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THE EFFECT OF NaOH CONCENTRATION IN DELIGNIFICATION PROCESS ON MICROCRYSTALLINE CELLULOSE FROM GREEN ALGAE (*Cladophora sp.*) AS THE RENEWABLE MARINE PRODUCT

PENGARUH KONSENTRASI NaOH PADA PROSES DELIGNIFIKASI TERHADAP SELULOSA MIKROKRISTAL DARI ALGA HIJAU (*Cladophora sp.*) SEBAGAI PRODUK BAHARI TERBARUKAN

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ABSTRACT

Research on marine natural resources as an excipient material of pharmaceutical product is still rare. One of the marine products is the green algae, Cladophora sp. High cellulose content causes Cladophora sp. which can be used as an alternative material of microcrystalline cellulose (SM). There are two stages to produce SM, namely delignification and hydrolysis. Delignification is the process of removing the lignin of complex compounds. The delignification process is carried out chemically in alkaline situation using a NaOH solution which dissolves lignin, carbohydrates, organic acids, and resins so that cellulose is released from its bonds. This is important because the presence of lignin may inhibit acid penetration prior to hydrolysis. Therefore, the purpose of this study is to investigate the effect of delignification by using NaOH solution at various concentrations (2, 4, and 6%) to cellulose content and physical character of microcrystalline cellulose from Cladophora sp. (SMC). In the hydrolysis process, 5% HCl solution was used. SMCs of various concentrations of NaOH were observed and the cellulose levels included alpha, beta and gamma levels. While the physical character observation is done on Scanning Electron Microscopy (SEM) test. Based on the cellulose content, the higher the concentration of NaOH used, the higher the alpha cellulose will increase. The opposite result occurs on the measurement of beta and gamma cellulose. Based on SEM test, it appears that there is no effect of increasing NaOH concentration on physical character of SMC.
Keywords: *Cladophora sp., delignification, microcrystalline cellulose, NaOH, SEM*

ABSTRAK

Penelitian mengenai pemanfaatan sumber daya alam bahari sebagai bahan eksipien sediaan farmasi masih jarang. Salah satu produk bahari adalah alga hijau Cladophora sp. Kandungan selulosa yang cukup tinggi menyebabkan Cladophora sp. dapat dijadikan sebagai alternatif bahan baku pembuatan selulosa mikrokristal (SM). Pembuatan SM dilakukan melalui dua tahapan yaitu proses delignifikasi dan hidrolisis. Delignifikasi merupakan proses penghilangan struktur lignin dari suatu senyawa kompleks. Proses delignifikasi dilakukan secara kimia dalam keadaan alkali menggunakan larutan NaOH yang berfungsi melarutkan lignin, karbohidrat, asam organik, dan resin sehingga selulosa terlepas dari ikatannya. Hal ini penting dilakukan karena adanya lignin dapat menghambat penetrasi asam sebelum dilakukan proses hidrolisis. Berdasarkan hal tersebut, tujuan penelitian ini adalah untuk mengetahui pengaruh penggunaan larutan NaOH pada berbagai konsentrasi (2; 4; dan 6%) terhadap kadar selulosa serta karakter fisik selulosa mikrokristal dari Cladophora sp. (SMC). Dalam proses hidrolisis, digunakan larutan HCl 5%. SMC dari berbagai konsentrasi NaOH dilakukan pengamatan kadar selulosa yang meliputi kadar alfa, beta dan gamma. Sedangkan pengamatan karakter fisik dilakukan berdasarkan pengujian Scanning Electron Microscopy (SEM). Ditinjau dari kadar selulosa, semakin tinggi konsentrasi NaOH yang digunakan, kadar alfa semakin meningkat. Hasil sebaliknya terjadi pada pengukuran kadar beta dan gamma selulosa. Berdasarkan pengamatan melalui uji SEM tampak bahwa tidak ada perbedaan karakteristik fisik seiring dengan peningkatan konsentrasi NaOH yang digunakan.
Kata kunci: *Cladophora sp., delignifikasi, selulosa mikrokristal, NaOH, SEM*

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INTRODUCTION

One example of Green algae, *Cladophora* sp., has a high growth rate. Uncontrolled growth can lead to "algae blooms" which can cause water ecosystem pollution characterized by changes in water color and produce unpleasant odor (Sze, 1993).

Mihranyan (2011) states that *Cladophora* sp. contains high cellulose. Cellulose is the main raw material of microcrystalline cellulose (SM). The dissolution of cellulose in strong alkali will produce alpha cellulose. The higher level of alpha cellulose will cause the higher purity level of microcrystalline cellulose that is produced (Gunam et al., 2010). The utilization of *Cladophora* sp. as the main raw source of cellulose has the advantage because this type of algae has not been widely used.

To be able to dissolve cellulose, lignin contained in the plant cell wall should be destroyed first. This process is called delignification. The current research on delignification process was carried out on rice straws. Prasetia et al. (2015), state that the use of NaOH solution in a delignification process can dissolve cellulose in to produce alpha cellulose. In the Mansur variety rice straws, the Balinese local rice, the use of 7.5% of NaOH is able to purify cellulose up to 82.96%. 15% of NaOH is required for rice straws IR-64 variety until 98.0% of alpha cellulose is obtained (Prasetia and Dewantara Putra, 2015). Different result is obtained by Dewantara Putra et al., 2016, where 5% of NaOH is required for the delignification process of Balinese local red rice straw. The different characteristics of plant cell wall will cause different solution of NaOH in the delignification process.

Based on this research, the researcher would like to examine the influence of using NaOH solution on various concentrations (2; 4; and 6 %) to the cellulose content and the physical characteristics of microcrystalline cellulose from *Cladophora* sp. (SMC).

METHODS

Material

Green algae of *Cladophora* sp. is obtained in Jimbaran coastal area in Badung Regency. The materials are NaOH (Pharmaceutical Grade, Bratachem), HCl (Pharmaceutical Grade, Bratachem), indicator of Ferroin (Pharmaceutical Grade, PT. Nusa Indah), potassium dichromate (Pharmaceutical Grade, Merck), ferrous ammonium sulfate (Pharmaceutical Grade, Merck) and distilled water (Pharmaceutical Grade, Waterone).

Equipment

The equipment used includes an analytical balance (*Adam® AFP-360L*), a desiccator, pH meter (*Oakton pH 510 series*), glass tools, mesh 60, a water bath (Memmert), an oven (Binder), SEM test equipment (JEOL-2200).

Sample Collection

The sample that is used is Cladophora sp. algae obtained in the coastal area in Jimbaran, Badung regency, Bali.

Plant Identification

The determination process was conducted in the Laboratory of the Department of Pharmacy Biology, Gadjah Mada University, Yogyakarta.

Sample Processing

The sample is sorted then baked in the oven at 60°C for 24 hours.

Delignification Process

One gram of sample is dissolved in 12 ml of NaOH into various concentrations of NaOH (2; 4; and 6%) at 60°C for 24 hours and then the result is filtered. The pulp is then separated and washed with distilled water until a neutral pH is obtained. Afterwards, the desiccation is conducted at room temperature for 24 hours (Mihranyan, 2011).

Hydrolysis Process

One gram of each formula is then soaked in 20 ml of 5% of HCL at 92°C. After that, let the pulp settle overnight at the room temperature. The pulp obtained is then filtered and washed with distilled water until the neutral Ph is reached. After the desiccation process is conducted at 60°C for 12 hours, the sample is sieved with a 60mesh sieve (Mihranyan, 2011).

Microcrystalline Cellulose Evaluation

SMC from various concentrations of NaOH is observed for identifying the cellulose content which includes alpha, beta and gamma content based on the method listed in the Indonesian National Standard (SNI) 2009. Meanwhile, the physical character observation is conducted based on the Scanning Electron Microscopy (SEM) test.

RESULT AND DISCUSSION

Cellulose Content Test

Cellulose content test was conducted by using the titration method which is marked by the formation of purple color (SNI, 2009). Alpha-cellulose is a long-chain cellulose with 600-1500 degree of polymerization and insoluble in 17.5% of NaOH solution or strong alkaline solution. Alpha-cellulose is used to determine the level of cellulose purity. Beta-cellulose is a short-chain cellulose with 15-90 degree of polymerization, can be dissolved in 17.5% of NaOH or strong base, can precipitate when it neutralized. Gamma-cellulose is a short-chain cellulose with polymerization degree less than 15, can be dissolved in 17.5% of NaOH or strong base and the main content is hemicellulose (Sumada et al., 2011). Gamma-cellulose test aims to identify hemicellulose contained in microcrystalline cellulose. The content of cellulose can be seen in the Table 2.

Figure 1 shows that alpha cellulose obtained is significantly increasing with the increasing NaOH that is used in the delignification process. However, the content of beta and gamma-cellulose produced is decreasing. The highest content of alpha-cellulose is produced in SMC-6. Conversely, with the same formula, the lowest price of beta and gamma-cellulose is obtained when compared to other SMCs.

Based on these data, an increase in NaOH concentration can increase alpha-cellulose content on microcrystalline cellulose produced. The higher concentration used, the higher the ability to damage lignin and cellulose. In addition, it also can cause the cellulose to be easily hydrolyzed so that the content of alpha-cellulose is getting higher (Gunam et al., 2010). The use of strong alkaline, NaOH, will be able to break the structure of hemicellulose and dissolve it. The more NaOH is used, the more hemicellulose content can be dissolved so that alpha-cellulose content increases.

Scanning Electron Microscopy (SEM) Test

The analysis of physical characteristics and surface morphology of a sample can be observed by using Scanning Electron Microscopy (SEM) method. The result can be seen in Figure 2. SEM from SMC-2, SMC-4 and SMC-6 shows that the fibrils intertwined with a smooth surface. It indicates that the morphologies produced are similar (Camacho et al., 2011). Therefore, in terms of physical characteristics, the increase in NaOH concentration has no significant effect.

Table I. NaOH Variations on Delignification Process in Making Microcrystalline Cellulose *Cladophora* sp.

Formula	NaOH Concentration (%)
SMC-2	2
SMC-4	4
SMC-6	6

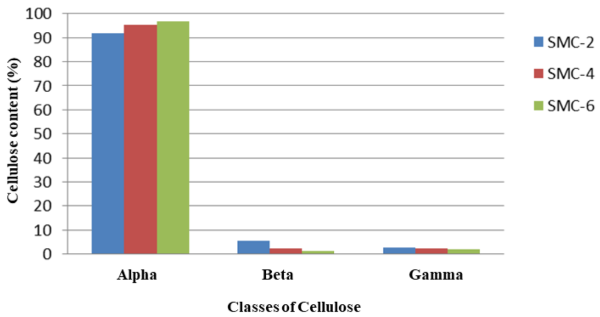

Figure 1. Graph of cellulose content in various SMCs

Table II. Observation Result of Cellulose Content in Various SMC Formula

Formula	Cellulose Content (%)		
	Alpha	Beta	Gamma
SMC-2	91.75 ± 0.55	5.60 ± 0.49	2.65 ± 0.06
SMC-4	95.41 ± 0.59	2.33 ± 0.56	2.25 ± 0.08
SMC-6	96.68 ± 0.36	1.32 ± 0.24	2.00 ± 0.19

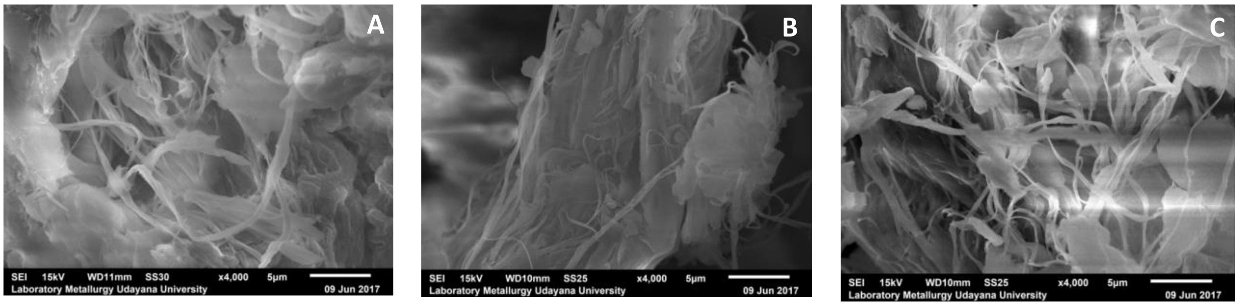

Figure 2. The SEM test result with 4000 times enlargement with various SMCs (A=SMC-2; B=SMC-4; and C=SMC-6)

CONCLUSION

Various concentrations of NaOH in the delignification process on *Cladophora* sp. affect the cellulose content that is produced but it does not affect the physical characteristic formed. This finding is based on the result of cellulose content test and SEM test.

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