

Original Article

CHARACTERIZATION OF KAPOK PERICARPIUM MICROCRYSTALLINE CELLULOSE PRODUCED OF ENZYMATIC HYDROLYSIS USING PURIFIED CELLULASE FROM TERMITE (*MACROTERMES GILVUS*)

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ABSTRACT

Objective: This study aimed to increase the yield of microcrystalline cellulose (MCC) from kapok pericarpium alpha-cellulose produced by enzymatic hydrolysis using purified cellulase from Termites (*Macrotermes gilvus*) and to compare the characteristics with the reference product.

Methods: In this research, MCC was prepared from kapok pericarpium powder through the chemical isolation process of alpha-cellulose, followed by enzymatic hydrolysis with purified cellulase from *Macrotermes gilvus*. The yield was improved by using purified cellulase in optimized temperature, pH, and hydrolysis time. Identification was carried out by using ZnCl and infrared spectrophotometry, followed by characterization of MCC include particle size analysis (PSA) and diffractogram pattern (X-Ray Diffraction). The results were compared with Avicel PH 101 as the reference product.

Results: Purified cellulase from *Macrotermes gilvus* showed high cellulose activity. Cellulose in the concentration of 11.743 U/ml formed 49 mm clear zone area with cellulolytic index 7.16 that similar to the formed clear zone area of *Trichoderma reesei* (50 mm), the optimum hydrolysis condition was achieved at 50 °C, pH 6.0, in 2 h, which produced 80% yield of MCC. Produced MCC was analyzed with ZnCl and FTIR spectrum resulting in positive results, similar to reference. The results of the organoleptic test, particle size analysis, and diffractogram pattern (X-Ray Diffraction) showed crystalline characteristics of MCC is similar to the reference (Avicel PH 101).

Conclusion: Cellulase *Macrotermes gilvus* yielded 80% MCC and higher enzymatic activity than *Trichoderma reesei*. Based on the organoleptic test, particle size analysis, and diffractogram pattern observation, MCC from kapok pericarpium has shown similar characteristics to reference (Avicel pH 101) and might be potential to be further developed.

Keywords: Cellulase purification, Enzymatic hydrolysis, Kapok pericarpium, *Macrotermes gilvus*, MCC characterization, Microcrystalline cellulose

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INTRODUCTION

Kapok fruits have a source of fiber that can be used as a basic material for mattresses, pillows, and clothing. The kapok rind can be used as a substitute for paper material while the bark is rich in potassium and the ashes can be used as fertilizer [1]. Kapok has been known to have high cellulose content consisted of 86.52 to 96.89% [2].

MCC is an additional ingredient commonly used in pharmaceutical, food, cosmetics, and other products. MCC is one of the most important tablet excipients because of the binding properties of tablets in the direct compression tablet creation method [3]. MCC can be synthesized by different processes such as acid hydrolysis and enzymatic hydrolysis. The enzymatic hydrolysis process is more interesting when viewed from energy use because it can be carried out at low temperatures, while chemical hydrolysis requires high temperatures and uses chemicals that can pollute the environment, but enzymatic hydrolysis takes longer [4].

Source of the enzyme for enzymatic hydrolysis can be obtained from fungi, mold, and animal (for example: insect). Termite is one of the insects that can produce cellulase. In common, termites can eat all of the materials that obtained cellulose. Preparation of MCC from kapok pericarpium powder by enzymatic reaction has already performed by Suryadi *et al.* [5]. In our previous study, the enzyme that used was still in the form of crude enzymes while in this study using the purified enzyme. Besides that, in previous studies used local isolates and *Trichoderma reesei* while in this study using *Macrotermes gilvus*. Enzymatic hydrolysis using termites has already

performed by Fagbohunka, Okonji, and Adenike [6]. In their study, cellulase was produced by *Ametermes eveuncifer* (Silverstri) Soldiers while in this study cellulase was produced by *Macrotermes gilvus* (different species). So, that it can be used as new knowledge about other types of mold and animals that can produce cellulase enzymes.

This paper reports the preparation of MCC from kapok pericarpium powder using an enzymatic method that used an enzyme from termites (*Macrotermes gilvus*) which was compared with reference (Avicel PH 101). Identification MCC was carried out by zinc iodinate and infrared spectrophotometry, followed by characterizations of microcrystalline cellulose compared to the reference, Avicel PH 101.

MATERIALS AND METHODS

Instruments

The instruments analysis used were autoclave (Hirayama), incubator (Mettler), oven (WTB Binder), analytical balance (Acculab), particle size analyzer (Cilas 1190), waterbath shaker, hotplate stirrer (Corning), pH meter (Eutech), centrifugator (Kubota 6800), UV-Vis spectrophotometer (Shimadzu), pH meter, and X-ray diffractogram (Rigaku), vacuum oven (Hotpack), blender (Maspion), sieve, filter paper, ose, tweezer, column, pipette volume and other glasswares commonly used in laboratories.

Raw material

Kapok pericarpium (*Ceiba pentandra*) obtained from Balai Penelitian Tanaman Rempah dan Obat (Balitro), Cimanggu, Ciwaringin district,

Central Bogor West Java, Indonesia, 16124 (6.5810 ° S, 106.7898 ° E). The mold used in this study was *Trichoderma reesei* and *Trametes versicolor* from Universitas Indonesia Culture Collection (UICC), Department of Biology, the Faculty of Mathematics and Natural Sciences, Universitas Indonesia. Termites used *Macrotermes gilvus* from trees in the Faculty of Pharmacy area, Universitas Indonesia.

Chemical material

The chemicals used in this study were Avicel PH 101 (aicma) as reference product, sodium hydroxide (Merck), sodium hypochlorite (Merck), sodium nitrite (Merck), sodium sulfite (Merck), Potato Dextrose Agar/PDA (Difco™), Potato Dextrose Broth/PDB (Merck), yeast extract (Himedia), tween 80 (Merck), Peptone (Difco™), ammonium sulfate (Merck, Germany), Diethylaminoethyl (DEAE)-Cellulose resin, Glucose (Merck), dinitrosalicylic acid (Himedia), nitric acid (Merck), acetic acid (Merck), KBr powder (Merck), zinc chloride (Merck), potassium iodide (Bratachem), distilled water (Merck), double distilled water (Otsuka).

Maintenance of mold isolates

Trichoderma reesei and *Trametes versicolor* was maintained in PDA medium, incubation at 27 °C for 7 d.

Isolation of alpha-cellulose

Biodelignification process was carried out using *Trametes versicolor* with modification [7, 8] with kapok pericarpium as substrate producing alpha-cellulose with lower lignin content, further delignized with NaOH 10% and NaOCl 3,5% to get a whiter result.

Extraction of cellulase enzyme

Termites were washed and rinsed with distilled water, the termites were carefully homogenized with 10 mmol natrium acetate buffer pH 5.0 and 1 mmol EDTA. The mixture was collected and centrifuged at 15000 rpm for 15 min at room temperature. The supernatant containing a crude enzyme was collected and stored in the refrigerator at 4 °C or min 10 °C [6].

Purification of cellulase

Cellulase was purified through precipitation with ammonium sulfate [6], followed by dialysis with selofan [7], and finally by DEAE-C column chromatography [9].

Characterization of cellulase

Cellulase characterization was performed by optimization of pH, temperature, and time of hydrolysis [6]. Each experiment was conducted in triplicate and the standard deviation for each experimental result was calculated.

Screening of cellulase activity based on clear zone

Cellulolytic microbial was determined using CMC media on petri dishes. The enzyme was dripped on paper disk by optimizing various volumes. After incubating for 24 h at room temperature, Lugol solution 0.1% (b/v) and 1% NaCl solution was added. The cellulolytic activity was determined by the cellulolytic index value which represents the difference between the diameter of the clear zone and the diameter of the colony [10]. For comparison,

Trichoderma reesei (molds that are often used in research for cellulase enzyme extraction), was also cultured. Cellulolytic index can be calculated with the following formula:

$$\text{Cellulolytic Index: } \frac{A-B}{B}$$

A = the diameter of the clear zone (mm); B = the diameter of paper disk (mm).

DNS method

Cellulase activity was determined based on the method conducted by Zhang *et al.* (2006) with Dinitro salicylic acid (DNS) reagent, with a slight modification, using 1% carboxymethyl-cellulose (CMC) as a substrate [6].

Preparation and characterization of MCC by enzymatic hydrolysis

Amount of 10 g of kapok pericarpium alpha-cellulose were dissolved into 100 ml acetate buffer (0.05 M, pH 4.8) and 3 ml of cellulase enzyme was added and stirred slowly. The mixed solution was stirred at 150 rpm at 48°C for 72 h on a water shaking incubator. The mixture was centrifuged at 10,000 rpm (at 7-10°C 20 min). The precipitated residue was washed with distilled water to remove the remaining enzymes on cellulose and then dried with an oven at 60°C for 48 h [8].

Identification with zinc iodinate

Zinc iodinate solution was prepared by dissolving 20 g of zinc chloride and 6.5 g of potassium iodide in 10.5 ml of water, follow by the addition of 0.5 g iodine, and shake for 15 min. About 10 mg of MCC was added to the watch glass and was then dissolved into 2 ml of zinc chloride solution [11]. The compound formed will produce a violet-blue color.

Infrared spectrophotometry (FTIR)

The amount of 99 mg KBr powder which has been dried at 105°C for 24 h was weighed carefully and was added with 1 mg MCC powder. The mixture was crushed and mixed until homogeneous, put into a disk or disc-shaped mold. KBr 100 mg was weighed for a blank and a baseline. A Scanning was performed in 4000 cm⁻¹ to 400 cm⁻¹ wave number area [12]. The MCC FTIR spectrum made from kapok pericarpium pulp was then compared to the FTIR spectrum from reference raw materials (Avicel PH 101).

Organoleptic examination

Samples of MCC were placed on a white base (parchment paper or other suitable paper) and then observed for the shape or appearance, the color, the taste, and the smell [13].

Size analysis and particle size distribution

Particle size distribution was determined using Particle Size Analyzer Mikro (CILAS1190 Liquid) [11].

X-ray diffraction analysis

Crystal analysis was performed using X-ray diffraction (XRD) (Rigaku Miniflex 600) [5].



Fig. 1: Kapok pericarpium powder (a), sterilized kapok pericarpium powder (b)

RESULTS**Preparation of alpha-cellulose**

Biodelignification alpha-cellulose was prepared before the process. Kapok pericarpium powder was moistened with distilled water, then sterilized using an autoclave (fig. 1a and 1b).

Biodelignification

Biodelignification is the process of removing lignin from substrate (kapok pericarpium) biomass using biological organisms. In this study, biodelignification was carried out using cellulase isolated from the mold (*Trametes versicolor*).

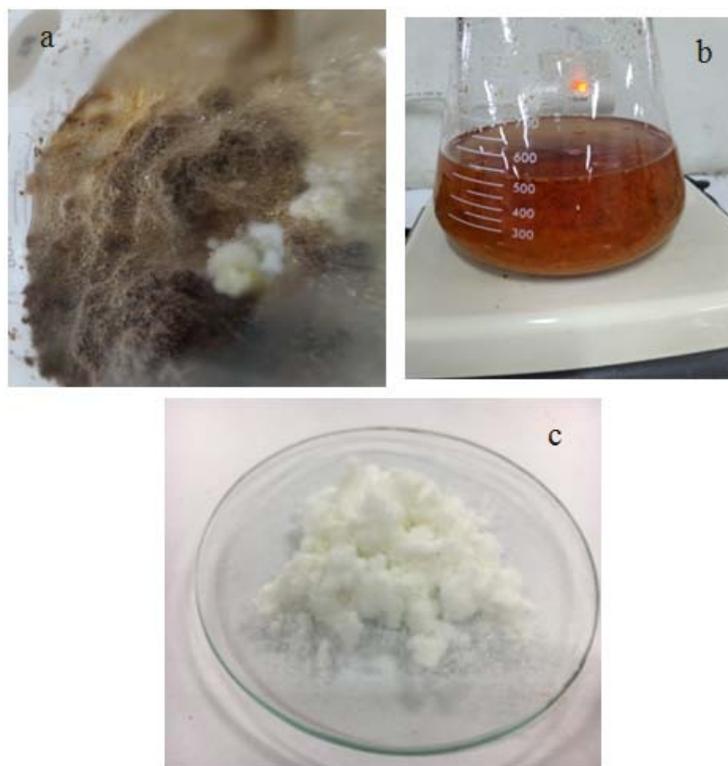


Fig. 2: Process of biodelignification (a), bleaching (b), and alpha cellulose obtained (c)

Biodelignification was carried out during 21 d using *Trametes versicolor*, white-colored mold covers the surface of the substrate (fig. 2a), further delignification (fig. 2b) has yellowish-white color, finally, the whitest color was obtained (fig. 2c). The result of α -cellulose isolation obtained from 5 g of substrate powder was 3.8 g (76.0%).

Extraction of cellulase enzyme

Extraction was carried out for hydrolysis enzymatic by mixing termites into buffer acetate solution pH 5.0 to adjust the stabilize conditions of the enzyme stored in the body of termite.

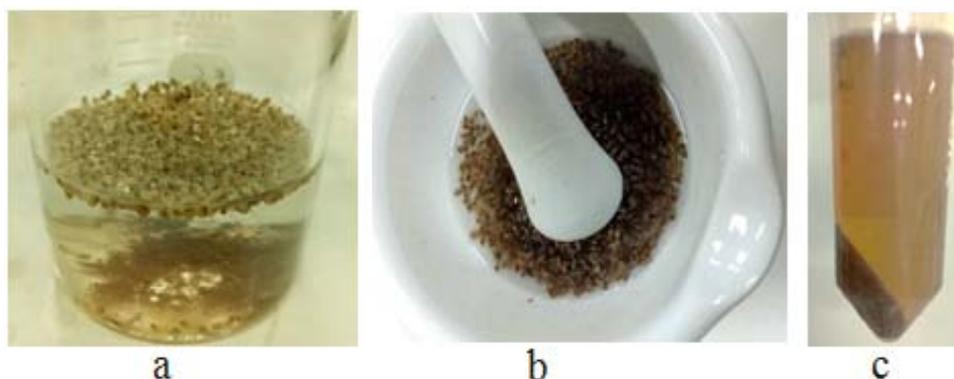


Fig. 3: (a) cleaning of termites from remaining soil on the body of termites; (b) homogenizing of termites with adding acetate buffer pH 5.0; (c) result of centrifugation (supernatant) as crude enzyme extract

Fig. 3a cleaning of termites from remaining soil on the body of termites, followed by homogenizing of termites with adding acetate buffer pH 5.0 (fig 3b), and finally centrifugation (supernatant) as crude enzyme extract was kept in 4 °C (fig. 3c).

Purification of cellulase

Purification process using Ammonium sulfate salt, dialysis, and column chromatography DEAE-C. The summary result was in table 1.

Table 1: Summary of the stages of cellulase purification

Steps	Volume (ml)	Protein (mg/ml)	Total protein (mg)	CMCase activity (mU/ml)	Total cellulose activity (mU)	Specific activity (mU/mg)	Purification fold	Yield (%)
CE	90	1703.33	153299.7	12593	1133370	7.393	0	100
Fractination with ammonium sulfate								
F1	10	1350	13500	11623	116230	8.610	1.165	10.255
F2	75	270	20250	TT	TT	TT	TT	TT
Dialysis								
Dt	10	1190	11900	12062	120620	10.136	1.371	10.643
Ion exchange chromatography (DEAE-Streamline)								
P1	5	1050	5250	11743	58715	11.184	1.513	5.181
P2	5	1030	5150	11150	55750	10.825	1.465	4.919
P3	5	970	4850	10600	53000	10.928	1.478	4.676
P4	5	850	4250	8663	43315	10.192	1.379	3.822
P5	5	830	4150	8200	41000	9.880	1.336	3.618
P6	5	250	1250	TT	TT	TT	TT	TT
P7	5	230	1150	TT	TT	TT	TT	TT

The highest activity during the purification process was the first fraction (P1) with the specific activity 11.184 mU/mg and purity grade 1.51 times compare to the crude enzyme.

Characterization of cellulase

Effect of pH variation

The activity of cellulase was determined at various pH ranges from 3.0-8.0 (fig. 4), the highest activity of cellulase was at pH 6.0 with the activity 7.29 U/ml.

Effect of temperature variation

The activity of cellulase was determined at various temperatures ranges from 30 to 60°C (fig. 5). The highest activity of cellulase was at 50°C with the activity 12.78 U/ml.

Optimization duration of hydrolysis

Optimization of hydrolysis duration was carried out during 10 h, the results indicate that the lowest glucose concentration was hydrolysis in 2 h incubation (fig. 6).

Screening of clear zone-based cellulase activity in medium CMC

The crude enzyme of *Macrotermes gilvus* showed a clear zone with a diameter of 42 mm and 49 mm and the cellulolytic index of 63.9 and 76.3, *Trichoderma reesei* showed a clear zone diameter of 50 mm and cellulolytic index 7.3 which means that *Macrotermes gilvus* has almost the similar results as *Trichoderma reesei* in term of hydrolysis activity

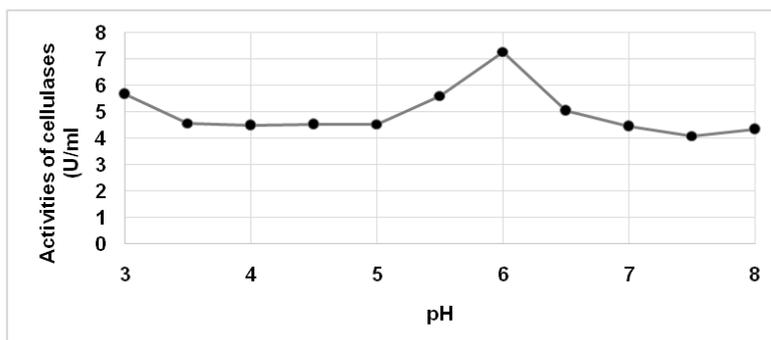


Fig. 4: Effect of pH on the activities of cellulases from *Macrotermes gilvus*, all values are reported as mean±SD (Standard deviation), triplicates

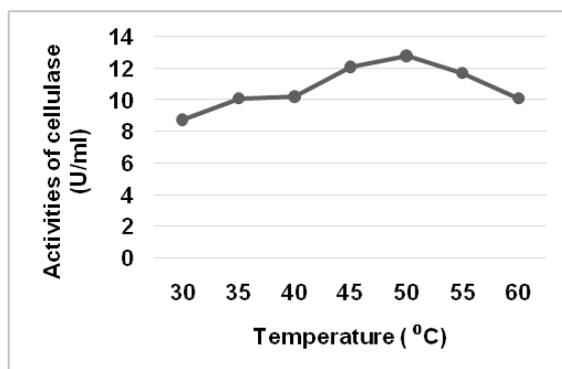


Fig. 5: Effect of temperature on the activities of cellulases of *Macrotermes gilvus*, all values are reported as mean±SD (Standard deviation), triplicates

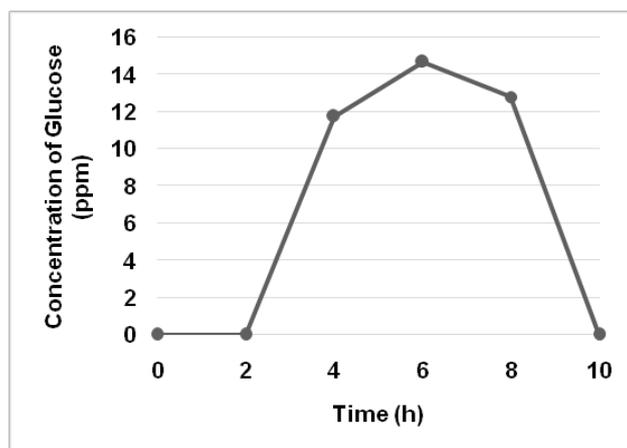


Fig. 6: Optimization duration of hydrolysis, all values are reported as mean±SD (Standard deviation), triplicates

Table 2: Results of screening of cellulase activities based on the formed clear zones in CMC media

Mold	The diameter of the clear zone (mm)	The diameter of the petri dish (mm)	Cellulolytic index
TR	50	6±0.00	7.3
MG (5 µl)	42	6±0.00	6
MG (10 µl)	49	6±0.00	7.16

TR= *Trichoderma reesei*, MG= *Macrotermes gilvus*, All values are reported as mean±SD (Standard deviation), triplicates

Quantitative test (DNS method)

The linear regression equation $y = 0,0099x - 0,4097$; $R^2 = 0,9963$ was then applied to calculate glucose concentration and cellulase enzyme activity in the sample.

Preparation and characterization of MCC by enzymatic hydrolysis

Alpha-cellulose, buffer, and purified enzyme were mixed and stored to incubator shaker settled at 50 °C, speed of 125 rpm for 2 h (according to the temperature, pH and optimal hydrolysis time) to produce MCC. Then, the obtained MCC was identified and characterized as below.

The qualitative identification resulted in a violet purple after ZnCl drops were added of both two samples that the samples contained cellulose was indicated. According to *Werner's Nomenclature of Color* by Syme (1821), the violet color of Avicel PH 101 has *Purples No. 40 (Imperial Purple)* and the sample has *Purples No. 41 (Auricula Purple)*.

FTIR

Although there were small differences between the reference and the sample, the FTIR spectrum showed a common characteristics spectrum of MCC (fig. 8).

Table 3: Results of the yield of MCC

Mold	Weight of alpha-cellulose	Weight of MCC	Yield
TR	10	8.75	87,5 %
CC	10	8	80 %

n=1

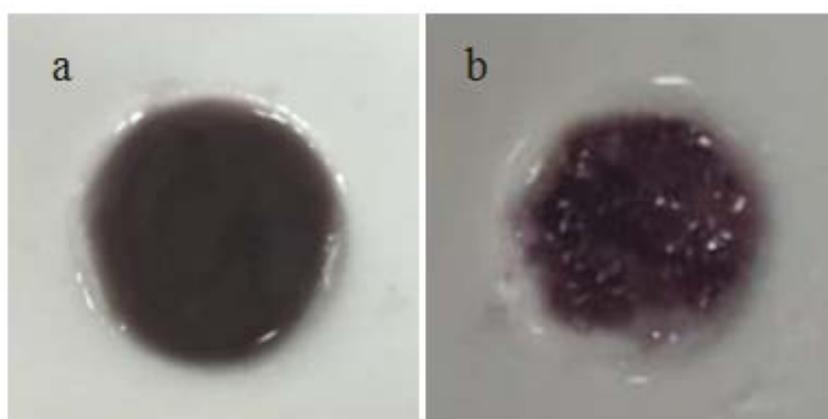


Fig. 7: Qualitative identification of samples using ZnCl (left: Avicel; right: sample)

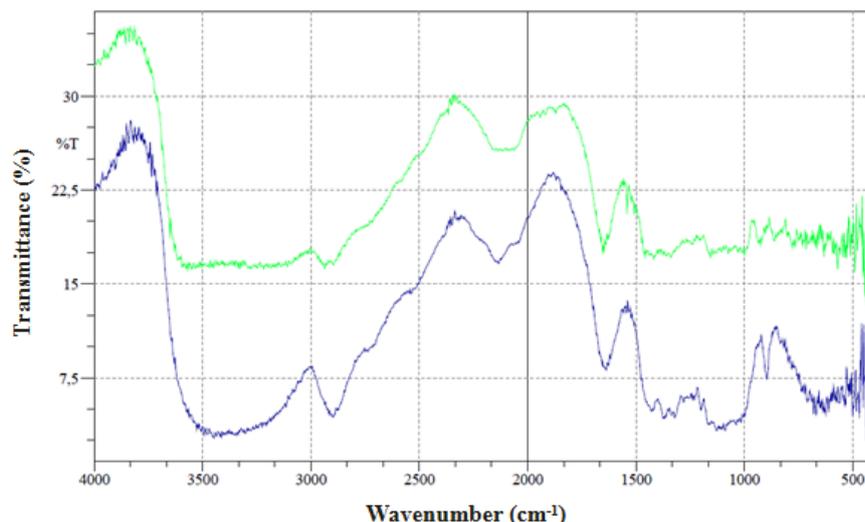


Fig. 8: Comparison of the results of avicel PH-101 (a, green) reference FTIR spectrum with the sample (b, blue)

MCC that produced in this study was characterized using FTIR. Characterization was using FTIR aimed to determine the functional groups in the sample. Fig. 8 shows a generated FTIR spectrum from the sample. The FTIR spectrum of the sample showed peaks at wavenumbers 3512.49, 2908.75, 1647.26, 1419.66, 1163.11, and 898.86. While the Avicel spectrum as a reference shows peaks at numbers 3576.14, 2937.68, 1647.26, 1419.66, 1155.4, 923.93. The peaks indicate the presence of O-H, C-H, C = O, C-O-C, and C-O stretching groups, and C-H₂ and C-H bending.

Based on the infrared spectrum, it can be seen some functional groups. Like at a wavelength of 3500 cm⁻¹ a wide band was showed the presence of OH groups. In the 2895.25 cm⁻¹ was shown a C-H aliphatic bond and in the wavelength 1647.26 cm⁻¹ was frequencies of deformation vibrations of CH₂. The presence of glycosidic C-O-C was showed in the 1419.66 cm⁻¹ band and 898.86 cm⁻¹ was β-

glycosidic. Based on literature for Avicel as a reference was showed the following vibration peaks of cellulose: 3445 cm⁻¹ corresponding to intramolecular OH stretching, including hydrogen bonds, 2898 cm⁻¹ due to CH and CH₂ stretching, 1650 cm⁻¹ corresponding to OH from absorbed water, 1430 cm⁻¹ due to CH₂ symmetric bonding, 1375 cm⁻¹ due to CH bending, 1330 cm⁻¹ due to OH in-plane bending, 1161 cm⁻¹ due to C-O-C asymmetric stretching, 1061 cm⁻¹ due to C-O-C stretching, and 898 cm⁻¹ corresponding to asymmetric (rocking) C1 (β-glycosidic linkage) out of the plane stretching vibrations.

Organoleptic test

From the produced MCC and comparison to the reference Avicel PH 101 (fig. 9), it can be seen that the color produced from the sample is almost the same as Avicel, except that the grain is still rather rough and not as smooth as Avicel PH 101, but the difference is not too significant.

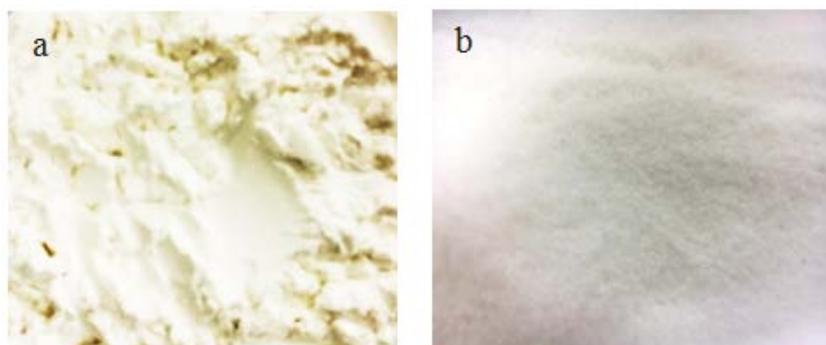


Fig. 9: The result of organoleptic on avicel PH-101 (a) and sample (b)

Table 4: Results of particle analysis on the sample with particle size analysis (PSA)

	dv10 (μm)	dv50 (μm)	dv90 (μm)	Mean (μm)
Reference Avicel PH-101	6.25	15.61	24.00	15.56
Sample	12.63	36.43	162.13	66.31

dv=diameter over volume, n=1 measurement

Size analysis and particle size distribution

Results of particle analysis on the sample using Particle Size Analyzer. Avicel PH 101 has 10% from the total of particle size 1,181 μm, 50% from the total of particle size 13,72 μm, and 90% from the total of particle size 30,52 μm with average 15,46 μm (table 4).

X-Ray diffraction analysis of MCC

From the result of XRD analysis, the crystal and the amorphous of the sample is shown on the value of 2θ (deg). In the hydrolysis result of the sample, there 2 peaks that indicated the existence of crystal character at 22.58 with intensity 634 and 21.85 with intensity 513.

For amorphous nature, there is 1 peak at 2θ (deg) value at 12.779 with intensity 418. The presence of crystalline and amorphous peak is influenced by the source of samples derived from kapok

pericarpium and the manufacture of α -cellulose using a strong base to remove lignin. The diffractogram from the sample is almost the same as Avicel, the difference is not too significant (fig. 10).

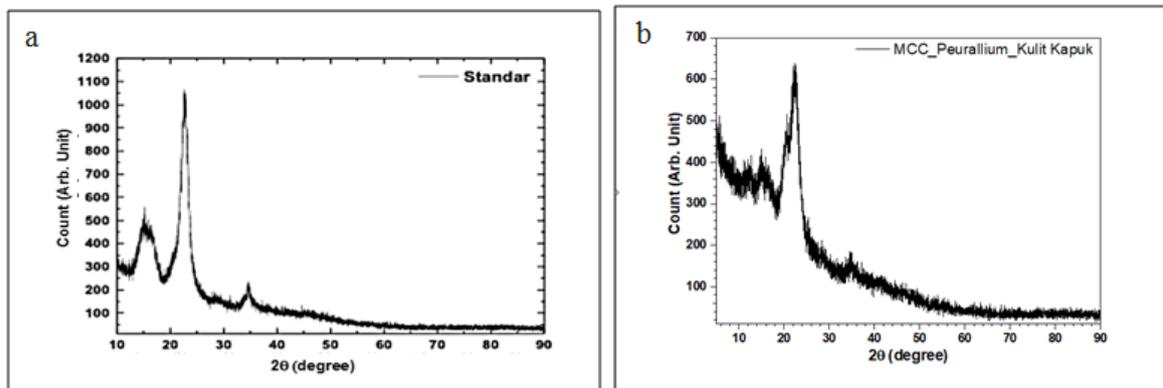


Fig. 10: Diffractogram of reference (a) and sample (b)

DISCUSSION

Biodelignification was carried out during 21 d using *Trametes versicolor*, the result of α -cellulose isolation obtained from 5 g of substrate powder was 3.8 g (76.0%). According to Balat, Balat, and Oz (2008) [14], biodelignification was *pre-treatment* for reducing the lignin component. Follow with the incubation process of kapok pericarpium and mycelium of *Trametes versicolor* in 30 d at 30 °C and for 21 d at 40 °C with the addition of nutrient solution. The mold of *Trametes versicolor* produces ligninolytic enzymes Laccase, Mn peroxidase (MnP), and Li peroxidase (LiP) [15, 16].

Purification process using Ammonium sulfate salt, dialysis, and column chromatography DEAE-C. In precipitation of ammonium sulphate, the process was carried out to purify the enzyme using the salting-out method assumed that at high salt concentrations (saturation) will increase the hydrophobic interaction between protein and water, where water will tend to be attracted by salt ions so that the solubility of the protein in water will decrease and protein deposition occurs. therefore these proteins will separate from each other depending on the different sizes of protein molecules contained in the solution [17].

The highest activity during the purification process was the first fraction (P1) from the column chromatography DEAE-C with the specific activity 11.1838 mU/mg and purity grade 1.51 times compare to the crude enzyme. Diethylaminoethyl cellulose (DEAE-C) is a positively charged resin [18]. P1 has the same ion charge as the matrix (resin DEAE-C) so that the enzyme can be get away from the column. The enzyme cellulase has a variation of ion charges when cellulase passed into the anion exchange matrix that has a positive charge, there will be fractionation based on affinity of ion charge [19].

The sample of cellulase used in this experiment is the supernatant of the crude enzyme from the isolate of the termites. The addition of Lugol as a dye to make it easier and measure that the clear zone formed. Lugol reagent will be bound at 1.4- β that will provide blue-black color of glycoside on cellulose. Meanwhile, the transparent color showed that cellulose has been hydrolyzed into monosaccharides, so that can not form a complex with iodine of Lugol. The ability to form a clear zone on a specific media indicates that the sample can produce the cellulolytic enzyme [20]. The change in pH affects the functional group ionization which can cause enzyme confrontation and its catalytic properties which will affect the hydrogen bonds contained in the enzyme. The change of enzyme confrontation can result in a decrease in enzyme activity caused by enzyme confrontation that is different from substrate confrontation. From the previous study, an optimum pH from the cellulose of *Macrotermes gilvus* of 8 was obtained for cellulose and temperature on the activity of cellulase showed the enzyme to have its optimum

temperature at 50 °C. In this study, optimum pH was 6.0 and the optimum temperature was 50 °C. This condition was used at reference, in which cellulases with pH values of 4.5 to 6.0 are common, and the optimum temperature was according to the previous study which was 50 °C [21].

Temperature is closely related to the energy needed by enzymes to react. The lower the temperature, the enzyme does not have enough energy to react. The optimum temperature of the enzyme can work optimally whereas if the enzyme is at a temperature optimum then the ability of the enzyme will decrease due to enzyme denaturation by heat. Optimum cellulase activity shows that environmental conditions (such as pH and temperature) are right for cellulase to react with the substrate at pH 6.0 and 50 °C [22]. All enzymes work at a certain temperature. Most enzymes have optimum activity in the temperature range of 20-50 °C. Whereas cellulase has optimum activity in the temperature range of 50-80 °C included in the thermozymic group or often referred to as thermolabel (heat resistant) [23]. Based on this study, cellulase obtained from *Macrotermes gilvus* belongs to the thermozymic group, stable at high temperatures.

Deng, Cui, Qi, Wang, Hou, and Zhu (2012) [24] reported the use of $ZnCl_2$ solution as a carbohydrate reaction medium which includes cellulose, glucose, sucrose, maltose, starch, and fructose. They confirmed that the $ZnCl_2$ solution with a concentration of 60% by weight can dissolve cellulose by forming a Zn-cellulose complex. So it can be concluded that the function of zinc iodinate is $ZnCl_2$ as a solvent for cellulose and iodine as a color modifier of cellulose to blackish blue-violet because iodine will give a violet-blue color to carbohydrate compounds and their derivatives.

Particle size and distribution analysis was performed using a micro Particle size analyzer (PSA) to determine the size and distribution of particles from the enzymatic hydrolysis samples from kapok pericarpium compared to the comparative sample Avicel PH 101. The results of particle size and distribution analysis depend on acid concentration, temperature, time, hydrolysis procedures, and mechanical processes [25].

The results of particle distribution analysis is known that the comparator Avicel PH 101 has 10% of the total particle size has a particle size of fewer than 1,181 μm , 50% of the total particle size has a particle size of less than 13.72 μm , and 90% of the total particle size has particle size is less than 30.52 μm with an average of 15.46 μm . The FTIR spectrum of the sample showed spectrum characteristics similar to microcrystalline cellulose.

Based on the XRD spectrum, Avicel has type I and the sample has type II. This is because the MCC sample comes from nature and under the heating process with sodium, forming sodium cellulose

causes a change in crystal type from I to II [26, 27]. The formation of crystals is also affected by the heating, where hydrolysis is carried out to cut cellulose into shorter chains, whereas the shape of the crystal depends on the heating temperature, the higher the temperature, the more the shape of the crystal [28].

CONCLUSION

As the conclusion, the highest yield of 93% of MCC was obtained from the hydrolysis of alpha-cellulose using cellulase from *Macrotermes gilvus*. The optimum Hydrolysis condition was achieved at 50°C, pH 6.0, in 2 h, which produced 80% yield of MCC. The *Macrotermes gilvus* was considered as the best source of cellulase with suitable activity for the preparation of MCC. Cellulase from termites *Macrotermes gilvus* has a high activity with clear zone area 49 mm. Organoleptic and qualitative test, FTIR spectrum, PSA and XRD, there was a similar characteristic of crystalline between MCC from kapok alpha-cellulose and Avicel PH 101 as a reference.

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Nil

AUTHORS CONTRIBUTIONS

All authors have equal contributions

CONFLICT OF INTERESTS

All authors declare there is no conflict of interest.

REFERENCES

- Friday ET, James O, Gabriel A. *In vitro* growth and inhibition studies of ceiba pentandra leaf: a common vegetable in nigeria. Int J Plant Physiol Biochem 2011;3:1.
- Astika JM, Chandrawati C, Hendrajaya I, Soemarno, Yogi O. The exploration of alpha-cellulose in kapok fruit as raw material for rocket propellant production. Agri Res Tech: Open Access J 2017;12:1-8.
- Hindi SSZ. Microcrystalline cellulose: the inexhaustible treasure for the pharmaceutical industry. Nanosci Nanotech Res 2017;4:17.
- Fuadi AM, Harismah K. Perbandingan efektifitas pembuatan glukosa dari kertas bekas secara hidrolisis asam dan enzim. Jurn Tek Bah Al 2017;1:6.
- Suryadi H, Sutriyo, Sari HR, Rosikhoh D. Preparation of microcrystalline cellulose from water hyacinth powder by enzymatic hydrolysis using cellulase of local isolate. J Young Pharm 2017;9:19-23.
- Fagbohunka BS, Okonji RE, Adenike AZ. Purification and characterization of cellulase from *Termite Ametermes eveuncifer* (Silverstri) soldiers. Int J Bio 2016;9:2-7.
- Archibalt FS, Bourbonnais R, Jurasek L, Paice MG, Reid ID. Kraft pulp bleaching and delignification by *Trametes versicolor*. J Biotech 1997;53:215-36.
- Suryadi H, Lestari YPI, Mirajunnisa, Yanuar A. Potential of cellulase of *Chaetomium Globosum* for preparation and characterization of microcrystalline cellulose from water hyacinth (*Eichhornia crassipes*). Int J Appl Pharm 2019;11:140-6.
- Begum MF, Absar N. Purification and characterization of intracellular cellulase from *Aspergillus oryzae* ITCC-4857.01. The Kor Soc of Myc 2009;37:121-7.
- Hasanah N, Saskiawan I. Aktivitas selulase isolat jamur dari limbah media tanam jamur merang. Pros Sem Nas Masy Biodiv Indon 2015;1:1110-5.
- United States Pharmacopoeial Convention. The United States Pharmacopeia, USP 30/The National Formulary, NF 25. Rockville, MD: U. S. Pharmacopoeial Convention. Inc; 2007. p. 2635.
- Harmita. Analisis Fisiko Kimia. Depok: Departemen Farmasi FMIPA Universitas Indonesia; 2006.
- British Pharmacopoeia Commission. British Pharmacopoeia. London: Stationery Office; 2008.
- Balat M, Balat H, Oz C. Progress in bioethanol processing. Prog Energy Combust Sci 2008;34:551-73.
- Moreira MT, Viacava C, Vidal G. Fed-batch decolorization of poly R-478 by *Trametes versicolor*. Braz Arch Biol Technol 2004;47:179-83.
- Knezevic A, Stajic M, Jovanovic VM, Kovacevic V, Cilerdzic J, Milovanovic I, et al. Induction of wheat straw delignification by *Trametes* species. Sci Rep 2016;6:1-12.
- Bollag DM, Edelman SJ, Protein methods willey-liss inc. New York; 1991.
- Rousseau RW, Ferrell JK, Reardon RF. Synthesis of diethylaminoethyl cellulose on cotton fabric. Indust Eng Chem Prod Res Dev 1986;3:250-2.
- Boyer RF. Modern experimental biochemistry. 2nd Edition. California: The Benjamin Cummings Publishing Company; 1993.
- Zhang YH, Himmel ME, Mielenz JR. Outlook for cellulase improvement: screening and selection strategies. Biotechnol Adv 2006;24:452-81.
- Mandels M, Reese ET. Introduction of cellulose in fungi by cellobiose. J Bact 1976;79:816-26.
- Whittaker JR. Principles of enzymology for the food sciences. 2nd Edition. New York: Marcel Decker; 1994. p. 67-70.
- Meryandini A, Widosari W, Maranatha B, Sunarti TC, Rachmania N, Satria H. Isolasi bakteri selulolitik dan karakterisasi enzimnya. Makara J Sci 2009;13:33-8.
- Deng T, Cui X, Qi Y, Wang Y, Hou X, Zhu Y. Conversion of carbohydrates into 5-hydroxymethylfurfural catalyzed by ZnCl₂ in water. Chem Commun 2012;48:5494-6.
- Das K, Ray D, Bandyopadhyay N, Ghosh T, Mohanty A, Misra M. A study of the mechanical: thermal and morphological properties of microcrystalline cellulose particles prepared from cotton slivers using different acid concentrations. Cellulose 2009;16:783-93.
- Mandal A, Chakrabarty D. Isolation of nanocellulose from wax sugarcane bagasse (SCB) and its characterization. Carbohydrate Polymer 2011;86:1291-9.
- Klemm D, Philipp B, Heinze U, Heinze T, Wegenknecht W. Comprehensive cellulose chemistry. Willey FCH 1998;2:31-43.
- Aulton ME. Aulton's pharmaceuticals: the design and manufacture of medicines. 3rd Edition. New York: Churchill Livingstone Elsevier; 2007. p. 412, 450.