Synthesis and Characterization of Chitosan Hydrogel Bioadsorbent Heavy Metals from Vannamei Shrimp Shell Waste

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Abstract. Heavy metal pollution from industrial wastewater is a serious problem for the environment. Adsorption using natural bioadsorbents is a sustainable and eco-friendly method for reducing heavy metal content in wastewater. This study aims to synthesize and characterize chitosan hydrogel as a bioadsorbent from vannamei shrimp shell waste and test its effectiveness in adsorbing heavy metals Cu and Ni. The synthesis process was carried out through the Knorr method to obtain shrimp shell chitosan (SSC) from chitin, which was then crosslinked with Tetraethylenepentamine (TEPA) into hydrogels, with molar ratio variations of TEPA and SSC (0.4; 0.5; 0.6; 0.7). Characterization was performed using FTIR and SEM to observe changes in functional groups and surface morphology. FTIR results showed that the molar ratio of 0.6 resulted in the most optimal formation of amide groups. Meanwhile, SEM results showed that the hydrogel structure at a molar ratio of 0.6 had the largest pore volume a value of 4.66×10^{10} and the most even pore distribution. Adsorption tests on electroplating industry wastewater showed that chitosan hydrogels were able to remove 99.17% of Cu and 98.77% of Ni, adsorption capacities of 1.071 mg/g and 2.9735 mg/g, respectively

1 Introduction

Industry is currently experiencing rapid progress along with rapid technological developments, causing many new industries to emerge. However, industrial growth also brings negative effects such as an increase in the amount of industrial wastewater that can pollute the environment. One of the contents contained in industrial wastewater is heavy metals. The main sources of heavy metal pollutants come from the industrial and mining sectors, such as iron-steel, electroplating, mining, packaging, and textiles.

Industrial wastewater will be able to cause serious pollution to the environment if the heavy metal content exceeds the set standard. Based on the Minister of Environment Regulation (Permen LH) No. 5 of 2014 on wastewater quality standards, the threshold levels of heavy metals in wastewater, are 2 mg/L for Cu metal and 0.6 for Ni (II) metal. Heavy metals have dangerous toxic properties and can cause serious illness if accumulated in the human body, so it is important to effectively treat industrial wastewater before it is discharged

into the surrounding environment. One alternative method to reduce the levels of heavy metals in industrial wastewater treatment is the batch adsorption method.

Adsorption is often used in wastewater treatment processes because it is more economical, non-toxic, and effective in reducing heavy metal levels in wastewater. Research on the application of the adsorption process to reduce heavy metal levels, one of which is Pb in laboratory wastewater. Activated carbon adsorbent was employed with a ratio of 1:10 while the stirring process was carried out within an hour inside a jar to reduce Pb levels from 1.1 mg/L to 0.7 mg/L [1]. Activated carbon as an adsorbent has been widely used to reduce heavy metal levels in wastewater in practice, but its application is limited due to high production costs, regeneration, percent removal and heavy metal adsorption capacity is not maximized so it must be further improved [2].

Adsorption usually uses conventional adsorbents such as activated carbon because it has a fairly good adsorption ability, but operational costs and regeneration are relatively expensive, so alternative adsorbents from natural materials are needed [3]. One of the adsorbents derived from natural materials is chitosan. Chitosan as a bioadsorbent can be synthesized with raw materials from vannamei shrimp shell waste. One of the areas in Indonesia that has the potential to provide of vannamei shrimp shell waste is Sumenep Regency, East Java.

Based on BPS (2023) the total national shrimp production reached 1.097 million tons/year, with most of it processed in factories processing frozen shrimp food. It is estimated that about 10% of shrimp processing leaves shrimp shell waste, which means it can produce about 109.7 million tons of shrimp shells/year. Shrimp shell waste can be utilized to produce chitosan, which is a deacetylated polysaccharide from chitin contained in shrimp shells (40-50% of shrimp body weight). The production of chitosan from shrimp shell waste will also increase the economic value.

Research on the synthesis and application of chitosan from shrimp shells as a bioadsorbent for heavy metal Zn in PLTD Monokwari. The mass of chitosan was varied as much as (0.1; 5; 1; and 2) grams in 100 mL of PLTD liquid waste. The best removal percent and adsorption capacity were 69.28% and 9.44 x 10^{-3} mg/g with chitosan mass of 2 gram [4]. However, these results are considered less than optimal for reducing heavy metals such as Zn, so the %removal & adsorption capacity on chitosan needs to be increased. One method is to react Shrimp Shell Chitosan (SSC) with active groups first in the form of Epichlorohydrin (ECH) as a bridge for further reaction with Tetraethylenepentamine (TEPA) to form a hydrogel. Therefore, this research aims to synthesize SSC which then reacted it with TEPA as cross linker and coordination site provider, expected to be dissolvable in water and formed a stable hydrogel. This research also investigates the structure and adsorption property of chitosan hydrogel as well as the efficiency of reducing heavy metal levels in electroplating industry wastewater. In addition, to investigate the advantages and feasibility of chitosan hydrogel as bioadsorbent for the removal of heavy metals Cu and Ni in electroplating industry wastewater.

2 Materials and Methods

2.1 Materials and Tools

Vannamei shrimp shells waste were taken from ponds in Sumenep, East Java (Indonesia). Epichlorohydrin (ECH) and tetraethylenepentamine (TEPA) were purchased Merck (Germany). Sodium Hydroxide (NaOH), Hydrochloric Acid (HCl), and Diklоromethane were purcased from SAP Chemical (Indonesia). Aquadest was used throughout the experiments.

Fourier transform infrared (FT-IR) spectra were obtained with a Thermo Scientific Nicolet iS10 FT-IR spectrometer from Thermo Fisher Scientific (USA). FTIR was used to identify functional groups and chemical changes that occur during the cross-linking process as well as determine the degree of deacetylization. Scanning Electon Microscope (SEM) (FEI Inspect S50, USA) was utilized to characterize the surface morphology and calculate the pore volume to be filled by chitosan hydrogel. Atomic Absorption Spectrophotometry (AAS) (Hitachi ZA3000 Series, Japan) was used to determine the concentration of Cu and Ni metals before and after treatment in electroplating wastewater.

2.2 Method

The research was conducted at the Industrial Waste Treatment Laboratory, Chemical Engineering Department, Faculty of Industrial Technology and Systems Engineering (FTIRS), Institut Teknologi Sepuluh Nopember, Surabaya (ITS), East Java, Indonesia. FTIR and AAS Analysis was carried out at the Water Technology Laboratory and Industrial Consulting, Chemical Engineering Department, FTIRS ITS. SEM Analysis of chitosan hydrogel was carried out at the Mechanical Engineering Department, FTIRS ITS.

Synthesis of Shrimp Shell Chitosan (SSC) was used Knorr method (pre-treatment, demineralization and deproteination) to produce chitin, and deacetylation to produce chitosan as well as characterization, such as moisture and ash content, organoleptic, identification of functional groups and calculation of deacetylization degree using FTIR. Then, SSC was reacted with Epichlorohydrin (ECH) as a bridge before crosslinked with TEPA based on variation of molar ratio of TEPA and SSC (0.4, 0.5, 0.6, 0.7) into ECH-SSC mixture to form chitosan hydrogel. Furthermore, it was further characterized by SEM and FTIR. The best result based on the effect of molar ratio of TEPA and SSC, was used for heavy metal removal by batch adsorption method on electroplating wastewater. Then the wastewater was analyzed by AAS to reveal the Ni and Cu concentrations comparing before and after treatment. The results of comparison described the capacity and percentage of heavy metal removal.

2.3 Data Analysis

Moisture content test is conducted to determine the amount of water present in chitosan, which can be known from the amount of water evaporated during oven heating. The maximum moisture content of chitin and chitosan is 12% [5]. The moisture content of chitosan was calculated using the following formula:

$$\% \text{ Moisture content} = \frac{x-y}{z} \text{ x } 100\% \qquad (1)$$

Where x is weight of the cup & initial sample (g), y is weight of the cup & dry ash (g), and z is weight of the initial sample (g)

Ash content test is conducted to know how well the dimeneralization process works & to know the mineral content present in the material as well as to show the purity of chitosan. The maximum ash content of chitin and chitosan is 5% [5]. The ash content of chitosan was calculated using the following formula:

$$\% \text{ Ash content} = \frac{x-y}{z} \text{ x } 100\% \qquad (2)$$

Where x is weight of the crust & initial sample (g), y is weight of the crust & dry ash (g), and z is weight of the initial sample (g)

Degree of deacetylization (DD) is a parameter that determines the quality of chitosan, where this value shows the percentage of acetyl groups that can be removed from chitin

compounds to produce chitosan compounds, using FTIR. Standard DD chitosan value more than equal to 75% [5]. DD chitosan was calculated using the following formula:

$$DD\ (\%) = [100 - (\frac{A_{1655}}{A_{3450}} \text{ x } \frac{100}{1,33})] \quad (3)$$

Where A_{1655} is absorbance of the amide group (1655 cm^{-1}) and A_{3450} is absorbance of the hydroxyl group (1655 cm^{-1})

Analysis of Cu and Ni levels was carried out with the final concentration of each substance, using Atomic Absorption Spectrometry (AAS). The removal of heavy metal Cu and Ni percentage was calculated using the formula as follows [6]:

$$\boldsymbol{\eta} = \frac{Co-C}{Co} \text{ x } 100\% \quad (4)$$

Where $\boldsymbol{\eta}$ is percent removal of substance (%), Co is initial Concentration (mg/L), and C is final concentration after wastewater is treated (mg/L)

The adsorption capacity was calculated using the formula as follows [7]:

$$Qe = \frac{(Co-Ce)V}{m} \quad (5)$$

Where Qe is adsorption capacity value (mg/gr), Co is initial concentration (mg/L), Ce is final concentration (mg/L), V is wastewater volume (L), and m is adsorbent mass (g)

3 Results and Discussion

3.1 Characterization of Chitosan Powder

The characteristics of chitosan are based on the Indonesian National Standard so that chitosan made needs to meet these standards before being used as adsorbent in an adsorption process. Some tests are analysis of moisture content, ash content, determination of deacetylization degree (DD) and identification of its functional groups using FTIR (Fourier Transform Infrared Spectroscopy). The analysis is needed to determine the content in chitosan that can affect its quality and ability to adsorb heavy metals.

Table 1. Analysis of Moisture Content, Ash Content, and Degree of Deacetylization

Parameter (%)	Standard SNI No. 7949:2013	Research Result
Moisture Content	≤ 12	0,35%
Ash Content	≤ 5	2,8%
Degree of Deacetylization	≥ 75%	83,01%

Based on the results obtained, the moisture content and ash content contained in shrimp shell chitosan were 0.35% and 2.8%. These results indicate that shrimp shell chitosan has met SNI 7949:2013 and is suitable for use as an adsorbent in the adsorption process. Moisture content analysis was conducted with the aim to determine the hygroscopic properties of a chitosan. The high water content of chitosan products affects the quality of chitosan especially against microorganism contamination [8]. Several things can affect the moisture content of chitosan, namely drying time, the weight of the dried sample, and the surface area of the drying medium [9].

Ash content analysis is used as a parameter to determine how well the demineralization process is successful and to determine the mineral content that exists in a material. In

addition, the ash content shows the purity of the chitosan produced. The higher the ash content in chitosan indicates that the higher the mineral content in it, which can affect the adsorption process because it causes blockages in the pores of the chitosan thus reducing ability of chitosan to reduce metal.

Analysis of the degree of deacetylization (DD) is a parameter that greatly determines the quality of chitosan, where this value shows the percentage of acetyl groups that can be removed from chitin compounds so that chitosan compounds are produced. The higher the degree of deacetylation of chitosan, the lower the acetyl group contained in the chitosan. Determination of the degree of deacetylation of chitosan using FTIR tools based on the base line method. Factors that affect the degree of deacetylization are the concentration of NaOH used, temperature and duration of the deacetylation process. In this study, the NaOH concentration used was 50% with a process temperature of 120 C for 4 hours, resulting in a deacetylization degree of 83.01%. This is in line with previous research that the highest deacetylization degree of 82.98% was obtained at a NaOH concentration of 50% [10].

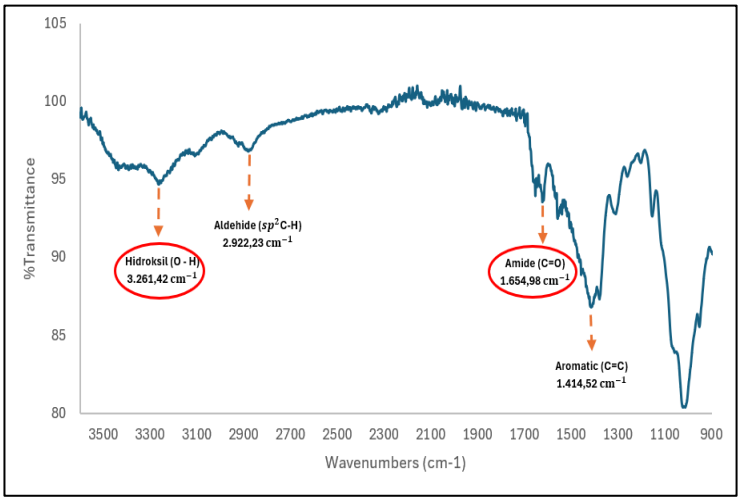

Fig. 1. FTIR Spectra of Shrimp Shell Chitosan

The functional groups confirmed by FTIR absorption indicate that chitosan was successfully synthesized from the deproteinization, demineralization, and deacetylization processes. The characteristic features of chitosan are the amide group and the hydroxyl group [11]. The location of typical absorption of amide groups at wave numbers 1655 - 1310 cm^{-1}, while hydroxyl groups are located at 3650 - 3200 cm^{-1}. The working principle of FTIR is to identify the functional groups of infrared absorbance compounds, so that some compounds can be distinguished and qualified. FTIR is also the best analysis to identify the type of chemical bond [12].

The next characterization is the chitosan organoleptic test which includes the shape and color of the chitosan. Good chitosan has a flake of powder form with light brown to white color [13]. The results of organoleptic analysis show that chitosan from vannamei shrimp shell (*Litopenaeus vannamei*) in this study is in the form of powder and white-brown in color **(Fig 2).** Based on standards, the organoleptic of the chitosan synthesis results above meet requirements. The synthesis of chitosan from other crustacean animals such as snails (*Achatina fulica*) produces a brownish white powder [14] and from crab shells produces brown powder [13].

Fig. 2. Chitosan Powder from Shrimp Shell

Furthermore, the result of the calculation of the % yield of chitosan obtained is of 17%. It is known that the initial amount of shrimp shell powder processed is 100 grams, while the chitosan obtained is 17 grams. There is no standard value for a good % yield. However, this result is slightly better than the % yield of chitosan produced from windu shrimp (*Penaeus monodon*) which is 15.26% [15] and windu shrimp chitosan from another study which is 14%. However, when compared to the yield of chitosan synthesis, the higher yield value indicates the easier the possibility of extracting chitosan from these biological resources.

Overall, the chitosan characterization results obtained have met the requirements set for each criterion. This chitosan characterization can still be developed again to provide maximum results for further development, for example by reacting shrimp shell chitosan with cross-linking agent in the form of Tetraethylenepentamine (TEPA) to form chitosan hydrogel.

3.2 Characterization of Chitosan Hydrogel

The characterization of these modified results can be done by Fourier transform infrared spectroscopy (FTIR), which is able to identify changes in functional groups based on typical absorption bands in the spectrum. The FTIR spectra displayed shows changes in the spectral profile at several wave number regions as a result of the variation in the molar ratio between chitosan and TEPA (0.4; 0.5; 0.6; and 0.7).

The wave number region between 3200-3650 cm^{-1}, characterized as hydroxyl (-OH) and amine (-NH) bands, showed a varying decrease in intensity. This indicates the involvement of -OH and -NH groups in the reaction process with TEPA, where the formation of new bonds causes vibrational changes in these groups. Meanwhile, in the region around 1655-1310 cm^{-1}, which are associated with amide group vibrations (C=O and N-H bending), there is an increase in intensity with increasing TEPA molar ratio. This increase indicates that more amide groups are formed as a result of the reaction between the amine group of TEPA and the carbonyl group of chitosan.

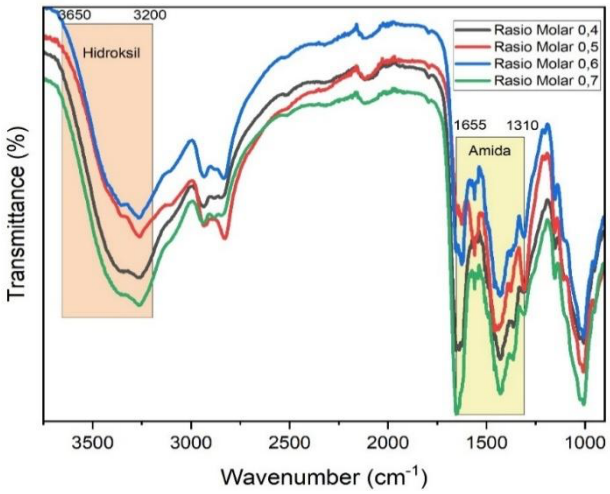

Fig. 3. Spectra FTIR Chitosan Hydrogel

Analysis based on **Fig. 3.** shows that at a molar ratio of 0.4, the amide peak is still weak, the hydroxyl peak is quite strong, indicating that the modification is not optimal. At a molar ratio of 0.5, the amide peak starts to appear more clearly, the hydroxyl peak is slightly reduced. At 0.6 molar ratio, the amide peak is very clear and strong and high, both at 1655-1310 cm^{-1}. A significant decrease in the hydroxyl group. This shows that the modification reaction is most successful. At a molar ratio of 0.7, the amide peaks are strong but begin to broaden or overlap, the hydroxyl peaks are very low, meaning that the reaction is very advanced, but there can be over-modification, which sometimes has a negative impact on the mechanical properties or stability of the material.

SEM Analysis was used to analyze the shape and surface morphology of pure chitosan and chitosan hydrogel based on vannamei shrimp shell waste. In **Fig. 4.a**, it appears that the pure chitosan surface is relatively smooth and compact, with few voids or cracks. The structure tends to be compact, indicating a low specific surface area. In addition, the pure chitosan shows very few pores, indicating a small pore volume that limits its ability to adsorb adsorbates. In **Fig. 4.b**, chitosan hydrogel with a molar ratio of 0.4 can be seen that the surface begins to appear cracks and small gaps, indicating the beginning of pore formation due to modification, but not yet optimal in creating a porous structure.

In **Fig. 4.c**, the chitosan hydrogel with a molar ratio of 0.5 looks rougher and irregularly textured, pores begin to spread on the surface. The number and size of pores increased, some pores began to look fused together. A significant increase in pore volume and active surface area, indicating that TEPA expanded the polymer network structure. In **Fig. 4.d**, the chitosan hydrogel with a molar ratio of 0.6 shows a very irregular and multilayered surface, indicating an increasingly open structure. There are many large and deep pores, partly merging with each other, the pore distribution spreads evenly across the surface. The structure is optimal for adsorption, with a balance between open morphology and mechanical stability. The pore volume is quite high, suitable for metal ion adsorption applications. In **Fig. 4.e**, the chitosan hydrogel with a molar ratio of 0.7 appears to have a rougher surface and the size of the pores is higher.

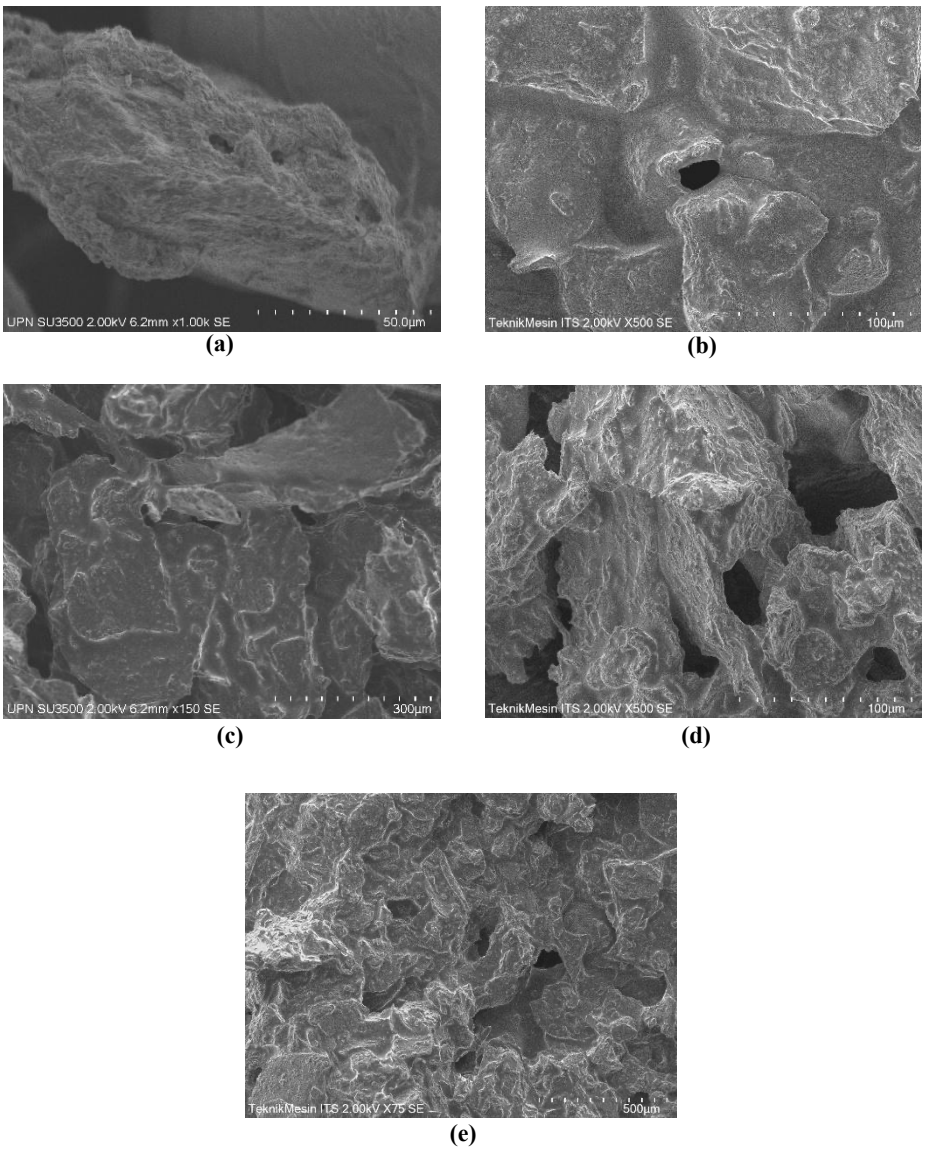

Fig. 4. SEM Test Results on: (a) Pure Chitosan and Chitosan Hydrogel molar ratio: (b) 0.4; (c) 0.5; (d) 0.6; (e) 0.7

Based on **Fig. 5**, the modification of chitosan with TEPA significantly increases the pore volume. Pure chitosan has the lowest pore volume due to its dense and unmodified structure. The molar ratio of 0.6 resulted in the largest pore volume and the most open structure, which is also consistent with the qualitative observation from the SEM image. After a ratio of 0.6, the increase in pore volume began to stagnate indicating the effective limit of the molar ratio between TEPA & SSC.

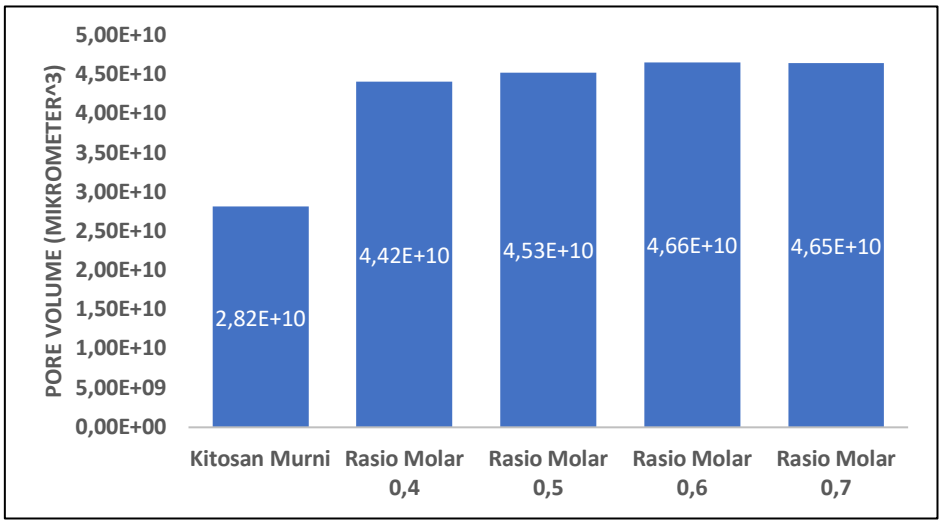

Fig. 5. Pore Volume of Pure Chitosan and Modified

Based on the results of FTIR analysis which showed that chitosan hydrogel (molar ratio 0.6) crosslinked reaction with TEPA occurred most successfully and SEM which showed the optimal structure and produced the largest pore volume a value of 4.66 x 10^{10} and most open structure, making it more suitable for heavy metal adsorption applications. Thus, chitosan hydrogel (molar ratio 0.6) was used for further testing in the form adsorption test of Cu and Ni in electroplating wastewater.

Adsorption test of heavy metals Cu and Ni in electroplating wastewater as much as 50 mL, with pH 3 and contact time 100 using 1 gram of chitosan hydrogel (molar ratio 0.6). The initial concentration of Cu and Ni were 21.60 mg/L and 60.21 mg/L. After treatment, there was a decrease concentration to 0.18 mg/L of Cu and 0.74 mg/L of Ni. Based on the adsorption test results, the % removal of Cu and Ni metals was 99.17% and 98.77% with an adsorption capacity of 1.071 mg/gr and 2.9735 mg/gr, respectively.

4 Conclusions

Modification of vannamei Shrimp Shell Chitosan (SSC) with TEPA through hydrogel formation significantly improved the pore volume and adsorption performance. The optimum molar ratio (0.6) provided a hydrogel structure with the largest pore volume of 4.66 x 10^{10} that favored an increase in adsorption capacity and heavy metal removal efficiency. Adsorption tests on electroplating industry wastewater showed that chitosan hydrogels were able to remove 99.17% of Cu and 98.77% of Ni, adsorption capacities of 1.071 mg/g and 2.9735 mg/g, respectively.

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