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MORPHOLOGICAL AND THERMAL PROPERTIES OF ALKALI TREATED BACTERIAL CELLULOSE FROM COCONUT WATER

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ABSTRACT

MORPHOLOGICAL AND THERMAL PROPERTIES OF ALKALI TERATED BACTERIAL CELLULOSE FROM COCONUT WATER. Alkali treatment is one of wide chemical treatment for removing non cellulosic materials and other impurities of bacterial cellulose (BC). Presently, BC produced by synthetic medium such as Schramm Hestrin or coconut water fermentation using pure culture of *Acetobacter xylinum* . In this research BC produced from coconut water fermentation using inoculums as suspension of mixture culture of bacteria *A. xylinum* in coconut water which commercialised in West Java (Cianjur). Purpose of this research is to know the native and the effect of alkali treatment on the BC (heterofermentive) with evaluation of morphological, elemental analysis and thermal properties by Scanning Electron Microscope (SEM)-Energy Dispersive X-ray Spectroscopy (EDX) and Thermo-Gravimetry Analysis (TGA), respectively. Observation on morphological surface of native BC showes that there were noncellulosic materials entangled on the network of cellulose fibrils which were identified by EDX analysis as sodium (Na), magnesium (Mg), aluminium (Al), silica (Si) and potassium (K) in addition to carbon (C) and oxygen(O). On thermal analysis, stepwise of decomposition are observed on the native BC indicating the presence of non-cellulosic components which decomposed at different temperatures. While the alkali- treated BC sheet shows a single decomposition stage. The amount of residue formed after alkali treatment was relatively lower than native BC. Thermal degradation properties of the BC produced from coconut water was higher than that of BC belonging to synthetic medium. Based on this data, it is concluded that alkali treated cellulose can be used as a reasonable substitute for various non biodegradable synthetic polymers for large scale industrial in specific application

Key words : *Bacterial cellulose, Acetobacter xylinum, Alkali treatment*

ABSTRAK

MORFOLOGI DAN SIFAT TERMAL SELULOSA BAKTERIA HASIL FERMENTASI AIR KELAPA SETELAH PERLAKUAN ALKALI. Penggunaan larutan alkali merupakan salah satu perlakuan kimia pada selulosa bakteria untuk menghilangkan material non selulosa dan pengotor lainnya. Selulosa bakteria pada umumnya merupakan produk fermentasi menggunakan medium sintetik (Hestrin & Schramm) atau air kelapa dengan menggunakan biakan murni *Acetobacter xylinum*. Dalam penelitian ini digunakan bakteri pembentuk nata berupa biakan dalam air kelapa dalam bentuk starter yang merupakan kultur campuran yang diperdagangkan di Cianjur, Jawa Barat. Tujuan dari penelitian ini untuk mengetahui pengaruh alkali pada selulosa bakteria (*heterofermentative*) melalui analisa morfologi dan sifat termalnya masing-masing menggunakan *Scanning Electron Microscope* (*SEM*)-*Energy Dispersive XRay Spectroscopy* (*EDX*) dan *Thermo-Gravimetry Analysis* (*TGA*). Hasil analisis morfologi permuka an pada selulosa bakteria native(tanpa perlakuan alkali) menunjukkan adanya material non selulosa berikatan dengan jaringan serat selulosa dari selulosa bakteria dan dari hasil identifikasi dengan EDX menunjukkan adanya unsur Natrium (Na), Magnesium (Mg), Aluminium (Al), Silika (Si) dan Potassium (K) disamping Carbon (C) dan Oksigen (O). Hasil analisis termal menunjukkan adanya beberapa tahap dekomposisi yang teridentifikasi pada selulosa bakteria native yang mengindikasikan adanya komponen non selulosa yang terurai pada beberapa tahap temperatur. Perlakuan dengan alkali menunjukkan hanya satu step dekomposisi. Jumlah residu yang terbentuk setelah proses alkali relative lebih rendah dibanding selulosa bakteri sintetis. Sedangkan degradasi termalnya lebih tinggi dibanding selulosa bakteri dari medium sintetis. Dari hasil analisis di atas menunjukkan perlakuan dengan alkali dapat digunakan sebagai pengganti dari polimer non-biodegradabel untuk aplikasi tertentu pada industri skala besar.

Kata kunci : Selulosa bakteria, *Acetobacter xylinum*, Perlakuan alkali

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INTRODUCTION

Bacterial cellulose (BC) is a natural biopolymer which is obtained from bacterial activity on a medium containing glucose as a carbon source. It is very unique and interesting material, because it has both great mechanical strength and biodegradability in comparison with green plant cellulose or synthetic polymers. Due to its high chemical purity and other excellent qualities such as high mechanical stability and strength, expectedly bacterial cellulose would be used as useful raw materials for newly design in a new field of biodegradable materials. Related to its unique properties, such as high mechanical strength, high crystallinity and a highly pure nanofibrill a network structure, bacterial cellulose is becoming a promising biopolymer for several applications. For example, due to its biocompatibility, BC has been extensively investigated in the biomedical field [1-3]. Other applications include its use as component for audio membranes [4-6], electronic paper [7], optically transparent composites [8-9], reinforcing agent for paper [10], and other polymeric materials and composites [11] among others.

Almost studies on the preparation and characterization of bacterial cellulose have been reported using synthetic medium such as Schramm Hestrin medium [7-11] as growth substrate by pure strain of *Acetobacter xylinum*, which is relatively expensive. Coconut water which is readily available as waste material in traditional market in Indonesia, shows as a potential raw material for producing BC, it is commonly used as a dessert food known as “nata de coco”. BC produced by using inoculum of acetic acid bacteria as suspension in coconut water in a static culture system in generally was called “starter”. By observation on the thickness of the growth BC during the fermentation process showed that the growth of BC in coconut water medium without any additional nutrient and innoculation by “starter” of Cianjur was better than using pure culture of *Acetobacter xylinum*. Three isolates of the bacteria : *Acetobacter aceti* (sp1), *Acetobacter pasteurianus* (sp2) and *Acetobacter aceti* (sp3) were investigated and concluded that BC fermentation using Cianjur “starter” was heterofermentative[12].

The BC obtained after fermentation is not pure, it is a composite structure, contains some impurities like protein, media ingredients and a large proportion of water, leading to problems in its application. The bacterial cellulose has to be purified to obtain pure cellulose. The most widely used process for purification of bacterial cellulose is alkali treatment. Alkali treatment of bacterial cellulose is importance, which capable for hydrolyzing and removing impurities present in the cellulose pellicle. It converts native cellulose into pure cellulose by removing entrapped non-cellulosic compounds like protein, nucleic acid, and others [13-16].

So far, no research relating on the characteristic of morphological surface ,elemental analysis and thermal analysis specially of BC heterofermentative obtained from coconut water fermentation using suspension of mix culture of bacteria *A. xylinum* from local farmer (Cianjur) has been reported. However, the use characteristic of cellulose involves plants , other cellulose from biomass and bacterial cellulose product fermentation from synthetic medium has been reported elsewhere and well documented.

Therefore, the purpose of study is to evaluate an effect of alkali treatment on the morphological and thermal properties of BC heterofermentative analyzed by Scanning Electron Microscope (SEM) and Thermal Gravimetric Analysis (TGA/DTG), respectively. Alkali concentration of 2% was used as a certain concentration to remove non cellulosic compound of BC without any structural destruction. To know the present of removal of non-cellulosic compound , Energy dispersive X-Ray spectroscopy (EDX) was used an analytical instrumentation to analyze impurities in BC.

EXPERIMENTAL METHOD

Coconut water and white sugar obtained from local market were used as raw materials in production of BC. Prior to fermentation process, coconut water and sugar were boiled and adjusted at pH 4 by addition of acetic acid glacial solution. After cooling, suspension of Acetobacter starter (20%) was purchased from local farmer in Cianjur was inoculated into the solution and then incubated at room temperature under static cultivation for 6-10 days. Washing and immersion of BC by running water were continuously conducted to remove a sour odor until pH neutral which is assigned as native BC. The further treatment of native BC was boiled in 2% of alkali solution for one hour. Neutralization of alkali-treated BC to pH 7 reached by soaking in water and frequently changed for several times until water pH is neutral. The sample assigned as alkali treated.

The native and alkali treated BC were squeezed to remove excess of water and then hotpressed at 100 kgf/cm^2 and 120 °C. BC sheet obtained was used for further analysis, morphological surface and thermal analysis.

Characterization

The morphology of native and alkali-treated BC sheet was observed by a Scanning Electron Microscope (SEM) JEOL JSM-6360LA. Previously, samples were coated with gold by sputter JFC 110 to create an electron conductive surface and prevent a surface charging. During observation on morphological surface of BC, impurities present in BC surface was analyzed by EDX to make sure a removal of impurities after alkali treatment. Thermal properties of BC was analyzed by thermo-gravimetry analyzer (TG/DTA 200) from Seiko Instruments SSC/5200 H station . Weight of samples about 5-10 mg was heated in open alumina pans from 30 to 500 °C under nitrogen atmosphere at flow rate of 260 mL/min and heating rate of 10 °C/min. The weight and derivative weight losses were plotted against temperature of native and akali treated samples. The computer system of TGA was employed to measure a change of material weight either as function of increasing temperature or time

RESULTS AND DISCUSSION

Morphological and Properties of Native and Alkali Treated Bacterial Cellulose

Alkali treatment on BC gave a considerable effect on morphological surface and thermal decomposition of BC. All samples were analyzed in dry state after hotpressed. Morphological surface of native BC shows cluster of bacterial cell pointed out in white circle and a dense fibrilar network pointed out in an white arrow whereas nonfibrillar material covered fibrilar structure of BC, shown in Figure 1. Otherwise stated , the surface of BC contained non-cellulosic impurities which entangled on the cellulosic fibril network.

Based on EDX analysis (Figure 2), element such as sodium (Na), magnesium (Mg), aluminium (Al), silica (Si), and potassium (K) could be clearly detected as chemical composition commonly present in coconut water [17] in addition to carbon and oxygen elements of cellulose. Silica appeared as elemental impurity, origin from coconut water. Morphological structure of alkali treated of BC (Figure 3) is quiete different from native BC. Sodium hydroxide successfully washed out non-cellulosic impurities making microfibril network are visibly appear. Visible fibril network reveals its topological and porous structure of BC. Vissually, alkali treatment caused BC sheet to be white and semitransparent indicating that the most of non-cellulosic impurities might be removed. EDX analysis (Figure 4) identifying that carbon and oxygen as the main element of the BC sheet. Absence of the elemental impurity corresponding to non cellulosic components reveals succesfull alkali treatment to remove non-cellulosic compound. This result were similar to result for BC treated with sodium hydroxide on native BC, where sodium hydroxide treatment is effective way to obtain a relatively pure of BC. [16,18].

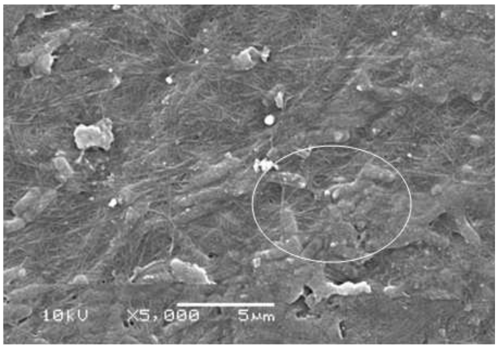

Figure 1. Morphological surface of native BC

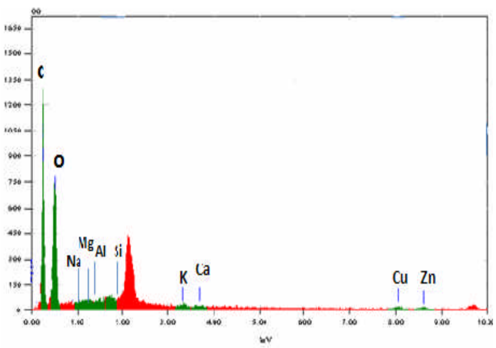

Figure 2. Elemental Analysis of native BC

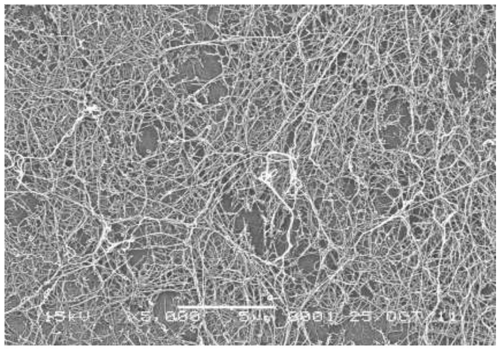

Figure 3. Morphological surface of alkali treated of BC

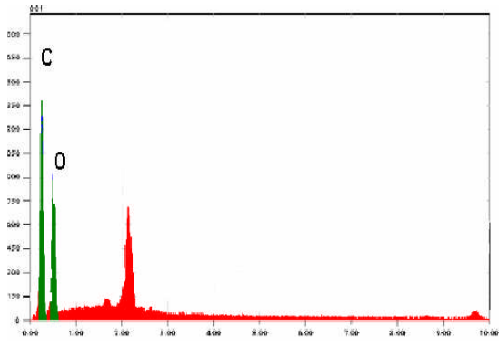

Figure 4. Elemental analysis of alkali treated BC

Thermal Properties of Native and Alkali Treated of BC Sheet

Different morphological properties and purity level of native and alkali-treated BC gives a distinctive thermal properties of them. TGA curve representated by plotting presentation of weight loss against temperature shows decomposition behavior and thermal stability. Figure 5 show thermal behavior of BC, percentage of weight loss against temperature. Prior to alkali treatment, three stepwise of weight reduction are clearly observed on native BC whereas non-cellulosic compound contributed on the thermal behavior. Gradual weight

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reduction 6.1% from 30 °C to 100 °C is observed as evaporation of water on the native BC curve. Heating up above 100 °C gave the second weight reduction of 28.66 % , from 93.9% up to 65.24% . The second stepwise decomposition corresponds to non-cellulosic material present in native BC whereas the highest losses occur at 206.25 °C as the maximum temperature decomposition (T_{max}) as shown in derivative thermal gravimetric (DTG) curve in Fig. 6.The next heating up to 500 °C gave the third weight reduction of 57.7 % with the highest reduction occur at 332.1 °C (Figure 6). This peak was attributed of degradation of cellulose. The amount of residue formed of native BC at final decomposition temperature of 500 °C was 25.24 %.

Degradation of alkali-treated BC shows two stepwise process of weight reduction, it means that thermal decomposition pattern changes completely after alkali treatment. The first weight reduction of 4.2 % from 30 °C to 100 °C is observed as evaporation of water and it was stable up to 211.6 °C. Second degradation , from 324.7 °C to 363.6 °C had weight degradation of 67.1 % with the highest reduction occur at 341.3 °C, as sharp

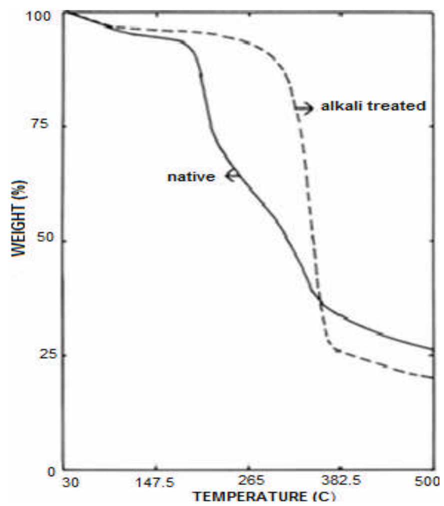

Figure 5. TG curve of native and alkali-treated BC

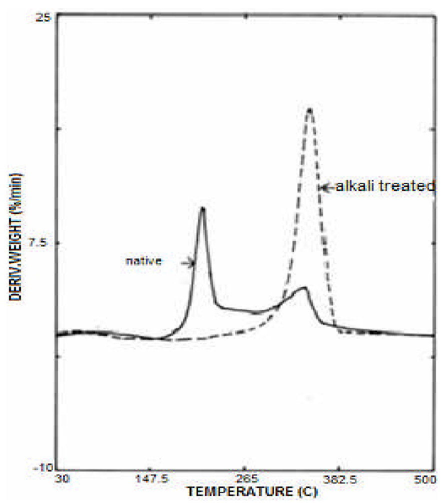

Figure 6. DTG curve of native and alkali-treated BC

peak at the derivative thermal gravimetric (DTG) curve in Figure 6. The increase in T_{max} of alkali-treated was found to be cumulative effect of such changes occurring as a result of exposure of alkaline [15].

The amount of residue formed of akali-treated at final decomposition temperature 500 °C was 20.2 %, it was relatively lower than native BC. Thermal degradation properties of the BC produced from coconut water was higher than that of BC belonging to synthetic medium Hestrin and Schramm [15-16].

CONCLUSION

Bacterial cellulose heterofermentative produced by fermentation of coconut water using "starter" from Cianjur was obtained. The morphology, elemental analysis and thermal properties of BC native and alkali treated were characterized using SEM/EDX and Thermal Gravimetry respectively. The amount of residue formed after alkali treatment was relatively lower than native BC. Thermal degradation properties of the BC produced from coconut water was higher than that of BC belonging to synthetic medium.

The results showed that alkali treatment plays an important role in removing most of the non-cellulosic materials, bacterial cell and others impurities to obtaining a relatively pure form and enhancing the thermal stability of bacterial cellulose heterofermentative . Based on this data, it is concluded that alkali treated cellulose can be used as a reasonable substitute for various non biodegradable synthetic polymers for large scale industrial in specific application.

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