Modeling and Statistical Optimization of Dilute Acid Hydrolysis of Eucalyptus Wood Chips using Response Surface Methodology.

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ABSTRACT

Dilute acid hydrolysis was applied for the pretreatment of eucalyptus wood chips to produce fermentable sugars. Chemical composition analysis of the wood chips revealed that the major components of the lignocellulosic material were cellulose, hemicellulose, and lignin (klason and acid soluble) accounting for 42.3, 15.2 and 24.1% respectively. A four variable central composite design for response surface methodology (RSM) was employed to evaluate the simultaneous effect of acid concentration, hydrolysis temperature, hydrolysis time, and liquid to solid ratio on the total reducing sugar concentration obtained during acid hydrolysis of eucalyptus wood chips. A validated quadratic statistical model was developed to relate the hydrolysis variables to the total sugar concentration. The optimal conditions of hydrolysis as obtained from RSM were as follows: acid concentration 0.59%w/w, hydrolysis temperature 130^{o}C, hydrolysis time 25 minutes and liquid to solid ratio 22.5 mL/g. Under these conditions, the maximum concentration of total reducing sugar was obtained to be 20.6 g/L. Validation of the model indicated no difference between predicted and observed values.

(Keywords: Eucalyptus, acid hydrolysis, lignocellulosic biomass, central composite design, optimization)

INTRODUCTION

As a result of the ever increasing prices of petroleum products, depletion of the world's crude oil reserves and the many environmental problems associated with the use of conventional petroleum based liquid fuels, the need to find sustainable and environmentally friendly alternatives has arisen (Amenaghawon et al., 2013; Hosseini and Shah, 2009; Mohan and Reddy, 2012). Bioethanol has been identified as a suitable and sustainable liquid fuel for road transportation. Bioconversion of lignocellulosic biomass to fermentable sugars and subsequent fermentation to bioethanol has been considered to be a cost effective process route for the production of bioethanol (Chen et al., 2009; Hahn-Hagerdal et al., 2006; Yang and Wyman, 2004).

Lignocellulosic biomass include naturally occurring materials such as waste paper and paper products, wood and wood wastes, agricultural and forest residues. These materials are an economical source of feedstock for bioethanol production as a result of their widespread availability, renewability and low cost (Agbro and Ogie, 2012; Canettieri et al., 2007; Shi et al., 2009). The capacity to economically and sustainably produce bioethanol from lignocellulosic biomass is limited by the ability to generate fermentable sugars from these materials in a cost effective manner (Lau and Dale, 2009). Lignocellulosic biomass compounds are complex materials consisting mainly of cellulose, hemicellulose, and lignin. The close association between these three components is a major factor that limits the utilization of these materials in the production of fermentable sugars (Emmel et al., 2003). In order to utilize these materials for bioethanol production, it is necessary to pretreat them to allow for the removal of lignin, hydrolysis of the hemicellulose and a reduction in the fraction of crystalline cellulose in order to improve the accessibility of the substrate to celluloytic enzymes (Amenaghawon et al., 2013; Ballesteros et al. 2008; Emmel et al., 2003; Hosseini and Shah, 2009).

Methods often adopted for the pretreatment of lignocellulosic biomass include physical (size

reduction), chemical (acid hydrolysis, alkaline pretreatment, ozone, steam, and solvent pretreatment) and biological (enzymatic hydrolysis) or some combination of the three (Alvira et al., 2010; Fang et al., 2010; Lau and Dale, 2009). Among these, dilute acid hydrolysis has been extensively studied and applied for the pretreatment of lignocellulosic biomass (Galbe and Zacchi, 2007; Martin et al., 2007; Mussato and Roberto, 2004; Saha et al., 2005; Shen et al., 2008; Zhao et al., 2008). It typically involves the use of dilute acids such as sulphuric acid, hydrochloric acid, phosphoric acid, and nitric acid to remove hemicellulose and enhance the digestibility of cellulose. It can be used as a pretreatment step to improve the performance of enzymatic hydrolysis or as a standalone hydrolysis process for the production of fermentable sugars.

When operated at high temperatures and low concentrations of acid, dilute acid hydrolysis can significantly improve cellulose hydrolysis and almost 100% hemicellulose removal can be achieved (Taherzadeh and Karimi, 2008). However, the implementation of high temperature acid hydrolysis is limited by the production of inhibitors such as furfural and hydroxy-methyl furfural, which are degradation products of pentose and hexose sugars respectively (Najafpour et al., 2007; Palmqvist and Hahn-Hagerdal, 2000).

Several factors such as hydrolysis time, lignocellulosic biomass particle size, hydrolysis temperature, acid concentration, etc. affect the yield of fermentable sugars during acid hydrolysis. Traditionally, optimizing these factors involves varying one factor at a time and keeping the others constant. This method is time consuming, cumbersome and often does not explain the effect of interaction between the various factors. Response surface methodology based on statistically designed experiments is a technique that has been found to be very useful in analysing the simultaneous effect of several factors on a process. It is employed for multiple regression analysis of quantitative data obtained from statistically designed experiments (Montgomery 2005).

In this work, the modelling and optimization of dilute acid hydrolysis of eucalyptus wood chips was studied. This feedstock is an agricultural residue typically left in the forest after the removal of the wood utilized for the production of paper. The objective of this study was to determine the optimum values of acid concentration, hydrolysis temperature, hydrolysis time and liquid-solid ratio and to determine their effect on the production of fermentable sugars. Central composite design for response surface methodology was adopted for optimizing the factors.

MATERIALS AND METHODS

Feedstock Preparation

Eucalyptus wood residue collected from a farm in Edo State, Southern Nigeria was used as the lignocellulosic biomass in this work. The residue was air dried, milled to 0.5 mm particles, homogenised in a single lot and stored under dry conditions prior to use. The moisture content of the wood samples was determined gravimetrically after drying at 105^{o}C for 20 minutes (Sluiter et al., 2008a).

Chemical Composition Analysis of Eucalyptus Wood Chips

The feedstock was analysed for structural carbohydrates, lignin, and ash content as well as extractives according to the National Renewable Energy Laboratory (NREL) standard analytical procedures. Structural carbohydrates were determined by quantitative acid hydrolysis of the extractive-free material through chromatographic quantification using a High Performance 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) (Sluiter et al., 2008b). Extractives were determined by ethanol extraction in a Soxhlet extraction apparatus (Sluiter et al., 2008c). Klason and acid-soluble lignin content were measured following the quantitative acid hydrolysis step (Sluiter et al., 2008b). The ash content was determined after combustion at 575^{o}C (Sluiter et al., 2008d).

Dilute Sulphuric Acid Hydrolysis

Dilute sulphuric acid hydrolysis of Eucalyptus wood chips was carried out in an autoclave. The operating conditions of the hydrolysis reaction were as follows: sulphuric acid concentration was varied between 0.1 to 0.75% (w/w), the

hydrolysis temperature was varied between 120 and 170°C, the hydrolysis time was varied between 10 and 30 minutes while the liquid to solid ratio was varied between 10 to 30 (mL/g). After acid hydrolysis, the solid residue was separated by centrifugation and the pH of the resulting supernatant was adjusted to 10 using 2N $Ca(OH)_2$ .The resulting precipitate was removed by centrifugation and the supernatant was adjusted to a pH of 6.5 using 10% H_2SO_4 (Silva et al., 1998).

Analytical Methods

The total reducing sugar content of the final hydrolysate was determined by the colorimetric method using glucose as standard (Miller, 1959). The reducing sugars were treated with 3,5-dinitro-salicylic acid (DNSA) 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 (PG Instruments model T70). 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: $KO_2CCH(OH)CH(OH)CO_2Na•4H2O$) 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 DNSA 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.

Experimental Design and Optimization by RSM

A four independent variable central composite design (CCD) for response surface methodology was used to develop a statistical model relating the response (total reducing sugar concentration) to the studied independent variables (acid concentration, hydrolysis temperature, hydrolysis time and liquid to solid ratio). The ranges of the variables varied over five levels are shown in Table 1. The CCD is a design that combines the vertices of the hypercube whose coordinates are given by a 2^n factorial design with star points (Box et al., 1978). The star points provide the estimation of curvature of the nonlinear response surface. The experimental design made up of 30 runs and the statistical model whose equation was determined by analysis of multiple regression was developed using Design Expert® 7.0.0 (Stat-ease, Inc. Minneapolis, USA). The 30 experimental runs were randomized to maximize the effects of unexplained variability in the observed responses due to extraneous factors. For statistical calculations, the independent variables were coded according to Equation (1).

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

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 centre point, and ΔX_i is the step change in X_i. The following generalized second order polynomial equation was used to correlate the independent variables and to estimate the response of the dependent variable as well as predicting the optimal point.

$$Y_i = b_o + \sum b_i X_j + \sum b_{ij} X_i X_j + \sum b_{ii} X_i^2 + e_i \quad \textbf{(2)}$$

where Y_i is the dependent variable or predicted response, X_i and X_j are the independent variables, b_o is offset term, b is the regression coefficient and e_i is the error term.

Table 1: Coded and Actual Levels of the Factors for Four Variable Central Composite Design.

Independent Variables	Sym.	Coded and Actual Levels				
		-2	-1	0	+1	+2
Temperature (°C)	X_1	120	132.5	145	157.5	170
Acid concentration (%w/w)	X_2	0.10	0.26	0.42	0.59	0.75
Time(min)	X_3	10	15	20	25	30
Liquid-Solid ratio (mL/g)	X_4	20	22.5	25	27.5	30

RESULTS AND DISCUSSION

Chemical Composition of Eucalyptus Wood Chips

The result of chemical composition analysis of eucalyptus wood chips is presented in Figure 1.

The major components of the lignocellulosic material were cellulose, hemicellulose and lignin (klason and acid soluble) accounting for 42.3, 15.2 and 24.1% respectively. Figure 1 also compares the results obtained in this study with the lignocellulosic biomass used for bioethanol production as reported by other researchers. The results show that the cellulose content of the feedstock used in this study was lower than that of eucalyptus wood chips and rice hull reported by Mendonca, (1997) and Martin et al. (2006) respectively. This observation can be attributed to the greater amount of leaves, barks and branches present in the residues. This also explains the relatively high ash content. Moreover, the sand present at the site of collection might have contributed to the high ash content (Canettieri et al., 2007). Generally, the composition of eucalyptus wood chips used in this study is comparable to that of other lignocellulosic feedstocks used for bioethanol production (Canettieri et al., 2007; Dien et al., 2006; Herrera et al., 2003; Lopez et al., 2010; Roberto et al., 2006; Sixta, 2006).

Statistical Analysis

The effect of hydrolysis temperature, acid concentration, hydrolysis time and liquid to solid ratio on the total reducing sugar concentration was evaluated. The results obtained from the 30 experimental runs carried out according to the central composite design are summarised in Table 2. A second order polynomial was fitted to the data presented in Table 2 using multiple linear regressions to determine the optimum conditions for the dilute acid hydrolysis of eucalyptus wood chips. The following second order polynomial was found to represent the relationship between the total reducing sugar concentration produced during acid hydrolysis and acid concentration, hydrolysis time, hydrolysis temperature and liquid to solid ratio.

$$Y = 184.36 + 0.124X_1 - 170.381X_2 + 0.584X_3 - 11.853X_4 + 0.159X_1X_2 - 0.000231X_1X_3 + 0.027X_1X_4 + 1.578X_2X_3 + 2.781X_2X_4 - 0.0359X_3X_4 - 0.00319X_1^2 + 59.467X_2^2 + 9.437X_3^2 + 0.139X_4^2 \quad \textbf{(3)}$$

The predicted response levels of total reducing sugar concentration using Equation (3) are also presented in Table 2. The fit of the statistical model for the total reducing sugar concentration was assessed by carrying out analysis of variance (ANOVA) and the results are presented in Tables 3 and 4.

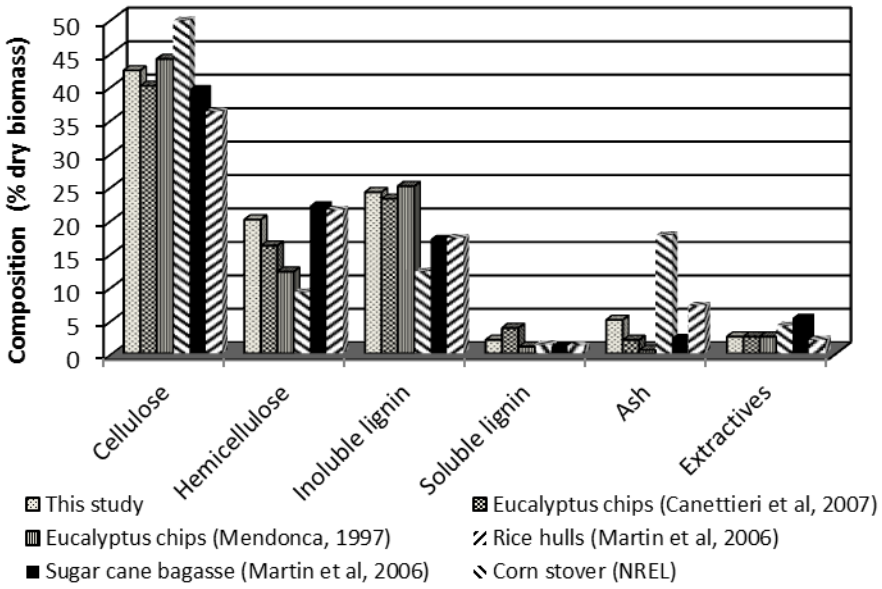

Figure 1: Chemical Composition of Eucalyptus Wood Chips Compared with Other Biomass Resources.

Table 2: Central Composite Design Matrix for the Optimization of Variables and the Response Values of Total Reducing Sugar Produced During Acid Hydrolysis.

Run No	Factors								Response	
	Coded levels				Actual values				Total sugar concentration	
	X_1	X_2	X_3	X_4	X_1	X_2	X_3	X_4	Observed	Predicted
1	-1	1	-1	1	132.5	0.59	15	27.5	12.38	13.17
2	0	2	0	0	145.0	0.75	20	25.0	9.23	10.18
3	1	-1	1	-1	157.5	0.26	25	22.5	7.84	8.08
4	1	-1	-1	1	157.5	0.26	15	27.5	9.99	9.40
5	-1	1	1	1	132.5	0.59	25	27.5	22.48	22.54
6	-1	-1	-1	-1	132.5	0.26	15	22.5	10.81	11.47
7	1	-1	-1	-1	157.5	0.26	15	22.5	18.76	19.60
8	0	0	0	0	145.0	0.42	20	25.0	17.72	17.84
9	1	1	-1	-1	157.5	0.59	15	22.5	7.89	8.37
10	0	-2	0	0	145.0	0.10	20	25.0	9.13	9.70
11	-1	1	1	-1	132.5	0.59	25	22.5	13.07	13.81
12	-1	-1	1	-1	132.5	0.26	25	22.5	5.93	6.46
13	1	-1	1	1	157.5	0.26	25	27.5	8.92	9.92
14	0	0	0	-2	145.0	0.42	20	20.0	12.81	11.17
15	0	0	0	0	145.0	0.42	20	25.0	17.87	17.52
16	0	0	0	0	145.0	0.42	20	25.0	19.47	18.09
17	0	0	0	0	145.0	0.42	20	25.0	10.94	10.56
18	0	0	0	0	145.0	0.42	20	25.0	7.76	7.14
19	-1	-1	1	1	132.5	0.26	25	27.5	17.65	15.72
20	1	1	-1	1	157.5	0.59	15	27.5	17.60	18.53
21	1	1	1	1	157.5	0.59	25	27.5	4.65	4.79
22	-1	1	-1	-1	132.5	0.59	15	22.5	19.93	18.78
23	0	0	2	0	145.0	0.42	30	25.0	19.63	18.10
24	0	0	0	2	145.0	0.42	20	30.0	10.01	11.54
25	2	0	0	0	170.0	0.42	20	25.0	8.91	8.85
26	0	0	0	0	145.0	0.42	20	25.0	8.52	9.85
27	-1	-1	-1	1	132.5	0.26	15	27.5	13.34	10.85
28	0	0	-2	0	145.0	0.42	10	25.0	11.86	10.85
29	1	1	1	-1	157.5	0.59	25	22.5	11.45	10.85
30	-2	0	0	0	120.0	0.42	20	25.0	11.01	10.85

Table 3: 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	529.96	14	38.65	4.42	0.0035
X_1–Temperature	17.37	1	17.37	2.03	0.0150
X_2 - Acid concentration	11.77	1	11.77	1.37	0.0255
X_3 – Time	294.22	1	294.22	34.34	<0.0001
X_4 – Liquid to solid ratio	46.13	1	46.13	5.38	0.0148
X_1X_2	1.67	1	1.67	0.19	0.0264
X_1X_3	3.34E-3	1	3.34E-3	3.89E-4	0.0185
X_1X_4	11.22	1	11.22	1.31	0.2704
X_2X_3	26.31	1	26.31	3.07	0.0011
X_2X_4	20.43	1	20.43	2.38	0.0134
X_3X_4	3.23	1	3.23	0.38	0.0487
X_1^2	6.84	1	6.84	0.79	0.3868
X_2^2	67.63	1	67.63	7.89	0.0132
X_3^2	1.53	1	1.53	0.18	0.6789
X_4^2	20.70	1	20.70	2.42	0.1410
Residual	128.53	15	8.57		
Lack of Fit	111.72	10	11.17	3.32	0.0986
Pure Error	16.81	5	3.36		
Cor Total	685.48	29			

Table 4: Statistical Information for ANOVA.

Source	Response
R-Squared	0.983
Adjusted R-Squared	0.953
Standard Deviation	1.084
C.V %	6.488
Adeq. Precision	7.636

The coefficient of determination (R^2) of the model was 0.983 as shown in Table 4. The closeness of this value to unity indicates that the model was able to adequately represent the actual relationship between the variables considered in this study. An R^2 value of 0.983 indicates that the model explains 98.3% of the variability in the response for the region studied while the remaining 1.7% was as a result of chance. The coefficient of variation (C.V.) obtained was 6.488%. The coefficient of variation indicates the degree of precision with which the runs were carried out. A low value of C.V. suggests a high reliability and reproducibility of the design (Mason et al, 1989; Montgomery, 2005). An Adequate precision value of 7.636 was obtained. Cao et al. (2009) reported that this parameter measures the signal to noise ratio and a value greater than 4 is generally desirable.

The results of ANOVA of the response model are presented in Table 3. Values of ''Prob. > F'' less than 0.05 indicate the model terms were significant. Values greater than 0.10 indicate the model terms were not significant. A model F-value of 4.42 and a very low probability value of 0.0035 imply that the response model was significant. Every model term was significant with the exception of X_1X_4, X_1^2, X_3^2, and X_4^2 but they were maintained in the final model to minimise the error determination. The "Lack of Fit" F-value of 3.32 implies that there was insignificant lack of fit. The "Lack of Fit" (Prob > F) value of 0.0986 implies that there is only 9.86% chance that the "Lack of Fit" F-value could occur due to noise.

Response Surface Optimization of Dilute Acid Hydrolysis of Eucalyptus Wood Chips

To determine the optimal levels of the variables that influence the acid hydrolysis of eucalyptus wood chips, response surface plots were generated according to Equation (3). The three-dimensional (3D) plots were generated by keeping two variables constant at the centre point and varying the others within the experimental range. The resulting response surfaces showed the effect of acid concentration, hydrolysis temperature, hydrolysis time and liquid to solid ratio on the total reducing sugar concentration.

Figure 2 shows the effect of acid concentration and hydrolysis temperature on the total sugar concentration. At low temperatures, the total reducing sugar concentration increased from 19.5 to 20.6 g/L with increase in acid concentration from 0.26 to 0.59 %w/w. The same trend was observed at high temperatures as the total sugar concentration also increased with increase in acid concentration. The trend observed may be as a result of the catalytic activity of the acid. Increasing the acid concentration during hydrolysis leads to a corresponding increase in the concentration of hydrogen ions which in turn increases the rate of the hydrolysis reaction and consequently the rate at which the glycosidic bonds are broken will increase resulting in a high conversion of hemicellulose fraction into fermentable sugars (Kumar et al., 2009; Mosier et al., 2002). Hu et al. (2010) investigated the acid hydrolysis of sugar maple wood extract at atmospheric pressure using dilute sulphuric acid. They observed that increasing the concentration of acid resulted in an increase in the concentration of fermentable sugars. This led them to conclude that the acid acted as a catalyst in the cleavage of the β (1–4) glycosidic linkages in the xylooligomers to yield xylose monomers. Lenihan et al. (2010) also reported that increasing the concentration of acid at mild temperatures resulted in an increase in the rate of the hydrolysis reaction.

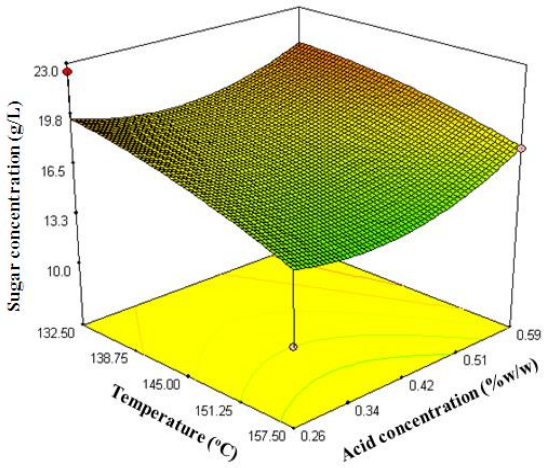

Figure 2: Response Surface Plot Showing the Effect of Acid Concentration and Hydrolysis Temperature on Total Reducing Sugar Concentration.

The effect of hydrolysis time and temperature on the total sugar concentration is presented in Figure 3. It was observed that there was a rapid, progressive and significant increase in the total sugar concentration with time in the course of the hydrolysis reaction. This trend was observed for the range of temperatures investigated. The maximum sugar recovery was obtained at a pretreatment time of 25 minutes. Romero et al. (2010) reported a similar trend for the dilute sulphuric acid hydrolysis of olive tree biomass.

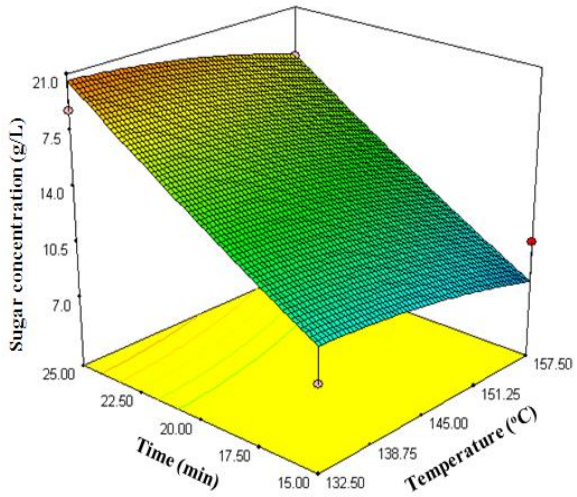

Figure 3: Response Surface Plot Showing the Effect of Hydrolysis Time and Temperature on Total Reducing Sugar Concentration.

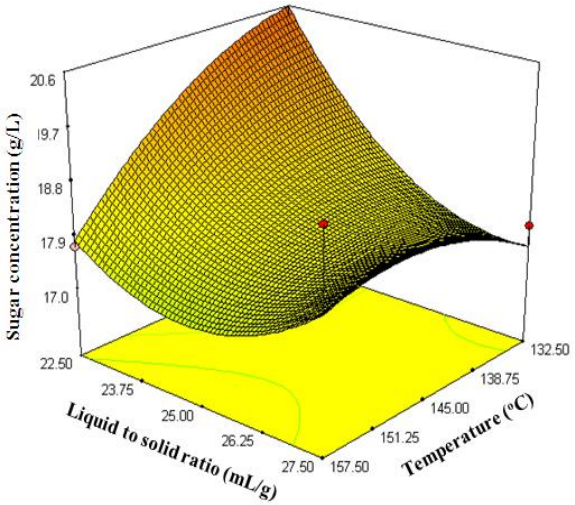

Figure 4: Response Surface Plot Showing the Effect of Liquid to Solid Ratio and Hydrolysis Temperature on Total Reducing Sugar Concentration.

Figure 4 shows the total reducing sugar concentration in the hydrolysate samples as a function of hydrolysis temperature and liquid to solid ratio. The total sugar concentration increased with decreasing liquid to solid ratio. The maximum sugar concentration was obtained at a liquid to solid ratio of 22.5 mL/g. Mansilla et al. (1998) evaluated the acid catalysed hydrolysis of rice hull for furfural production. They reported that a liquid to solid ratio of 25 mL/g resulted in the optimum production of furfural. Similar results were reported by Riera et al. (1991) for the acid catalysed hydrolysis of corn cobs.

It can be observed from Figures 2, 3, and 4 that there was a reduction in the total sugar concentration when the temperature was increased beyond 133^{o}C. This is probably as a result of the degradation of fermentable sugars to by-products such as furfural and hydroxyl methyl furfural (Najafpour et al., 2007; Palmqvist and Hahn-Hagerdal, 2000). Lenihan et al. (2010) reported diminishing reducing sugar yields beyond an optimum temperature of 135^{o}C as a result of the degradation of sugars to furfural and hydroxyl methyl furfural.

Figure 5 shows the effect of the interaction between hydrolysis time and acid concentration on the total reducing sugar concentration produced at a hydrolysis temperature of 132.5 ^{o}C and a liquid to solid ratio of 22.5 mL/g. The trend observed is similar to that presented in Figure 3.

For the entire range of acid concentration investigated, the concentration of total reducing sugars produced generally increased with increase in hydrolysis time. This suggests that the hemicellulose fraction of the lignocellulosic biomass was being broken down to produce fermentable sugars.

The maximum sugar concentration was obtained at a hydrolysis time of 25 minutes and an acid concentration of 0.59 %w/w. Lu et al. (2007) investigated the dilute acid hydrolysis of corn stover making use of sulphuric acid concentrations of 2, 4 and 6%w/w and temperatures of 80, 100 and 120 ^{o}C. They reported optimum hydrolysis conditions of 2%w/w acid concentration, 43 minutes hydrolysis time and an hydrolysis temperature of 120 ^{o}C.

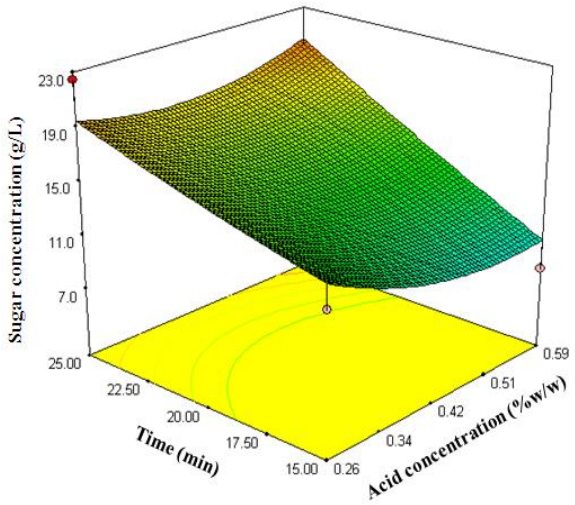

Figure 5: Response Surface Plot Showing the Effect of Hydrolysis Time and Acid Concentration on Total Reducing Sugar Concentration.

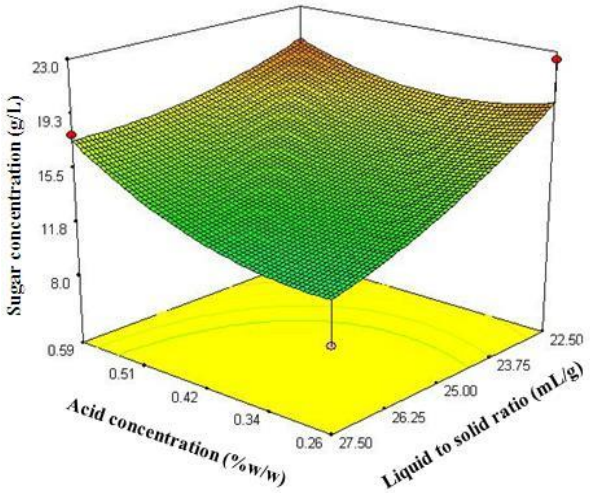

Figure 6: Response Surface Plot Showing the Effect of Liquid to Solid Ratio and Acid concentration on total reducing sugar concentration

The effect of the interaction between liquid to solid ratio and acid concentration is presented in Figure 6. The total sugar concentration produced during hydrolysis increased with an increase in acid concentration and a decrease in liquid to solid ratio.

With respect to the acid concentration, the trend observed for the total sugar concentration is similar to that presented in Figure 2. Also, it is observed that the maximum sugar concentration was obtained at low liquid to solid ratios just like the case presented in Figure 4. Similar observations were reported by Ferrer et al. (2013) who investigated the effect of sulphuric acid concentration, liquid to solid ratio, hydrolysis temperature and time on the hydrolysis of palm empty fruit bunches. They further observed that maximum values of the dependent variables (reducing sugar concentration) were obtained using low liquid to solid ratio ratios.

Figure 7 displays the effect of the interaction between hydrolysis time and liquid to solid ratio. Irrespective of the value of the liquid to solid ratio, the total sugar concentration increased with increase in hydrolysis time with the maximum sugar concentration resulting at a hydrolysis time of 25 minutes and a liquid to solid ratio of 22.5 mL/g.

To determine the optimum values of acid concentration, hydrolysis temperature, hydrolysis time and liquid to solid ratio that resulted in the maximum reducing sugar concentration, the statistical model of Equation (3) was optimized. The result of optimization by response surface methodology indicated a maximum reducing sugar concentration of 20.6 g/L. The optimum conditions of hydrolysis that resulted in this value are as follows: acid concentration 0.59%w/w, hydrolysis temperature 130^{o}C, hydrolysis time 25 minutes and liquid to solid ratio 22.5 mL/g.

Canettieri et al. (2007) obtained the following optimum conditions for the dilute acid hydrolysis of hemicellulose fraction of *Eucalyptus grandis*: sulphuric acid 0.65%, temperature 157 ^{o}C and residue/acid solution ratio of 1/8.6 and reaction time 20 minutes. Under these conditions, a total reducing sugar concentration of about 16.85 g/L was obtained. Lenihan et al. (2010) investigated the dilute acid hydrolysis of potato peels. They reported optimum hydrolysis conditions of 135^{o}C for temperature, 8 minutes for hydrolysis time, 10%w/w for acid concentration and a total sugar concentration of 55.2g per 100g biomass. Amenaghawon et al. (2013) obtained the following optimum conditions for the dilute sulphuric acid hydrolysis of corn stover: acid concentration; 1.68 % (w/w), hydrolysis temperature; 152^{0}C and hydrolysis time; 33 minutes. Under these conditions, the total reducing sugar concentration was obtained to be 21.14g/L.

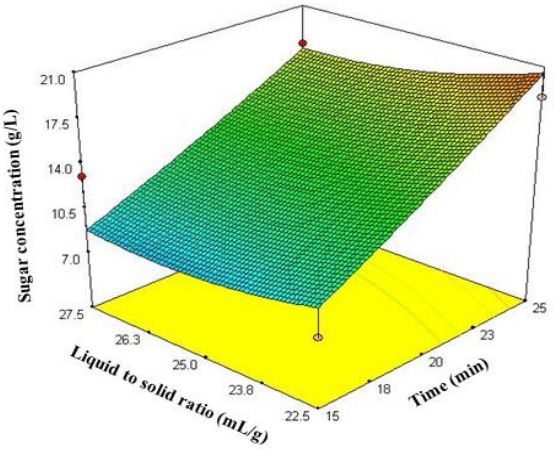

Figure 7: Response Surface Plot Showing the Effect of Liquid to Solid Ratio and Hydrolysis Time on Total Reducing Sugar Concentration.

To confirm the validity of the results predicted by the statistical model, experiments were performed in triplicates under the established optimal hydrolysis conditions. The results obtained showed that the total sugar concentration (20.04 g/L) obtained was close to the predicted value (20.6g/L). The excellent correlation between the predicted and measured values of these experiments indicates the validity of response model.

CONCLUSION

Eucalyptus wood chips were subjected to dilute sulphuric acid hydrolysis for the purpose of producing fermentable sugars. Results of chemical composition analysis indicate that cellulose, hemicellulose and lignin were the major constituents of the wood chips. The hydrolysis process was affected by the acid concentration, hydrolysis time, hydrolysis temperature and liquid to solid ratio. These variables were related to the total sugar concentration by a validated quadratic statistical model. The model was able to predict to a high level of confidence, the concentration of total reducing sugar produced during hydrolysis. The optimum conditions of hydrolysis are as follows: acid concentration 0.59%w/w, hydrolysis temperature 130^{o}C, hydrolysis time 25 minutes and liquid to solid ratio 22.5 mL/g. Under these conditions, the maximum concentration of total reducing sugar was obtained to be 20.6 g/L. This was close to the value (20.04 g/L) obtained from repeated experiments carried out under the optimized conditions.

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