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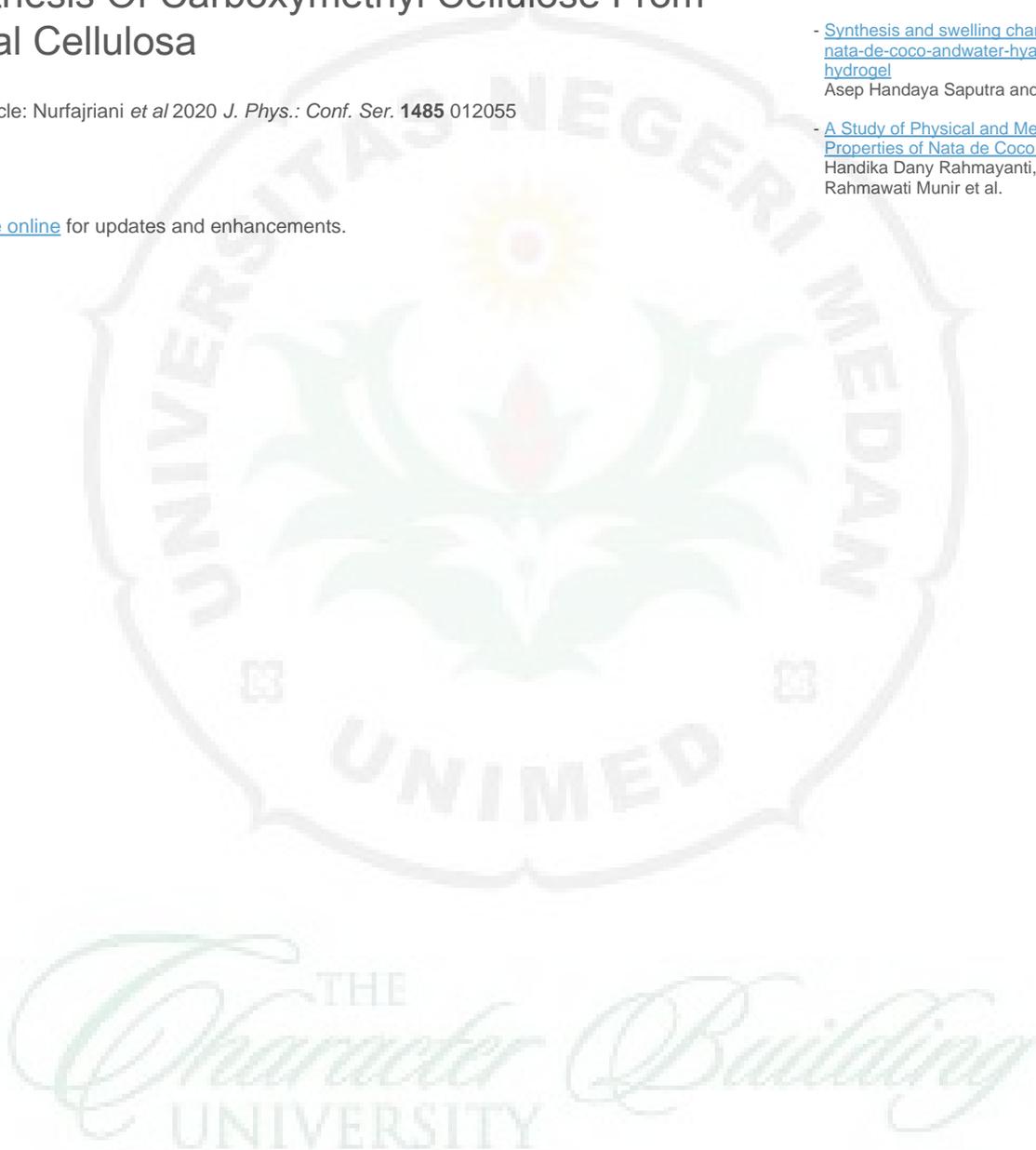# The Effects Of Sodium Hydroxide Concentrations on Synthesis Of Carboxymethyl Cellulose From Bacterial Cellulosa

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The Effects Of Sodium Hydroxide Concentrations on Synthesis Of Carboxymethyl Cellulose From Bacterial Cellulosa

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Abstract. This investigation aimed to create Carboxymethyl Cellulose (CMC) from the nata de coco cellulose. The nata de coco was produced by fermentation method of coconut water used *acetobacter xylinum*. Bacterial cellulose (BC) was isolated by furification nata de coco with 1% NaOH and 1% acetic acid solutions respectively. In the process of making CMC, dried BC was immersed in isopropanol medium, then alkalised with 10%, 15% and 20% NaOH solutions respectively for 1 hour at a temperature of 55 oC. Then BC reacted with 18 grams of chloroacetic acid. FTIR analysis results show that CMC was successfully synthesized from BC. The increase in NaOH concentrations in the CMC preparation caused an increase in the degree of substitution (DS). DS is one of the main parameters of the success of the CMC synthesis process from the initial raw material for cellulose. At a concentration of 20% NaOH, the resulting CMC synthesized with DS 0.743 was better than 10% NaOH with DS 0.371.

1. Introduction

In general, carboxymethyl cellulose (CMC) can be produced from cellulose derived from plant products such as cotton and plant parts, such as tree trunks, twigs, leaves, and other parts. In addition to plants, CMC can also be produced through synthesis by microorganisms such as bacteria (*Acetobacter, Rhizobium, Agrobacterium, and Sarcina*) - fungi and algae [1]. Gea [2] has reported that the bacterium *Acetobacter xylinum* is a species of bacteria capable of producing cellulose called Bacterial Cellulose (BC). Klemm et al [3] reported that BC extracted from *A. xylinum* bacteria produced pure BC gel with a mass composition of 99.8% water and 0.2% inert polysaccharides, which did not contain lignin, hemicellulose, pectin, and wax. In its growth, *A. xylinum* is a good bacterium for the biosynthesis of glucose into cellulose, one of which is by using coconut water media.

Coconut water contains a substrate such as glucose and some minerals and protein which is 2% - 5% wet weight of coconut water [4]. Based on statistical data obtained from the Directorate General of Plantations of the Ministry of Agriculture (2016), specifically in the area of North Sumatra Province

coconut production per year includes fixed figures starting from 2015 to 2017, there are 88,844 tons with plant area reaching 85,808 ha. Therefore there is an abundant amount of coconut water that can be used as a medium for bacterial growth to produce nata de coco, which contains fiber which can be a source of cellulose raw material for manufacturing CMC.

Studies on the use of coconut water as a growth medium of *A. Xylinum* have been carried out by Awalludin et al [1] who used nata de coco purified into BC for CMC synthesis with high purity. In general, the process of making CMC includes two stages, namely the alkalization stage and the etherification stage. At the stage of the alkalinization reaction, sodium hydroxide is used as a reagent whose amount must be excessive to increase the reactivity of cellulose. NaOH serves to damage the crystalline structure of cellulose by breaking the hydrogen bonds in cellulose, thereby opening up cellulose fibers to be reacted into cellulose-Na and can facilitate the entry of carboxymethyl groups in the etherification process that adds a solution of monochloroacetic acid [5].

One important parameter that determines the nature of CMC is the value of the degree of substitution (DS). The degree of substitution of CMC is the average number of hydroxyl groups in the cellulose structure that are subsidized by other groups. The greater the degree of substitution, the better the quality of CMC, because of the greater water solubility [6]. Therefore in this study, we will examine more deeply the influence of the concentration of NaOH used in the synthesis of CMC from BC sourced from nata de coco.

2. Experimental

2.1. Materials
Coconut water, inoculum *A. xylinum*. Sucrose, ammonium sulphat, acetic acid, NaOH, Sodium monochloroacetat (SMCA) isopropanol (p.a Merck), and methanol 80%.

2.2. Preparation of Nata de coco
Produce of the nata de coco, an amount of 100 g of sugar and 4 g $(NH_4)_2SO_4$ is dissolved in 1 L of coconut water, then heated for 20 minutes until boiling at 120 C then cooled and poured into the tray, and added 10 mL CH_3COOH (pH 4 - 5), followed by the addition of a starter as much as 100 mL, covered with paper cover. Furthermore, the tray is stored in a flat, stable place and allowed to stand for 1-2 weeks at room temperature to produce nata de coco.

2.3. Preparation and Purification of BC
Purification and preparation of BC, freshly harvested nata de coco is washed with running water then pressured until almost all the water comes out, soaked in 1% (w / v) NaOH solution for 1 week, then neutralized with 1% CH_3COOH (v / v) for 24 hours, followed washing with water. At the BC preparation stage, the nata is dried in an oven at 60 ^{o}C, then crushed with a blender and filtered using a sieve ($\pm$ 60 mesh) to obtain BC solid powder.

2.4. Syntheisis of CMC
An amount of 5.5 g of BC was added with 100 ml of isopropanol and stirred with a magnetic stirrer at room temperature for 15 minutes, then added 40 ml of NaOH with variations in the concentration of 10%, 15%, and 20%. The mixture was left for 1 hour then added 18 g of monochloroacetic acid and stirred for 4 hours at a temperature of around 55 °C. Furthermore, filtering and washing with 80% methanol is followed, followed by a neutralization process with the addition of acetic acid at room temperature. The resulting CMC was washed with absolute methanol, and dried at 60 °C [1].

2.5. Analysis and Characterization of CMC
The synthesized CMC is determined the DS value and characterization using infrared spectroscopy (FT-IR) and Scanning Electrons Microscop (SEM).

The concept of determining DS is acid-base titration based on the method used by Lin et al [7]. The CMC sample was first dried in a vacuum of 110 ^{o}C to ensure the CMC was free of water. As much as 0.1 g of CMC was dissolved in 50 mL of deionized water to ensure the sample was not contaminated with other metals. then added 30 mL of HCl 0.105 M. After 30 minutes, the mixture was titrated using 0.095 M NaOH solution using 1-2 drops of phenolphthalein as an indicator until the solution was violet. The DS calculation follows the following equation:

$$D = \frac{162\,m}{m - 58B}$$

where B represents the molar weight of sodium hydroxide reacted with carboxyl group is equal to (0.095V- 0.105 x 30) x 10^{-3} and m represents the weight of CMC [7].

3. Result and Discussion

In this study BC was prepared from nata de coco which is the result of fermented coconut water using *A. xylinum* bacteria. The nata de coco product produced is shown in **Figure 1a and t**he BC produce show in **Figure 1b**. .
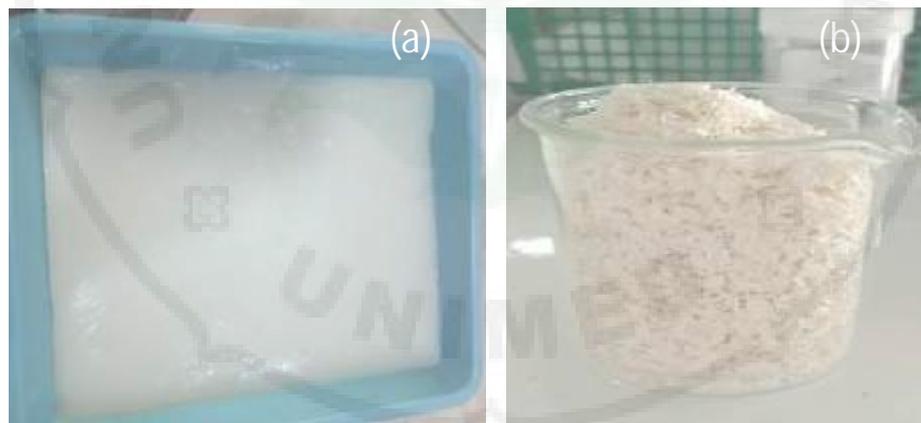

Figure 1. Nata de coco products from incubation for 2 weeks (a) and purified dried BC (b).

The process of preparation and purification of BC from nata de coco using 1% NaOH solution for 1 week to separate nata from the content of sugar, fat and protein produced by bacteria [8]. The loss of the content is marked by the appearance of a brownish or dark yellow color in the soaking of nata in 1% NaOH solution. This brownish color is sugar, fat and protein which are degraded by alkaline solutions.

CMC products produced in the variation of 10%, 15% and 20% NaOH are shown in **Figure 2**. The CMC obtained is slightly yellowish white. In the process of formation of CMC consists of two stages, at the stage of planting the alkylation process of BC is carried out with NaOH. The concentration used <20%. The use of NaOH as a reagent to increase cellulose reactivity and to damage the crystalline structure of cellulose by breaking the hydrogen bonds in cellulose, thereby opening up the cellulose fibers to be reacted to form cellulose-Na and can facilitate the entry of carboxymethyl groups in the etherification process at a later stage, and can increase the degree of substitution [9]. In the second stage, etherification was carried out using monochloroacetic acid or sodium monochloroacetic (SMCA) reagents. The carboxymethylation process can be carried out using sodium monochloroacetate reagents, in this process the -OH group on cellulose is replaced by ClCH$_2$COONa. At this stage, carboxylic groups adhere to the cellulose structure. In addition, in this process also

occurs the formation of side products in the form of sodium glycolic. Alkalization and etherification reactions are given in **Figure 3**.
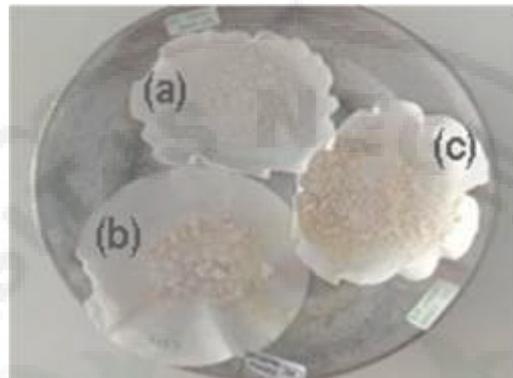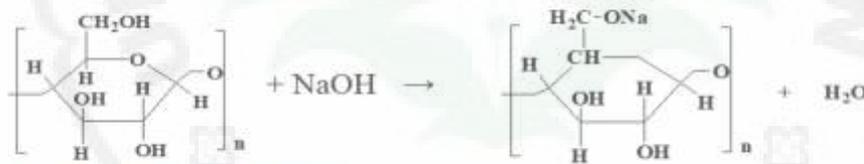

Figure 2. CMC products are synthesized with NaOH (a) 10%, (b) 15% and (c) 20%.
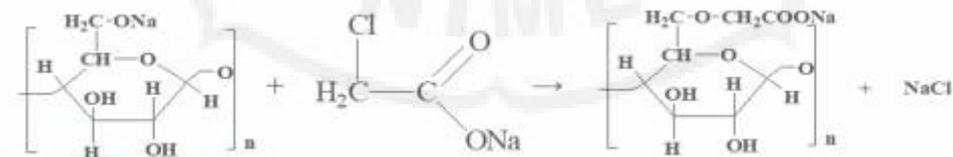

Figure 3. Alkalization and etherification reaction on CMC formation from BC nata de coco.

3.1. Value of Substitution (DS)

The DS value of the CMC product produced in the variation of NaOH concentration is presented in **Figure 4**. In the figure it can be seen that the DS value of the CMC has increased with increasing NaOH levels. This is consistent with the results conducted by Bisht et al [10] that with increasing base concentration, DS will also increase. It can be understood that the use of NaOH can increase the reactivity of cellulose and damage the crystalline structure of cellulose by breaking the hydrogen bonds in cellulose to form Na-cellulose. This condition facilitates the entry of carboxymethyl groups in the etherification process at a later stage, and can increase the degree of substitution [9]. In figure 4 it can be seen that the highest DS is obtained at a concentration of 20% NaOH namely 0.743 and the lowest DS is obtained at a concentration or 10% NaOH level that is 0.371. DS CMC values generated from this study have met Indonesian National Standards (SNI) and FAO standards. According to SNI the degree of substitution of CMC quality 1 is in the range of 0.7 - 1.2, while according to the FAO standard that is 0.2-1.5 [11].
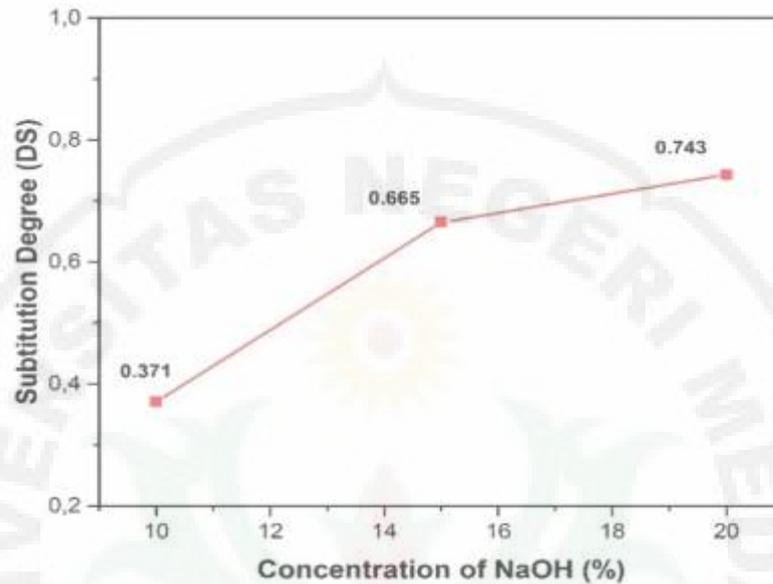
Figure 4. Graph of the relationship of DS CMC at different NaOH levels

3.2. FTIR analysis

The FTIR spectra of BC are shown in **Figure 5**. The characteristic absorption of the BC functional groups was observed at 2893.71 cm-1 and 1159 cm-1 which were the uptake of the asymmetric C-H and C-O-C groups. OH, group uptake was observed at 3338.52 cm-1. Meanwhile, the absorption peaks at 1800-1500 cm-1, 1300-1150 cm-1, and 1100-1050 cm-1, respectively showed the presence of lipids, proteins and nucleic acids in BC [12]. This FTIR spectra also show that the cellulose obtained really comes from the activity of microorganisms.
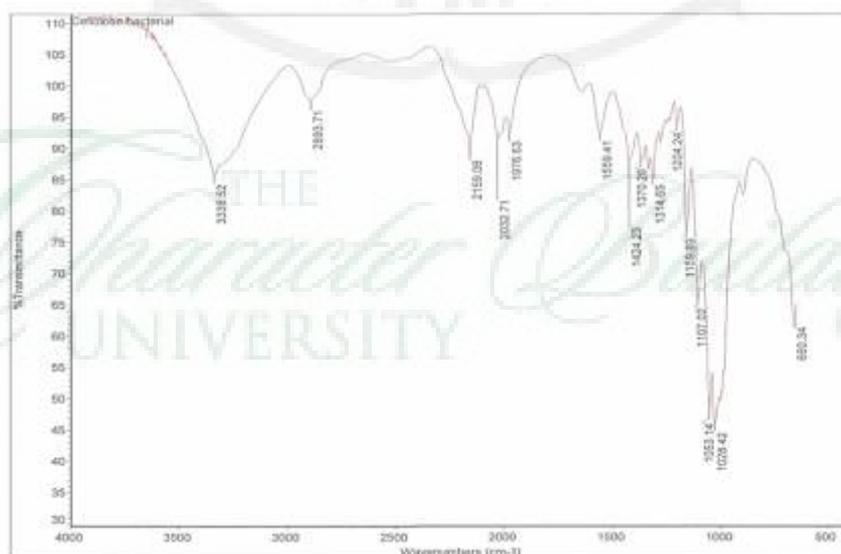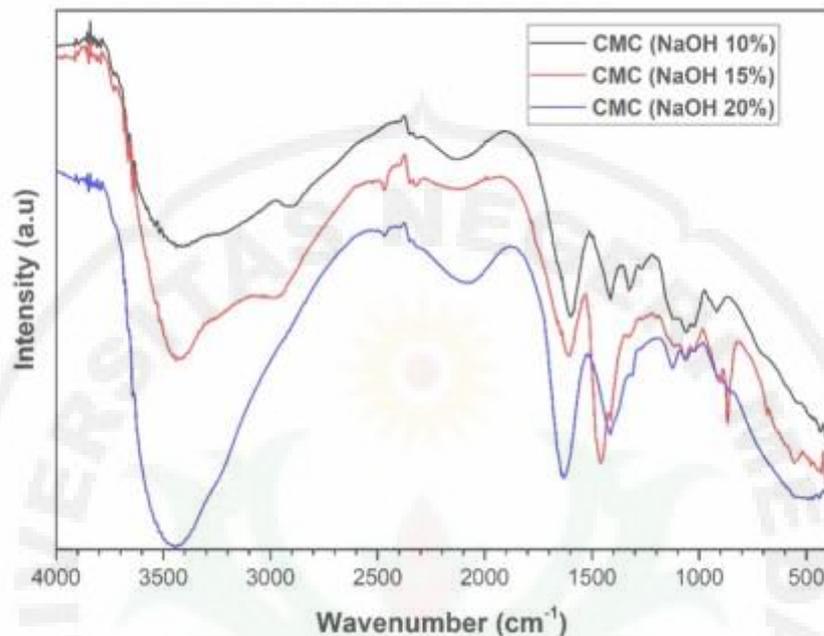

Figure 5. FT-IR spectra of BC.

Figure 6. Comparison of FT-IR spectra of CMC synthesized with variations of 10%, 15% and 20% NaOH in the range 4000-400 cm^{-1}.

Comparison of FT-IR spectra of CMCs that were synthesized with variations of 10%, 15% and 20% NaOH concentrations are given in Figure 6. The CMC spectra showed absorption at around 1631.78 cm-1 which is a characteristic of C = O or COO- [10][13] and absorption at regional wavelength ranges from 1415.75 - 1462.02 cm-1 as a characteristic of -CH2. The absorption intensity of the carboxyl group C = O or COO- increases with the greater of NaOH used. Meanwhile OH group uptake was observed in the range 3402.43 -3444.87 cm-1 with the peak sharper along with the increase in NaOH concentration. Similar results were reported by Yeasmin and Mondal [14]. The absorption intensity of the OH group was observed to be sharper and higher with the increase in the concentration of NaOH used. These results correlate with the increase in DS values in **Figure 4**. These data indicate that the formation of carboxyl group bonds in the cellulose structure. The higher the concentration of NaOH used the more the number of carboxyl groups bound by cellulose and increase the value of DS.

3.3 SEM analysis

 SEM analysis was carried out to determine morphological differences between BC and CMC surface specimens as a result of synthesis [15]. Comparison of surface morphology of BC and CMC is shown in Figure 7.

 The surface morphology of BC (figure 7a) looks very tight and dense, and is a characteristic of BC that distinguishes it from plant cellulose which has space and is hollow [1]. The surface morphology of BC is microfibrils intertwined with one another, tightly fused and very strong. While CMC surface morphology (figure 7b) looks very rough with many pores on the surface, resembling a honeycomb. The destruction of the form of BC is caused by the carboxymethylation process which is the use of strong alkali during synthesis as reported by Zhang et al [16]. When compared with the SEM results from the study of Lin et al. in BC morphological testing [7] and Yue L et al [17] in CMC morphological testing, the resulting image is similar to the CMC bottom surface of BC.
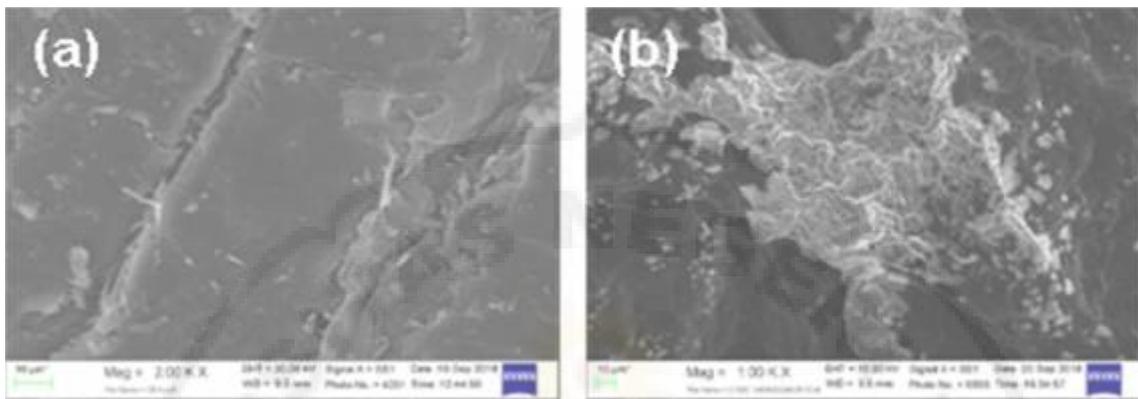
Figure 7. Surface morphology from (a) BC, (b) CMC synthesized

4. Conclussion

Nata fiber produced from *A. xylinum* culture in coconut water media can be used as a good source of BC cellulose for CMC synthesis. The degree of CMC substitution resulting from the synthesis increases with increasing NaOH concentration used. DS CMC values from BC nata de coco are by SNI and FAO standards.

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