sequential fermentation with L. *plantarum* followed by B. *thuringiensis.* The resulted biochitin could adsorb anionic heavy metal species, such as Cr(VI) in the diluted electroplating waste at pH 5, with the adsorption efficiency up to 51.99%±0.41. Modification of biochitin can be conducted to increase the adsorption efficiency for Cr(VI) anions. N donor atom, as well as -NH and -SH groups of dithizone are attached to biochitin to perform electrostatic attraction with the anionic Cr(VI) species. Objective of this presented study was to optimize the parameters for dithizone immobilization into biochitin. The investigated parameters were the mass of dithizone and the reaction time during reflux (then referred as reflux time). Adsorption efficiency of Cr(VI) was used as the dependent variable to determine the optimum conditions for the biochitin modification method.

2. Methods

Equipment used for preparing biochitin and modification in this study were: Laminar Air Flow (LAF), cool boxes, ovens, blenders, and 80 mesh sieves. Batch adsorption was conducted by shaking the mixture of adsorbent-adsorbate with a shaker. Universal pH meter was used to measure pH of solution. Shimadzu 1601 UV-Vis spectrophotometer was used to measure the absorbance of Cr(VI)-diphenylcarbazide complex compound. A 8400S Shimadzu FTIR Spectrophotometer was used to determine the functional groups of biochitin (ditizone modified and unmodified). Scanning Electron Microscopy (SEM) and Particle Size Distribution 1090 Dry Rinse were used to capture the surface morphology images of biochitin and its particle size. All reagents and standard solutions were prepared using clean glass apparatus.

Raw materials to produce biochitin in this study were white shrimp (*L.vannamei*) shell from PT. Sekar Katokichi in Sidoarjo Indonesia, *L. plantarum* was purchased from the Biology Department of Brawijaya University in Malang Indonesia, while *B. thuringiensis* bacteria was from the Soil Department of Brawijaya University in Malang Indonesia. All chemicals used for biochitin extraction and modification were in analytical grade and purchased from Merck, including MRSA (deMann Rogosa Sharpe Agar), glucose, NA (Nutrient Agar), NaOH (Merck 106498.1000), HCl, NaOCl (Merck 105614), dithizone (1,5diphenylthiocarbazone), toluene (Merck 108325), and ethanol (Merck 100983). Chemicals used for preparing standard solution of Cr(VI) and analysis were all in analytical grade and purchased form Merck, including diphenylcarbazide crystal (Merck 103091), acetone (Merck 100012), $K_2Cr_2O_7$ crystal (Merck 104865), and H_2SO_4 (Merck 109073). For adsorption test, electroplating wastewater samples was collected from a local electroplating industry in Malang Indonesia.

3. Procedure

3.1 Extraction of Biochitin

Biochitin used in this presented study was extracted through sequential fermentation method as described by Rosmawati *et al*[11]. As a starter, the culture of 15 hours *L. plantarum* in the de Man, Rogosa, and Sharpe Agar (MRSA) modified with Shrimp Head Extract Shell (SHES) in 2% glucose was used. A 500 mL starter was then added with 500 g of shrimp shell flakes (1:1) [19]. Next, 10% w/w of glucose 1 M and 1% w/w of NaCl 1 M were added. The first fermentation was performed for 30 hours at 37° C. The produced solid of demineralized shrimp shells were then separated, washed, and dried in oven at 80° C for overnight. A total of 3% (w/v) of demineralized chitin was then added with 10% (v/v) of the 24-hour

culture of *B. thuringiensis* in Nutrient Agar (NA)-SHES. The mixture was incubated at 37° C for 81 hours with agitation at 200 rpm. Afterward, the mixture was filtered. Solid product of biochitin was then washed with water and dried in the oven for overnight at 80° C.

3.2 Modification of Biochitin with Dithizone

The method to modify biochitin with dithizone was adapted from Mudasir *et al* [9]. In general, as much as 1.0 g of the biochitin was added to 40 ml toluene and then mixed with dithizone at certain mass in a 500 mL flask. The mixture was refluxed at certain time at 70º C. After the reflux was completed, the mixture was filtered and the product was washed several times using toluene, ethanol, and water sequentially to remove the excess of dithizone on the surface of modified biochitin. The solid product was then dried at 60º C for 12 hours. The modified biochitin was then grounded and sieved through 80 mesh sieving.

3.3. Optimization of Biochitin Modification

Two parameters of biochitin modification were optimized in this study, including the reflux time and the mass of dithizone. The mixture of biochitin-dithizone in toluene was refluxed for 2, 4, and 6 hours. Meanwhile, the mass of dithizone was varied at 0.005; 0.010; 0.015; and 0.020 g/g biochitin. The optimum conditions for biochitin modification were determined based on the optimum adsorption efficiency of Cr(VI). Adsorption was conducted in batch method. About 0.5 g of modified biochitin was added into 25 ml of Cr(VI) 50 mg/L (in the form of $Cr_2O_7^{2-}$) in a 100 mL Erlenmeyer. The pH of the diluted solution was about 5.0. The mixture was then shaken for 6 hours and then filtered. The adsorption was repeated three times (triplo). Initial and final concentration of Cr(VI) were analyzed followed procedure as described at section 3.6. Further, adsorption efficiency of Cr(VI) was calculated using Eq. (1) as shown below:

$$\textit{Adsorption efficiency (\%)} = \frac{m_0 - m_t}{m_0} \; x \; 100\% \quad (1)$$

Where, m_0 is the amount of initial mass of Cr(VI) (g) and m_t is the amount of Cr(VI) final mass (g).

3.4. Characterization of Modified Biochitin

Characterization was conducted to the modified biochitin which was produced at optimum conditions. To confirm the successfulness the modification of biochitin, functional groups of the modified biochitin was analyzed using FTIR spectrophotometer at wavenumbers 400-4000 cm^{-1}. Furthermore, the morphology of modified biochitin was captured with Scanning Electron Microscopy (SEM) which aims to determine the microstructure morphology and surface shape. Particle Size Analyzer (PSA) was also used to determine particle size and distribution of the modified biochitin.

3.5 Adsorption Test for Cr(VI) in Electroplating Waste

The dithizone modified biochitin (DiCh) was used to adsorb Cr(VI) in electroplating waste. Electroplating waste comprises high concentration of Cr(VI). Therefore, for the adsorption test with the modified biochitin, the electroplating waste was diluted several times to about 100 mg/L. The pH of the diluted waste was around 4.6. In this step, 0.4 g of adsorbent was mixed with 25 ml of diluted electroplating waste in a 100 ml Erlenmeyer. The mixture was then shaken for two hours and then filtered. Adsorption was conducted three times (triplo). The adsorption efficiency was then calculated with Eq.1. Adsorption of Cr(VI) was also performed using other adsorbents, including raw shrimp shell (KU), chemical extracted biochitin (KK), and unmodified biochitin (LPBT). Raw shrimp shell was produced by drying the head shell of white shrimp in oven at 105º C for 12 hours to remove water. Dried shell was then grounded and sieved at 80 mesh. Chemical extracted chitin was produced from powder of dried head of shrimp shell through deproteination and demineralization. Deproteination was conducted by using NaOH 40% at ratio 1:1. Deproteination was performed at 80º C for 1 hour. Meanwhile, demineralization was carried out by using HCL 1 M and heated at 80º C for 1 hour. The final product was then dried in oven at 60^0 C [20].

3.6. Spectrofotometric Method for Cr(VI) Analysis

Initial and final concentration (after adsorption) of Cr(VI) in the solution was determined based on the absorbance measurement of Cr(VI)-diphenylcarbazide complex compound at 540 nm [21]. In general, 1 mL of sample containing Cr(VI) (electroplating waste and Cr(VI) standard solutions) was added by 10% of sulfuric acid in a 50 ml volumetric flask. The pH of solution was adjusted at 2.0. Afterward, 1 ml of 1,5 diphenylcarbazide solution was added. The mixture was left for at least 8 minutes before measuring the absorbance. Concentration of Cr(VI) in the analyte was calculated based on the standard curve of Cr(VI) standard. Cr(VI) standard solutions were prepared from $K_2Cr_2O_7$ powder.

4. Results and Discussion

Biochitin had been extracted from white shrimp shells through deproteination and demineralization using two strains of bacteria sequentially. It produced light brown

powder, as shown in Figure 1(a). The color is still brown because the depigmentation process was not conducted. As reported by Rosmawati *et al* [11], the biochitin extracted by sequential fermentation method has acetylation degree (DA) of 86.22%. It was higher than chemically extracted chitin. It means, the biochitin consists mostly secondary amine in the acetamide groups which is responsible for adsorption.

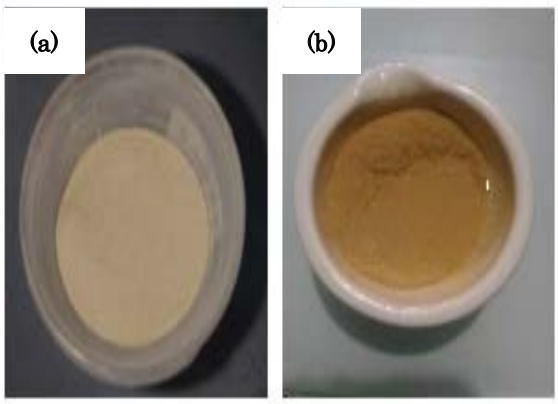


Fig. 1: Physical performance of (a) unmodified biochitin powder and (b) dithizone modified biochitin.

Modification of biochitin was performed by immobilization of dithizone into biochitin. It could be reached by refluxing biochitin with dithizone in toluene medium. Toluene was chosen as a solvent instead of water to avoid the hydrogen bond formation between the -OH groups in glucosamine rings with water molecules, which can reduce the ability of biochitin to interact with dithizone. The resulted product was washed with several solvents to remove the excess dithizone in the surface of the modified biochitin. The modified biochitin has dark brown color (Figure 1.b) due to the presence of dithizone immobilized in the biochitin.

4.1 Optimization of Dithizone for Biochitin Modification

In this study, two parameters had been optimized for modifying biochitin. Figure 2 shows the adsorption ability of modified biochitin produced at various time of reflux. It can be seen from Figure 2 that all modified biochitins have high adsorption efficiency to Cr(VI) for more than 85%. It can be seen from the graph that the reflux time seems not differ the ability of modified biochitin as adsorbent for Cr(VI).

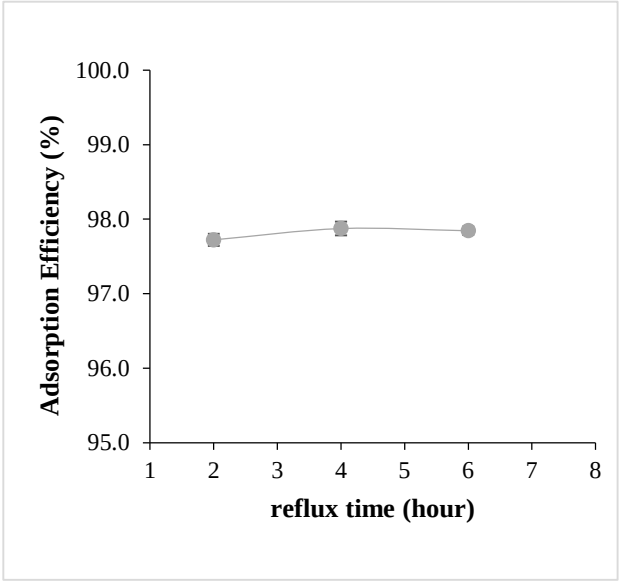


Fig. 2: The influence of reflux time for the modified biochitin to the adsorption efficiency for Cr(VI).

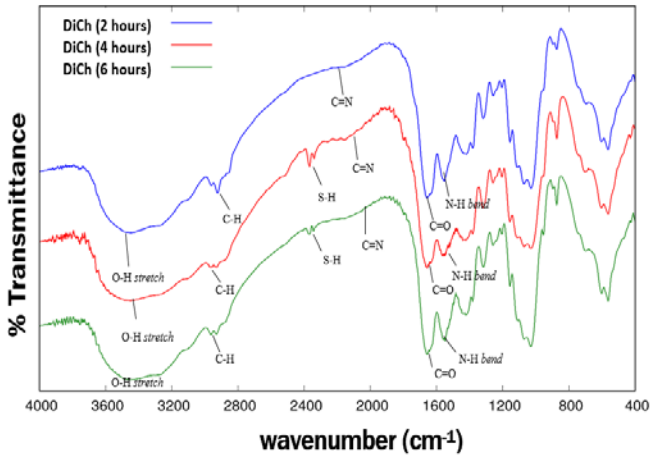


Fig. 3: IR spectra of dithizone modified biochitin at various reflux time.

Hence, to determine the optimum reflux time, the IR spectra of modified biochitins were measured. From the IR spectra as shown in Figure 3, it can be seen that modification was not completed when reflux was performed for 2 hours, indicated by the absence of -SH spectra at around 2400 cm^{-1}. Increasing the reflux time could assure complete reaction during dithizone immobilization, as noted by the presence of -SH spectra when immobilization conducted for 4 and 6 hours. In contrast, longer reaction time means longer heating time which could oxidize the active sites of dithizone bound to the surface of biochitin, indicated by the low intensity of -SH spectra as shown in Figure3. Mudasir *et al*[9] found the similar phenomena in the modification of chemical extracted chitin by dithizone ligand. Based on this result, the optimum reflux time for modification of biochitin by dithizone was at 4 hours.

The second optimizaation was performed by varying the mass of dithizone ligand. The optimum mass of immobilized dithizone in biochitin can be determined from the adsorption efficiency of Cr(VI)[22]. Based on Figure 4, the highest adsorption efficiency was achieved by using biochitin modified with 0.015 g dithizone/g biochitin. About 98.49% ± 0.065 of Cr(VI) could be adsorbed on the surface of the modified biochitin. Addition the amount of dithizone on the biochitin means increasing the adsorption sites on the surface of modified biochitin. In contrast, from the Figure 4 it can be seen that the adsorption efficiency of Cr(VI) was decreased when using biochitin modified by 0.020 g dithizone. It could be due to all N atom in acetamide group and -OH group in glucosamine ring have all attached by dithizone thus decreases the number of active sites on the surface of modified biochitin. Therefore, it can be concluded that the optimum mass of dithizone for modifying biochitin was 0.015 g/ g biochitin.

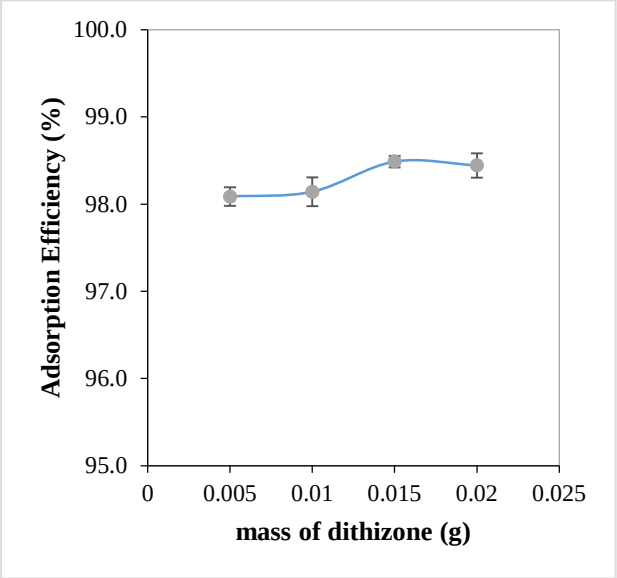


Fig. 4: The influence of dithizone mass for the modifying biochitin to the adsorption efficiency of Cr(VI).

4.2. Characterization of Dithizone Modified Biochitin

Chitin structure is a linear homopolysaccharide composed of N-acetylglucosamine groups in the beta chain and has a monomer in the form of glucose molecules with nitrogen-containing branches. As proposed by Mudasir *et al* [9], modification of biochitin by dithizone could occur through two possible mechanisms. The first mechanism is by attack of N lone pair electron attack of dithizone to the -OH groups of glucosamine ring. The second possible mechanism is through electrostatic interaction between lone pair electron of N atom in dithizone with the protonated carbonyl groups of acetamide group.

It can be seen from Figure 5 that the dithizone modified biochitin (DiCh) has specific spectra compared to the spectra of unmodified biochitin (LPBT). Spectra at wave number of 3404.13 cm^{-1} refers to aliphatic -OH group of glucosamine ring. Low intensity of this spectra in DiCh (red line) is due to the formation of hydrogen bond between -OH of glucosamine ring with N atom of dithizone. However, the spectra of -OH aliphatic is broad indicating that DiCh consists high water content. The weak band at 2931.60 cm^{-1} refers to stretching of C-H olefin. Presence of dithizone in the modified biochitin could be shown from weak bands at 2376.13 cm^{-1} that indicates -SH of dithizone and bands at 2279.14 cm^{-1} that indicates C=N of dithizone (red line spectra). Bending spectra of NH acetamide groups appeared at 1552.59 cm^{-1}. Vibration bands at wave number of 1589.23 cm^{-1} indicates C=C of aromatic skeleton of phenylic group which is supported by bands at 873.69 and 738.69 cm^{-1} which correspond to vibration of C-H aromatic. All the wave numbers mentioned indicate that dithizone has been successfully immobilized on biochitin.

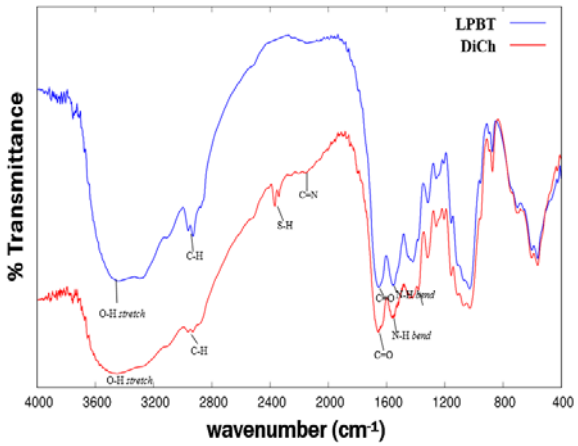
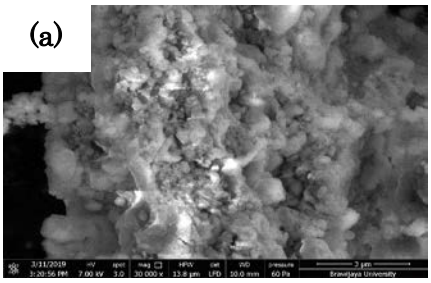


Fig. 5: IR spectra of LPBT (blue line) and DiCh produced at optimum conditions (red line).

Figure 6 shows microphotograph LPBT (a) and DiCh (b). Chitin has an inhomogeneous structure so both samples have immature particles of various shapes and sizes [14,23]. Modification of biochitin with dithizone resulted in adsorbent with numbers of uniform pores. Hence, the modified biochitin should have high adsorption capacity.



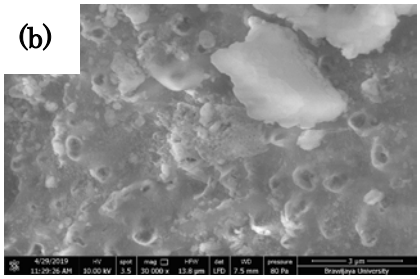


Fig. 6: SEM photographs of LpBt (a) and DiCh (b) with 30000x magnification.

As an adsorbent, the modified biochitin should have small particle size. Based on the particle size analysis, the average particle size of modified biochitin, were 20.74; 106.60; and 220.79 µm with the cumulative values of 10%, 50%, and 90% respectively (Figure 7). As shown in Figure 7, particle size of the modified biochitin produced at optimum conditions in the range of 90 - 200 µm has a high frequency.

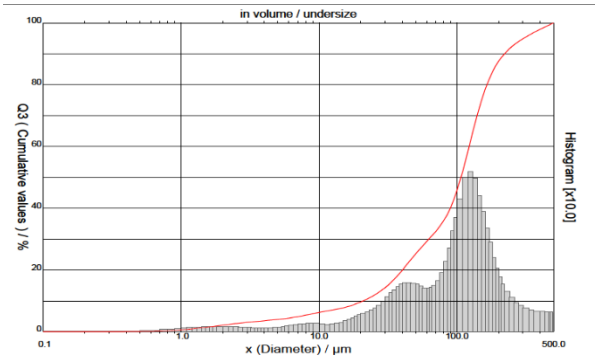


Fig. 7: Histogram of the dithizone modified biochitin particle diameter distribution pattern.

4.3. Adsorption of Cr(VI) from Electroplating Waste by Modified Biochitin

Electroplating waste consists high concentration of Cr(VI) in the form of chromate or dichromate depending on pH of solution. In this study, the pH of electroplating waste was 3.5. Dilution of the samples was carried out to decrease Cr(VI) concentration suitable for adsorption. The diluted sample solution had pH about 4.6. At this pH, Cr(VI) is in the form of $Cr_2O_7^{2-}$ and $HCrO_4^-$. Adsorption of these species on the surface of chitin based adsorbent could be occurred through electrostatic attraction between the negative charge of Cr(VI) species with the positive charge of protonated N atoms in the adsorbent. The samples that were adsorbed using adsorbents-based chitin. As shown in Figure 8, the Cr(VI) from electroplating waste could be adsorbed efficiently by using the dithizone modified biochitin (DiCh). It was reached 83.45% ± 0.40. It could be due to the high numbers of N atoms, either from acetamide groups and from dithizone.

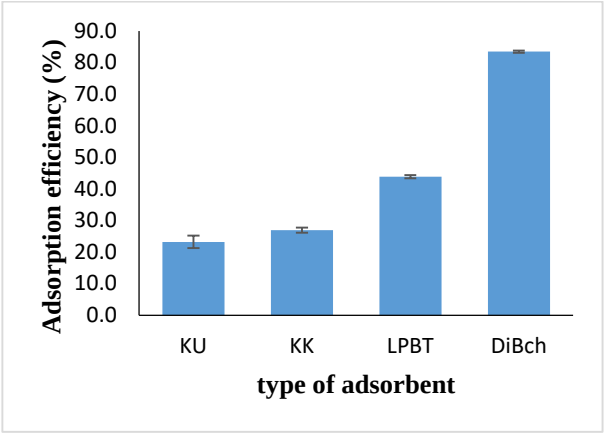


Fig. 8: The influence of adsorbent type to the adsorption percentage of Cr(VI) (Caption: KK is chemical chitin, LPBT is unmodified biochitin, KU is shrimp shell, DiCh is dithizone modified biochitin).

5. Conclusion

Modification of biochitin by dithizone had been performed by optimizing reflux time and mass of dithizone. The optimum conditions for modifying biochitin were reached when the mixture of biochitin and dithizone was refluxed for 4 hours in toluene medium by adding 0.015 g dithizone/g biochitin. The modified biochitin showed higher performance to adsorb Cr(VI) from electroplating waste ss much as 83.45%.

Acknowledgements

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