



Research Article

Optimization of Microwave-Assisted Alkali Pretreatment for Enhancement of Delignification Process of Cocoa Pod Husk

Maktum Muharja^{*}, Rizki Fitria Darmayanti, Bekti Palupi, Istiqomah Rahmawati, Boy Arief Fachri, Felix Arie Setiawan, Helda Wika Amini, Meta Fitri Rizkiana, Atiqa Rahmawati, Ari Susanti, Ditta Kharisma Yolanda Putri

Department of Chemical Engineering, Faculty of Engineering, University of Jember, Kalimantan 37, Jember 68121, Indonesia.

Received: 5th September 2020; Revised: 15th January 2021; Accepted: 16th January 2021
Available online: 17th January 2021; Published regularly: March 2021



Abstract

In this study, the optimization of microwave-assisted alkaline (MAA) pretreatment is performed to attain the optimal operating parameters for the delignification of cocoa pod husk (CPH). The MAA performance was examined by heating the CPH solid with different particle sizes (60–120 mesh) and NaOH solution with a different sample to a solvent (SS) ratio (0.02–0.05 g/L), for short irradiation time (1–4 min). Box-Behnken Design (BBD) was utilized to optimize the percentage of lignocellulose composition changes. The results show that by enlarging particle size, the content of lignin and cellulose decreased while hemicellulose increased. By prolong irradiation time, the content of lignin and hemicellulose decreased while cellulose elevated. On the other hand, increasing the SS ratio was not significant for hemicellulose content changes. From FTIR and SEM characterization, the MAA drove the removal of lignin and hemicellulose of CPH and increased cellulose slightly. Supported by kinetic study which conducted in this work, it was exhibited that MAA pretreatment technology is an effective delignification method of CPH which can tackle the bottleneck of its commercial biofuel production.

Copyright © 2021 by Authors, Published by BCREC Group. This is an open access article under the CC BY-SA License (<https://creativecommons.org/licenses/by-sa/4.0>).

Keywords: alkaline; biofuel; cocoa pod husk; delignification; microwave

How to Cite: M. Muharja, R.F. Darmayanti, B. Palupi, I. Rahmawati, B.A. Fachri, F.A. Setiawan, H.W. Amini, M.F. Rizkiana, A. Rahmawati, A. Susanti, D.K.Y. Putri. (2021). Optimization of Microwave-Assisted Alkali Pretreatment for Enhancement of Delignification Process of Cocoa Pod Husk. *Bulletin of Chemical Reaction Engineering & Catalysis*, 16(1), 31-43 (doi:10.9767/bcrec.16.1.8872.31-43)

Permalink/DOI: <https://doi.org/10.9767/bcrec.16.1.8872.31-43>

Introduction

Lignocellulosic biomass is considered as the most promising raw material to produce green energy to reduce dependence on fossil fuels [1]. Conversion of the abundant, renewable, and non-edible lignocellulosic biomass into energy

and fuels has attracted excellent deal attention over the past few years [2,3]. In a tropical country like Indonesia, the crop residues have great potential to be exploited because of their sustainable and environmentally friendly characteristic [4]. One of the prospective lignocellulose biomass in Indonesia is coconut pod husk (CPH). The composition of CPH, which can be potentially utilized are cellulose, hemicellulose, and lignin with the composition of 29.93%,

* Corresponding Author.

Email: maktum@unej.ac.id (M. Muharja)

Telp: +62 331-330224, Fax: +62 331-337422, 339029

10.94%, and 11.64%, respectively [5]. Despite having potential, however, the rebellious nature of lignocellulosic biomass harshly hinders the further biological process, namely enzymatic hydrolysis and fermentation, becoming a significant drawback of lignocellulose utilization for biofuel production [6]. The development of cost-effective technology for effective conversion is urgently needed for the biofuel commercialization [7].

The pretreatment process is crucial to damage the complex structure of lignin, facilitating the release of monomeric sugars in biochemical conversions into bioenergy products [8–11]. Some pretreatment processes, for example, physical and chemical, Physico-chemical, biological processes, have been developed previously or combinations of these processes [12]. Each of these processes has advantages and limitations that depend on the nature of the biomass [13]. Among these methods, alkaline pretreatment exhibited a more effective and beneficial delignification performance because it uses inexpensive and widely available chemicals, compared to other chemicals such as deep eutectic solvents (DES) and ionic liquid (IL) [6,9,12,14]. However, the long delignification time is a drawback in this method. The application of microwaves has considered an alternative to conventional heating of alkali pretreatment [15].

Microwave-assisted delignification has been demonstrated to be a promising method with maximum removal of lignin and less time processing required [8,16]. Microwave facilitated indirect absorption of energy by molecules, increased heat transfer, and accelerated the rupture of biomass structure through the molecular collision triggered by the dielectric polarization. Polar particle movement produces fast heating resulting in lower process temperatures and times [17]. Despite some previous works using microwave heating for biomass pretreatment has been reported, the application of alkaline in microwave heating for lignin fractionation and depolymerization was still limited with a different operational condition for the different varieties of lignocellulosic feedstock. From literature studies, there are no works reported before about the combination of the microwave with the chemical process as a potential method for the delignification of CPH.

From the problems mentioned above, in this study, the optimization of microwave-assisted alkaline pretreatment was carried out to obtain the optimal operating condition for enhancing the delignification of CPH. The physicochemical properties were conducted by using SEM

and FTIR analysis. It was hypothesized that the alkaline pretreatment combined with microwave irradiation gives a higher efficiency of lignin depolymerization than conventional heating.

2. Material and Methods

2.1 Materials

CPH was collected from the plantation area in Jember District, East Java, Indonesia. It was firstly dried under the sunlight for 2 days, then milled and screened to obtain a particle size of 60–120 mesh. Analytical grade of sodium hydroxide (NaOH) was purchased from Sigma Aldrich, Japan.

2.2 Methods

2.2.1 Microwave-Assisted Alkaline (MAA) Pretreatment

The MAA process was run by mixing 5 g of cocoa pod husk and 3% NaOH solution with a sample to a solvent ratio of 0.02–0.05 g/L in the beaker glass. Then, the mixture was heated in the microwave (100 W) during particular irradiation time, 1–4 min. The same process was conducted for the different particle sizes of 60–120 mesh. After the pretreatment process, the pretreated solid was washed, filtered, and dried in the oven at 45 °C for 1 day. Finally, the sample was stored at 4 °C before analyzed. The process was run under batch operation in the triplicate experiment.

2.2.2 Analytical Methods

The compositions of lignin, hemicellulose, and cellulose at the untreated and MAA-treated conditions were analyzed using the Chesson method [18]. The solid of native and MAA treated were characterized by Scanning Electron Microscopy (SEM) (Evo MA 19, Carl Zeiss, England) analysis and Fourier Transform Infrared Spectroscopy (FTIR) (Nicolet iS10, Thermo Scientific, USA) analysis. The analysis was employed to investigate chemical composition changes after the MAA pretreatment applied.

2.2.3 Design of experiment

In this study, a Box-Behnken design (BBD) was utilized to optimize the operating conditions of MAA delignification towards the percentage of lignocellulose composition changes. Parameters, such as: particle size (60–120 mesh), feed to volume ratio (0.02–0.05 g/L), and delignification time (1–4 min) were investi-

gated. Experimental design of BBD of MAA pretreatment was shown in Table 1. According to statistical modeling generated by Design Expert 10 software (Stat-Ease), 17 runs were required to analyze the data. Each run was carried out in duplicate and data was showed in average. Table 1 shows the experimental design of this work with a randomized run. The second-order nonlinear polynomial equation was fitted for the experimental data as Eq. (1) follows:

$$Y = \beta_0 + \sum_{i=1}^n \beta_i X_i + \sum_{i=1}^n \beta_{ii} X_i^2 + \sum_{i=1}^{n-1} \sum_{j=i+1}^n \beta_{ij} X_i X_j + \varepsilon \quad (1)$$

where Y denotes the lignocellulose percentage as response, β_0 denotes the intercept term, n denotes the number of variables, β_i denotes the liner effect term, β_{ii} means the square effect term, β_{ij} means the interaction effect term, X_i and X_j means the independent variables, and ε means the random error.

2.2.4 Kinetics analysis

The kinetics of the lignocellulosic components conversion was modelled as the equation of reaction rate. The reaction rate was expressed as the simple function of substrate concentration (C_A) with the certain reaction rate constant and reaction order as the following Eq. (2).

$$-r_A = kC_A^n \quad (2)$$

The order of the reaction and the reaction the reaction rate constant was determined by frac-

tional time method. The data was plotted as graphic and the required time to remain 80% ($t_{0.8}$) substrate was observed from the plot for each concentration data. The fractional time was defined as the following Eq. (3):

$$t_F = \frac{(0.8)^{1-n} - 1}{k(n-1)} C_{A0}^{1-n} \quad (3)$$

Then, the required data was plotted linearly with $\log t_F$ as the y-axis and $\log C_{A0}$ as the x-axis.

$$\log t_F = \log \left(\frac{(0.8)^{1-n} - 1}{k(n-1)} \right) + (1-n) \log C_{A0} \quad (4)$$

From the linearized plot as Eq. (4), the value of reaction order and reaction rate constant was calculated.

3. Results and Discussion

3.1 Experimental Design and Statistical Analysis

Statistical analysis for the regression model of lignin, cellulose, and hemicellulose using Box-Behnken Design is shown in Tables 2–4. From the ANOVA result of the regression model of lignin (Table 2), there is a 72.17% chance of data could occur due to noise. The model p-value of 0.7217 indicates that the model was statistically insignificant and demonstrates the unreliability of the models. As shown in Table 2, all model terms, utilizing the linear coefficients (A , B , C), interaction coefficients (AB ,

Table 1. Experimental design of Box-Behnken design of MAA pretreatment.

Std	Run	Factor A: Particle Size (mesh)	Factor B: Sample to a solvent ratio (g/L)	Factor C: Irradiation time (min)
4	1	120	0.050	2.5
10	2	90	0.050	1
1	3	60	0.020	2.5
3	4	60	0.050	2.5
6	5	120	0.035	1
7	6	60	0.035	4
8	7	120	0.035	4
2	8	120	0.020	2.5
9	9	90	0.020	1
15	10	90	0.035	2.5
14	11	90	0.035	2.5
13	12	90	0.035	2.5
16	13	90	0.035	2.5
11	14	90	0.020	4
17	15	90	0.035	2.5
12	16	90	0.050	4
5	17	60	0.035	1

Table 2. Analysis of variance for the regression model of lignin content.

Source	Sum of Squares	df	Mean Square	F-value	p-value
Model	1.05	9	0.1171	0.6650	0.7217
A-Particle size	0.1791	1	0.1791	1.02	0.3467
B-Ratio	0.0194	1	0.0194	0.1102	0.7496
C-Time	0.0047	1	0.0047	0.0265	0.8754
AB	0.4180	1	0.4180	2.37	0.1672
AC	0.0615	1	0.0615	0.3494	0.5730
BC	0.0608	1	0.0608	0.3452	0.5753
A ²	0.0980	1	0.0980	0.5566	0.4799
B ²	0.0402	1	0.0402	0.2283	0.6473
C ²	0.1719	1	0.1719	0.9765	0.3560
Residual	1.23	7	0.1760		
Lack of Fit	0.2479	3	0.0826	0.3358	0.8017
Pure Error	0.9843	4	0.2461		
Cor Total	2.29	16			

Table 3. Analysis of variance for the regression model of hemicellulose content.

Source	Sum of Squares	df	Mean Square	F-value	p-value
Model	14.04	9	1.56	3.19	0.0703
A-Particle size	0.5539	1	0.5539	1.13	0.3228
B-Ratio	0.7260	1	0.7260	1.48	0.2627
C-Time	8.97	1	8.97	18.33	0.0036
AB	0.0410	1	0.0410	0.0838	0.7806
AC	0.4343	1	0.4343	0.8872	0.3776
BC	0.3405	1	0.3405	0.6956	0.4318
A ²	0.3567	1	0.3567	0.7287	0.4216
B ²	0.3270	1	0.3270	0.6681	0.4406
C ²	2.31	1	2.31	4.71	0.0666
Residual	3.43	7	0.4895		
Lack of Fit	0.7053	3	0.2351	0.3456	0.7954
Pure Error	2.72	4	0.6803		
Cor Total	17.47	16			

Table 4. Analysis of variance for the regression model of cellulose content.

Source	Sum of Squares	df	Mean Square	F-value	p-value
Model	13.93	9	1.55	2.75	0.0980
A-Particle size	1.90	1	1.90	3.38	0.1084
B-Ratio	0.6430	1	0.6430	1.14	0.3205
C-Time	1.63	1	1.63	2.89	0.1328
AB	2.65	1	2.65	4.70	0.0668
AC	2.19	1	2.19	3.90	0.0890
BC	0.1314	1	0.1314	0.2336	0.6436
A ²	0.2004	1	0.2004	0.3563	0.5694
B ²	3.27	1	3.27	5.81	0.0468
C ²	1.51	1	1.51	2.68	0.1457
Residual	3.94	7	0.5625		
Lack of Fit	0.4124	3	0.1375	0.1560	0.9206
Pure Error	3.53	4	0.8813		
Cor Total	17.86	16			

AC , BC), and quadratic coefficients (A^2 , B^2 , C^2), had no significant effect ($p > 0.05$). The lack of fit p-value of 0.8017 means the lack of fit is not significant relative to the pure error.

As shown in Tables 3 and 4 for ANOVA of hemicellulose and cellulose content, the Model p-value of 0.0703 and 0.098, respectively, revealed that model terms are insignificant when the significance level of 95% was used. However, when the significance level of 90% was applied, the terms are significant. In Table 3, the variable of exposure time (C) is a significant model term. In Table 4, the quadratic term of ratio (B^2) is a significant model term. The lack of fit p-value of 0.7954 and 0.9206 demonstrated that the lack of fit of hemicellulose and cellulose, respectively, is not significant relative to the pure error. The R-squared of the hemicellulose and cellulose model are 0.8039 and 0.7796, respectively. They indicate that the models have a quite good agreement with the experimental data. It also represents that the models are acceptable and can satisfy 80.39% and 77.96%, respectively, of the variability in the delignification process.

3.2 Fitting of Models

In this work, a second-order polynomial mathematical equation presented the relationship between the independent variables and the responses. The developed model equations are provided in Equations (5-7), where A represents particle size (mesh), B represents the ratio (g/L), and C represents time (min).

$$\begin{aligned} \text{Lignin}(Y_1) = & 0.6776 - 0.149625A + 0.04925B \\ & - 0.024125C + 0.32325AB + 0.124AC \\ & - 0.12325BC - 0.15255A^2 + 0.0977B^2 \\ & - 0.20205C^2 \end{aligned} \quad (5)$$

$$\begin{aligned} \text{Hemicellulose}(Y_2) = & 2.7284 + 0.263125A \\ & - 0.30125B - 1.05913C - 0.10125AB \\ & - 0.3295AC - 0.29175BC + 0.29105A^2 \\ & - 0.2787B^2 + 0.74005C^2 \end{aligned} \quad (6)$$

$$\begin{aligned} \text{Cellulose}(Y_3) = & 7.7454 - 0.48775A \\ & + 0.2835B + 0.451C + 0.81325AB \\ & + 0.74025AC - 0.18125BC + 0.218175A^2 \\ & + 0.880675B^2 - 0.598325C^2 \end{aligned} \quad (7)$$

3.3 Diagnostic of Model Adequacy

The percentage of normal probability plot of residuals was typically scattered near along the straight line, which denotes a tolerable deviation of variance.

A good agreement between the model and the experimental data observed can be qualitatively justified by using a Predicted vs. Actual plot (parity plot) [19]. The accuracy of the model can be understood by evaluating the normal distribution with the predicted and actual results, as exhibited in Figure 1; Figures 1a–b for lignin, Figures 1c–d for hemicellulose, and Figures 1e–f for cellulose. Except for the lignin plot (Figure 1b), the actual values of hemicellulose and cellulose are moderately close to the predicted line, which was obtained from the developed model in previous Eq. (5–7). This fact implies the significance and reliability of the model used in this study (see Figures 1d and f).

3.4 The Effect and Optimization of Variables

3.4.1 The effect of variables on lignin content

Figure 2 shows a three-dimensional response plot of the effect of variables on the changes of lignin composition after MAA pretreatment. For the particle size parameter, the lignin content decreases continuously (delignification increases) with increasing particle size from 60 to 120 mesh, as shown in Figures 2a and b. In microwave heating, different from the heat transfer concept of conventional heating, heat transfer was induced throughout with more selectivity to higher polar parts. This condition generates hotspots within the complex structures of lignocellulosic biomass [20].

For the ratio effect parameter, lignin content decreases with increasing ratio when interacting feed to volume ratio variables (see Figure 2a). Conversely, lignin content increases with increasing ratio when interacting with time variables, as shown in Figure 2c. It was revealed that the delignification process was strongly affected by solvent volume. The delignification increased linearly as the increase of liquid to the solid ratio (LSR). This phenomenon may be attributed to the larger of the biomass swelling in larger solvent volume. This condition facilitates the absorption of microwaves straightly on the matrix, leading to degradation of the lignin compound [16]. The large portion of lignin content underwent the delignification process by breaking of aryl ether linkages after being subjected by alkaline solution [21]. Furthermore, hydrogen bonds that connect between lignin with cellulose and hemicellulose were ruptured. Sodium and hydroxide ions are formed during the process, then the ions strike ester-carbohydrate bonds in the lignocellulose [22].

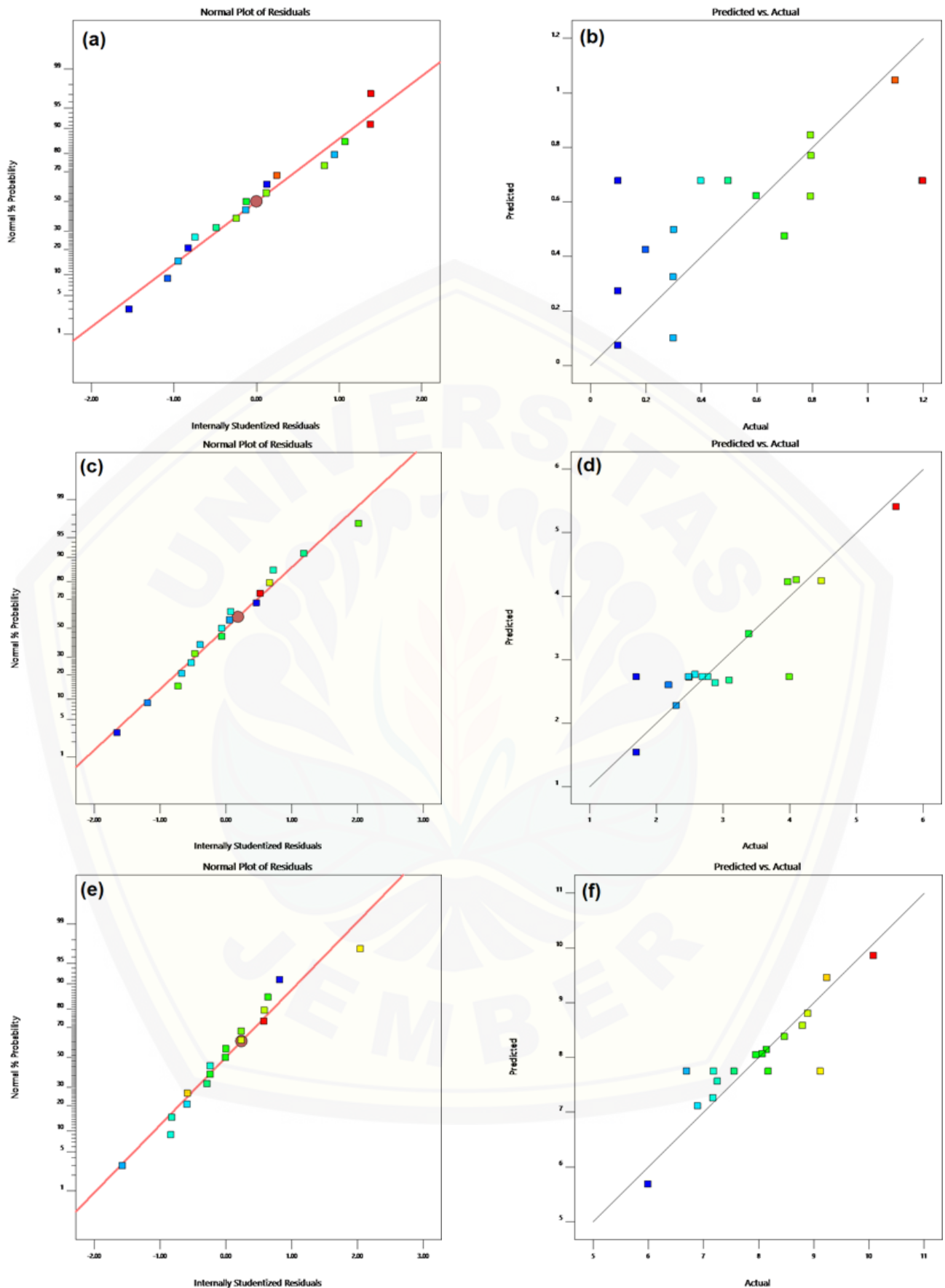


Figure 1. Normal Plot and Predicted *vs.* Actual. (a) Normal Plot of Lignin, (b) Predicted *vs.* Actual of Lignin, (c) Normal Plot of Hemicellulose, (d) Predicted *vs.* Actual of Hemicellulose, (e) Normal Plot of Cellulose, (f) Predicted *vs.* Actual of Cellulose.

Increasing the concentration of alkaline to a certain level may have a positive impact on the swelling of biomass. This swelling increases the surface area of the complex nature of biomass. The instability of holocellulose at a higher alkaline concentration in microwave heating may be caused by the dissolution of polysaccharides and lignin simultaneously [1]. However, a further increase in ratio had no significant enhancement during delignification. This phenomenon may be due to the difficulty of attaining the activation energy as the solvent volume escalates, subsequently leading to lower efficiency. This condition may be due to the solution gets saturated with the substrate, thereby giving a significant reduction in the mass transfer rate [16].

In this study, the highest delignification performance of MAA was obtained at mild condition, namely particle size of 90 mesh, SS ratio of 0.035, and irradiation time of 2.5 min. This condition resulted in the lignin content of

1.698%. Before the delignification process, the lignin content in cocoa pod husk was 21.4%. After the delignification process, the lignin content decreased to 0.099%, as shown in Figure 2c. Gazliya and Aparna [1] reported a high delignification result, *i.e.* from 20.11% to 9.43% at 5 N after MAA treatment.

On the irradiation time consideration, the lignin content rises to the optimum point and then drops dramatically by prolonging the irradiation time (Figure 2b). This trend may occur due to the collision phenomena between the molecules. Moreover, the disruption of intermolecular forces between the molecules stimulated by electromagnetic irradiation occurred. Furthermore, the prolonging irradiation time assisted the thermal accumulation, which caused a rapid breakdown of lignin [23,24]. Alexander *et al.* [16] reported that the delignification continuously elevated with extend in exposure time, yielding 73.21% delignification after 4.40 min.

Furthermore, conceptually, longer reaction times give an increase in the rate of reaction, which was presented by higher delignification yield. This condition provides a higher possibility of a sufficient collision frequency among the molecules which generate immense intramolecular heat energy [16]. However, there were no significant differences in delignification yields between 2 min and 3 min and reached a plateau in 2.2 min (See Figure 2a). This phenomenon may be attributed to kinetic factors, indicating that from 2.2 min to higher reaction times, the amount of extracted lignin is nearly constant. The prolonged delignification process beyond 3 min of irradiation time had an insignificant effect.

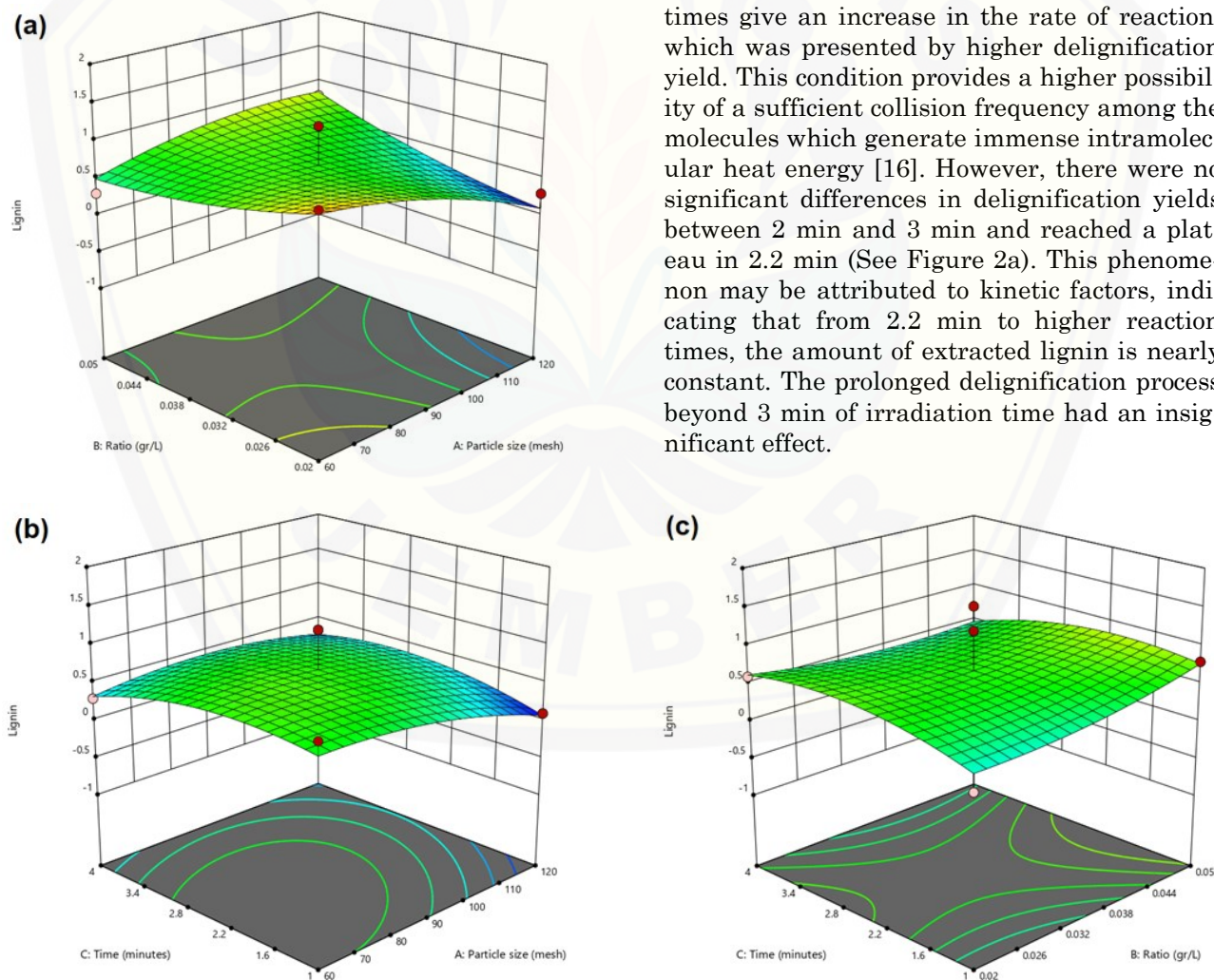


Figure 2. The effect of ratio and particle size (a), time and particle size (b), and time and ratio (c), on the lignin content.

From the other work utilizing microwave for biomass pretreatment, Gazliya and Aparna [1] reported that MAA pretreatment gave higher delignification results (*i.e.* 53.10% *vs.* 48.63%) using lower NaOH concentration (*i.e.* 5 N *vs.* 7.5 N) compared to alkaline pretreatment using conventional heating. This fact indicated that MAA gave a better result from performance and environmentally friendly aspects. Moreover, the physicochemical properties observed after the delignification process showed that MAA pretreatment increased cellulose content and surface area compared to alkaline pretreatment. Kamalini *et al.* [15] also found that MAA was sufficient for the biomass delignification process. It was reported that lignin content decreased from 19.33% to 14.59% after MAA applied.

In comparison with alkaline pretreatment, the delignification of softwood pellets using diluted sulfuric acid was performed by Zhou *et al.*

[25]. It was obtained a lignin yield of 82.31 % at 190 °C for 10 min. By comparing this result with those reported studies, this work obtained delignification with comparable and higher yield under milder alkaline conditions and shorter time.

3.4.2 The Effect of variable on hemicellulose content

Figure 3 shows a three-dimensional response plot of the effect of variables on the changes of hemicellulose composition after MAA pretreatment. Before the pretreatment process, the hemicellulose content in cocoa pod husk was 3.8%. After the pretreatment process, the most effective hemicellulose content decreased from 3.8% to 1.195% by 68.55%, which can be seen in Figures 3a–c. The most effective reduction of hemicellulose in the experiments was carried out at 80 mesh particle size, a ratio of 0.035, and a time of 2.5 min. Kamalini *et al.* [15] reported that the hemicellulose underwent lower solubilization during MAA pretreatment, which declined from 32.30% to 27.15%. Thus, the decrease in hemicellulose levels in this study can be said to be better than previous studies. Hemicellulose content increases significantly with increasing particle size (Figures 3a and b). On the other hand, changes in hemicellulose content were not too significant with an increase in the ratio (see Figures 3a and c).

Hemicellulose content decreases significantly with the longer heating time (Figures 3b and c). Liu *et al.* [11] found that the more prolonged chemical treatment significantly gave a positive contribution to the hemicellulose separation. Moreover, the higher temperature also

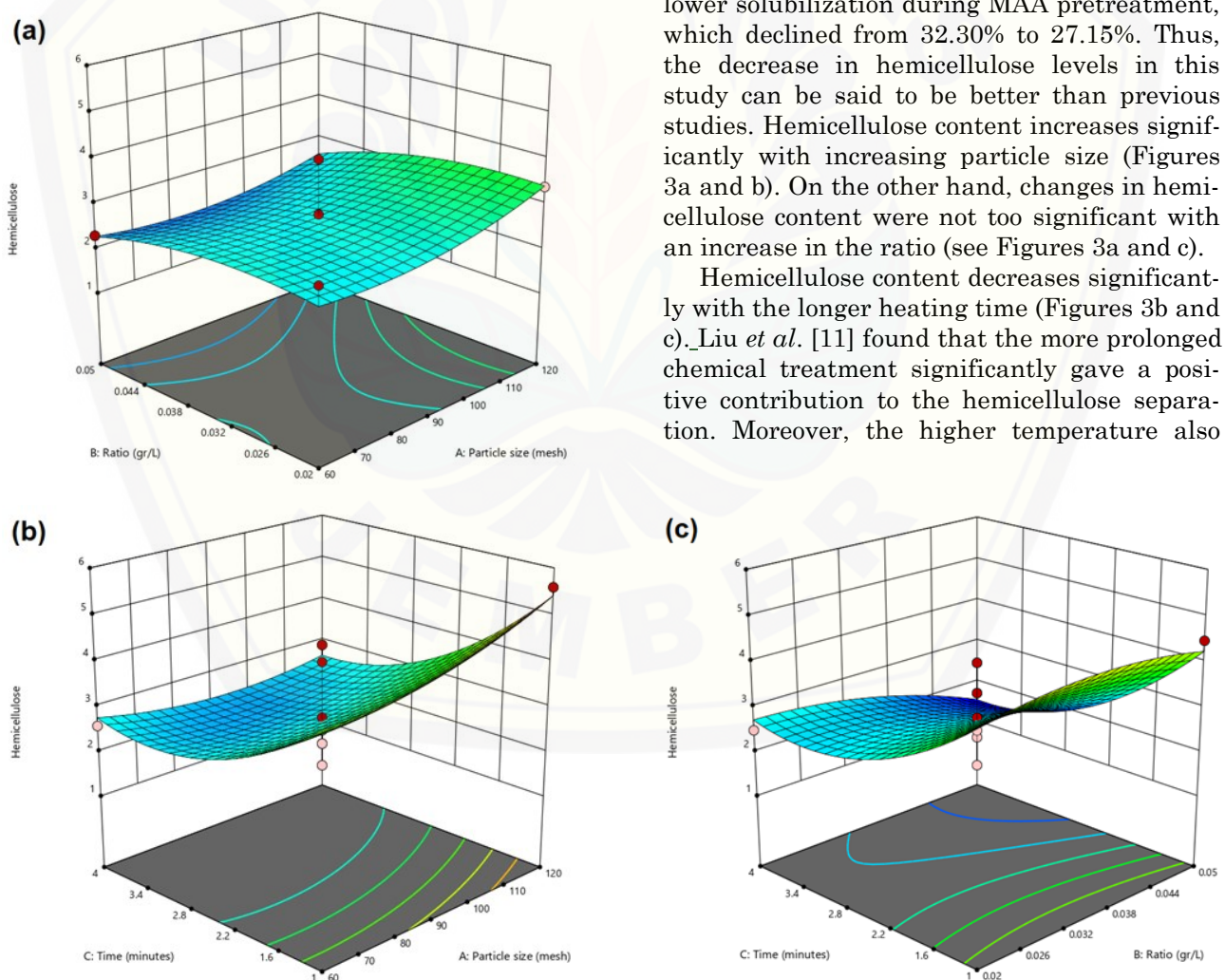


Figure 3. The effect of ratio and particle size (a), time and particle size (b), and time and ratio (c) on the hemicellulose content.

provided higher hemicellulose yield than the low temperature during the alkaline treatment process. This phenomenon might be attributed to the synergistic work between microwave irradiation and alkaline treatment. The straight interaction of biomass with the electromagnetic field volumetrically and rapidly occurred when the microwave irradiated the biomass. This interaction significantly removed hemicelluloses from the substrate, leading to the high hemicelluloses yield [11]. The successful performance of microwave heating for xylan removal was also reported by Panthapulakkal *et al.* [26]. It was reported that the xylose removal was more significant 6.2% than the conventional heating. For the severe heating conditions, the combined microwave and alkaline treatment contributed positive effects on the escalation of removal of lignin and hemicelluloses. The separation was presented by high hemicelluloses yield and improvement on hemicelluloses molecular weight [11].

3.4.3 The effect of variables on cellulose content

As shown in Figure 4, Cellulose content decreased significantly with increasing particle size (see Figures 4a and b). The lowest cellulose content was obtained at the midpoint of the condition (see Figures 4a and c). Cellulose content increases with prolonged reaction time. The highest cellulose content is in a severe condition (Figures 4b and c). From this work, the cellulose concentration was discovered to be elevated following the MAA process. Due to the effect of microwave heating, the cellulose content was remarkably increased during the alkaline treatment. The microwave irradiation cracked the compact structure of biomass, simultaneously stimulated the alkaline penetration into the inner biomass structure, leading to the promotion of the cellulose content [11].

3.5 Structural Changes of SCW Pretreated Solid

3.5.1 Fourier Transform Infrared Spectroscopy (FTIR)

FTIR characterization was carried out to determine the functional groups contained in the detached and delignified cocoa shells. In general, the functional groups found in the two types of cocoa husk arise from lignin, hemicellulose, and cellulose [27]. Typical FTIR peaks that appear include 3410.15 cm^{-1} (O-H group stretching vibrations), $3000\text{--}3100\text{ cm}^{-1}$ and $675\text{--}870\text{ cm}^{-1}$ (aromatic C-H), 2924.09 cm^{-1} (C-H alkane group), 1851 cm^{-1} (carbonyl group C=O), 1635.64 cm^{-1} (C=C alkenes), 1519.91

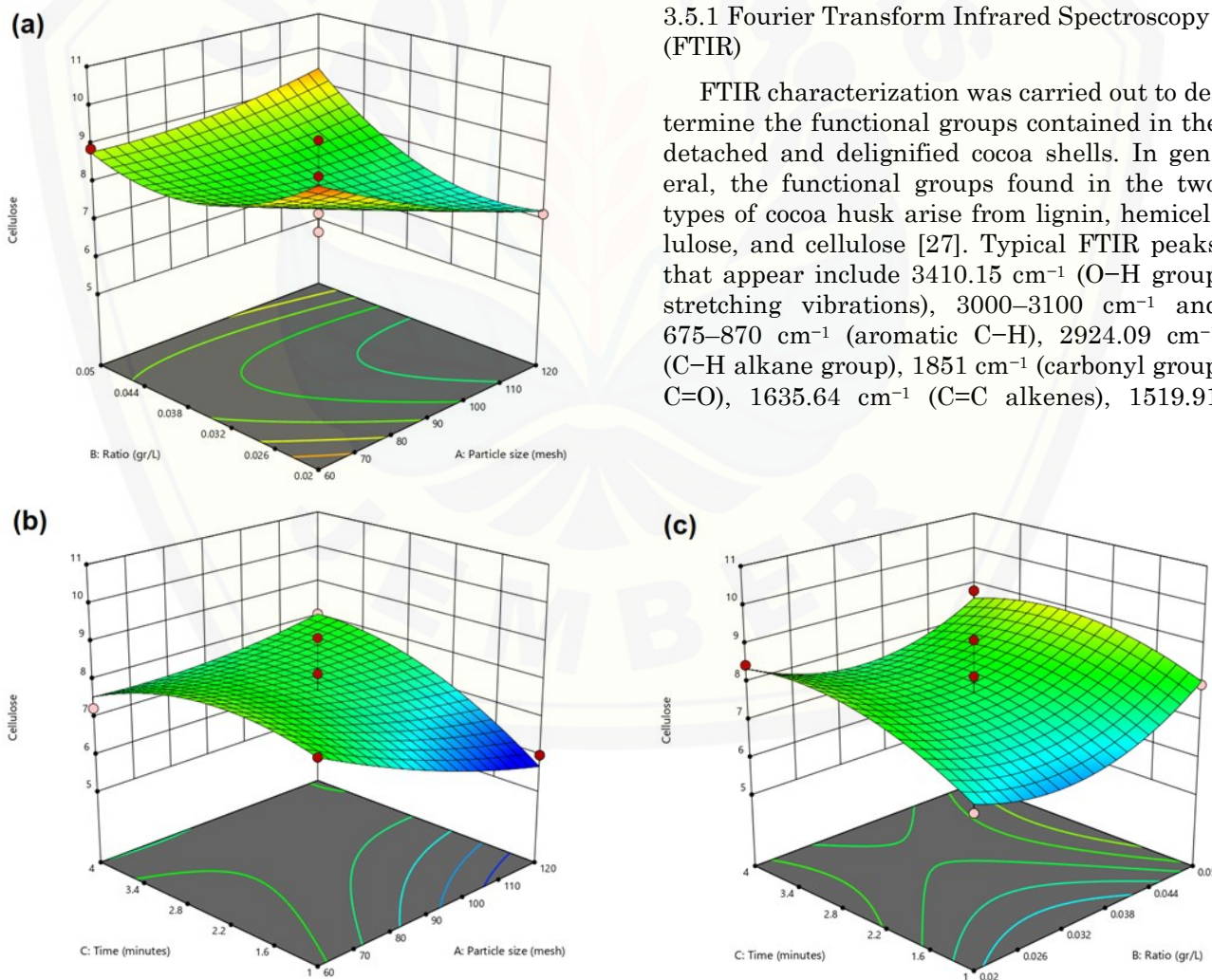


Figure 4. The effect of ratio and particle size (a), time and particle size (b), time and ratio (c), on the cellulose content.

cm^{-1} (C=C stretching aromatic ring), 1201 cm^{-1} (C–O ester), 894 cm^{-1} (C–H alkenes group).

Cocoa depectination (without delignification test) contains lignin, cellulose, and hemicellulose. As shown in Figure 5, the results of the FTIR spectrum of the detected cocoa had O–H groups at wave number 3410.15 cm^{-1} , group C=O at wave number 1743.65 cm^{-1} , and double bonds (C=C alkene and C=C aromatics) with each wave number is 1651.07 cm^{-1} and 1527.62 cm^{-1} . This result indicates that the detached cocoa (without delignification test) contains lignin compounds. Besides, depectination cocoa also has cellulose content which is characterized by the emergence of CH alkane and C–H alkene groups with wave numbers 2924.09 cm^{-1} and 900 cm^{-1} and the appearance of wave numbers 1242.16 cm^{-1} indicating the presence of C–O ester groups and the presence of C–O ester groups aromatic CH group with a wave-number of 856.39 cm^{-1} . These results indicate

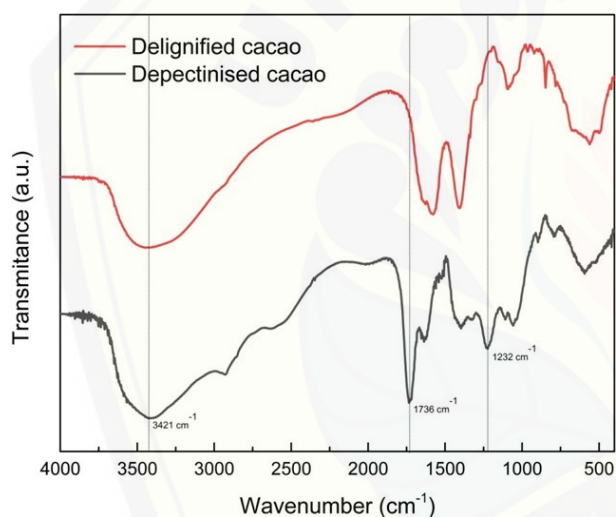


Figure 5. FTIR spectra of cocoa pod husk.

that depectination cocoa contains cellulose and lignin compounds that bind to each other.

The content of lignin, hemicellulose, and cellulose in cacao depectination was changed when the sample underwent the delignification process. One of the changes is that there are a shift and decrease in peak intensity in the functional group. Delignified cocoa showed a shift in absorption peaks from several functional groups such as O–H group from 3410.15 cm^{-1} to 3450.65 cm^{-1} , which indicated that lignin levels increased, significantly decreased the peak of C=O group (1743.65 cm^{-1}), as well as the decrease in C=C of the alkene in the delignification process to 1639.49 cm^{-1} . Also, the loss of C–H alkanes and C=C aromatic groups indicates a change in hemicellulose. The cellulose content in the depectinated cocoa, which has undergone a delignification process, also decreased the peak absorption of the C–O ester group (1238 cm^{-1}). So, it can be said that the delignified cocoa has decreased lignin and hemicellulose content (see in Figures 2 and 3, respectively), but there is a slight increase in cellulose content. FTIR results in this work were found in agreement with the increase in cellulose content as shown in Figure 4.

3.5.2 Scanning Electron Microscopy (SEM)

SEM is a method used to analyze pore structure, morphology and can be used to analyze changes in particle size in samples. SEM on cocoa depectination and delignification shows a significant difference in particle size. Figure 6 illustrates the morphology and particle of cocoa before being delegated (depectination) and cocoa that has been delegated. Morphology shows that delignified cocoa has a smaller cavity compared to depectative

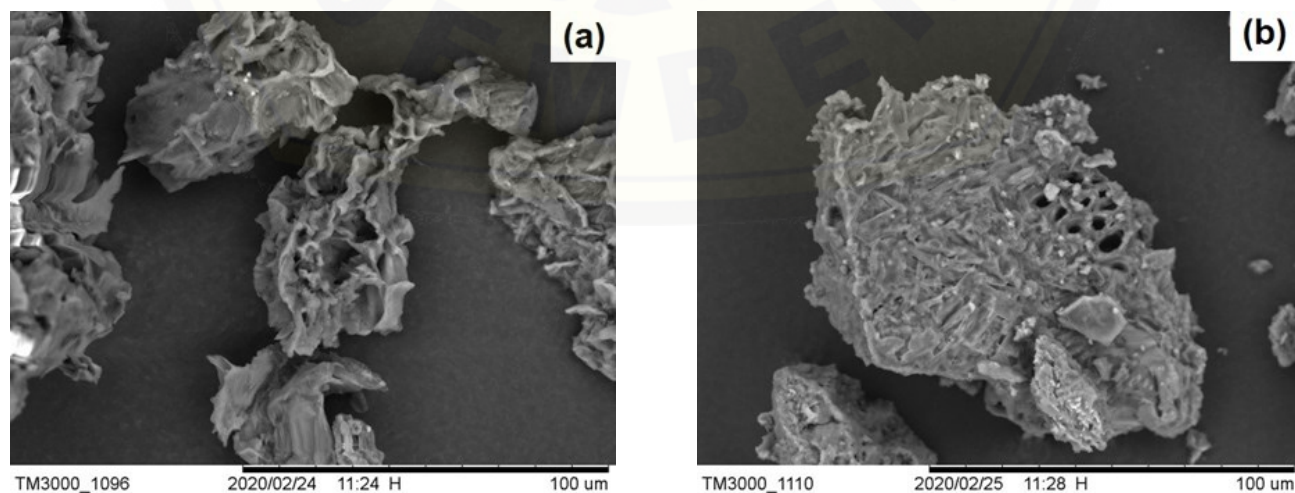


Figure 6 SEM images of (a) depectinised and (b) delignified cocoa pod husk.

cocoa, in Figure 2b, having a thicker and rougher surface to protect the lignocellulosic content contained in fiber [28]. The significant difference in the two samples lies in the particle size. Delignified cocoa (Figure 2a) has a much smaller particle size and is barely noticeable, in contrast to depectination cocoa, which has massive particle and cavity sizes. The smaller the particle size, the more cellulose is produced, and the proportion of lignin decreases as the particle size decreases. This condition may be because lignin binds cellulose in acidic conditions so that it will condense and precipitate [29]. So, delignification cocoa has lower lignin content compared to depectination cocoa.

3.6 Kinetic Study

Lignocellulosic contains majorly of lignin, cellulose, and hemicellulose. In this study, the kinetics of microwave assisted alkali (MAA) pretreatment were studied for lignin, as the major target of delignification process which would degrade to inhibitor during fermentation without any pretreatment. Kinetics of cellulose conversion in the pretreatment was also studied as the hexose, fermentable sugars were derived from cellulose. Meanwhile, hemicellulose was not discussed because the pentoses as the main product, was not preferable as fermentable sugar by many type of strain [30].

In this study, the alkali was assumed to be exceeding reactant, so the concentration of lignocellulosic component was the driving force, and the reaction was independent to each other. The result from the kinetics analysis was shown in Eqs. (8–9) below.

$$-r_{\text{lignin}} = 1.241 \left(\frac{L^{0.264}}{\text{mol}^{0.264} \text{s}} \right) C_{\text{lignin}}^{1.264} \quad (8)$$

$$-r_{\text{cellulose}} = 1.029 \left(\frac{L^{0.312}}{\text{mol}^{0.312} \text{s}} \right) C_{\text{cellulose}}^{1.312} \quad (9)$$

This result was obtained using fractional time method with 80% fraction. The R^2 value of the fractional time linear plot was 0.999 and 0.997 for lignin and cellulose, respectively. These values showed the fit sufficiency of this model to the existing data from the experiment. Using MAA, the pretreatment of CPH performed faster conversion rate for lignin with k_{lignin} of $1.241 \text{ L}^{0.264}/\text{mol}^{0.264}\text{s}$ and n_{lignin} of 1.264, compared with cellulose with lower $k_{\text{cellulose}}$ of $1.029 \text{ L}^{0.312}/\text{mol}^{0.312}\text{s}$ and only slightly higher order $k_{\text{cellulose}}$ of 1.312. This process effectively degraded lignin and simultaneously favouring cellulose degradation which both may be beneficial to reduce the requirement of next processing after delignification, saccharification.

The kinetics of delignification was not yet studied for CPH using MAA. Keshwani *et al.* [31] studied the MAA for switchgrass using time dependent kinetics modelling, resulting reaction constant of 0.499 for lignin and 0.011 for cellulose. This study for CPH resulted in higher reaction constant, means that this process could be performed faster than previous study mentioned. For further research, it is still crucial to conduct the economic review of MAA pretreatment for application in the pilot and industrial scale.

4. Conclusions

In this work, the alkaline pretreatment under microwave heating was sufficient for the delignification process within a less time irradiation process. The optimal results were obtained on the mild condition; particle size of 90 mesh, SS ratio of 0.035, and a delignification time of 2.5 min. The integrated treatment was characterized by decreased lignin and hemicellulose content, but there is a slight increase in cellulose content. In this work, the best condition of MAA delignification was attained at mild condition of particle size, SS ratio, and irradiation time, namely 90 mesh, 0.035, and 2.5 min, respectively, resulted in the 1.698% decrease of lignin content. From kinetic study, using MAA, the pretreatment of CPH performed faster conversion rate for lignin with kinetic constants of lignin (k_{lignin}) and cellulose ($k_{\text{cellulose}}$) are 1.241 and 1.029, respectively. Furthermore, kinetic order found for lignin (n_{lignin}) and cellulose ($n_{\text{cellulose}}$) were 1.264 and 1.312, respectively. This study presents that MAA process is an effective pretreatment method to degrade lignin and simultaneously favouring cellulose degradation.

Acknowledgment

Authors thank the Directorate General of Resources for Science, Technology, and Higher education, Ministry of Research, Technology, and Higher Education of Republic Indonesia for funding this work.

References

- [1] Gazliya, N., Aparna, K. (2019). Microwave-assisted alkaline delignification of banana peduncle. *Journal of Natural Fibers*, 1–10, doi: 10.1080/15440478.2019.1645786.
- [2] Gollakota, A.R.K., Kishore, N., Gu, S. (2018). A review on hydrothermal liquefaction of biomass. *Renewable and Sustainable Energy Reviews*, 81, 1378–1392, doi: 10.1016/j.rser.2017.05.178.
- [3] Muharja, M., Fadhilah, N., Nurtono, T., Widjaja, A. (2020). Enhancing enzymatic digestibility of coconut husk using nitrogen assisted-subcritical water for sugar production. *Bulletin of Chemical Reaction Engineering & Catalysis*, 15(1), 84–95, doi: 10.9767/brec.15.1.5337.84-95.
- [4] Muharja, M., Junianti, F., Ranggina, D., Nurtono, T., Widjaja, A. (2018). An integrated green process: Subcritical water, enzymatic hydrolysis, and fermentation, for biohydrogen production from coconut husk. *Bioresource Technology*, 249, 268–275, doi: 10.1016/j.biortech.2017.10.024.
- [5] Darmayanti, R.F., Amini, H.W., Rizkiana, M.F., Setiawan, F.A., Palupi, B., Rahmawati, I., Susanti, A., Fachri, B.A. (2019). Lignocellulosic material from main Indonesian plantation commodity as the feedstock for fermentable sugar in biofuel production. *ARPJ Journal of Engineering and Applied Sciences*, 14(20), 3524–3534.
- [6] Rajeswari, G., Arutselvy, B., Jacob, S. (2020). Delignification of Aloe Vera Rind by Mild Acid Associated Microwave Pretreatment to Persuade Enhanced Enzymatic Saccharification. *Waste and Biomass Valorization*, 11, 5965–5975, doi: 10.1007/s12649-019-00830-7.
- [7] Muharja, M., Fadhilah, N., Darmayanti, R.F., Sangian, H.F., Nurtono, T., Widjaja, A. (2020). Effect of severity factor on the subcritical water and enzymatic hydrolysis of coconut husk for reducing sugar production. *Bulletin of Chemical Reaction Engineering & Catalysis*, 15(3), 786–797, doi: 10.9767/brec.15.3.8870.786-797.
- [8] Kohli, K., Katuwal, S., Biswas, A., Sharma, B.K. (2020). Effective delignification of lignocellulosic biomass by microwave assisted deep eutectic solvents. *Bioresource Technology*, 303, 122897, doi: 10.1016/j.biortech.2020.122897.
- [9] Muley, P.D., Mobley, J.K., Tong, X., Novak, B., Stevens, J., Moldovan, D., Shi, J., Boldor, D. (2019). Rapid microwave-assisted biomass delignification and lignin depolymerization in deep eutectic solvents. *Energy Conversion and Management*, 196, 1080–1088, doi: 10.1016/j.enconman.2019.06.070.
- [10] Muharja, M., Umam, D.K., Pertiwi, D., Zuhdan, J., Nurtono, T., Widjaja, A. (2019). Enhancement of sugar production from coconut husk based on the impact of the combination of surfactant-assisted subcritical water and enzymatic hydrolysis. *Bioresource Technology*, 274(November 2018), 89–96. <https://doi.org/10.1016/j.biortech.2018.11.074>.
- [11] Liu, Y., Sun, B., Zheng, X., Yu, L., Li, J. (2018). Integrated microwave and alkaline treatment for the separation between hemicelluloses and cellulose from cellulosic fibers. *Bioresource Technology*, 247, 859–863, doi: 10.1016/j.biortech.2017.08.059.
- [12] Sorn, V., Chang, K.L., Phitsuwan, P., Ratanakhanokchai, K., Dong, C.D. (2019). Effect of microwave-assisted ionic liquid/acidic ionic liquid pretreatment on the morphology, structure, and enhanced delignification of rice straw. *Bioresource Technology*, 293, 121929, doi: 10.1016/j.biortech.2019.121929.
- [13] Muharja, M., Junianti, F., Nurtono, T., Widjaja, A. (2017). Combined subcritical water and enzymatic hydrolysis for reducing sugar production from coconut husk. *AIP Conference Proceedings*, 1840(1), 30004, doi: 10.1063/1.4982264.
- [14] Avelino, F., da Silva, K.T., de Souza Filho, M.S.M., Mazzetto, S.E., Lomonaco, D. (2018). Microwave-assisted organosolv extraction of coconut shell lignin by Brønsted and Lewis acids catalysts. *Journal of Cleaner Production*, 189, 785–796, doi: 10.1016/j.jclepro.2018.04.126.
- [15] Kamalini, A., Muthusamy, S., Ramapriya, R., Muthusamy, B., Pugazhendhi, A. (2018). Optimization of sugar recovery efficiency using microwave assisted alkaline pretreatment of cassava stem using response surface methodology and its structural characterization. *Journal of Molecular Liquids*, 254, 55–63, doi: 10.1016/j.molliq.2018.01.091.
- [16] Alexander, R.A., Innasimuthu, G.M., Rajaram, S.K., Jeganathan, P.M., Chellam Somasundarar, S. (2020). Process optimization of microwave-assisted alkali pretreatment for enhanced delignification of *Prosopis juliflora* biomass. *Environmental Progress and Sustainable Energy*, 39(1), 1–11, doi: 10.1002/ep.13289.
- [17] Avelino, F., Marques, F., Soares, A.K.L., Silva, K.T., Leitão, R.C., Mazzetto, S.E., Lomonaco, D. (2019). Microwave-Assisted Organosolv Delignification: A Potential Eco-Designed Process for Scalable Valorization of Agroindustrial Wastes. *Industrial and Engineering Chemistry Research*, 58(25), 10698–10706, doi: 10.1021/acs.iecr.9b01168.

- [18] Datta, R. (1981). Acidogenic fermentation of lignocellulose-acid yield and conversion of components. *Biotechnology and Bioengineering*, 23(9), 2167–2170, doi: 10.1002/bit.260230921.
- [19] Fachri, B.A., Sari, P., Yuwanti, S., Subroto, E. (2020). Experimental study and modeling on supercritical CO₂ extraction of Indonesian raw propolis using response surface method: Influence of pressure, temperature and CO₂ mass flowrate on extraction yield. *Chemical Engineering Research and Design*, 153, 452–462, doi: 10.1016/j.cherd.2019.11.014.
- [20] Zhu, Z., Macquarrie, D.J., Simister, R., Gomez, L.D., McQueen-Mason, S.J. (2015). Microwave assisted chemical pretreatment of Miscanthus under different temperature regimes. *Sustainable Chemical Processes*, 3(1), 15, doi: 10.1186/s40508-015-0041-6.
- [21] Subhedar, P.B., Gogate, P.R. (2014). Alkaline and ultrasound assisted alkaline pretreatment for intensification of delignification process from sustainable raw-material. *Ultrasonics Sonochemistry*, 21(1), 216–225, doi: 10.1016/j.ultsonch.2013.08.001.
- [22] Kim, J.S., Lee, Y.Y., Kim, T.H. (2016). A review on alkaline pretreatment technology for bioconversion of lignocellulosic biomass. *Bioresource Technology*, 199, 42–48, doi: 10.1016/j.biortech.2015.08.085.
- [23] Aguilar-Reynosa, A., Román, A., Rodríguez-Jasso, R.M., Aguilar, C.N., Garrote, G., Ruiz, H.A. (2017). Comparison of microwave and conduction-convection heating autohydrolysis pretreatment for bioethanol production. *Bioresource Technology*, 243, 273–283, doi: 10.1016/j.biortech.2017.06.096.
- [24] Asomaning, J., Haupt, S., Chae, M., Bressler, D.C. (2018). Recent developments in microwave-assisted thermal conversion of biomass for fuels and chemicals. *Renewable and Sustainable Energy Reviews*, 92, 642–657, doi: 10.1016/j.rser.2018.04.084.
- [25] Zhou, L., Budarin, V., Fan, J., Sloan, R., Macquarrie, D. (2017). Efficient Method of Lignin Isolation Using Microwave-Assisted Acidolysis and Characterization of the Residual Lignin. *ACS Sustainable Chemistry & Engineering*, 5(5), 3768–3774, doi: 10.1021/acssuschemeng.6b02545.
- [26] Panthapulakkal, S., Kirk, D., Sain, M. (2015). Alkaline extraction of xylan from wood using microwave and conventional heating. *Journal of Applied Polymer Science*, 132(4), 41330, doi: 10.1002/app.41330.
- [27] Adjin-Tetteh, M., Asiedu, N., Dodoo-Arhin, D., Karam, A., Amaniampong, P.N. (2018). Thermochemical conversion and characterization of cocoa pod husks a potential agricultural waste from Ghana. *Industrial Crops and Products*, 119, 304–312, doi: 10.1016/j.indcrop.2018.02.060.
- [28] Chun, K.S., Husseinayah, S., Osman, H. (2014). Development of biocomposites from Cocoa pod husk and Polypropylene: Effect of filler content and 3-aminopropyltriethoxysilane. *Polymers from Renewable Resources*, 5(4), 139–156, doi: 10.1177/204124791400500401.
- [29] Wang, H., Chen, C., Fang, L., Li, S., Chen, N., Pang, J., Li, D. (2018). Effect of delignification technique on the ease of fibrillation of cellulose II nanofibers from wood. *Cellulose*, 25(12), 7003–7015, doi: 10.1007/s10570-018-2054-2.
- [30] Noguchi, T., Tashiro, Y., Yoshida, T., Zheng, J., Sakai, K., Sonomoto, K. (2013). Efficient butanol production without carbon catabolite repression from mixed sugars with *Clostridium saccharoperbutylacetonicum* N1-4. *Journal of Bioscience and Bioengineering*, 116(6), 716–721, doi: 10.1016/j.jbiosc.2013.05.030.
- [31] Keshwani, D.R., Cheng, J.J. (2010). Modeling changes in biomass composition during microwave-based alkali pretreatment of switchgrass. *Biotechnology and Bioengineering*, 105(1), 88–97, doi: 10.1002/bit.22506.