

Research Article

Application of Box-Behnken Design in Optimization of Glucose Production from Oil Palm Empty Fruit Bunch Cellulose

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Received 23 July 2013; Accepted 8 October 2013

Academic Editor: Yulin Deng

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Oil palm empty fruit bunch fiber (OPEFB) is a lignocellulosic waste from palm oil mills. It contains mainly cellulose from which glucose can be derived to serve as raw materials for valuable chemicals such as succinic acid. A three-level Box-Behnken design combined with the canonical and ridge analysis was employed to optimize the process parameters for glucose production from OPEFB cellulose using enzymatic hydrolysis. Organosolv pretreatment was used to extract cellulose from OPEFB using ethanol and water as the solvents. The extracted cellulose was characterized by thermogravimetric analysis, FTIR spectroscopy, and field emission scanning electron microscopy. Hydrolysis parameters including amount of enzyme, amount of cellulose, and reaction time were investigated. The experimental results were fitted with a second-order polynomial equation by a multiple regression analysis and found that more than 97% of the variations could be predicted by the models. Using the ridge analysis, the optimal conditions reaction time found for the production of glucose was 76 hours and 30 min, whereas the optimum amount of enzyme and cellulose was 0.5 mL and 0.9 g, respectively. Under these optimal conditions, the corresponding response value predicted for glucose concentration was 169.34 g/L, which was confirmed by validation experiments.

1. Introduction

Bioconversion of lignocellulosic waste materials to chemicals and fuels are receiving interest as they are low cost, renewable, and widespread in nature [1]. Malaysia is well acknowledged for its potential in renewable resources of lignocellulosic materials such as oil palm waste, sugar cane bagasse, and rice straw. At present Malaysia is the largest exporter and producer of palm oil and its production accounts approximately 40–60% of world total palm oil over the 25 years [2, 3]. In the process of extraction of palm oil from oil palm fruit, a lignocellulosic material, namely, OPEFB, is generated as a main solid residue, with every ton of fresh fruit bunches producing ~0.22 ton of OPEFB [4]. In Malaysia, ~17 million tons of OPEFB is produced every year. Commonly, this biomass is burnt in incinerators, and it does not only create environmental pollution problems but it also offers limited value to the industry. Several approaches have been developed to utilize OPEFB fibres to produce valuable materials

including paper pulp, composite boards, thermoset polymer, and activated carbon [5].

OPEFB consists of 67% of holocellulose (cellulose and hemicellulose) and 24% of lignin [6]. Cellulose (β -glucan) is a polymer of glucose [7] and can be used as a substrate for the production of a wide variety of compounds by chemical and biochemical processes. One such compound is succinic acid, which is extensively used in food, pharmaceuticals, and manufacturing of biodegradable polymers [8]. Production of glucose from OPEFB cellulose increased the yield and concentration of glucose produced compared to using acid or base pretreated OPEFB as reported before. Enzymatic hydrolysis using cellulase from *Trichoderma reesei* (a mixture of endoglucanases, exoglucanases, and β -glucosidase) is widely used for the degradation of cellulose into soluble oligo-glucan/oligomers, cellobiose, and glucose [9]. In this project, a mixture of two enzymes was used to carry out the reaction. Novozyme Celluclast 1.5 L is a brand for cellulase enzyme that is capable to break the cellulose chains, particularly at middle

and chain end sections into cellobiose (the dimer of glucose). Whereas, Novozyme 188 that mainly contains β -glucosidase was used to optimize the glucose production since it breaks the cellobiose chains into glucose.

Optimization of parameters in reaction is one of the most important stages in the development of an efficient and economic production of high value products from renewable sources. The traditional "one-factor-at-a-time approach" is time consuming, and moreover the interactions between independent variables are not considered. Response surface methodology (RSM) is an effective optimization tool wherein many factors and their interactions can be identified with fewer experimental trials [10].

RSM has been widely used in various fields including food process operations, new product development, biotechnology-media composition, and bioprocessing such as enzymatic hydrolysis and fermentation [10]. The RSM is a collection of mathematical and statistical techniques for designing experiments, building models, evaluating the effects of factors, and searching optimum condition of factors for desired responses. The optimization process of this methodology involves studying the response of statistically designed combinations, estimating the coefficients by fitting it in mathematical model that fits best the experimental conditions, predicting the response of the fitted model, and checking the adequacy of the model. Central composite design (CCD) and Box-Behnken design (BBD) are amongst the most commonly used in various experiments [11]. Box-Behnken design allows calculation of the response to be made at intermediate levels which were not experimentally studied. A three-level Box-Behnken design was employed in the present study and the optimal conditions were determined through a minimal experiment number compared with other designs [12].

The objective of this study is to optimize the glucose production from OPEFB cellulose via enzymatic hydrolysis with the help of Box-Behnken design, followed by canonical and ridge analyses. OPEFB was treated by organosolv method for lignin and hemicellulose removal. In this context, reaction time, amount of enzyme, and amount of substrates were optimized. Enzymatic hydrolysis was also carried out on commercially available cellulose for comparison purpose.

2. Materials and Methods

2.1. Raw Material. OPEFB fibers were collected from local palm oil mill (United Oil Palm Industries SdnBhd, Malaysia), sun dried, and ground to particles with diameter of <1 mm. The OPEFB biomass was then oven dried at 105°C overnight and was analyzed following the standard method for determination of its main composition [13].

2.2. Chemicals and Enzymes. Two types of standard cellulose from Sigma-Aldrich were used in this study, namely, fiber cellulose and microcrystalline cellulose. Solvent and other chemicals were obtained from R&M Chemicals. Celluclast 1.5 L (cellulase) and Novozyme 188 (cellobiase) were obtained from Novozymes Malaysia SdnBhd.

2.3. Autolytic Pretreatment. Autohydrolysis pre-treatment was conducted for the delignification process of OPEFB. OPEFB fibers (30 grams) were loaded into 4 L stainless steel reactor (98 kPa, 120°C) and were supplemented with appropriate amount of deionized water. The autohydrolysis was carried out at 120°C for 1-2 hours.

2.4. Organosolv Treatment. Autohydrolyzed OPEFB (10 grams) was milled and mixed with 80% aqueous ethanol (EtOH/H₂O: 8/2 v/v) and 0.2% w/w sulphuric acid as catalyst [14]. The mixture was heated at 120°C for 1 hour and filtered and washed with methanol [14]. This was followed by treatment with hydrogen peroxide (H₂O₂) 2% for 4 hours at 50°C to obtain cellulose.

2.5. Enzymatic Hydrolysis. Enzymatic hydrolysis experiment was carried out in a 100 mL shaking flask at 40°C and 145 rpm inside an incubator shaker. The cellulase-catalysed hydrolysis of different cellulose substrates (untreated or pretreated) was carried out in a stirred flask. In a typical hydrolysis reaction, 500 mg of cellulose was added to 9 mL acetate buffer (50 mm, pH 4) and incubated for 2 hours (40°C; 145 rpm). After this preincubation step, hydrolysis was initiated by adding 0.1 mL of 10 mg/mL cellulase (activity: 700 EGU/g). The reaction medium was withdrawn repeatedly at a specific time interval to determine the progress of the reaction which was stopped by incubating the withdrawn sample at 90°C for 20 min [15]. Then, the sample was diluted in ultrapure water and filtered (0.2 μ m) prior to analysis by high performance liquid chromatography (HPLC).

The same procedure was also performed for the cellulose extracted from OPEFB. The reaction was carried out at 40°C, for 48–94 hours. The amount of cellulose and enzyme used was varied from 300 mg to 500 mg and from 0.3 mL to 0.7 mL, respectively.

2.6. Box-Behnken Design. Based on the preliminary one-factor-at-a-time experiments, variables such as duration of reaction, amount of samples, and total enzymes were identified to have strong effects on the response. Therefore, these factors were selected as the variables tested in the 15-run experiment of the Box-Behnken design experiment.

Table 1 shows the Box-Behnken design matrix of the experiment of 15 trials. As shown in Table 1, the three factors chosen for this study were designated as X_1 , X_2 , and X_3 and prescribed into three levels, coded +1, 0, -1 for high, intermediate, and low value, respectively. Three test variables were coded according to the following equation:

$$x_i = \frac{X_i - X_o}{\Delta X} \quad i = 1, 2, 3, \quad (1)$$

where x_i is the coded value of an independent variable; X_i is the actual value of an independent variable; X_o is the actual value of an independent variable at centre point; and ΔX is the step change value of an independent variable. All experiments were carried out in triplicate, and the averages of glucose concentration were taken as response [12].

TABLE 1: Levels and code of variables chosen for Box-Behnken design.

Variables	Symbol		Coded levels		
	Uncoded	Coded	-1	0	+1
Hydrolysis reaction time (h)	X_1	x_1	48	76	94
Amount of enzymes (mL)	X_2	x_2	0.1	0.2	0.3
Amount of samples (g)	X_3	x_3	0.3	0.5	0.7

For predicting the optimal point, a second-order polynomial model was fitted to correlate the relationship between independent variables and response (glucose concentration). The equation for three factors is stated as follows:

$$Y = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \beta_3 x_3 + \beta_{12} x_1 x_2 + \beta_{13} x_1 x_3 + \beta_{23} x_2 x_3 + \beta_{11} x_1^2 + \beta_{22} x_2^2 + \beta_{33} x_3^2, \quad (2)$$

where Y is the predicted response; β_0 is model constant; x_1 , x_2 , and x_3 are independent variables; β_1 , β_2 , and β_3 are linear coefficients; β_{12} , β_{13} and β_{23} are cross-product coefficients; and β_{11} , β_{22} , and β_{33} are the quadratic coefficients. The quality of fit of the polynomial model equation was expressed by the coefficient of determination R^2 .

2.7. Statistical Data Analysis. The analyses of regression and variance were carried out using the RSREG procedure of the Statistical Analysis System (SAS) (Version 9.03, SAS Institute Inc., Cary, NC, USA). Both canonical and ridge analyses were also carried out.

2.8. Characterization. Fourier transform infrared spectra of raw and treated OPEFB fibers were recorded using Perkin Elmer Fourier Transform Infrared (FTIR) model GX. The powdered samples for each type were mixed with KBr and compressed into pellets. Samples were scanned from 400 to 4000 cm^{-1} . Thermogravimetric analysis (TGA) was carried out using a Mettler Toledo model TGA/SDTA 851^c. Samples of approximately 6 mg were placed in alumina pans and heated from 30 to 800°C at 10°C min^{-1} , under a dynamic flow of nitrogen (50 mL min^{-1}). Morphology of the samples was examined by using Zeiss Field Emission Scanning Electron Microscope (FESEM) model Supra 46VP. FESEM images were recorded using an accelerating voltage of 3–5 kV.

The glucose concentration was determined by using High Performance Liquid Chromatography (HPLC) and glucose meter. The system used for HPLC was Waters HPLC system, and evaporative light scattering detectors (ELSD) was used as detectors. Glucose was determined using a Zorbax NH_2 column and acetonitrile: water 80:20 as the mobile phase at a flow rate of 1 mL/min. The retention time of glucose is 13.32 min (rsd \pm 0.56%). Quantification was based on calibration curves established using standard glucose purchased from Sigma-Aldrich.

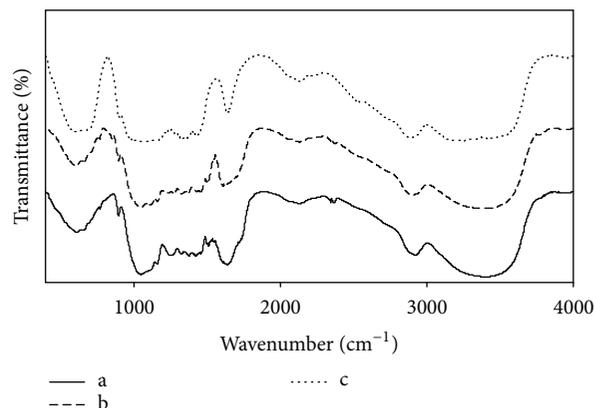


FIGURE 1: FTIR spectra for (a) raw OPEFB, (b) delignified OPEFB, and (c) OPEFB cellulose.

3. Results and Discussion

3.1. Characterization of OPEFB Cellulose

3.1.1. FTIR Spectroscopy. Figure 1 shows the FTIR spectra of cellulose extracted from OPEFB (OPEFB cellulose), raw OPEFB, and delignified OPEFB. The peaks ascribed to lignin appeared at 1501 and 1512 cm^{-1} that are due to C=C stretching and C=C aromatic skeletal vibration of lignin, respectively. However, the two peaks of lignin were not observed in the FTIR spectra of the delignified OPEFB (Figure 1,b) and OPEFB cellulose (Figure 1,c). The peak attributed to hemicellulose was observed in the spectra of the raw OPEFB and delignified OPEFB (Figure 1,b) at 1732–1735 cm^{-1} due to C=O stretching. However, the corresponding peaks were disappeared in the spectrum of OPEFB cellulose (Figure 1,c). This observation indicated that the organosolv treatment was able to remove the lignin as well as hemicellulose from OPEFB fibers. The peak observed at 898 cm^{-1} is attributed to the presence of β -glucoside linkage between glucose units in cellulose [16]. All FTIR spectra in this study showed a β -glucoside linkage peak including the FTIR spectrum for the obtained OPEFB cellulose (Figure 1,c).

3.1.2. Thermal Analysis of OPEFB Cellulose. Figure 2 presents the TGA thermograms and the corresponding DTG curves of the raw OPEFB, delignified OPEFB, and OPEFB cellulose. A clear “shoulder” at around 250–300°C is normally assigned to the thermal decomposition of hemicellulose [17]. The high temperature “tails” around 400–600°C were normally ascribed to degradation of lignin. For the raw OPEFB (Figure 2(c)), hemicelluloses shoulder peaks were not obvious because it is overlapped with main peaks of cellulose. In the thermogram for delignified OPEFB (Figure 2(b)), the first peak appears at around 300°C (shoulder) resembling the thermal decomposition of hemicellulose. There is no tail appears within the range of 400–600°C indicating that the delignification process was successful. In the case of cellulose obtained from organosolv and H_2O_2 treatments, there is no shoulder and tails present in the thermogram indicating that

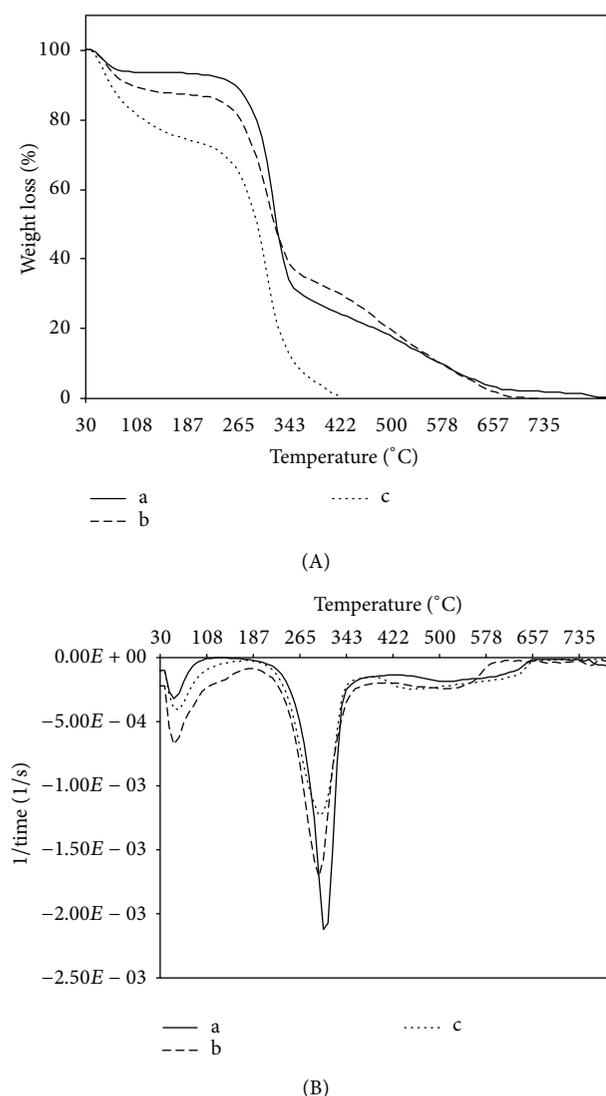


FIGURE 2: TGA thermograms and DTG curves of (a) OPEFB cellulose, (b) delignified OPEFB, and (c) raw OPEFB.

lignin and hemicellulose were successfully removed. Thermal decomposition at around 310°C showed that OPEFB cellulose was successfully obtained (Figure 2(a)).

3.1.3. Morphology of OPEFB Fibers. SEM micrographs of raw OPEFB, delignified OPEFB, and obtained cellulose (OPEFB cellulose) are shown in Figure 3. A rigid appearance can be seen for raw OPEFB material (Figure 3(a)). The figure shows that raw OPEFB fibers exhibit stiff and hard surfaces. Delignification of OPEFB reduced the stiff appearance and some parts of the fibers were split and became more refined. The images also showed that some fibers were broken. Figure 3(c) shows the SEM image of obtained cellulose (OPEFB cellulose) after delignification and organosolv treatment. The appearance is quite different and the length and diameter of fibers were reduced. This is probably due to the decrease in spiral angle around the fibre axis and increase in molecular orientation after pretreatment. A fair amount of randomness

TABLE 2: Effect of different parameters on enzymatic hydrolysis of OPEFB cellulose.

Run	Amount of substrate (g)	Total enzymes (mL)	Reaction time (Hours)	Glucose concentration (g/L)
1	0.7	0.2	48	91.8
2	0.5	0.1	94	97.2
3	0.5	0.3	48	97.2
4	0.5	0.3	94	93.6
5	0.3	0.2	48	55.8
6	0.3	0.2	94	39.6
7	0.7	0.2	48	122.4
8	0.7	0.2	94	167.4
9	0.3	0.1	76	21.6
10	0.7	0.3	76	97.2
11	0.7	0.1	76	48.6
12	0.7	0.3	76	100.8
13	0.5	0.2	76	52.2
14	0.5	0.2	76	54.0
15	0.5	0.2	76	55.8

is introduced to the orientation of the crystallites due to removal of noncellulosic matters [15] leading to the formation of fibers with smaller diameter and length.

3.2. Effect of Pretreatments on Enzymatic Hydrolysis of Cellulose. Effect of pretreatments on enzymatic hydrolysis of various cellulose substrates is shown in Figure 4. Plots in Figure 4 show that glucose concentration increased with reaction time. Figure 4 also indicated that organosolv treated OPEFB cellulose produced the highest glucose concentration followed by acetic acid treated OPEFB, microcrystalline cellulose, cellulose fiber, and delignified OPEFB. The concentration of glucose produced from the organosolv treated OPEFB cellulose was 36.5 g/L with 48 hours reaction time and used minimum amount of enzyme. The sugar recovery for organosolv treated OPEFB cellulose is 0.73 g/g.

Table 2 shows the effect of different parameters on enzymatic hydrolysis of cellulose extracted from OPEFB. It was observed that model run number 8 produced the highest amount of glucose, that is, 167.4 g/L. The lowest glucose concentration was produced by run number 9 with concentration of only 21.6 g/L. The glucose concentration increased with the increasing amount of substrate. The longer reaction time also increased the glucose concentration. The best total enzyme to produce high glucose concentration is 0.2 mL. This means that the use of the same amount of novozyme 188 and celluclast (0.1 mL : 0.1 mL) can improve the activity of the two enzymes as catalyst.

3.3. Statistical Analysis

3.3.1. Model Building and Statistical Significance Test. A 15-run Box-Behnken design with three factors and three levels, including three replicates at the centre point, was used for

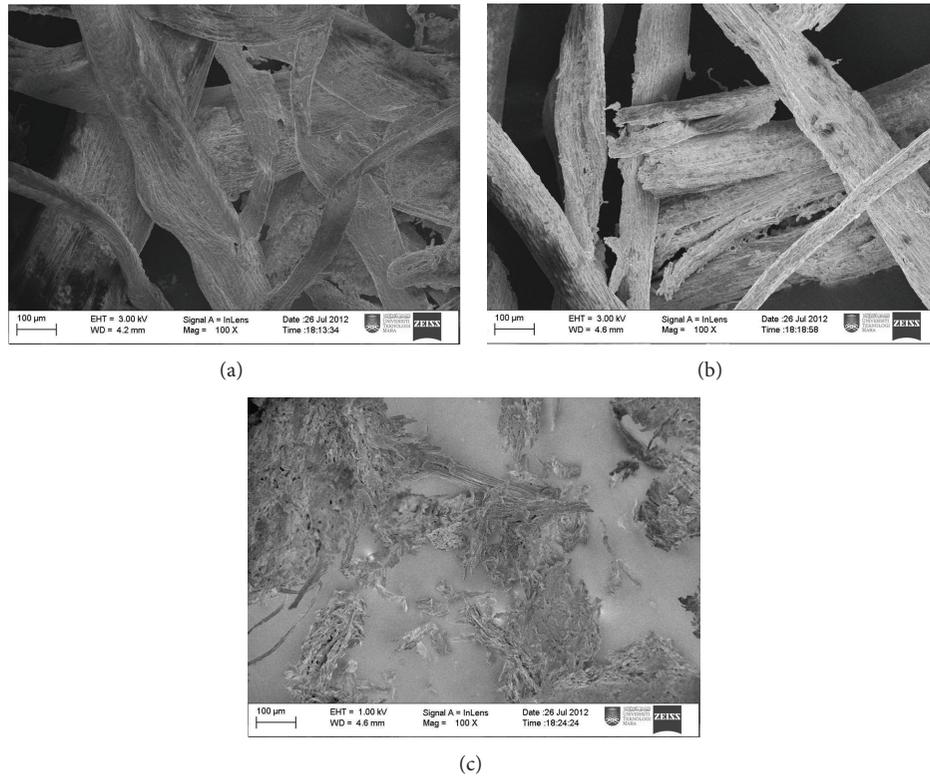


FIGURE 3: SEM images of (a) raw OPEFB fibers (b) delignified OPEFB (c) OPEFB cellulose.

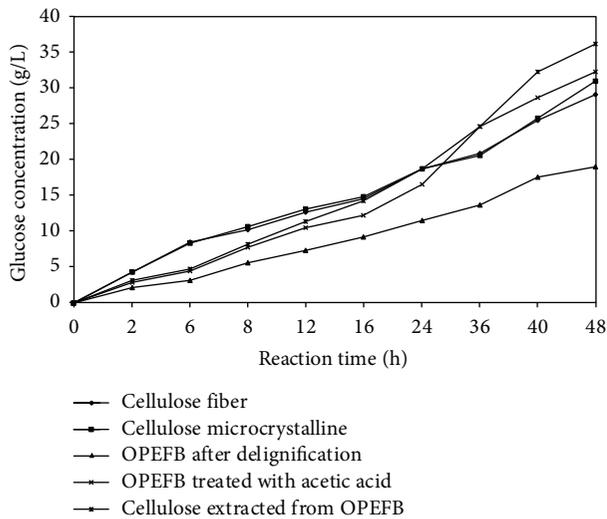


FIGURE 4: Effect of pre-treatments on enzymatic hydrolysis of various cellulose substrates.

appropriate second-order responses surface. The three centre point runs were added to provide as a measure of process stability and inherent variability. The considerable variation in the glucose concentration produced from OPEFB cellulose under different conditions is shown in Table 3. The glucose concentration predicted by the final quadratic model along with corresponding values observed was given too. The

TABLE 3: Box-Behnken design with experimental and predicted values of glucose concentration.

Run	x_1	x_2	x_3	Glucose concentration (g/L)		
				Predicted	Experimental	Difference
1	-1	-1	0	94.95	91.8	-3.15
2	-1	-1	0	94.95	97.2	2.25
3	1	1	0	94.95	97.2	2.25
4	1	1	0	94.95	93.6	-1.35
5	-1	0	-1	65.00	55.8	-9.20
6	1	0	-1	34.40	39.6	5.20
7	-1	0	1	127.60	122.4	-5.20
8	1	0	1	158.20	167.4	9.20
9	0	-1	-1	22.35	21.6	-0.75
10	0	1	-1	107.95	97.2	-10.75
11	0	-1	-1	22.35	48.6	26.25
12	0	1	1	115.55	100.8	-14.75
13	0	0	0	54.00	52.2	-1.80
14	0	0	0	54.00	54.0	0.00
15	0	0	0	54.00	55.8	1.80

agreement between the yield predicted by the model and the experimental data is very strong, with a small difference in glucose concentration.

Furthermore, the model capability was checked using the *F*-test and determination coefficient R^2 . The analysis of variance (Table 4) showed that this regression model was

TABLE 4: Analysis of variance in the regression model for optimisation of glucose production from OPEFB cellulose.

Source of variations	Degree of freedom	Sum of square	Mean square	F value	Determination coefficient (R^2)
Regression	8	19264	240.75	31.14**	0.9765
Residual	6	464.04	77.34		
Pure error	14	19728.04	170.72		
Linear	3	11072.00	3690.67	47.72**	
Quadratic	2	2868.57	1434.29	18.55**	
Cross-product	3	5323.12	1774.37	22.94**	
Lack of fit	1	72.00	72.00	0.92	
Total error	5	392.35	78.47		

$F_{0.05}(8,6) = 4.15$; $F_{0.01}(8,6) = 8.10$; $F_{0.05}(3,5) = 5.41$; $F_{0.01}(3,5) = 12.06$; $F_{0.05}(2,5) = 5.79$; $F_{0.01}(2,5) = 13.27$; $F_{0.05}(1,5) = 6.61$; $F_{0.01}(1,5) = 16.26$.

**Significant at 1% level.

TABLE 5: Regression analysis of a full second-order polynomial model for optimisation of glucose production from OPEFB cellulose.

Variables	Standard error	t value	P-value	Coefficient based on coded value
Intercept	5.077401	10.64	0.0001**	54.000000
x_1	3.877929	2.37	0.0553	9.200000
x_2	4.635012	-2.32	0.0595	-10.750000
x_3	3.877929	12.02	0.0001**	46.600000
$x_1 \times x_1$	6.716770	6.30	0.0007**	42.300000
$x_2 \times x_1$	10.154802	-5.82	0.0011**	-59.100000
$x_2 \times x_2$	8.028076	7.19	0.0004**	57.750000
$x_3 \times x_1$	4.397158	3.48	0.0132*	15.300000
$x_3 \times x_2$	6.388923	-6.70	0.0005**	-42.800000
$x_3 \times x_3$				0.000000

**Significant at 1% level.

*Significant at 5% level.

highly significant ($P < 0.01$) with F value of 31.14. The suitability of model was further confirmed by a satisfactory value of the determination coefficient, which was calculated to be 0.9765 indicating that 97.65% of the variability in the response could be predicted by the model.

The regression coefficients along with the corresponding P values for the model of glucose concentration produced from OPEFB cellulose are shown in Table 5. The P values were used as a device to check the significance of each coefficient, and the P values showed that x_1^2 and x_2^2 as well as two cross products (x_1x_3 and x_2x_3) were significant.

The polynomial model for glucose concentration (Y) was regressed by considering only the significant terms and shown in the following equation:

$$Y = 54 + 46.6x_3 + (42.3x_1^2) - (59.1x_2x_1) + (57.75x_2^2) + (15.3x_3x_1) - (42.8x_3x_2). \quad (3)$$

3.3.2. Canonical and Ridge Analysis. The canonical analysis of the response surface was performed with SAS to determine the shape of the fitted response and the estimated stationary point. According to the model, the predicted response at the

TABLE 6: Canonical analysis on the coded and actual value.

Variables	Coded value	Actual value	Predicted response
X_1	0.245784	76.245784	
X_2	1.176647	1.376647	109.03 g/L
X_3	2.584741	3.084741	

TABLE 7: Eigenvalues and eigenvectors.

Eigenvalues	Eigenvectors		
	X_1	X_2	X_3
86.04	0.57	-0.78	0.24
21.18	0.81	0.52	-0.23
-7.17	0.05	0.33	0.94

stationary point ($X_1 = 76.25$, $X_2 = 1.38$, and $X_3 = 3.08$, shown in Table 6) was 109.93 g/L.

The three eigenvalues had different signs, indicating that the stationary point for this model was a saddle point as shown in Table 7. Therefore, the estimated surface did not have a unique optimum, and a ridge analysis was performed to determine the optimum.

The results of ridge analysis (Table 8) indicated that all variables tested, that is, hydrolysis reaction time, total of enzymes, and amount of substrate, were positively related to the response, and the optimal level of them was found as 76 hours and 30 min reaction time, 0.5 mL total enzymes, and 0.9 g of substrate (OPEFB cellulose), respectively, with a predicted glucose concentration of 169.34 g/L.

In order to confirm the predicted values, three further experiments using the optimum parameters found above were conducted and the value of 165.24–172.5 g/L (mean value 168.71 g/L) of glucose concentration was obtained.

The strong relationship between the yield predicted by the final quadratic model and the experimental results (Table 2) and the variance analysis of the second-order polynomial model and the value of lack of fit (Table 3) showed that the accuracy and overall ability of the polynomial model are very good. The analysis of response trends using the model is considered to be acceptable.

Kunamneni and Singh [18] (2005) used a central composite design (CCD) which is one of the models from response

TABLE 8: Results of ridge analysis.

Radii	Yield (g/L)	Standard error	Actual value		
			X_1	X_2	X_3
0.0	54.000000	5.077401	76.000000	0.200000	0.500000
0.1	59.135102	5.088990	76.027497	0.162679	0.588606
0.2	65.148586	5.111521	76.071382	0.099157	0.657274
0.3	72.467739	5.161894	76.123974	0.023639	0.708632
0.4	81.311717	5.312751	76.179672	0.055354	0.750024
0.5	91.774748	5.661294	76.236446	0.135154	0.785946
0.6	103.899910	6.293774	76.293615	0.215023	0.818662
0.7	117.709117	7.258911	76.350935	0.294764	0.849361
0.8	133.214604	8.565148	76.408309	0.374337	0.878709
0.9	150.423721	10.197117	76.465695	0.45375	0.907111
1.0	169.341156	12.132559	76.533077	0.533021	0.934823

surface methodology (RSM) to optimize the effect of four variables (precooking-amylase dose, postcooking-amylase dose, glucoamylase dose, and saccharification temperature) in the optimization of glucose production from maize starch [18]. As comparison, they did 28 experiments for 4 factors and finally obtained a coefficient determination of 0.9558, whereas in this project only 3 factors with 15 experiments were used and the coefficient determination value obtained is 0.9765.

Although many experiments are completed at the canonical analysis, the ridge analysis is very useful to find out the maximum response which may occur in the experiment when the results of the canonical analysis shows a saddle, stationary point, and no unique optimum in the estimated surface [19]. Based on the ridge analysis, the optimal reaction time, total of enzymes, and amount of samples for glucose production were determined in this study with a good predicted hydrolysis reaction rate without further experimental work. An average glucose concentration of 168.71 g/L was obtained in three further validation experiments using the optimum reaction parameters. As compared to those of experimental (167.4 g/L) and predicted value (169.34 g/L), there is a good correlation amongst those three results verifying the validity of the response model and the actuality of optimal point.

4. Conclusions

Enzymatic hydrolysis of OPEFB cellulose was successfully carried out at 40°C under various amounts of samples, total enzymes, and reaction time (48–94 hours). Box-Behnken design was shown to be useful in investigating those three critical variables. Through the canonical and ridge analyses of the second-order polynomial model, a maximum glucose concentration of 168.71 g/L was obtained under the following conditions: 76 hours and 30 minutes hydrolysis reaction time used 0.5 mL enzymes and 0.9 gram samples/OPEFB cellulose. In summary, within the reaction conditions tested the concentration of glucose produced is proportional to the reaction time, total enzymes, and amount of samples.

Acknowledgments

The authors express their acknowledgements to Universiti Kebangsaan Malaysia and Malaysia Government for the financial support of this work (UKM-AP-2011-20).

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