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CROSSLINKER EFFECT ON CHARACTERISTICS OF BACTERIAL CELLULOSE-EXTRACTED BINAHONG LEAF (*A. CORDIFOLIA* TEN. STEENIS) COMPOSITES

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ABSTRACT
This study aims to determine the effect of adding crosslinkers 1,3 and 5% on the characteristics of bacterial cellulose composites from Binahong Leaf Extract (*Anredera cordifolia* (Ten.) Steenis). *Acetobacter xylinum* is used in the 14-day fermentation of a mixture of coconut water, sugar, and urea to produce bacterial cellulose (BC). The identification of the characteristics of BC, BC-EBL, and BC-EBLC according to the standard cartilage value was carried out by testing water content, tensile strength, and functional group analysis using FTIR. Using a 5% crosslinker, the BC water content test results can be decreased from 99.29% to 97.94%. The tensile strength value from the results of testing the mechanical properties of KSB-EDBC 5% is higher than KSB-EDBC 3%, KSB-EDBC 1%, KSB-EDB, and SB whose respective values are 121.35 MPa, 72.08 MPa, 66.58 MPa, 49.81 MPa and 41.97 MPa. The results of the FTIR spectrum showed that bacterial cellulose only experienced a shift in the wave number of the functional group.It was concluded that with the addition of a 5% crosslinker, the characteristics of the bacterial cellulose composite of binahong leaf extract according to cartilage standards were maintained.

Keywords: Bacterial Cellulose; Binahong; Composite; Crosslinker

1. INTRODUCTION

Bacterial cellulose (BC) has characteristics that can be applied to several bio-medical applications, for example applications for wound dressings and modeling for cartilage [1-3]. In in vivo studies applied to mice, pure cellulose from BC (*nata de coco*) has been shown to have high compatibility properties. The test results showed that there was no hypertrophy in the tissues and the body showed no signs of a reaction to foreign objects in the mice [4-6].

Cellulose is a biopolymer which is an important component in various research and biomedical applications because of its widespread presence in nature and has a long and linear chain molecular structure, multi-chirality, hydrophilicity, and biocompatibility [7], high tensile strength of the ultrafine

network structure and has a high value of Young's modulus [8], and susceptibility to chemical and physical modifications during the cultivation process as well as in the post-purification process [9-11].

Acetobacter xylinum is one of the most effective gram-negative bacteria in the production of cellulose and has a higher thickness than other organisms [12-15]. This is because *A. xylinum* has the potential as an oxidizing agent for various types of alcohols and sugars whose oxidation results are in the form of acetic acid [16-18]. During the production process, a gel-like membrane is formed on the surface of the culture medium other than acetic acid. After identifying the film it was found that this material is cellulose. Expressed as bacterial cellulose (BC) because it is produced by bacteria [19]. BC consists of β-1,4-glycoside units bonded to the first and fourth carbon atoms, which are called β-glycosidic bonds [20].

BC is a secondary metabolite product of Acetobacter xylinum, while the primary metabolite product is acetic acid. The more levels of nutrients provided [21], the greater the opportunity to grow these bacteria, the more *Acetobacter xylinum* and the more cellulose produced. The intensity of SB synthesized is not only based on the type of species of the bacteria itself, but also many other influencing factors, such as culture method, pH [22], temperature, oxygen availability and contamination with the presence of other bacteria due to the aerobic nature of *Acetobacter xylinum* [23].

Researchers are interested in researching BC based on the facts stated above. Applications of BC in biomedical sciences have drawbacks and limitations, such as a low compressive modulus or the ratio of compressive strength to strain [24-27]. In addition, BC has low elasticity qualities that make it suitable for use as cartilage material [28].

Due to these problems, to increase the value of elasticity, a study was carried out to combine SB with a new material called composite [29]. Researchers have improved the elastic properties of SB by making and modifying it with other materials. Research by Putra, namely the preparation of SB and Polyacrylamide N,N''-methylene bisacrylamide (pAAM) composite materials using double-network hydrogel, which can be used as ligaments [30]. In addition to using synthetic materials, composite materials can also be made from natural materials.

In this research, the material to be combined is BC (as a matrix) with natural ingredients, namely binahong leaves (*Anredera cordifolia* (Ten.) Steenis) as filler. Binahong Leaf Extract (*Anredera cordifolia* (Ten.) Steenis), which will be combined with BC to form a Bacterial Cellulose Composite Binahong Leaf Extract (BC-EBL), is a natural herbal plant ingredient that is widely available in Indonesia. To make the use of composites from BC-EBL more effective in their application, researchers will also use starch which also functions as a crosslinker (BC-EBLC).

Addition of other materials with modifications to improve the elastic characteristics of BC. The materials used are natural materials, herbal plants that are widely available in Indonesia, namely

Binahong Leaf Extract (*Anredera cordifolia* (Ten.) Steenis) which will be combined with SB to form a Bacterial Cellulose Composite of Binahong Leaf Extract (BC-EBLC).

Figure 1. Binahong Leaf (Walters & Africa, 2015)

Binahong leaves are used as fillers because they are hydrocolloid and contain secondary metabolites such as saponins, tannins, alkaloids, triterpenoids, flavonoids, phenolics, steroids, glycosides [31], and polyphenols [32]. Besides, binahong leaves are also widely used as traditional medicine [33]. This is due to the compounds of tannins, saponins and flavonoids which are included in the phenol group of compounds which are strong antibacterial substances.

The purpose of this study was to determine the effect of adding 1%, 3%, and 5% crosslinker variations affected the physical properties (water content), mechanical properties (tensile strength), and structural analysis of functional groups using FTIR instruments.

2. EXPERIMENTAL SECTION

2.1. Bacterial Cellulose (BC) Preparation

As much as 600 mL of old coconut water is filtered and put into a 5 liter stainless steel pan for heating using a stove. Then, in the pan, combine 6 grams of $CO(NH_2)_2$ and 60 grams of sugar. This solution is heated to boiling. After the solution boils, the acidification step is with cka acid (CH_3COOH) 12 mL until it reaches a pH of 4-4.3. The mixture is then poured into a sterilized fermentation container measuring 24174 cm and covered with sterilized newsprint. The mixture is allowed to reach room temperature (28˚C). After that, *Acetobacter xylinum* starter was added aseptically and fermented at room temperature until SB with a thickness of 1 cm was formed. Once formed, BC can be harvested.

2.2. Purification of Bacterial Cellulose

The stages of washing and purification of bacterial cellulose (SB) using running water for ± 24 hours. The purification process carried out by SB action was immersed in a 2% NaOH solution for ±24 hours, this was done with the aim of removing impurities and bacterial cells. The next process, the SB was washed again with running water until it was clean, then the SB was soaked with distilled water and stored in a closed container. The soaking water is changed ± 1 time a day, to keep the SB fresh.

2.3. Production of Binahong Leaf Extract

The binahong leaves are washed and air-dried until the binahong leaves are clean so that the dirt that sticks out is gone. Weigh 150 grams of binahong leaves to be extracted. Add 100mL of water, then blend until smooth (±5 minutes) to produce binahong leaf extract. The resulting EBL is filtered with a filter cloth to produce a filtrate, this filtrate is used as a filler for making BC-EBL. Save the EBL in a bottle for the next stage of the process.

2.4. BC-EBL Preparation

The stored SB was cut into 2×2×1 cm and 15×2×1 cm sizes. After cutting, SB was immersed in 300mL EDB in a plastic container for 4 days and subjected to UV light. During the immersion process, the samples were shaken using a shaker located in the UV box modification of the material science laboratory for the manufacture of BC-EBL. The BC-EBL sample was removed and the surface side was dried with a tissue so that the remnants of chemical compounds on the surface of the sample no longer existed.

2.5. BC-EBL Characterization

2.5.1 Physical Properties Test (Water Content)

Samples of SB, KSB-EDB, and KSB-EDBC 1, 3, and 5% were cleaned and adjusted to a size of 2×2×1 cm to be tested for water content (Wc) using an analytical balance. The sample was weighed to get the initial weight value of the sample (W0), then oven at 105˚C until the sample was dry. The obtained samples that have been dried are weighed again (W1).

$$\%W_c = \frac{W_0 - W_1}{W_0} \times 100$$

Where: Wc = Water content (%)

W_0 = Initial mass of the sample, and

W_1 = Final mass of the sample.

2.5.2 BC-EBL Mechanical Properties Test (Tensile Strength)

The tensile strength test was carried out with a Tensile Strength tool so that the sample was pulled until it broke.

2.5.3 Functional Group Analysis Using FTIR

The samples used in this analysis were BC, BC-EBL, BC-EBLC 5% which had been removed from the oven and analyzed using FTIR (Fourier Transform Infra Red).

3. RESULTS AND DISCUSSION

3.1 Bacterial Cellulose Preparation

During the preparation process for the manufacture of bacterial cellulose (SB) is influenced by several factors, such as temperature, pH, source of nutrients and the type of bacteria used for the fermentation process. In the preparation process using an aseptic method (sterile) which aims to avoid contamination by other bacteria that affect the formation of SB. The fibers formed on the surface of the culture media came from the bacteria used, namely Acetobacter xylinum. The culture medium for bacterial growth contained coconut water and sugar which was used as a source of glucose for the formation of SB during the fermentation process, in the medium also contained urea which served as a nitrogen source in the formation of SB. The optimum growth of cellulose fiber grows at a pH ranging from 4-5 with optimum temperature conditions ranging from 28˚C-31˚C.

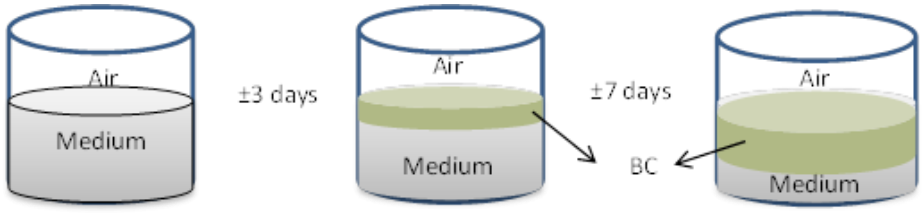

Figure 2. BC Fermentation Illustration

3.2 Purification of Bacterial Cellulose

SB that has been well formed and not moldy is washed with running water by soaking it in running water for ± 24 hours so that on the surface of the SB, there are remnants of dirt that stick to it. After soaking for 1 day, purification was carried out, by soaking the SB in a 2% NaOH solution for ± 24 hours to remove the remnants of the medium that was still attached to the surface of the SB. This 2% NaOH solution has a function to remove non-cellulose components that can affect hydrogen bonds between cellulose chains. If the hydrogen bonds between the cellulose molecular chains are disturbed, the mechanical properties of the cellulose will decrease and SB cannot be used as a matrix (composite building material). And also to remove the remaining bacteria that are still on the surface of the SB which will continue to work with nutrients.

Figure 3. NaOH solution 2% (a), SB immersed in 2% NaOH solution (b)

3.3 Binahong Leaf Extract (EDB) Preparation

Preparation and results of binahong leaf extract (EDB) which has been powdered first by means of binahong leaves in an oven at a temperature of ±105˚C, this is done to avoid rotting of binahong leaves. Then the binahong leaf powder was dissolved in distilled water with a ratio of sample weight and solvent volume of 1:10. The result obtained from this process is a dark green EDB solution on which there is foam on the surface of the gel.

Figure 4. EDB results in distilled water

3.4 BC-EBL Preparation

EBL is used as a filler in the formation of BC-EBL because it has thick, rubbery properties which are expected to be SB fillers and improve and improve the mechanical, thermal, chemical, and biological properties of SB composites. Preparation of BC-EBL was done by soaking SB in EBL for ±4 days using UV light and without UV light which during the soaking process BC-EBL was also shaker. Soaking for 4 days is the optimum time for EBL (filler) to be absorbed into the BC (matrix).

The use of a shaker in this immersion is to maximize the EBL (filler) that enters the BC (matrix), so that a physical absorption process occurs in the matrix. This absorption by absorption can be proven by cutting BC-EBL and looking at the inside which has the same color as the surface. In addition to using a shaker, UV light is also used in the immersion process with the aim of increasing the binding of free radicals between the filler and the matrix so that the resulting BC-EBL has better mechanical properties than BC-EBL without UV. The wavelength of the UV lamp used is 380-315 nm. The preparation of BC-EBL was carried out in a UV box covered with a black cloth, which aims to maximize the intensity of UV rays during the shaker process.

Figure 4. BC-EBL immersion results

3.5 Immersion of KSB-EDB with 1.3 and 5% Amylum Crosslinker

The next step is soaking the BC-EBL in a crosslinker solution for ±3 days which is called BC-EBLC, where the addition of the crosslinker for ±3 days is the optimum time for absorption of the crosslinker into the BC-EBL. The crosslinker used was starch obtained from tapioca flour with concentrations of 1%, 3%, and 5%. The use of a crosslinker is used as a liaison or adhesive between the matrix and the filler, so that the filler that was originally contained in the matrix is only absorbed physically and chemically becomes bound because it is connected by a crosslinker. Immersion of BC-EBL into the crosslinker is a way to improve the mechanical properties of BC-EBL.

Figure 5. BC-EBLC Result

Crosslinker acts as a binder between the matrix and filler. So, initially the filler is only physically absorbed in the matrix in the presence of a crosslinker, the matrix with the filler becomes bonded. The crosslinker is divided into two fractions that can be separated by hot water, namely the soluble fraction called amylose and the insoluble fraction called amylopectin. The water-soluble amylose is continued for the immersion process with cellulose which will result in a cross-linking process to form polysaccharides that are useful for increasing elasticity. Immersion of BC-EBL with this crosslinker can improve the mechanical properties of BC-EBLC.

3.6 Characteristics of BC-EBL

3.6.1 Water Content

The water content in this test is calculated by calculating the ratio of the total amount of water contained in a material to the dry weight of the material. Determination of water content aims to determine the amount of water contained in BC, BC-EBL UV, BC-EBLC 1%, 3%, and 5% with a predetermined temperature and time during the drying process using the oven. Based on the research results, the water content of SB is 99.29%. The average percentage of water content of BC-EBL with UV obtained 98.56%.

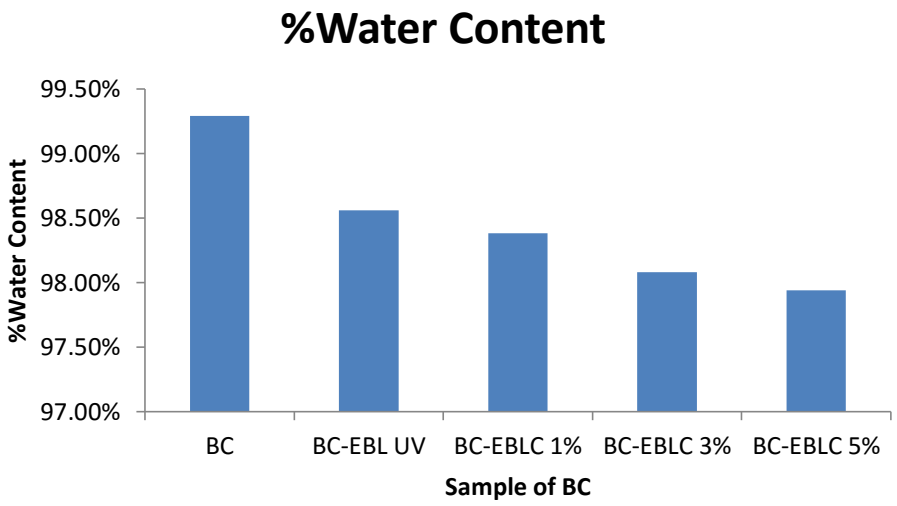

Figure 6. Sample water content test chart

Based on the graph above, it can be seen that the percentage of water content of BC-EBL with the addition of 1%, 3%, and 5% tapioca flour crosslinker, respectively, experienced a decrease in water content with increasing concentrations. The results of the water content obtained from each addition of the crosslinker were 98.38%, 98.08%, and 97.94%. The addition of crosslinker concentration in BC-EBL can reduce the water content, so that this condition is expected to strengthen the mechanical properties of BC-EBL.

3.6.2 BC-EBL Mechanical Properties Test (Tensile Strength)

The tensile strength value is the maximum tensile force applied to a material until the material breaks. This tensile strength test will determine the quality of SB, BC-EBL, and BC-EBLC.

Figure 8. The process of testing the tensile strength of the sample with the instrument

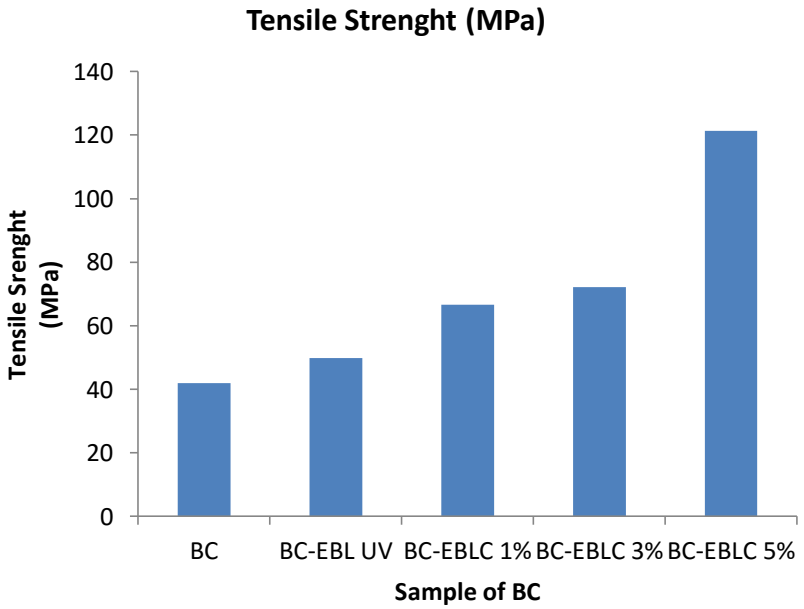

Figure 7. Comparison of sample tensile strength values

About the comparison of the tensile strength of the sample. The results obtained that the tensile strength value of BC-EBL was higher than that of SB, which were 49.81 MPa and 41.97 MPa. And with the addition of tapioca flour crosslinker in the sample with the addition of a concentration of 1%, 3%, and 5% has a greater tensile strength value. BC-EBL with the addition of 1% crosslinker with a tensile strength value of 66.58 MPa, the addition of a 3% crosslinker obtained a tensile strength value of 72.08 MPa, and the addition of a 5% crosslinker obtained a tensile strength value of 121.36 MPa. This can prove that the addition of a crosslinker to the BC-EBL sample can affect the mechanical properties of bacterial cellulose.

3.6.3 Functional Group Analysis (FTIR)

The use of a spectrophotometer for the analysis of bacterial cellulose functional groups. The wave number used for the analysis of the sample functional groups is in the range of 4000-600 cm^{-1}. The FTIR test used samples of BC, BC-EBL, and BC-EBL which had been soaked with 5% crosslinker (BC-EBLC 5%). The obtained results of the FTIR spectrum were analyzed to determine the functional groups of the sample.The results of the FTIR analysis in this study are close to the results of previous research conducted by Sartika, 2016, where BC, BC-EBL, and BC-EBLC 5% showed OH absorption at a vibration wave number of 3550-3200 cm-1, and CO absorption (β-glycosidic bond) was around 1500-1000 cm^{-1}. Table 1. Shows the wavenumber functional group of BC, BC-EBL, BC-EBL5%.

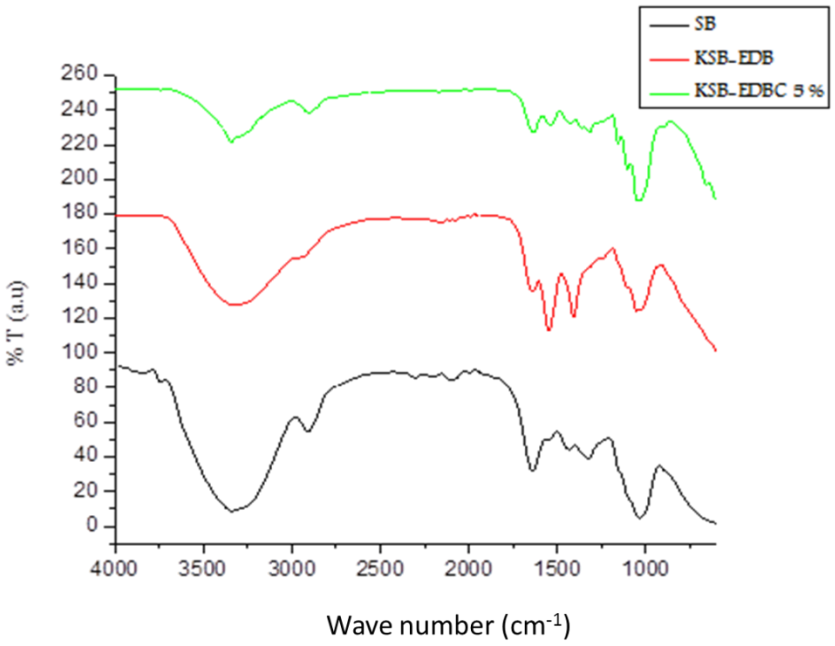

Figure 8. FTIR spectrum

Table 1. Vibration wave number in each functional group

Sample	Peak (cm-1)			
	OH	**CH**	**C=C**	**CO**
SB	3340.63	2910.89	1640.78	1035.67
KSB-EDB	3343.03	2163.15	1640.28	1054.54
KSB-EDBC 5%	3342.27	2903.38	1633.69	1031.04

Based on the functional group analysis data obtained, the SB composite with the addition of binahong leaf extract filler and the SB composite with the addition of a tapioca flour crosslinker did not produce new functional groups, only experienced a shift in the functional group within the same wave number range. This shift in functional groups was caused by the addition of filler of binahong leaf extract and a crosslinker in the form of tapioca flour in SB. This statement can also be said that the process that occurs in SB is only in the form of physical absorption.

4. CONCLUSION

The Based on the results of the research that has been done. The addition of tapioca flour solution as a crosslinker in BC-EBL can reduce the percentage of water content of BC-EBL which was initially 98% to 97%. A good concentration of tapioca flour solution to improve the mechanical properties of BC-EBL is 5%. The addition of tapioca flour as a crosslinker did not change the structure of BC-EBL, only affected the location of the functional groups in the same wave number range in BC-EBL.

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