

# OPTIMIZATION OF PURIFIED CELLULOSE EXTRACTION FROM CORN COB AND CHARACTERIZATION OF THE ISOLATED PRODUCT

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This study reported the isolation of cellulose from corn cob by using microwave-assisted alkaline treatment and bleaching. The Central Composite Design (CCD) approach of Response Surface Methodology (RSM) was applied to study the effect of sodium hydroxide (NaOH) concentrations (1.5-2.5M), duration of alkaline treatment (4-8 min), hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) concentrations (7-9 wt%) and bleaching time (3-7 min) on the removal percent of non-cellulosic components and impurities from corn cob. The optimized conditions obtained from this study were 2.37M NaOH, 9 wt% H<sub>2</sub>O<sub>2</sub>, alkaline treatment time of 4 min, and bleaching time – 4.20 min. The non-cellulosic part and the impurities removed under the optimized conditions in microwave-assisted alkaline treatment and bleaching amounted to 72.26 ± 0.15%. The cellulose content in the thus purified corn cob material was determined as 92.47%. The isolated corn cob cellulose was characterized by scanning electron microscopy (SEM), Fourier transform infrared spectroscopy (FTIR), X-ray powder diffraction (XRD), and the thermogravimetric analysis (TGA). These characterizations confirmed that the alkaline treatment and microwave-assisted bleaching under the optimized conditions achieved the optimal removal percent of the non-cellulosic components and impurities from the corn cob biomass to achieve highly purified cellulose.

**Keywords:** cellulose, corn cob, microwave-assisted, isolation, optimization

## INTRODUCTION

Biomass wastes are widely available as they are inexpensive and abundant in nature. There are five basic categories of biomass materials: virgin wood, energy crops, agricultural residues, food waste, and industrial waste.<sup>1,2</sup> Agricultural residues, such as rice straw, wheat straw, rice husk, and corn stover, may be used as fodder, but are also commonly left in the fields after harvesting the crops or burned in the open air.

Cellulose is the main constituent in plant cell walls, it is surrounded by a monolayer of hemicelluloses, and is embedded in a matrix of hemicelluloses and lignin. Cellulose is a large-

molecule polymer composed of repeating units of D-glucose units joined by β-1,4-glycosidic bonds. Hydrogen bonds form an extensive network within the intramolecular and intermolecular cellulose, directly contributing to its crystalline structure.<sup>5</sup> Hemicelluloses are short-chain polysaccharides, heteropolymers composed of several water-soluble sugars, such as mannose, glucose, xylose, and others.<sup>3</sup> Lignin is a crosslinked phenolic polymer with aliphatic and aromatic structures, such as p-coumaryl alcohol, coniferyl alcohol and sinapyl alcohol.<sup>4</sup>

Different sources of lignocellulosic biomass have varied cellulose, hemicelluloses, and lignin contents.

Cellulose fibers isolated from biomass have many applications, such as in paper products, composites, textile materials, *etc.* Also, cellulose derivatives can be obtained by the chemical modification of cellulose, which can further widen its applicability, making it more robust, recyclable and biocompatible.<sup>6</sup>

Corn cob has been the most often studied among agricultural wastes, due to its high global production, with approximately 1.9 billion tons in 2021-2022.<sup>7</sup> Meanwhile, it represents 55% of the weight of corn, making it highly abundant after each harvesting period.<sup>8</sup> Considering that corn cob contains roughly 36–46% cellulose content, it can be transformed into more valuable materials than just being used as animal feed, left to degrade or burned in the open field, negatively impacting the environment.<sup>9</sup> However, it has been reported that different species of corn cob have varied cellulose contents.<sup>10,11,12</sup> Still, considering its non-toxicity, biodegradability and environmental friendliness, it can gain important economic value.

Alkaline hydrolysis is used to solubilize lignin through the cleavage of lignin-carbohydrate linkages, which leads to an easy breakdown of the lignocellulosic matrix. The mechanism of alkali hydrolysis involves the hydroxide ( $\text{OH}^-$ ) diatomic anions, weakening hydrogen bonds between cellulose and hemicelluloses.<sup>13</sup> Diluted sodium hydroxide (NaOH) was reported to swell woody fiber raw materials; as a result, it increased the internal surface area and decreased the degree of polymerization and crystallinity.<sup>13</sup> When high alkaline doses (6–20%) were applied, the dissolution of non-degraded polysaccharides occurred.<sup>14</sup> Various alkaline solutions, such as sodium hydroxide, potassium hydroxide, calcium hydroxide, and magnesium hydroxide, were reported to remove lignin and most of hemicelluloses. Among the alkali, sodium hydroxide was reported to be the most effective in the solubilization of large amounts of lignin.<sup>15–18</sup> In diluted alkaline treatments, hemicelluloses remained intact along with cellulose, but they could be solubilized when the concentration of NaOH was increased by more than 10%.<sup>19</sup> Besides, saponification of the ester-linked hemicelluloses and lignocellulosic materials and the removal of acetyl and uronic acid from the

hemicelluloses could occur during the alkaline pretreatment.<sup>19</sup>

Thus, the conditions of alkaline hydrolysis, such as alkali concentration, treatment time, and temperature, affect the subsequent yields, which also depend on differences in biomass compositions. The effects of the alkali concentration and alkaline treatment time were reported in studies on cellulose extraction with alkaline hydrolysis assisted by microwave irradiation,<sup>15,16,20–23</sup> and without.<sup>24–29</sup> Other assisting methods in the extraction of cellulose have also been studied, such as ultrasound,<sup>30,31</sup> and steam explosion.<sup>16</sup> However, ultrasound has not been proved to be very efficient for the objectives of this study, while steam explosion required specialized equipment, capable of withstanding high-pressure conditions. Hence, the microwave-assisted treatment was preferred in this study.

The objective of the present study has been to obtain highly purified cellulose from corn cob, after removing most of the non-cellulosic components and the impurities naturally found in biomass through several treatment steps. As reported previously, besides cellulose, corn cob also contains major non-cellulosic components, such as hemicelluloses and lignin, as well as impurities, such as fats, starch and proteins, in contents of 0.3%, 0.67%, and 4.26%, respectively.<sup>11</sup> Considering the objective of this work, for the sake of simplicity, in this paper, the term “impurities” will be used to include all these major and minor non-cellulosic elements. In order to isolate the cellulose, microwave-assisted alkaline treatment and bleaching of the initial raw materials was performed. To ensure an efficient process, the optimum extraction conditions were determined by RSM. Thus, NaOH concentration (1.5–2.5M), alkaline treatment time (4–8 min),  $\text{H}_2\text{O}_2$  concentration (7–9 wt%), and bleaching time (3–7 min) were chosen as factors in RSM to examine their effect on the response variable of interest, *i.e.*, the removal percent of impurities from the corn cob biomass. A central composite design (CCD) coupled with RSM was used to optimize the response variable. The cellulose isolated under the optimized conditions from the corn cob was then characterized by various techniques, such as field emission scanning electron microscopy (FESEM), Fourier transform infrared spectroscopy (FTIR), particle size analysis, thermogravimetric analysis (TGA) and X-ray powder diffraction (XRD).

## EXPERIMENTAL

### Materials

Corn cob (Taiwan Species, code: 518) was locally sourced from Malim Nawar, Perak, Malaysia. Sodium hydroxide (NaOH) was purchased from R&M Chemicals Sdn Bhd, hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>, 35%), and hexadecyltrimethylammonium bromide (CTAB, ≥97%), were purchased from Merck, and sulfuric acid (H<sub>2</sub>SO<sub>4</sub>, 96%), was purchased from Fisher Scientific (M).

### Pretreatment of corn cob

The corn cob was dried in an oven at 105 °C overnight until it reached a constant weight.<sup>40</sup> Then, it was cut into smaller pieces and ground in a grinding machine (RT-N08, Rong Tsong, Taiwan). The obtained corn cob powder was sieved using a 500 μm sieve. Before storage, the moisture content of corn cob was determined using a moisture content analyzer (MX-50, A&D, Japan), by placing 5 g of corn cob in the moisture content analyzer's pan and heating it to 170 °C. The temperature was set at 170 °C to ensure complete moisture removal, considering that the water boiling point is at 100 °C, while the lowest decomposition temperature of the corn cob components was reported to start around 160 °C.<sup>41</sup> The moisture content of the corn cob in this study was found to be 10.57%, below the 15% limit for the inhibition of anaerobic microbial activity.<sup>42</sup> The corn cob was stored in a desiccator with desiccant until further processing.

### Optimization study of alkaline treatment and bleaching conditions

Design Expert Software (Stat-Ease Inc., Version 6.0) and RSM were employed to optimize the parameters for the alkaline treatment and bleaching for removing hemicelluloses, lignin and impurities, from the corn cob powder. The four chosen factors were: NaOH concentration (1.5–2.5M), alkaline treatment time (4–8 min), H<sub>2</sub>O<sub>2</sub> concentration (7–9 wt%) and bleaching time (3–7 min). A designed experiment consisting of 28 runs was proposed. The response variable was set to be the removal percent of impurities. For a typical run, 5 g of corn cob powder was measured and pre-dried in the oven for 1 hour at 105 °C. After pre-drying, it was added to 250 mL of NaOH

solution in a 500 mL beaker covered with a watch glass and magnetically stirred. The mixture of corn cob powder with NaOH solution was irradiated at high power (800 W) using a household microwave oven (R-218H, Sharp, Japan). After heating, it was cooled and washed with distilled water to remove the residual NaOH solution, assisted by a centrifuge machine (CT15RT, Techcomp, UK) up to six times, at room temperature and 8000 rpm. The washing step lasted for 15 min. The treated corn cob powder was mixed and added to 250 mL of H<sub>2</sub>O<sub>2</sub> solution. The mixture of corn cob powder with H<sub>2</sub>O<sub>2</sub> solution was again irradiated at high power (800 W) using the same microwave oven. After heating, it was cooled and washed by a similar procedure as described above to remove the residual NaOH solution.

### Design of experiments

RSM was applied to determine the optimum conditions (NaOH concentration, time of alkaline treatment, H<sub>2</sub>O<sub>2</sub> concentration and bleaching time) of the reactions from the experiments and obtain the maximum value of the response variable (removal percent of impurities) using Design Expert Software 6.0. The coded level of each variable is presented in Table 1. The second-order polynomial model equation was fitted to each factor as in Equation (1):

$$\hat{y} = \beta_0 + \beta_1x_1 + \beta_2x_2 + \beta_3x_3 + \beta_4x_4 + \beta_{12}x_1x_2 + \beta_{13}x_1x_3 + \beta_{14}x_1x_4 + \beta_{23}x_2x_3 + \beta_{24}x_2x_4 + \beta_{34}x_3x_4 + \beta_{11}x_1^2 + \beta_{22}x_2^2 + \beta_{33}x_3^2 + \beta_{44}x_4^2$$

where  $\hat{y}$  represents the impurities removal percentage, while  $x_1$  is NaOH concentration (A),  $x_2$  – alkaline treatment time (B),  $x_3$  – the H<sub>2</sub>O<sub>2</sub> concentration (C) and  $x_4$  – bleaching time (D);  $\beta$  represents the constant coefficient ( $\beta_0$  – a constant,  $\beta_i$  – the linear coefficient,  $\beta_{ij}$  – the interaction coefficient, and  $\beta_{ii}$  – the quadratic coefficient). The coefficient of variation and statistical significance were used in the analysis of variance (ANOVA) to assess the quality of fit to the polynomial model equation.

The optimized conditions of NaOH concentration, alkaline treatment time, H<sub>2</sub>O<sub>2</sub> concentration, and bleaching time were calculated using the numerical optimization function in the Design Expert software. The optimized conditions for the maximum hemicelluloses and lignin removal were repeated five times to obtain the reliability and repeatability of the developed model.

Table 1  
Coded levels of variables

Variable	Code	Symbol	Level		
			Low (-1)	Intermediate (0)	High (+1)
NaOH concentration (mol/L)	A	$x_1$	1.5	2.0	2.5
Alkaline treatment time (min)	B	$x_2$	4	6	8
H <sub>2</sub> O <sub>2</sub> concentration (wt%)	C	$x_3$	7	8	9
Bleaching time (min)	D	$x_4$	3	5	7

**Determination of cellulose content**

The cellulose content of the raw corn cob and isolated corn cob cellulose was determined using the AOAC methods (AOAC 973.18-1990)<sup>43</sup> for acid detergent fiber (ADF) and acid detergent lignin (ADL). The ADF solution was prepared using CTAB dissolved in sulfuric acid (1 N) in a 1 L volumetric flask, while the ADL solution was prepared in 72% sulfuric acid. The cellulose content can be determined by subtracting ADL from ADF.

**Characterizations of isolated corn cob cellulose**

**Field emission scanning electron microscopy (FESEM)**

FESEM images were taken to elucidate the morphologies of untreated corn cob powder, and corn cob cellulose isolated under the optimized conditions using a JEOL JSM-6701F SEM. The samples were fixed on a metal stub with carbon tape, and the surface of the samples was coated with platinum by an ion sputter coater. Images were taken at a 4.0 kV accelerating voltage and x5,000 magnification.

**Fourier transform infrared spectroscopy (FTIR)**

The spectra of untreated corn cob powder, and corn cob cellulose isolated under the optimized conditions were obtained by FTIR (model Spectrum RX1) to examine any changes in the functional groups after the samples were submitted to the various treatment conditions. The samples were ground and prepared as potassium bromide (KBr) pellets for FTIR analysis. 32 scans were performed from 400 cm<sup>-1</sup> to 4000 cm<sup>-1</sup>, at the resolution of 4 cm<sup>-1</sup>. The spectrum of microcrystalline cellulose (Sigma-Aldrich, 2022) was also included for reference.

**X-ray powder diffraction (XRD)**

The crystallinities of untreated corn cob powder, and corn cob cellulose isolated under the optimized conditions were determined using a Shimadzu 6000 XRD. The samples were scanned at a rate of 2°/min from 10° to 80° (2θ), using a Cu K<sub>α</sub> radiation (λ = 1.540600 Å) at 40 kV and 30 mA, while the scanning rate was 2°/min. The crystallinity index (CrI) was calculated using Equation (2), where I<sub>002</sub> is the maximum intensity of the principal peak while I<sub>am</sub> is the diffraction intensity of the amorphous cellulose:

$$CrI = \frac{I_{002} - I_{am}}{I_{002}} \times 100 \tag{2}$$

**Thermogravimetric analysis (TGA)**

In order to assess the thermal degradation of untreated corn cob powder and isolated corn cob cellulose, thermogravimetric analysis (TGA) was carried out using a TGA/DSC3+ Star System. The weight loss of each sample was measured as a function of temperature, which was increased from 30 °C to 600 °C, at a heating rate of 10 °C/min in a nitrogen environment.

**RESULTS AND DISCUSSION**

**Statistical analysis**

According to the RSM design, 28 experiments were conducted under the specified conditions, and the results are summarized in Table 2. From the experimental results obtained, the second-order polynomial model (Eq. 1) was fitted based on the responses, which resulted in Equation (3), which represents the response model with the fundamental factors in their original units, where y is the response for the removal percent of impurities. The values of the removal percent of impurities obtained from Equation (3) are presented in Table 2.

$$\hat{y} = 72.54 + 1.30x_1 + 0.44x_2 + 0.18x_3 - 0.11x_4 - 0.42x_{12} + 0.32x_{13} - 0.22x_{23} - 1.36x_1^2 + 0.18x_2^2 + 0.41x_3^2 - 0.19x_4^2 \tag{1}$$

Regression analysis and analysis of variance (ANOVA) were used to fit the model and determine the terms' statistical significance. A summary of the ANOVA is presented in Table 3. First, the ANOVA demonstrated that the quadratic regression model (Eq. 3) is highly significant, as the F-test had a very low probability value (p < 0.0001). Table 3 also presents the estimated regression coefficients of the quadratic polynomial model of the response surface, the regression coefficient of determination (R<sup>2</sup>), and the proportion of variation in the response characteristic. In addition, the adjusted R<sup>2</sup> values were referred to in order determine the model's accuracy. It has been suggested that a well-fitted model's R<sup>2</sup> value should not be less than 0.8, as a lower R<sup>2</sup> value indicates the model's unsuitability for explaining the relationship between variables.<sup>44</sup>

Table 2  
Responses for parameters used in central composite design

Run	NaOH concentration (mol/L)	Alkaline treatment time (min)	H <sub>2</sub> O <sub>2</sub> concentration (wt%)	Bleaching time (min)	Predicted removal percent of impurities (%)	Actual removal percent of impurities (%)
1	1.5	4	7	3	69.61	69.04
2	2.5	4	7	3	72.29	72.46
3	1.5	8	7	3	71.60	71.41
4	2.5	8	7	3	72.61	72.62
5	1.5	4	9	3	69.73	69.20
6	2.5	4	9	3	73.68	74.02
7	1.5	8	9	3	70.85	71.28
8	2.5	8	9	3	73.13	73.13
9	1.5	4	7	7	69.07	68.96
10	2.5	4	7	7	71.99	71.78
11	1.5	8	7	7	71.38	71.26
12	2.5	8	7	7	72.64	73.07
13	1.5	4	9	7	69.26	69.47
14	2.5	4	9	7	73.45	73.53
15	1.5	8	9	7	70.70	70.42
16	2.5	8	9	7	73.23	74.01
17	1.0	6	8	5	64.50	65.13
18	3.0	6	8	5	69.71	68.96
19	2.0	2	8	5	72.36	72.72
20	2.0	10	8	5	74.13	73.65
21	2.0	6	6	5	73.82	74.17
22	2.0	6	10	5	74.53	74.06
23	2.0	6	8	1	72.00	72.22
24	2.0	6	8	9	71.56	71.23
25	2.0	6	8	5	72.54	72.53
26	2.0	6	8	5	72.54	72.29
27	2.0	6	8	5	72.54	72.47
28	2.0	6	8	5	72.54	72.87

Table 3  
ANOVA results for the quadratic model – Equation (3)

Sources	Removal percent of impurities (%)		
	F-value	P value	Significance
Model	40.14	<0.0001	**
A ( $x_1$ )	157.70	<0.0001	**
B ( $x_2$ )	18.16	0.0006	**
C ( $x_3$ )	2.90	0.1076	
D ( $x_4$ )	1.13	0.3043	
AB ( $x_{12}$ )	10.75	0.0047	**
AC ( $x_{13}$ )	6.21	0.0241	**
BC ( $x_{23}$ )	2.97	0.1041	
A <sup>2</sup> ( $x_1^2$ )	171.99	<0.0001	**
B <sup>2</sup> ( $x_2^2$ )	2.87	0.1096	
C <sup>2</sup> ( $x_3^2$ )	15.50	0.0012	**
D <sup>2</sup> ( $x_4^2$ )	3.34	0.0864	
Lack of fit	5.17	0.1011	Not significant
R <sup>2</sup> = 0.9650		Adjusted R <sup>2</sup> = 0.9410	
CV = 0.71		Predicted R <sup>2</sup> = 0.8593	
Standard deviation = 0.51		Mean = 71.71	

Nevertheless, the  $R^2$  values obtained from ANOVA revealed 0.9650 for the removal percent of impurities, indicating that the model successfully described the correlation between the factors. On the other hand, the adjusted  $R^2$  obtained from this model was high, 0.9410, which also fulfills the requirement that the difference between the predicted  $R^2$  and adjusted  $R^2$  is within 0.2. Moreover, the model's 'lack of fit' did not result in a significant p-value for the studied variables, which was 0.1011 ( $p > 0.05$ ), indicating the model was not significantly related to the error rate and sufficiently accurate to predict relevant responses.

It is suggested that the model utilized in this study (Eq. 3) can determine the optimal operating conditions for the selective isolation of cellulose from corn cob. A model's coefficient of variance (CV) is usually lower than 10, which means the model has good repeatability.<sup>38</sup> The CV of this model was 0.71, further indicating that the model exhibited an excellent ability to predict the observed data.

According to Table 3, NaOH concentration is the most significant factor affecting the removal percentage of impurities due to its highest coefficient value and lowest P value. In contrast, the alkaline treatment time,  $H_2O_2$  concentration, and n time of bleaching were less significant. Based on Equation (3), the coefficients with one factor represented the effect on that factor, while the coefficients with two factors and with second-order terms represented the interaction between the two factors and the quadratic effect,

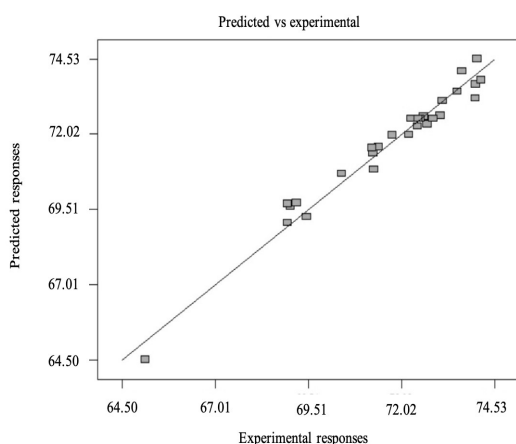


Figure 1: Graph of y-predicted vs. y-experimental responses

Figure 2 (a, c, and e) demonstrate that NaOH concentration and alkaline treatment time had the most significant effect on the removal percent of

impurities. The positive sign before the terms indicated a synergistic effect, while the negative sign indicated an antagonistic effect. Hence, the model allowed us to define the optimal composition for the percentage of impurities removal and showed the combined effects of the four factors studied.

Figure 1 shows the predicted responses from the empirical model and the actual responses from the experiments. From the plot obtained, the data points are close to the actual points from experiments, which leads to an  $R^2$  close to unity. While the normal probability plot specifies that the normal percent probability distribution follows the residuals, the points fitted on a straight line and obtained in Figure 2 indicated that the quadratic polynomial model satisfied the assumptions of the analysis of variance to get optimized conditions in the isolation of cellulose.<sup>45</sup>

### Analysis of response surface plots

The 3D surface plots and contour plots of the combined effects of the variables of NaOH concentration (A), the alkaline treatment time (B),  $H_2O_2$  concentration (C), and bleaching time (D) on the removal percent of impurities were shown in Figure 2. In this study, 3D response surfaces were obtained by keeping one of the variables constant at a zero level, while varying the other variables. Figure 2 shows a quadratic effect of NaOH concentration, the alkaline treatment time,  $H_2O_2$  concentration, and bleaching time on the removal percent of impurities.

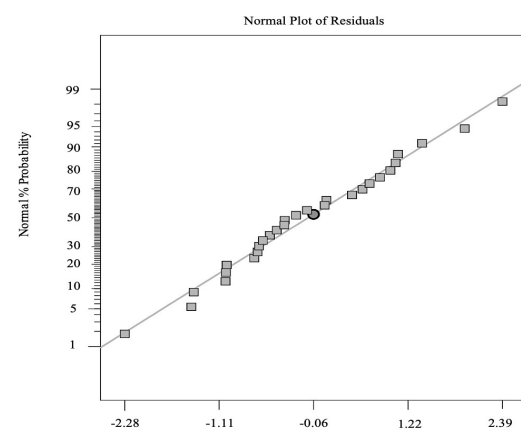


Figure 2: Normal probability plot of residuals

impurities. The effects of these four variables on the best removal conditions' percentage of impurities were illustrated using a three-

dimensional representation of the response surface. The 3D response surfaces further verify the significance of each of the terms in Equation (3) from Table 3, in which A, B, AB, AC,  $A^2$ , and  $C^2$  were significant terms, while C, D, AD, BC, BD, CD,  $B^2$ , and  $D^2$  were insignificant terms in this study. One point is worth noting: as these four parameters were studied using RSM, they interact with each other, and the response outcome was not affected by one factor, but all.

### Effect of alkaline concentration

NaOH concentration is the most significant factor in the whole reaction. It played a prominent role in removing impurities from corn cob to isolate cellulose. In the 3D surface plot, NaOH concentration was shown to be a significant variable correlated with the data provided in Table 3. From the surface plot in Figure 3 (a), a curve slope indicated an optimal point within the studied range, which indicated the removal percent of impurities increased when alkaline concentration and alkaline treatment time increased. Although the interaction of A-B was significant, it suggests that a more apparent response was obtained when both variables were studied: A at around 2.25 M of NaOH concentration, and B at a higher level. From Figure 3 (a and c), it was interesting to note that NaOH concentration significantly impacted the response. Hence, it gave a distinct curve slope in every 3D surface plot that involved NaOH

concentration. This behavior might be attributed to the excessive swelling of the cellulose in the presence of 2.25M–2.5M NaOH, which might prevent hemicelluloses from further separating from the fiber structure, as it swelled extensively.<sup>46</sup>

### Effect of alkaline treatment time

As the duration of alkaline treatment is also one of the significant factors in this reaction, it affected the removal percentage of impurities in the same way as NaOH concentration. The longer the reaction time, the higher the removal percent of impurities. In Figure 3 (a and b), a linear slope was obtained for the alkaline treatment time. The observation indicated that the surface response might be possible to obtain an optimized response in a higher range. At the same time, the trend suggested that the reaction might need a longer reaction time to allow the breakdown of the  $\alpha$ -ether linkage of hemicelluloses and lignin by NaOH.

### Effect of $H_2O_2$ concentration

$H_2O_2$  concentration was insignificant in this study because the alkaline treatment was the one that mainly removed the impurities and depressed the effect of  $H_2O_2$  in the cellulose isolation. It showed a concave curve slope in Figure 3 (b).

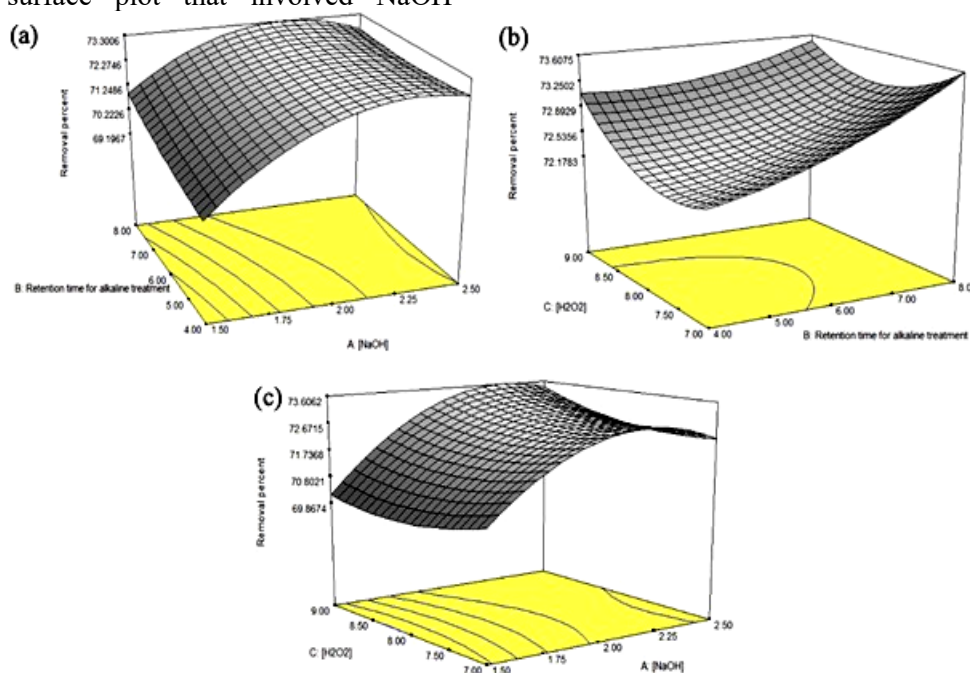


Figure 3: 3D surface plots of removal percent against (a) A-B (b) B-C (c) A-C

This trend was attributed to the interaction of H<sub>2</sub>O<sub>2</sub> concentration with the other three variables having different effects on the response. The slope obtained for H<sub>2</sub>O<sub>2</sub> concentration decreased at the lower concentration and then increased after 8 wt% H<sub>2</sub>O<sub>2</sub>. It was worth noting that as the maximum points felt at two sides, 7 wt% and 9 wt%, the surface response was still outside the region studied, and a higher level can be evaluated for the positive factor.

### Effect of bleaching time

The bleaching duration is insignificant in this study, as it is tightly connected with H<sub>2</sub>O<sub>2</sub> concentration. The reason for this was mentioned in the discussion regarding the effect of H<sub>2</sub>O<sub>2</sub> concentration. However, other researchers' findings reported that the H<sub>2</sub>O<sub>2</sub> concentration and bleaching time were substantial factors when the biomass was bleached without alkaline treatment.<sup>38</sup> Interestingly, while each studied factor is significant, not all combined factors examined are dominant. Based on this study, the dominant factors were NaOH concentration and alkaline treatment time.

### Optimization of treatment conditions

The optimization criteria were aimed to maximize the removal percent of impurities, with the four significant factors set to be within the range to construct desirability indices. According to Equation (3), the best pretreatment conditions were obtained, which included NaOH concentration (2.37M), alkaline treatment time (4 min), H<sub>2</sub>O<sub>2</sub> concentration (9 wt%) and bleaching time (4.36 min). The predicted value of the removal percent of impurities obtained from these optimized conditions was 73.86%. After performing the extraction under the optimal treatment conditions five times, the verification result was  $72.26 \pm 0.15\%$ , reinforcing the correctness and reliability of the established response model (Eq. 3).

### Characterization of isolated corn cob cellulose

#### Cellulose content determination

The cellulose content of the untreated corn cob and isolated corn cob cellulose was determined using ADF and ADL, and the results were listed in Table 4. The cellulose content in the isolated corn cob cellulose was higher than that reported by other researchers, implying a purified cellulose.<sup>20,21</sup>

### Morphology of isolated corn cob cellulose

The SEM images of untreated corn cob and isolated cellulose are shown in Figure 4. As seen in Figure 4 (a and b), the surface of the untreated corn cob was smooth, possibly due to the fact that its surface was covered with many non-cellulosic components. After the treatment with the alkaline solution and bleaching, the surface of the corn cob became rougher, and some pores can be seen (Fig. 4 (c and d)). Non-cellulosic components, such as pectin, lignin, and hemicelluloses, found in the amorphous regions, acted as a "natural binder" of cellulose, while their removal by the chemical treatments revealed a rough and porous surface.

Also, the particle sizes of the extracted corn cob cellulose were smaller than those of the untreated material. This also confirms the efficiency of the microwave-assisted chemical treatments in removing the outer layer of non-cellulosic components, thus decreasing the particle size from 500  $\mu\text{m}$  to an average of 70  $\mu\text{m}$  (Fig. 5), in each set of the experiments, as determined by the particle size analysis (Malvern, Masterizer, Hydro 2000 MU).

### Fourier transform infrared spectroscopy

The spectra of untreated corn cob and isolated corn cob cellulose analyzed by FTIR are shown in Figure 6. The spectrum of microcrystalline cellulose (Sigma-Aldrich, 2022) was included for reference. The peak around 3400  $\text{cm}^{-1}$  present in all the spectra was due to the stretching vibration of hydroxyl groups. The intense and broad peaks between 3550 and 3200  $\text{cm}^{-1}$  indicated the presence of hydrogen-bonded hydroxyl groups with intermolecular bonding in their structure. While the peak around 2900  $\text{cm}^{-1}$  represented the stretching vibration of saturated C-H in cellulose and hemicelluloses, the peak at 1643  $\text{cm}^{-1}$  in the spectra was attributed to the absorbed water H-O-H bending.

The peak at 1429  $\text{cm}^{-1}$  corresponded to the symmetric CH<sub>2</sub> bending vibration, while 1280  $\text{cm}^{-1}$  corresponded to C-O-C stretching in cellulose, residual hemicelluloses, and lignin. The peak at 896  $\text{cm}^{-1}$  corresponds to the  $\beta$ -glycosidic linkages between anhydroglucose rings on cellulose.<sup>16</sup>

In the spectrum of untreated corn cob material, the peak at 1732  $\text{cm}^{-1}$  corresponds to the stretching vibration of carbonyl groups (C=O), which represents the acetyl and uronic ester groups or ether linkage of carboxylic groups of



acetic, ferulic, and p-coumaric acid, which are the dominant constituents of hemicelluloses and lignin. The peaks detected at  $1515\text{ cm}^{-1}$  and  $1255\text{ cm}^{-1}$  corresponded to the C=C stretching of the aromatic ring and the C-O stretching of the ether linkages in hemicelluloses and lignin, respectively. These peaks disappeared in the spectrum of

isolated corn cob cellulose, and the intensity of the peak intensity at  $896\text{ cm}^{-1}$  increased, indicating the ester bond and carboxylic groups in hemicelluloses and lignin were cleaved and were removed after the microwave-assisted chemical treatment.<sup>47</sup>

Table 4  
Cellulose content of samples

Samples	Cellulose content (%)
Corn cob	33.37
Isolated corn cob cellulose	92.47

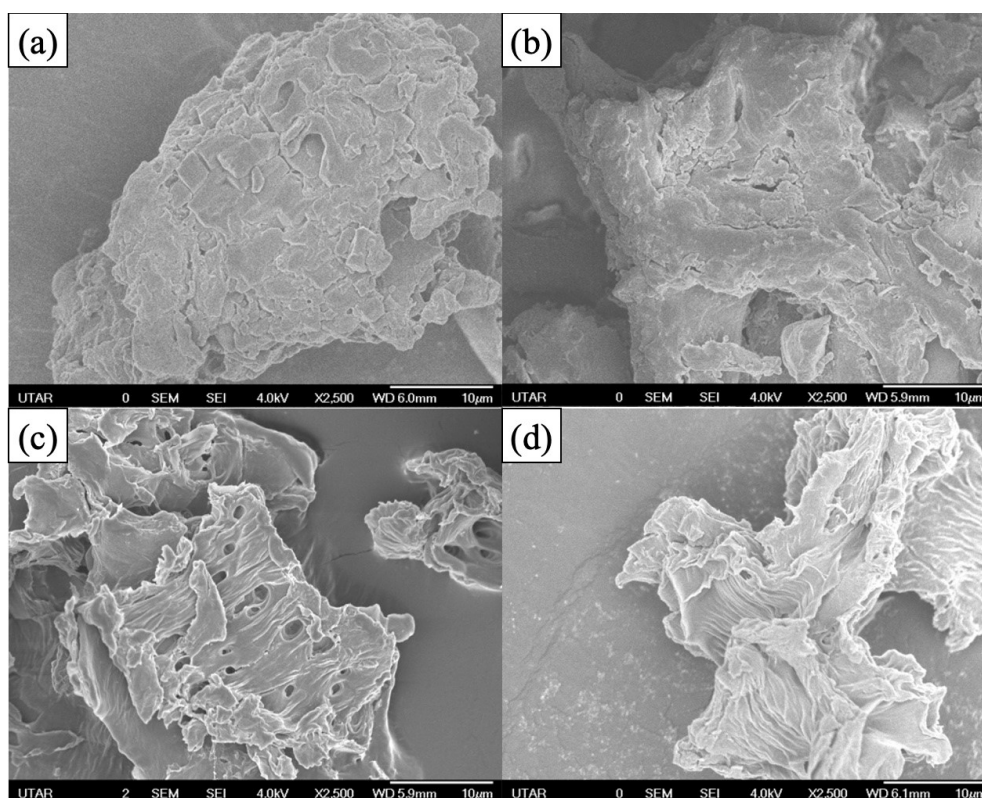


Figure 4: SEM images of (a), (b) untreated corn cob; (c), (d) cellulose isolated from corn cob under optimized conditions

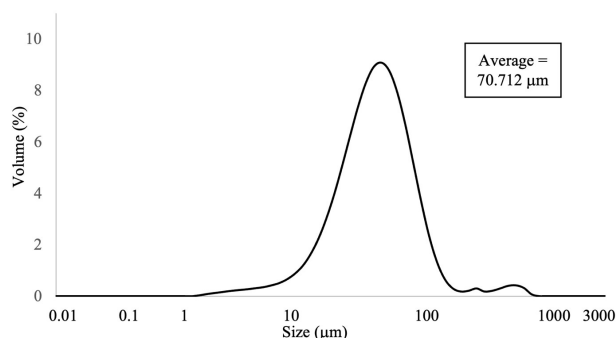


Figure 5: Particle size distribution of isolated cellulose using optimized conditions

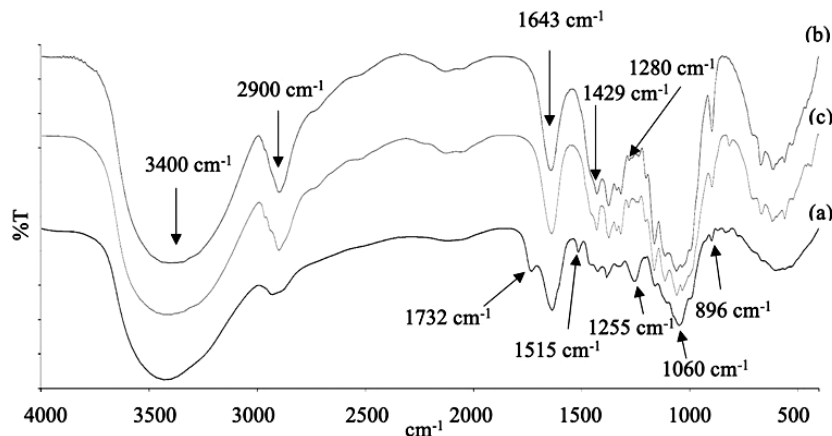


Figure 6: FTIR spectra of untreated corn cob (a), isolated corn cob cellulose (b), and commercial microcrystalline cellulose (c)

### *X-ray powder diffraction*

The diffractograms of untreated corn cob and isolated corn cob cellulose are shown in Figure 7. The typical peak of cellulose in the XRD diffraction pattern was recorded at  $2\theta = 15.7^\circ$ ,  $22.6^\circ$  and  $35.19^\circ$ .<sup>29</sup> Both samples showed a peak around  $2\theta = 15.4^\circ$  and  $22.1^\circ$ , indicating that the crystal integrity in the isolated corn cob cellulose remained intact. A sharper peak obtained in isolated corn cob cellulose at  $2\theta = 22.1^\circ$ , compared to that for the untreated corn cob, indicated an increase in crystallinity in its structure due to the removal of non-cellulosic constituents. Cellulose molecules' crystal-like properties caused them to be arranged in a typically ordered structure within their hydrogen bonds.<sup>38</sup> Naturally, cellulose's crystalline lattice is a monoclinic formation surrounded by amorphous hemicelluloses and embedded in a lignin matrix. Hence, the crystallinity of cellulose increased when the amorphous hemicelluloses and lignin matrix were removed by the microwave-assisted chemical treatments.

The crystallinity index (CrI) of untreated and isolated corn cob cellulose was calculated. The CrI of an untreated corn cob was 50.35%, while the isolated corn cob cellulose was 74.83%. The increase in the percentage of crystallinity indicated an increase in the cellulose rigidity, which had an ordered and compact molecular structure that would produce a high material strength.<sup>21</sup>

### *Thermogravimetric analysis*

Figures 8 and 9 show the thermogravimetric (TG) and differential thermogravimetric (DTG) curves of untreated corn cob and isolated corn cob cellulose, respectively. An initial slight weight loss is noted in both curves in the temperature range from  $30^\circ\text{C}$  to  $105^\circ\text{C}$ , corresponding to the loss of the absorbed moisture in the samples. However, the decomposition trend of the untreated corn cob was different from that of the isolated corn cob cellulose. This indicates that the untreated corn cob was composed of various components that decomposed at different temperatures. The maximum decomposition temperature ( $T_d$ ) for the untreated corn cob sample was  $220^\circ\text{C}$  and the onset of degradation was at  $200^\circ\text{C}$  ( $T_s$ ). Such observation can be attributed to the presence of lignin, which decomposes between  $150^\circ\text{C}$  and  $900^\circ\text{C}$ . The peak occurred at  $288^\circ\text{C}$  due to the decomposition of hemicelluloses, while the degradation of cellulose occurred at  $345^\circ\text{C}$ . Waters *et al.*<sup>48</sup> reported cellulose decomposition occurred between  $300^\circ\text{C}$  to  $400^\circ\text{C}$ , while hemicellulose decomposition occurred between  $220^\circ\text{C}$  to  $315^\circ\text{C}$ . On the other hand, Figure 9 shows that purified cellulose shows only one thermal degradation slope ( $343^\circ\text{C}$ ), which falls between  $300^\circ\text{C}$  and  $400^\circ\text{C}$ . This observation confirms that the hemicelluloses and lignin were successfully removed from the corn cob.

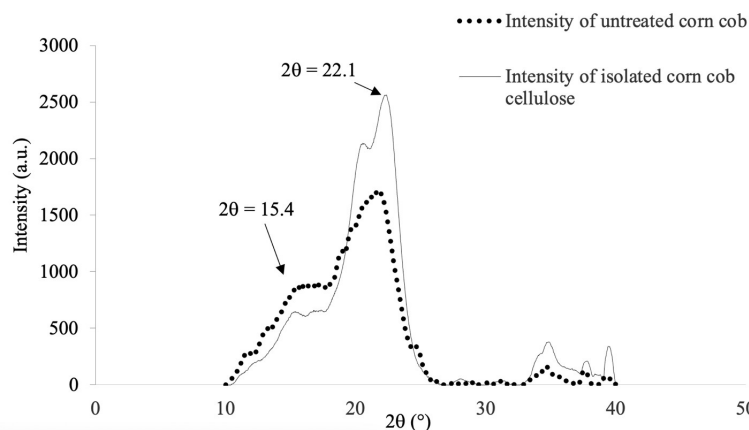


Figure 7: XRD diffraction patterns of untreated corn cob and isolated corn cob cellulose

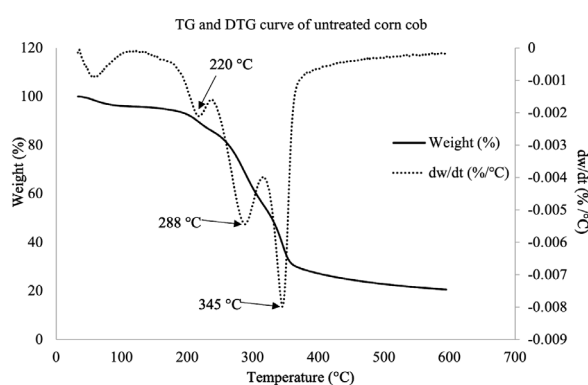


Figure 8: TG and DTG curves of untreated corn cob

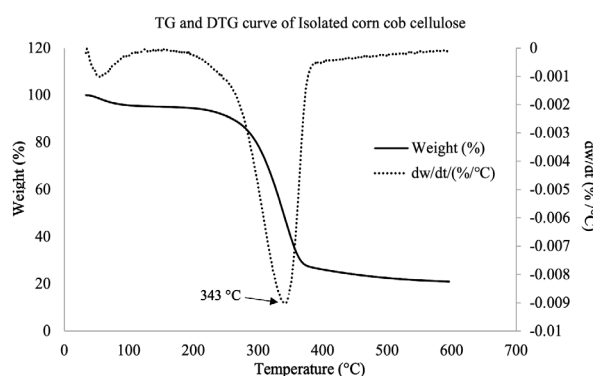


Figure 9: TG and DTG of isolated corn cob cellulose

## CONCLUSION

This study successfully develops a highly reliable and accurate response model using RSM to determine the optimum conditions that maximize the removal percentage of impurities from corn cob. The results show that the NaOH concentration and time of alkaline treatment are the significant factors, while  $H_2O_2$  concentration and time of bleaching are less significant in the response model. The maximum and verified removal percent of impurities was reported as 72.26% using the microwave-assisted alkaline treatment and microwave-assisted bleaching under the optimized conditions of 2.37M NaOH, 4 min of alkaline treatment time, 9 wt%  $H_2O_2$  and 4.20 min of bleaching time. Moreover, the efficacy of removing impurities from the raw material is high, as supported by the determination of the cellulose content, which was found to be of 92.47% in the isolated corn cob cellulose. At the same time, the morphology, chemical structure, crystallinity, and thermal properties of untreated corn cob and isolated corn cob cellulose were characterized using SEM, FTIR, XRD, and TGA. All analyses revealed that

the amorphous components, *i.e.*, hemicelluloses and lignin, as well as other impurities, were successfully removed.

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