

Optimization of acetic acid pretreatment of corn stover for bioethanol production

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Abstract- It has been found that different pretreatment of corn stover produces varying quantities of reducing sugars. Therefore, this study experimentally investigated the optimum conditions with respect to concentration of acetic acid, temperature and time. Response surface methodology (RSM) was employed for the analysis of the simultaneous effect of acid concentration, pretreatment temperature and time on the resulting total reducing sugar concentration obtained. A three-variable, five-level Central Composite Rotatable Design (CCRD) of experiment was used to develop a statistical model for the optimization of the process variables. The findings showed that the optimum total reducing sugar were acetic acid concentration; 91.89%, temperature; 150 °C and time; 5 hours. Under these conditions, the total reducing sugar concentration obtained was 21.09 mg/ml. Validation of the model indicated insignificant difference (± 1.2) between predicted and observed values. This study found that corn stover has a potential of producing substantial amount of reducing sugars which are the major raw materials in the production of bioethanol.

Index Terms- Acetic acid, Corn stover, Reducing sugars, bioethanol, Response surface methodology (RSM)

I. INTRODUCTION

Corn stover is lignocellulosic biomass that can be converted into fermentable sugars for the production of second generation biofuels such as cellulosic ethanol [1, 2]. The conversion process is facilitated by use of either enzymatic or chemical hydrolysis. Chemical pretreatment processes include the use of acids, alkalis, catalyzed steam-explosion, Ammonia fiber/freeze explosion (AFEX), pH-controlled liquid hot water, Ionic liquids (ILs) and organic solvents, among others [3].

Currently the world is faced with crisis of fossil fuel depletion and environmental degradation. Demirbas [4] indicated that the oil crisis and the continuous increase in oil prices have led countries to investigate new and renewable fuel alternatives.

The major organic components of biomass are celluloses, hemicelluloses and lignin [1, 4-6].

There are three ways to use biomass. It can be burned to produce heat and electricity, changed to gas-like fuels such as methane, hydrogen and carbon monoxide or converted to a liquid fuel. Biofuels (liquid or gaseous fuels produced from biomass) are predominantly two forms of alcohol: ethanol and methanol. Biofuels generally offer many benefits including sustainability,

reduction of greenhouse gas emissions, regional development, reduction of rural poverty and fuel security [4, 7].

The most commonly used biofuel is ethanol. This is generally produced from sugarcane, corn, and other grains. Gasoline and ethanol blends are already used in cities with high air pollution. However, ethanol made from biomass is currently more expensive than gasoline. Therefore, there is a need to find out the less expensive ways to produce ethanol from other biomass crops. Ethanol feedstocks are classified into three types:

- i. Sucrose-containing feedstocks.
- ii. Starchy materials.
- iii. Lignocellulosic biomass.

Lignocellulosic biomass, such as agricultural residues (corn stover and wheat straw), wood and energy crops, is attractive materials for ethanol fuel production since it is the most abundant reproducible resource on Earth. Production of renewable biofuels can be classified based on the production technologies employed as illustrated in **Table 1**

Table 1: Classification of renewable biofuels based on their production technologies[4]

Generation	Feedstock	Example
First generation biofuels	Sugar, starch, vegetable oils or animal fats	Bioalcohols, vegetable oil, biodiesel, byosngas, biogas
Second generation biofuels	Non-food crops, wheat straw, corn, wood, solid waste, energy crops	Bioalcohols, bio-oil, bio-DMF, biohydrogen, bio-Fischer-Tropsch diesel, wood diesel.
Third generation biofuels	Algae	Vegetable oil, biodiesel
Fourth generation biofuels	Vegetable oil, biodiesel	Biogasoline

Lignocellulose materials are broken down into individual sugars followed by fermentation to ethanol.

The prerequisite in the utilization of lignocellulose for ethanol production is to efficiently yield fermentable hydrolyzates rich in glucose from the cellulose content present in the feedstock

[3]. A pretreatment process is employed to breakdown the lignocellulose to fermentable sugars. This is the key process of production of second-generation biofuels. Various pretreatment techniques have been developed during last decades, which increase cellulose accessibility by modifying chemical compositions or cell wall structures. The prevailing challenge is the accessibility of these polymers and how to convert them into fuels and building blocks for utilization. The flow diagram given in **Figure 1** illustrates a generalized production of biofuels from lignocellulosic biomass.



Figure 1: The schematic diagram showing the conversion of lignocellulosic biomass to fuel.

II. MATERIALS AND METHODS

2.1 Raw materials

Corn stover samples were collected from the farm around Moi University. Collection of raw materials was done by random sampling method. These samples were prepared according to Laboratory Analytical Procedure (LAP) of National Renewable

2.2 Pretreatment and hydrolysis

Energy Laboratory (NREL) "Preparation of samples for compositional analysis"[8].

The pretreatment of corn stover was performed in batch reactors at chemical laboratory in Moi University. This was carried out at a constant ratio of solids to liquids of 1:10 for all experimental runs.

In order to come up with reducing sugars, the insoluble solids after pretreatment were subjected to dilute hydrochloric acid hydrolysis. Appropriate conditions for dilute HCl hydrolysis are; 2.5% HCl concentration, 120°C and hydrolysis time of 1hr [9, 10].

2.3 Analysis of reducing sugars

The total reducing sugars of the hydrolyzates was determined by UV-Vis Spectrophotometer, at 540nm wavelength using 3, 5-dinitrosalicylic acid (DNS reagent) with glucose as standard[11].

In order to get the overall pretreatment/hydrolysis yields, having found the optimum yield, calculations were done according to equation 1 [12].

Overall yields =

$$\frac{\text{Total reducing sugars concentration} \times \text{Dilution}}{\text{Weight of cellulose and hemicellulose in raw biomass}} \times 100\% \quad (1)$$

2.4 Experimental design

In order to come up with optimal conditions for pretreatment of corn stover to yield fermentable sugars, full factorial central composite rotatable design (CCRD) was used. The independent pretreatment factors to be determined were; acetic acid concentration (X_1 , %), pretreatment temperature (X_2 , °C) and pretreatment time (X_3 , hours). Reducing sugars was the

dependent response (Y , mg/ml). Milled corn stover is maintained at 10% [13] solids to liquid ratio in all experiments.

As a result, the design involved, $F=2^k$ factorial points, $2k$ axial points, and n_0 centre points. In this case k was the number of independent variables. The two axial points were on the axis of each design variable at a distance of α from the design centre. For rotatable CCD, α was calculated according to Equation 2:

$$\alpha = F^{1/4} \quad (2)$$

Therefore, $\alpha = (2^3)^{1/4} = 1.682$

The total number of experimental runs N is given by $N = F + 2k + n_0$

This gives 20 runs i.e. ($N = 2^3 + 2 \times 3 + 2 \times 3 = 20$, $n_0 = 2k$).

The variables were coded according to the Equation 3.

$$X_i = \frac{x_i - x_0}{\Delta x} \quad (3)$$

Where, X_i was the coded value of variable i , x_i the uncoded real value of an independent variable, x_0 the value of X_i at the center point and Δx was the step change between levels 0 and 1. The behaviour of the system was explained by second order polynomial- Equation 4.

$$Y = \beta_0 + \sum_{i=1}^3 \beta_i X_i + \sum_{i=1}^3 \beta_{ii} X_i^2 + \sum_{i,j=1(i \neq j)}^3 \beta_{ij} X_i X_j \quad (4)$$

Where, Y was the fermentable sugars yield, β_0 the offset term, β_i the coefficient of linear effect, β_{ii} the coefficient of squared effect, β_{ij} the coefficient of interaction effect and X_j was the coded value of variable j . **Table 2** indicates the independent variables of pretreatment conditions both in terms of coded levels and actual values.

Table 2: Independent variables of pretreatment conditions

Variables	Coded levels/Actual levels				
	-1.682	-1	0	1	+1.682
Acetic acid concentration, X_1 (%)	68.18	75	85	95	99.97
Pretreatment temperature, X_2 (°C)	132.95	150	175	200	217.05
Pretreatment time, X_3 (hrs)	0.977	2	3.5	5	6.02

Acetic acid pretreatment parameters of lignocellulosic biomass were: concentration 75-95%, pretreatment temperature 150-200°C and pretreatment time 2-5 hours according to [14].

2.6 Preparation of standard/calibration curve

In determination of total reducing sugar concentration after pretreatment followed by hydrolysis, glucose was used as the standard. The recommended range of glucose concentration is 1.2-24mg/ml [15].

III. RESULTS, ANALYSIS AND DISCUSSIONS

3.1 Calibration curve

The absorbances of different concentrations of glucose were found as indicated in the **Table 3**.

Table 3: Results for Calibration curve

Test tube No. :	Concentration of glucose (mg/ml)	Volume of DNSA added (ml)	Keep in boiling water bath for 15 minutes	Volume of Rochell's Salt added (ml)	Absorbance at 540nm
1	4	25		25	0.135
2	8	25		25	0.267
3	12	25		25	0.543
4	16	25		25	0.782
5	20	25		25	1.022
6	24	25		25	1.161

A plot of glucose concentration against absorbance was plotted using Microsoft excel as shown in **Figure 2**. The curves equation was $y = 0.0545x - 0.1117$ with a regression analysis of 0.9908. This indicated that there was adequate correlation of the observations made in **Table 3**.

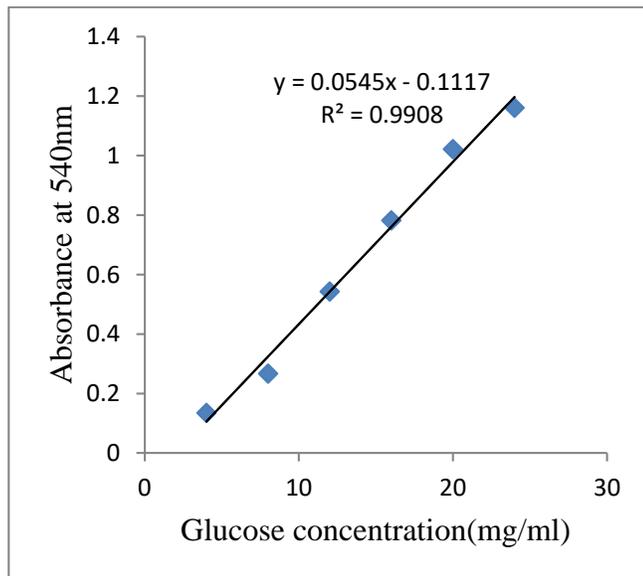


Figure 2: Glucose standard curve

3.2 Experimental results and Statistical analysis

Table 4 shows experimental results (actual response and predicted response) obtained from different sets of pretreatment conditions. Actual response was obtained by applying Beer-Lambert law together with calibration curve of **Figure 2**. Predicted response was obtained by use of second-order polynomial obtained from actual responses.

Design Expert 11 version software was used to analyze the data obtained. By applying multiple regression analysis to experimental data, the following second degree polynomial (**Equation 5**)

Table 2: Actual and predicted responses of pretreatment

Ru n No.	X ₁ (%)	X ₂ (°C)	X ₃ (hrs)	Actual Response Y (mg/ml)	Standard deviation	Predicted response (mg/ml)
1.	-1	-1	-1	10.48	0.98	16.38
2.	1	-1	-1	12.21	0.36	10.04
3.	-1	1	-1	13.11	0.78	17.81
4.	1	1	-1	18.82	0.1	18.22
5.	-1	-1	1	19.03	0.44	16.38
6.	1	-1	1	21.17	0.16	20.21
7.	-1	1	1	18.67	0.38	16.38
8.	1	1	1	17.58	0.34	19.64
9.	-	1.682	0	12.82	0.08	13.29
10.	1.682	0	0	18.43	0.24	19.91
11.	0	-	1.682	18.91	0.42	16.38
12.	0	1.682	0	19.2	0.47	16.38
13.	0	0	-	9.67	0.04	9.45
14.	0	0	1.682	20.53	0.29	18.79
15.	0	0	0	16.8	0.55	13.48
16.	0	0	0	17.19	0.09	17.71
17.	0	0	0	17.38	0.03	17.58
18.	0	0	0	16.32	0.01	16.38
19.	0	0	0	15.6	0.83	21.05
20.	0	0	0	14.92	0.25	13.4

was found to represent relationship between reducing sugar concentration and acetic acid concentration, pretreatment time and temperature:

$$Y = 16.38 + 1.31X_1 + 0.4231X_2 + 2.94X_3 + 0.0938X_1X_2 - 0.7988X_1X_3 - 1.65X_2X_3 - 0.3099X_1^2 + 0.9028X_2^2 - 0.4955X_3^2$$

(5)

Equation 4 in terms of coded factors was used to make predictions about the response for given levels of each factor. By default, the high levels of the factors were coded as +1 and the low levels are coded as -1. Predicted reducing sugar concentrations are indicated in **Table 4** along with the observed reducing sugar concentration. In order to access the significance of the quadratic model fit to the results, model summary statistics and analysis of variance (ANOVA) was carried out and presented in **Table 5** and **6** respectively.

Table 3: Model summary statistics

Source	Std. Dev	R ²	Adjusted R ²	Predicted R ²	PRESS
Quadratic	1.2	0.9293	0.8658	0.5515	91.25

Table 4: Analysis of Variance (ANOVA) for Quadratic Model for Reducing Sugar concentration

The Model F-value of 14.62 implies the model is significant and that there is only a 0.01% chance that F-value this large could occur due to noise.

The model terms X₁, X₃, X₂X₃, X₂² were significant since their P-values were less than 0.05.

The Lack of Fit F-value of 2.14 implied that Lack of Fit was not significant relative to the pure error. There was a 21.14% chance that a "Lack of Fit F-value" this large could occur due to noise.

Table 5: Fit Statistics

Std. Dev.	1.2
Mean	16.44
C.V. %	7.29
R ²	0.9293
Adjusted R ²	0.8658
Predicted R ²	0.5515
Adeq. Precision	13.6901

The coefficient of determination (R²) of the model was 0.9293 (**Table 7**), which indicated that the model adequately represented the real relationship between the variables under consideration. An R² value of 0.9293 means that 92.93% of the variability was explained by the model and only 7.07% was as a result of chance. The coefficient of variation (C.V.) obtained was 7.29%. The Coefficient of Variation (C.V) indicated the degree of precision with which the treatments were carried out. A low value of C.V suggests a high reliability of the experiment [16]. Adequate precision value (13.6901) measures the signal to-noise ratio, and a ratio greater than 4 is generally desirable [17].

The Predicted R² of 0.5515 was not as close to the Adjusted R² of 0.8658. The difference was more than 0.2. This suggested that the model may have fit random noise[18].

3.2.1 Model adequacy checking

In order to proceed with optimization of a fitted response surface, it was important that the model provided an adequate fit [18]. This involved examining the fitted model to ensure that it provided an adequate approximation of the true system. Residuals analysis was an important tool in checking model adequacy.

A normal probability plot of residuals was done to check normality assumption, as in **Figure 3** the residuals plot were approximately along a straight line and therefore normality assumption was satisfied and there was no apparent problem with normality revealed [18].

A plot of Externally Studentized Residuals versus predicted response and a plot of the externally studentized residuals versus run number **Figure 4** and **5** respectively did not reveal any model inadequacy. This was because all the externally studentized residuals were in the interval of $-3 \leq \text{residuals} \leq 3$. According to Myers, *et al* [18], any observation outside this interval (outlier) was potentially unusual with respect to the observed response.

Source	Sum of Squares	df	Mean Square	F-value	p-value
Model	189.1	9	21.01	14.62	0.0001
X ₁ -Acetic acid concentration	23.53	1	23.53	16.37	0.0023
X ₂ - Temperature	2.44	1	2.44	1.7	0.2215
X ₃ -Time	117.71	1	117.71	81.88	< 0.0001
X ₁ X ₂	0.0703	1	0.0703	0.0489	0.8294
X ₁ X ₃	5.1	1	5.1	3.55	0.0889
X ₂ X ₃	21.75	1	21.75	15.13	0.003
X ₁ ²	1.38	1	1.38	0.9628	0.3496
X ₂ ²	11.75	1	11.75	8.17	0.017
X ₃ ²	3.54	1	3.54	2.46	0.1477
Residual	14.38	10	1.44		
Lack of Fit	9.8	5	1.96	2.14	0.2114
Pure Error	4.58	5	0.9151		
Cor Total	203.47	19			

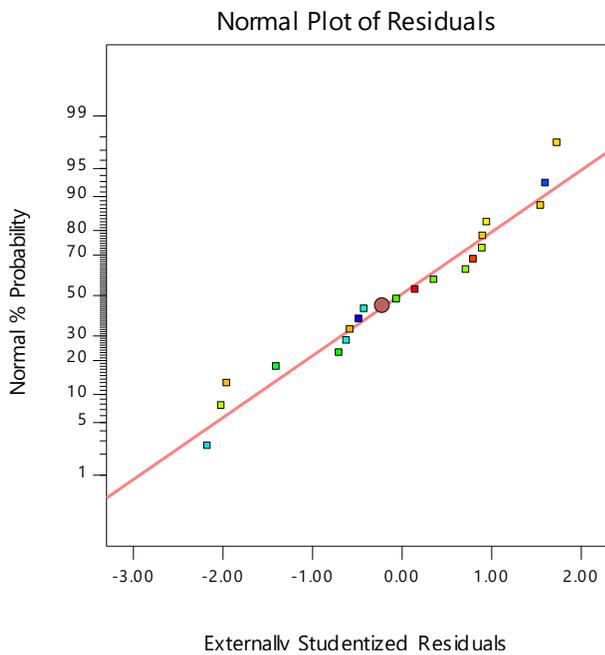


Figure 3: Normal probability plot of Externally Studentized Residuals

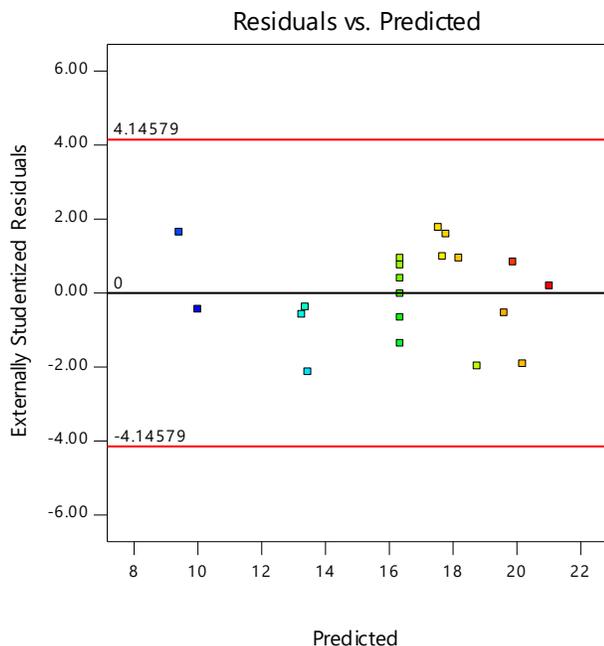


Figure 4: Plot of Externally Studentized Residuals versus predicted response

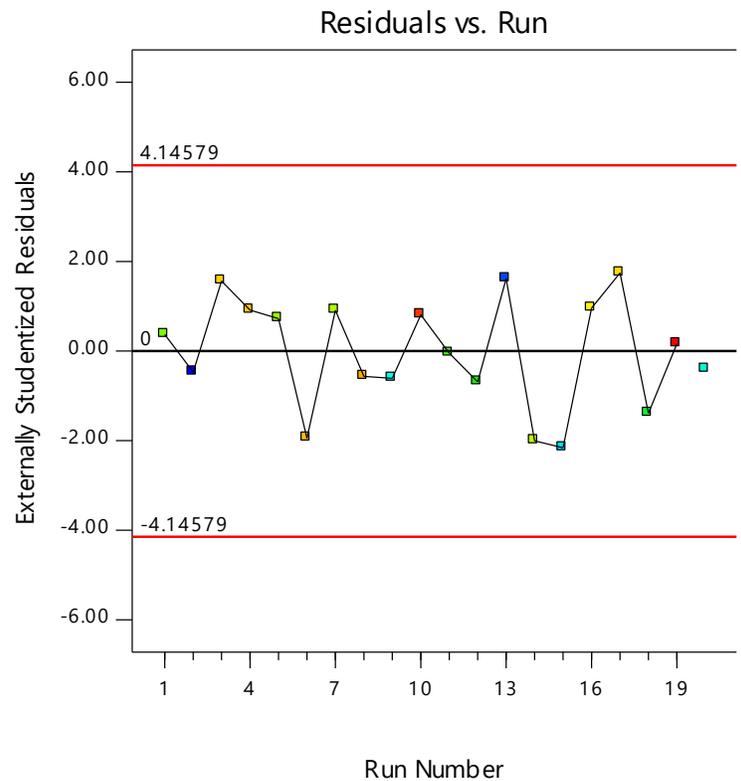


Figure 5: Plot of the externally studentized residuals versus run number

3.3 Optimization analysis

Response surface plots were generated in order to optimize the variables that influence acetic acid pretreatment of corn stover. The three-dimensional (3D) plots were generated by keeping one variable constant at the centre point and varying the others within the experimental range[19].

Figure 6 shows the response surface and corresponding contour plot (**Figure 7**) for total reducing sugar concentration as a function of acetic acid concentration and pretreatment temperature. From the two plots, it can be inferred that as acetic acid concentration increased with pretreatment temperature, the total reducing sugar concentration increased. Reducing sugar concentration reached maximum value of 18.79mg/ml at 95% acetic acid concentration and at temperature of 200°C as shown by the trend of the surface plot.

The trend observed in **Figure 6** may be attributed to the catalysing activity of the acid. Increasing the concentration of acid implied a corresponding increase in the number of hydrogen ions present in the solution hence the hydrolysis process will occur more rapidly. Consequently, the rate at which the glycosidic bonds are broken will increase resulting in a high conversion of hemicellulose fractions into fermentable sugars [20]

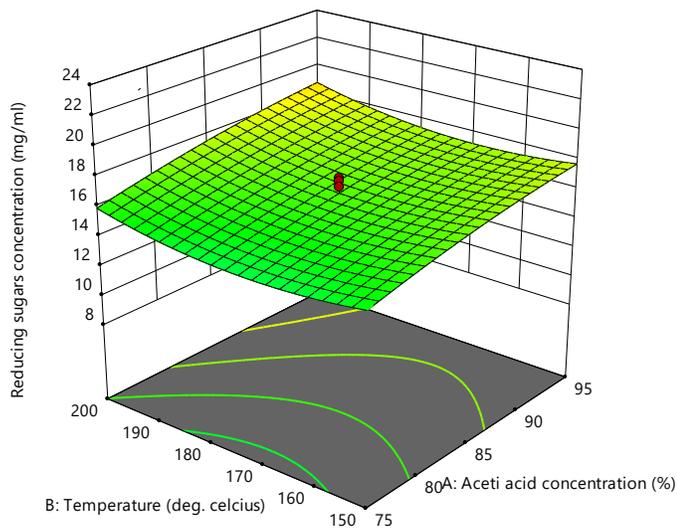


Figure 6: Acetic acid concentration vs. temperature response surface plot

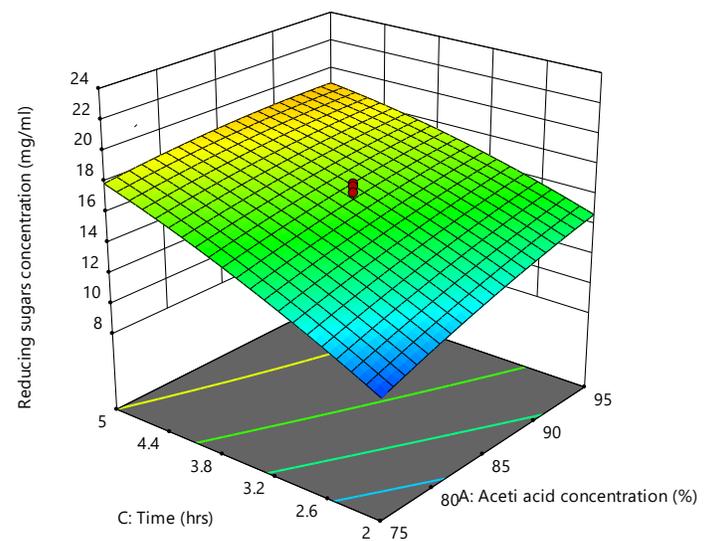


Figure 8: Acetic acid concentration vs. time response surface plot

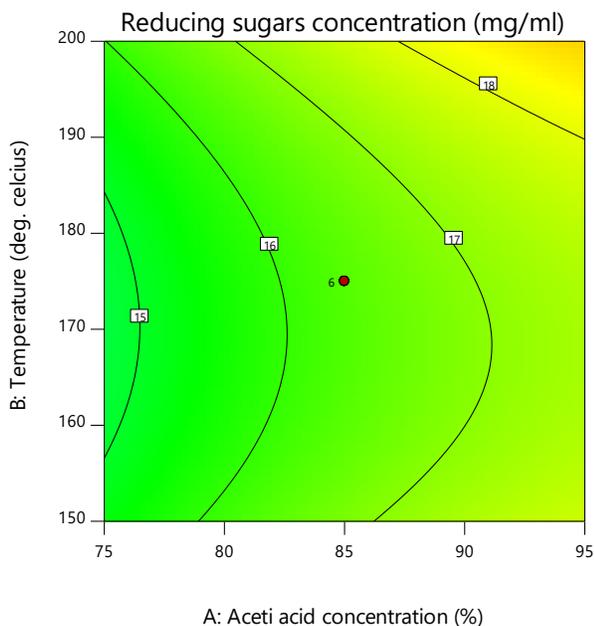


Figure 7: Acetic acid concentration vs. temperature contour plot

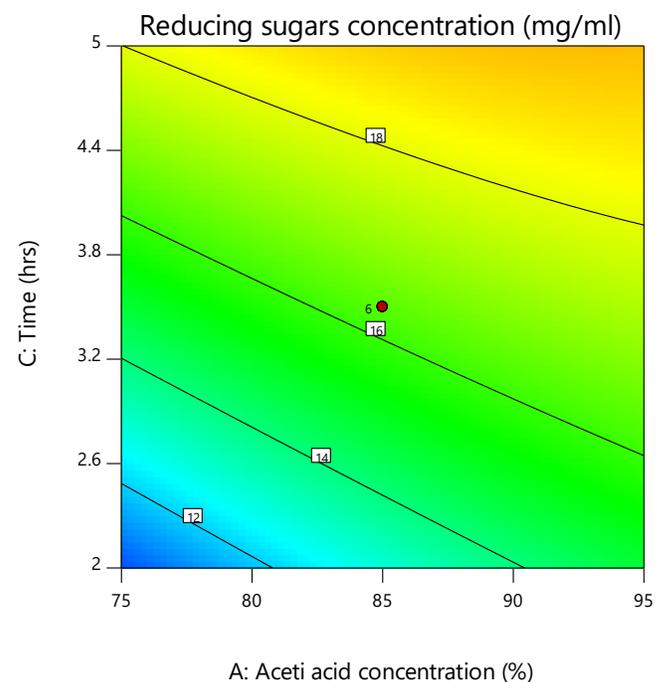


Figure 9: Acetic acid concentration vs. time contour plot

Figure 8 shows the response surface and corresponding contour plot (**Figure 9**) for total reducing sugar concentration as a function of acetic acid concentration and pretreatment time. The effect of interaction of the two variables was that as acetic acid concentration increased with time, the total reducing sugar concentration increased reaching maximum value of 19.02mg/ml at 95% acetic acid concentration and at time of 5hrs as shown by the trend of the surface plot below.

At 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 [20].

The effect of interaction between temperature and time was shown in **Figure 10** and **11** and it was seen that maximum reducing sugar concentration of 20.93mg/ml was obtained at a temperature of 150°C and at a time of 5 hours.

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. This suggests that temperature plays a very important role in the hydrolysis of lignocellulosic materials [20].

yield was found to be 64.4%. According to [21], corn stover was pretreated using various pretreatment methods and then subjected to standard evaluation techniques, the yields of sugars was found to be more or less the same. It was found out that the overall yield of sugars was 70-80% of untreated corn stover. This indicated that the value of reducing sugars from this research was lower. This difference could be attributed to random errors that occur during lab experiments. In addition, the observed difference in sugar yield indicates that possibly there might be noticeable differences in terms of sugar yields from different types of corn stover. Different types of analytical equipment have also different accuracies and reliabilities. This could possibly be another factor that brought out the difference in sugars yields to those from literature.

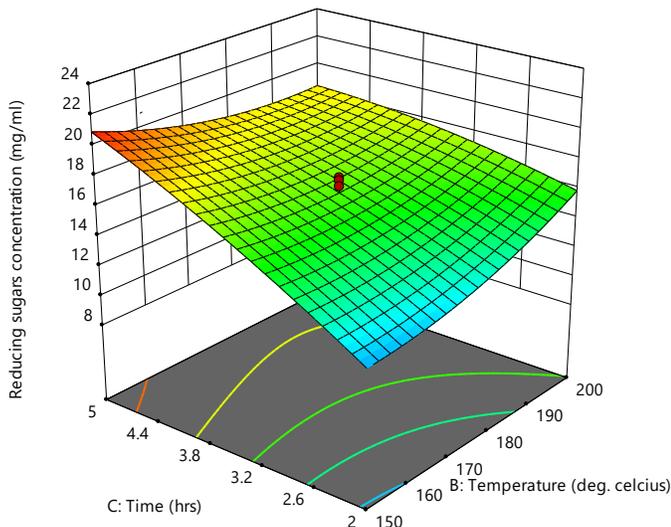


Figure 10: Temperature vs. time response surface plot

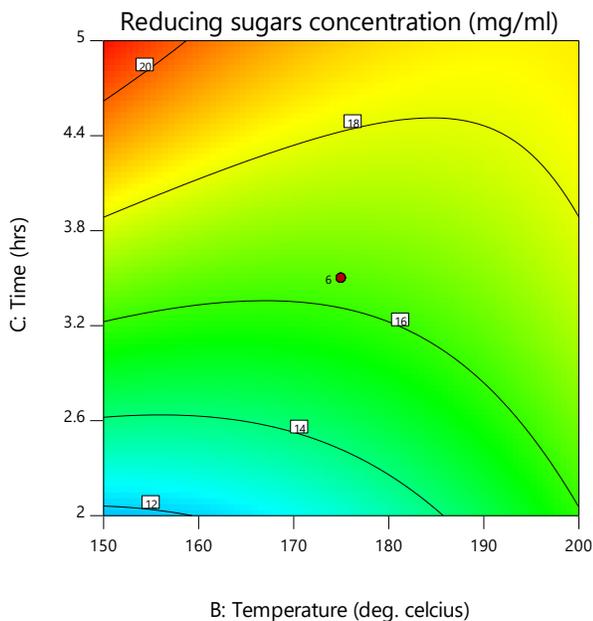


Figure 11: Temperature vs. time contour plot

In order to get optimum point in response surface optimization, a saddle point has to be found from the response surface plots and contour plots. If a saddle point is not found, partial differentiation of the fitted regression equation with respect to the independent variables is done.

Using Design Expert 11 software, optimum sugar concentrations was 21.09mg/ml at optimum pretreatment conditions of 91.89% acetic acid concentration, 150°C and 5hrs. This was arrived at by partial differentiation of regression equation with respect to independent variables. Validation of the model indicated insignificant difference (± 1.2) between predicted and observed values.

The overall reducing sugars yield was calculated using **Equation 1**, with the composition of untreated corn stover and the optimum yield of reducing sugars. The overall reducing sugars

IV. CONCLUSIONS AND RECOMMENDATIONS

4.1 Conclusions

In this work, acetic acid pretreatment of corn stover followed by dilute HCl hydrolysis was studied quantitatively over five levels using a three variable central composite rotatable design for response surface methodology. From the study, the following conclusions can be drawn;

- It was concluded that acetic acid pretreatment of corn stover was influenced by the concentration of acid, pretreatment temperature and time.
- The optimum pretreatment conditions were found to be 91.89% Ethanoic acid concentration, 150°C and 5hrs. Total reducing sugars under these conditions was 21.09mg/ml.
- Pretreatment of corn stover as carried out in this study, yielded substantial amounts reducing sugars which can be fermented to produce bioethanol.
- This study leads to extended utilization of corn stover beyond being used as animal feed (which has low nutrient content) to industrial utilization and hence reduction in utilization of food crops such as starches for bioethanol production.

4.2 Recommendations

From this research, the following recommendations can be made;

- Since lignin is a complex polymer which forms a protective layer around cellulose and hemicellulose fibres, analysis of its removal is recommended to further determine the effectiveness of pretreatment.
- Analysis of major specific reducing sugars to determine the right microorganism to use during fermentation.
- Techno-economic analysis of corn stover to be carried out to determine uses of corn stover beyond animal feeds.
- Analysis of fermentation of reducing sugars obtained from corn stover pretreatment and hence its industrial application in bioethanol production.

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