Science and Technology Indonesia
e-ISSN:2580-4391 p-ISSN:2580-4405
Vol. 8, No. 2, April 2023

Check for updates

Research Paper

Isolation of Cellulase from Selected Fungal Strains and Its Use for Manufacture Microcrystal Cellulose from Kapuk Cortex (*Ceiba Pentandra* (L.) Gaertn)

Mi'rajunnisa[1], Herman Suryadi[2*], Sutriyo[2], Yulianita Pratiwi Indah Lestari[1]

[1]*Faculty of Pharmacy, University of Muhammadiyah Banjarmasin, Banjarmasin, 70653, Indonesia*
[2]*Faculty of Pharmacy, Universitas Indonesia, Depok, 16424, Indonesia*
***Corresponding author**: hsuryadi@farmasi.ui.ac.id

Abstract
This study aims to obtain cellulase enzymes from selected molds for microcrystalline cellulose preparation from α-cellulose of kapok cortex. Alpha-cellulose was obtained by biodelignification, and the purified cellulase was obtained from the selected mold. The Microcrystalline cellulose obtained from enzymatic hydrolysis was then identified FTIR and DSC, followed by characterization of microcrystalline cellulose, Particle Size and Distribution Analysis (PSA), and Scanning Electron Microscope-Energy Dispersive X-ray (SEM-EDX), Loss on drying, pH, bulk density, tapped density, and flow rate. Biodelignification produced 14.88% α-cellulose, *Penicillium sp.* the selected mold had the highest cellulase activity, with a cellulolytic index of 4.83. FTIR identification was similar to Avicel PH 101 with a melting point of 244.580°C. Loss on drying was 3.74%, pH was 7.0, particle size ranged from 13.06 to 196.79 μm, bulk density and tapped density were 0.11 g/cm^3 and 0.23 g/cm^3, respectively flow rate character is quite good. SEM-EDX was showed that the morphological shape of the microcrystalline cellulose of the kapok cortex is elongated. Microcrystalline cellulose has shown a quite similar in character and can be furthered.

Keywords
Cellulase, Cellulose, Enzymatic Hydrolysis, Kapok, Microcrystalline Cellulose

Received: 1 December 2022, Accepted: 3 March 2023
https://doi.org/10.26554/sti.2023.8.2.227-234

1. INTRODUCTION

Microcrystalline cellulose is an additive used in pharmaceuticals, food, cosmetics, and other industries (Hindi, 2017). Several articles claim that microcrystalline cellulose is an exceptional excipient and still the most commonly used direct compression excipient. It can be a dry solid binder, tablet disintegrant, absorbent, filler or diluent, lubricant, and anti-adherent (Ahmad and Akhtar, 2020). Other cellulose derivatives include methylcellulose, ethylcellulose, carboxymethyl cellulose, hydroxypropyl methylcellulose, and cellulose acetate, all of which have extensive commercial use in the pharmaceutical, cosmetic, food, paper, textile, and engineering industries. Microcrystalline cellulose is one of these derivatives (Adeleye et al., 2022).

In addition to algae, acetobacter, and rhizobium, other organisms also make cellulose, a structural component of the cell walls of green plants. The average cellulose content of all plant materials is around 33%. Cellulose can be extracted from its raw biomass sources (Aziz et al., 2019). There are four distinct polymorphs of cellulose: I, II, III, and IV. Native cellulose (NC), the most prevalent type of cellulose in nature, can be transformed into Cellulose II (α-cellulose) through alkali treatment or regeneration. While cellulose IV can be produced by treating cellulose III with glycerol, cellulose III, which is amorphous, can be produced by treating cellulose I or II with amines (Adeleye et al., 2022).

In addition to hemicellulose, lignin is a significant component of lignocellulosic biomass, and its interaction results in a resilient and recalcitrant lignocellulosic structure (Fu et al., 2018). In addition to hemicellulose, lignin is a significant component of lignocellulosic biomass, and its interaction results in a resilient and recalcitrant lignocellulosic structure (Goshadrou et al., 2011). Further enzymatic hydrolysis using complexes including ligninolytic (laccase, MnP, LiP) and cellulases enzymes may answer this issue (Pal et al., 2013). The most effective lignin degraders are white rot fungi (WRF), probably the best organisms to use in an industrial process that calls for the delignification of lignocellulosic substrates. The primary ligninolytic enzymes of WRF that are directly involved in the breakdown of lignin in lignocellulosic substrates are lignin peroxidase (LiP) E.C. 1.11.1.14, manganese peroxidase (MnP) E.C. 1.11.1.13, and laccase E.C. 1.10.3.2 (Asgher et al., 2013).

A source of MCC has also been produced from lignocellulosic non-wood materials, such as cotton fiber, cotton stalks, patchwork, cotton cloth waste, citrus peels, soybean skins, corn cobs, water hyacinth, and shredded seeds from milkweed pods (*Calotropis procera*) and bushes (Ahmad and Akhtar, 2020). Kapok was one of the sources of α-cellulose used in producing microcrystalline cellulose. In Indonesia, kapok grows readily, especially in tropical regions like the island of Java (Putri et al., 2022). In the tropics, kapok, one kind of plant, is frequently farmed. The fiber in the fruit of the kapok plant is the component that is utilized the most frequently. This fiber is typically used as a filler for pillows, bolsters, and mattresses. However, using kapok cortex fiber leaves waste that is not used. Astika et al. (2017) previously reported that the peel of kapok contains α-cellulose was > 90%. The content of α-cellulose is relatively high in the kapok rind, indicating its potential as a source of microcrystalline cellulose material.

The hydrolysis process (acid hydrolysis and enzymatic hydrolysis) and microcrystalline cellulose drying are the two steps that can be used to create microcrystalline cellulose from cellulose (after the hydrolysis process). Two different hydrolyses: acid hydrolysis and enzymatic hydrolysis, can be used to hydrolyze microcrystalline cellulose. Because the acid hydrolysis method takes less time than other techniques, it is frequently utilized. According to Li et al. (2014), chemical hydrolysis of microcrystalline cellulose using 2.46 M HCl was carried out at 88.28 C for 64.02 minutes. In contrast, enzymatic hydrolysis has numerous benefits, including its low-intensive, no-chemical, and mild-condition requirements (Wang et al., 2020).

For this reason, we hydrolyzed microcrystalline cellulose by enzymatic hydrolysis using pure cellulase from selected fungal, and α-cellulose was carried out microbiologically using *Trametes versicolor* suspension. The resulting microcrystalline cellulose was then identified and characterized. The objectives of this research are delignification and hydrolysis processes use microbes, making them more environmentally friendly.

2. EXPERIMENTAL SECTION

2.1 Materials

Kapok cortex powder from the Jemari Pati kapok farms served as the study's primary source of raw materials. It was dried and powdered at the Indonesian Spice and Medicinal Plants Research Institute (BALITRO). The chemicals used in this study were Avicel PH 101 (FMC) as a reference, nitric acid (Merck), acetic acid (Merck), sodium hydroxide (Merck), sodium hypoch lorite (Merck), sodium nitrite (Merck), sodium sulfite (Merck), potassium bromide (Merck), potato dextrose agar (DifcoTM), potato dextrose broth (Merck), yeast extract (Himedia), peptone (DifcoTM), glucose (Merck), zinc chloride (Merck), aquadestilata (Merck), aquabidestillata (Otsuka). The molds used in the study were *Chaetomium globosum*, *Fusarium oxsporum*, *Ganoderma sp*, *Paecillomyces niveus*, *Phanaerochaete chrysosporium*, *Penicillium verniculatum*, *Trametes versicolor*, (Department of Biology, Faculty of Mathematics and Natural Sciences, University of Indonesia & IPB Culture Collection (IPB CC), Department of Biology, Faculty of Mathematics and Natural Sciences, Bogor Agricultural University).

2.2 Methods

2.2.1 Mold Screening for Cellulose Activity Based on Clear Zones on CMC Agar Medium

A micropipette was used to transfer 5 L of crude enzyme extract on a 6 mm paper disc in a petri dish with CMC media. The Petri dishes were then incubated for 3 to 7 days at room temperature (27 to 28°C). Lugol staining helped to clarify the transparent zoning. Creating clear zones around the CMC medium can be used to identify fungal isolates with cellulolytic activity. The acquired cellulolytic fungal isolates were then chosen by contrasting the diameters of the clear zone and colony (Kharismi and Suryadi, 2018).

2.2.2 Cellulase Purification

Purification of cellulase enzymes using ammonium sulfate with saturation variations of 0-40%, 40-80%, and 80-95% followed by separation using a DEAE column (Megha et al., 2019). Cellulase activity was determined using the DNS sugar reduction method with visible spectrophotometry. Determination of the calibration curve using glucose standards with several concentrations, namely 60 ppm, 75 ppm, 90 ppm, 105 ppm, 120 ppm, and 135 ppm, measured at a wavelength of 510-540 nm. Data for each concentration were processed using MS excel and determined linear regression y=bx + a (Suryadi et al., 2018).

2.2.3 Biodelignification

As much of 30 g of kapok cortex is boiled in 150 mL of distilled water, heated to 100°C for 1 hour and filtered. The residue was put in Erlenmayer, added 45 mL of nutrient broth solution and sterilized using an autoclave at 121°C for 15 minutes. For 5.42 mL of Trametes versicolor suspension was incubated at 40°C for 21 days (Lestari et al., 2020). The incubated kapok peel powder was then followed by the Kraft process with the following conditions: ratio of 10% NaOH solution to 30 g of oven-dry weight, L: W = 1:5 (L=weight of kapok cortex powder, W= 10% NaOH solution) (Bicu and Mustata, 2013).

2.2.4 Hydrolysis of Microcrystalline Cellulose

20 mL of 0.1 M acetate buffer with a pH of 5.0 was used to dissolve the α-cellulose of kapok rind, and 0.4 mL of pure cellulase was added to the solution. The combined solution was agitated for one hour over a shaking incubator at 160 rpm, 50°C, and then centrifuged for 20 min at 10,000 rpm. The precipitated residue was washed with distilled water (Suryadi et al., 2019).

2.2.5 Identification of Microcrystalline Cellulose

Identification of microcrystalline cellulose of kapok cortex used identified using Differential scanning calorimetry (DSC) and FTIR (Kuthi et al., 2016; Suryadi et al., 2019). For FTIR

© 2023 The Authors.

identification, KBr powder and microcrystalline cellulose were dried at 105°C for 24 hours and scanned in the wave number area of 400 per cm to 4000 per cm (Suryadi et al., 2019). The IR spectrum of microcrystalline cellulose from kapok rind was compared to the IR spectrum of Avicel PH 101. Samples were placed in an aluminum DSC pan and examined. To remove water, samples were first equilibrated in the DSC for 10 minutes at 120°C. Then, samples were equilibrated for 10 minutes at 5°C, and finally, samples were heated from 50°C to 1000°C at 10°C per minute Kuthi et al. (2016) with modification.

2.2.6 Characterization of Microcrystalline Cellulose

Microcrystalline cellulose is characterized as follows organoleptic examination, pH, ash content test, loss on drying, particle size and distribution analysis (PSA), bulk density, tapped density, compressibility index, flow rate test, angle of rest test, Scanning Electron Microscope-Energy Dispersive X-Ray (SEM-EDX) (Crouter and Briens, 2014; Commission, 1970; Zuliahani et al., 2016; Suryadi et al., 2019; Kharismi and Suryadi, 2018; Suryadi et al., 2018; United States Pharmacopeial Convention, 2019)

3. RESULTS AND DISCUSSION

3.1 Cellulase Mold Selection

Each cellulase mold was cultured on PDA and made into suspension using nutrient broth. The enzyme suspension obtained was then tested for cellulase activity in solid-state fermentation (SSF), namely CMC media. When a continuous gaseous phase and a little amount of water are present in the spaces between the solid particles, SSF involves the growth of microorganisms on the particles. There can be a thin film of water on the surface in addition to a few drops of water in between the particles. The gas phase fills most of the particle space because the water phase between the particles is not continuous. The majority of the water is absorbed by the wet particles. According to Steudler et al. (2019), Every method of fermentation involving solids, including the suspension of solid particles in a continuous liquid phase and even drip filters, is referred to as "solid substrate fermentation." SSF fermentation is therefore categorized as a kind of solid-substrate fermentation.

The results of cellulase activity screening (Table 1 and Figure 1) showed clear zones with the following diameters, namely: *Chaetomium globosum* 3.3 cm; *Fusarium oxysporum* 3.2 cm; *Ganoderma sp*. does not produce a clear zone; *Paecilomyces niveus* shows no clear zone; *Penicillium sp*. 3.5 cm; *Phanaerochaetae chrysosporium* 5 cm (partial); Meanwhile, the cellulase activity itself shows the following results:

Based on the test, it is known that *Penicillium sp* has the most excellent cellulase activity with a clear zone diameter of 3.5 cm and a cellulolytic index of 4.83.

3.2 Cellulase Purification

Standard glucose solution was prepared at several serial concentrations, including 60 ppm, 75 ppm, 90 ppm, 105 ppm, 120 ppm, and 135 ppm, to derive the calibration curve's linear

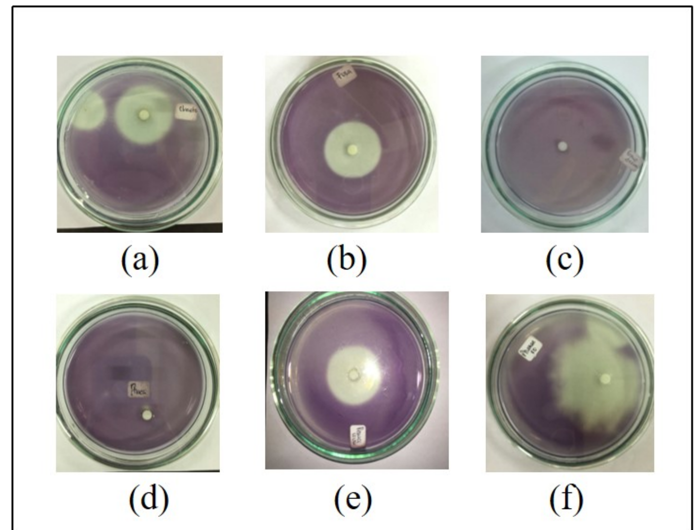

Figure 1. Cellulase Activity Screening Results Based on Clear Zones Using CMC Media: (a) *Chaetomium globosum* (b) *Fusarium oxsporum* (c) *Ganoderma sp*. (d) *Paecilomyces niveus* (e) *Penicillium sp*. (f) *Phanerochaete chrysosporium*

Table 1. Cellulase Activity of Enzyme Extract

Ammonium Sulphate (%)	Cellulase Activity (U/mL)
0-40	0.51
40-80	0.53
80-95	0.52

regression equation (Figure 2). The glucose concentration in the sample is then determined using the linear regression equation. The derived equation for linear regression is y = 0.0099x - 0.4097 with R^2= 0.9925.

The crude enzyme extract that has been obtained is then added with ammonium sulfate salt (Figure 4) to precipitate the cellulase enzymes with concentrations of 0-40%, 40-80%, and 80-95%. Precipitation of cellulase enzymes aims to increase enzyme activity by reducing the content of other compounds from the crude extract of the enzymes. Ammonium sulfate dissolved in water will cause salting out so that the protein will precipitate.

The highest cellulase activity was obtained from the enzyme extract, which was given an ammonium sulfate concentration of 40-80%, as shown in Table 1.

The enzyme precipitate was then dialyzed using a 0.01 M phosphate buffer pH 7.0 to remove the salt in the residue. Dialysis is one way to remove residual ammonium sulfate, which is still bound to enzymes (Kavya et al., 2019). In dialysis, the tiny salt molecules will exit through the dialysis bag's pores, during the enzymes/proteins, because they have a much larger size, will remain in the bag (Bańkowski, 2013).

Dialysis was carried out for 24 hours and three times the replacement of the phosphate buffer solution in cold condi-

© 2023 The Authors.

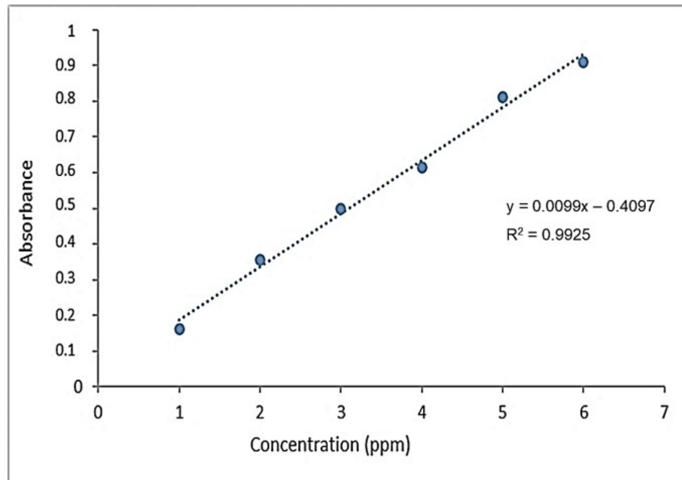

Figure 2. Curve Calibration of Glucose (Reference)

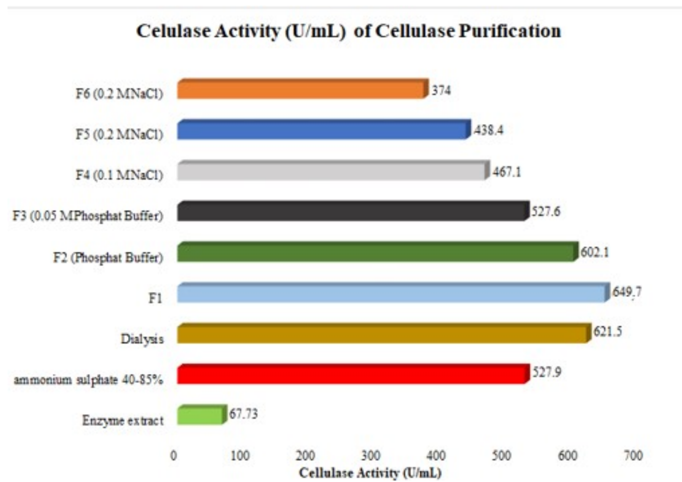

Figure 3. Results of Cellulase Purification

tions so as not to damage the enzyme and to use a magnetic stirrer to assist the diffusion process, the lower concentration of phosphate buffer solution outside the cellophane bag will cause ammonium sulfate residue in the bag Cellophane leaves the enzyme (Bańkowski, 2013) after the dialysis process it is known that the enzyme activity is 0.62 U/mL.

A stepwise gradient of 0.05 M, 0.1 M, and 0.2 M NaCl was used in DEAE column chromatography to purify the dialyzed enzyme precipitate. It uses a flow rate of 30 mL per hour. A UV-Vis spectrophotometer was used to test the fractions' activity after they had been collected. The first column diffraction revealed the highest cellulase activity, measuring 649.7 mU/mL (Figure 3).

3.3 Biodelifnification

Biodelignification of kapok cortex using *Trametes versicolor* myce lium has been inoculated in PDB, according to Yang et al. (2017). Biodelignification was incubated at 40°C for 21 days. After the 21-day incubation period ended, decontamination was carried out using an autoclave to kill *Trametes versicolor*, and 10% NaOH solution was added to increase lignin degradation. NaOH compounds will cause swelling of the biomass, leading to lignin degradation. During the delignification process using NaOH, hydroxide ions from NaOH attack the carbon ester bonds that exist between lignin and hemicellulose or cellulose (Abraham et al., 2011).

Figure 4. α-cellulose of Kapok Cortex

To determine the purity of cellulose, α-cellulose of biodelignification was left for 30 minutes at 20°C in a hot plate stirrer, then washed using distilled water and filtered. The resulting residue was added with 8.3% NaOH and followed by washing using aquadestillata. The residue was washed using distilled water with a pH of 7.0 and 10% acetic acid. For 48 hours, the residue is dried at 50°C until dry. An amount of 8.93 g of α-cellulose (Figure 4) with a purity of 96.52% and a yield of 14.88% was produced from 60 g of kapok cortical powder.

3.4 Hydrolysisi of Microcrystalline Cellulose

The results of enzymatic hydrolysis of microcrystalline cellulose from kapok cortex are shown in Figure 5.

The enzymatic hydrolysis of 5 g of α-cellulose from the kapok cortex yielded 3.8 g microcrystalline cellulose or 78%. The resulting microcrystalline cellulose is white and in powder form. The organoleptic produced follows United States Pharmacopeial Convention (2019) organoleptic from microcrystalline cellulose in the form of white powder, odorless and tasteless.

3.5 Identification of Microcrystalline Cellulose

FTIR was identified to determine whether the resulting microcrystalline cellulose showed similarities to Avicel PH 101 (Figure 6) in the wavelength range of 400 per cm to 4000 per cm.

The FTIR results of the samples and standards show similar spectra even though the % transmittance is different. However, the spectrum results show similarities in the functional groups found in the samples and standards (Table 2).

© 2023 The Authors.

Figure 5. Microcrystalline Cellulose of Kapok Cortex

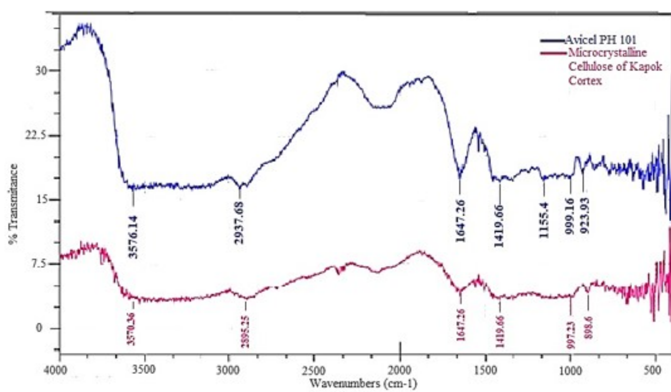

Figure 6. FTIR Spectrum of Avicel PH 101 and Microcrystalline Cellulose of Kapok Cortex

Based on the results of the DSC analysis, it can be seen that the melting point of Avicel PH 101 is 331.97°C (Table 3 and Figure 7) while the microcrystalline cellulose of kapok cortex is 244.580°C.

The TGA diagram shows that Avicel PH 101 began to lose weight at 267.630°C while the microcrystalline cellulose of kapok cortex began to lose weight at 217.54°C.

Characterization of microcrystalline cellulose from kapok cortex was showed in Table 4. The obtained microcrystalline cellulose is yellowish white (white color no.4) and odorless. The organoleptic obtained from the microcrystalline cellulose powder of kapok cortex complies with the requirements of microcrystalline cellulose by United States Pharmacopeial Convention (2019), where the organoleptic microcrystalline cellulose is white, odorless, and tasteless.

Microcrystalline cellulose obtained from α-cellulose of kapok cortex has a pH of 7.0. The pH obtained met the requirements of microcrystalline cellulose from The United States Pharmacopoeia 2019, where the pH of microcrystalline cellulose ranged from 5.0 to 7.5. The results of the ash content

Table 2. Functional Groups in Avicel PH 101 and Microcrystalline Cellulose of Kapok Cortex

Functional Groups	Avicel PH 101 (cm^{-1})	Microcrystalline Cellulose of Kapok Cortex (cm^{-1})
O-H	3576.14	3570.36
C-H	2937.68	2895.25
CH_2	1419.66	1647.26
C-O-C glicosidic	1155.4	1419.66
B-glicosidic	923.93	898.96

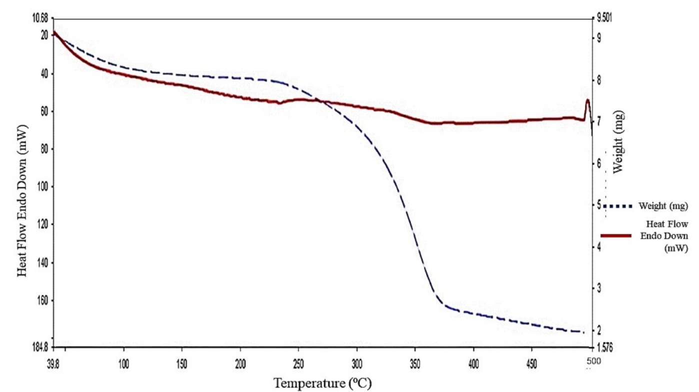

Figure 7. DSC Analysis Result of Microcrystalline Cellulose from Kapok Cortex

test showed that the microcrystalline cellulose of kapok cortex was 0.46%. The results of the ash content test showed that the ash content of the microcrystalline cellulose of kapok rind still did not meet the requirements set by the (Commission, 1970). The ash content of microcrystalline cellulose should be at most 0.1%.

Loss on drying test is a test for determining the number of volatile compounds under specified conditions. The loss on drying test was carried out twice to obtain a constant weight. The drying shrinkage of microcrystalline cellulose from kapok cortex is 3.74%, and this result meets the requirements of The National Formulary 30 that microcrystalline cellulose is less than 7% or in a lower percentage.

To compare samples produced by the enzymatic hydrolysis of the kapok cortex to the standard Avicel pH 101 sample, particle size analysis and particle dispersion were performed using a microparticle size analyzer (PSA) in a water medium. According to the particle size analysis, 10% of the total particle size in Avicel PH 101 is less than 6.25 m, 50% is less than 15.61 m, and 90% of the total particle size is less than 24.00 m. 10% of the total microcrystalline cellulose in the kapok cortex had particles smaller than 13.06 m, which made up 50% of the total.

Bulk density is the ratio of the powder's uncompressed mass to its volume, considering the volume of the spaces between

© 2023 The Authors.

Table 3. Results of Differential Scanning Calorimetry (DSC) Analysis

Sample	Transition Glass Temperature (T_g)	Crystalization Temperature (T_c)	Melting Temperature (T_m)
Avicel PH 101	274.140°C	301.410°C	331.97°C
Sampel	216.100°C	219.580°C	244.580°C

Table 4. Characterization of Microcrystalline Cellulose Kapok Cortex

Characterization	Microcrystalline Cellulose of Kapok Cortex
Organoleptic	Yellowish White, Odorless, Tasteless
Particle Size Distribution	dv 10: 13.06 μm dv 50: 39.40 μm, dv 90: 196.79 μm
pH	7.0
Melting Point	456.250°C
Loss on Drying	20.46%
Water Content	3.75%
Bulk Density	0.11 g/cm^3
Tapped Density	0.15 g/cm^3
Flow Rate Test	0.04 g/s
Test Angle of Repose	92
Compressibility Index	1.12
SEM-EDX	Needle-like and Elongated

the particles. The density of the powder particles and their arrangement determine the bulk density. The microcrystalline cellulose of the kapok cortex is 0.11 g/cm^3, and the sample test results do not meet the literature requirements. Tapped density is the level of compacted powder density obtained by mechanically tapping a measuring cup or measuring vessel containing powder. The density of microcrystalline cellulose compressed powder of kapok cortex is 0.23 g/cm^3.

The compressibility index is the ability of granules to reduce their volume (compress themselves) at a specific pressure. The compressibility index is affected by the particles' density, size, and shape. The smaller the percent compressibility index of the powder or granule, the better the flow properties. Conversely, the greater the powder or granule's compressibility index, the worse the flow properties. Microcrystalline cellulose of kapok cortex has a reasonable flow rate of 1.12.

The mass per time flowing from the type of receptacle is frequently used to calculate the flow rate through an orifice (cylinder, funnel, & hopper). In pharmaceutical industry, adequate powder flow is a prerequisite for successful tablet manufacture. Poor powder flow can lead to large variations in tablet weight, poor content uniformity, and inconsistent tablet properties such as breaking strength, disintegration time, and sometimes dissolution rate (Sun, 2010). The flow rate test results for microcrystalline cellulose of the kapok cortex were 0.041 g/s. The results showed that the reference (Avicel PH 101) had a better flow rate than MCC of kapok cortex. It can be concluded that the standard characteristic of standard had better flow rate characteristic than MCC obtained.

Figure 8. Particles from Microcrystalline Cellulose of Kapok Cortex

An angle of repose test was carried out to determine the ability of microcrystalline cellulose to flow. The smaller the angle formed, the better the ability to flow a substance. According to United States Pharmacopeial Convention (2019), microcrystalline cellulose has an angle of repose that ranges from around 34.49°. The angle of repose of microcrystalline cellulose from the kapok cortex is 92°, because of this large

© 2023 The Authors.

angle of repose, the flow properties become smaller, making it difficult for microcrystalline cellulose to flow. Scanning Electron Microscope- Energy Dispersive X-Ray (SEM-EDX) is a characterization that will show the shape of microcrystalline cellulose particles from kapok cortex.

The 500× magnification on SEM-EDX shows that the shape of microcrystalline cellulose tends to be needle-like and elongated (Figure 8) and contains carbon (C), oxygen (O), aluminum (Al), and silica (Si).

4. CONCLUSION

Penicillium sp. is a potential mold with cellulase activity with a cellulolytic index of 4.83 FTIR identification and the melting points of Avicel PH 101 and kapok cortex microcrystalline cellulose were different. Characteristics: The organoleptic microcrystalline cellulose sample of kapok cortex is slightly more yellowish and the powder is coarser when compared to Avicel PH 101 which tends to be fine powder and white in color; the particle size of microcrystalline cellulose is larger than Avicel PH 101; SEM-EDX showed differences in morphology with Avicel PH 101; pH, loss on drying, bulk density, tapped density, flow rate and angle of repose show differences with the standard; compressibility index and Hausner's ratio is quite good.

5. ACKNOWLEDGMENT

We want to thank the lecturers and the manager of the Laboratory of Microbiology, University Indonesia, for supporting the establishment of this research.

REFERENCES

Abraham, E., B. Deepa, L. A. Pothan, M. Jacob, S. Thomas, U. Cvelbar, and R. Anandjiwala (2011). Extraction of Nanocellulose Fibrils from Lignocellulosic Fibres: A Novel Approach. *Carbohydrate Polymers*, **86**(4); 1468–1475

Adeleye, O. A., O. A. Bamiro, D. A. Albalawi, A. S. Alotaibi, H. Iqbal, S. Sanyaolu, M. N. Femi Oyewo, K. O. Sodeinde, Z. S. Yahaya, and G. Thiripuranathar (2022). Characterizations of Alpha-Cellulose and Microcrystalline Cellulose Isolated from Cocoa Pod Husk as a Potential Pharmaceutical Excipient. *Materials*, **15**(17); 5992

Ahmad, U. and J. Akhtar (2020). *Pharmaceutical Formulation Design: Recent Practices*. BoD Books on Demand

Asgher, M., Z. Ahmad, and H. M. N. Iqbal (2013). Alkali and Enzymatic Delignification of Sugarcane Bagasse to Expose Cellulose Polymers for Saccharification and Bio-ethanol Production. *Industrial Crops and Products*, **44**; 488–495

Astika, J., C. Chandrawati, L. Hendrajaya, and P. Yogi (2017). The Exploration of Alpha Cellulose in Kapok Fruit as Raw Material For Rocket Propellant Production. *Agricultural Research & Technology: Open Access Journal*, **12**(4); 1–8

Aziz, T., H. Fan, X. Zhang, and F. U. Khan (2019). Synergistic Impact of Cellulose Nanocrystals and Calcium Sulfate Fillers on Adhesion Behavior of Epoxy Resin. *Materials Research Express*, **6**(11); 1150b7

Bańkowski, E. (2013). *Biochemistry Workbook*. Białystok: Medical University of Białystok

Bicu, I. and F. Mustata (2013). Optimization of Isolation of Cellulose From Orange Peel Using Sodium Hydroxide and Chelating Agents. *Carbohydrate polymers*, **98**(1); 341–348

Commission, B. P. (1970). *British pharmacopoeia*. London: The Stationary Office

Crouter, A. and L. Briens (2014). The Effect of Moisture on The Flowability of Pharmaceutical Excipients. *Aaps PharmSciTech*, **15**; 65–74

Fu, Y., T. Luo, Z. Mei, J. Li, K. Qiu, and Y. Ge (2018). Dry Anaerobic Digestion Technologies for Agricultural Straw and Acceptability in China. *Sustainability*, **10**(12); 4588

Goshadrou, A., K. Karimi, and M. J. Taherzadeh (2011). Bioethanol Production From Sweet Sorghum Bagasse by Mucor Hiemalis. *Industrial Crops and Products*, **34**(1); 1219–1225

Hindi, S. S. Z. (2017). Microcrystalline Cellulose: The Inexhaustible Treasure for Pharmaceutical Industry. *Nanoscience and Nanotechnology Research*, **4**(1); 17–24

Kavya, P., S. Bhat, S. Siddappa, A. Rao, and K. Kadappu (2019). When to Avoid Dialysis during Protein Purification. *Advance in Industrial Biotechnology*, **2**(9); 1–5

Kharismi, R. R. A. Y. and H. Suryadi (2018). Preparation and Characterization of Microcrystalline Cellulose Produced From Betung Bamboo (dendrocalamus Asper) Through Acid Hydrolysis. *Journal of Young Pharmacists*, **10**(2s); S79

Kuthi, F. A. A., N. R. Norzali, and K. H. Badri (2016). Thermal Characteristics of Microcrystalline Cellulose From Oil Palm Biomass. *Malaysian Journal of Analytical Sciences*, **20**(5); 1112–1122

Lestari, Y. P. I., H. Suryadi, W. Mangunwardoyo, and A. Yanuar (2020). Characterization of Kapok Pericarpium Microcrystalline Cellulose Produced of Enzymatic Hydrolysis Using Purified Cellulase From Termite (Macrotermes Gilvus). *International Journal of Pharmacy and Pharmaceutical Sciences*, **2**(3); 7–14

Li, J., X. Zhang, M. Zhang, H. Xiu, and H. He (2014). Optimization of Selective Acid Hydrolysis of Cellulose for Microcrystalline Cellulose Using FeCl3. *BioResources*, **9**(1); 1334–1345

Megha, S., S. Maragathavalli, S. Brindha, V. Karthikeyan, B. Annadurai, and S. Gangwar (2019). Isolation and Purification of Cellulase. *International Journal of Science and Nature*, **3**(4); 474–479

Putri, R. W., M. Rendana, U. Waluyo, and T. Andrianto (2022). Effect of Plasticizer and Concentration on Characteristics of Bioplastic Based on Cellulose Acetate from Kapok (Ceiba pentandra) Fiber. *Science and Technology Indonesia*, **7**(1); 73–83

Steudler, S., A. Werner, and T. Walther (2019). It Is the Mix That Matters: Substrate-specific Enzyme Production From Filamentous Fungi and Bacteria Through Solid-state

© 2023 The Authors.

Fermentation. *Solid State Fermentation: Research and Industrial Applications*, **3**(5); 51–81

Sun, C. C. (2010). Setting the Bar for Powder Flow Properties in Successful High Speed Tableting. *Powder Technology*, **201**(1); 106–108

Suryadi, H., M. Angeline, and M. W. Murti (2018). Characterization of Microcrystalline Cellulose Obtained from Enzymatic Hydrolysis of Alpha-cellulose and its Application. *Journal of Young Pharmacists*, **10**(2s); S87

Suryadi, H., Y. P. I. Lestari, and M. A. Yanuar (2019). Potential of Cellulose of Chaetomium Globosum for Preparation and Characterization of Microcrystalline Cellulose From Water Hyacinth (eichhornia Crassipes) Inter. *International Journal of Applied Pharmaceutics*, **11**(4); 140–146

United States Pharmacopeial Convention (2019). *The United States pharmacopeia USP42, the national formulary NF37 (First edition.)*. United States Pharmacopeial Convention

Wang, X., S. Cheng, Z. Li, Y. Men, and J. Wu (2020). Impacts of Cellulase and Amylase on Enzymatic Hydrolysis and Methane Production in the Anaerobic Digestion of Corn Straw. *Sustainability*, **12**(13); 5453

Yang, S. O., H. Sodaneath, J. I. Lee, H. Jung, J. H. Choi, H. W. Ryu, and K. S. Cho (2017). Decolorization of Acid, Disperse and Reactive Dyes by *Trametes versicolor* CBR43. *Journal of Environmental Science and Health, Part A*, **52**(9); 862–872

Zuliahani, A., R. Nurul Nadhirah, A. Rozyanty, W. I. Nawawi, and A. Nor Hanani (2016). Crystallinity, Tapping and Bulk Density of Microcrystalline Cellulose (MCC) Isolated from Rice Husk (RH). *Applied Mechanics and Materials*, **835**; 272–276

© 2023 The Authors.