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Optimization Study and Characterization of Microcrystalline Cellulose Isolated from Coconut Husk via Hydrolysis using Ionic Liquid

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ABSTRACT

This paper reports the optimization study and characterization of microcrystalline cellulose (MCC) isolated from coconut husk via hydrolysis using ionic liquid. Three process parameters, namely solvent concentration, temperature and reaction time were optimized using response surface methodology. The responses were measured in terms of percentage of solubility. Analysis of variance was carried out to obtain the most significant parameter influencing the solubility of MCC. It has been observed that the optimum solubility of MCC produced is 37.0±2.0% at optimum conditions of 90.9 °C, 92.3 minutes and solvent concentration of 65.6 wt %. Fourier transform infrared spectra have further confirmed the removal of lignin and hemicellulose from coconut husk, suggesting that almost pure MCC has been produced. Bulky form of MCC in micro-size ranging from 10-100 µm was observed from scanning electron microscopy micrographs, probably been caused by the inappropriate drying method. It is expected that a smaller size of crystalline cellulose could be prepared if a proper drying method such as freeze dryer was implemented.

Keywords: Microcrystalline Cellulose, Coconut Husk, Ionic Liquid, Response Surface Methodology.

1. INTRODUCTION

Cellulose is the most common organic polymer that available abundantly across the world. It represents about 1.5 x 10^{12} tons of the total production of biomass annually and is considered as the most promising source of renewable material to fulfill the increasing demand for environmentally-benign and safer products [1]. Cellulose can be categorised into three types; microcrystalline cellulose (MCC), microfibrillated cellulose (MFC) and bacterial microcellulose (BMC) [2]. Among them, MCC attracted the most attention due to its remarkable physical and optical properties, easiness to prepare and a wide range of applications. Cellulose has been reported to be produced from a myriad of materials including wood, plant, algae, tunicate, bacterial and lignocellulosic biomass [3].

Lignocellulosic biomass is cheap, abundant and easily available, hence gaining a reputation as the most suitable source for MCC preparation. Numerous lignocellulosic materials are reported to consist high cellulose content including coir fibres, corn cob, pineapple leaf fibres, rice straw, sugarcane bagasse and coconut husk [3]. Coconut husk has been reported to consist up of 69 wt % of cellulose, which makes it an ideal source for the production of MCC [4]. MCC has been commonly produced via acid hydrolysis using strong acids such as sulphuric acid, hydrochloric acid and hydrobromic acid. However, this conventional hydrolysis method faces various issues

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and problems including time-consuming method, the difficulty for solvent recovery, hazardous and requiring corrosion resistant reactors. These drawbacks motivate researchers to discover new environmentally-friendly solvent for MCC preparation. Ionic liquid (IL) is a new class of solvent that recently received much attention as safer and environmentally-benign solvent [5]. It has been touted as the promising replacement for traditional volatile organic solvents due to its fascinating properties such as non-volatility, non-flammability, high thermal stability and easily recyclable [6]. Thus, one type of IL namely 1-butyl-methylimidazolium chloride (BmimCl) is used in this study for the preparation of MCC. BmimCl containing halide (Cl^-) and it is widely reported to have high ability to dissolve cellulose efficiently by breaking down the strong intra- and inter-molecular interactions that are present in the cellulose structure [7]. Although there are extensive works that have been carried out for the preparation of MCC using IL, the optimization study in the dissolution process is still lacking. Hence, the optimum operating conditions for MCC preparation were determined by investigating three process parameters; solvent concentration, temperature and reaction time. The as-produced MCC was then characterised using Fourier transform infrared (FTIR) and scanning electron microscopy (SEM), respectively to confirm the removal of lignin and hemicellulose from coconut husk, and to explore the morphology of the MCC.

2. RESEARCH METHODOLOGY

2.1 Materials

Unripe coconut husk fibres were collected from fruit stalls in Padang Besar, Perlis, Malaysia. The chemicals such as BmimCl and sodium hydroxide (NaOH) were acquired from Sigma Aldrich, Malaysia and used as received.

2.2 Preparation of Raw Material and Pre-Treatment

The coconut husk was cut into smaller pieces and dried at 80 °C for 4 hours in an oven (Model UF110, Memmert, Schwabach). Next, the dried-husk was ground using a grinder to reduce its size to fine powder form. 10 g of ground-fibres were dispersed in 250 mL distilled water for 10 min. Then, the suspension was stirred for 2 hours at 60 °C and filtered in order to eliminate the soluble extractives in water. This step was repeated for one more time. Next, the remaining residue was dispersed in 500 mL of 2% (w/v) NaOH solution and continuously stirred at 700 rpm and 80 °C by using a heating plate (Model 11-102-50SH, Fisher Scientific, Malaysia). The solution was filtered again and the residue was washed with distilled water for 3-5 times. Next, the pre-treated husk was dried at 80 °C for 2 hours and subsequently kept in a desiccator until further use.

2.3 Hydrolysis Using IL

5 g of pre-treated coconut husk was dissolved in 50 mL of BmimCl at concentration ranged from 50-90 wt %. This mixture was heated using heating plate between 60 and 120 minutes at temperatures ranging from 50°C to 110 °C. Next, the mixture was left to cool to room temperature before it was centrifuged at 7,500 rpm for 30 minutes to isolate the crystalline cellulose particles. The supernatant was filtered and washed 3-5 times using distilled water to recover the IL. The remaining residue of MCC was then dried in drying oven to produce MCC powder.

2.4 Optimization Study Using Design of Experiment (DOE) Software

The optimization study and the subsequent data interpretation for the MCC solubility were conducted using central composite design (CCD) in DOE software [8]. A reduced factorial design

with three process parameters; solvent concentration, temperature and reaction time was set up using specific solvent concentration ranged from 50-90 wt %, the temperature range between 50-110 °C and reaction time ranged from 60-120 minutes [9]. The experiments were conducted according to the CCD design, and the impact of a different factor on the solubility of MCC was then evaluated. The final solubility was determined using Eq. (1) for each experiment.

$$\text{Solubility, } S = \frac{\text{weight of MCC sample, } W_i}{\text{weight of initial dry sample, } W_o} \times 100\% \quad (1)$$

2.5 Fourier Transform Infrared (FTIR) Spectroscopy

The FTIR spectra of the as-produced MCC, raw material and pre-treated samples were recorded using Shimadzu IR spectrophotometer model Prestige-21. The mixture of the samples and potassium bromide (KBr) was pelletized before it was scanned in the spectrometer range of 400 cm^{-1} to 4000 cm^{-1}.

2.6 Scanning Electron Microscopy (SEM)

The surface morphology and size of MCC samples were evaluated by scanning electron microscope (Model Zeis Supra 35VP). Prior to analysis, the samples were coated with platinum using a vacuum sputter coater.

3. RESULTS AND DISCUSSION

3.1 Optimization Study

The solubility of MCC prepared at different preparation conditions is shown in Table 1. The three process parameters (i.e. solvent concentration, temperature and reaction time) investigated in this study were denoted as A, B and C, respectively. The highest solubility of MCC (37.9 %) was obtained at 80.0 °C, 90.0 min, and solvent concentration of 65.0 wt % (run 2). Whilst, the lowest MCC solubility was recorded at preparation conditions of 50.0 °C, 90.0 min and solvent concentration of 65.0 wt % (run 3).

Table 1 Optimization of MCC with different preparation conditions

Run	Process Parameter			Response
	Solvent concentration, A (wt %)	**Temperature, B (°C)**	**Reaction time, C (min)**	**Solubility(%)**
1	50.1	97.8	72.2	29.2
2	**65.0**	**80.0**	**90.0**	**37.9**
3	65.0	50.0	90.0	8.3
4	65.0	80.0	90.0	35.2
5	65.0	80.0	90.0	33.3
6	90.0	80.0	90.0	23.2
7	79.9	62.2	107.8	22.2
8	65.0	80.0	60.0	32.7
9	65.0	80.0	90.0	35.9
10	65.0	110.0	90.0	29.8
11	50.1	62.2	107.8	15.1
12	65.0	80.0	120.0	37.4

13	79.9	97.8	72.2	31.1
14	65.0	80.0	90.0	34.1
15	79.9	97.8	107.8	37.2
16	79.9	62.2	72.2	18.9
17	40.0	80.0	90.0	27.8
18	50.1	97.8	107.8	32.5
19	65.0	80.0	90.0	35.2
20	50.1	62.2	72.2	16.4

3.2 Analysis of Variance (ANOVA)

The analysis of variance (ANOVA) was used to measure the precision of the model from the result obtained. The statistical significance of the ratio of mean square variation and mean square residual error were verified using ANOVA. From Table 2, it was observed that experimental R-squared value is 0.9607 and the adjusted R-squared value is 0.9252. The predicted R-squared value of 0.7564 is in reasonable agreement with the aforementioned adjusted R-squared value.The low values of coefficient of variation (C.V.) (8.12%) as well as standard deviation (2.33) are reflecting the reproducibility of the model. The signal to noise ratio in term of adequate precision was determined to be at 18.161, hence validating the precision of the model. This is in agreement with the conclusion made by Hamid and his co-workers which stated that the value of adequate precision should be larger than 4 in order to obtain the effective determination of the model [10].

Table 2 Statistical data for ANOVA

Parameter	Value	Parameter	Value
Std. Dev.	2.33	R-Squared	0.9607
Mean	28.67	Adj R-Squared	0.9252
C.V. %	8.12	Pred R-Squared	0.7564
PRESS	335.49	Adeq Precision	18.161

Table 3 shows the result of ANOVA for the response surface quadratic mode. The result indicates that the considered process parameters were significantly affecting the percentage solubility of MCC, as implied by the model F-value of 27.13. There was only a 0.01% (p-value) chance that a model with F-value this large could occur due to noise [11]. In addition, the result also showed that the model terms were significant. The result also revealed that the individual effect of temperature (B) is most significantly influencing the solubility of MCC, as shown by very high F-value of 117.88. Besides, B, C, A^2, and B^2 were also concluded as significant model terms as all their p-values are less than 0.1000 [11]. If there are too many insignificant model terms, the model reduction may improve the model. The F-value for lack of fit (3.35) implies that the lack of fit is not significant relative to the pure error. There is a 10.55% chance that a lack of fit with the F-value this large could occur due to noise. In other words, non-significant lack of fit is desired as the model is presenting a good relationship between independent variables and the response [12].

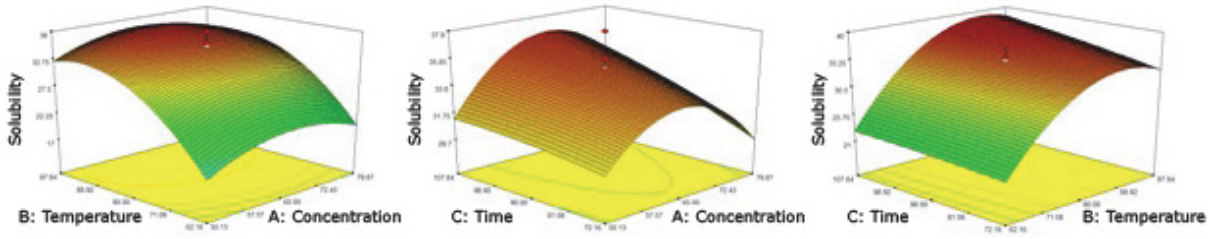
Table 3 ANOVA for response surface quadratic mode

Source	Sum of Square	df	Mean Square	F-Value	p-value Prob>F	
Model	1323.25	9	147.03	27.13	< 0.0001	significant
A-Concentration	5.24	1	5.24	0.97	0.3488	
B-Temperature	638.92	1	638.92	117.88	< 0.0001	
C-Time	27.55	1	27.55	5.08	0.0478	

AB	1.07	1	1.07	0.20	0.6669	
AC	6.88	1	6.88	1.27	0.2861	
BC	7.03	1	7.03	1.30	0.2813	
A^2	186.96	1	186.96	34.49	0.0002	
B^2	498.04	1	498.04	91.89	< 0.0001	
C^2	0.70	1	0.70	0.13	0.7271	
Residual	54.20	10	5.42			
Lack of Fit	41.73	5	8.35	3.35	0.1055	Not significant
Pure Error	12.47	5	2.49			
Cor Total	1377.45	19				

3.3 Response Surface Analysis

Three dimensional and contour plots were used to indicate the relationship between independent variables and the response. The overall shape of the curve can be analyzed from the plots. Figure 1(a-c) portray three-dimensional response surfaces with contour plots of solubility of MCC prepared at a different combination of process parameters, by setting the other parameter to be constant at a time. It has been observed from Figure 1 that the solubility of MCC is less affected by the combined effects of two process parameters. In Figure 1(a) and (c), the individual effect of temperature (B) has by far eclipsing the effect of the other process parameter. However, it is also worth to notice that the solubility of MCC has been decreasing at higher temperatures (> 95 °C), potentially due to the degradation of cellulose [13]. Meanwhile, Figure 1(b) has shown that reasonably high solubility of MCC could be achieved by employing moderate solvent concentration coupled with the moderate value of reaction time.



(a) (b) (c)

Figure 1. 3D response surfaces with contour plot of the combined effects of (a) solvent concentration and temperature, (b) solvent concentration and reaction time, and (c) temperature and reaction time for MCC preparation.

3.4 Optimization Validation

In order to optimize the preparation conditions of MCC, the solubility was set as the maximum value for the response. The values of the three process parameters (i.e. solvent concentration, temperature and reaction time) were set in the ranges being studied. The sample was prepared under the proposed optimum conditions and the experimental results were compared with the predicted values. The percentage deviation between the predicted and experimental conditions was evaluated and presented in Table 4. The average percentage error between actual and predicted solubility (3.1 %) was revealed to be less than 5.0 %, hence the quadratic model used in the prediction model was accepted reasonably [11].

Table 4 Experimental validation for optimization of MCC production

Solution no.	Concentration (wt %)	Temperature (°C)	Reaction Time (min)	Predicted solubility (%)	Actual solubility (%)	Percentage error (%)
1	65.6	90.9	92.3	38.2	37.2	2.6
2	65.6	90.9	92.3	38.2	36.8	3.7
3	65.6	90.9	92.3	38.2	37.1	2.9
Average					**37.0**	**3.1**

3.5 Fourier Transform Infrared Spectroscopy (FTIR)

Figure 2 shows the FTIR spectra of the coconut husk fibres for untreated raw material, pre-treated coconut husk and the as-produced MCC. From Figure 2, the absorption peaks between 3412-3413 cm^{-1} and 2916-2919 cm^{-1} regions corresponded to O-H stretching and C-H stretching, respectively. Whilst, the peaks located in the range between 1720-1740 cm^{-1} represents the stretching of hemicellulose and lignin. The peaks at 1620-1633 cm^{-1}were attributed to C=C aromatic skeletal vibration of lignin or O-H deformation due to the presence of water. Peaks in 1509-1516 cm^{-1} region indicate that only C=C aromatic skeletal vibration of lignin occur. The peaks at 1057-1059 cm^{-1} and 850-890 cm^{-1} were attributed to the C-O stretching of hemicellulose or lignin and C-H deformation of hemicellulose or cellulose, respectively. Meanwhile, ring bend of lignin can be indicated by peaks at 580-615 cm^{-1} [14, 15].

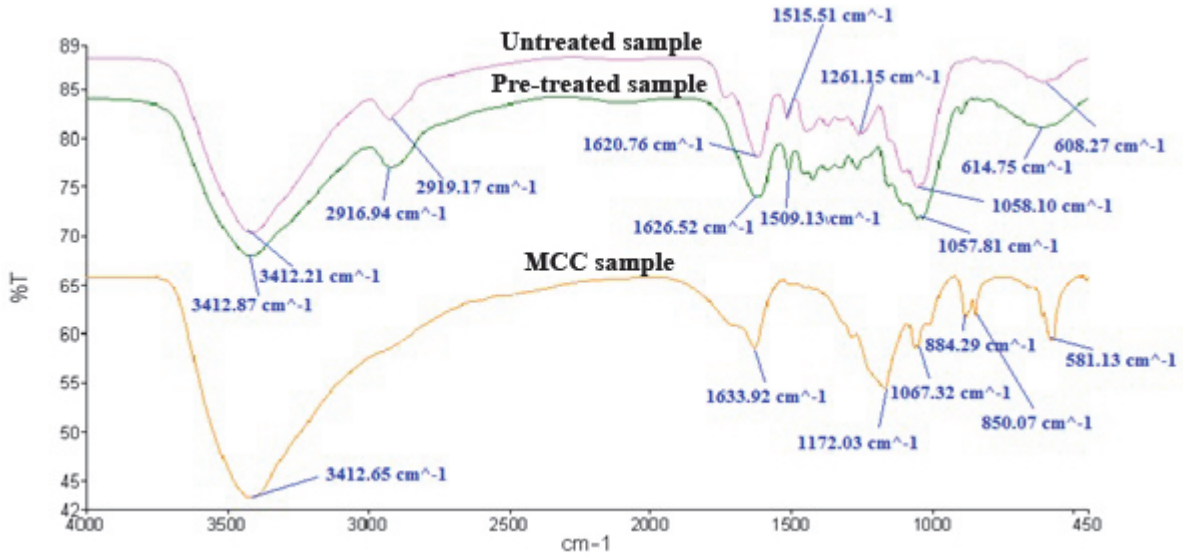


Figure 2. FTIR spectra of an untreated sample of coconut husk, pre-treated sample and MCC sample.

No significant vibration peak in the region of 1720-1740 cm^{-1} was observed for pre-treated and MCC samples, although this peak was observed in the untreated raw material sample. This peak was assigned to the C=O stretching in acetyl and uronic ester groups of hemicellulose or ester carbonyl groups in the *p*-coumaric units of the lignin [14]. The absence of this peak from the spectra of pre-treated and MCC samples proof that the alkaline treatment has successfully eliminated hemicellulose from the coconut husks. Besides, the disappearance of vibration peak at 1509-1516 $cm^{-1}$for MCC sample has confirmed the ability of pre-treatment to successfully remove lignin from coconut husk fibres [15].

3.6 Scanning Electron Microscopy (SEM)

SEM analysis was carried out to investigate surface morphology of untreated sample, pre-treated sample and MCC sample. Figure 3(a-c) portray the SEM micrographs of an untreated

sample of coconut husk fibres, pre-treated sample and MCC sample at similar resolution. Figure 3(a) shows that the untreated fibres originally have a rougher surface as compared to the pre-treated sample (Figure 3(b)). The smooth surface of the pre-treated sample might result from the successful removal of lignin and hemicellulose via alkaline pre-treatment. It was observed from Figure 3(c) that the MCC had been agglomerated and forming bulk particles with size ranged between 10 and 100 μm. The agglomeration was speculated to be due to the inappropriate drying method [16]. In addition, it is also difficult to determine the actual size of the as-produced MCC due to the inconsistent bulk size of the agglomerated particles.

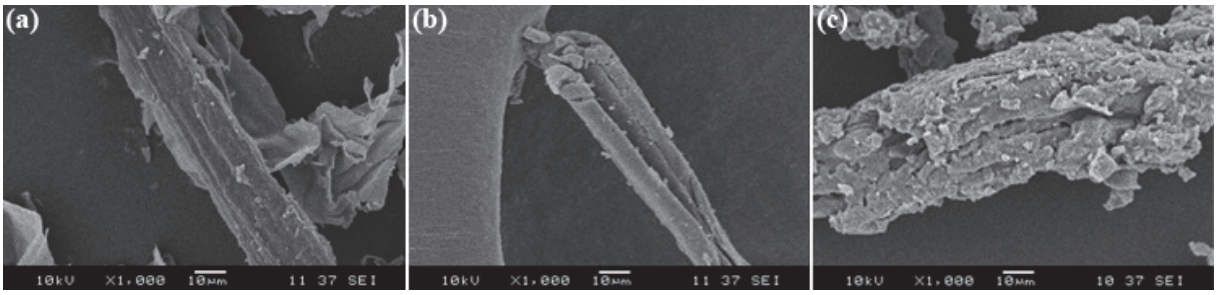


Figure 3. SEM micrographs of (a) untreated sample of coconut husk fibres, (b) pre-treated sample and (c) MCC sample at similar resolution.

4. CONCLUSION

The MCC had been successfully isolated from coconut husk via hydrolysis using IL. The optimum solubility of MCC of 37.0 % had been achieved at optimum conditions of 90.9 °C, 92.3 minutes and at a solvent concentration of 65.6 wt %. FTIR analysis had revealed that the lignin and hemicellulose could be successfully removed from the coconut husk via alkaline pre-treatment and hydrolysis using BmimCl. This was supported by the absence of vibration peaks in the region of 1720-1740 cm^{-1}, 1509-1516 cm^{-1} and 1261 cm^{-1} for the MCC sample. SEM micrographs also show that the bulk and agglomerated MCC particles with size ranging from 10-100 μm were produced, highly probably due to inappropriate drying method. However, it is expected that smaller size of MCC in nano-size could be produced by implementing appropriate drying method such as freeze-drying in the MCC preparation process.

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