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# *Preparation and Characterization of Composite Bacterial Cellulose - Mimosa Pudica Leaf Extract*

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**Abstract— The aim of this research was to prepare the composite Bacterial Cellulose of Mimosa pudica Leaf Extract (BC-MPLE) through a the process of merging matrix (SB) with filler (MPLE) . The preparation of BC using coconut water, a starter A.xylinum and fermented at room temperature until BC . The extract of MPLE was gained from Mimosa pudica. The preparation of BC-MPLE was performed by immersing BC in MPLE with a variation of immersion time for 7, 14 and 21 days without and using UV light radiation. Results of BC-MPLE had better physical, mechanical and structural characteristics than BC and MPLE. The water content of BC was 98,84 % and EDPM was 90,38%. Whereas the water content of BC-MPLE was lower than BC, which was98,75 %. Result of the swelling degree BC-MPLE was higher than BC. The maximum percentage of BC-MPLE swelling degrees was obtained in by 7th day MPLE using UV. BC-MPLE has better elasticity, tensile strength and compressive strength than SB. Furthermore, the FTIR spectrum from BC-MPLE was a composite spectrum of BC and MPLE. The degree of crystallinity of KSB-EDPM was higher than BC (BC = 84.470% and BC-MPLE UV = 84.962%).**

**Keywords— BC, MPLE, BC-MPLE, UV Light, non UV Light.**

## I. INTRODUCTION

Cellulose Bacterial (BC) was cellulose produced with the of bacteria such as *Acetobacter xylinum* (*A.xylinum*). This bacterial cellulose has unique properties when compared to cellulose produced by plants which has high purity, high crystallinity, high elastic modulus, non-toxic and others (Petrauskaite, 2013). Cellulose synthesized by bacteria is known as Cellulose Bacterial (BC) (Phillips, 2000).

Cellulose Bacterial (BC) has a high level of purity, has a high water absorbing capacity, high crystallinity, biocompatibility, high degree of polymerization, large elasticity and non allergenic (Singh, 2016) so that many studies on BC are carried out. BC is widely applied in non-medical and medical fields. The applications of BC in the non-medical field are as matrix in composites, reinforcement for fine structures, supercapacitors, biosensors in hydrogen peroxide and formaldehyde (Rajwade et al., 2015). Where as in the medical field BC can be applied as an anti-microbial wound dressing material, bone growth material and bone tissue engineering (Picheth *et.al,* 2017).

BC has a high tensile strength along the direction of the fiber layer, but has a compressive modulus value or a low ratio of compressive strength and strain if pressed from an angle perpendicular to the direction of the stack. This causes the water in the BC to be easily broken out of the BC just by being given a small pressure such as being pressed using a finger, after that BC cannot return to its original shape because BC is not elastic (Putra *et al*., 2010).

Nakayama (2004) and Putra (2010) in the studies that have been done prove that combining BC with other materials such as gelatin and *polycrilamide* can produce composites that have characteristics such as compressive strength, tensile strength and higher elasticity than pure SB. Therefore, researchers are looking for other alternatives to improve the elasticity characteristics of BC through modification with the addition of another material which later BC obtained can be applied in the

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biomedical field as drug material and bone growth material. The material used is a natural material that has medicinal properties such as herbal plants that are widely available in Indonesia, especially in the area of West Sumatra, one of which is the Putri Malu leaf (*Mimosa pudica L*). Addition of *Mimosa pudica* leaf extract which acts as a *filler* in the composit.

*Mimosa pudica* were plants that were considered as plants that are not useful, as impurities and must be eradicated and thrown away. But by the community there is already been used to reduce fever. In addition, *M.pudica* leaves have many health benefits, namely treating diabetic wounds, thinning phlegm, promoting blood circulation, overcoming asthma to prevent diarrhea and others. The chemical content of the *M.pudica* leaf extract is *flavonoids* and leaf *methanol* extract. *Flavonoids* are compounds that have anti-inflammatory, anti-viral and anti-fungal properties (Marina, 2009).

In this study, the composites were prepared by soaking BC in *M.pudica* leaf extract (MPLE) with a variation of immersion time for 7, 14, 21 days without and with UV light irradiation. This treatment is expected to 900˚C according to the hardness of the raw material used [14].This combustion process causes the decomposition of organic compounds that make up the structure of produce a new composite material that is better, stronger and of high elasticity.

## II. Experimental

### *Materials*

The equipment needed to characterize BC-MPLE is glassware (measuring flask, beaker, measuring cup), stirring rod, funnel, watch glass, analytic balance. In addition, the equipment needed is universal pH paper, *shaker*, blender, *Compressive Strength* and *Tensile Strength* ASTM 638 type V*, glass, Fourrier Transform Infra Red, X-ray Difraction*, UV lamps, screw micrometers, irons, plastic containers, *stainless steel* pans, stoves, knives, scissors, filters, fabrics, plastics, washcloths, newspapers, rubber bands, tissues, UV *boxes* and label papers.

The ingredients needed include coconut water, urea ($CO(NH_2)_2$),$C_6H_{12}O_6$, *Mimosa pudica,* acetic acid ($CH_3COOH$), *starter A.xylinum*, NaOH teknis, aquades and water.

### *BC Preparation*

Coconut water 600,00 mL was put into a stainless steel pan and then heated . Then add 60,00 grams of sucrose and 6,00 grams of urea (CO(NH2)2). This solution was heated to boiling. Furthermore, acidified with 25% acetic acid (CH3COOH 25%) were 12,00 mL until the pH reaches 4-4,5. After that, in hot conditions the solution was transferred to a plastic container measuring 24 cm x 17 cm x 4 cm 600.00 mL and covered with newsprint that has been sterilized until the media reaches room temperature between 25-30°C. Then in the media inoculated 10% v/v starter A.xylinum aseptically and fermented at room temperature until BC formed with thickness ± 1.00 cm . After SB is formed ± 1.00 cm SB is harvested (Sartika, 2016 and Febrianta, 2015).

### *Purification of Bacterial Cellulose*

Harvested BC were washed using running water for ± 24 hours. Then SB was immersed in 2% (w/v) NaOH solution for ±24 hours (Nugraha, 2016). BC which has been immersed in 2% NaOH followed by washing using water again. After that BC can be stored in water until SB will be used. BC immersion water was replaced every 6 hours (Sartika, 2016).

### *MPLE Preparation*

Mimosa pudica leaf were washed with running water, then dried using an oven to dry. Then the dried leaves were mashed using a blender and sifted until the powder was obtained. The powder was dissolved using distilled water (10: 100 w/v) until it was obtained by the extract of the M.pudica leaf (MPLE) which will be used as a filler in making BC-MPLE.

### *BC-MPLE Preparation*

BC that had been arranged to a certain size were then assembled in MPLE for 7, 14, 21 days without and using UV light. During the immersion process the sample was shaked using a shekr to be processed BC-MPLE

### *BC-MPLE characterization*

*a. Water content measurement*

BC-MPLE the water content was determined by means of the sample being ovened at 105oC, then weighed using an analytical balance. The percentage of water retention can be calculated using the following equation.

$$W_c(\%) = \frac{W_b - W_k}{W_b} x100$$

*b. Degree of swelling measurement*

The dried BC gel was measured in thickness. The sheet was weighed initially (Wd) and soaked in distilled water at a certain time. Then the surface of the sample was dried and immediately weighed. This weighing was carried out every 24 hours until a constant weight is obtained (Ww). The percentage of degree of swelling can be calculated using the following equation.

$$DP(\%) = \frac{Ww - Wd}{Wd} \times 100\%$$

*c. Compressive strength measurement*

Compressive strength test was performed using Compressive Strength tool Test

*d. Tensile strength measurement*

KSB-EDPM tensile strength test done using a *tensile* tool *test* .

*e. Analyst a functional structure using FTIR*

The sample used for functional structure analysis was an oven- dried sample. The sample was put on sample holder, then measured absoption using FTIR.

*f. Degree of crystallinity Analysing using XRD*

The sample used to calculate the degree of crystallinity was sample that has been dried using an oven. The percentage of the degree of crystallinity can be calculated using the weighing method. The relationship between mass and cellulose crystallinity is as follows.

$$\%\ crystallinity = \frac{m_{kristal}}{m_{kristal} + m_{amorf}} x\ 100\ \%$$

## III. RESULT AND DISCUSSION

*A. Preparation of A.Xylinum starter*

Starter of *A.xylinum* used the basic ingredients of pineapple fruit because naturally there are already existed or already living *A.xylinum* bacteria in pineapple fruit . But still need to be added with nutrients by adding sucrose as food to be fermented by *A.xylinum* bacteria. In the growth of *A.xylinum* bacteria, several factors must be considered in the growth of *A.xylinum* which include the amount of carbon and nitrogen, the acidity (pH) with the optimum pH range of bacteria was 4.5-5, the temperature at which bacteria can grow in a temperature range of 25-30˚C, the place used must be sterile, flat and free from shocks. *A.xylinum* was a Gram negative, aerobic and can produce cellulose. The presence of a starter of Activated carbonis heated to a temperature of $310^0$C in the furnace. After the temperature is reached, the carbon is allowed to coolin the furnace by not relating to outside air. After cool is put into the desiccator and weighed.

*A.xylinum* bacteria was very necessary in making Bacterial Cellulose (BC). In the absence of this bacterium, the BC layer cannot be formed (*Lapuz et al. ,* 1967 ).

*B. Preparation of Bacterial Cellulosa (BC)*

BC preparation was processed of SB formation with the help of A.xylinum bacteria . A.xylinum bacteria will be able to form cellulose if grown in coconut water that has been enriched with carbon (C) and nitrogen (N) through a controlled process. Under these conditions bacteria can produce entracellular enzymes that can arrange sugar into thousands of fiber chains or cellulose. From the tiny millions that grow in coconut water, it will produce millions of pieces of cellulose threads which eventually appear white to transparent solids called bacterial cellulose (BC).

BC formed was certainly diverse because it was influenced by several factors in the growth and activity of *A.xylinum* used. If the ratio between carbon and nitrogen was adjusted optimally and the process is well controlled, then all liquids will form SB without leaving any residue at all.

Coconut water used in BC preparations must come from cooking coconut water, not too old or too young. Additional ingredients needed by bacteria were simple carbohydrates, sources of nitrogen and acetic acid. In general, simple carbohydrate compounds as a supplement in making SB in this study are from sucrose. The nitrogen source used wass from inorganic nitrogen such as Urea. Acetic acid or vinegar was used to lower the pH or increasing the acidity of coconut water wass glacial acetic acid (99,8%), low concentrations of acetic acid can be using, but to achieve the desired level of acidity that was 4.5-5. Apart from the additional material that must be considered, the place used in the preparation must be flat, sterile, free from shocks and the room temperature must be at an optimum temperature of 25-30˚C. So that the bacteria used remain active so that the formation of cellulose fibers can occur quickly.

*C. Purification of BC*

The purification process aims to remove non-cellulose components and the remaining bacteria that are still trapped in the BC. The non-cellulose component and the rest of the bacteria will block the hydrogen bonds that occur between the cellulose molecule chains, which results in a decrease in the cellulose mechanical strength. In addition, the bacteria trapped also reduce mechanical strength because these bacteria can still move by using nutrients in the BC (Widyaningsih, 2008) .

(a) (b)

Figure 2. (a) BC before washing and refining (b) BC after washing and purification

The BC washing process was carried out by soaking BC in water and also flowing it with running water for ± 24 hours, then purification is continued by immersion using 2% (w/v)

NaOH for ± 24 hours at room temperature. Purification using 2% NaOH (w/v) is done because it can increase the purity of cellulose produced so that the relationship between the chains in cellulose gets stronger through hydrogen bonds between chains and cellulose structure becomes more tightly (Piluharto, 2003). As a result, the BC obtained has good mechanical properties.

Based on the results of washing and purification of the SB that have been carried out, it can be seen in Figure 2, BC which was initially yellowish white becomes whiter and the thickness of the BC layer was reduced. The reduced thickness of the BC layer was due to the non-cellulose components trapped already being eroded by NaOH 2% (w/v).

*D. MPLE extraction*

MPLE preparation aims to obtain MPLE which functions as a filler component in the composite. MPLE preparation was started by collecting crushed daughter leaves powder using a blender and soaking for ±15 minutes in hot water (temperature 90˚C) with a comparison between shy princess leaf powder and water (10:100 w/v). According to Hagerman (1998) there are tannin compounds contained in M.pudica leaf that are easily soluble in water, especially hot water, hydrolyzed tannin compounds are usually in the form of amorphous compounds, which are yellowish-brown hygroscopic which were usually used as natural dyes (Darmayanti, 2001) which causes MPLE to be colored yellowish brown. The preparation step can be seen in Figure 3 .

(a) (b) (c) (d) (e)

Figure 3. (a) The leaves of the *M.pudica* leaf who have been cleansed and dried (b) Blending of them *M.pudica* leaves was embarrassed (c) Dissolving the leaves of *M.pudica* using distilled water (d) Filter the solution of the *M.pudica* leaf to shame (e) *M.pudica* leaf extract was ready for use.

*E. BC-MPLE Preperation*

BC-MPLE preparation was a process of combining BC (matrix) with MPLE (filler) to form a composite. This composite formation aims to produce a new material that has better physical, mechanical and structural properties than BC (matrix) and MPLE (filler).

BC-MPLE preparation was carried out by merging BC in MPLE with variations of time 7, 14 and 21 days without and using UV light. During immersion the sample was rocked using a shaker. The *purpose was to use the shaker so that MPLE (filler*) enters the BC cavity. If you do not use a shaker, MPLE will settle at the bottom of the soaking container, so that there was no adsorption or absorption into the BC (*matrix*).

Based on the research conducted, it can be seen that BC soaked in EDPM for 7 , 14 , and 21 days can be seen in Figure 4. Color changes that occur after immersion with MPLE because *M.pudica* leaves contain tannin compounds that turn yellowish brown when *M.pudica* leaf powder was dissolved in hot water (90˚C). As a result BC-MPLE turned brown.

(a) (b)

Figure 4. (a) BC which measures 2x2 cm and 15x2x1cm before soaking with MPLE(b) BC which measures 2x2 cm and 15x2x1 cm after soaking with MPLE

*F. Water content measurement*

The effect of BC immersion time in MPLE on the percentage of water content can be show in Figure 5. In figure 5.

It percentage of the water content of the BC was obtained at 98.84%. This shows that the water content of BC is very high. This result was in accordance with the research conducted by Ciechanska (2004) that the high water content of bacterial cellulose is 98-99%. And this result was also in accordance with the research conducted by Pa'e (2014) which states that the normal BC water content is more than 90%.

Based on the results of the research it can be seen that the absorption process during BC immersion in MPLE occurred because the components of MPLE acting as *filler* were absorbed into the *matrix* and replaced the water inside the *matrix.* At BC-MPLE 7$^{th}$ day UV BC-MPLE was heavier than BC-MPLE TUV with a greater percentage of water content BC-MPLE UV of 99.15% than TUV of 99.05%, means more water was contained in BC-MPLE UV 7th days compared to MPLE which is tied to BC-MPLE 7th day. However on the 14th and 21st day the weight of BC-MPLE TUV was heavier than BC-MPLE UV with the percentage of BC-MPLE TUV water content of 98.85% lower than using UV at 99% this

means that there were also many MPLE attached to the BC soaked for 14 and 21 TUV. So there was an influence given by UV light during immersion using MPLE because it can make *filler* (MPLE) was less attached to the *matrix* (BC). Perhaps because UV light when the *shaker* makes the *filler* entering from the surface over time fall to the base of the *matrix* causing at least the MPLE attached to the BC .

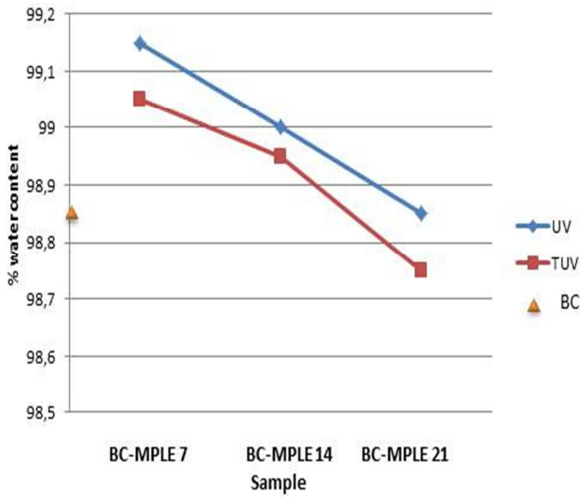


Figure 5.The effect of Immersion Time on percentage of Water content

*G. Degree of Swelling of BC-MPLE*

Based on research on the percentage of swelling degrees obtained by BC, which is 200.6%, 7th day BC-MPLE (UV and TUV) obtained a 7-fold increase in percentage compared to SB and then decreased at BC-MPLE 21 (UV and TUV). Graph of swelling can be seen in Figure 15. However , in general, the longer the immersion of BC in MPLE, the more fillers entering the matrix so that the degree of swelling from BC-MPLE increases. With the filler (MPLE) that enters,BC-MPLE pores with the longest immersion will be smaller according to regular interactions. According to Pandey, et al. (2013), the high interconnected SB pores into this area are easier for the diffusion of water molecules so that the water absorbing capacity (swelling) becomes higher.

The effect of the BC immersion time in MPLE on persentage of water content can be show in Figure 6.

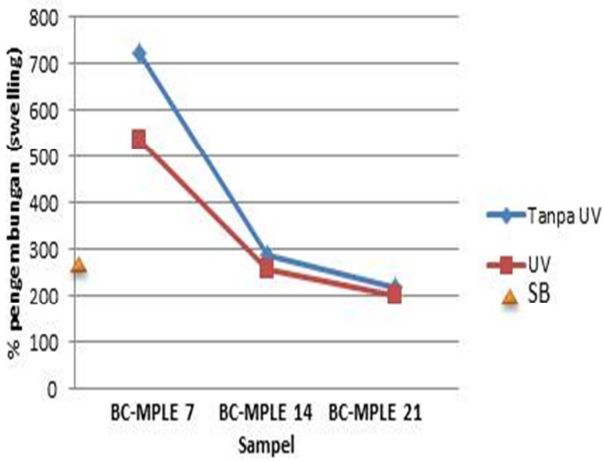


Figure 6.The effect of immersion time on percentage of swelling

*H. Compressive Strength BC-MPLE*

Testing the compressive strength *(compressive strength)* was a very important parameter in designing or making a material structure, because this test can determine the quality of the resulting BC (Urbaik *et al*, 2006). The measurement results of BC-MPLE compressive strength can be seen in Figure 7 the following. Based on figure 7. it can be seen that the BC samples immersed in optimun compressive strength MPLE are found in BC-MPLE14 days using UV and TUV which is 1.03 kN (UV) and 1.08 kN (TUV). But the longer the immersion time of the compressive strength of BC-MPLE should be higher. Because the increase in the compressive strength of BC-MPLE caused by the amount of *filler* entering the *matrix* replaces water during immersion time. The more water that was replaced by *filler* , the structure of the *matrix* will be stronger, so a larger compressive force was needed to flatten the BC-MPLE sample.

BC-MPLE which was irradiated using UV has a higher compressive strength than the sample TUV irradiation, so it is concluded that the UV light gives an effect on the increase in the compressive strength value of BC-MPLE .

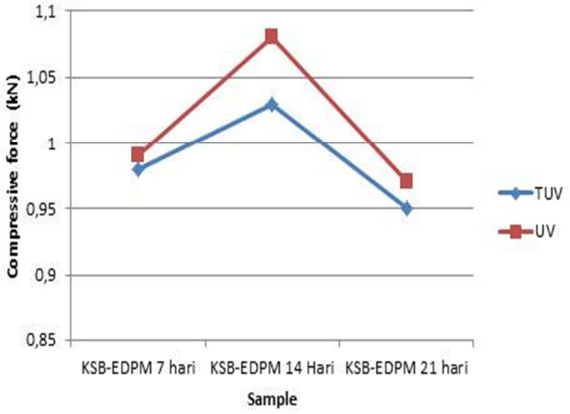


Figure 7. Effect of immersion time on compressive force

*I. Tensile Strength test*

The value of tensile strength is defined as a change in force on the cross-sectional area of the area subject to this force. Figure 8 shows that the tensile strength of BC is 12,5 $N/m^2.10^{-3}$. BC-MPLE tensile strength value decreases with increasing immersion time. The greatest tensile strength values were in the BC-MPLE sample of 7$^{th}$ day UV, then the tensile strength values decreased in the BC-MPLE 14 and 21 (UV and TUV) samples. This was caused by the filler found in BC which was immersed in MPLE for 14 and 21 days, the amount of filler contained in the matrix was still small and the interaction between filler and matrix was not accompanied by a bond formed between the two but only the physical absorption process occurs. found in the detached matrix, so that the measurement does not significantly affect the sample when pulled. This was also influenced by the shaking process using a shaker that helps the process of removing the filler from the matrix. While in the BC-MPLE sample of 7th day the amount of filler absorbed quite a lot replaces the water that fills the pores of the matrix , thus causing an increase in the tensile strength value in the sample shown in figure 8 (a).

Giving UV light also affects the value of tensile strength . BC-MPLE UV samples have a much lower tensile strength than the BC-MPLE TUV sample. This was because *filler* was difficult to glue on the *matrix*, so the need for adding a *crosslinker* to make the bond between the *filler* and *matrix* occur .

Strain was defined as the ratio of changes in the length of an object to its initial length due to a force with a direction parallel to that length odf change.The strain comparison data with the duration of BC immersion in MPLE was shown in Figure 8(b), indicating that the strain value in the BC-MPLE UV sample is increasing, soaking time decreases while in the BC-MPLE TUV sample the time increases. The lowest strain value is in the 21th day BC-MPLE UV sample of 0.014. The sample quality was to be good if the strain value of the sample is getting smaller. Then the 21th day BC-MPLE UV sample is the best sample quality, because it has the lowest strain value.

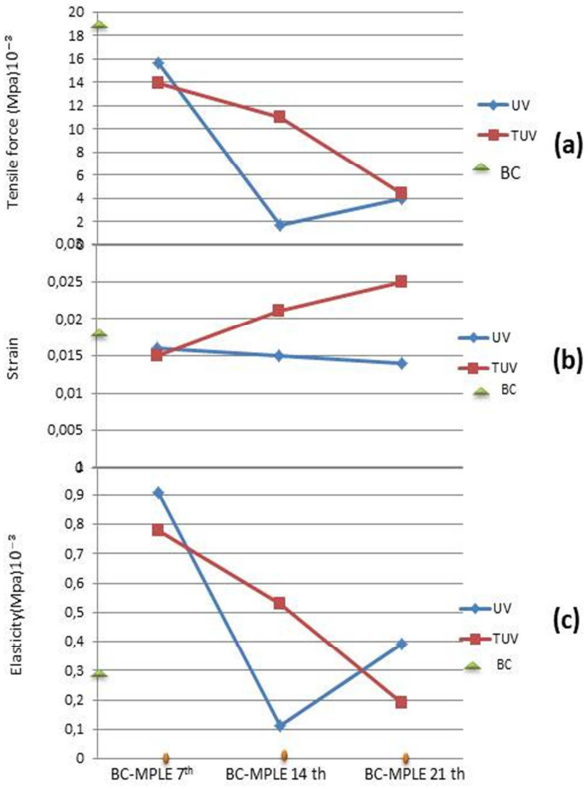


Figure 8. The effect of immersion time on (a) tensile force, (b) strain and (c) elasticity.

Each of the tensile strength values, the elasticity value of BC-MPLE was increasing every day tends to decrease, but increases in the 21th day BC-MPLE UV sample 21th day UV BC-MPLE elasticity value of 0,39 $MPa.10^{-3}$ . The decrease and increase in the elasticity value of the BC-MPLE sample were also due to the *filler* contained in *the* sample *matrix*.

*J. function structure of BC-MPLE*

The results of the functional group analysis using FTIR can be seen in Figure 9. Based on Figure 9 functional groups and wave numbers generated from the spectra can be seen in Table 1.

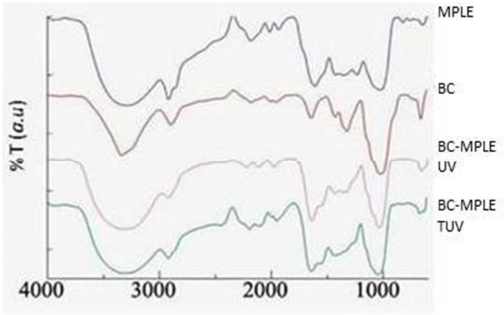


Figure 9. FTIR spectra from MPLE , BC, BC-MPLE UV and BC-MPLE TUV

Based on the description of the test data an above can be obtained, that the functional group differences between BC and BC-MPLE (UV and TUV) can be seen from the addition of functional groups found in the BC-MPLE spectrum. It can be seen in the BC-MPLE spectrum (UV and TUV) that was formed was a combination of spectrum consisting of spectra BC and MPLE.

Table 1. The peak of the wave number in each functional structure

| Sample | Peak ($cm^{-1}$) | | | | |
|---|---|---|---|---|---|
| | O-H | C-H | N=C=S | C-O-C | C=C |
| MPLE | 3284.95 | 2919.06 | 2016.62 | 1028.38 | 1613.06 |
| BC | 3337.87 | 2897.19 | - | 1029.18 | 1645.05 |
| BC-MPLE UV | 3336.67 | 2920.28 | 2115.56 | 1038.05 | 1639.11 |
| BC-MPLE TUV | 3319.67 | 2919.29 | 2101.62 | 1038.03 | 1635.59 |

*K. The degree of crystallinity of BC-MPLE*

The results of the analysis of the degree of crystallinity using XRD can be seen in Figure 10 below.

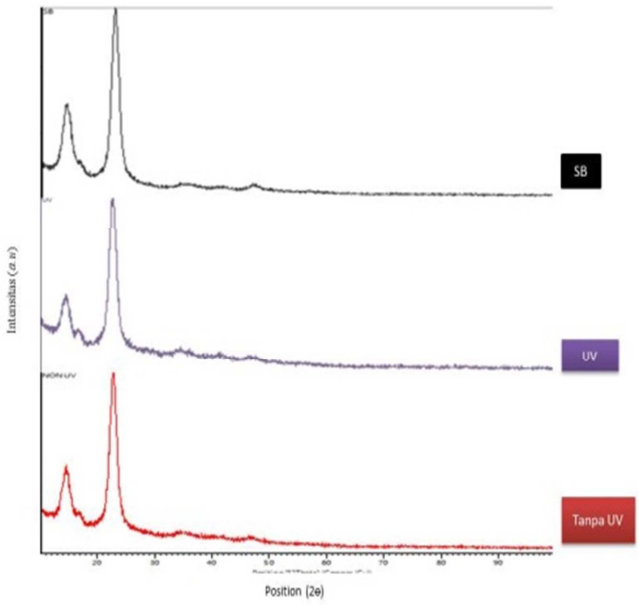


Figure 10. Diffractogram BC, BC-MPLE UV and BC-MPLE TUV.

The test results using XRD were obtained fragmented in Figure 10. shows that there was no difference from the sample BC, BC-MPLE UV, BC-MPLE TUV. Where the peaks were formed and the intensity of each peak looks the same . This result can be seen from the BC peaks that appear at 2Θ ranging from 14 ° , 17° and 23 ° while the peak of BC-MPLE UV and BC-MPLE TUV that appeared at 2Θ ranged from 15°, 17° and 23.5° . Based on the peaks formed, it can be concluded that BC and BC-MPLE were type I cellulose (Yue *et al*., 2013). From the results of the above diffractogram , the degree of crystallinity can be determined by BC-MPLE and BC. The degree of crystallinity of BC-MPLE in this study can be determined using the method *Hermans - Weidingermethod* (Terinte, 2011) that was by using the following equation.

$$\%crystalinity = \frac{W\ Crystal}{W\ Total} x100\%$$
$$= \frac{W\ crystal}{W\ (kristal + amorf)} x100\%$$

Table 2. The Crystallinity degree of BC-MPLE

| Sample | Total Weight (grams) | Crystal Weight (gram) | Amorphous Weight (grams) | % degree of crystallinity |
|---|---|---|---|---|
| BC | 0.117 | 0.1 | 0.017 | 84, 470 % |
| BC-MPLE UV | 0.133 | 0.113 | 0.020 | 84, 962 % |
| BC-MPLE TUV | 0.127 | 0.106 | 0.021 | 83, 464 % |

The results of the calculation of the BC and BC-MPLE samples obtained from this equation can be seen in Table 2 that the degree of crystallinity obtained by BC is 84.470 % means it has an amorphous structure of 15.530%. BC-MPLE UV has the highest degree of crystallinity which is 84.962 % with an amorphous structure of 15.038%. BC-MPLE TUV has a crystallinity degree of 83.464% with an amorphous structure of 16.536%. This result is not much different from the results of the BC sample , so it can be said that irradiation with UV light during the BC-MPLE immersion process can increase the degree of crystallinity. The higher the degree of crystallinity, the less elasticity of a material.

## IV. CONCLUSIONS

Results of the research that has been done, it can be concluded :

a. The longer the immersion time of BC in MPLE, the lower the percentage of water content produced.
b. The degree of swelling of BC-MPLE is higher than BC. The maximum percentage of swelling degree of BC-MPLE is obtained in BC immersion in 7th day MPLE.
c. Mechanical properties (tensile strength and compressive strength) as BC immersion increases in MPLE decreases the mechanical value produced.
d. The FTIR spectrum of BC-MPLE is a composite spectrum of BC and MPLE.
e. The degree of crystallinity of BC-MPLE is lower than BC.

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