International Journal of Research and Review
Vol.7; Issue: 7; July 2020
Website: www.ijrrjournal.com
Research Paper *E-ISSN: 2349-9788; P-ISSN: 2454-2237*

Manufacture and Characterization of Microcrystalline Cellulose of Water Hyacinth (*Eichhornia crassipes* (Mart.) Solms) by Enzymatic Process

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ABSTRACT

Research on manufacture and characterization of microcrystalline cellulose from water hyacinth (*Eichhornia crassipes* (Mart.) Solms) has been carried out enzymatically using cellulase enzymes produced from *Trichoderma Viride* fungi. The water hyacinth leaves are cut small and washed and then dried. The dried water hyacinth leaves were mashed and the water hyacinth powder was obtained then macerated with 2 liters of ethanol for 24 hours. Then maceration powder was extracted with 17.5% NaOH for 5 hours, then filtered and washed with distilled water. After it was dried in the oven and then obtained alpha cellulose. The alpha cellulose obtained was then hydrolyzed enzymatically using the cellulase enzyme so that the hydrolysis results were microcrystalline cellulose compounds. The resulting MCC was tested for FTIR, SEM, DSC. Based on the results of the study obtained the results of the FTIR test obtained functional groups O-H, C-H, C = C-H. Same with the comparison of Vivacel PH 102. The SEM results are looking at the surface morphology of a sample with a magnification of 1000 obtained a crystal form. The results of the Vivacel DSC thermogram PH 102 are melting points (85.406^{o}C). MCC that was made was obtained the average melting point (93.196^{o}C). The results showed that water hyacinth has the potential to show characterization similar to the reference microcrystalline cellulose (Vivacel PH 102).

Keywords: Microcrystalline Cellulose, Water Hyacinth, Cellulase Enzyme.

INTRODUCTION

Microcrystalline cellulose (MCC) is widely used in printing by the method of direct compression tablet as a dry binder, tablet disintegrant, absorbents, fillers, lubricants, and anti-adherent. MCC has been widely used as additives in direct compression as flowability, compatibility, and compressibility good. Moreover, in the pharmaceutical industry, MCC is also an important ingredient in the cosmetics industry, food, and other industries (Ngozi, et al., 2014; Haque, et al., 2015).

MCC was first introduced in the early 1960s as an excipient material that is binder, filler and a destroyer in the manufacture of tablets by direct printing, resulting in tablets with good hardness, not brittle and have a short disintegration time and can improve the flow properties of the granules (Bhimte & Tayade, 2007).

Halim (2002) reported the MCC in Indonesia is all derived from abroad so that Indonesia began thinking MCC production. Because the price of MCC that comes from abroad is expensive, is also a reason to find the source of the MCC, one source of MCC was derived from agricultural waste.

Hydrolysis of cellulose can be chemically and enzymatically, chemical hydrolysis can be performed using acid, which is a strong acid of low concentration and high concentration of weak acids (Octavian, 2013).

Enzymatic hydrolysis was carried out using the cellulase enzyme. Cellulase catalyzes the hydrolysis of cellulose with three types, namely: *endoglucanase, cellobiohydrolase, and β- glucosidase* (Li, et al., 2009).

This study aims to obtain MCC of water hyacinth (*Eichhornia crassipes* (Mart.) Solms) by enzymatic hydrolysis method using cellulase enzymes and to determine the characterization of MCC of water hyacinth by hydrolysis method by enzymatically using cellulase enzymes in accordance with Vivacel PH 102 standard®.

EXPERIMENTAL METHODS

Instruments and Materials

The instruments were used in this research are autoclave, Fourier Transforms Infrared (FTIR), Scanning Electron Microscopys (SEM) (Phenom world), Differential Scanning Calorimetry (DSC) (Setaram, 131-Evo), centrifuges, laminar air flow, an analytical balance (Precisa), blender (Philips), a water bath, an oven, a pH meter (Hanna Instruments), a sieve No. 40, light spirits, a loopful, flask, test tube, stirring rod, a measuring cup, beaker glass, pipette, scissors, paper filter, porcelain, cotton, and sterile gauze.

Materials used in this study were water hyacinth leaves, Vivacel PH 102® distilled water, ethanol (C_2H_5OH) (Brataco®), hydrogen peroxide (H_2O_2), sodium hydroxide (NaOH) (Brataco®), Trichoderma viride, wheat bran, alcohol 70%, zinc chloride ($ZnCl_2$) (Merck®), potassium iodide (KI), iodine (I) (Merck®), monopotassium phosphate (KH_2PO_4) (Merck®), magnesium sulfate ($MgSO_4$) (Merck®), calcium nitrate ($CaNO_3$) (Merck®).

Research procedure

Sample Determination

Identification of water hyacinth plants was carried out at the Herbarium of the Department of Biology, Faculty of Mathematics and Natural Sciences, Andalas University, Padang, Indonesia. Samples were taken for identification is the entire plant.

Sample Preparation

Water hyacinth leaves were taken as 1 kg of area Jl. Gajah Mada, North Padang, Padang, Indonesia in sorting wet with the aim of separating dirt and other foreign materials such as soil and grass, then do washing to remove soil and impurities the other attached to the leaves of water hyacinth, with clean running water. Then a chopping was done to simplify the drying process. The leaves of the water hyacinth were cut into small pieces ± 3 cm after which they were air-dried at room temperature. Water hyacinth leaves that has been dried subsequently comminuted in a blender and then sieved with a sieve 40 to obtain a fine powder. After that the water hyacinth powder was weighed and processed into alpha-cellulose by removing the lignin it contains (Halim, 2002). The powder was water hyacinth weighed as much as 500 grams, then macerated with 2 liters of ethanol for 24 hours at room temperature.

Maceration aims to eliminate the lignin content of the water hyacinth. After the powder results of maceration soaked with hot water which aims to remove the remaining ethanol and then dried.

Cellulase Enzyme Production

Hatchery preparation

The hatchery medium used in this study was Potato Dextrose Agar (PDA). PDA powder weighed as much as 3.9 grams, was dissolved in 100 ml of distilled water, then heated on a heater, and stir until completely dissolved and colored clear, then closed with cotton stoppers wrapped with sterile gauze. Furthermore sterilized by autoclaving at 121^oC temperature, the pressure of 15 lbs, for 15 minutes (Gupta, 2015).

Fungi Trichoderma Viride Isolates Rejuvenation

The isolated and purified Trichoderma viride fungi were transferred with the help of an ose needle onto the PDA

media. The execution was done aseptically in a laminar air-flow, then incubated at 28ºC for 7-10 days, then stored at 4°C when the spores has been formed (Li, et al., 2009).

Cellulase Enzyme Production

Enzyme production was carried out in a 250 ml Erlenmeyer containing mineral salt media (KH_2PO_4 0.05%, $CaNO_3$ 0.05% and $MgSO_4$ 0.05%), and 5 grams of wheat bran. The ratio of solids and liquids is 1:3. Erlenmeyer closed and sterilized at a temperature of 121^{o}C for 20 minutes. Once cool, 0.5 ml of spore suspension was inoculated into the fermentation medium and incubated for 3 days (Gupta, 2015).

Enzyme Extraction

The enzyme was extracted by adding 25 ml of 0.05M phosphate buffer to the Erlenmeyer. The mixture was stirred for 45 minutes at a speed of 150 rpm and filtered with filter paper. The extract was centrifuged for 10 minutes at a speed of 10,000 rpm. The supernatant formed is a source of cellulase enzymes (Gupta, 2015).

Starch Test

A total of 10 mg of powder was added to 90 mL of distilled water and heated for 15 minutes. Then filtered while hot. Added to the filtrate 0.1 mL of 0.05 M iodine, shapeless blue (British Pharmacopoeia, 2002).

Analysis of Fourier Transform Infrared spectroscopy (FT-IR)

MCC Infrared spectrum of water hyacinth compared with 102 Vivacel using FTIR. Tests were carried out on samples with ATR technique (attenuated total Reflectant). Plat form horizontal ATR crystal plate prism shape (ZnSe) mounted on a FT-IR instrument then measuring the background. Samples were placed on the surface of the plate and measured (Watson, 2009).

Analysis of Scanning Electron Microscopy (SEM)

Powder samples were placed in an aluminum holder with a thickness of 10 mm. samples were then observed a wide range of magnification SEM tool. Kept to a minimum voltage at 20 kV 12 mA current. This analysis will show the morphological shape of the particles of the compound MCC (Goldstein, et al., 1992).

Analysis of Differential Scanning Calorimetry (DSC)

DSC analysis was performed on MCC samples made enzymatically of water hyacinth. MCC accurately weighed 5 mg in 30 mL aluminum plate with a perforated lid. DSC instrument 45-400^{o}C with programmed over a temperature range of heating speed of 10°C min, the nitrogen flow rate of 20 mL/min. The endothermic and exothermic process was recorded on a monitor (Vora & Shah, 2015).

RESULTS AND DISCUSSION

Research has been conducted on the manufacture and characterization of MCC of water enzymatically results of characterization of an organoleptic fine powder, white, odorless and tasteless and for the identification of colors obtained is blue-violet and drying shrinkage loss should not be more than 6% and the solubility in water should not exceed 0.25% and 5-7.5 pH test and blue color test starch is not formed in accordance with the basic standards of Vivacel pH 102.

Lignin is one of the main components of water hyacinth in addition to cellulose and hemicellulose. Lignin liberation of complex compounds is one of the important pre-treatment carried out prior to the hydrolysis process. This process is important before the hydrolysis of cellulolytic material because lignin is a sturdy wall attached to cellulose and hemicellulose fibers so that a plant becomes hard and can stand firm. Lignin can inhibit the penetration of the acid or enzyme hydrolysis takes place (Gunam, et al., 2010).

The process of making MCC, there is a delignification process. It aims to damage the structure of lignin so that the cellulose can be extracted. Delignification lignin is a process of liberation of a complex compound. Delignification can be done using a base, one of them with a solution of NaOH (Herawan, et al., 2013). The powder

was water resulting from maceration weighed as much as 200 grams and in the delignification with NaOH solution 17.5% w/v by heating for 5 hours at a temperature above 90^{o}C water bath. Aims to eliminate lignin of water hyacinth powder. The powder was water hyacinth delignification results with 17.5% NaOH filtered and washed with distilled water, then dried in the oven at 60°C so that alfacellulose was obtained.

MCC manufacture of water hyacinth using enzymatic hydrolysis method using three concentrations of cellulase enzymes which can result *Trichoderma viride* fungal isolation, the concentration of enzyme used is 0.4 ml (MCC 1), 1.2 ml (MCC 2), and 2 ml (MCC 3), respectively.

Furthermore, the characterization was done by organoleptic tests, drying shrinkage, identification, solubility in water, the pH, the absence of a starch test, FT-IR, SEM, and DSC. The results showed that the MCC produced to meet the requirements of the British Pharmacopeia (2009). The results obtained showed similarities to one another that meets the requirements.

Analysis of Fourier Transform Infrared spectroscopy (FT-IR)

MCC was generated in this study compared with Avicel PH 102 using FT-IR spectroscopy analysis. The infra-red spectrum showed that the resulting spectrum has transmission peaks located at the wavenumbers generated close together. The MCC spectrum does not look very different from each other.

Infrared spectroscopic analysis was conducted to identify the functional groups. Each absorption band at a particular wavenumber describes the presence of a specific functional group.

Tabel 1. Type bond FTIR results of MCC

Wavenumbers (cm^{-1})	Type bond
3448.14	O-H, N-H
2360.80	-C≡C-, C≡N bending
1735.11	C=C (acids, aldehi, ketones, amides, esters, anhydrides) bending
1420.05	C-H bending
873.96	C=C-H, Ar-H bending
668.88	C=C-H, Ar-H bending

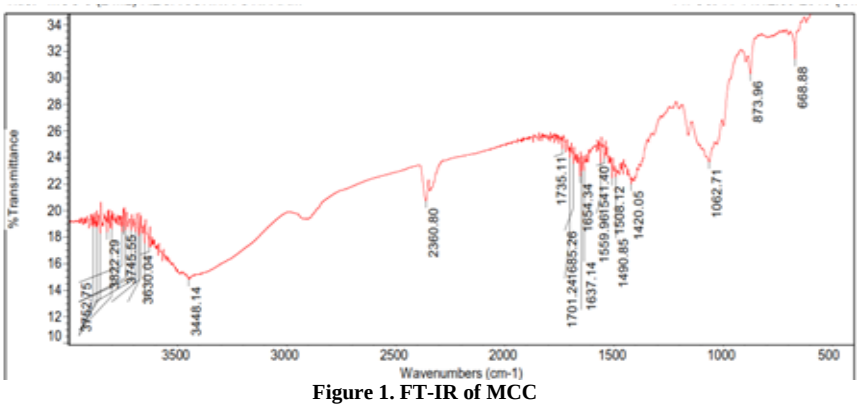

Figure 1. FT-IR of MCC

The results of the MCC 3 spectrum test (Figure 1, Table 1) show that there are OH, NH functional groups in the wavenumber 3448.14 cm^{-1}. The strain group functions –C≡C-, C≡, N wave number 2360.80 cm^{-1}, and at wave number 1735.11 cm^{-1} existence stretch functional group C=O (acids, aldehydes, ketones, amides, ester, anhydride) and the wave number 1420.05 CH bonds are the types of bending and 873.96 cm^{-1} wave number of functional groups C=CH, Ar-H bending and wave number 668.88 cm^{-1}, and the functional group C=CH, Ar-H bending. The results of the FT-IR spectroscopic analysis of vivacel PH 102® has more functional group similarities than the different functional groups that were made with MCC, namely MCC 1, MCC 2, MCC3.

Analysis of Scanning Electron Microscopy (SEM)

SEM analysis aimed to examine the surface morphology of a microscopic sample and provide information about the

texture of the surface of the sample (Figure 2). The morphology of the sample can be viewed from three sides, namely the top surface, side surfaces, and surfaces in the space (Whalley & Langway, 1980). MCC made enzymatically of water hyacinth visible crystalline solid at 1000 times magnification. The results of this SEM showed that microcrystalline made to resemble crystalline forms of vivacel pH102® crystalline form.

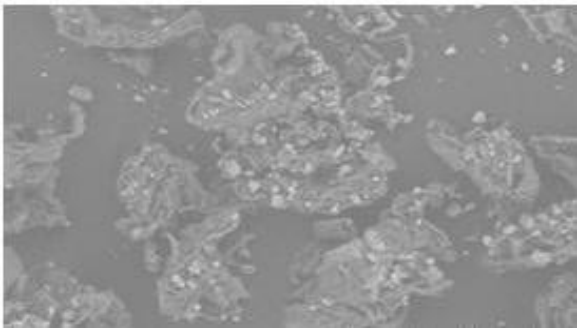

Figure 2. Results Scanning Electron Microscopy of MCC.

Differential Scanning Calorimetry (DSC)

Thermal analysis using a DSC meter. Thermal analysis is a technique of measurement of physical properties and chemistry of a sample as a function of temperature or upon changing the response. In general, this analysis technique to observe the effect of a material that was heated. In the thermal analysis, the temperature rises during physical or chemical property measurements were done programmatically.

DSC analysis is one thermal analysis method that can be used to determine the heat capacity and enthalpy of a sample (Figure 3). DSC was used quite widely in the field of pharmacy to obtain the identity and purity, can also be used to determine the degree of heat of fusion and useful to know polymorphism (Martin, et al., 2000).

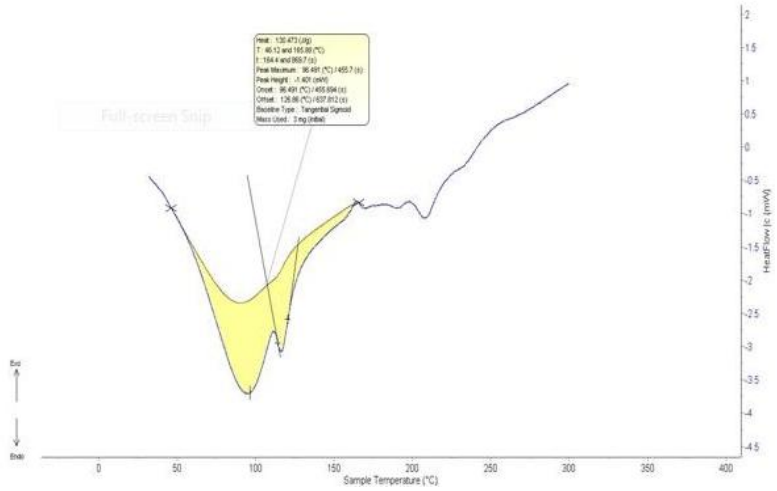

Figure 3. Results Differential Scanning Calorimetry of MCC.

DSC thermogram of comparison vivacel PH 102 shows a melting point at 85.406^{o}C requiring melting energy/enthalpy of 267.77J/g. The vivacel high PH 102 thermogram shows endothermic peak at -2.204 mW with 3 mg of sample. Vivacel PH 102 begins to melt at a temperature of 33.07°C and ends at a temperature 163.13°C. So based on the results of DSC of MCC made of water hyacinth can be concluded that the higher the concentration of cellulase enzymes employed in the process of making MCC with enzymatic hydrolysis the lower the melting point but not at MCC 3 that requires a higher melting point. At MCC 1 with a concentration of 0.4 ml enzyme melting point (92.447°C), MCC 2 enzyme concentration of 1.2 ml (90.62°C) and MCC 3 enzyme concentration of 2 ml (96.491°C) and melting energy or enthalpy also lower. Energy enthalpy at MCC 1 (331.511 J/g), MCC 2 (292.314 J/g), and the MCC 3 of (130.473 J/g).

CONCLUSION

From the research results can be concluded as follows: MCC can be made from water hyacinth (*Eichhornia crassipes* (Mart.) Solms) with the enzymatic hydrolysis method. MCC obtained meets the requirements of the British Pharmacopoeia for organoleptic, identification, pH, solution in water, drying shrinkage, and testing for the absence of starch.

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How to cite this article: Yetti RD, Zulharmita, Junita R. Manufacture and characterization of microcrystalline cellulose of water hyacinth (*Eichhornia crassipes* (mart.) solms) by enzymatic process. International Journal of Research and Review. 2020; 7(7): 384-389.

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