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Optimization of Acid Concentration and Hydrolysis Time in the Isolation of Microcrystalline Cellulose from Water Hyacinth (*Eichornia crassipes* solm)

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ABSTRACT

Water hyacinth (*Eichornia crassipes*) is an aquatic plant that can disrupt aquatic ecosystems. This plant contains high cellulose and has the potential to be a source of microcrystalline cellulose (MCC); therefore, it has high economic value. This study aims to determine the optimal hydrolysis conditions to isolate MCC from water hyacinth. The optimum conditions were designed with a 3^2 factorial design with acid concentration and hydrolysis time as independent variables and MCC physical properties as dependent variables. Based on the DX®10 analysis, the optimum conditions were obtained at an acid concentration of 1.5 M HCl for 30 minutes. Under this optimum condition, the yield of MCC was 91.70% with the angle of repose of 20.695 and moisture absorption capacity of 3.24%. Furthermore, the FTIR and X-ray spectra indicated that MCC of water hyacinth had the same peaks like that of Avicel® PH101.

Keywords: Water hyacinth, Microcrystalline cellulose, Hydrolysis, Avicel.

Introduction

Water hyacinth (*Eichornia crassipes*) is one of the fastest growing-aquatic plants which is considered as weed. The existence of water hyacinth (WH) in the water surface causes several environmental problems. However, WH potentially adsorbs pollutants from the water. It's ability to adsorb water pollutants such as, Cadmium, lead and copper, and dyes have been reported in other studies.[1–4] Aside from being an adsorbent, WH also shows pharmacological activities such as antioxidant, antimicrobial, antifungal and anticancer.[5–7]

Water hyacinth can be an economic problem because it has a negative impact on aquatic ecosystem, slowing or preventing water flow, inhibiting irrigation and slowing hydroelectric power generation.[8] Various strategies have been adapted to control this weed, and most promising approach is the effective utilization of the plant for commercial purpose and for contributing to solve environmental problems. [9]

Previous study has shown that, chemical content of WH fibers are around 60% cellulose, 8% hemicellulose and 17% lignin.[10–12] Based on its cellulose content, WH has the potential source of microcrystalline cellulose (MCC) which has high economic value.[13] Microcrystalline cellulose is widely used in the pharmaceutical, cosmetic and food industries.[14] MCC is also used as an excipient in tablet manufacturing because of its neutrality, nontoxic, hygroscopicity and can produce tablets with a high level of hardness but short disintegration time.[15-16] Previous studies have successfully produced nano crystalline cellulose (NCC) from WH and the isolation was carried out under one hydrolysis condition with 5 M HCl.[12] Hydrolysis is a suitable method for producing MCC. The time and acid concentration factors can be optimized.[17] The acid hydrolysis method is preferred because the reaction conditions are easier to control and with a shorter production process-time per batch.[14] Different conditions of the hydrolysis process will produce the MCC with different properties.[18] Therefore, this study aims to optimize the acid concentration and hydrolysis time of the MCC isolation from water hyacinth. The optimal conditions were determined by using a 3^2 factorial design with the hydrolysis time and acid concentration as independent variables. The physicochemical properties of microcrystalline cellulose yield as dependent variable were compared to Avicel® PH101.

Materials and Methods

Material

Water hyacinth was collected from Indralaya village, South Sumatra, Indonesia in January 2019. The plant was identified in Indonesian Institute of Sciences. The voucher specimen (Voucher number: HBSU/1009/18) was deposited at Biology Department of Sriwijaya University. Avicel® PH101 was purchased from Sigma Aldrich and all chemicals were analytical grades.

Determination percentages of cellulose, hemicellulose and lignin

The percentages of cellulose, hemicellulose and lignin in the water hyacinth were determined using Chesson-Datta method.[19] Lignin removal and alpha cellulose extraction from the water hyacinth was based on the pulping procedures.[20]

Microcrystalline cellulose preparation

MCC preparation was carried out in nine conditions based on variations in acid concentration and hydrolysis time determined with a 3^2 factorial design. Variations in acid concentration and hydrolysis time are shown in Table 1. A 50 g α-cellulose mass was hydrolyzed under the conditions as presented in Table 1. The hot mixture was poured into cold water while stirring vigorously with a spatula and allowed to stand overnight. After the microcrystalline cellulose was washed, it was then dried in an oven at 57-60^{o}C for 60 minutes. Finally, it was milled and sieved by using a mesh sieve no. 25.[20]

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Characterization of microcrystalline cellulose (MCC)
The physicochemical and powder properties of microcrystalline cellulose were characterized by using a procedure as presented on a microcrystalline cellulose study of groundnut water.[20]

Statistical analysis
DX®10 software (Stat-Ease Inc. USA) was used for MCC analysis on the nine formula conditions. Minitab®17 and SPSS®17 (one sample t-test) were used for the analysis of the optimum formula and percentage calculation of residual standard error (RSE%).

Optimum hydrolysis condition
The optimum conditions of MCC hydrolysis from WH were determined based on the highest desirability value of the specified parameters. Comparative tests were carried out by using the Minitab® 17 program between predictive data (from the DX® 10 program) and actual data (from the research). Calculation of residual standard error (RSE) or the percentage of error was done to ensure the accuracy of predictive data using equation (1).

$$\%\,RSE = \frac{research\ data - prediction\ data}{prediction\ data} \cdot 100\% \quad \text{.....(1)}$$

The optimum conditions of MCC from WH suggested by the DX®10 program were compared to Avicel® PH101. Furthermore, MCC obtained from the optimum conditions was characterized to analyze the crystallinity index and IR spectra.

Crystallinity index determination
The crystallinity index was determined using a Xray Diffractometer (Rigaku® Mini Flex 600 USA) at a voltage of 40 kV and a current of 35 mA. Samples were measured in a solid form.

The crystallinity index was determined by Segal's equation (Eq.2).[21]

$$\%\,Cristallinity\ Index = \left(\frac{I_{22} - I_{am}}{I_{22}}\right) \cdot 100 \quad \text{.....(2)}$$

I_{22} = the maximum intensity of the 002 lattice diffraction ($2\theta \sim 22^{o}$)

I_{am} = the maximum intensity diffraction of amorphous part ($2\theta \sim 18^{o}$)

Analysis of functional groups
Microcrystalline cellulose from optimum conditions were analyzed by using IR spectrophotometer (Thermo Scientific®) to determine functional groups. The MCC spectrum was compared to the spectrum of Avicel® PH101.

Results and Discussions

Percentage of cellulose, hemicellulose and lignin
Water hyacinth contains biomass components of cellulose, hemicellulose and lignin. The analysis showed that the content of cellulose, hemicellulose and lignin in the WH sample were 46.15%; 13.46% and 23.07% respectively. The percentages of cellulose, hemicellulose and lignin in our research were different from those reported in previous research.[10,12] This difference may be due to the different types and concentrations of solvents used.
The isolation of microcrystalline cellulose was performed using the acid hydrolysis method. The acid hydrolysis can damage the amorphous regions of cellulose and produce crystalline.[22] Microcrystalline cellulose produced through this process were in the form of white powder. The transformation from alpha cellulose to microcrystalline cellulose occurred due to termination of the amorphous chain to produce cellulose with a micro size that has a high crystalline index.[23]

Characteristics of microcrystalline cellulose
Microcrystalline characteristics obtained from nine conditions indicate organoleptic properties as specified in British Pharmacopeia 2004 in the form of odourless fine white powder (Figure 1). The characteristics of MCC powder from 9 treatments is summarized in Table 2. The MCC samples have the Carr's index in the range of 24−36 %. This index indicates that the samples have poor flow properties The smaller size of particles causes greater compressibility, and consequently the powder is more difficult to flow.[24] Based on ANOVA analysis, the acid concentration had a significant effect on all MCC characteristics ($p<0,05$). However, the hydrolysis time and the interaction between the two factors had no significant effect on the Car's index response ($p>0,05$). DX®10 analysis showed that the acid concentration and hydrolysis time could increase the response of Carr's index (Equation 3). The Hausner ratio has a value that is directly proportional to the Carr's index. MCC from hydrolysis at conditions 1-3 has poor flow properties because the value was more than 1.5.[25] Beside increasing the Carr's index response, the acid concentration could also increase the response of Hausner ratio. Contrary, hydrolysis time caused a decrease in the Hausner ratio (Equation 4).

Table 1: Variation in Acid Concentration and Hydrolysis Time

Treatment	Acid concentration HCl (N)	Time (minutes)
F1	1	30
F2	1	45
F3	1	60
F4	1.5	30
F5	1.5	45
F6	1.5	60
F7	2	30
F8	2	45
F9	2	60

Figure 1: Microcrystalline cellulose from water hyacinth in nine hydrolysis conditions

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Table 2: Characteristics of MCC from water hyacinth

No	Ph	True Density (g / ml)	Relative Density (g/ml)	Carr's Index (%)	Hausner Ratio (%)	Hydration Capacity (%)	Swelling Capacity (%)	Moisture sorption capacity	Angle of repose	Yield (%)
1	6.81 ± 0.03	0.26 ± 0.00	0.42 ± 0.00	36.20 ± 0.34	1.57 ± 0.01	27.76 ± 0.02	51.07 ± 1.86	3.50 ± 0.01	26.60 ± 0.82	94.24 ± 0.43
2	6.75 ± 0.03	0.28 ± 0.00	0.46 ± 0.00	34.60 ± 0.58	1.53 ± 0.01	29.90 ± 0.01	50.40 ± 0.70	3.35 ± 0.01	24.28 ± 0.75	92.63 ± 0.53
3	7.16 ± 0.02	0.31 ± 0.00	0.46 ± 0.01	33.60 ± 0.47	1.50 ± 0.01	30.43 ± 0.03	50.79 ± 1.37	3.32 ± 0.00	22.46 ± 1.03	92.51 ± 0.82
4	7.26 ± 0.03	0.36 ± 0.00	0.52 ± 0.00	30.91 ± 0.49	1.44 ± 0.01	31.12 ± 0.02	21.66 ± 2.88	3.22 ± 0.01	20.69 ± 0.08	91.770.26
5	6.86 ± 0.03	0.42 ± 0.02	0.60 ± 0.02	29.50 ± 1.05	1.41 ± 0.02	31.42 ± 0.02	26.66 ± 2.88	3.22 ± 0.02	19.63 ± 0.29	88.54 ± 0.36
6	7.22 ± 0.04	0.47 ± 0.01	0.65 ± 0.02	28.73 ± 1.66	1.40 ± 0.03	31.67 ± 0.06	25.00 ± 0.00	3.21 ± 0.01	18.34 ± 0.21	87.80 ± 0.26
7	7.11 ± 0.02	0.53 ± 0.03	0.73 ± 0.02	27.57 ± 2.84	1.38 ± 0.05	32.35 ± 0.03	25.00 ± 0.00	3.19 ± 0.01	17.21 ± 0.13	86.64 ± 0.36
8	6.95 ± 0.02	0.65 ± 0.07	0.89 ± 0.06	26.77 ± 2.81	1.36 ± 0.05	32.79 ± 0.02	12.50 ± 0.00	3.04 ± 0.00	16.22 ± 0.63	81.81 ± 0.40
9	6.94 ± 0.02	0.75 ± 0.07	1.00 ± 0.04	24.75 ± 4.16	1.33 ± 0.07	33.00 ± 0.01	16.66 ± 0.00	2.93 ± 0.00	14.74 ±	69.43 ± 0.17
Avicel PH101	7.35 ± 0.03	0.35 ± 0.00	0.48 ± 0.00	27.91 ± 0.22	1.38 ± 0.00	31.14 ± 0.01	20.00 ± 0.00	3.22 ± 0.08	20.16 ± 0.44	-

$Y_1 = 30.29 + 4.51A[1] - 0.57A[2] + 1.27B[1] -1.71B[2] + 13A[1]B[1] - 0.071A[2]B[1] - 0.20A[1]B[2]-0.21A[2]B[2]$ ……(3)

$Y_2 =11.99+0.39A[1]-0.06A[2] + 0.11B[1]-3.10B[2]+0.024A[1]B[1] - 8.35A[2]B[1] - 0.01A[1]B[2] - 0.01A[2]B[2]$ …..…….(4)

$Y_3 = 31.16 - 1.80A[1] + 0.24A[2] - 0.75B[1] + 0.21B[2] - 0.85A[1]B[1] + 0.47A[2]B[1] + 0.33A[1]B[2] 0.20A[2]B[2]$……..…….(5)

$Y_4 = 3.22 + 0.16A[1] +1.90A[2] + 0.08B[1] - 0.01B[2] + 0.02A[1]B[1] - 0.07A[2]B[1] - 0.02A[1]B[2] + 0.02A[2]B[2]$……..…….(6)

$Y_5 = 20.02 + 4.42A[1] - 0.46A[2] + 1.48B[1] + 0.02B[2] + 0.67A[1]B[1] - 0.34A[2]B[1] - 0.18A[1]B[2] + 0.05A[2]B[2]$……...(7)

$Y_6 = 87.06 + 5.85A[1] + 2.17A[2] + 3.66B[1] + 0.33B[2] - 2.46A[1]B[1] - 1.19A[2]B[1] - 0.84A[1]B[2] - 1.131A[2]B[2]$……..……...(8)

Where;
Y_1 = Carr's index response; Y_2 = Hausner ratio response ; Y_3 = Hydration capacity response; Y_4 = Moisture sorption capacity response; Y_5 = Angle of repose response; Y_6 = Yield percentage response A = Acid concentration; B = Hydrolysis time.

The hydration capacity value increased with the increasing acid concentration and hydrolysis time (Equation 5). The lowest hydration capacity (at 27.757%) was as a result of amorphous samples. This characteristic causes the MCC to more easily attract ions from the water; and it thus becomes more soluble. Consequently, the weight of the sediment produced decreased. The hydration capacity of condition 4 was 31.117% which had similar value with Avicel®PH101 result of 31.146%.

The value of moisture absorption capacity and the angle of repose decreased with increasing acid concentration and hydrolysis time (equation 6 and 7). The increasing of two factors would increase the crystalline form, making it difficult for water to penetrate into the powder structure, consequently reducing the moisture absorption capacity. The angle of repose of MCC on the condition 4 showed a similarity to Avicel® PH101. Based on the relationship between the angle of repose and flow properties, both of them have excellent flow properties because they had an angle of repose less than 25°. The yield measurement aims to find out the total MCC under certain hydrolysis conditions. The yield of MCC was calculated by comparing the weight of hydrolyzed microcrystalline cellulose to the dry weight of α-cellulose. An increase in acid concentration and hydrolysis time dissolved more glucose monomers during washing so that they decreased microcrystalline yield. The high acid concentrations and long reaction times led to excessive degradation of cellulose allowing the acid to penetrate more rapidly into the tissue layer to hydrolyze the cellulose and then hydrolyze the amorphous regions of the cellulose crystals.[26]

Determination of Optimal Conditions

The optimum conditions were determined based on three parameters, namely the repose angle, the yield percentage, and the water sorption capacity. These parameters were considered the most important priority responses in the MCC manufacturing process. The percentage of yield has importance (+++++) because it affects the number of MCC generated. The repose angle is related to the flow properties of the MCC in tablet manufacturing. While the moisture sorption plays an important role to stabilize the MCC and cellulose-based tablets stored in humid conditions.[20]

Design Expert®10 analysis using a factorial design showed that the condition had the desired specifications of 0.986. A desirability value indicates that the acid concentration and hydrolysis time predicted from the program will produce an MCC with the desired criteria. The results of the optimal conditions from the DX® 10 program were acid concentration of 1.5 N and hydrolysis time of 30 minutes. This condition may produce MCC with a yield of 91.71%, the moisture sorption capacity of 3. 24 and angle of repose 20.69.

Comparative analysis of Microcrystalline Cellulose

Observed data was compared to predicted data from DX® 10 by using one sample t-test. There was no significant difference between these data. The results of the analysis are shown in the table 3. Residual Standard Error (RSE) analysis was performed to assess the accuracy of the data. The smaller % RSE value indicates more accurate prediction.[27] The % RSE values of all responses were less than 1%. These results indicate that the observed data has a good agreement with predicted data.

FTIR Spectrophotometry

Measurement by FTIR (Thermo Scientific®) spectrophotometry was carried out on MCC produced under optimal conditions compared with commercial microcrystalline cellulose Avicel ® PH101. The results of the analysis can be seen in Figure 2. The infrared spectrum indicates the same absorption pattern in the functional group region between WH microcrystalline cellulose and Avicel PH101.

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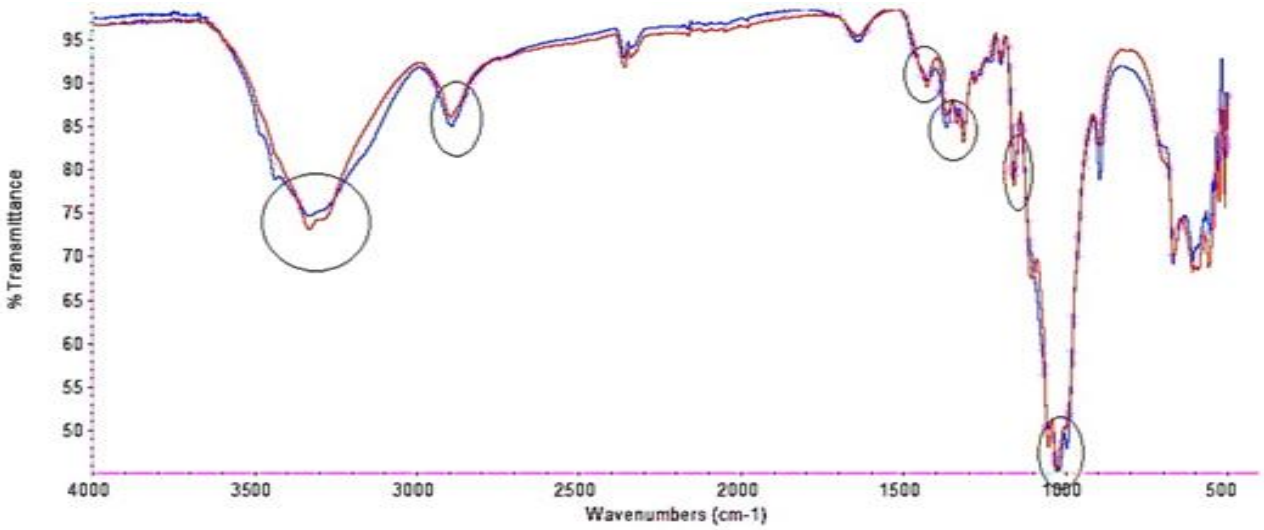

Figure 2: The FTIR spectra of the microcrystalline cellulose from water hyacinth and Avicel ® PH-101. Red and blue lines denote Microcrystalline cellulose of WH and Avicel ® PH-101, respectively.

Table 3: Comparative Analysis of Prediction Data and Observed Data

The response	Prediction	Research ± SD	% RSE	*p-value*
Yield	91.71	91.77 ± 0.26	0.063	0.99
Angle of repose	20.69	20.69 ± 0.07	0.002	0.99
Moisture absorption capacity	3.24	3.22 ± 0.01	0.002	0.97

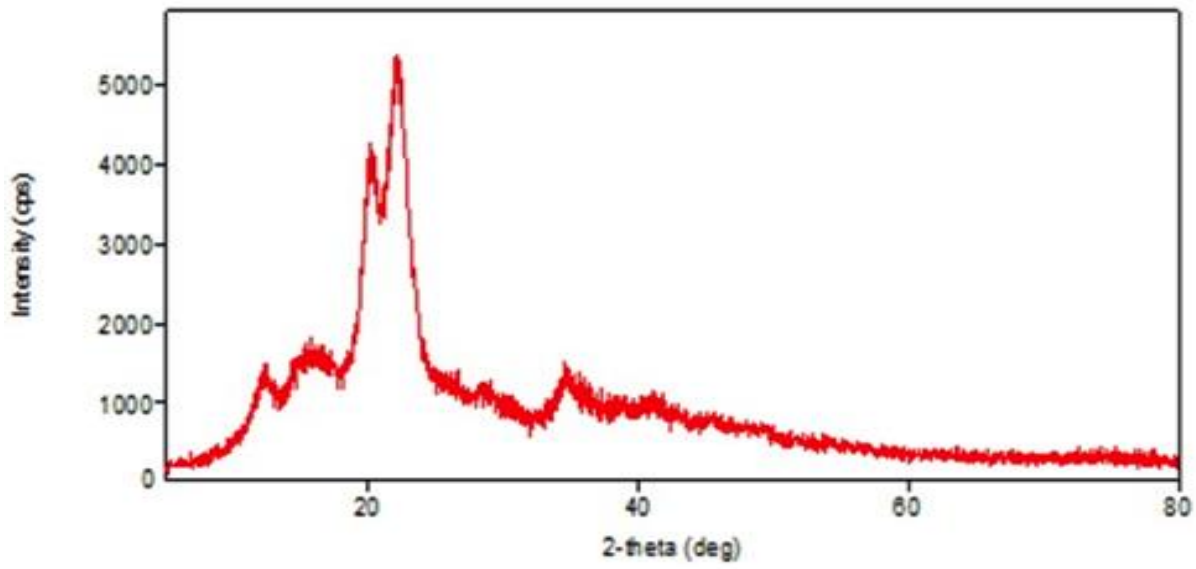

Figure 3: XRD microcrystalline cellulose from water hyacinth

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The FTIR spectroscopy peaks of the microcrystalline cellulose at 2900- 2800 cm^{-1} and 3350 -3300 cm^{-1} are related to the stretching of aliphatic C-H bonds and O-H bonds, respectively. The peaks in a range of 1470-1360 cm^{-1} indicate that there are C-H stretching. While the peak at 1200-1000 cm^{-1} is associated with C-O-C stretching. The FTIR spectrum shows that there are no peaks around 1700 cm^{-1}. This indicates that after the chemical process of the cellulose, the non-cellulose fiber content were lost because they had been dissolved by the solvent.[28]

Crystallinity index

Figure 3 shows that the MCC of the optimal conditions had a higher peak at the value of $2\Theta = 22.17^{O}$. Meanwhile, the lattice (002) and amorphous were at the value of $2\Theta = 22.17^{O}$ and $2\Theta = 15.83^{O}$ with intensities of 2637 and 574 respectively. The peaks in the MCC spectrum showed more clearly that the acid hydrolysis process was able to remove some amorphous material from cellulose alpha.[29] From our calculation, the crystallinity index of MCC was 78.23%. This value indicates that the sample belongs to microcrystalline cellulose because the value of the normal crystallinity index ranges from 55 to 80 % depending on the source of cellulose and reaction conditions.[30,31] Avicel®PH101 has a crystallinity index of 77.27%.[21] Some studies reported the percentage value of MCC crystallinity are different from various sources: kenaf is 70%; peanut shell is 74%, bagasse is 76%, rice straw 78%, cotton stalks 77% and sisal fiber (*Agavae sisalana Perrine*) 60%.[32-35] Empty Fruit Bunch Palm Oil 73%.[36] The crystalline index value of MCC from WH was not much different than that of the Avicel® PH101 and of previous studies.

Comparison of optimal MCC with Avicel® PH 101

The optimized microcrystalline cellulose of WH samples were compared with Avicel ®PH101 to ensure that the MCC produced was in accordance with the standard of commercially available microcrystalline cellulose such as Avicel ® PH101. The results of the evaluation of the two microcrystalline cellulose can be seen in Table 4. Table 4 shows that MCC produced from the optimization was insignificantly different from that of Avicel® PH101.

Conclusion

The optimal conditions for the isolation of microcrystalline cellulose from water hyacinth were at 1.5 N acid concentration and 30-minute hydrolysis time. The characteristics of microcrystalline cellulose from water hyacinth produced under those conditions had similar characteristics to Avicel®PH101. The XRD analysis showed a crystalline index value of 78.23% similar to Avicel® PH101 with a crystalline index of 77.27 %.

Conflict of interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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