The effect of amino acids extracted from tofu wastewater on chitosan performance for methylene blue removal from water

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Abstract. In this study, we have modified chitosan using amino acids to form a chitosan-amino acid film. Amino acids were extracted from the tofu wastewater of the tofu producer, Timbul Jaya in Banda Aceh. The amino acid loaded on chitosan was varied (1, 0.5, 0.25, and 0.1 mL). The best chitosan-amino acid films were obtained at 0.5 mL amino acid loading. Chitosan-amino acid films were characterized using XRD (X-Ray Diffraction), TGA (Thermogravimetric Analysis), DSC (Differential Scanning Calorimetry), and FT-IR (Fourier Transform Red Spectroscopy). XRD data showed that amino acid loading did not affect the crystallinity of chitosan. Based on TGA and DSC data, the addition of amino acids slightly decreased the thermal properties of chitosan. FTIR data confirmed the amount of amino acids loaded can increase the amine group in chitosan. Adding amino acids to chitosan film was able to increase the adsorption capacity of chitosan.

1. Introduction

Chitosan is a biopolymer that is widely used when removing contaminants from water. The most common application of chitosan is in the membrane preparation process and the adsorption process [1]. This is due to the presence of functional groups amine and hydroxyl as these functional groups play an important role in the adsorption process of a substance. In addition, chitosan is also environmentally friendly, biodegradable, and biocompatible [2]. Although chitosan is a useful adsorbent, it has a relatively low adsorption capacity. Previous scholars have conducted research about how to improve the adsorption performance of modified chitosan [3].

Chitosan can be modified through several methods, such as: adding functional groups, adding photocatalysts, and adding crosslinked agents. For instance, Ren [4] modified chitosan through amino acid addition to increase the adsorption of Pb²⁺ ions in water. The research results indicated a 91% increase in adsorption efficiency at an adsorption temperature of 30°C, pH 4, and contact time of 20 hours. Boggio et al. [5] modified chitosan with amino acids and copper to increase endoglucanase adsorption. The results showed a 40% increase in endoglucanase adsorption compared with unmodified chitosan. Based on the previous research, the addition of amino acids during chitosan modification improved the adsorption capacity of chitosan. However, most researchers who have
modified chitosan with amino acids did so using commercial amino acids. No one has yet modified chitosan with amino acids from tofu wastewater.

Currently, Aceh’s tofu manufacturers dump tofu wastewater into the river which pollutes the water. Tofu wastewater contains organic substances that can cause rapid microbial growth in water, which causes the oxygen level in the water to drop dramatically. Additionally, tofu wastewater contains suspended particles and substances which make the water dirty or turbid [6]. Chua and Liu [7] stated that tofu wastewater contains high concentrations of protein, fat, and carbohydrates. If the organic compounds decompose within the water, methane gas, carbon dioxide, and other gases will be produced. The high levels of protein and other organic compounds cause BOD (Biochemical Oxygen Demand) and COD (Chemical Oxygen Demand) levels in the water to increase. To produce 1 ton of tofu or tempeh, 3,000-5,000 liters of wastewater are produced [6]. One of the potential strategies to decrease the impact of water pollution due to tofu wastewater is by repurposing the wastewater as an amino acid source. Therefore in this research, tofu wastewater from the Tahu Sumedang Timbul Jaya manufacturing company in Banda Aceh was used to produce amino acids. The resulting amino acids were used to modify chitosan and applied as methylene blue adsorbent.

2. Materials and methods

2.1. Materials

The tofu wastewater (TW, pH 5.6) was obtained from Tahu Sumedang Timbul Jaya (T. Umar street, Bandar Raya, Banda Aceh, Aceh, Indonesia). The chitosan was purchased from Tokyo Chemical Industry Co., Ltd. in Japan and was manufactured from shrimp shell with 75.0-85.0% deacetylation degree. The HCl 37% used during analysis, NaOH and Glacial Acetic Acid were obtained from Merck, Germany. Methylene Blue and Distilled Water were also used in the process.

2.2. Extraction of amino acids from tofu wastewater

In order to extract the Amino acids from the wastewater, 200 mL of tofu wastewater was combined with NaOH 2 N. Thus, the solution yielded a pH of 10 and was heated at a temperature of 50˚C for 1 hour. It was filtered and then the resulting filtrate was combined with HCl 2 N to generate a pH of 4.5 (isoelectric pH) and stored at a temperature of 10˚C for 1 night. The next day, it was centrifuged at 4˚C for 20 minutes. The pellets obtained from centrifugation were washed using distilled water and dried at 50˚C for 5 hours using an oven. The proteins were then extracted and weighed [8]. The proteins extracted from the tofu wastewater were combined with HCl 6 N with an excess volume (0.5 mL/mg) [9]. The solution was heated at a temperature of 70˚C for 4 hours in closed conditions and stirred using a magnetic stirrer. Finally, The solution was exposed to a temperature of 55˚C and a vacuum of 45 mbar for 20 minutes so the excess HCl would evaporate. The resulting amino acids were stored in a freezer.

2.3. Preparation of chitosan-amino acid film

In order to prepare the chitosan-amino acid film, 1 gram of chitosan was dissolved into 99 mL of 2% acetic acid for 2 hours using a magnetic stirrer. Next, 1 mL of amino acid was added and stirred for 1 hour. Finally, the homogeneous mixture was poured into a 17x12 cm film mold and dried at 40˚C in an oven for 48 hours. The procedure was repeated with various volumes of acetic acid and amino acids of (99.9: 0.1), (99.75: 0.25), and (99.5: 0.5).

2.4. Methylene blue adsorption study

0.025 grams of chitosan-amino acid film that had been cut 1x1 cm was added into Erlenmeyer containing 20 mL of methylene blue. The erlenmeyer flask was put on the shaker for 30 minutes. Methylene blue was filtered and analyzed its absorbance using a spectrophotometer UV-Vis at the maximum wavelength.
3. Result and discussion

3.1. Amino acids extracted from tofu wastewater

The first stage of this research was protein extraction from tofu wastewater by adding NaOH 2N to obtain a pH of 10. Alkaline conditions during the protein extraction process can cause most amino acids negatively charged at pH above the isoelectric point. Amino acids with the same charge, typically repel each other, resulting in minimal interaction between the acid residues and amino acids. This means that there is an enhancement of purity in protein [8].

In order to reach an isoelectric point, HCl 2N was added until the solution reached a pH of 4.5 during protein extraction. At the isoelectric point, the total charge on each amino acid in the protein is equal to zero [8]. The extracted protein in this research weighed 1 gram, had a white colour as shown in Figure 1.

The most important aspect of protein hydrolysis in various substances is obtaining a hydrolysate solution with high levels of amino acids or according to the levels of amino acids in the sample. Thus, in the protein hydrolysis process carried out on materials containing proteins, it can completely break down the amino acids and reduce the amino acid degradation during protein hydrolysis.

In general, protein hydrolysis using HCl 6N is carried out at a temperature of 110˚C for 24 hours [10]. However, based on the results obtained in this research, this method cannot be used for protein hydrolysis with tofu wastewater, because the protein hydrolysate was black and contained black powders such as ash that was estimated to cause amino acid damage in the protein hydrolysis process. This can be seen in Figure 2.

![Figure 1. The extracted protein is obtained from tofu wastewater.](image1)

![Figure 2. The results of protein hydrolysate of tofu wastewater using general protein hydrolysis (110˚C, 24 hours).](image2)

The important parameters in protein hydrolysis are temperature, hydrolysis time, and composition of the hydrolysis mixture (volume of HCl and amount of protein) [11]. In this case, we tried to hydrolyze the proteins created from tofu wastewater at a temperature of 70˚C. According to the research of Lamp et al. [10], protein hydrolysis was carried out between 2-140 hours. In this research, we used a protein hydrolysis time of 4 hours.

Protein hydrolysis from tofu wastewater using HCl 6 N with an excess volume of HCl 6N aims to reduce the concentration of carbohydrate components in the hydrolysis medium. This is because the tofu wastewater sample also contains much carbohydrates. The carbohydrates will decompose during the hydrolysis process to form humin compounds in the form of particles that can absorb certain amino acids. The protein hydrolysis process is also carried out in a closed state because oxygen from the air can oxidize amino acids [9]. The visual results of protein hydrolysate can be seen in Figure 3.
According to the FT-IR spectrum, the protein hydrolysate (Figure 4) that was hydrolyzed at a temperature of 70°C showed a distinctive absorption band with amide I at wavenumbers 1654 cm$^{-1}$. Wavenumbers 1543 cm$^{-1}$ shows a distinctive absorption band with amide II and wavenumbers 1462 cm$^{-1}$ shows a unique absorption band with amide III [13]. In addition, the appearance of absorption bands at wavenumbers 1165 cm$^{-1}$ indicates the presence of polysaccharides [12].

3.2. Adsorption study
Chitosan that has been modified with amino acids has a better adsorption capacity than chitosan without amino acid modification. This proves that amino acids have been successfully added as new active groups in chitosan. The adsorption capacities obtained from the methylene blue adsorption experiments using chitosan and chitosan-amino acids film are shown in Figure 5.
Based on Figure 5, the best adsorption capacity was obtained using chitosan-amino acids film prepared with 0.5 mL of amino acids, which was 1.908 mg/g. The chitosan-amino acids film containing 1 mL of amino acid in its preparation shows the lowest adsorption capacity because there were too many active groups in the chitosan that had bound with the groups on the amino acids. Therefore, the ability of chitosan to adsorb methylene blue slightly reduced.

3.3. The characterization of chitosan-amino acids film
The chitosan-amino acid films were characterized by XRD (X-Ray Diffraction), TGA (Thermogravimetric analysis), DSC (Differential Scanning Calorimetry) and FT-IR (Fourier Transform-Red Spectroscopy).
Based on the XRD data, the typical diffractogram peak of chitosan appears at 2θ of 19.5˚ as shown in Figure 6. The sharp peaks of the diffractogram in both samples showed high crystallinity. The addition of amino acids did not significantly affect the crystallinity of chitosan.

![Figure 7. DSC and TGA curves of chitosan film.](image)

![Figure 8. DSC and TGA curves of chitosan-amino acids film.](image)

The DSC curves are consistent with the results of the TGA analysis. The thermogram of the chitosan film (Figure 7) and chitosan-amino acid film (Figure 8) show a two-stage thermal event. The first stage occurs between 50-100˚C, and is associated with the loss of water within the sample. The second stage, starting at 279˚C for chitosan film and 238.78˚C for chitosan-amino acid film, was associated with the degradation of the sample. Endothermic peaks approaching 100˚C can be attributed to water loss. At the exothermic peak around 315.30˚C for the chitosan film and 238.35˚C for the chitosan-amino acid films are related to the decomposition of chitosan [5]. The introduction of amino acids to chitosan film slightly reduces the thermal stability of chitosan due to the loss of hydrogen bonds.

![Figure 9. FTIR Spectra of chitosan film (a) and chitosan-amino acids film (b).](image)

Based on the FT-IR data in Figure 9, chitosan modified with amino acids had no significant change compared to the functional groups of chitosan without modification such as the O-H and N-H groups. In unmodified chitosan, the IR spectrum showing the O-H group and N-H stretching vibrations were around 3485 cm⁻¹, while in the chitosan modified with amino acids, the stretching vibrations were
around 3448 cm\(^{-1}\). The wider peak about 3400 cm\(^{-1}\) of chitosan-amino acids film indicated the formation of -NH\(_3^+\). The peak increase of about 1100 cm\(^{-1}\) of chitosan-amino acids film, indicated that loading amino acids can increase the amine group in chitosan [14].

4. Conclusion
Based on the research, it was determined that Tofu wastewater is able to become an amino acid source in chitosan modification. Chitosan that has been modified with amino acid extracted from tofu wastewater has a greater adsorption capacity than unmodified chitosan. However, the addition of amino acids to chitosan slightly decreased the thermal properties of chitosan.

5. References
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