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Protein Adsorption on Modified Bacterial Cellulose

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Abstract. The protein adsorption was interesting study, especially in the biological fluidic application. In the present study, we study the protein adsorption behavior on the bacterial cellulose and modified bacterial cellulose. In here, bacterial cellulose was modified by acid hydrolysis using hydrochloric acid. The contact time and pH were used as variable to study protein adsorption behavior on the modified bacterial cellulose. As the results, based on functional group analysis, there are not different between bacterial cellulose and modified bacterial cellulose. However, after modification, there was increasing of crystallinity of bacterial cellulose from 84.5% to be 87.7%. In the protein adsorption study, increasing the contact time increase percent adsorption until contact time of 90 minutes, however the further contact time relatively constant. The protein adsorption on both of bacterial cellulose and modified bacterial cellulose decreases, following the increase of pH.

Keywords: Bacterial Cellulose, Protein Adsorption, Acid Hydrolysis

Introduction

Bacterial Cellulose (BC) is cellulose that biosynthesized by certain bacteria, e.g, Rhizobium spp., Agrobacterium spp., Acetobacter spp., and Alcaligenes spp. Compare with cellulose from plants, BC have advantageous properties such as high purity, high crystallinity, and high tensile strength. As a result of its advantageous properties, BC have been widely used in various field applications such biomedical, food industry and paper industry [1-3].

Nanometric dimension of BC fibres lead to high surface area and potential to be adsorbent material. Using BC as adsorbent have been used for dyes, heavy metals and protein [3,4]. Protein adsorption is interested study, especially for biomedical applications. Various chemically modified of BC have been developed for protein adsorption, Phosphorylation Bacterial Cellulose (PBC), Querterner Ammonium Bacterial Cellulose (QABC), and Carboxymethyl Bacterial Cellulose (CBC) and acid hydrolysis-base were some modified of BC for protein adsorption [5-8]. Acid hydrolysis is one of the modified cellulose that have many used to generate Cellulose Nano Crystal (CNC) with high crystallinity and high surface area [9].

The aim in this work is modied bacterial cellulose by acid hydrolysis and used it as adsorbent for protein adsorption. In here, hydrochloric acid was used as hydrolysis agent. Analysis structure, crystallinity and surface charge group were used to observed modified bacterial cellulose. Study adsorption was carried by batch method with various contact time and pH.

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Methods

Preparation of Bacterial Cellulose

Bacterial Cellulose is produced from Nata de coco with a 10-day fermentation period. Nata de coco 10 kg is cut into 2 x 2 cm small boxes to make boiling easier. Nata de coco is boiled with 2% NaOH (w/v) at 70 °C for 1 hour to remove acetic acid, urea, and residual fermented sugar [10]. Nata de coco is then washed with water repeatedly and the pH of the washing water is measured using pH meter to pH 7 (neutral). Nata de coco is blended with maximum speed until it becomes porridge. Nata de coco porridge is filtered and dried in an oven at 95 ^{o}C for 8 hours. The Nata de coco plate produced from the oven process is blended until smooth. Nata de coco powder is sieved with a 60 mesh sieve and the powder is taken through the sieve.

Modification of Bacterial Cellulose

HCl 6 M of 480 mL is put into a round bottom flask and stirred with the stirrer to a temperature of 70 °C. Then, 8.0009 grams of Bacterial Cellulose powder are added to a round bottom flask [11]. The mixture is stirred with the stirrer for 2 hours. The suspension is added with distilled water to a volume of 1000 mL to stop the reaction. The suspension is centrifuged at 10,000 rpm for 10 minutes. The pH of the supernatant after centrifugation was measured using a universal pH indicator to pH 7 (neutral) [11]. Neutralizing Bacterial Cellulose was then 15 minutes ultrasounded with Ultrasonic Branson Sonifer-250 Power 250 W, Electrical: 100 V, 50/60 Hz, 3 A. Then centrifuged and the resulting pellet was dried in an oven at 50 ^{o}C for 30 minutes. The result is further refined with mortar and pestle [12].

Conductometric Titration

Bacterial Cellulose powder of 0.5000 grams modified are put into a 1000 mL three-neck flask, then 250 mL of 0.001 M. NaCl is added to the mixture and then added to 5 mL of 0.05 M HCl solution. The solution is stirred with the stirrer and its conductivity is measured with a conductometer that has been calibrated with 0.01 M KCl solution. The solution is added NaOH gradually with an initial volume of 0.5 mL 0.01 M NaOH to approach the turning point. When approaching the turning point of 0.1 M as much as 0.1 M in addition to know the exact initial volume when HCl runs out. The addition of NaOH is carried out gradually until the value of conductivity is constant. Measurement of conductivity at room temperature under nitrogen gas pressure [13].

The number of carboxyl charge groups (COO-) can be calculated from the titration curve using equation 1.

$$\mathrm{COO-(mmol/kg)} = \frac{C \times (V2 - V1)}{m} \quad (1)$$

Where, C is concentration of NaOH solution (mmol L-1), m is sample dry weight (kg), V1 is the volume of NaOH is consumed at the first intersection point (L) and V2 is the volume of NaOH is consumed at the second intersection point (L) [13].

Adsorption Test

Adsorption experiment carried out by batch technique to examine effect of contact time and pH. The other, batch technique also used to kinetic study. In here, bovine serum albumin (BSA) was selected as model protein in the present study.

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Effect of Contact Time on BSA Adsorption

BSA solution of 400 mg/L is added into a 100 mL Erlenmeyer. Then 0.1000 g of adsorbent was added [8]. The mixture is shaken with a shaker at a speed of 100 rpm to avoid protein damage [14]. Shuffle is done with a time range of 60, 90, 120, 150 and 180 minutes. The suspension is filtered with filter paper. Filtrate was taken 50 µL and put in a cuvette. The solution was added to 2.5 mL of Bradford reagent and allowed to stand for 5 minutes. The absorbance solution was measured by a visible spectrometer at maximum wavelength [15].

Effect of pH on BSA Adsorption

Protein solution of Bovine Serum Albumin (BSA) concentration of 400 mg/L was made by taking 20 mL of Bovine Serum Albumin (BSA) solution of 1000 mg/L and put it in a 50 mL volumetric flask. The solution was diluted with citrate-phosphate buffer pH 3, 4, 5 and 6. BSA solution 400 mg/L from a 50 mL volumetric flask was then put into a 100 mL Erlenmeyer. Solution added 0.1000 g of adsorbent [8]. The mixture is shaken with a 100 rpm speed shaker to avoid protein damage [14]. Shaking is done as long as the time that has been generated when there is equilibrium. The suspension is filtered with filter paper. Filtered filtrate was taken 50 µL and put in a cuvette. The solution was added to 2.5 mL Bradford reagent and allowed to stand for 5 minutes. The absorbance solution was measured by a visible spectrometer at maximum wavelength [15].

Results and Discussion

Functional Group Analysis by FTIR

Figure 1 shows FTIR-spectra for both of BC and MBC have similar bands, indicate that after acid hydrolysis treatment did not change structure on BC. Broad bands at 3346 represent to O-H stretching vibration, the band 2897 cm^{-1} to C-H stretching vibration, 1431 cm^{-1} to C-H bending vibration, and 1058 cm^{-1} to C-O-C bending vibration.

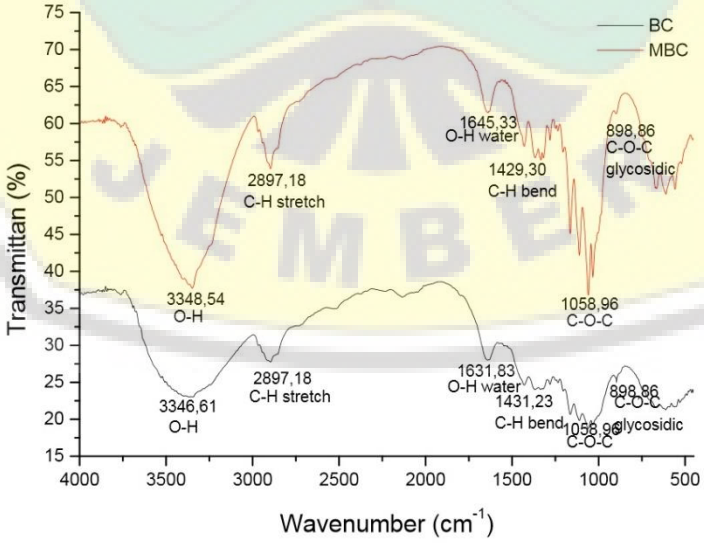

Figure 1. FTIR Spectra of BC and MBC

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Crystallinity by XRD and Surface Charge Group

XRD analysis was used to determine changes in the degree of crystallinity of BC after acid hydrolysis treatment. Figure 2 shows that there is increasing crystallinity from 84.5% (BC) to 87.7% (MBC). Increasing degrees of crystallinity due to remove amorphous region in the BC after acid hydrolysis treatment. During hydrolysis, hydrochloric acid breaks the 1.4 beta glycoside bond in the amorphous region [10]. Surface charge group of MBC was determined by conductometric titration. Charge group is sulfat group that attach on the BC structure. As the result, surface charge group of MBC is 9 mmol/kg.

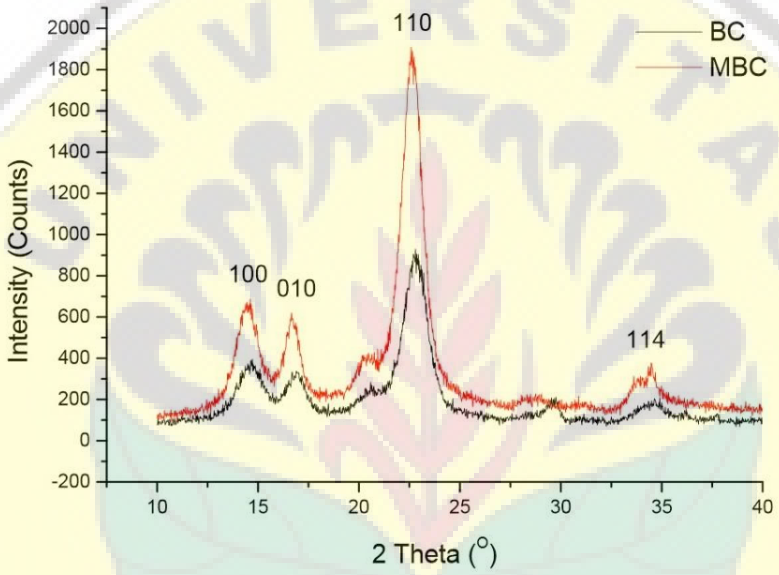

Figure 2. X-Ray Diffraction pattern of BC and MBC

Effect of Contact Time

Figure 3 shows effect of contact time for protein adsorption show that adsorption equilibrium is reached within 90 minutes for both BC and MBC, and further increase of contact time, it was not change significantly. Compare with BC, MBC have higher protein adsorption capacity. It can be explained that after acid hydrolysis treatment, MBC have surface charge group that play important role in increasing adsorption capacity.

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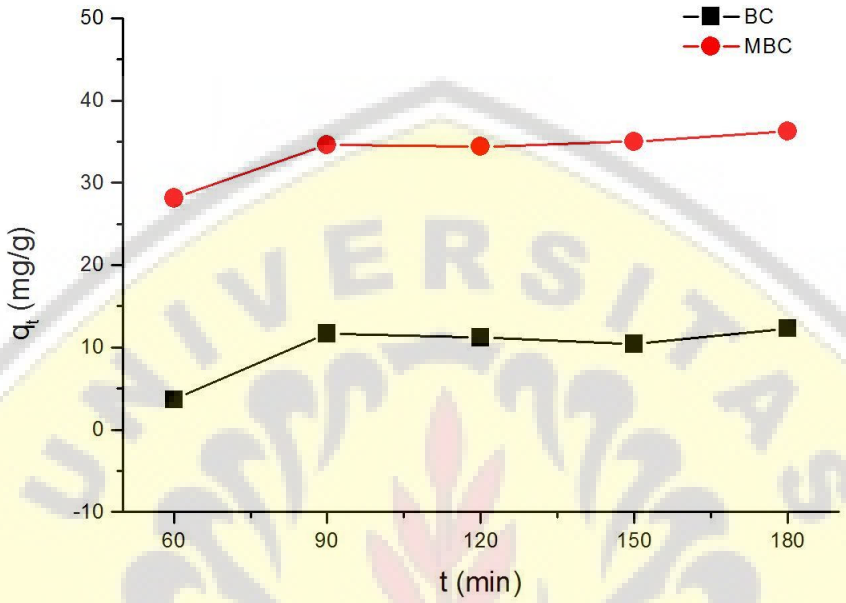

Figure 3. Effect of time on protein adsorption

Effect of pH

Figure 4 shows that the protein adsorption capacity on both of BC and BC decreased with increasing pH. Protein adsorption is highest in the pH of 3. It can be explained that pH below the isoelectric point lead to positively charge of protein that generate high interaction with MBC that have negatively charge. In the other hand, pH above the isoelectric point lead to negatively charge of protein, consequently electrostatic repulsion take place [8].

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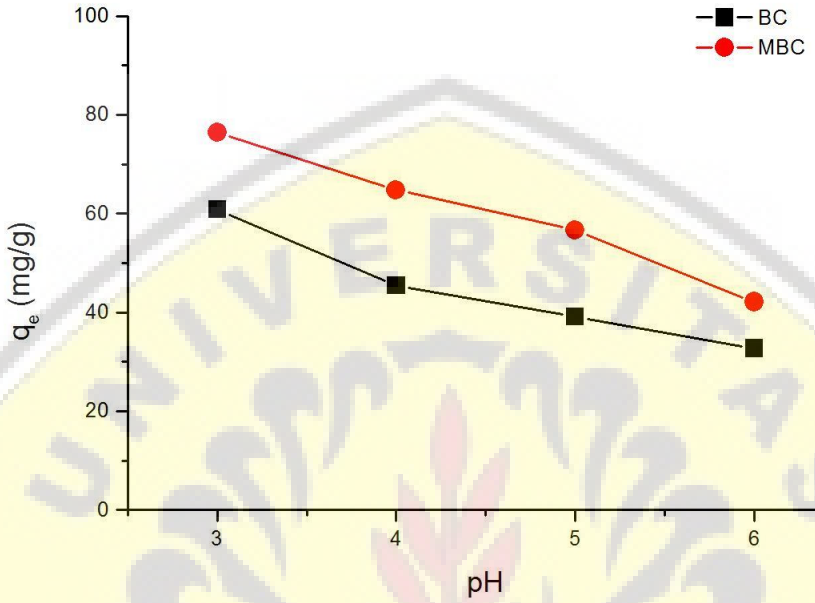

Figure 4. Effect of pH on protein adsorption

Kinetic study

In here, pseudo second order model was selected as kinetic study in this present study. pseudo second order model described binding capacity was proportional to the number of active sites occupied on the sorbent (Equation 2) [16].

$$\frac{t}{q_t} = \frac{t}{q_e} + \frac{1}{q_e^2 k} \quad (2)$$

Where, k is the equilibrium rate constant of pseudo second order adsorption kinetics [g/mg min], q_e the amount of metal ion adsorbed at equilibrium [mg/g], q_t the amount of absorbate on the surface of sorbent at any time t [mg/g]

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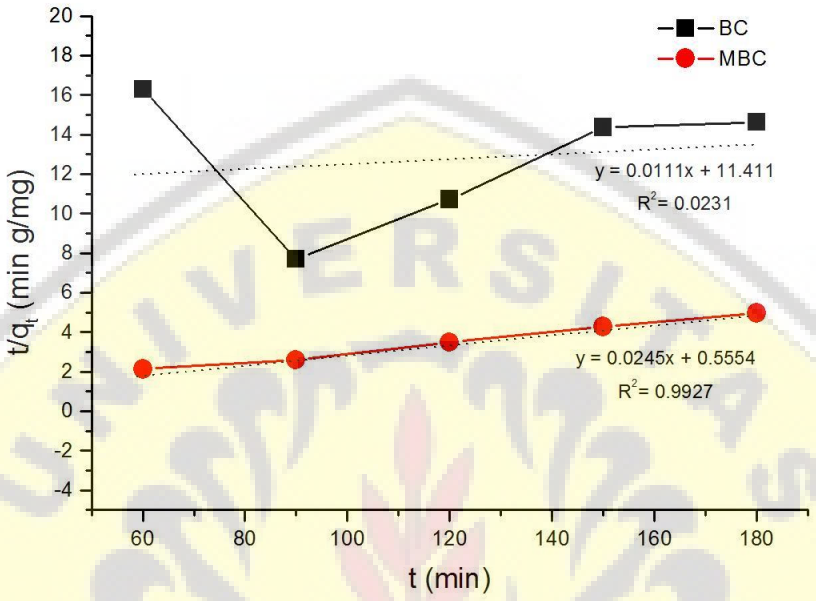

Figure 5. Pseudo second order model of protein adsorption on BC and MBC

Based on Figure 5, the pseudo second order model is suitable for MBC but not for BC. The result of the constant values obtained is 3.03.

Conclusions

Modified Bacterial Cellulose (MBC) by acid hydrolysis has been successfully prepared as adsorbent for protein. Compare with Bacterial Cellulose (BC), BC have higher in adsorption capacity. Protein adsorption below the isoelectric point is higher for both of BC and MBC. It is due to electrostatic interaction between adsorbent and adsorbat. Based on kinetic study using pseudo second order kinetic model, kinetic constant is 3.03.

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