Journal of Engineering Science and Technology
Vol. 9, No. 4 (2014) 443 - 454

MODELLING AND OPTIMISATION OF DILUTE ACID HYDROLYSIS OF CORN STOVER USING BOX-BEHNKEN DESIGN

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Abstract

Response surface methodology (RSM) was employed for the analysis of the simultaneous effect of acid concentration, hydrolysis time and temperature on the total reducing sugar concentration obtained during acid hydrolysis of corn stover. A three-variable, three-level Box-Behnken design (BBD) was used to develop a statistical model for the optimisation of the process variables. The optimal hydrolysis conditions that resulted in the maximum total reducing sugar concentration were acid concentration; 1.72% (w/w), temperature; 169.26^{0}C and pretreatment time; 48.73 minutes. Under these conditions, the total reducing sugar concentration was obtained to be 23.41g/L. Validation of the model indicated no difference between predicted and observed values.

Keywords: Corn stover, Acid hydrolysis, Lignocellulosic biomass, Box-Behnken design, Optimisation.

1. Introduction

The inevitable depletion of the world's crude oil reserves, increasing prices of petroleum products, and environmental concerns regarding fossil fuel usage have motivated the development of sustainable alternative sources of energy [1-4]. Bioethanol as liquid fuel for road transportation has received most of the attention in recent years.

First generation bioethanol is produced from starch containing feedstock such

Nomenclatures

b_1	Linear coefficient term for acid concentration
b_2	Linear coefficient term for temperature
b_3	Linear coefficient term for time
b_{11}	Quadratic term for acid concentration
b_{22}	Quadratic term for temperature
b_{33}	Quadratic term for time
b_{12}	Interaction term between acid concentration and temperature
b_{13}	Interaction term between acid concentration and time
b_{23}	Interaction term between temperature and time
X_1	Actual value of acid concentration, %w/w
X_2	Actual value of temperature, ^oC
X_3	Actual value of time, min.
Y	Total reducing sugar concentration, g/L

Abbreviations

ANOVA	Analysis of variance
BBD	Box-Behnken design
C.V	Coefficient of variation
RSM	Response surface methodology

as corn, cassava, wheat, potatoes, etc. [5]. However, there are ethical concerns relating to the use of potential food resources for biofuel production [6]. Bioethanol produced from lignocellulosic feedstocks is potentially sustainable as lignocellulosic materials, which are mainly agricultural and forestry residues, have the potential to be an economical source of feedstock as a result of their widespread availability, sustainable production and low cost [7, 8].

Lignocellulosic biomass such as corn stover is recognised as one of the most abundant of all naturally occurring feedstock for cellulosic ethanol production [9]. The conversion of corn stover to ethanol is more challenging due to the complex structure of the plant cell wall. It is necessary to pretreat the corn stover to alter its structural and chemical composition to facilitate rapid and efficient hydrolysis of carbohydrates to fermentable sugars [1, 10-12]. Amongst the pretreatment methods often adopted, acid hydrolysis has been extensively studied and used for pretreating lignocellulosic biomass [13-17]. Acid hydrolysis is a direct hydrolysis method used for biomass conversion. A single stage with dilute acid (less than 5% acid concentration) is typically used rather than concentrated acid. A rather high temperature is needed to achieve a maximum conversion for a short reaction time. However, the implementation of high temperature is limited by the production of inhibitors such as furfural and hydroxy-methyl furfural, which are degradation products of pentose and hexose sugars respectively [18,19]. Canattieri et al. [20] applied response surface methodology to the optimisation of acid hydrolysis of the hemicellulosic fraction of Eucalyptus grandis residue. The optimum conditions they determined were: H_2SO_4 concentration of 0.65%, temperature of 157^oC and residue/acid solution ratio of 1/8.6 with a reaction time of 20 min. Under these conditions, 79.6% of the total xylose was recovered. Diluted hydrolysis of α-cellulose with high temperature was investigated by Xiang et al.

[21]. They reported that at temperatures above 215°C, the chemical reaction was more sensitive to temperature than the physical factors of cellulose.

The yield of fermentable sugars during acid hydrolysis is affected by factors such as hydrolysis time, particle size, hydrolysis temperature, acid concentration, etc. The classical method of optimisation involves varying one factor at a time and keeping the others constant. This is often useful but does not elucidate the effect of interaction between the various factors under consideration. Response surface methodology is an empirical statistical technique employed for multiple regression analysis of quantitative data obtained from statistically designed experiments by solving the multivariate equations simultaneously [22, 23]. By making use of design of experiment for response surface methodology, the input levels of each factor as well as the level of the selected response can be quantified. The central composite, Box-Behnken and Doehlert designs are among the common designs used for response surface methodology.

In this work, the modelling and optimisation of dilute acid hydrolysis of corn stover was studied. The objective of this study was to optimise the effect of acid concentration, hydrolysis temperature, and hydrolysis time levels. Using Box-Behnken design of experiments, a mathematical correlation between acid concentration, hydrolysis temperature, and hydrolysis time was developed to obtain maximum fermentable sugar concentration.

2. Materials and Methods

2.1. Subtrate

The corn stover used in this study was obtained from a farm in the Nigerian Institute for Oil Palm Research (NIFOR), Benin City, Edo State, Nigeria. The corn stover was washed thoroughly with tap water to remove sticky clay and then air-dried (over a stream of air at 35^{o}C). The dried corn stover was milled and screened to 2 mm particles to increase its surface area and make the cellulose readily available for hydrolysis. It was then stored at room temperature (27±2^{o}C) for subsequent use.

2.2. Dilute acid hydrolysis

Acid hydrolysis of corn stover was carried out in an autoclave with a solid-liquid ratio of 5% (g dry weight to g solution). The sulphuric acid concentration range was 0.4%-2.0% (w/w), the hydrolysis temperature range was 140–200^{o}C and the hydrolysis time range was 5–60 minutes. After acid hydrolysis, the solid residue was separated by centrifugation and the pH of the resulting supernatant was adjusted to 11 using 2N $Ca(OH)_2$ .The resulting precipitate was centrifuged off and the supernatant was adjusted to neutral pH using 2N HCl [24].

2.3. Chemical composition analysis

The carbohydrate content in the raw corn stover was determined according to the National Renewable Energy Laboratory (NREL) standard procedure no. 2CS. This was done using a High Perfromance Liquid Chromatography (HPLC) system equipped with an Aminex HPX-87 P column (Bio-Rad, USA) and refractive index (RI) detector (Refracto Monitor® III, Model 1109, LDC/Milton Roy, USA). The amount of protein contained in the raw corn stover was determined using LECO

CHN-600 protein determinator. Klason and acid-soluble lignin contents were determined as described in NREL standard procedure no. 03. The ash content of raw corn stover was determined as described in the NREL standard method no. 05.

2.4. Fermentable sugar analysis

The total reducing sugar content of the final hydrolysate was determined by the colorimetric method using glucose as standard [25]. The reducing sugars were treated with 3, 5-dinitro-salicylic acid (DNS) which is reduced to 3-amino-5-nitro-salicylic acid. The latter was quantified by measuring absorbance at a wavelength of 540 nm using a UV-Vis spectrophotometer, (Cecil 1000). The DNSA reagent consisted of 1 g DNS dissolved in 20 mL 2M NaOH and 50 mL distilled water. Thirty grams of Rochelle salt (potassium sodium tartarate tetrahydrate: $KO_2CCH(OH)CH(OH)CO_2Na \cdot 4H_2O$) was added and distilled water was added to make up the volume to 100 mL. The reducing sugars were measured as follows: To a test tube were added the following; 0.2 mL reducing sugar solution, 1.8 mL distilled water and 2 mL DNS reagent. The mixture in the test tube was boiled for 5 min in a water bath followed by cooling to room temperature and diluting to 24 mL. A standard curve was prepared using known concentrations of glucose from which the concentration of reducing sugar was determined.

2.5. Design of experiment

A three variable Box-Behnken design for response surface methodology was used to study the combined effect of acid concentration, hydrolysis temperature and time on total reducing sugar concentration over three levels. The other parameters that could affect the process of hydrolysis include pH, agitation speed, moisture content and particle size. These factors were excluded from the experimental design because they have been confirmed by previous workers [1, 9, 26, 27]. It was assumed that the relationship between the chosen response (i.e., total sugar concentration) and the selected factors was quadratic in nature hence the choice of the Box-Behnken design for formulating the model for the purpose of optimisation.

The range and levels of the variables optimised are shown in Table 1. The Box-Behnken design is suitable for exploration of quadratic response surfaces and generates a second degree polynomial model, which in turn is used in optimising a process using a small number of experimental runs. This design requires an experimental number of runs according to $N = k^2 + k + c_p$. k is the factor number (3) and c_p is the number of replications at the center point (5). The design which was developed using Design Expert® 7.0.0 (Stat-ease, Inc. Minneapolis, USA), resulted in 17 experimental runs as shown in Table 2. The 17 experimental runs were randomized to maximize the effects of unexplained variability in the observed responses due to extraneous factors. The levels of the independent variables as shown in Table 1 were selected based on preliminary experiments. The relation between the coded values and actual values are described as follows:

$$x_i = \frac{X_i - X_o}{\Delta X_i} \quad (1)$$

where x_i and X_i are the coded and actual values of the independent variable respectively. X_o is the actual value of the independent variable at the center point, and ΔX_i is the step change of X_i. A second degree polynomial was fitted to the experimental data using the statistical package Design Expert® 7.0.0 to estimate the response of the dependent variable and predict the optimal point. The second degree polynomial was expressed as follows:

$$Y = b_o + b_1X_1 + b_2X_2 + b_3X_3 + b_{12}X_1X_2 + b_{13}X_1X_3 + b_{23}X_2X_3 + b_{11}X_1^2 + b_{22}X_2^2 + b_{33}X_3^2 \quad (2)$$

where Y is predicted response, X_1, X_2 and X_3 are independent variables, b_0 is offset term, b_1, b_2, b_3 are linear effects, b_{11}, b_{22}, b_{13} are interaction terms.

Table 1. Coded and Actual Levels of the Factors for Three Factor Box-Behnken Design.

Independent Variables	Symbols	Coded and Actual Levels		
		-1	0	+1
Acid Concentration (%w/w)	X_1	0.4	1.2	2.0
Temperature (°C)	X_2	140	170	200
Time (min.)	X_3	5	33	60

Table 2. Three Factor Box-Behnken Design with Experimental as well as Predicted Responses of Dependent Variables (Total Sugar Concentration g/L).

Run No.	Factors						Response	
	Coded values			Actual values			Total sugar concentration (g/L)	
	X_1	X_2	X_3	X_1	X_2	X_3	Observed	Predicted
1	0	0	0	1.2	170	33	20.57	20.47
2	+1	0	+1	2.0	170	60	21.15	22.91
3	+1	+1	0	2.0	200	33	21.09	22.91
4	+1	0	-1	2.0	170	5	8.53	9.03
5	+1	1	0	2.0	140	33	20.89	20.31
6	0	0	0	1.2	170	33	20.97	20.47
7	-1	1	0	0.4	200	33	15.88	16.66
8	0	-1	-1	1.2	140	5	6.33	6.01
9	-1	0	+1	0.4	170	60	20.36	20.08
10	0	+1	+1	1.2	200	60	20.62	19.71
11	0	0	0	1.2	170	33	23.13	22.47
12	-1	0	-1	0.4	170	5	7.11	6.56
13	-1	-1	0	0.4	140	33	15.70	17.17
14	0	-1	+1	1.2	140	60	20.90	20.32
15	0	0	0	1.2	170	33	19.77	20.47
16	0	+1	- 1	1.2	200	5	8.24	8.62
17	0	0	0	1.2	170	33	20.34	20.47

3. Results and Discussion

3.1. Composition of raw corn stover

The raw corn stover was subjected to complete chemical composition analysis prior to dilute acid hydrolysis. The major components of corn stover were glucan, xylan, and lignin. The weight percent of each component is summarized in Table 3. The ratio of glucan to xylan was 1.7. Lignin (klason and acid soluble) accounted for about 20% of the raw corn stover used in this study. Other minor components were proteins, acetyl groups, and extractives and these accounted for about 11% of corn stover. The values indicated in Table 3 can be seen to be similar to those obtained by the NREL.

Table 3. Composition of Raw Corn Stover.

Component	Weight Percent (This work)	Weight Percent (Data from NREL)
Klason lignin	17.3	17.2
Acid soluble lignin	2.4	
Glucan	36.6	36.1
Xylan	21.3	21.4
Proteins	3.8	4.0
Ash	7.4	7.1
Others (Arabian, mannan, galactan, acetyl groups, and extractives)	11.2	14.2

3.2. Statistical analysis

The results obtained from the 17 experimental runs carried out according to the Box-Behnken design are summarised in Table 2. The proposed second degree polynomial was fitted to the data presented in Table 2 using multiple linear regressions to determine the optimum conditions for the acid hydrolysis of corn stover that resulted in the maximum value of total reducing sugar concentration.

Figure 1 shows a plot of the predicted and observed (actual) values of total sugar concentration as a function of experimental run number. The plot has been displayed with standard error bars to indicate the level of deviation between the predicted and observed level of total sugar concentration. The small magnitude of the error bars as shown in Fig. 1 indicates that the predicted values are close to the observed values. This result will be verified by carrying out analysis of variance (ANOVA).

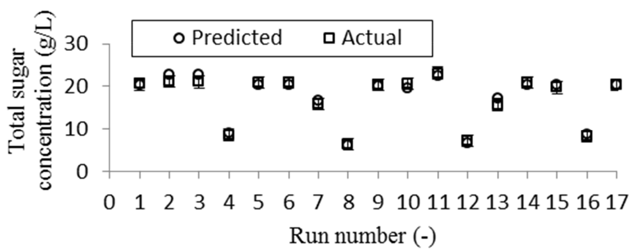

Fig. 1. Comparison between Predicted and Observed Levels of Total Sugar Concentration with Error Bars Indicated.

The effects of acid concentration, hydrolysis time and hydrolysis temperature were quantitatively evaluated using response surface curves. By applying multiple regression analysis on the experimental data, the following second degree polynomial was found to represent the relationship between the total reducing sugar produced and acid concentration, hydrolysis time and hydrolysis temperature adequately.

$$Y = -50.309 + 2.157X_1 + 0.588X_2 + 0.842X_3 + 0.000272X_1X_2 - 0.00722X_1X_3 - 0.0000668X_2X_3 - 1.794X_1^2 - 1.644X_2^2 - 7.377X_3^2 \quad (3)$$

The predicted levels of total reducing sugar using Eq. (3) are given in Table 2 along with experimental data. The significance of the fit of the second-order polynomial for the concentration of total reducing sugar was assessed by carrying out analysis of variance (ANOVA) as shown in Tables 4 and 5.

The coefficient of determination (R^2) of the model was 0.971 (Table 4), which indicated that the model adequately represented the real relationship between the variables under consideration. An R^2 value of 0.971 means that 97.1% of the variability was explained by the model and only 2.90% was as a result of chance. The coefficient of variation (C.V.) obtained was 8.64%. The Coefficient of Variation (C.V) indicates the degree of precision with which the treatments were carried out. A low value of C.V suggest a high reliability of the experiment [23, 28]. Adequate precision value (14.386) measures the signal to- noise ratio, and a ratio greater than 4 is generally desirable [29].

Table 4. Statistical Information for ANOVA.

Source	Response Value
R-Squared	0.971
Adjusted R-Squared	0.935
Standard Deviation	1.480
C.V %	8.640
Adeq. Precision	14.386

Table 5 presents results obtained after carrying out ANOVA. Values of ''Prob. > *F*'' less than 0.05 indicate the model terms are significant. Values greater than 0.10 indicate the model terms are not significant. A model F-value of 24.60 and a very low probability value [(Prob > *F*) less than 0.0001] imply significant model fit. From the regression model of total reducing sugar concentration, the model terms X_1, X_2, X_3, X_1^2, X_2^2, X_3^2 were significant with a probability of 95%. The term X_1X_3 was also significant indicating that there was interaction between acid concentration and hydrolysis time. The interaction between the terms X_1, X_2 and X_2, X_3, however had no significant effect on the total reducing sugar produced during acid hydrolysis. The "Lack of Fit" F-value of 1.75 implies that there is insignificant lack of fit. The "Lack of Fit" (Prob > *F*) value of 0.2951 implies that there is only 29.5% chance that the "Lack of Fit" *F*-value could occur due to noise.

Table 5. Analysis of Variance (ANOVA) for Quadratic Model for Total Sugar Concentration.

Sources	Sum of Squares	df	Mean Squares	*F* value	*p*- value [Prob >*F*]
Model	515.21	8	64.40	24.60	< 0.0001
X_1 –Acid concentration	19.85	1	19.85	7.59	0.0249
X_2 – Temperature	0.50	1	0.50	19.10	0.0072
X_3 - Time	348.48	1	348.48	133.26	< 0.0001
X_1X_2	1.709×10^{-4}	1	1.709×10^{-4}	6.532×10^{-5}	0.9937*
X_1X_3	0.10	1	0.10	6.19	0.0130
X_2X_3	1.22	1	1.22	0.46	0.5148*
X_{12}	5.55	1	5.55	5.53	0.0156
X_{22}	9.23	1	9.23	2.51	0.0271
X_{32}	131.41	1	131.41	50.24	0.0001
Residual	20.93	8	2.62		
Lack of Fit	14.28	4	3.57	1.75	0.2951
Pure Error	6.65	4	1.66		
Cor Total	536.13	16			

*not significant

3.3. Optimisation of dilute acid hydrolysis

In order to optimise the variables that influence the acid hydrolysis of corn stover, response surface plots were generated from the regression model. The three-dimensional (3D) plots were generated by keeping one variable constant at the centre point and varying the others within the experimental range. The resulting response surfaces showed the effect of acid concentration, temperature, and hydrolysis time on the total reducing sugar concentration.

Figure 2 shows the response surface and corresponding contour plot for total sugar concentration as a function of hydrolysis temperature and acid concentration. An increase in the acid concentration with temperature resulted in an increase in the total reducing sugar concentration until an optimum value of about 23.41 g/L, i.e., 169.26°C temperature and 1.72% (w/w) acid concentration. Any further increase in the acid concentration was found to be unfavourable for the production of reducing sugar as explained by the decreasing trend observed.

The upward trend observed in Fig. 2 may be attributed to the catalysing activity of the acid. Increasing the concentration of acid implies a corresponding increase in the number of hydrogen ions present in the solution hence the hydrolysis process will occur more rapidly [30, 31]. Consequently, the rate at which the glycosidic bonds are broken will increase resulting in a high conversion of hemicellulose fraction into fermentable sugars.

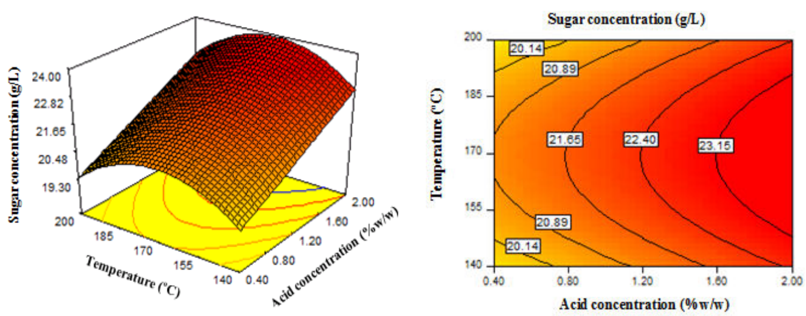

Fig. 2. Response Surface Plot and the Corresponding Contour Plot Showing the Effects of Hydrolysis Temperature and Acid Concentration on Total Reducing Sugar Concentration.

The effect of hydrolysis time and acid concentration on the total sugar concentration is presented in Fig. 3. An increase in hydrolysis time along with a steady increase in acid concentration resulted in an increase in total reducing concentration until an optimum value of about 23.41 g/L, i.e., 48.73 minutes hydrolysis time and 1.72% (w/w) acid concentration. Further increase had a reverse effect on product formation as explained by the slight decline in trend observed.

Under conditions of high acid concentration, the cellulose fraction is disrupted and glucose is produced. Since the acid acts as a catalyst, high concentration of acid may therefore increase the rate of the hydrolysis reaction rate consequently increasing the sugar concentration [31].

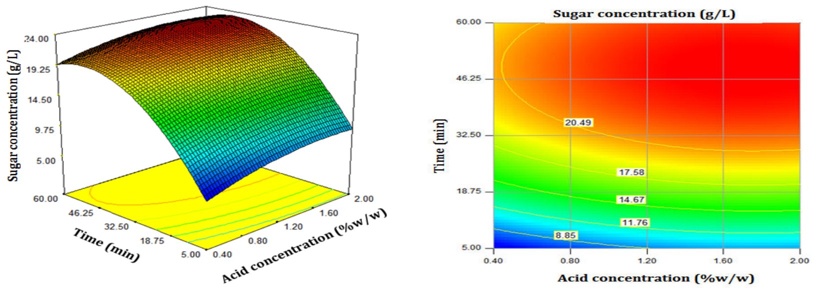

Fig. 3. Response Surface Plot and the Corresponding Contour Plot Showing the Effects of Hydrolysis Time and Acid Concentration on Total Reducing Sugar Concentration.

Figure 4 shows the effect of the interaction between hydrolysis time and temperature on total sugar concentration. The centre point of Fig. 4 reveals the optimal values of hydrolysis time and temperature that may be combined to obtain optimal concentration reducing sugar. This was revealed to be 48.73 minutes hydrolysis time and 169.26 ^{o}C temperature. Any further increase in both the hydrolysis time and temperature led to no appreciable effect on the production of reducing sugars. The rate of the hydrolysis reaction was observed to be higher at high temperatures than at low temperature as is evident in the high values of total sugar concentration recorded at high temperatures. However, the sugar concentration was observed to decrease slightly at elevated temperatures (greater than the optimum 169.26^{o}C) while making use of high acid concentration. Sustaining the hydrolysis reaction (i.e., longer reaction time) at high temperatures may result in the degradation of the fermentable sugars to furfural and hydroxyl methyl furfural [18,19]. This suggests that temperature plays a very important role in the hydrolysis of lignocellulosic materials as reported by Kim et al. [32].

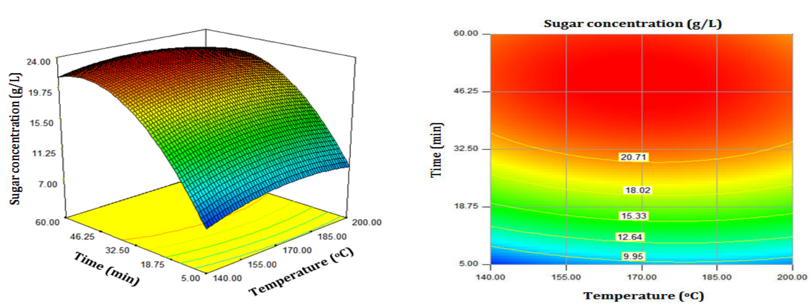

Fig. 4. Response Surface Plot and the Corresponding Contour Plot Showing the Effects of Hydrolysis Time and Temperature on Total Reducing Sugar Concentration.

In order to select the optimum conditions and their respective levels, the model was analysed. The maximum response predicted from the model was a total reducing sugar concentration of 23.41 g/L. The final optimised hydrolysis conditions obtained with RSM were 1.72% (w/w) (acid concentration), 169.26^{o}C (temperature) and 48.73 minutes (hydrolysis time).

The validity of the results predicted by the regression model, was confirmed by carrying out repeated experiments under optimal hydrolysis conditions (i.e., acid concentration; 1.72% (w/w), temperature; 169.26°C and hydrolysis time; 48.73 minutes). The results obtained from three replications demonstrated that the average of the maximum total sugar concentration (23.04 g/L) obtained was close to the predicted value (23.41 g/L). The excellent correlation between the predicted and measured values of these experiments justifies the validity of response model.

4. Conclusions

In this work, the dilute acid hydrolysis of corn stover was studied quantitatively over three levels using a three variable Box-Behnken design for response surface methodology. The following conclusions can be drawn from the study.

- The use of response surface methodology to determine the conditions leading to the efficient pre-treatment of corn stover using acid hydrolysis has been demonstrated.
- Dilute acid pre-treatment of corn stover is influenced by the concentration of acid, hydrolysis time and temperature.
- The concentration of total reducing sugars produced during dilute acid hydrolysis is related to acid concentration, hydrolysis time and temperature by a validated quadratic regression model.
- The quadratic regression model equation developed was able to predict to a high level of confidence, the concentration of total reducing sugar produced during hydrolysis.
- The combination of optimum process conditions were an acid concentration of 1.72% (w/w), hydrolysis temperature of 169.26°C, and hydrolysis time of 48.73 minutes. Under these conditions, the maximum concentration of total reducing sugar was obtained to be 23.41 g/L. This was close to the value (23.04 g/L) obtained from repeated experiments carried out under the optimised conditions.

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