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STATISTICAL MODELLING AND OPTIMIZATION OF THE CONCENTRATION OF BIO-ETHANOL FROM WASTE PEELS OF *MANIHOTESCULENTA CRANTZ* USING RESPONSE SURFACE METHODOLOGY

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ABSTRACT

In this study, the optimization of the concentration of bio-ethanol from waste peels of Manihotesculenta Crantz (cassava) was carried out. The acid hydrolysis process was optimized using Response Surface Methodology (RSM). Central Composite Design (CCD) was employed to study the effect of hydrolysis temperature, pH, and acid concentration and also, for optimization of the bio-ethanol concentration from the peels of Manihotesculenta C. The anaerobic fermentation process was carried at room temperature (≈30^{0}C) for four days. Prior to this, the fermentation media was prepared by culturing yeast to ferment the sugar rich liquid. A quadratic statistical model was developed for the acid hydrolysis process and then validated. The model gave a significant p-value < 0.05 and also showed an insignificant lack of fit. The model predicted that at optimum acid concentration of 1.2 % v/v, temperature of 131.8 ^{0}C and pH of 5.3, a maximum bio-ethanol concentration of 24.48 g/L should be obtained. The prediction of the model was validated by a triplicate set of experiments carried out at the predicted optimum parameters which yielded an average value of 24.41 g/L for the bio-ethanol concentration. The results obtained indicate the viability of Manihotesculenta Crantz peels as a bio-fuel feedstock and corroborates the efficiency of CCD in determining the optimum values of the process parameters for the acid hydrolysis step of the bio-ethanol production process.

***Keywords**: Hydrolysis, bio-ethanol, optimization, Response Surface Methodology, model*

1.0. INTRODUCTION

Bio-ethanol is the most widely used bio-fuel worldwide, partially able to replace fossil fuels, reducing the environmental impact of greenhouse gas emissions Balat (2011); Cunha *et al*. (2018). There has been growing research on viable feedstocks for use in bio-ethanol production. First generation bio-ethanol is produced mainly from C_6 sugars such as sugar beets, cereals, and sugarcane while second-generation bio-ethanol is produced from renewable lignocellulosic biomass and industrial by-products or residues Ho *et al.* (2014); Naik *et al.* (2010). The second generation production of ethanol derived from lignocellulosic materials is being tested in pilot plants Taherzadeh & Karimi, (2007a); Taherzadeh & Karimi, (2007b); Taherzadeh & Karimi, (2008). The steps involved in the production of bio-ethanol include; pre-treatment, hydrolysis, fermentation and finally distillation process. Each of this process is specific that is, it has its own functionality and rationale. The hydrolysis process is important as it involves the breaking down of polysaccharide compounds such as lignin, hemicelluloses, cellulose to simple fermentable sugars which are further fermented using yeast (*Saccharomyces cerevisae*) to produce ethanol and carbon dioxide which is further distilled to separate the alcohol water mixture. The choice of feedstock for production of ethanol is important because it's not merely a matter of which one has the greatest yield, but also a question of economics. A model is simply a representation usually in terms of mathematical equations of a process so as to predict the behaviour and interactions of the process variables under varying conditions. Modelling involves generating a representation defined by a set of mathematical equations that conforms closely in reality to what actually takes place Levenspiel (1998); Otoikhian *et al.* (2017). As an important subject in the statistical design of experiments, the Response Surface Methodology (RSM) is a collection of mathematical and statistical techniques useful for the modeling and analysis of problems in which a response of interest is influenced by several variables and the objective is to optimize this response (Montgomery, 2013).In this work, bio-ethanol from waste peels of *Manihot esculenta Crantz* is produced and the Response Surface Methodology feature of the Design Expert version 7.0 (Statease inc.,

Minneapolis) is used to perform statistical modelling and optimization of the process parameters viz; temperature, pH and acid concentration for the hydrolysis step of the process. This work will serve to contribute to the knowledge on production of bio-ethanol from Lignocellulosic biomass in general and waste peels of *Manihot esculenta Crantz* in particular.

2.0. MATERIALS AND METHODS

2.1. Experimental Procedures

2.1.1. Feedstock Preparation and Pre-treatment

Waste cassava peels were collected from a local famer in Ekosodin, Benin City, Nigeria. The brownish part was removed and the whitish layer with starch content was carefully washed to remove all sand and dirt present. The cassava peel was then sun dried for approximately three days to remove extra moisture. The sample was ground to powder form of about 0.5-1mm using a grinding machine so as to increase the sample surface area. It was sieved to get a homogenous powder. The ground sample was subjected to pre-treatment, hydrolysis, fermentation and distillation processes to obtain bio-ethanol. The prepared feedstock was stored in an air tight container before usage. Cassava peels pre-treatment was carried out using an autoclave. 100 mL of 0.5 % sulfuric acid was added to 30 g of the sample to remove the lignin, reduce cellulose crystallinity and increase porosity of the material. The mixture was heated to 120 ^{o}C under a pressure of 25 psi for 1 hour (Mishra *et al.* 2011)

2.1.2. Acid Hydrolysis Process

The Acid hydrolysis process for optimization of bio-ethanol concentration was conducted using Design Expert 7 software. 50 g 0f grounded cassava peel was used for the experiment and variables for hydrolysis includes: dilute hydrochloric acid concentration in the range 0.5-2.5 % v/v, temperature (100-140 ^{o}C), and at time duration of 15 minutes. The pH of the hydrozylate was neutralized to 6.7. The solid residue was separated using filter paper to obtain the sugar- rich liquid after the hydrolysis process to separate the non – fermentable cellulose and lignin. The resulting precipitate was poured into an air tight container where the pH range of 4 – 7 for optimization studies was adjusted for each experimental run according to the design of experiment.

2.1.3. Fermentation Process

The fermentation process was carried out in an anaerobic condition (air tight container) at a temperature of 30 ^{o}C which is approximately room temperature. The fermentation process was carried out for period of four days. Prior to this, the fermentation media was prepared by culturing of the yeast which was used in this case to ferment the sugar rich liquid. The nutrient media was prepared by adding 5 g of NH_4SO_4, 1.5 g of KOH and 0.2 g $CaCl.2H_2O$ to 500 mL of distilled water and dissolving completely. The media was autoclaved for 3 minutes at a temperature of 120 ^{o}C and pressure of 10 pounds per square inch.

2.2. Experimental Design

Response Surface Methodology (RSM) was used to optimize bio-ethanol production process from cassava peels and investigate the influence of different process variables on the bio-ethanol production. The central composite design was applied to study process variables. A total of twenty (20) experimental runs for the three identified design independent variables, namely: hydrolysis, acid concentration (A), pH (*B*), hydrolysis temperature (*C*), with low (−Alpha) and high (+Alpha) level were selected. Factorial points were augmented with five (5) replicates at the center point to assess the pure error. Response selected was bio-ethanol concentration. The levels were selected based on preliminary study results. The design factors (variables) with low (−1) and high (+1) levels and the central values (zero levels) chosen for experimental design are presented in Table 1 for *A*, *B*, *C*, respectively.

Table 1: Variables in the experimental design

Factors	Symbol	Coded and Actual levels				
		-α	**-1**	**0**	**+1**	**+α**
Acid hydrolysis concentration	*A*	0.5	0.9	1.5	2.1	2.5
Hydrolysis Temperature	*B*	100	108.1	120	131.9	140
pH	*C*	4	4.6	5.5	6.4	7

2.2.1. Statistical Analysis

Once the experiments were preformed, the next step was to perform a response surface experiment to produce a prediction model to determine curvature, detect interactions among the design factors (independent variables), and optimize the process, that is, determine the local optimum independent variables with maximum concentration of bio-ethanol. The model used in this study to estimate the response surface is the quadratic polynomial represented by the following equation:

$$Y = \beta_o + \sum \beta_i x_j + \sum \beta_{ij} x_i x_j + \sum \beta_{ii} {x_i}^2 + e_i \quad (1)$$

Where Y is the bio-ethanol concentration (g/L), β_o is the value of the fixed response at the centre point of the design, and β_i, β_{ij}, and β_{ii} are the linear, interactive, and quadratic coefficients, respectively, e_i is the error term, $x_i$and x_j are the independent variables (factors) under study.

The statistical software Design Expert 7.0 was used for design of experiments, regression, graphical, statistical analysis, Analysis of Variance (ANOVA) and optimization of the hydrolysis process.

2.3. Bio-ethanol Concentration Determination:

Colour Reaction and Colorimetry

The concentration of bio-ethanol in the fermentation sample was determined using a spectrophotometric method. To an aliquot of standard stock solution containing 1.6 mg/mL, 5 mL of sodium dichromate solution, 5 mL of acetate buffer pH 4.3 and 25 mL of 1N sulphuric acid was added in 50 mL of volumetric flask. The mixture was shaken gently for 1 minute and allowed to stand for 120 minutes as incubation period at room temperature resulted in formation of green coloured reaction product. Following incubation period the absorbance at 600 nm was read on 562 UV-Vis spectrophotometer model 752.

This procedure was followed for each of the samples prepared. Software supplied with the instrument was used to read the concentration of the samples from the plot concentration curve for standard and concentration of sample was calculated using equation (2) (Sumbhate *et al*. 2002).

$$Percentage\ of\ ethanol\ in\ sample\ (\%) = C_s \times \frac{A_u}{A_s} \times 100 \quad (2)$$

Where; $C_s\ is$ Concentration of standard, $A_u\ is$ Absorbance of standard and $A_s\ is$ Absorbance of sample.

3.0. RESULTS AND DISCUSSION

3.1. Central Composite Design of Experiment for the Optimization Studies.

In the experimental procedure carried out, three input variables; acid concentration, hydrolysis temperature, and pH were studied to determine the optimum of bio-ethanol concentration produced from cassava peels using the central composite design.

3.1.1.Model Fitting and Analysis of Variance

The central composite design was used to analyse the different effect of variables; acid concentration, temperature and pH, this resulted in 20 experimental runs with the various range of values, as shown in Table 2 with the response or dependent variable chosen as bio-ethanol concentration. Equations (3) and (4) are the quadratic statistical models in terms of actual and coded variables that were obtained after applying multiple regression analysis to the experimental data; this was used to calculate the predicted bio-ethanol concentration from experimental runs.

$$Y = 351.01911 + 74.58564A + 2.86552B + 46.96746C - 0.33358AB - 0.99702AC - 0.025102BC - 10.87848A^2 - 7.82119 \times 10^{-3}B^2 - 3.99932C^2 \quad (3)$$

$$Y = 20.13 - 2.12A + 4.16B - 1.37C - 2.36AB - 0.53AC - 0.27BC - 3.85A^2 - 1.11B^2 - 3.18C^2 \quad (4)$$

Where A, B and C are the acid concentration, temperature, pH respectively, in order to check the analysis of variance (ANOVA) and to check the adequacy of the model obtained the statistical analysis was performed. The ANOVA results and the statistical information for the for the second order response surface model are shown in Tables 3 and 4 respectively.

The model F-value of 264.50 and very low value of (<0.0001) showed that the model was significant. Each term in the model was also checked for significance.

Value of prob<0.05 is an indication that the model term is significant. Values greater than 0.1000 are insignificant model terms. The adequacy of the model was further checked. There was an insufficient lack of fit. According to Montgomery & Runger (2010) the lack of fit is an indication of the failure of the model represnting the experiment data at which some points

not included in the regression or variance in the model cannot be acounted for by random errors. This therefore implies that if there is a significant lack of fit, the model is discarded. From the ANOVA, the lack of fit F-value of 0.89 implies that it not significant relative to pure error. There is a 54.73 % chance that the lack of fit F-value this large could be due to noise.

Table 2 Central composite design for the optimization of variables and the response values.

Std	Run	Factor 1 A: Acid conc. $(v/v\,\%)$	Factor 2 B: temp. ℃	Factor3 B: pH	Response Ethanol conc. (g/L)	predicted Ethanol conc. (g/L)
1	13	0.9	108.1	4.6	8.21	8.15
2	6	2.1	108.1	4.6	9.36	9.69
3	14	0.9	131.9	4.6	22.08	21.72
4	9	2.1	131.9	4.6	13.81	13.83
5	3	0.9	108.1	6.4	7.21	7.00
6	16	2.1	108.1	6.4	6.26	6.43
7	10	0.9	131.9	6.4	20.03	19.51
8	20	2.1	131.9	6.4	9.63	9.50
9	4	0.5	120.0	5.5	12.24	12.80
10	2	2.5	120.0	5.5	6.02	5.67
11	19	1.5	100.0	5.5	10.24	9.98
12	5	1.5	140.0	5.5	23.52	23.98
13	17	1.5	120.0	4.0	13.49	13.42
14	8	1.5	120.0	7.0	8.53	8.81
15	18	1.5	120.0	5.5	19.85	20.11
16	11	1.5	120.0	5.5	20.04	20.11
17	1	1.5	120.0	5.5	19.92	20.11
18	15	1.5	120.0	5.5	21.23	20.11
19	7	1.5	120.0	5.5	19.67	20.11
20	12	1.5	120.0	5.5	20.08	20.11

Table 3: Analysis of Variance (ANOVA) for response surface quadratic model

Source	Sum of squares	Degree of freedom	Mean Square	F value	p-value prob ≥F
Model	701.25	9	77.92	264.58	<0.0001 significant
X_1	61.29	1	61.29	208.11	< 0.0001
X_2	236.60	1	236.60	803.44	< 0.0001
X_3	25.53	1	25.53	86.69	< 0.0001
X_1X_2	44.51	1	44.51	151.14	< 0.0001
X_1X_3	2.24	1	2.24	7.59	0.0203
X_2X_3	0.57	1	0.57	1.93	0.1954
X_1^2	213.18	1	213.18	723.90	< 0.0001
X_2^2	17.63	1	17.63	59.87	< 0.0001

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Source	Sum of squares	Degree of freedom	Mean Square	F value	p-value prob ≥F
X_3^2	145.86	1	145.86	495.31	< 0.0001
Residual	2.94	10	0.29		
Lack of Fit	1.39	5	0.28	0.89	0.5473 Not significant

Table 4: Statistical information for ANOVA

Std. Dev.	**0.54**	R^2	**0.9958**
Mean	14.57	Adj R^2	0.9921
C.V. %	3.72	Pred R^2	0.9818
PRESS	12.79	Adeq Precision	47.7240

The quality of the model developed was evaluated based on the correlation coefficient, R^2 value. A model developed should be best at low standard deviation and high R^2 statistics which is closer to unity as it will give predicted value closer to the actual value for the response (Ahmad, Hameed, & Ahmad 2009; Lenihan et al. 2010; Rahman et al. 2007).

In this work, R^2 value was 0.9958. This indicated that 99.58 % of the total variation in the final concentration was attributed to the experimental variables studied. The high R^2 equally showed that the predicted value would be more accurate and closer to its actual value (Mohd & Rasyidah 2010). The standard deviation for the model was 0.54 which indicated that the predicted values for this model are still considered as suitable to correlate the experimental data. The adequate precision which measured the signal to noise ratio was 47.7240 which indicated an adequate signal. Also the "pred R^2 of 0.9818 was in reasonable agreement with the "Adj R^2 of 0.9921.

3.2. Diagnostic Plots

The quality of the model developed was further assessed using residual plots. Residual is the difference between the experimental value and value predicted by the model. Some of the residual plots used were: normal plot of residuals which indicates whether the residuals follow a normal distribution, and plot of predicted vs.

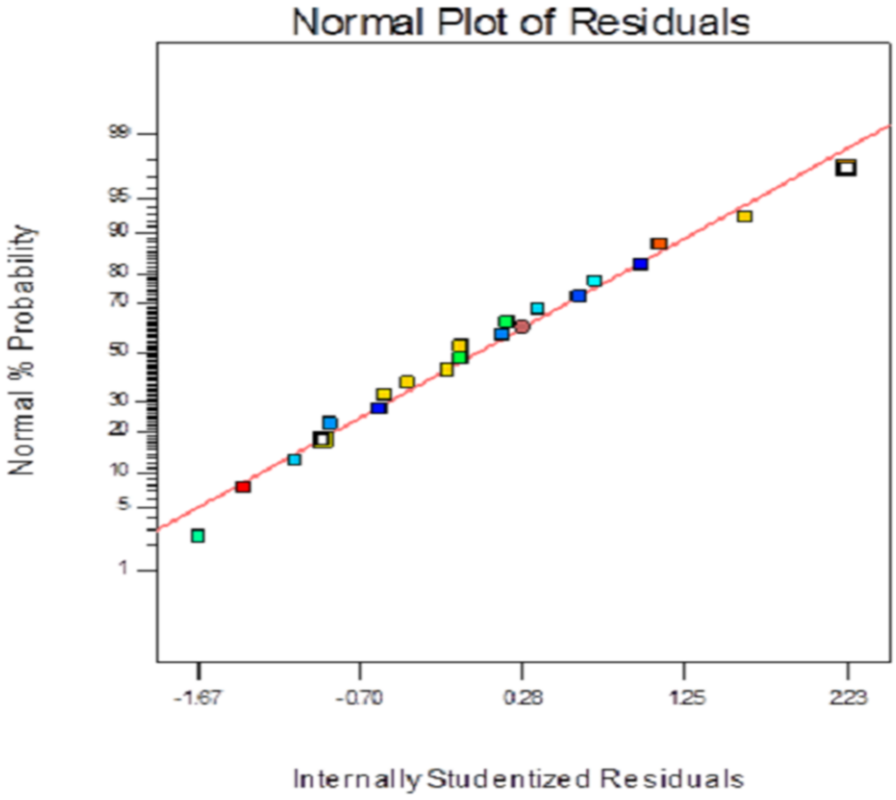

Figure 1: Residual Plots Normal Plot of Residuals

Actual response values which helps to detect a value, group of values that are not easily predicted by the model (Onuigbo, 2017). The residual plots are shown in Figures 1 and 2.

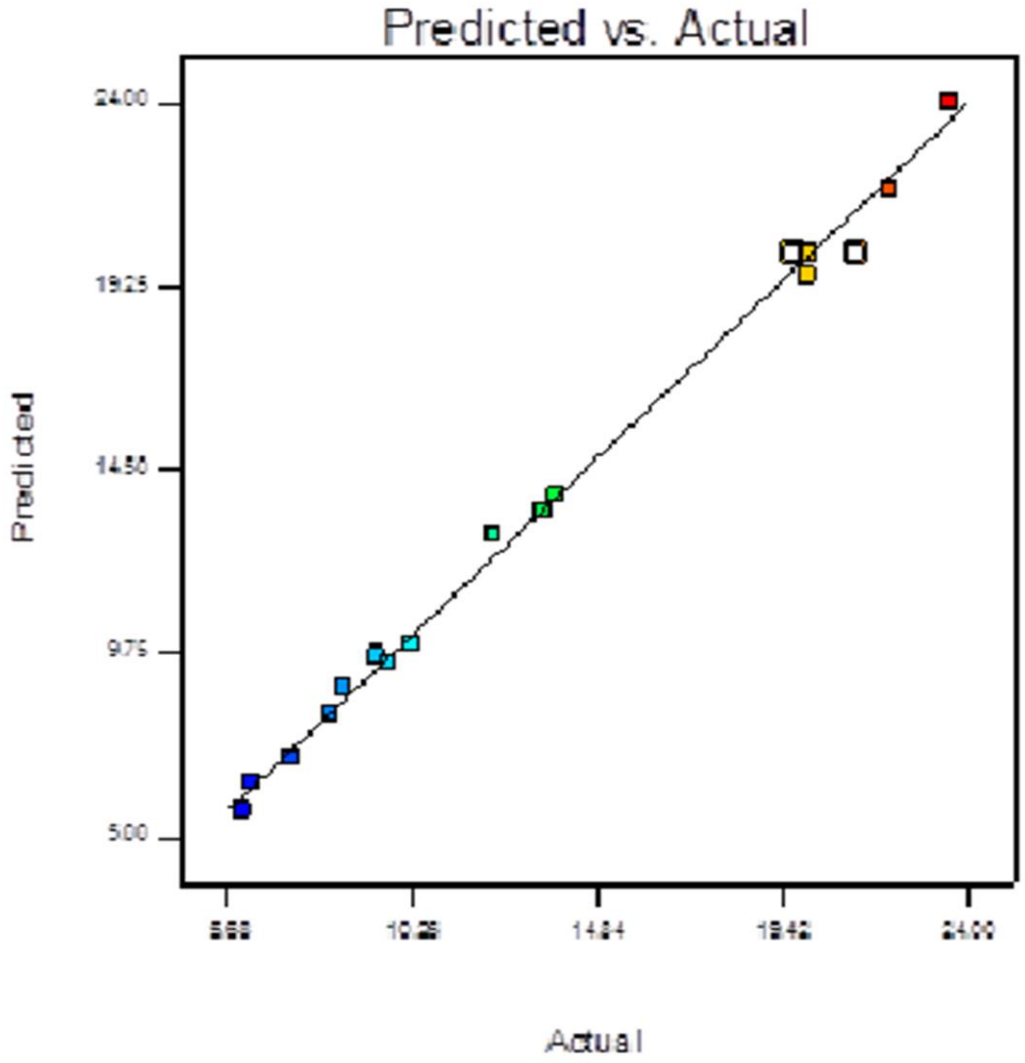

Figure 2: Residual Plot of Predicted vs. Actual Response Values.

3.3. Combined Effects of Different Variables on Bio-ethanol Concentration Produced

Response surface curves were plotted to examine the effect of the interaction between the independent variables and to determine the optimum levels of the variables.

3.3.1. Combined Effect of pH And Acid Concentration on Bio-ethanol Concentration

Figure 3 below shows the interaction between independent variables, pH and acid concentration keeping temperature constant at a value of 120 ^{0}C. From the plot it is observed that at a constant acid concentration of (0.9 % v/v), decreasing pH from 6.4-4.6 causes an increase in ethanol concentration, this effect being as a result of increase in the acidic medium as pH decreases. Increasing acid concentration from 0.9-2.1, at a high pH of 6.4 and at a low pH of 4.6,

shows similar effect as bio-ethanol concentration increases to an optimum before a gradual decrease. This trend observed may be attributed to the catalytic activity of the acid. Increasing the rate of the fermentation medium, results in the release of more hydrogen ion that eventually acts as catalytic agent during hydrolysis.

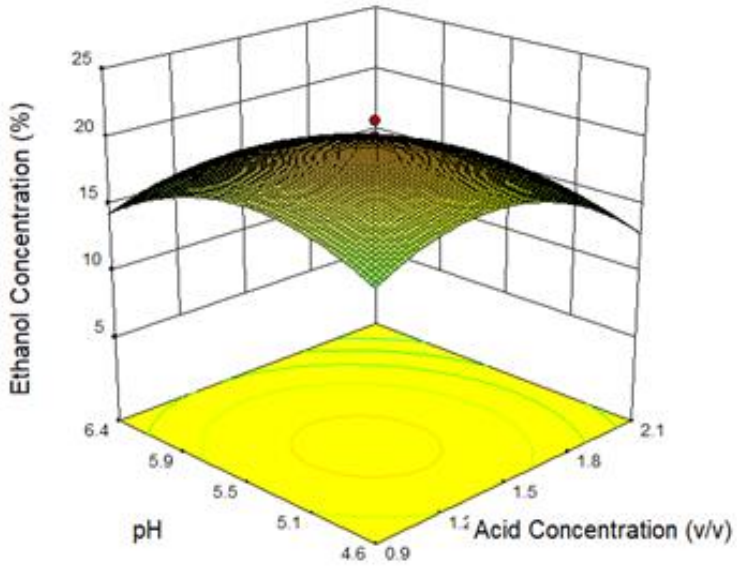

Figure 3: Response surface model plot of simultaneous effect of pH and hydrolysis acid concentration on bio-ethanol concentration produced.

3.3.2. Combined Effect of Hydrolysis pH and Temperature on Bio-ethanol Concentration

Figure 4 shows the combine effect of temperature and pH keeping acid concentration constant at a value of 1.5, from the plot it is observed that at low and high values of pH 4.6 and 6.4 respectively, the same effect is observed, which is a progressive increase in rate of ethanol produced with respect to increase in temperature from 108 ^{0}C to 131.9 ^{0}C. This effect could be associated with acidic medium of pH since acidic medium favours Fermentation process.

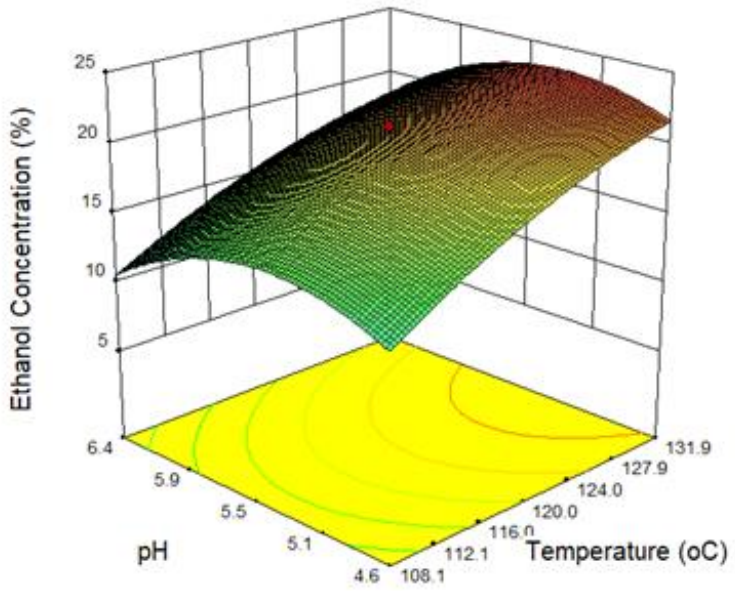

Figure 4: Response surface model plot of simultaneous effect of pH and temperature on bio-ethanol concentration produced.

At low temperature of 108.1, increase in pH causes an initial increase in ethanol concentration to an optimum before a gradual decrease; also at high temperature of 131.9 ^{0}C the same effect is also noticed. This decrease in concentration could be as a result of low activity of the yeast as pH increases.

3.3.3. Combine Effect of Acid Hydrolysis Concentration and Hydrolysis Temperature on Bio-ethanol Concentration

Figure 5 below shows the effect of acid concentration and hydrolysis temperature on ethanol concentration when pH is maintained constant at a value of 5.5, from the plot it is observed that increasing the temperature of the medium led to significant increase in ethanol production at low value of acid concentration (0.9 % v/v), this could be attributed to increase in the rate of collision of the molecules of the reacting species.

At a high value of acid concentration (2.1 % v/v) increase in temperature from 108 ^{0}C - 131.9 ^{0}C led to an initial increase in ethanol concentration up to an optimum after which further increase led to a decrease. This could be as a result of unfavorable temperature of the reacting medium.

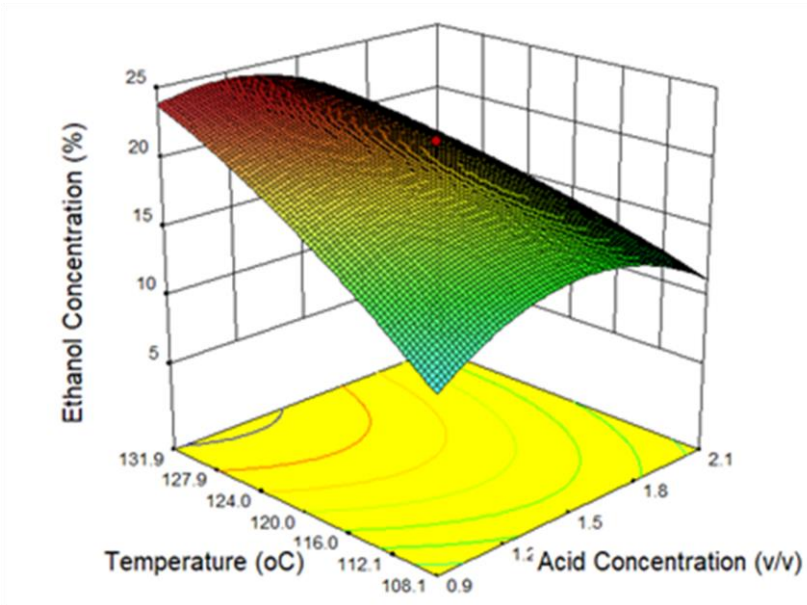

Figure 5: Response surface model plot of simultaneous effect of temperature and hydrolysis acid concentration on the concentration of the ethanol produced.

At low temperature of 108.9 ^{0}C increase in acid concentration from 0.9 - 1.5 % v/v resulted to an increase in ethanol concentration produced this could be attributed to the catalytic activity of the acid during hydrolysis process resulting in high yield of simple sugar and thus increase in the fermentation of simple sugar to bio-ethanol. At high temperature of 131.9 ^{0}C, increase in acid concentration led to a decrease in ethanol concentration. This effect could be as a result of elevated temperature which is not favourable.

3.4. Validation of Statistical Model

To confirm the validity of the statistical model, three confirmation experimental runs were performed at the chosen optimum hydrolysis conditions indicated in Table 6. The result shows that maximum experimental ethanol concentration of 24.48 g/L obtained was close to the predicted value of 24.41 g/L. The excellent correlation between the predicted and measured values of these experiments shows the validity of statistical model.

Table 6: Solution for optimum conditions

Acid Conc. $(v/v\,\%)$	Temperature ℃	pH	Ethanol Conc. (g/L)
1.2	131.8	5.3	24.48

4.0. CONCLUSION

Drawing from the results obtained in this work, it is seen that the model developed using the Central Composite Design (CCD) in Response Surface Methodology

(RSM) to represent the acid hydrolysis step for the production of ethanol from cassava peels is valid as it gave a significant p-value < 0.05 and also showed an insignificant lack of fit. A triplicate set of experiments carried out at the optimum values predicted by the model yielded an average value of 24.41 g/L for the ethanol concentration which was very close to the predicted value of 24.48 g/L running at optimum acid concentration of (1.2 % v/v), temperature of 131.8 ^{0}C and pH of 5.3.

5.0. ACKNOWLEDGEMENT

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