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Ethanol Production from Cassava Root Sieviate

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Abstract- Two stage dilute acid hydrolysis was carried out on Cassava root sieviate (CRS). The proximate analysis revealed a high composition of cellulose and hemicellulose. The first stage hydrolysis gave a glucose yield of 20.4g/100 g dry weight. In the second stage hydrolysis, 10% v/v acid concentration gave an optimum glucose yield of 23.59g/ dry weight (d.w) at 140oC, 120 min. An optimum glucose yield of 24.38g/d.w was obtained at 170oC and 30 mins for 15% acid concentration. Activation energy (Ea) of 29.96 KJ and 40.99 KJ was obtained for k1 and k2 respectively, for 10% acid concentration while activation energy of 38.380KJ and 10.799KJ was obtained for k1 and k2 respectively, for 15% acid concentration.

Optimization of the second stage hydrolysis gave optimum glucose yield of 25g/d.w at acid concentration of 13.36%, 110.3oC and 44.1 mins. After 36hrs of fermentation, ethanol yield of 95mg.L was obtained. These work shows that CRS an agricultural waste can be hydrolysed and fermented into ethanol, a useful product.

Keywords- *Agricutural waste, Dilute-acid hydrolysis, Optimization, Fermentation*

I. INTRODUCTION

Nigeria is the largest producer of cassava in the world with about 45 million metric tons [1]. Cassava is also produced in other African countries like the Democratic Republic of the Congo, Ghana, Madagascar, Mozambique, Tanzania and Uganda etc.

Cassava is traditionally consumed in Nigeria as garri, fufu, edible starch, tapioca cakes. It is industrially used in the production of starch, flour, chips, pellets and ethanol.

The cultivation of cassava for ethanol production has been intensified and this according to [2] may affect the development of rural Africa both in a positive and negative way. The positive effect includes poverty reduction as sells made from cassava by local farmer provides money, increase in trade activities and provision of employment. But on the other hand, Cassava has been a staple food for 500 million people in the humid tropics; increase in its price caused by a rising cassava demand for ethanol production are likely to have effects on the food access of poor people which do not produce cassava themselves [2].

This competition informs the need to look for alternative feed stocks available for ethanol production. Wastes generated from cassava processing such as garri sieviate, cassava root sieviate, cassava whey and cassava peels can be used for ethanol production this way the competition between food and ethanol production from cassava can be avoided.

Agriculture waste biomass consists of cellulose, hemicellulose, lignin and other materials called extractive [3]. This cellulose can be hydrolyzed to produce glucose for human needs, which can further be used as substrates for fermentative production of useful products like alcohols [4], [5]. This conversion can be achieved by enzyme hydrolysis or acid hydrolysis.

Dilute acid hydrolysis can be carried out as one stage process or two-stage process. The main drawback of dilute acid hydrolysis process, particularly in one stage is degradation of sugars in hydrolysis reaction and formation of undesirable by-products [6]. Therefore it is suggested that the hydrolysis process be carried out in at least two stages, the first stage at relatively milder conditions during which the hemicellulose fraction is hydrolyzed and second stage can be carried out by enzymatic hydrolysis or dilute acid hydrolysis at higher temperatures during which the cellulose is hydrolyzed [7].

In this work, a two stage dilute acid hydrolysis was conducted using cassava root sieviate for ethanol production using phosphoric acid and the resulting hydrolysate was fermented using Saccharomyces cerevisiae. The second stage hydrolysis process was statistically optimized using the central composite design.

II. MATERIALS AND METHODS

A. Raw Materials and Characterization.

The Cassava Root Sieviate (CRS) was collected from local cassava processors in Ohaji Egbema in Owerri Imo state, Nigeria. The substrate was sun dried for 3 days and afterwards grinded and sieved using a 1 mm mesh size sieve. The sievates were stored in a cool dry place for subsequent use. The organic composition of the CRS was identified using Fourier transform Infra-Red (FTIR) Spectrophotometer (BUCK Scientific Infrared Spectrophotometer Model 530). The lignin, ash and moisture composition of cassava root sieviate were quantified using standard analytical procedure for proximate analysis by

AOAC [8] while the hemicellulose and cellulose composition were quantified using Crampton and Maryrand method [9].

B. First stage pre-hydrolysis

The batch hydrolysis studies were carried out using 4% (v/v) phosphoric acid concentration at 121^{o}C using 20% substrate to acid solution ratio. The substrates are first dissolved in acid and then placed in an autoclave at 121 ^{o}C for 30 minutes. At the end of this period the autoclaved substrate was placed inside an ice cold water to stop reaction; afterwards it was filtered and oven dried at 60 ^{o}C for 2hours. The solid residue was stored in a cool place for subsequent use.

C. Second stage hydrolysis

50g of the solid residue from pre-hydrolysis process was dissolved in a 500ml of acid solution. The solution was stirred with magnetic stirrer at 150rpm and temperature at 140 ^{o}C. Samples were drawn at intervals of 30minutes for 2.5 hours. Each sample was placed in an ice cold water to quench the reaction process, filtered and the hydrolysate obtained was analysed for its glucose concentration. The process was repeated for 150 ^{o}C, 160 ^{o}C, 170 ^{o}C and 180 ^{o}C. Acid concentration of 10% (v/v) and 15% (v/v) phosphoric acid was used.

D. Kinetic Model

Saeman model [10] was used to fit the experimental data. This model was designed for the hydrolysis of cellulose from fir using sulphuric acid, and it assumes the reaction proceeds according to equation 1.

$$\text{Cellulose+ wate} \xrightarrow{K_1} \text{glucose} \xrightarrow{K_2} \text{decomposition products} \quad (1)$$

Where k_1 is the rate of conversion of cellulose to glucose and k_2 is the rate of decomposition of glucose. Both have units of the reciprocal of time (min^{-1}). Both reactions were considered to be first order and irreversible. Assuming homogeneous first-order reactions with excess water, and solving equation 1 gives equation 2:

$$G = \left(\frac{k_1 C_0}{k_2 - k_1}\right) \quad (e^{-k_1 t} - e^{-k_2 t}) + G_0 e^{-k_2 t} \quad (2)$$

Where C_o the initial cellulose concentration, gL^{-1}; G_o is the initial glucose concentration gL^{-1}. Assuming that the initial glucose concentration to be approximately equal to 0, then Equation (2) becomes

$$G = \left(\frac{k_1 Co}{k_2 - k_1}\right) \quad (e^{-k_1 t} - e^{-k_2 t}) \quad (3)$$

It is assumed that all the cellulose hydrolyses to glucose; therefore the initial cellulose concentration, C_O (in gL^{-1})$_{is}$ equal to the potential concentration of glucose Gn_0 obtainable from the cellulose. Equation (3) becomes:

$$G = \left(\frac{k_1 Gno}{k_2 - k_1}\right) \quad (e^{-k_1 t} - e^{-k_2 t}) \quad (4)$$

Gn_0 can be determine analytically [11] by equation (5)

$$Gn_0 = \frac{FZ\rho}{WSR} \quad (5)$$

Where F is stoichiometric factor due to hydration of molecule during the hydrolysis, ρ is the density of hydrolysis, z is composition of the raw material for the polysaccharides and WSR is the water to solid ratio.

The temperature dependence of reaction rates can be described by Arrhenius equation, equation (6)

$$k_i = k_{i0} e^{\frac{-E_a}{RT}} \quad (6)$$

Where,

ki = Kinetic coefficient (*i* = 1 or 2) (min-1)

ki0 = Pre-exponential factor (*i* = 1 or 2) (min-1)

Ea = Activation Energy (kJ mol^{-}1)

R = Gas Constant, 8.314 (kJ mol^{-}1 K^{-}1)

T = Temperature (K)

Linearizing equation 6, gives equation (7)

$$Ink_i = \frac{-E_a}{R}\frac{1}{T} + Ink_{i0} \quad (7)$$

Plotting Ink versus $1/T$ allows for the calculation of the activation energy.

E. Statistical Optimization

To statistically determine the optimum conditions for peak glucose yield from the CRS, the impact of the following independent variables were investigated; the acid concentration (%v/v), temperature (^{0}C), and time (min). In order to examine the combined effect of these 3 factors on the % change in the responses and derive a model, a Central Composite Factorial Design of $2^3 = 8$, plus 6 centre points and (2 x 3 = 6) star points leading to a total of 20 experiments were analysed.

Experimental ranges of independent variables were selected based on experimental values obtained from the second stage hydrolysis process. Table 1 shows the experimental range of the independent variables at different levels for the acid hydrolysis of pre-hydrolysed Cassava Sieviate to glucose and Table 2 the experimental design matrix for the Central Composite Design.

TABLE I. EXPERIMENTAL RANGE OF THE INDEPENDENT VARIABLES AT THEIR DIFFERENT LEVELS FOR THE ACID HYDROLYSIS OF PRE-HYDROLYSED CASSAVA SIEVIATE TO GLUCOSE

Independent Variable	Coded Levels				
	-1.68	-1	0	+1	+1.68
	Actual Levels				
Acid Conc, (%V/V) X_1	6.64	8	10	12	13.36
Temp(^{0}C)-X_2	106.4	120	140	160	173.6
Time (Min) X_3	26.4	40	60	80	93.6

TABLE II. EXPERIMENTAL DESIGN MATRIX FOR THE CENTRAL COMPOSITE DESIGN FOR CASSAVA SIEVIATE

Exptl Run	Factors						Response
	Coded Values			Actual Values			Y_1 (Glucose Conc.)
	X_1	X_2	X_3	X_1	X_2	X_3	
1	-	-	-	8	120	40	
2	+	-	-	12	120	40	
3	-	+	-	8	160	40	
4	+	+	-	12	160	40	
5	-	-	+	8	120	80	
6	+	_	+	12	120	80	
7	-	+	+	8	160	80	
8	+	+	+	12	160	80	
9	-1.68	0	0	6.64	140	60	
10	+1.68	0	0	13.36	140	60	
11	0	-1.68	0	10	106.4	60	
12	0	+1.68	0	10	173.6	60	
13	0	0	-1.68	10	140	26.4	
14	0	0	+1.68	10	140	93.6	
15	0	0	0	10	140	60	
16	0	0	0	10	140	60	
17	0	0	0	10	140	60	
18	0	0	0	10	140	60	
19	0	0	0	10	140	60	
20	0	0	0	10	140	60	

F. Fermentation

The hydrolysate obtained from the first hydrolysis process was added together with the hydrolysate from the hydrolysis process carried out at the optimum conditions obtained from the statistical optimization. This was heated at 100^{o}C for 15mins and calcium hydroxide was added to adjust the pH to 4.0 then, centrifuged to harvest filtrate. The filtrate was heated at 60^{o}C and allowed to cool down. 10 g l^{-1} of dry cell weight of yeast (*Saccharomyces cerevisiae*) was added to the hydrolysate. The fermentation was carried out at room temperature under aerobic conditions for 36hrs.

G. Product Analysis

The glucose composition of the hydrolysate were analysed using the DNS (3, 5- Di nitro Salicylic Acid) method with glucose as standard [12]. Absorbance was measured with the UV spectrophotometer at 540nm. The ethanol yield were analysed using Buck 530 Gas chromatogram equipped with on-column, automatic injector, flame ionization detector, HP 88 capillary column.

III. RESULT AND DISCUSSION

A. FTIR Spectroscopy

According to spectra interpretation table by Coates [13], the spectrum of CRS shows that the polysaccharide is mainly made up of saturated aliphatic (alkane/alkyl) and hydroxyl compounds (figure 1). Aromatic ring (aryl), alkene, carbonyl compounds, thio substituted compounds and nitrogen multiple and cumulated double bond are also present in the cassava root sieviate lignocellulosic material. Table 3 shows the band positions, the band assignments and group frequencies of the CRS.

TABLE III. THE BAND POSITIONS, BAND ASSIGNMENTS AND GROUP FREQUENCIES OF THE CASSAVA ROOT SIEVIATE

Band Position Cm^{-1}	Band Assignment.	Group frequencies
685.4634	Aromatic C-H out-of-plane bend	Aromatic ring (aryl)
796.3154	Aromatic C-H 1,3-Disubstitution (meta)	Aromatic ring (aryl)
1031.603	Cyclohexane ring vibrations	Saturated aliphatic (alkane/alkyl)
1195.559	Methyne (CH-)Skeletal C-C vibrations	Saturated aliphatic (alkane/alkyl)
1310.916	Vinylidene C-H in-plane bend	Olefinic (alkene)
1470.365	Methylene C-H bend	Saturated aliphatic (alkane/alkyl)
1640.504	Alkenyl C=C stretch	Olefinic (alkene)
1896.282	Aromatic combination bands	Aromatic ring (aryl)
2035.575	Transition metal carbonyls	Carbonyl compound
2232.934	Aromatic cyanide	Nitrogen multiple and cumulated double bond
2520.342	Thiols (S-H stretch)	Thiols and thio-substituted
2730.757	Aldehyde	Carbonyl compound
2869.441	Methyl C-H sym. Stretch	Saturated aliphatic (alkane/alkyl)
3014.056	Medial, cis- or trans-C-H stretch	Olefinic (alkene)
3109.838	Aromatic C-H stretch	Aromatic ring (aryl)
3211.098	Normal ‘‘polymeric’’ OH stretch	Alcohol and hydroxyl compound
3356.236	Normal ‘‘polymeric’’ OH stretch	Alcohol and hydroxyl compound
3540.126	Internally bonded OH stretch	Alcohol and hydroxyl compound

B. Proximate analysis of CRS

The lignocellulosic fraction composition of CRS was obtained as: cellulose 58.9%, hemicellulose 30.88%, lignin 2.4%, ash 1.33% and moisture 6.67%. Agu et al [14] carried out proximate analysis on cassava grate waste (waste from garri production process), and obtained a cellulose content of 58 %.

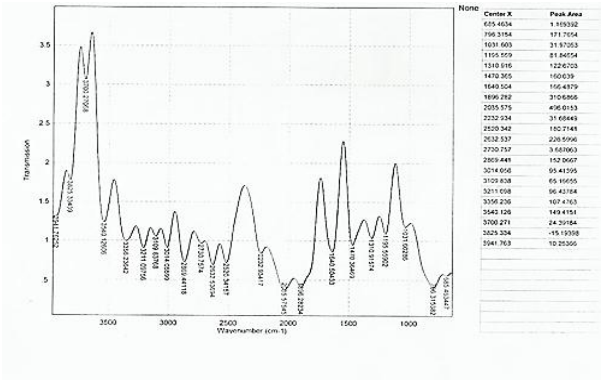

Figure 1. Spectrum of CRS

C. Pre- hydrolysis

After pre-hydrolysis , the proximate analysis of the lignocellulosic fractions shows that the hemicellulose contained in cassava root sieviate were reduced by 39.2% while the cellulose component was reduced by 10.909%. The result is shown in table 4. This means that at the operating conditions of this stage hemicellulose was not completely hydrolysed and cellulose composition was slightly affected. The glucose obtained from the pre-hydrolysis of CRS is 20.441 g/100g dry weight.

D. D. Effect of process parameters on hydrolysis of CRS

1) Effect of temperature and time on Glucose yield.

Figs. 2 and 3 shows the various yields of sugars produced at different temperatures when the acid concentration is 10% (v/v) of phosphoric acid. At 140^0C, the rate of glucose generation was slower and takes a longer period to reach its peak than at the other temperatures. The reaction at this temperature also experiences a lower level of glucose decomposition which was occurring simultaneously with glucose yield. This remains high for a longer period of time than at other temperatures. At this temperature, the maximum yield of 23.59 g of glucose/100 g of dry CRS was obtained after 120 minutes of reaction before the rate of the decomposition reaction surpasses the rate of glucose production.

At 150^0C, the rate of glucose generation reaches its peak much earlier at a time of 60 minutes with a yield of 21.28 g of glucose/100 g of dry CRS. The reaction became more aggressive as it reaches its peak earlier and also decomposes more rapidly resulting in a low yield of 9.92 g of glucose/100 g of dry CRS after 150 minutes.

At 160^0C, the trend obtained was similar to that observed at 150^0C with a glucose yield peak value of 19.25 g of glucose/100 g of dry CRS reached at 60 minutes of reaction time. The glucose produced was also rapidly decomposed that by the end of the reaction only 37% of it is left. A glucose yield peak value of 20.47 g of glucose/100 g of dry CRS was obtained at 60 minutes reaction time for the hydrolysis at 170^0C. The glucose generation at 180^0C had the highest reaction rate and reached its peak value of 22.25 g of glucose/100 g of dry CRS after 30 minutes of reaction. Since this is the first sampling time, it is possible that a higher glucose yield may be obtained before this time. Aggressive glucose decomposition also occurs at this temperature resulting in a loss of 81% of the glucose produced by the end of the reaction.

TABLE IV. THE LIGNOCELLULOSIC FRACTION COMPOSITION

Composition (%)	Proportion	
	Initial composition	After pre-hydrolysis
Moisture	6.67	2.00
Ash	1.33	4.49
Lignin	2.40	22.23
Hemicellulose	30.88	18.77
Cellulose	58.94	52.51

Fig. 4 and 5 shows the glucose yield produced from acid hydrolysis of CRS at different temperatures using 15% v/v Phosphoric acid. At 140^0C a peak value of 17.34 g of glucose/100 g of dry CRS was obtained at 60 minutes reaction time with 34% of this left by the end of the reaction. For temperatures of 150^0C to 180^0C, the peak values of the glucose yields were all obtained at 30 minutes reaction time. Since this is the first sampling time, it is possible that a higher glucose yield may be obtained before this time. At 150^0C a peak value of 19.18 g of glucose/100 g of dry CRS was obtained with 23% of this left by the end of the reaction. At 160^0C a peak value of 20.63 g of glucose/100 g of dry CRS was obtained with 12 % of this left by the end of the reaction. The glucose generation at 170^0C had the highest reaction rate with a peak value of 24.38 g of glucose/100 g of dry CRS but with only 5.6 % of this left by the end of the reaction. At 180^0C a peak value of 23.95 g of glucose/100 g of dry CRS was obtained at 30 minutes of reaction. The reaction also decomposed rapidly with only 4.5% of this glucose yield remaining by the end of the reaction. It was observed that the rate of decomposition increases with increasing temperature. These trends described above for the two acid concentrations, agrees with Lenihan et al [15] and Choi and Matthews [16].

2) Effect of acid concentration on Glucose yield

Figs 6-10 shows the effects of Phosphoric acid concentration on glucose yield for Cassava Root Sieviate hydrolysed at 140^0C to 180^0C. Within this temperature range, the glucose yield for the reaction using 15% Phosphoric acid was more than that obtained with 10% acid concentration at 30 minutes reaction time. The reaction at 15% however experiences rapid decomposition as the glucose yield became less than that obtained from the 10% concentration at reaction times of 60 to 150 minutes**.** This result agrees with that obtained by Lenihan et al [15], in which the highest yield was obtained at the highest acid concentration of 10% (w/w) of phosphoric after a time of 8mins while 7.5 %(w/w) reached its optimum after 15min. This shows that with higher acid concentration, optimum glucose yield is achieved much earlier.

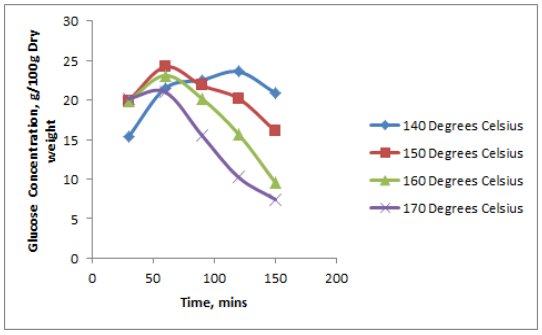

Figure 2. Effect of temperature and time on glucose yield from CRS hydrolysed at 140^{0}C - 170^{0}C with 10% v/v Phosphoric acid

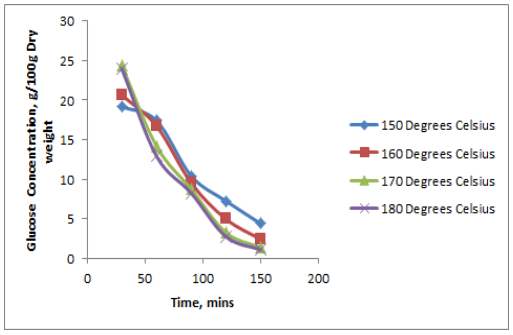

Figure 5. Effect of temperature and time on glucose yield from CRS hydrolysed at 150^{0}C - 180^{0}C with 15% v/v Phosphoric acid

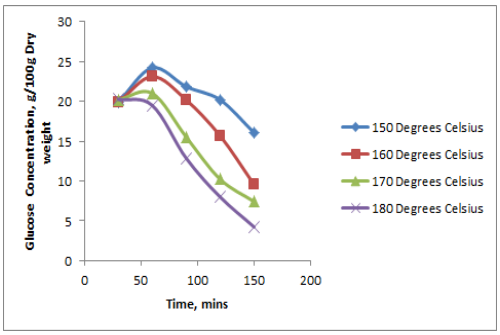

Figure 3. Effect of temperature and time on glucose yield from CRS hydrolysed at 150^{0}C - 180^{0}C with 10% v/v Phosphoric acid

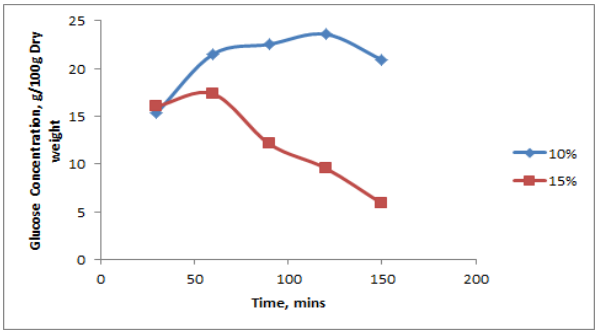

Figure 6. Effect of acid concentration on glucose yield from CRS hydrolysed at 140^{0}C with Phosphoric acid

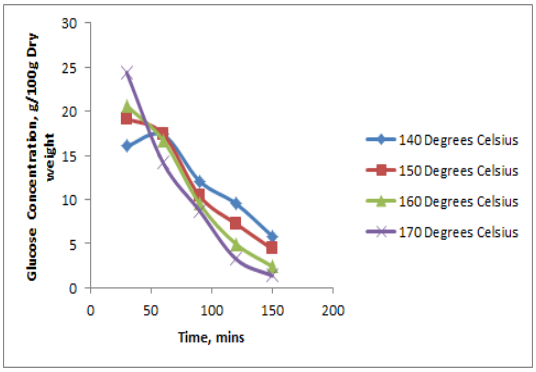

Figure 4. Effect of temperature and time on glucose yield from CRS hydrolysed at 140^{0}C - 170^{0}C with 15% v/v Phosphoric acid

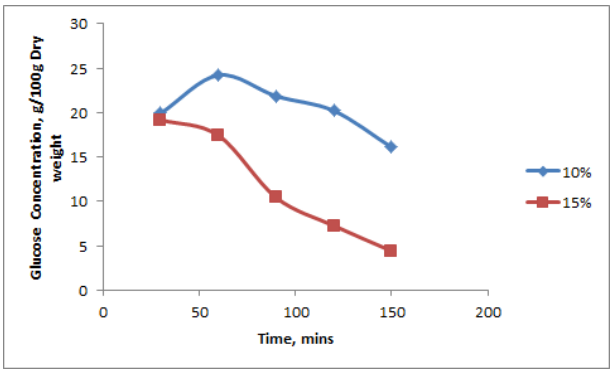

Figure 7. Effect of acid concentration on glucose yield from CRS hydrolysed at 150^{0}C with Phosphoric acid

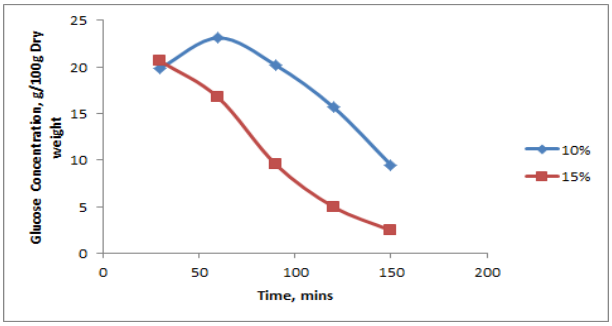

Figure 8. Effect of acid concentration on glucose yield from CRS hydrolysed at 160^{0}C with Phosphoric acid.

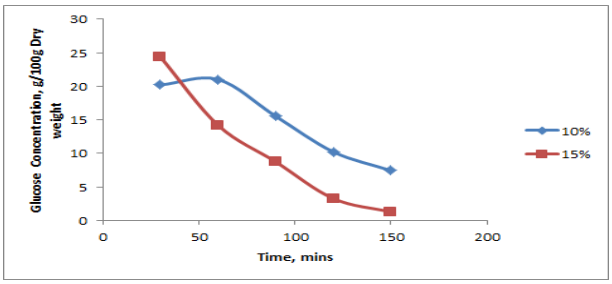

Figure 9. Effect of acid concentration on glucose yield from CRS hydrolysed at 170^{0}C with Phosphoric acid

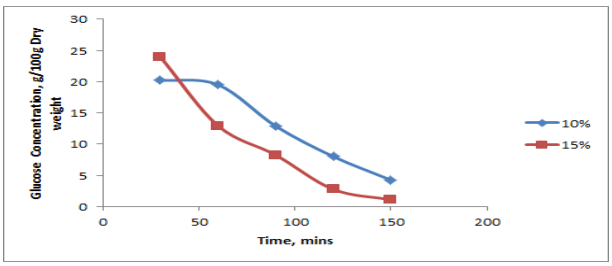

Figure 10. Effect of acid concentration on glucose yield from CRS hydrolysed at 180^{0}C with Phosphoric acid

E. *Kinetic study*

Equation (5) gives the potential glucose concentration

$$Gn_0 = \frac{FZ\rho}{WSR} \quad (5)$$

Where F is stoichiometric factor due to hydration of molecule during the hydrolysis and is given by 180/162 for hexoses such as glucose [11], ρ is the density of hydrolysate (10 g/l), z is composition of the raw material for the polysaccharides (52.51g/100g of raw material dry basis) and WSR is the water to solid ratio used (10.636g/g). From equation (5), the potential composition of glucose in the liquors was obtained as 56.2g glucose/l.

The kinetic parameters of Saeman model for 10% and 15% phosphoric acid concentration are shown in Table 5 and 6. The values show that the Saeman model can be used to describe the experimental data obtained; this is based on the R-square values which are close to one. Figures 11 and 12 shows the relationship between the natural log values of the rate constants and the inverse of temperature according to the linearized Arrhenius equation for glucose formation from Cassava Root Sieviate hydrolysed at 140^{0}C - 180^{0}C with 10% v/v and 15% Phosphoric acid respectively. The high values of R^2 as seen in the figures 11 and 12 indicate that the model follows the Arrhenius equation. Activation energy (Ea) of 29.96 KJ and 40.99 KJ was obtained for k_1 and k_2 respectively for 10% acid concentration. This shows that the rate of glucose formation was higher than the rate of degradation reaction. The activation energy of 38.380KJ and 10.799KJ was obtained for k_1 and k_2 respectively for 15% acid hydrolysis. This values shows that the rate of formation of degradation products is higher than the rate of glucose formation. These values shows close range agreement with values obtained for glucose formation by Ajani et al [17].

F. *Statistical optimization of acid hydrolysis process*

The experimental results obtained from the phosphoric acid catalysed hydrolysis are shown in table 7, while the results of the statistical analysis of this data done with Design Expert 9.0.1 (Statease inc., USA) are given in tables 8 and 9, and figures 13-16.

TABLE V. KINETIC PARAMETERS OF SAEMAN MODEL FOR 10% V/V H_3PO_4 HYDROLYSIS OF CRS

Temperature (˚C)	K_1	K_2	R^2
140	0.01203	0.00914	0.95540
150	0.01804	0.01266	0.92400
160	0.02117	0.01662	0.09781
170	0.02364	0.02140	0.99450
180	0.02656	0.02527	0.99690

TABLE VI. KINETIC PARAMETERS OF SAEMAN MODEL FOR 15% V/V H_3PO_4 HYDROLYSIS OF CRS

Temperature (˚C)	K_1	K_2	R^2
140	0.01869	0.02578	0.98300
150	0.02540	0.02840	0.98960
160	0.03125	0.03080	0.99200
170	0.04517	0.03209	0.99080
180	0.04643	0.03395	0.98580

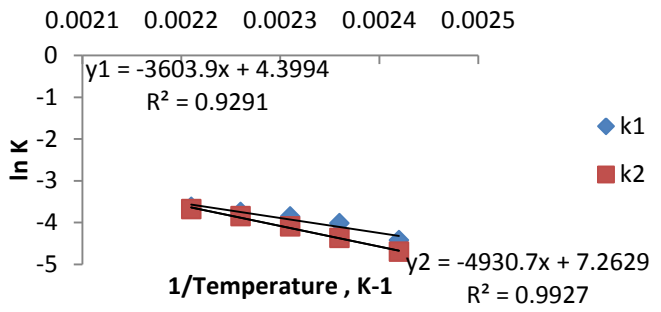

Figure 11. Arrhenius plot for glucose formation from CRS hydrolyzed at 140^0C - 180^0C with 10% v/v H_3PO_4

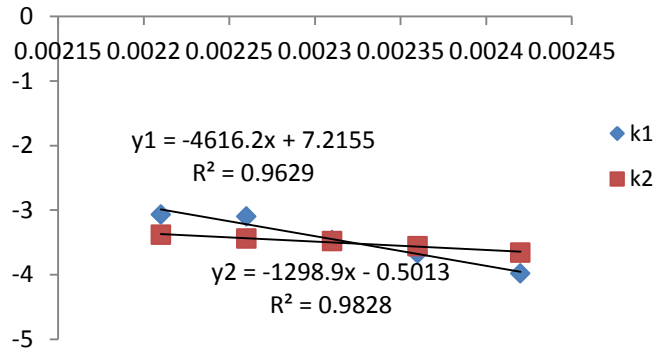

Figure 12. Arrhenius plot for glucose formation from CRS hydrolysed at 140^0C - 180^0C with 15% v/v H_3PO_4

An analysis of the experimental results showed that the data is best represented by a cubic model fit. A cubic model fit of the experimental results for the effects of acid concentration (X_1), temperature (X_2) and time (X_3) on glucose yield (GY) gave the following final equation in terms of coded factors:

$$GY = 21.5 + 0.34X_1 - 1.03X_2 + 3.04X_3 + 0.21X_1X_2 + 1.2X_1X_3 + 0.84X_2X_3 - 0.081{X_1}^2 - 0.18{X_2}^2 - 1.34{X_3}^2 - 0.34X_1X_2X_3 + 2.24{X_1}^2X_2 - 3.93{X_1}^2X_3 + 2.07X_1{X_2}^2 \quad (6)$$

The model Coefficient of Determination (R^2) and Adjusted R^2 values are 0.9968 and 0.9899 respectively.

Equation 6 gives the cubic model fit of the experimental results for the effects phosphoric acid concentration, temperature and time on the glucose production from CRS. The equation is in terms of the coded factors and can be used to make predictions about the response for given levels of each factor. The equation is also useful for determining the relative impact of the factors by comparing the factor coefficients. From equation 6, it can be inferred that within the factor range investigated, the reaction time has a greater impact on glucose yield than reaction temperature and acid concentration. The ANOVA result for the model is shown in table 8. From the table, the model is significant with a p-value < 0.0001 and also has an insignificant "lack of fit" which is desirable (p=0.7410). The significant model terms as indicated by their p-values are X_1, X_2, X_3, X_1X_3, X_2X_3, ${X_3}^2$, $X_1X_2X_3$, ${X_1}^2X_2$, ${X_1}^2X_3$, and $X_1{X_2}^2$ removing the insignificant terms from the model, equation 6 becomes:

$$GY = 21.5 + 0.34X_1 - 1.03X_2 + 3.04X_3 + 1.2X_1X_3 + 0.84X_2X_3 - 1.34{X_3}^2 - 0.34X_1X_2X_3 + 2.24{X_1}^2X_2 - 3.93{X_1}^2X_3 + 2.07X_1{X_2}^2 \quad (7)$$

The model coefficient of determination (R^2) and adjusted R^2 values are 0.9968 and 0.9899 respectively. These values are close to 1 and show that the model is a good representation of the experimental data. This is also confirmed by the ANOVA result which stated that the model has an insignificant "lack of fit". The normality plot of the predicted versus actual response shown in figure 13 indicates that the predicted and actual response have fairly good agreement. Figure 14 gives the Response surface and contour plots for glucose yield from phosphoric acid hydrolysis of CRS showing interaction of acid concentration and temperature at reaction time of 60 minutes. From this plot, an increase in glucose yield is observed as both the acid concentration and the temperature moves from its -1 to +1 value. The response surface and contour plot for glucose yield showing interaction of acid concentration and time at a reaction temperature of 140^0C shown in figure 15. The glucose yield increase slightly and then decreases with time from -1 to +1, and it also first decreases and then increases slightly with acid concentration. From the response surface and contour plots for glucose yield showing interaction of reaction temperature and time at an acid concentration of 10% v/v as shown in figure 16, it is seen that change in temperature and time from their -1 to +1 value results in a decrease in glucose yield. An increase in glucose yield is observed with time from its -1 to +1 value. Based on the cubic model, the maximum glucose concentration as given by the software obtainable from CRS hydrolyzed with Phosphoric acid is 25.012 g/ 100 g dry weight and this was obtained at an acid concentration of 13.36 % v/v, temperature of 110.3 ^{0}C, and reaction time of 44.1 minutes as shown in table 9.

TABLE VII. GLUCOSE YIELD FROM EXPERIMENTS RUN ACCORDING TO THE FACTORIAL DESIGN FOR PHOSPHORIC ACID HYDROLYSIS OF CRS

Exptl Run	Factors						Response
	CODED VALUES			ACTUAL VALUES			Y_1 (Glucose Conc.)
	X_1	X_2	X_3	X_1	X_2	X_3	
1	-	-	-	8	120	40	19.750
2	+	-	-	12	120	40	12.050
3	-	+	-	8	160	40	19.372
4	+	+	-	12	160	40	22.890
5	-	-	+	8	120	80	13.197
6	+	_	+	12	120	80	20.664
7	-	+	+	8	160	80	17.542
8	+	+	+	12	160	80	24.448
9	-1.68	0	0	6.64	140	60	20.739
10	+1.68	0	0	13.36	140	60	21.870
11	0	-1.68	0	10	106.4	60	22.763
12	0	+1.68	0	10	173.6	60	19.293
13	0	0	-1.68	10	140	26.4	12.623
14	0	0	+1.68	10	140	93.6	22.843
15	0	0	0	10	140	60	21.460
16	0	0	0	10	140	60	21.532
17	0	0	0	10	140	60	21.521
18	0	0	0	10	140	60	20.970
19	0	0	0	10	140	60	21.991
20	0	0	0	10	140	60	21.502

TABLE VIII. ANOVA FOR RESPONSE SURFACE CUBIC MODEL OF GLUCOSE YIELD FROM CRS HYDROLYZED WITH H_3PO_4

Source	Sum of Squares	df	Mean Square	F-Value	P-Value Prob > F	Inference
Model	167.67	13	12.90	144.17	<0.0001	significant
X_1	0.64	1	0.64	7.15	0.0368	
X_2	6.02	1	6.02	67.30	0.0002	
X_3	52.22	1	52.22	583.77	<0.0001	
$X_1 X_2$	0.36	1	0.36	4.02	0.0917	
$X_1 X_3$	11.50	1	11.50	128.53	<0.0001	
$X_2 X_3$	5.63	1	5.63	62.93	0.0002	
X_1^2	0.095	1	0.095	1.06	0.3435	
X_2^2	0.46	1	0.46	5.15	0.0638	
X_3^2	26.02	1	26.02	290.86	<0.0001	
$X_1 X_2 X_3$	0.94	1	0.94	10.48	0.0177	
$X_1^2 X_2$	16.55	1	16.55	185.03	<0.0001	
$X_1^2 X_3$	51.30	1	51.30	573.50	<0.0001	
$X_1 X_2^2$	14.17	1	14.17	158.43	<0.0001	
Residual	0.54	6	0.089			
Lack of Fit	0.013	1	0.013	0.12	0.7410	not significant
Pure Error	0.52	5	0.10			
Cor Total	168.21	19				

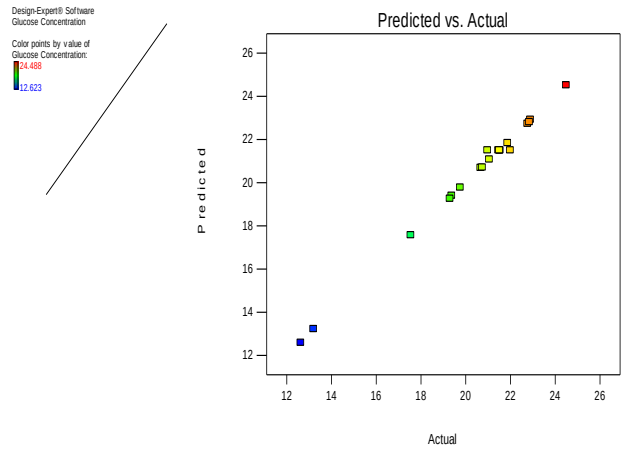

Figure 13. Normal probability plot of predicted versus actual response for glucose yield from cassava root sieviate hydrolysed with Phosphoric acid

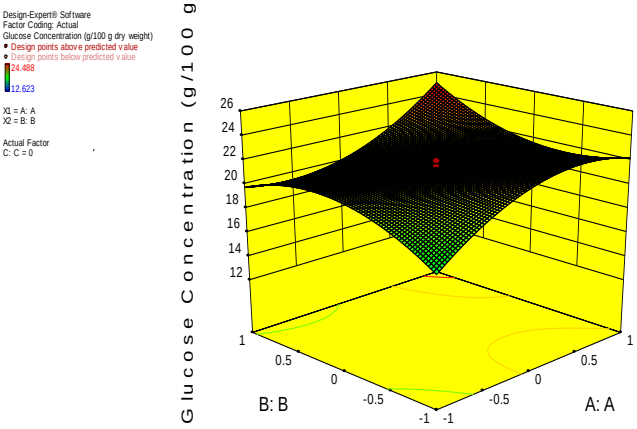

Figure 14. Response surface and contour plots for glucose yield showing interaction of acid concentration and temperature at reaction time of 60 minutes

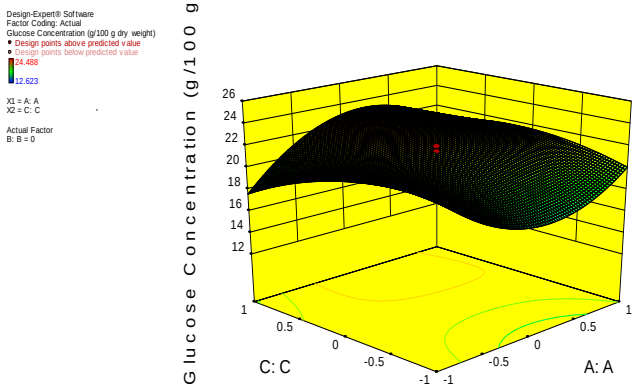

Figure 15. Response surface and contour plots for glucose yield showing interaction of acid concentration and time at reaction temperature of 140^0C

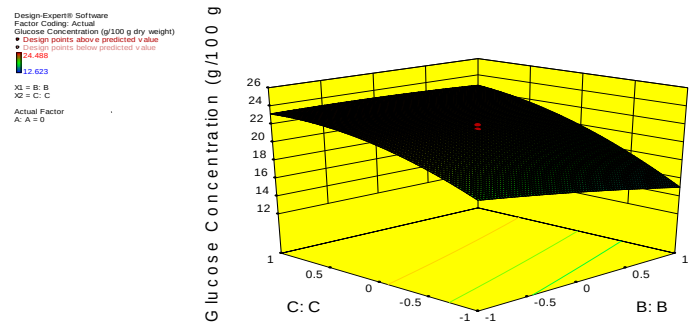

Figure 16. Response surface and contour plots for glucose yield showing interaction of reaction temperature and time at an acid concentration of 10% v/v

TABLE IX. CONDITIONS FOR OBTAINING THE MAXIMUM GLUCOSE FROM CRS HYDROLYSED WITH PHOSPHORIC ACID

Factor	Coded Value	Real value
X_1, Acid Concentration (%v/v)	1.68	13.36
X_2, Temperature, ^{0}C	-1.485	110.3
X_3, Time, mins	-0.795	44.1

G. Fermentation

The fermentation of the hydrolysate gave an ethanol yield of 95mg/L. See figure 17 for gas chromatogram result. 10.6g/cm^3 of ethanol was obtained from cassava peels fermented with saccharomyces cerevisiae by Oyeleke et al [18]. Agu et al [14] obtained 3.5 (v/v %) after yeast fermentation of cassava grate waste.

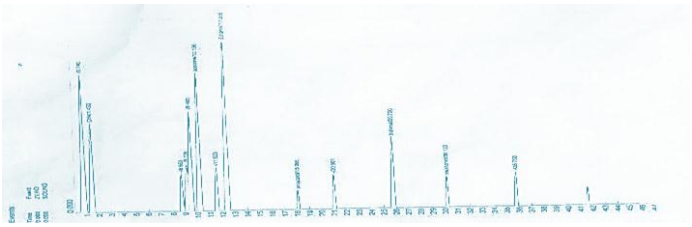

Figure 17. Gas chromatograph analysis result

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