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RESPONSE SURFACE METHODOLOGY FOR OPTIMIZING ALKALINE HYDROGEN PEROXIDE PRETREATMENT OF CORN STOVER

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ABSTRACT

Pretreatment is a critical step in the conversion of lignocellulosic biomass to fermentable sugars. In this study, alkaline hydrogen peroxide pretreatment was evaluated for the purpose of obtaining fermentable sugars from corn stover. The effect of alkaline hydrogen peroxide concentration, residence time, and temperature was investigated and optimised using response surface methodology (RSM). Pretreatment was carried out with the following range of the independent variables: temperature (60 °C - 90 °C), time (20 min - 40 min) and AHP concentration (2.5 w/v% -7.5w/v%) resulting in 17 experimental runs. The optimum operating conditions predicted by RSM was AHP concentration of 7.43w/v%, time of 22 min and temperature of 68.40 °C with a sugar yield of 30.16 g/L.

1. INTRODUCTION

One of the most important prerequisites for sustainable development is the production of appropriate fuels, which can be applied as alternative to the current fossil fuels (Saha and Cotta, 2006). Currently, first generation starch and sugar crops (like corn and sugarcane) are being used to produce the liquid transportation fuel ethanol as a substitute for gasoline. Nearly 40% of the corn produced in the United States is converted to fuel ethanol (Yang and Wyman, 2008). Sugar and starch are easily fermented into ethanol. However, using these crops as a renewable feedstock for ethanol puts the fuel supply in direct competition with the food supply.

One proposed alternative is the use of lignocellulose as a renewable feedstock to supply energy demands. Lignocellulose is the most abundant and renewable source of carbon on the planet, being the main structural component of plants (Garcio et al., 2006). Harnessing the energy stored in lignocellulose has been tipped as one solution for meeting the growing energy demands without decreasing the food supply. Lignocellulose is the primary building block of plant cell walls. Plant biomass is mainly composed of cellulose, hemicellulose, and lignin, along with smaller amounts of pectin, protein, extractives (soluble nonstructural materials such as nonstructural sugars, nitrogenous material, chlorophyll, and waxes) (Besombes and Mazeau, 2005). The lignocellulosic materials such as agricultural residues (e.g., wheat straw, sugarcane bagasse, corn Stover), forest products (hardwood and softwood), and dedicated crops (switch grass, Salix) are renewable sources of energy. These raw materials are sufficiently abundant and generate very low net greenhouse emissions. Several pretreatment processes have been investigated to change the crystalline structure of these materials (Curreli et. al., 1997). It has frequently been proven that by decreasing the crystallinity, one can increase the accessibility of cellulose to enzymatic attack and improve the yield of subsequent enzymatic hydrolysis (Esteghlaluan et al., (1997). Alkaline pretreatment (e.g. NaOH, $Ca(OH)_2$, H_2O_2) is one of the most effective processes among the different proposed pretreatment methods to change the crystalline structure of these material, hence this study which investigates the alkaline hydrogen peroxide pretreatment of corn stover.

2. MATERIALS AND METHODS

2.1. Materials Preparation

The corn stover used in this study was collected from a model farm at the Nigerian Institute for Oil Palm Research (NIFOR), Benin City, Edo State, Nigeria. The corn stover was air dried, milled and screened to a particle size of 2mm. It was subsequently stored at room temperature for further use.

2.2. AHP Pretreatment

The alkaline peroxide pretreatment was performed using sodium hydroxide and hydrogen peroxide at 2.5-7.5% concentration. The corn stover in AHP was subjected to thermal treatment at different temperature (60, 75 and 90 °C) for 20, 30 and 40 minutes. The tests were carried out in 500 mL round bottom flask using 20 g of the corn stover and 200 mL of the AHP solution. The 500 mL round bottom flask containing the reaction mixture was mounted on a thermostat heating mantle and heated to the required temperature for the required duration. On completion, the round bottom flask was removed from heat source, and cooled to room temperature. The solid was separated from the liquid using a filter paper. The filtrate was subsequently analysed for fermentable sugars.

2.3. Analytical Methods

The total reducing sugar content of the final hydrolysate was determined by the colorimetric method using glucose as standard (Miller, 1959). A standard curve was prepared using known concentrations of glucose from which the concentration of reducing sugar was determined.

2.4. Experimental Design

A three-level-three-factor Box-Behnken design was used to study the response pattern and to determine the optimum combination of AHP concentration, temperature and time and this resulted in 17 experimental runs. The coded and actual levels of the factors are shown in Table 1. Equation 1 is a quadratic response model which was used to fit the experimental data and this was achieved by using multiple regression analysis to estimate the values of the coefficients of the model. Analysis of variance (ANOVA) was then used to assess the quality and significance of the model.

$$Y_i = b_o + \sum b_i X_i + \sum b_{ij} X_i X_j + \sum b_{ii} X_i^2 + e_i \tag{1}$$

Y_i is the predicted response or dependent variable, X_i and X_j are the independent variables, b_o is the offset term, b_i and b_{ij} are the single and interaction effect coefficients and e_i is the experimental error term.

The low, middle, and high levels of each variable were coded as -1, 0, and +1, respectively. The factors were coded according to Equation 2.

$$x_i = \frac{X_i - X_o}{\Delta X_i} \tag{2}$$

Where x_i and X_i are the coded and actual values of the factors respectively. X_o is the actual value of the factors at the centre point and ΔX_i is the step change in the actual value of the factors. Design Expert® 7.0.0 (Stat-ease, Inc. Minneapolis, USA), a statistical software used for the experimental design, regression analysis and analysis of variance (ANOVA). The predictive capacity of RSM was evaluated using the R^2 values. The R^2 value is an indication of the fit of a mathematical model. An R^2 value close to unity is indicative of a good fit between model and experimental data (Qi et al., 2009).

Table 1: Coded and actual levels of the factors for three factor BBD

Variables	Symbol	Coded and actual levels		
		-1	0	+1
AHP Concentration (w/v%)	X_1	2.5	5.0	7.5
Temperature (°C)	X_2	60	75	90
Time (min)	X_3	20	30	40

3. RESULTS AND DISCUSSION

3.1. Statistical Analysis

The results of the 17 BBD experiments are shown with the observed and predicted responses in Table 2. Equation 3 is the model equation which relates the response (sugar concentration) to the factors in terms of actual values. The equation represents sugar concentration (Y) as a function of AHP concentration (X_1), temperature (X_2) and time (X_3).

$$\begin{aligned} Y = &-17.99 + 2.75X_1 + 0.43X_2 + 0.54X_3 - 0.028X_1X_2 + 0.0014X_1X_3 \\ &+ 0.0028X_2X_3 + 0.067X_1^2 - 0.010X_2^2 - 0.0038X_3^2 \end{aligned} \tag{3}$$

The actual and predicted values shown in Table 2 were plotted (Figure 1) to analyze the correlation between them. It was observed from the plot that the data points are distributed near the 45° line indicating that the quadratic regression model was able to predict the sugar concentration obtained from pretreatment process to a high level of accuracy or confidence. Hence this equation can be used for both predictive and design purposes.

The precision of a model is a function of its determination coefficient (R^2) value. The R^2 value of 0.9826 obtained in this study indicates that the model is accurate enough to represent the real relationships among the studied pretreatment variables. This directly implies that 98.26% of the sample variation is attributed to the factors. In other words, about 1.74% of total variations of sugar yield was not satisfactorily explained by the model. This unexplained value is presented in terms of residual error in the values. The coefficient of variation (CV) is the standard deviation expressed as a percentage of the mean. The experimental data is usually considered reproducible if the CV is not greater than 10%. A value of 2.96% obtained in this case, indicates reliability of the experiments. The adequate precision value measures signal to noise ratio and a ratio greater than 4 is desirable. A value of 31.951 obtained in this case indicates an adequate signal meaning that the model can be used to navigate the design space (Montgomery, 2005).

Table 2: BBD matrix showing actual and coded values along with the experimental values and predicted sugar concentration

	Factors						Response	
Run	Coded levels			Actual values			Sugar Concentration (g/L)	
no	X_1	X_2	X_3	X_1	X_2	X_3	Observed	Predicted
1	1	0	-1	7.50	30	60	28.10	28.03
2	-1	0	1	2.50	30	90	16.30	16.40
3	1	-1	0	7.50	20	75	30.06	30.69
4	1	0	1	7.50	30	90	30.06	29.93
5	0	-1	1	5.00	20	90	22.92	22.48
6	0	0	0	5.00	30	75	22.35	22.70
7	0	0	0	5.00	30	75	22.83	22.70
8	0	0	0	5.00	30	75	22.88	22.70
9	1	1	0	7.50	40	75	27.28	26.91
10	0	0	0	5.00	30	75	22.09	22.70
11	0	-1	-1	5.00	20	60	22.05	21.52
12	-1	0	-1	2.50	30	60	14.55	14.72
13	0	1	1	5.00	40	90	20.39	20.95
14	-1	1	0	2.50	40	75	15.51	14.91
15	0	0	0	5.00	30	75	23.27	22.70
16	0	1	-1	5.00	40	60	17.86	18.33
17	-1	-1	0	2.50	20	75	15.49	15.86

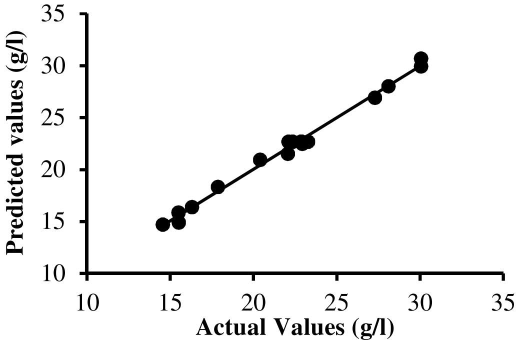

Figure1: Parity plot showing actual experimental and predictive values for sugar concentration

Table 3: Analysis of variance for response surface

Source	Sum of	df	Mean	F	p- value
Model	388.58	9	43.18	101.68	<0.0001
X_1	360.06	1	360.06	847.94	<0.0001
X_2	11.19	1	11.91	26.34	0.0013
X_3	6.32	1	6.32	14.88	0.0062
X_1X_2	1.99	1	1.99	4.68	0.0672
X_1X_3	0.011	1	0.011	0.026	0.08765
X_2X_3	0.69	1	0.69	1.62	0.2434
X_1^2	0.75	1	0.75	1.76	0.2434
X_2^2	4.43	1	4.43	10.43	0.0145
X_3^2	3.07	1	3.07	7.22	0.0312
Residual	2.97	7	0.42		
Lack of fit	2.10	3	0.70	3.24	0.1432
Pure Error	0.87	4	0.22		
Corr Total	391.56	16			

Table 5: Statistical information for ANOVA

Standard Deviation	Mean	Coefficient of variance (%)	PRESS	R^2	Adjusted R^2	Predicted R^2	Adequate Precision
0.65	22	2.96	35.03	0.9924	0.9826	0.9105	31.951

3.2. Optimization of Pretreatment of Corn Stover

In order to determine the optimal levels of the independent variables affecting the pretreatment of corn stover, three-dimensional (3D) response surface and contour plots were constructed according to the regression model (Equation 3). The 3D plots were generated by keeping two factors at their optimum point and varying the other two factors within their experimental ranges. The plots show how alkaline hydrogen peroxide concentration, residence time and temperature affected the amount of the sugar produced. Sugar production was enhanced at high levels of AHP concentration and low reaction time as shown in Figure 2. This suggests that the higher the concentration of hydrogen peroxide, the more radicals are produced resulting in more delignification. This agrees with similar observation made by Amenaghawon et al. (2014).

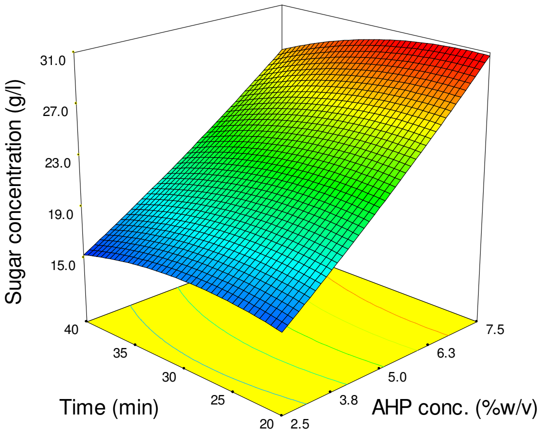

Figure 2: Response surface plot showing the effect of time and AHP concentration on sugar yield

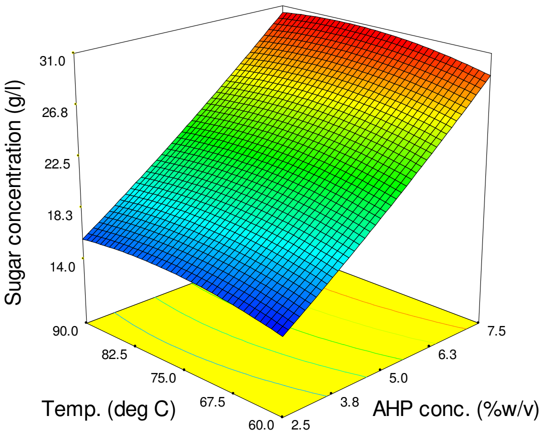

Figure 3: Response surface plot showing the effect of temperature and AHP concentration on sugar yield

Figure 3 shows that medium levels of temperature was suitable for the pretreatment process. Higher temperatures have been linked with the degradation of sugars to inhibitory products such as furfural (Zheng et al., 2009).

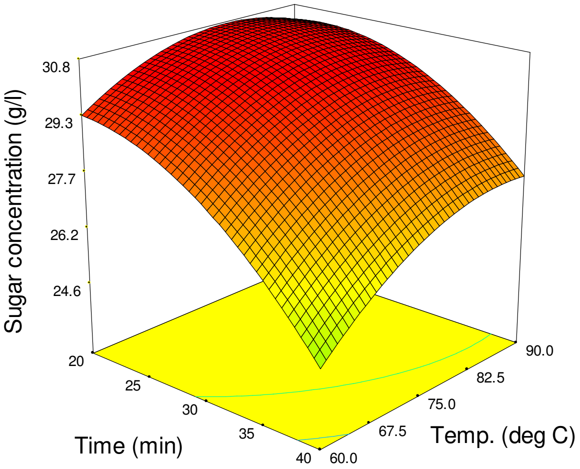

Figure 4: Response surface plot showing the effect of time and temperature concentration on sugar yield

The Design-Expert software was used to numerically optimise the statistical model (Equation 3) to determine the optimum reducing sugar production conditions. The results showed that a maximum sugar concentration of 30.80 g/l was obtained. The corresponding values of the independent factors were AHP concentration (7.5 %w/v), time (21.51 min), temperature (80.18 °C). The optimum sugar concentration predicted by the model was validated by carrying out repeated experiments at the optimum conditions. The mean of the observations was obtained as 30.51 g/l.

4. CONCLUSION

The use of statistical like BBD with RSM is useful in optimization of process conditions. This study serves as a step towards further understanding of the mechanism of AHP pretreatment to enhance enzymatic digestibility of lignocellulose biomass. AHP concentration, temperature and time affect the efficiency of the pretreatment process thus influencing the concentration of sugar produced. These variables are related to the sugar concentration by a validated quadratic regression model. These results find application in pilot scale AHP pretreatment of corn stover targeted at fermentable sugar production.

5. CONFLICT OF INTEREST

There is no conflict of interest associated with this work.

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