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Statistical Optimization of the Total Reducing Sugars Yield from the Dilute Acid Hydrolysis of Sugarcane Bagasse

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Abstract*—The dilute acid hydrolysis of sugarcane bagasse using sulphuric acid at different concentrations (2.32-5.68% w/w), hydrolysis times (18-52 min) and hydrolysis temperatures (76-144°C) were studied. The total reducing sugar present in the hydrolysate was quantified using DNS Method. A 2^3 five level Central Composite Design (CCD) was used to develop a statistical model for the optimization of process variables which are acid concentration, hydrolysis time and hydrolysis temperature. Response Surface Methodology (RSM) was employed for the optimization of the dilute acid hydrolysis conditions. The optimal hydrolysis conditions that resulted in the maximum total reducing sugar concentration were acid concentration of 4.99% (w/w), hydrolysis temperature of 90.41°C and hydrolysis time of 44.84 minutes. Under these conditions, the total reducing sugar concentration was obtained to be 21.7383 g/L. Quadratic model selected for the analysis was then validated.*

***Keywords*—** *Sugarcane bagasse, reducing sugar, acid hydrolysis, surface response, statistical modelling and optimization.*

I. INTRODUCTION

The increasing costs of oil based products, the unavoidable depletion of the world's crude oil reserves, along with the environmental contamination caused by non-renewable energy sources like fossil fuels has prompted the need to create economical and sustainable sources of energy. Bioethanol has been identified as a potentially sustainable liquid fuel for road transportation hence a good alternative to fossil fuels. First generation bioethanol, manufactured from starch containing biological materials like corn, cassava, wheat, etc. has been discovered not to be sustainable as a result of the ethical concerns relating to the use of potential food resources for biofuel production (Amenaghawon et al., 2014).

Sugarcane bagasse is currently utilized as the fundamental source of energy required in sugar mills. However, an important part of the bagasse produced is underused as the energy requirement of the sugar mill plant is satisfied with only half of the produced bagasse (de Moares Rocha et al., 2010). The excess bagasse can be utilized in applications like ethanol production, pulp and paper, boards, animal feed and furfural.

The yield of fermentable sugars during acid hydrolysis is influenced by variables like pre-treatment time, pre-treatment temperature, particle size, acid concentration etc. (Amenaghawon et al., 2014). Hence, the focus of this study was on optimizing the yield of the fermentable sugars via dilute acid hydrolysis of the lignocellulosic biomass, sugarcane bagasse.

Over the years, before process optimization received attention, a lot of resources and time have been wasted, unnecessary costs have been incurred while trying to achieve a process objective. This is so because the optimal conditions for the completion of the process were not determined, hence trial and error methods, and other time wasting methods were used. Agricultural and industrial residues over the years have always been discarded, underused and have caused a great nuisance in terms of solid waste pollution to the environment if not properly disposed. The main problem this research work aims at solving is the optimization of the hydrolysis process. Determining these conditions would help minimize cost, maximize efficiency, save a lot of time and also help in industrial decision making and economical estimation.

This study was limited to the production of reducing sugars from lignocellulosic biomass only. It did not go further to ferment these sugars to produce bioethanol. The research study was a small scale laboratory study, hence only small measured quantities were studied.

II. MATERIALS AND METHODS

2.1 Materials

2.1.1 Reagents and raw materials

- Sugarcane Bagasse
- Dilute Sulphuric Acid (2.32 to 5.68 %w/v)
- Distilled water
- 3,5 dinitrosalicylic Acid
- Analytical Glucose
- 2M Sodium hydroxide
- Rochelle salt (Potassium sodium tatarate)

2.1.2 Equipment and apparatus

- Grinding machine

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- Scout Pro Electronic weighing balance SPU20001, S/N 7129151748
- Vision scientific Oven Model LDO-201-E
- Filter paper
- Jenway 6405 UV- Vis Spectrophotometer S/N 3976
- Stuart UC152 Hot plate magnetic stirrer S/N R60000548
- Funnel
- Test Tubes and Holder
- Beaker
- Conical Flask
- Thermometer (0 -360 degree Celsius)
- Pipette and Pipette sucker
- Measuring Cylinder
- Volumetric flask
- Reagent Bottles
- Round and flat bottom flasks
- Wash bottle

2.2 Methods

2.2.1 Pretreatment of substrate

The substrate (Sugarcane bagasse) was air dried for 2 weeks, after which it was ground and sieved.

2.2.2 Preparation of DNS reagent

1g of DNS Acid was dissolved in 20ml of 2M Sodium Hydroxide and 50ml distilled water. To this mixture, 30g of Rochelle salt was added after which distilled water was added to make the volume up to 100ml. The solution was then filtered off to remove any precipitates.

2.2.3 Dilute acid hydrolysis

Dilute acid hydrolysis of substrate was carried out using sulphuric acid concentration range of 2.32%-5.68%, hydrolysis temperature range of (76-144) °C and an hydrolysis time range of (18-52) minutes. In a beaker 1.5g of substrate was weighed to which 50ml of sulphuric acid added. The mixture was placed on a hot plate magnetic stirrer with the magnet inserted and set to the hydrolysis temperature. The mixture was left to hydrolyze for a time range of 18 to 52 minutes after which the mixture removed and filtered. The filtrate (also known as the hydrolysate) was then analyzed for total reducing sugar by calorimetric method using a UV Spectrophotometer.

2.2.4 Total reducing sugar analysis by calorimetric method

2.2.4.1 Preparation of glucose standard curve

5 glucose solutions of the concentrations of 20g/L, 40g/L, 60g/L, 80g/L and 100g/L were prepared. To a test tube, 1.8ml of distilled water was added to 0.2 ml of 20g/L glucose solution. 2ml of DNS Reagent was added and the mixture was then boiled for 5 minutes in a water bath, after which it was cooled to room temperature and then diluted to 24ml as shown in plate 3. The absorbance of the resulting solution was then measured at wavelength of 540 nm using a UV spectrophotometer. The process was repeated for glucose concentrations of 40g/L, 60g/L, 80g/L and 100g/L.

The resulting absorbance gotten for the different glucose solutions were then plotted against their concentrations.

2.2.4.2 Determination of total reducing sugar content of hydrolysate

To a test tube, 0.2ml of hydrolysate (reducing sugar solution), 1.8ml of Distilled water and 2ml of DNS Reagent were added. The mixture was then boiled for 5 minutes in a water bath, after which it was cooled to room temperature and then diluted to 24ml. The absorbance of the resulting solution was then measured at a wavelength of 540nm using a UV Spectrophotometer. The absorbance gotten from the spectrophotometer was used to obtain the concentration of total reducing sugars from the Glucose standard concentration curve. The process was repeated for all the hydrolysates gotten after each hydrolysis.

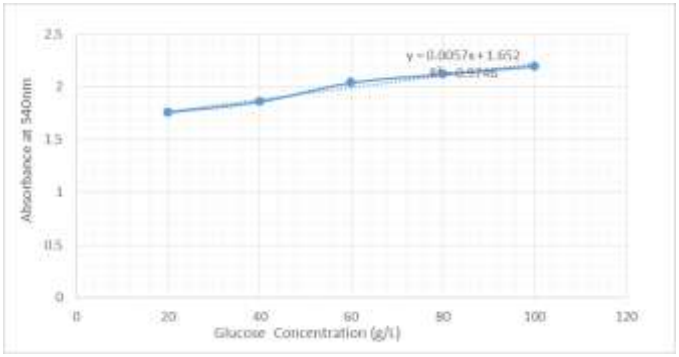

Fig. 2.1. Glucose standard concentration curve.

2.2.5 Design of experiment

A Central Composite Design (CCD) with three factors was used to examine the response pattern and to determine the optimum combination of acid concentration, hydrolysis temperature and hydrolysis time for maximizing the sugar recovery from sugarcane bagasse. The range and levels of variables optimized are as shown in table 2.1. The Central Composite Design combines the vertices of the hypercube whose coordinates are given by a 2^n factorial design with star points. The star points provide the estimation of curvature of the nonlinear response surface (Amenaghawon et al., 2013). The experimental design was developed using Design Expert® 7.0.0 and it resulted in 20 runs as shown in table 2.2. The 20 experimental runs were randomized to maximize the effects of unexplained variability in the responses observed.

TABLE 2.1. Coded and actual levels of the factors for the 3 factor central composite design.

Independent Variables	Symbols	Coded levels				
		-1.68	-1	0	1	1.68
		Actual Levels				
Acid Concentration (%w/v)	X_1	2.32	3	4	5	5.68
Hydrolysis temperature (°C)	X_2	76	90	110	130	144
Hydrolysis Time (min)	X_3	18	25	35	45	52

III. Results and Discussion

3.1 Result Presentation

3.1.1 Linear model fit

Final Equation in terms of the coded values:

$$\text{Sugar Concentration} = 16.56+1.16X_1 + 0.66X_2 + 3.42X_3 \quad 3.1$$

Final Equation in terms of actual factors:

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$$\text{Sugar Concentration} = -3.69999 + 1.15592X_1 + 0.033175X_2 + 0.34239X_3 \quad 3.2$$

TABLE 3.1. Experimental results for the dilute acid hydrolysis of sugarcane bagasse.

Runs	Factors						Response
	Coded Values			Actual Values			Total Reducing Sugar Concentration (g/L)
	X_1	X_2	X_3	X_1	X_2	X_3	Y_1
1	1	1	1	5	130	45	21.59
2	1	1	-1	5	130	25	15.34
3	1	-1	1	5	90	45	21.32
4	1	-1	-1	5	90	25	13.55
5	-1	1	1	3	130	45	18.96
6	-1	1	-1	3	130	25	15.02
7	-1	-1	1	3	90	45	19.78
8	-1	-1	-1	3	90	25	9.99
9	-1.68	0	0	2.32	110	35	13.08
10	1.68	0	0	5.68	110	35	17.68
11	0	-1.68	0	4	76	35	17.32
12	0	1.68	0	4	144	35	19.01
13	0	0	-1.68	4	110	18	9.86
14	0	0	1.68	4	110	52	21.29
15	0	0	0	4	110	35	16.92
16	0	0	0	4	110	35	15.49
17	0	0	0	4	110	35	16.98
18	0	0	0	4	110	35	16.68
19	0	0	0	4	110	35	16.28
20	0	0	0	4	110	35	14.99

TABLE 3.2. ANOVA table for the linear model fit for the hydrolysis of sugarcane bagasse.

Source	Sum of Squares	Df	Mean Square	F value	p-value Prob. > F	Inference
Model	185.86	3	61.95	38.82	< 0.0001	Significant
X_1	18.25	1	18.25	11.44	0.0038	
X_2	6.07	1	6.07	3.80	0.0690	
X_3	161.54	1	161.54	101.23	< 0.0001	
Residual	25.53	16	1.60			
Lack of Fit	22.20	11	2.02	3.03	0.1154	Not significant
Pure Error	3.33	5	0.67			
Cor Total	211.39	19				

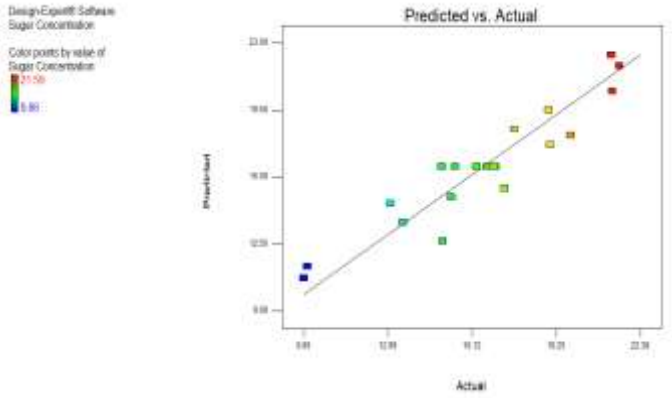

Fig. 3.1. Predicted vs Actual response values for the linear model fit for the dilute acid hydrolysis of sugarcane bagasse.

TABLE 3.3. Data for the linear model fit.

R squared	0.8792
Adj R squared	0.8566
Pred. R squared	0.7910
Adeq Prec	20.606
Standard deviation	1.26
Mean	16.56

3.1.2 Two factor interaction model fit

Final Equation in terms of the coded values

$$\text{Sugar Concentration} = 16.56 + 1.16X_1 + 0.66X_2 + 3.42X_3 - 0.27X_1X_2 + 0.036X_1X_3 - 0.92X_2X_3 \ldots. \quad 3.3$$

Final Equation in terms of actual factors

$$\text{Sugar Concentration} = -26.83905 + 2.50717X_1 + 0.28414X_2 + 0.83458X_3 - 0.013438X_1X_2 + 3.62500\text{E-}003X_1X_3 - 4.60625\text{E-}003X_2X_3 \ldots\ldots \quad 3.4$$

TABLE 3.4. ANOVA table for the two factor model fit for the hydrolysis of sugarcane bagasse

Source	Sum of Squares	Df	Mean Square	F value	p-value Prob. > F	Inference
Model	193.23	6	32.21	23.06	< 0.0001	Significant
X_1	18.25	1	18.25	13.07	0.0031	
X_2	6.07	1	6.07	4.34	0.0574	
X_3	161.54	1	161.54	115.68	< 0.0001	
X_1X_2	0.58	1	0.58	0.41	0.5312	
X_1X_3	0.01	1	0.01	0.01	0.9322	
X_2X_3	6.79	1	6.79	4.86	0.0461	
Residual	18.15	13	1.40			
Lack of Fit	14.83	8	1.85	2.78	0.1372	Not significant
Pure Error	3.33	5	0.67			
Cor Total	211.39	19				

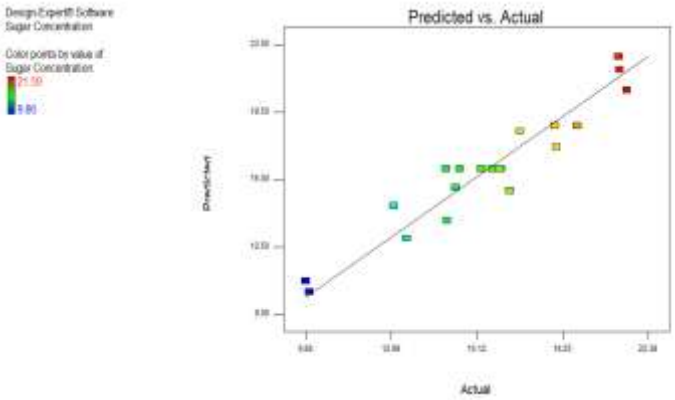

Fig. 3.2. Predicted vs Actual response values for the two factor interaction model fit for the dilute acid hydrolysis of sugarcane bagasse.

TABLE 3.5. Data for the two factor model fit.

R squared	0.9141
Adj R squared	0.8745
Pred. R squared	0.7469
Adeq Prec	17.476
Standard deviation	1.18
Mean	16.56

3.1.3 Quadratic model fit

Final Equation in terms of the coded values

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Sugar Concentration = 16.21 + 1.16X_1 + 0.66X_2 + 3.42X_3 – 0.27X_1X_2 + 0.036X_1X_3 - 0.92X_2X_3 -0.18X_1^2 + 0.79X_2^2 -0.11X_3^2 3.5

Final Equation in terms of actual factors

Sugar Concentration = -7.51366 + 3.92425X_1 - 0.18519X_2 + 0.91039X_3 – 0.013438X_1X_2 + 3.62500E-003X_1X_3 – 4.60625E-003X_2X_3 - 0.17714X_1^2 + 1.96972E-003X_2^2 -1.08307E-003X_3^2 ……. 3.6

TABLE 3.6. ANOVA table for the quadratic model fit for the hydrolysis of sugarcane bagasse.

Source	Sum of Squares	Df	Mean Square	F value	p-value Prob. > F	Inference
Model	203.91	9	22.66	30.29	< 0.0001	Significant
X_1	18.25	1	18.25	24.40	0.0006	
X_2	6.07	1	6.07	8.11	0.0173	
X_3	161.54	1	161.54	215.99	< 0.0001	
X_1X_2	0.58	1	0.58	0.77	0.4001	
X_1X_3	0.01	1	0.01	0.01	0.9080	
X_2X_3	6.79	1	6.79	9.08	0.0131	
X_1^2	0.45	1	0.45	0.60	0.4552	
X_2^2	9.25	1	9.25	12.37	0.0056	
X_3^2	0.17	1	0.17	0.23	0.6392	
Residual	7.48	10	0.75			
Lack of Fit	4.15	5	0.83	1.25	0.4073	Not significant
Pure Error	3.33	5	0.67			
Cor Total	211.39	19				

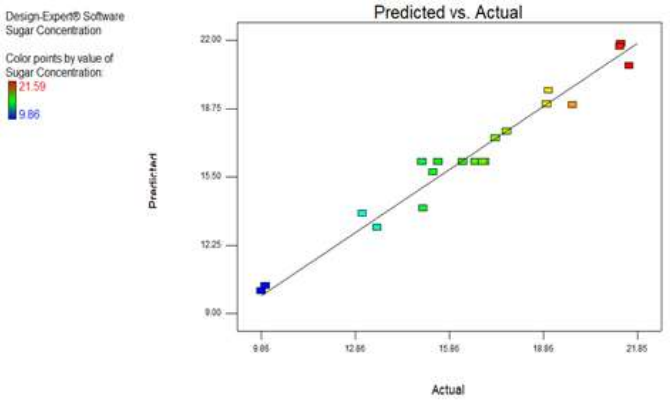

Fig. 3.3. Predicted vs Actual response values for the quadratic model fit for the dilute acid hydrolysis of sugarcane bagasse.

TABLE 3.7. Data for the quadratic model fit.

R squared	0.9646
Adj R squared	0.9328
Pred. R squared	0.8123
Adeq Prec	19.260
Standard deviation	0.86
Mean	16.56

3.1.4 Cubic model fit

Final Equation in terms of the coded values

Sugar Concentration = 16.21 + 1.37X_1 + 0.5X_2 + 3.36X_3 – 0.27X_1X_2 + 0.036X_1X_3 - 0.92X_2X_3 -0.18X_1^2 + 0.79X_2^2 -0.11X_3^2 +0.54$X_1X_2X_3$ +0.29$X_1^2X_2$ +0.11$X_1^2X_3$ -0.36$X_1X_2^2$ 3.7

TABLE 3.8. ANOVA table for the cubic model fit for the hydrolysis of sugarcane bagasse.

Source	Sum of Squares	Df	Mean Square	F value	p-value Prob. > F	Inference
Model	207.00	13	15.92	21.77	0.0006	Significant
X_1	10.58	1	10.58	14.46	0.0089	
X_2	1.43	1	1.43	1.95	0.2118	
X_3	65.32	1	65.32	89.30	< 0.0001	
X_1X_2	0.58	1	0.58	0.79	0.4083	
X_1X_3	0.01	1	0.01	0.01	0.9085	
X_2X_3	6.79	1	6.79	9.28	0.0226	
X_1^2	0.45	1	0.45	0.62	0.4620	
X_2^2	9.25	1	9.25	12.64	0.0120	
X_3^2	0.17	1	0.17	0.24	0.6423	
$X_1X_2X_3$	2.34	1	2.34	3.20	0.1237	
$X_1^2X_2$	0.28	1	0.28	0.38	0.5617	
$X_1^2X_3$	0.04	1	0.04	0.05	0.8264	
$X_1X_2^2$	0.43	1	0.43	0.59	0.4710	
$X_1X_3^2$	0.00	0				
$X_2^2X_3$	0.00	0				
$X_2X_3^2$	0.00	0				
X_1^3	0.00	0				
X_2^3	0.00	0				
X_3^3	0.00	0				
Residual	4.39	6	0.73			
Lack of Fit	1.06	1	1.06	1.59	0.2626	Not significant
Pure Error	3.33	5	0.67			
Cor Total	211.39	19				

TABLE 3.9. Data for the cubic model fit.

R squared	0.9792
Adj R squared	0.9343
Pred. R squared	-0.1174
Adeq Prec	16.565
Standard deviation	0.86
Mean	16.56

3.1.5 Optimization of the dilute acid hydrolysis of sugarcane bagasse

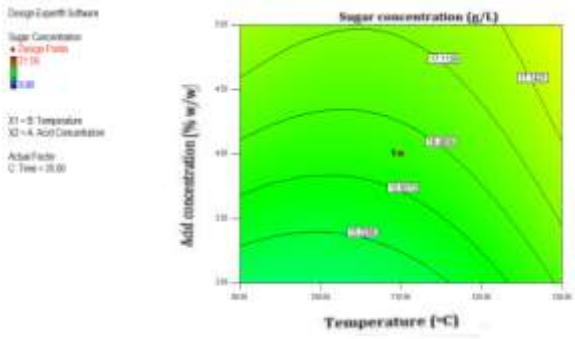

Fig. 3.4a. Contour plot showing the effect of acid concentration and temperature on the total reducing sugar concentration.

Kamalu C.I.O., Dairo M., Obijiaku J.C., Okere P.C., Egbufor U.C., and Chukwu M.M., "Statistical optimization of the total reducing sugars yield from the dilute acid hydrolysis of sugarcane bagasse," *International Research Journal of Advanced Engineering and Science*, Volume 3, Issue 3, pp. 127-134, 2018.

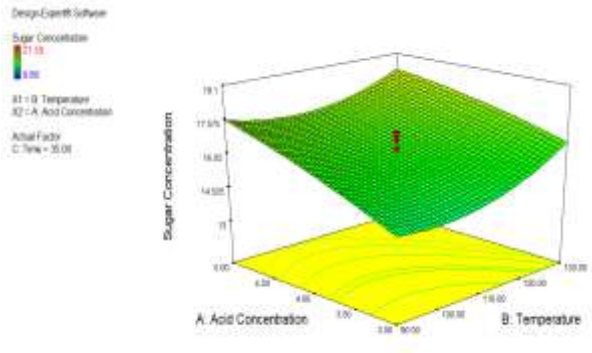

Fig. 3.4b. Response surface plot showing the effect of acid concentration and temperature on the total reducing sugar concentration.

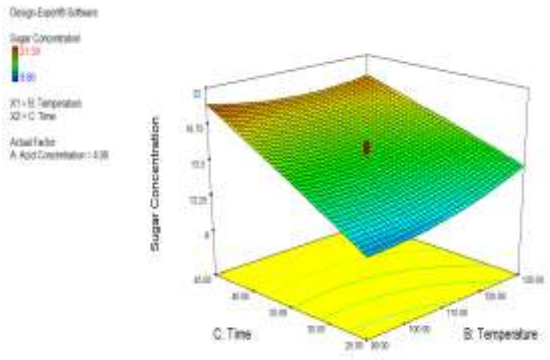

Fig. 3.6b. Response surface plot showing the effect of reaction time and hydrolysis temp. on the total reducing sugar concentration.

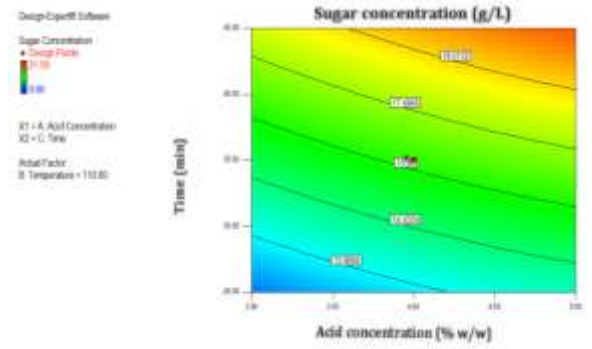

Fig. 3.5a. Contour plot showing the effect of reaction time and acid concentration on the total reducing sugar concentration.

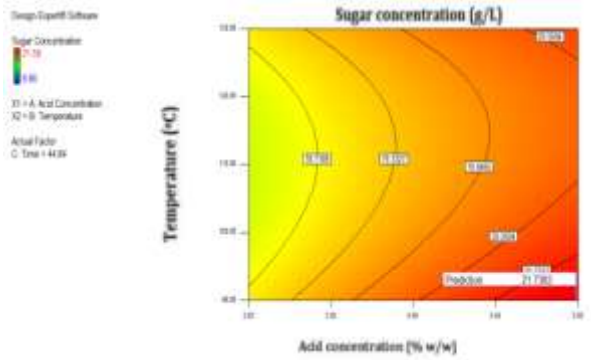

Fig. 3.7a. Optimum reaction time for the hydrolysis of sugarcane bagasse.

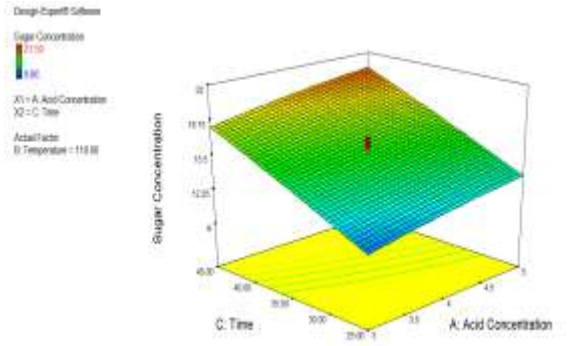

Fig. 3.5b. Response surface plot showing the effect of reaction time and acid concentration on the total reducing sugar concentration.

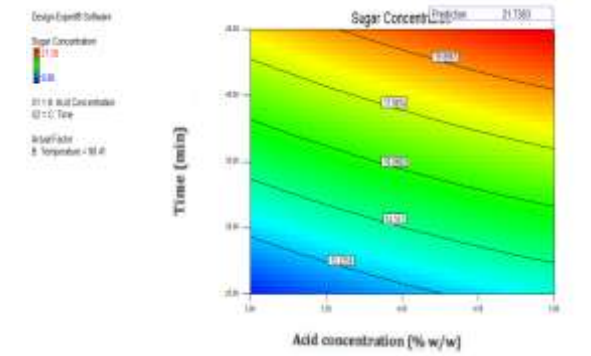

Fig. 3.7b. Optimum reaction temperature for the hydrolysis of sugarcane bagasse.

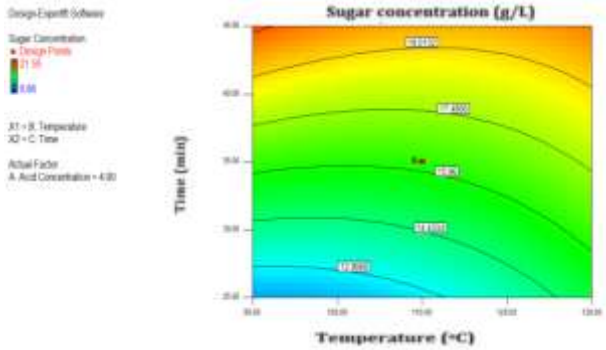

Fig. 3.6a. Contour plot showing the effect of reaction time and hydrolysis temperature on the total reducing sugar concentration.

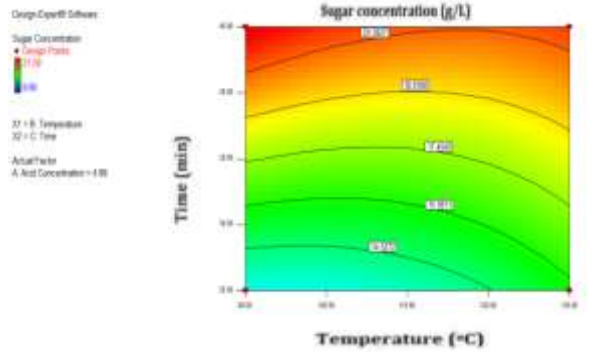

Fig. 3.7c. Optimum acid concentration for the hydrolysis of sugarcane bagasse.

Kamalu C.I.O., Dairo M., Obijiaku J.C., Okere P.C., Egbufor U.C., and Chukwu M.M., "Statistical optimization of the total reducing sugars yield from the dilute acid hydrolysis of sugarcane bagasse," *International Research Journal of Advanced Engineering and Science*, Volume 3, Issue 3, pp. 127-134, 2018.

TABLE 3.10. Optimum conditions for the hydrolysis of sugarcane bagasse.

Factor	Value
X_1 - Acid Concentration (%w/w)	4.99
X_2 - Temperature (°C)	90.41
X_3 - Time (min)	44.84

3.2 Discussion

3.2.1 Statistical analysis of the results obtained from the dilute acid hydrolysis of sugarcane bagasse

The statistical analysis of the results obtained for the dilute acid hydrolysis of sugarcane bagasse was done using Design expert software version® 7.0.0. The response variable (total reducing sugar concentration) was analyzed using a linear, two factor, quadratic and cubic models. The ANOVA was used to evaluate the statistical significance of the models and the experimental factors based on their p-values, model coefficients of determination and model lack of fit. Model graphs were also plotted to enable further statistical inference to be drawn.

Table 3.1 shows the experimental results for reducing sugar concentrations obtained for the dilute acid hydrolysis of sugarcane bagasse. It was observed that the highest reducing sugar concentrations were obtained at very high acid concentrations, very high reaction times and moderately high temperatures.

3.2.2 Linear model fit

The linear model fit for the experimental results obtained for the dilute acid hydrolysis study of the effects of acid concentration, hydrolysis temperature and hydrolysis time on the amount of reducing sugar produced equations 3.1 and 3.2 in terms of the coded and actual values.

The model coefficient of determination (R^2), adjusted R^2, predicted R^2 and adequate precision values obtained were 0.8792, 0.8566, 0.7910 and 20.606 respectively (Table 3.3). The R^2 value of 0.8792 indicated that the model could explain 87.92% of the variability of the response data around its mean. The adjusted R^2 and predicted R^2 values of 0.8566 and 0.7910 showed that both parameter values are in reasonable agreement since the values are within 0.2 of each other. An adequate precision value of 20.06 indicates an adequate signal and a desirable ratio (a ratio greater than 4), hence the model can be used to navigate the design space.

A standard deviation of 1.26 and mean of 16.56 were also obtained for the model. Table 3.2 shows the ANOVA table for the linear model fit. From table 3.2, Model F-value of 38.82 implies the model is significant. There is only a 0.01% chance that a "Model F-Value" this large could occur due to noise. A "Prob. > F" less than 0.05 indicates that the model terms are significant while values greater than 0.1000 indicates the model terms are insiginifcant. The linear model generated has a p-value less than 0.0001, hence X_1 and X_3 are significant model terms. The lack of fit F-value of 3.03 obtained implied that the lack of fit is not significant relative to the pure error. The lack of fit p-value of 0.1154 obtained implied that there is a 11.54% chance that a lack of fit F-value this large could occur due to noise.

Figure 3.1 shows the plot of the predicted response values versus the actual response values. From the plot, it is observed that the actual values are not so far apart from the predicted values.

3.2.3 Two factor interaction model fit

The two factor model fit for the experimental results obtained for the dilute acid hydrolysis study of the effects of acid concentration, hydrolysis temperature and hydrolysis time on the amount of reducing sugar produced equations 3.3.and 3.4 in terms of their actual and coded values.

From Table 3.5, the model coefficient of determination (R^2), adjusted R^2, predicted R^2 and adequate precision values obtained were 0.9141, 0.8745, 0.7469 and 17.476 respectively. The R^2 value of 0.9141 shows the model could explain 91.41% of the variability of the response data around its mean. Compared to the linear model fit, the R^2 value and the adjusted R^2 value increased while predicted R^2 and adequate precision values decreased. The adjusted R^2 and predicted R^2 values of 0.8745 and 0.7469 showed that the predicted and adjusted R^2 values are in reasonable agreement since their values are within 0.2 of each other. Even though the adequate precision value decreased (comparing it with the linear fit model), it is still greater than 4, hence indicating an adequate signal and a desirable signal to noise ratio, hence the model can be used to navigate the design space.

A standard deviation of 1.18 and mean of 16.56 were also obtained for the model. Table 3.4 shows the ANOVA table for the Two Factor Interaction Model Fit. From the ANOVA table, Model F-value of 23.06 implies the model is significant. X_1, X_3 and X_2X_3 are significant terms. The lack of fit F-value of 2.78 implies that the lack of fit is not significant relative to the pure error and there is a 13.72% chance that a "Lack of Fit F-value" this large could occur due to noise. Compared to the linear model fit, the "Lack of Fit F-value" obtained was lower, but its probability of occurrence was higher.

The two factor interaction model was much more complex than the linear model, as it expressed the interactions between the independent variables. This model is much more significant than the linear model, as the R^2 values obtained were closer to 1 than that obtained for the linear model. It shows the effects of the interactions of two independent variables on the response. From the ANOVA table, the interaction between X_2 and X_3 (i.e X_2X_3) had significant effect on the total reducing sugar (response) produced during the dilute acid hydrolysis while the interaction between the terms X_1, X_2 and X_1X_3 had no significant effect on the total reducing sugar produced during the dilute acid hydrolysis.

Figure 3.2 shows the plot of the predicted response values versus the actual response values. From the plot, it is observed that the actual values are closer to the predicted values, compared to the linear model.

3.2.4 Quadratic model fit

The quadratic fit for the experimental results obtained for the dilute acid hydrolysis study of the effects of acid concentration, hydrolysis temperature and hydrolysis time on the amount of reducing sugar produced equation 3.5 and 3.6 in terms of the coded values and actual values.

From table 3.7, model coefficient of determination (R^2), adjusted R^2, predicted R^2 and adequate precision values of

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0.9646, 0.9328, 0.8123 and 19.26 respectively, were obtained. The R^2 value of 0.9646 indicates the model could explain 96.46% of the variability of the response data around its mean, it also implies that the model is significant. Compared to the linear and the two factor model fits, the R^2 and adjusted R^2 values increased. The predicted R^2 value obtained from this model was greater than those of linear and two factor models. The adequate precision value obtained for this was higher that the value for the two factor model but lower than that of linear model. The adjusted R^2 and predicted R^2 values of 0.9328 and 0.8123 showed that the predicted and adjusted R^2 values are in reasonable agreement since their values are within 0.2 of each other. The adequate precision has a value greater than 4, hence indicating an adequate signal and a desirable signal to noise ratio, hence the model can be used to navigate the design space.

A standard deviation of 0.86 and a mean of 16.56 were obtained for this model. Table 3.6 shows the ANOVA table for the Quadratic Model Fit. From table 3.6, Model F-value of 30.29 implies the model is significant. There is only a 0.01% chance that a "Model F-Value" this large could occur due to noise. A "Prob. > F" less than 0.05 indicates that the model terms are significant while values greater than 0.1000 indicate the model terms are insiginifcant. X_1, X_2, X_3, X_2X_3 and X_2^2 are significant terms. The lack of fit F-value of 1.25 implies that the lack of fit is not significant relative to the pure error and that there is a 40.73% chance that a "Lack of Fit F-value" this large could occur due to noise. Compared to the linear and two factor model fits, the "Lack of Fit F-value" obtained was lower, but its probability of occurrence was higher. This indicates an inverse relationship between the "Lack of Fit F-value" and "Lack of Fit Prob. > F value".

The quadratic model is more significant than both the linear and the two factor model, as the R^2 values obtained were closer to 1 than those of linear and two factor models. It shows the effects of the interactions of two independent variables and the quadratic effects of each of the independent variables on the response. From the ANOVA table, the interaction between X_2 and X_3 (i.e X_2X_3) had significant effect on the total reducing sugar (response) produced during the dilute acid hydrolysis while the interactions between the terms X_1, X_2 and X_1X_3 had no significant effect on the response. Also the quadratic effect of the independent variable, X_2 $(i.e\ X_2^2)$ had a significant effect on the total reducing sugar produced.

Figure 3.3 shows the plot of the predicted response values versus the actual response values. From the plot, it is observed that the actual values are more closer to the predicted values, compared to those of linear and two factor interaction models.

3.2.5 Cubic model fit

The cubic model fit for the experimental results obtained for the dilute acid hydrolysis study of the effects of acid concentration, hydrolysis temperature and hydrolysis time on the amount of reducing sugar produced equation 3.7 in terms of the coded values. The Final Equation in terms of the actual values was not available because some of the model terms were aliased with one another.

Model coefficient of determination (R^2), adjusted R^2, predicted R^2 and adequate precision values of 0.9792, 0.9343, -0.1174 and 16.565 were obtained respectively (Table 3.9). The R^2 value of 0.9792 indicates the model could explain 97.92% of the response data variability, and implies the model is significant. The negative predicted R^2 value implied that the overall mean is a better predictor of the response than the current model. The model's R^2 and predicted R^2 values were the highest compared to the previous models studied. The adjusted R^2 and predicted R^2 values obtained were not in reasonable agreement since their values were not within 0.2 of each other. The adequate precision has a value greater than 4, hence indicating an adequate signal and a desirable signal to noise ratio, hence the model can be used to navigate the design space.

A standard deviation of 0.86 and a mean of 16.56 were obtained for this model. Table 3.8 shows the ANOVA table for the Cubic Model Fit. From the table, the Model F-value of 21.77 implies the model is significant. X_1, X_3, X_2X_3 and X_2^2 are significant terms. The lack of fit F-value of 1.59 implies that the lack of fit is not significant relative to the pure error, and there is a 26.26% chance that a "Lack of Fit F-value" this large could occur due to noise.

Also from the table, the model terms $X_1X_3^2$, $X_2^2X_3$, $X_2X_3^2$, X_1^3, X_2^3, and X_3^3 did not have any values because $X_1X_3^2$ aliased $X_1X_2^2$, $X_2^2X_3$ aliased $X_1^2X_3$, $X_2X_3^2$ aliased $X_1^2X_2$, X_1^3 aliased X_1 and $X_1X_2^2$, X_2^3 aliased X_2 and $X_1^2X_2$, X_3^3 aliased X_3 and $X_1^2X_3$. Alias takes place when the estimate of an effect includes the influence of one or more other effects. The implication of this is that the least square parameters for the aliased models will not be unique and the contour plots obtained will be misleading.

Although the cubic model studied the effects of interaction of the three independent variables, and their cubic effects, and gave the highest coefficient of determination (R^2) and the adjusted R^2 values, it cannot be suitable for this optimization problem because some of the model terms were aliased with one another, meaning that the least square parameters for the aliased models will not be unique and the contour plots obtained will be misleading.

3.2.6 Optimization of the dilute acid hydrolysis of sugarcane bagasse

The quadratic model was selected for the optimization because it had a very high coefficient of determination (R^2) and had no alias between its model terms. To obtain the optimum conditions for the hydrolysis of sugarcane bagasse that will yield the maximum total reducing sugar concentration, response surface plots were generated for the quadratic model. The 3-D plots and contour plots were obtained by keeping one variable constant at the centre while varying the other two variables. The response generated from these plots expressed the effects of acid concentration, hydrolysis temperature and hydrolysis time, on the total reducing sugar concentration.

Figures 3.4a and 3.4b show the contour plot and response surface plot for the effect of acid concentration and temperature, on the total sugar concentration.

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From figure 3.4b, the lines at the base of the surface plot indicate high interaction and proportionality between acid concentration and temperature because they are curves. The more curvature, the more interaction. The figures show that regardless of temperature, the total reducing sugar concentration increased from 13 to 17.575 as the acid concentration increases from 3 to 5%w/w. Maximum sugar recovery was obtained at an acid concentration of 5%w/w.

Figures 3.5a and 3.5b show the contour plot and response surface plot for the effect of reaction time and acid concentration, on the total sugar concentration. From figure 3.5b, the lines at the base of the surface plot indicate minimal interaction between reaction time and acid concentration and also inverse proportionality. Figures 3.5a and 3.5b show that as the acid concentration increased with the reaction time, the total reducing sugar also increased. Maximum sugar recovery was obtained at a time of 45 minutes.

Figures 3.6a and 3.6b show the contour plot and response surface plot for the effect of reaction time and temperature, on the total sugar concentration. From figure 3.6b, the lines at the base of the surface plot indicate interaction and proportionality between reaction time and temperature. Figures 3.6a and 3.6b show that for all the temperatures studied, the total reducing sugar concentration increased with reaction time.

To select the optimum conditions for the hydrolysis reaction using the quadratic model, the model was analyzed and the maximum response predicted as shown on figures 3.7a, 3.7b and 3.7c respectively.

According to figures 3.7a, 3.7b and 3.7c, the predicted maximum total sugar concentration was 21.7383g/L and it was achieved under the conditions stated in table 3.7.

The maximum total reducing sugar concentration obtained from the dilute acid hydrolysis of sugarcane bagasse at the optimum conditions in table 3.10 from a laboratory test was 21.61g/L which was quite close to the predicted value of 21.7383g/L. The result obtained from the laboratory test shows that there is an excellent agreement between the laboratory result and predicted result, hence confirming the validity of the model.

IV. CONCLUSION

In this work, the dilute acid hydrolysis of sugarcane bagasse was studied quantitatively using a 2^3 central composite design for response surface methodology. The following conclusions were drawn from the study:

- Sugarcane bagasse is a good feedstock for bioethanol production as its acid hydrolysis produced reducing sugars which can be fermented to produce bioethanol.
- Acid concentration, hydrolysis time and hydrolysis temperature all significantly influenced the dilute acid pre-treatment of the cellulosic feedstock, and the concentration of total reducing sugars produced.
- Reaction time has more impact on the total reducing sugar yield from sugarcane bagasse compared to acid concentration and reaction temperature.
- A validated quadratic model fully expressed the relation between the total reducing sugar concentration produced during the acid hydrolysis relative to the acid concentration, hydrolysis temperature and hydrolysis time.
- Based on the results obtained, sugarcane bagasse is a good source of total reducing sugar, and the substrate produced an optimum yield of total reducing sugars at the factor values of 5%w/w, 90°C and 45 minutes respectively.

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