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Preparation and Characterization of Nanocrystalline Cellulose from *Cladophora sp. Algae*

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Abstract— **Nanomaterial is one of the recent technological advancements that attracts the attention of many scientists. Many advanced properties could emerge from the utilization of materials on this scale. Nanocrystalline cellulose (NCC) is a cellulose-based nanomaterial currently gaining attention because of its remarkable properties. NCC was prepared by removing the amorphous part of cellulose through acid hydrolysis. Cladophora is a type of algae with a fiber-like body, that could grow rapidly and widely available in the Indonesian coastal area, which is considered a nuisance and has no known high economic value. Cladophora can be used as an alternative source of cellulose because it has a high amount of cellulose. Cellulose could be extracted from Cladophora using 17.5% of NaOH at 100°C, 1 M sulfuric acid hydrolysis at 100°C, and bleaching with 5% hydrogen peroxide at 100°C. Nanocrystalline cellulose could be extracted by sulfuric acid hydrolysis at 100°C, with a concentration of 2 M, 3 M, and 5 M. The duration of the process for each concentration, varied for 5, 10, and 15 hours. X-Ray Diffraction (XRD), Particle Size Analyzer (PSA), Transmission Electron Microscopy (TEM), and Fourier Transform Infrared (FTIR) spectroscopy were used to characterize the resulting hydrolyzed product. The result showed that the increase in acid concentration and processing duration increased the crystallinity while decreased particle length and cellulose diameter. The highest crystallinity and smallest particle size were 96.36% and 189.2 nm, obtained by acid hydrolysis in 5 M sulfuric acid for 15 hours.**

Keywords— ***Algae***; ***Cladophora***; **crystallinity: nanocrystalline cellulose; particle size.**

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I. Introduction

The development and usage of nanomaterial-based or related products have drawn more attention to many scientists in these recent years. Attention to nanomaterial research is driven by unique properties that can emerge from the utilization of the material in this scale [1], [2]. Awareness of the current environmental problems also influences the development of more environmentally friendly nanomaterials. The utilization of biological resources, as an alternative to the synthetic nanomaterials, such as cellulose, is getting more attention since it is renewable and less expensive to produced [3]. Cellulose is one of the naturally occurred polymers which can be found in nature. Cellulose is also known as the most abundant, naturally occurred polymer. It is estimated that 1.5 $x10^{12}$ tons of cellulose are generated annually in nature [4]. This number virtually makes cellulose as an almost inexhaustible biological-based resource [5]. Cellulose is a linear polysaccharide in which the repeat unit consists of two glucose molecules linked by β-glycosidic bond [6]. The repeat unit of cellulose is also called as cellobiose. The linearity of the cellulose chain allows cellulose to form relatively regular structures or semicrystalline polymers, technically [7]. Cellulose is found in nature on the form of bundles of cellulose chains known as microfibrils, consisting of several cellulose chains with 10 – 30 nm in diameter and length, at several micrometers [8]. Cellulose microfibrils consist of two regions, which are regularly arranged crystalline regions and randomly arranged amorphous regions.

Nanocrystalline cellulose (NCC) is one of the products that could be derived from cellulose. NCC could be defined as a nano-sized crystalline part of the cellulose obtained by selectively hydrolyzing the chemically susceptible amorphous part of cellulose microfibrils [6]. Strong acid, such as sulfuric acid, is usually used for the hydrolysis process toward cellulose to obtain the NCC, with concentration as high as 62.4 – 64 wt% [7], [9], [10]. Some research on the NCC grows in recent years because of the nature of cellulose, which is abundant, renewable, and biodegradable, as a bio-based polymer to develop a "greener" nanomaterial [7], [9]. The NCC has a relatively low density (1.59 g/cm^3) and has experimental strength and modulus of 7.5 GPa and 150 GPa, respectively [11], [12]. It also has a low thermal expansion [5], biocompatibility, and low toxicity [6]. The NCC has modifiable surface chemistry to give additional functionalities due to abundant hydroxyl groups [12]. Extraordinary properties of NCC encourage its utilization in various fields. Some studies utilized NCC as reinforcement material to improve the composite's mechanical properties [7], [13]. The potential utilization of NCC in tissue engineering scaffolds, biosensors, drug delivery, and electronic devices [9], [12, [14].

The NCC can be extracted from various cellulose sources, which include plants, bacteria, and algae. In some previous research, the NCC is extracted from Eucalyptus globulus, rice straw [6], wood, cotton linters, cattail, red algae [9], doum (*Chamaerops humilis*) leaves [7], and cassava bagasse [10]. Purification of cellulose from these resources is the essential requirement before the NCC is extracted. The NCC characteristics vary in the crystallinity, molecular weight, morphology, and dimensions depending on the source, even extracted with the same preparation method [9].

Cladophora is a type of green algae with a fiber-like body structure, which can be found in various coastal areas around the world, including Indonesia. It is relatively abundant because of its rapid growth rate [15], but it almost has no economic value since it is regarded as a nuisance, especially in the algae cultivation region [16], [17]. The best-known application of this alga after their removal from the cultivation area is for cattle fodder.

In this research, Cladophora sp. was used as the source of cellulose to be further processed into the NCC, as Cladophora sp. algae have a relatively high cellulose content around 20 – 30% [18]. Despite its potential, utilization of Cladophora sp. as a source of cellulose or NCC is not widely studied. Sucaldito [12] used HBr to obtain the NCC from Cladophora rupestris algae cellulose, which was then utilized to reinforce starch-based plastics. Sulfuric acid is used in this research to obtain the NCC from purified Cladophora sp. cellulose. Through the result of this research, we aim to contribute to the variation development of both cellulose and the NCC sources and give an alternative utilization of Cladophora sp. algae to a more added-value application as a valuable source of cellulose.

II. Material and Method

A. Materials

The source of cellulose used in this research is Cladophora algae which obtained from algae cultivation in Krakal Beach, Gunung Kidul Regency, the Special Region of Yogyakarta, Indonesia. Sodium hydroxide flakes (98% purity) and Hydrogen peroxide, which were used in the cellulose extraction process from Cladophora algae, were procured from PT Asahimas Chemical and Central Kimia, Bandung. Meanwhile, analytical grade sulfuric acid (97% purity), which was used in the cellulose and NCC extraction process, was purchased from SMART LAB.

B. Instrumentation

XRD (BRUKER D8 Advance), PSA (Horiba SZ-100), and TEM (Hitachi HT770) characterization of the cellulose and NCC samples were conducted in Research Centre for Nanoscience and Nanotechnology (RCNN) Institut Teknologi Bandung. FTIR spectra were recorded using Shimadzu Prestige 21 in wavelength ranged 500 – 4000 cm^{-1} in the Chemistry Science Study Program, Faculty of Mathematical and Natural Science (FMIPA), Institut Teknologi Bandung.

C. Procedure

1) Cellulose Extraction from Cladophora sp.: The extraction of cellulose from Cladophora sp. was conducted by alkali treatment, acid hydrolysis, and bleaching successively. Alkali treatment was conducted using 17.5% of NaOH solution at 100°C. The alkali-treated algae were then filtered and washed to neutral pH with deionized water. After dried, acid hydrolysis by 1 M sulfuric acid at 100°C was conducted to the dried alkali-treated algae. Then, the washed and dried products obtained from this stage were bleached using 5% of hydrogen peroxide at 100°C to obtain the purified cellulose of Cladophora algae.

2) Preparation of the NCC from Cladophora sp. Cellulose: The extraction of the NCC from Cladophora sp. cellulose by hydrolysis was conducted using sulfuric acid at 100°C. The acid concentration and hydrolysis duration of the hydrolysis process are shown in Table 1. The obtained suspension was then diluted to 2% of concentration and dialyzed to deionized water until neutral pH. Later, the neutralized suspension was filtered and dried.

TABLE I
THE ACID CONCENTRATION AND HYDROLYSIS DURATION OF THE HYDROLYSIS PROCESS SIZES FOR PAPERS

Sample Code	Acid Concentration (M)	Hydrolysis Duration (Hour)
PH	Pre-hydrolysis sample	
2M15H	2	15
3M15H	3	15
5M5H	5	5
5M10H	5	10
5M15H	5	15

3) Sample Characterization: Morphology of the obtained NCC from various samples were observed using TEM to investigate the effect of the processing parameter on the morphology of the obtained NCC. The diameter of NCC was determined from the result of TEM images by using image processing software. Changes in the crystallinity of samples were determined from the diffraction pattern obtained by XRD characterization. The samples' crystallinity value was determined as the Crystallinity Index (CR. I) obtained through Segal's method [7]:

$$Cr.I = \frac{I_{002} - I_{am}}{I_{002}} \quad (1)$$

I_{002} is the intensity of the crystalline portion at 2Θ=22°, and I_{am} is the amorphous portion at 2Θ=18°. The particle size of the samples was obtained through PSA characterization. Both changes in crystallinity and particle size were characterized to analyze the effect of the variation of acid hydrolysis parameters on the properties of the obtained hydrolyzed products. Moreover, this characterization was to confirm these results so that the results could be categorized as the NCC. FTIR characterization was performed to obtain the value of Total Crystallinity Index (TCI). TCI value is utilized to confirm the change of crystallinity resulted from XRD characterization. Total Crystallinity Index from FTIR was calculated using equation 2 [19].

$$TCI = \frac{A_{1375}}{A_{2900}} \tag{2}$$

In equation (2), A_{1375} and A_{2900} are referred to as the absorption values in wavelength of 1375 cm^{-1} and 2900 cm^{-1}, respectively.

III. Results and Discussion

A. The Effect of Sulfuric Acid Concentration to the Crystallinity and Particle Size of Hydrolyzed Product

The hydrolysis of cellulose obtained from Cladophora sp. was conducted using sulfuric acid in various concentrations. Pre-hydrolysis samples (PH) and hydrolysis products from 2M (2M15H), 3M (3M15H), and 5M (5M15H) sulfuric acid concentration with each conducted for 15 hours were characterized to evaluate the effect of acid concentration to the properties of resulted in NCC.

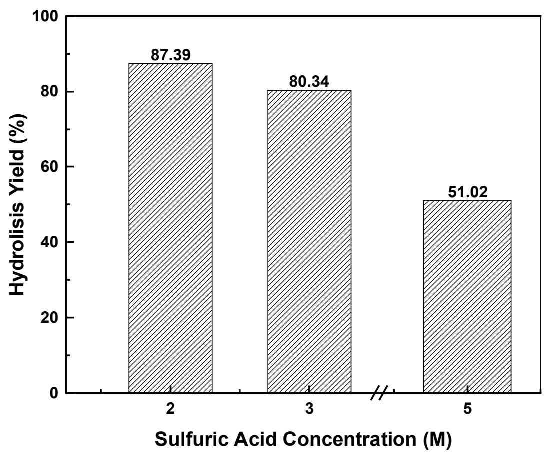


Fig. 1 Distribution of yield after hydrolysis as the effect of acid concentration

The yield of the post-hydrolysis product was obtained by comparing the weighted sample of before and after processing. The result showed that the yield of post-hydrolysis products decreased along with the increase of sulfuric acid concentration, as presented in Figure 1. The higher acid concentration provided more hydrolysis reaction to occur and allowed more acid susceptible regions in cellulose microfibrils to be removed and resulting in a lower yield of the product. The removal of the acid susceptible region also allows a smaller particle to be produced, which could have higher the probability of the particles passing through the filter, which then contributes to a lower yield of cellulose trapped in the filter papers.

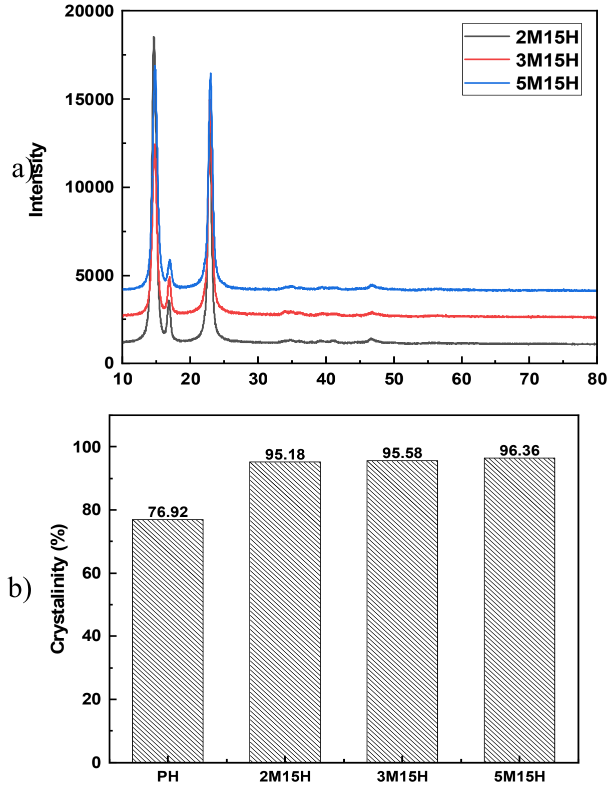
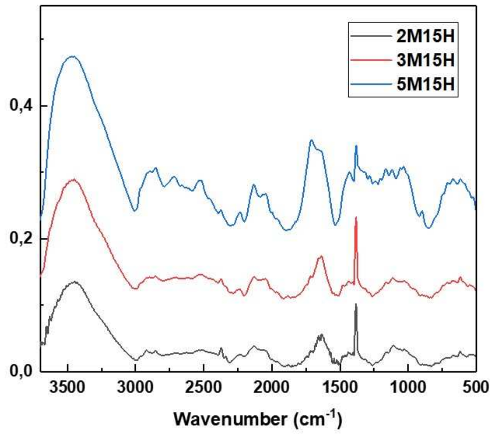


Fig. 2 XRD result on NCC obtained from various acid concentration a) Diffraction pattern of samples from XRD characterization, b) Crystallinity of samples calculated from XRD.

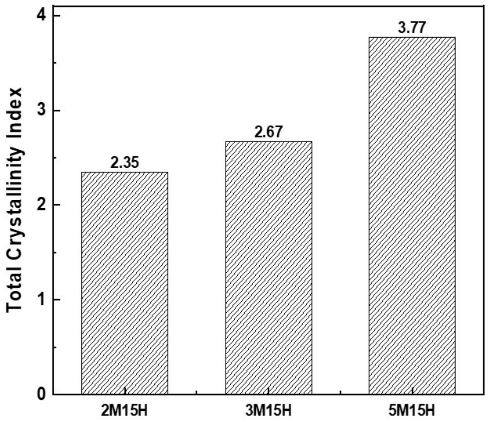
The result from XRD characterization showed that the increase of sulfuric acid concentration increases the crystallinity of the obtained hydrolyzed product, as presented in Figure 2b. The crystallinity of the obtained NCC was increased from 76.92% before hydrolysis to 96.36% in the highest sulfuric acid concentration of 5 M.

Spectra from FTIR characterization of 2M15H, 3M15H, and 5M15H samples are shown in Figure 3a. TCI value calculated from resulted FTIR spectra shows an increase of TCI value as an increase of sulfuric acid concentration (Figure 3b). A similar trend of TCI value to the XRD characterization result confirms the increased crystallinity of NCC as an increase of sulfuric acid concentration.

The increase of crystallinity was caused by the removal of the amorphous part of cellulose microfibril with acid hydrolysis. This was possible because the amorphous part of cellulose could be easily penetrated by acid molecules, which then reacted to the glycosidic bond in the cellulose chain. Further hydrolysis would convert the repeat units into glucose molecules. The removal of the amorphous part then would leave a more resistant crystalline part of cellulose intact; thus, it increased the regularity of the system, as illustrated in Figure 4. The higher concentration of sulfuric acid provides more hydrogen to the system to allow more hydrolysis reaction to occur in the amorphous part of cellulose.



(a)



(b)

Fig. 3 a) FTIR Spectra of the obtained samples, b) Total Crystallinity Index Value by the increase of Acid Concentration

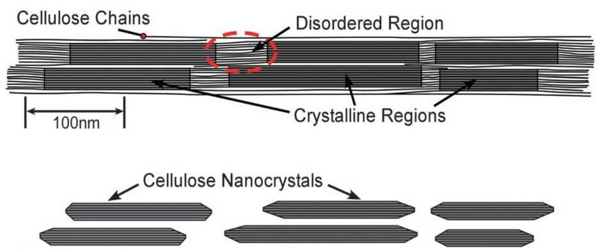


Fig. 4 Illustration of amorphous part removal of cellulose microfibrils [8]

Particle length of the samples was investigated using PSA characterization. As presented in Figure 5, the result showed that the increase of sulfuric acid concentration would decrease the particle size of the obtained hydrolyzed product. The smallest NCC length obtained was 189.2 nm which resulting from hydrolysis with 5M sulfuric acid

The decrease of the particle size is caused by the removal of the cellulose microfibril amorphous part. The removal process involves breaking of β-1,4 glycosidic bond and resulting in smaller cellulose particles after hydrolysis. Higher sulfuric acid concentration allowed a more amorphous part in cellulose microfibrils to be removed, resulting in smaller particles.

The red dashed line in Figure 5 represent the average particle size of NCC obtained in previous studies which are up to 300 nm [7],[9],[10],[12],[20], which means the result below this line is having an appropriate particle size of an NCC.

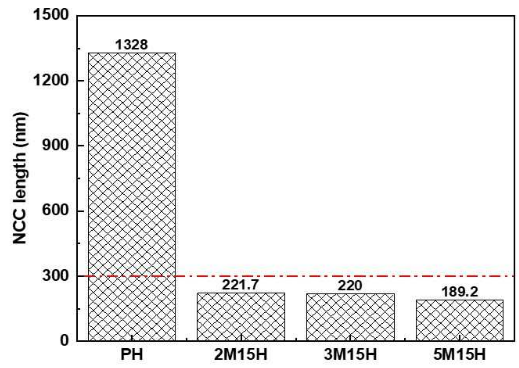
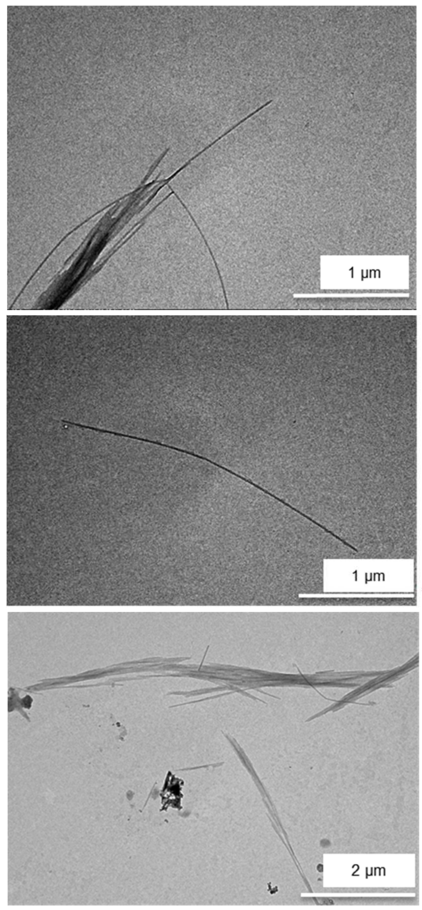


Fig. 5 Distribution of particle length of samples from PSA characterization as the effect of acid concentration, red dashed line is average particle NCC length from references

The morphological investigation of the NCC obtained from hydrolysis with various concentration were conducted using TEM characterization. The result showed that all samples of the obtained NCC particles have rod-like or whisker-like morphology (Figure 6), which is the typical morphology of NCC mentioned in previous researches. Rod or whisker-like morphology of NCC is caused by a parallelly aligned cellulose chain of the crystalline region left after the removal of the amorphous part of cellulose microfibril during the acid hydrolysis [8]. These results also confirmed the removal of the amorphous part of cellulose microfibril is the effect of the conducted hydrolysis process.



a
b
c

Fig. 6 TEM micrograph of the obtained NCC from various acid hydrolysis duration a) 5M5H; b) 5M10H; c) 5M15H

The diameter of the obtained NCC was measured by image analysis on the TEM result. The results show that the diameter of NCC tends to decrease as the increase of acid concentration, as shown in Figure 7.

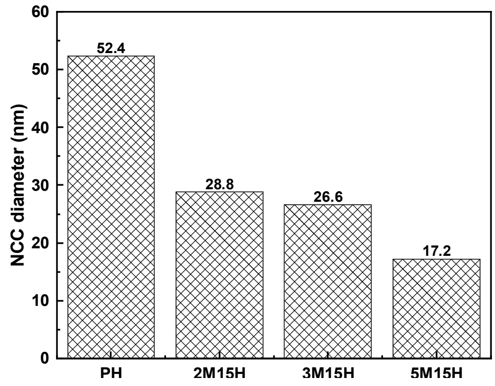


Fig. 7 Variation of the diameter of NCC as the effect of acid concentration measured from TEM micrograph

The result of the characterization of NCC in various sulfuric acid concentrations shows that higher acid concentration leads to higher crystallinity and smaller NCC size despite the lower yield. The NCC with the highest crystallinity and smallest size (length and diameter) are obtained in 5M of sulfuric acid concentration.

B. The Effect of Hydrolysis Duration to the Crystallinity and Particle Size of Hydrolyzed Product

The significance of hydrolysis duration to the properties of the resulting NCC is evaluated using the highest sulfuric acid concentration of 5 M with duration varied in 5 hours (5M5H), 10 hours (5M10H), and 15 hours (5M15H). The yield of product obtained after hydrolysis in various duration are showed to decrease along with the increase of duration, as showed in Figure 8. The longer duration allows a higher chance of hydrolytic reaction of cellulose molecules in the more amorphous region, thus resulting in a smaller amount of cellulose left after the process.

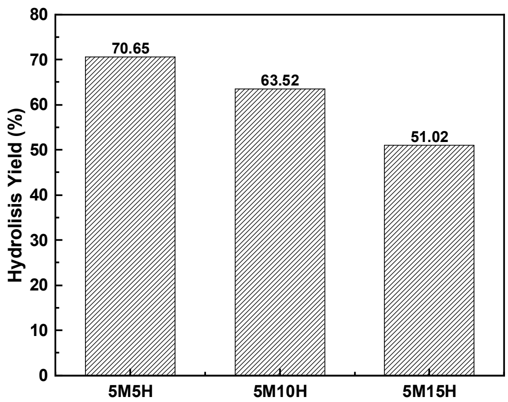


Fig. 8 Distribution of yield after hydrolysis as the effect of acid concentration

Prolonging hydrolysis duration is found to increase the crystallinity percentage, as shown in Figure 9. The crystallinity percentage of samples increase from 76.92% as PH up to 96.46% in 15 hours hydrolysis. The total crystallinity index value calculated from the FTIR result also shows similar trends, as shown in Figure 10. The crystallinity index obtained is as high as 4.07 after 15 hours of hydrolysis.

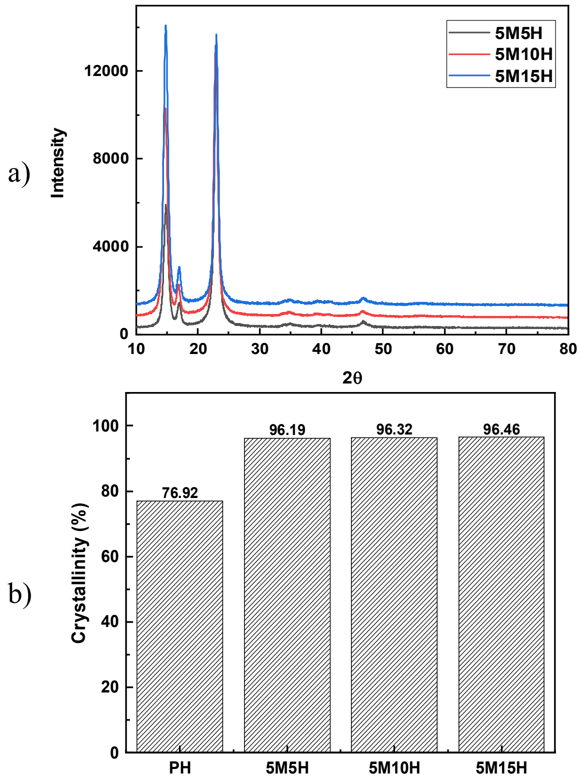


Fig. 9 XRD result on NCC obtained from various hydrolysis duration a) Diffraction pattern of samples from XRD characterization, b) Crystallinity of samples calculated from XRD

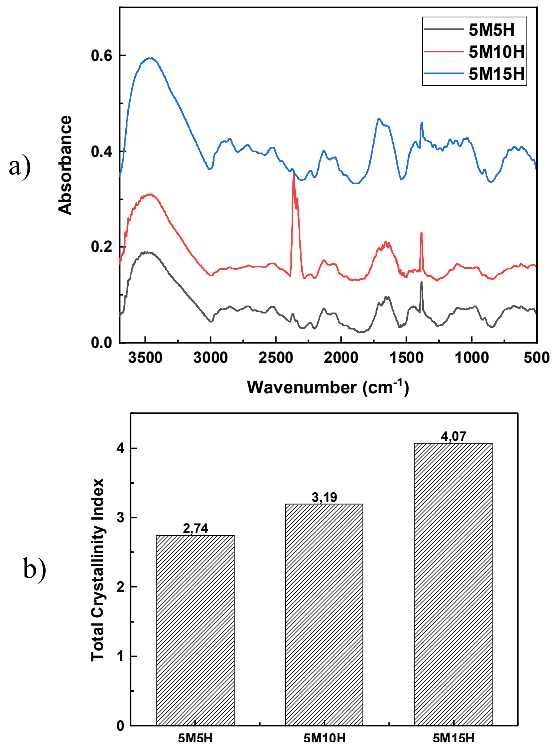


Fig. 10 a) FTIR Spectra of the obtained samples, b) Total Crystallinity Index Value by the increase of hydrolysis duration.

The increase of crystallinity is caused by a longer time available for acid molecules to react and remove more glycosidic bonds in the amorphous region of cellulose. These phenomena are also driving the decrease of length of the obtained NCC as evaluated using PSA shown in Figure 11 as more cellulose in the susceptible regions in the ends of whiskers are removed and resulting in shorter whiskers. Evaluation of the NCC's diameter through the micrograph showed that the longer hydrolysis duration resulted in, the thinner diameter of NCC, as shown in Figure 12, which was caused by more cellulose in whisker surface removed during the process.

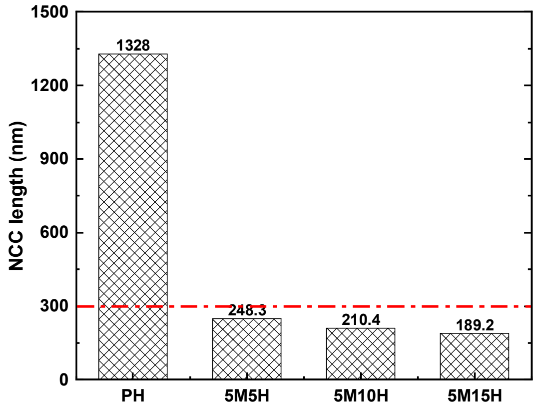


Fig. 11 Distribution of particle length of samples from PSA characterization as the effect of hydrolysis duration, red dashed line is average particle NCC length from the reference

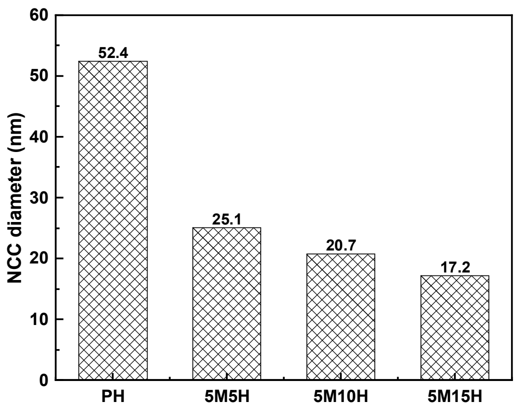


Fig. 12 Variation of the diameter of NCC as the effect of hydrolysis duration measured from TEM micrograph

C. Influence of Processing Parameters to properties of Resulted NCC

The evaluation of sulfuric acid concentration and hydrolysis duration showing a similar trend as both causing the yield decreased, increase of crystallinity, and decreased length and diameter of NCC whisker. Based on characterization results, sulfuric acid concentration shows a more significant effect on NCC characteristics and properties, as summarized in Table 2.

The combination of the highest acid concentration of 5M and the most prolonged duration of 15 hours resulted in the smallest diameter obtained, which is around 17.2 nm with length and crystallinity 189,2 nm and 96,36%, respectively. The obtained NCC whisker diameter is the following mentioned in previous research, which is around 3 – 30 nm [6]–[8]. The reduction of the NCC whisker diameter along with the increase of the hydrolysis acid concentration or duration is caused by the hydrolysis of the cellulose on the outermost region of the cellulose whisker, which readily available to contact with acid, thus more prone to hydrolysis. Higher concentration or longer duration causing more of these outermost celluloses to be hydrolyzed and resulting in the smaller diameter of NCC whiskers.

Based on XRD and PSA characterization result, 2M15H; 3M15H; 5M5H; 5M10H; and 5M15H meet the condition to be categorized as NCC since the crystallinity percentage and particle size of the samples above 80% and below 300 nm respectively [7],[9],[10],[12],[20].

TABLE II
SUMMARY OF THE RESULT

	2M 15H	**3M 15H**	**5M 5H**	**5M 10H**	**5M 15H**
Yield (%)	87.35	80.34	70.65	63.52	51.02
Crystallinity (%)	95.18	95.48	96.16	96.32	96.36
Particle Size (length) (mm)	220	221.7	248.3	210.4	189.2
Diameter (nm)	28.8	26.6	25.1	20.7	17.2

IV. CONCLUSION

Nanocrystalline cellulose was obtained from Cladophora algae cellulose by hydrolysis with sulfuric acid in elevated temperatures. The crystallinity of the acid hydrolyzed cellulose increased along with the increase of sulfuric acid concentration, while the particle size decreased with the increase of sulfuric acid concentration and longer hydrolysis time. Cladophora cellulose hydrolyzed with 2 M and 3 M concentration of sulfuric acid for 15 hours, and 5 M concentration for 5, 10, and 15 hours could be categorized as the NCC. The highest crystallinity, smallest particle length, and diameter obtained were 96.36%, 189.62 nm, and 17.2 nm, respectively, which resulted from Cladophora cellulose hydrolyzed in 5 M sulfuric acid for 15 hours.

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