

Original Research Article

STATISTICAL MODELLING OF ENZYMATIC HYDROLYSIS OF BANANA PEELS FOR BIOETHANOL PRODUCTION

ABSTRACT

This study investigated the use of banana peel (BP) for bioethanol production and the optimisation of the process parameters. Characterization of BP was done by Fourier Transform Infrared Spectroscopy (FTIR) and proximate analysis. *Aspergillus niger* isolated from infected cassava tubers was used for the hydrolysis of BP in a separate hydrolysis and fermentation process (SHF). Industrial *Saccharomyces cerevisiae* was used for the fermentation of the hydrolytes. Statistically significant quadratic regression models ($p = .05$) were developed for reducing sugars and bioethanol yields prediction. Optimal condition values for the processes parameters were established by response surface methods (RSM). The FTIR results showed that BP had strong peaks for alcohols, phenols and carboxylic acids functional groups. The proximate analysis revealed that BP contains cellulose (65.5 %), fibre (15.4 %) and protein (6.0 %) majorly. The optimum conditions for reducing sugar yields from the hydrolysis process were the temperature of 34 °C, pH of 6.5 and a period of 5 days with a yield of 122 mg/ml. While the optimum conditions for bioethanol yield from the fermentation process were the temperature of 34 °C, pH of 6.0 and a period of 5 days with a yield of 8.1 % volume weight. This work shows that optimisation of hydrolysis and fermentation processes parameters respectively improve their yields and also *Aspergillus niger* isolated from rotten cassava is effective in the hydrolysis of BP for bioethanol production.

Key Words: Banana peel, enzymatic hydrolysis, Fermentation, Distillation, Optimization

1. Introduction

The global production of banana is estimated to be around 96 million tons with approximately third of it produced in African, Asia-Pacific and North American regions [1]. The peels about 30 % of the total fruit composition are often discarded. These peels are generated in huge quantities by fruit processing industries, restaurants and households [2]. Hydrolysis of the peels into an economically valuable product like reducing sugars will not only help reduce environmental pollution but also provide a cheap base material for bioethanol production.

Production of ethanol from lignocellulosic materials like BP can be achieved by hydrolysis of the cellulose content of the materials to reducing sugars and fermentation of the hydrolysate. Ethanol is then recovered by the distillation process. The main difference between the process alternatives is the hydrolysis steps, which can be performed by dilute acid, concentrated acid or enzymatically. The choice of a hydrolysis method depends on a number of factors: cost, nature of material and toxicity of chemicals involved [3].

Enzymatic hydrolysis is a method in which cellulases are utilised for the hydrolysis process. This is a quite a new approach when compared to concentrated-acid and dilute-acid hydrolysis. The significant advantages of enzymatic hydrolysis are high ethanol yield and safer operating conditions. Furthermore, the by-product formation is low. The primary disadvantage is the low reaction rate and the high cost of enzymes [4]. Common cellulases producing fungi species include *Trichoderma*, *Penicillium* and *Aspergillus niger* [5].

44 Production of bioethanol from BP has generated interests among researchers, and different methods
45 have been used. The use of organic acid for the hydrolysis process in a separate hydrolysis and
46 fermentation process was carried out by Chongkhon [6]. Also Barve and Tarfe [7] investigated the
47 efficiency of bioethanol production from co-cultures of *Aspergillus niger* and *Saccharomyces*
48 *cerevisiae*. Pre-treatment of BP using hot water and steam was reported by Vaitheki and Deepa [8]
49 and Alula [9] respectively.

50 In order to achieve high products yield from the hydrolysis and fermentation processes, it is important
51 to optimise the variables that significantly affect the processes [10]. Therefore the objective of this
52 present study is to optimise the process variables of temperature, pH and time using response
53 surface methodology (RSM) in order to achieve high products yield. Mathematical correlations were
54 also developed to understand better the effects of the process variables on the yields of reducing
55 sugars and ethanol.

56

57 **2. Materials and Methods**

58 **2.1 Collection and Preparation of Banana Peel**

59 Banana peels were obtained from a local market in Mgbakwu, Awka in Anambra State. The peels
60 were washed and dried in a cabinet oven at 50 °C for 24 hours. The dried samples were milled into
61 fine flour using a milling machine, sieved and packaged in an air-tight polyethene bag and labelled for
62 analysis.

63 **2.2 Composition of Banana Peel**

64 A Shimadzu Analytical FTIR-8400S was used to determine the functional groups present in BP and
65 proximate composition of BP was determined by standard methods.

66 **2.3 Determination of proximate composition**

- 67 i. The moisture content was determined based on ASTM standard method [11].
68 ii. The ash content was determined using ASTM standard method [11].
69 iii. The fibre content was determined according to the standard method of ASTM [12].
70 iv. The lipid contents of the peels were determined by Soxhlet Fat Extraction method [13].
71 v. The total protein content was determined by the sum of the total nitrogen using the micro
72 Kjeldahl procedure. The amount of protein obtained was multiplied by the nitrogen content of
73 6.25 (a constant factor) [14].
74 vi. The cellulose content is calculated using equation 2.1 [2].

$$75 \text{ \% Cellulose} = 100 - (\text{\% Protein} + \text{\% Moisture} + \text{\% Ash} + \text{\% Fat} + \text{\% Fibre}) \quad (2.1)$$

76

77 **2.4 Enzyme preparation**

78 **2.4.1 Substrate preparation**

79 Potato Dextrose Agar (PDA) cultivation medium was prepared using a standard method [15]. 200.0 g
80 of potato was peeled, minced and boiled in 800 ml of deionised water for 30 minutes with the addition
81 of 20 g glucose and 16 g agar, without pH adjustment (initial 6.4). The mixture was sterilised by
82 autoclaving at 121 °C for 20 minutes to obtain the PDA cultivation medium as isolation substrate.

83 **2.4.2 Identification of microorganism (*Aspergillus niger*)**

84 *Aspergillus niger* was originally isolated from rotten cassava tubers and maintained on PDA slant at
85 4°C. The isolation was carried out by serially diluting 0.1g of rotten cassava tuber in 1ml of sterile
86 water, followed by plating out 0.1ml of 10⁻⁴ dilution on PDA substrate in a Petri dish. The culture was
87 cultivated upside down for 3 days under 37°C. Furthermore, a single colony was separated and
88 inoculated on plate medium cultivated for another 3 days. A pure colony of *Aspergillus niger* was then
89 obtained and stored on PDA at 4°C [15].

90 **2.4.3 Inoculum development**

91 The inoculum was developed using a standard method [16]. Three (3) agar plugs from the Petri
92 dishes were inoculated into one litre Erlenmeyer flask containing 500ml of sterile fermentation
93 medium. The fermentation medium comprises 20g/l raw potato starch, 16g/l peptone, 10g/l (NH₄)
94 HPO₄, 1g/l NaCl and 1g/l MgSO₄·7H₂O in de-ionized water.

95 **2.5 Inoculums preparation**

96 **2.5.1 Hydrolysis inoculums**

97 The inoculum (5 %) of the multiplied *A. niger* from a PDA slant was prepared by aseptically
98 transferring 50 g of the pure and screened *A. niger* from the slant to a 1liter volumetric flask. Distilled
99 water autoclaved at 121°C for 15 minutes was added to make the mark of the flask. The mixture was
100 left for 10 minutes at 150 rpm. The inoculums size was set to have a cell concentration of 1.0 × 10⁸
101 cells per ml [17].

102 The whole of the hydrolysis experiment was carried out using this inoculum.

103 **2.5.2 Fermentation inoculums**

104 100 ml of distilled water was heated to 40 °C in a shaker flask and 0.5 % (w/w) of *Saccharomyces*
105 *cerevisiae* yeast was added to the warm water. The mixture was left at 150 rpm for 10 minutes. The
106 inoculums size was set to have a cell concentration of 5.3 × 10⁷ cells per ml, dilution of the inoculums
107 was done when the concentration of the cells was too high [18].

108 **2.6 Hydrolysis, Fermentation and Distillation processes**

109 **2.6.1 Enzymatic hydrolysis with *Aspergillus niger***

110 The enzymatic hydrolysis was carried out at different temperatures, for the different time interval and
111 at different pH levels. This was done using a 250 cm³ conical flask containing 50 cm³ of 5% inoculums
112 of *A. niger* and 11 g of the peel sample. The mixture was incubated on a shaker with an agitation rate
113 of 300 rpm and subsequently filtered. The concentration of reducing sugar in the filtrate of hydrolysis
114 was determined by 3, 5-dinitro salicylic acid (DNS) method using a glucose solution as standard [19].

115

2.6.2 Fermentation using *Saccharomyces cerevisiae*

A 250 cm³ conical flask containing 30cm³ of the medium obtained from the enzymatic hydrolysis was used to carry out the fermentation. The medium was inoculated with 5% (v/v) growth medium containing the activated *Saccharomyces cerevisiae* and incubated on a shaker with an agitation rate of 300 rpm at various temperatures, time and pH [20]. A simple distillation process recovered the product. The quantity was determined by multiplying the volume of the distillate by the density of ethanol (0.00008033 mg/ml) and expressed a percentage of the loaded substrate [21].

2.7 Optimization of the Hydrolysis and Fermentation Processes

They variables temperature, pH and time were optimised using the central composite design (CCD) of the response surface methodology (RSM). They variables were varied at five different levels (- α , -1, 0, +1, + α). A set of 20 experiments were performed for the hydrolysis and fermentation processes respectively. Each study of a process consists of 6 centre points or null points. The distance of the star-like point α used was 1.35. In order to avoid systematic error, the experiments were performed at random.

The upper and lower limits of the independent variables for hydrolysis and fermentation processes are presented in Tables 1. While the design matrix for the hydrolysis and fermentation processes with the responses is presented in Tables 2.

Table 1: Upper and lower limits for the optimisation of the hydrolysis and fermentation processes

Parameter	Hydrolysis		Fermentation	
Temperature (^o C)	30	40	30	70
pH	3.5	7.5	3.5	7.5
Time (day)	1	9	1	9

Table 2: Design matrix for the optimization of the hydrolysis and fermentation processes

Std	X ₁ : Temp °C	X ₂ : pH	X ₃ : Time Day	Reducing Sugars Yield mg/ml	Std	X ₁ : TEMP °C	X ₂ : pH	X ₃ : Time Day	Ethanol Yield (BP) %
1	30	3.5	1	116.4	1	30	3.5	1	4
2	40	3.5	1	116	2	70	3.5	1	3
3	30	7.5	1	119.4	3	30	7.5	1	7.1
4	40	7.5	1	118.6	4	70	7.5	1	3
5	30	3.5	9	119.8	5	30	3.5	9	7
6	40	3.5	9	119.3	6	70	3.5	9	6.3

7	30	7.5	9	118.8	7	30	7.5	9	5.1
8	40	7.5	9	118.1	8	70	7.5	9	3.1
9	30	5.5	5	120.3	9	30	5.5	5	8
10	40	5.5	5	119.6	10	70	5.5	5	5.6
11	35	3.5	5	120.4	11	50	3.5	5	7.2
12	35	7.5	5	120.7	12	50	7.5	5	7.2
13	35	5.5	1	119	13	50	5.5	1	5.8
14	35	5.5	9	119.5	14	50	5.5	9	6.2
15	35	5.5	5	121	15	50	5.5	5	7.3
16	35	5.5	5	121.1	16	50	5.5	5	7.13
17	35	5.5	5	121.2	17	50	5.5	5	7.23
18	35	5.5	5	121.3	18	50	5.5	5	7.45
19	35	5.5	5	121.4	19	50	5.5	5	7.53
20	35	5.5	5	122	20	50	5.5	5	7.63

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140

141 3. RESULTS AND DISCUSSION

142 3.1 Composition Analysis Results

143 The result of the FTIR study of BP is presented in Figure 1. The result of the FTIR study was analyzed
144 based on the standard peaks presented by Silvestein et al [22] for various functional groups. It can be
145 seen from Figure 1 that BP contain OH group for alcohols, phenols, acids and carboxylic acids which
146 are the essential functional groups commonly found in cellulose materials used for the production of
147 ethanol.

148 The results from the proximate analysis of the BP is presented in Table 2a. From the table, it can be
149 seen that BP contained 6 % protein. Feumba et al., [23] reported a close protein content of 10.44 %.
150 The fibre content of the peels was found to be 15.4 %. Abubakar et al., [24] also reported a similar
151 fibre content of 14.8 %.

152 BP contained 0.72 % crude fats. Feumba et al., [23] reported a higher fat content of 8.4 %. The
153 variations could be due to species type and treatment procedure.

154 The lignin content of 0.8 % was low enough for easy hydrolysis of the peel.

155 The cellulose content of the peel was calculated to be 65.5 %. Feumba et al., [23] also reported a
156 high cellulose content of 43.4 %. The high cellulose content makes the peel an adequate feedstock
157 for bioethanol production.

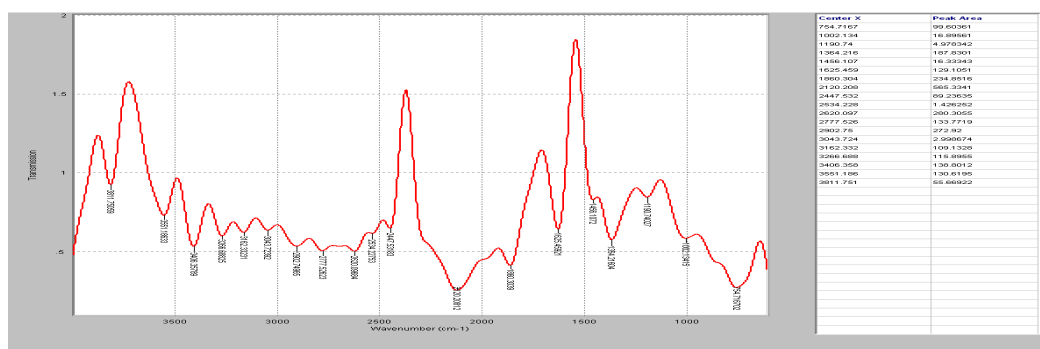


Fig.1: FTIR result of banana peels

Table 2a. Proximate composition of BP (%)

Parameters	Composition (%)
Protein	6.0±0.10
Cellulose	65.5±0.10
Fibre	15.40±0.10
Fats	3.20±0.10
Tannins	1.52±0.10
Moisture content	5.05±0.10
Lignin	0.80±0.10
Ash	3.50±0.10

3.2 Statistical Modeling and Optimization of the Hydrolysis process

3.2.1 Analysis of Variance (ANOVA)

The Statistical Analysis of Variance (ANOVA) was carried out to assess the significance of the selected model as well as the significance of individual terms and their interaction on the chosen response. It also identifies the important factors in a multi-significant model. ANOVA justifies the adequacy and significance of the selected model through the Fisher's (F-test) and the probability values. The F-statistic is simply a ratio of two variances. Variances are a measure of dispersion. The ANOVA results are presented in Tables 4 and 5.

F-values of 29.5 and 54.2 was obtained for the hydrolysis and fermentation processes respectively, this implies that the models were significant with a 99 % confidence.

Lack of fit measures the failure of a model to represent the data in the experimental domain at points which were not included in the regression. Tables 4 and 5 show that the lack of fit p-values were greater than 0.05 for the hydrolysis and fermentation models developed. The p-value (probability of error value) is used to check the significance of each regression coefficient and interactions between

the test variables [25]. The larger the magnitude of F-test value, the smaller the magnitude of p-values and the higher the significance of the corresponding coefficient. The p-values obtained for lack of fits indicate that the experimental data fitted well to the models and the models were adequate for predicting the response variables.

Residual is the difference between the observed value of the dependent variable and the predicted value. Residual values are used for residual plots (Figures 4 and 5) to identify abnormalities in the models. Pure error represents random variation in the response variable. It is the difference between different observations on the same response for the same treatment combination (Draper and Smith, 1981). Pure values were used to generate the normal % probability values (Figures 2 and 3).

More so, Coefficient of Variation (CV) is a standard deviation expressed as a percentage of the mean. The CV value shows the ratio between standard error of estimate and the mean value of the observed response as a percentage. A CV value less than 10 % indicates that the model is reproducible. In the present study CV values of 0.35 % and 5.25 % were obtained for the hydrolysis and fermentation processes respectively (Tables 4 and 5). The results suggested a good precision.

Predicted Residual Error Sum of Squares (PRESS) provides a summary measure of the fit of a model to a sample of observations that were not used in estimating the model [26]. Models with low press values are considered more adequate than those with high press values. PRESS values of 7.11 and 12.95 obtained for the hydrolysis and fermentation processes respectively (Tables 4 and 5) suggested that the models were adequately fit.

The coefficient of Regression (R-squared) measures the amount of variation around the mean explained by a model [25]. R-squares values of 0.96 and 0.98 were generated for BP/CP hydrolysis and fermentation processes respectively (Tables 4 and 5). This implies that 96 % and 98 % of the variability in the response can be explained by the models. This suggests that the prediction of the experimental data is quite satisfactory.

Adjusted R-Squared ($\text{Adj } R^2$) measures the amount of variation around the mean explained by the model adjusted for the number of terms in the model. The value of $\text{Adj } R^2$ decreases as the number of terms in the model increases if those additional terms don't add value to the model [26]. In this study $\text{Adj } R^2$ values of 0.93 and 0.96 were obtained for the hydrolysis and fermentation processes respectively (Tables 4 and 5). This indicates a high degree of correlation between the experimental and predicted values.

Predicted R-Squared ($\text{Pred } R^2$) is used to determine how well a regression model can make predictions. $\text{Pred } R^2$ can identify cases where a model is able to provide a good fit for the existing data but can't make a good prediction [26]. The variations between the $\text{Adj-}R^2$ and $\text{Pred } R^2$ were less than 20 % for the hydrolysis and fermentation models developed in this study (Tables 4 and 5). This implies that the model can make good predictions.

Adequate Precision compares the range of predicted values at the design points to the average prediction error. A model with an adequate precision value greater than 4 is considered adequate. In the present study, adequate precision values of 17.47 and 21.73 were obtained for hydrolysis and fermentation processes respectively (Tables 4 and 5). These values were quite above 4 indicating the adequacy of the developed models.

Figures 2 and 3 give the normal probability plots of the models. The normal probability plot is a graphical technique to identify and substantiate departures from normality. The normal probability plot indicates whether the residuals follow a normal distribution, in which case the points follow a straight line. The plot is necessary in order to make the resulting image look close to a straight line if the data are approximately normally distributed. Deviations from the straight line suggest a deviation from normality [26]. Figures 2 and 3 show that the data were closely distributed along the straight line of the plots. There were few points away from the normality line but they were not totally out of range for adequate models.

The predicted Vs Actual plots (Figure 4 and 5) were used to assess the correlation between the experimental and the predicted values. It is seen that there is a close correlation between the experimental response and the predicted response. From the plots (Figures 4 and 5), the points were closely distributed to the straight line of the plot, this confirms the good relationship between the experimental values and the predicted values of the responses. These plots equally confirm that the selected model was adequate in the prediction of the variables responses in the experimental values.

Table 4: ANOVA for Response Surface Quadratic model (Hydrolysis Process)

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F	
Model	46.71623	9	5.190692	29.94735	< 0.0001	Significant
X ₁ -Temp.	0.961	1	0.961	5.544425	0.0403	
X ₂ -pH	1.369	1	1.369	7.898353	0.0185	
X ₃ -Time	3.721	1	3.721	21.46806	0.0009	
X ₁ X ₂	0.045	1	0.045	0.259624	0.6214	
X ₁ X ₃	0.052	1	0.053	0.344533	1.0000	
X ₂ X ₃	7.605	1	7.605	43.87653	< 0.0001	
X ₁ ²	2.434602	1	2.434602	14.04627	0.0038	
X ₂ ²	0.319602	1	0.319602	1.843924	0.20431	
X ₃ ²	7.404602	1	7.404602	42.72035	< 0.0001	
Residual	1.733273	10	0.173327			
Lack of Fit	1.099939	5	0.219988	1.736746	0.2797	Not significant
Pure Error	0.633333	5	0.126667			

Cor Total	48.4495	19		
Std. Dev.	0.416326		R-Squared	0.964225
Mean	119.695		Adj R-Squared	0.932028
C.V. %	0.347822		Pred R-Squared	0.853179
PRESS	7.11341		Adeq Precision	17.46619

Table 5: ANOVA for Response Surface Quadratic model (Fermentation Process)

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F	
Model	50.71681	9	5.635201	54.20713	< 0.0001	Significant
X ₁ -Temp.	10.404	1	10.404	100.08	< 0.0001	
X ₂ -pH	0.4	1	0.4	3.847751	0.0272	
X ₃ -Time	2.304	1	2.304	22.16305	0.0008	
X ₁ X ₂	2.42	1	2.42	23.2789	0.0007	
X ₁ X ₃	0.72	1	0.72	6.925953	0.0251	
X ₂ X ₃	8.405	1	8.405	80.85088	< 0.0001	
X ₁ ²	1.463651	1	1.463651	14.07941	0.0038	
X ₂ ²	0.298651	1	0.298651	2.872833	0.1209	
X ₃ ²	6.433651	1	6.433651	61.88772	< 0.0001	
Residual	1.039568	10	0.103957			
Lack of Fit	0.860818	5	0.172164	4.815766	0.5548	Not significant
Pure Error	0.17875	5	0.03575			
Cor Total	51.75638	19				
Std. Dev.	0.322423			R-Squared	0.979914	
Mean	6.1425			Adj R-Squared	0.961837	
C.V. %	5.249057			Pred R-Squared	0.750122	
PRESS	12.95381			Adeq Precision	21.72964	

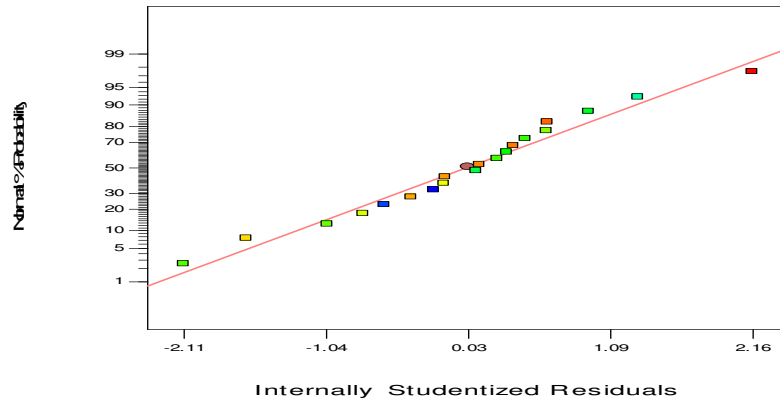


Fig.2: Normal Plots of Residuals (hydrolysis process)

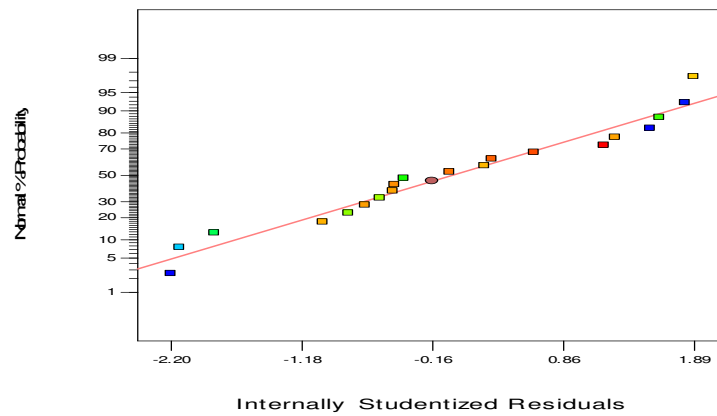


Fig.3: Normal Plots of Residuals (fermentation process)

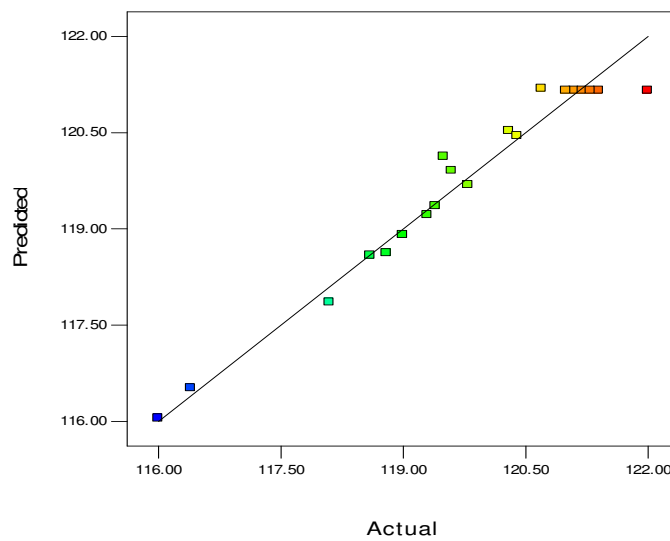


Fig.4: Plot of Predicted vs Actual (hydrolysis process)

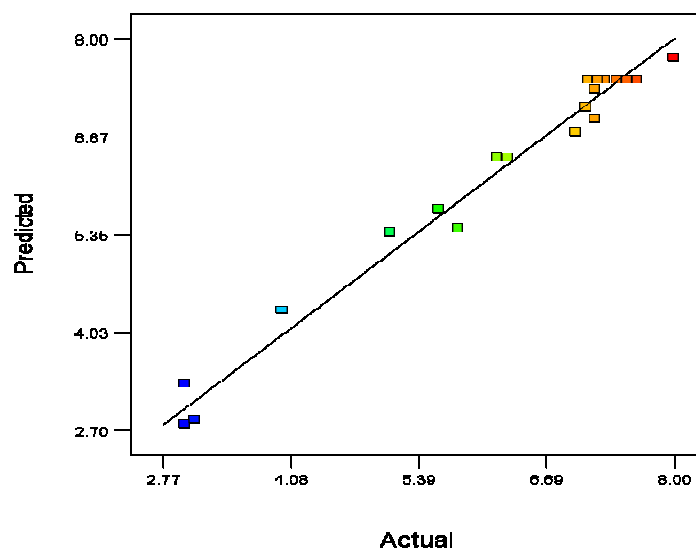


Fig.5: Plot of Predicted Vs Actual (fermentation process)

3.2.2 CCD Regression models for reducing sugars and ethanol yields

Models are used to show how the response variables relate to the factors (independent variables) which affect their outcome. Multiple regression analysis was used to correlate the responses with the independent variables using a second order polynomial equation.

The quadratic regression models obtained for reducing sugars and ethanol yield from the hydrolysis and fermentation processes are represented by Equations 1 and 2. The letters X_1 , X_2 and X_3 represent the independent variables of temperature, pH and time respectively.

In a regression equation, when an independent variable has a positive sign it means that an increase in the variable will cause an increase in the response. While a negative sign denotes a decrease in the response. The coefficients with one factor represent the effect of that particular factor on the expected response. Also, the coefficients with two factors represented the combined interactive effects between the two factors.

$$\text{Reducing sugar yield (mg/ml)} = 121.16 - 0.31X_1 + 0.37X_2 + 0.61X_3 - 0.075X_1X_2 + 0.00X_1X_3 - 0.98X_2X_3 - 0.94 X_1^2 - 0.34 X_2^2 - 1.64 X_3^2 \quad (1)$$

$$\text{Ethanol Yield (\%)} = 7.44 - 1.02 X_1 - 0.20 X_2 + 0.48 X_3 - 0.55 X_1X_2 + 0.30 X_1X_3 - 1.02 X_2 X_3 - 0.73 X_1^2 - 0.33X_2^2 - 1.53X_3^2 \quad (2)$$

The p - value (Probability of error value) is used to check the significance of each regression coefficient and the interactions between the test variables. Coefficients with p - values less than

0.05 (Tables 4 and 5) indicate that the model term is significant [21]. Eliminating insignificant model terms from Equations 1 and 2, the final model equations are represented by Equation 3 and 4.

$$\text{Reducing sugar yield (mg/ml)} = 121.16 - 0.31X_1 + 0.37X_2 + 0.61X_3 - 0.98X_2X_3 - 0.94 X_1^2 - 1.64 X_3^2 \quad (3)$$

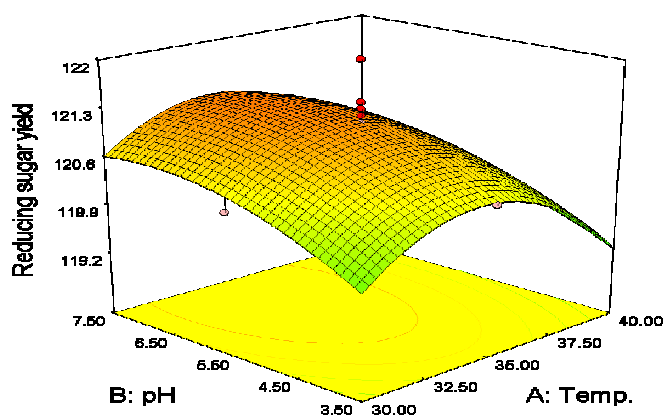
$$\text{Ethanol Yield (\%)} \text{ from BP} = 7.44 - 1.02 X_1 + 0.48 X_3 - 0.55 X_1X_2 - 1.03 X_2 X_3 - 0.73 X_1^2 - 1.53 X_3^2 \quad (4)$$

3.2.3 Three dimensional (3D) response surface plots

The 3-D response surface plots are presented in Figures 6-11. The 3-D response surface plots are a graphical representation of the interactive effects of any two variables. Response surface plot is a function of two factors at a time, maintaining all other factors at fixed levels. They are helpful in understanding the relationship between the two independent variables and the response variables. The nature of the response surface curves shows the interaction between the variables.

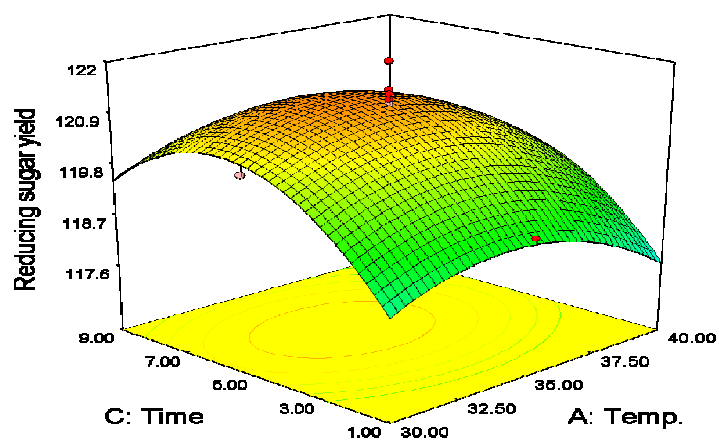
Figures 6-8 show the 3-D response surface plots for the hydrolysis process. Figure 6 gives the interactive effect of pH and temperature. A significant increase in pH and temperature increased reducing sugar yield. It can be deduced that temperature has more influence on the shape as interactions with high-temperature values gave low yields of reducing sugars. Figure 7 showed that time and temperature had strong interactive effects on the yield of reducing sugars. The shape indicates that high temperatures and longer hydrolysis time yielded less reducing sugars. This is because enzymes are denatured at high temperatures and fewer nutrients are available over a period of time. The shape of the interaction between pH and time (Figure 8) shows the weak influence pH have on the yield of reducing sugars. This is because there is no exponential decline in the yield of reducing sugars at high pH values.

Figures 9-11 show the 3-D surface plots for the fermentation process. Figure 9 gives the interactive effect of pH and temperature. The interaction indicated a strong combined influence on ethanol yield from the fermentation process. But temperature had a stronger influence because the increase in pH had low effect on ethanol yield than an increase in temperature. This is because of enzymes denture fasts at high temperatures. Figure 10 showed a similar temperature effect but it interaction with time was not significant. The interaction of time with pH was however significant (Figure 11). Increase in pH and time increased ethanol yield from the fermentation process. The interactive influence of the two variables can be explained by the fact that the enzyme's surface has charges that react to changes in pH over time.



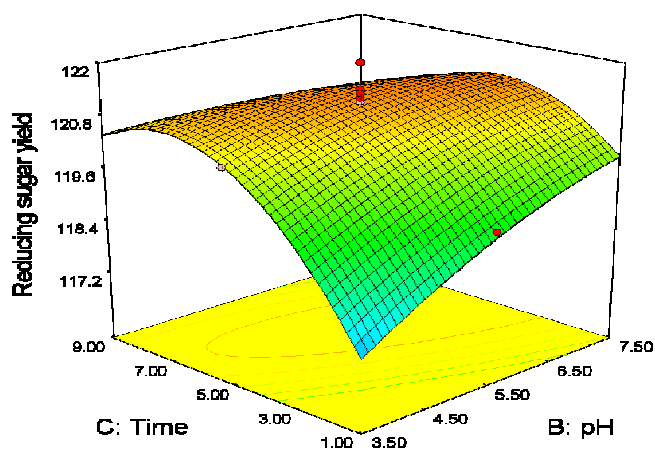
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313 **Fig.6: pH Vs temperature 3D plot for hydrolysis**



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315 **Fig.7: Time Vs temperature 3D plot for hydrolysis**



316

317 **Fig.8: Time Vs pH 3D plot for hydrolysis**

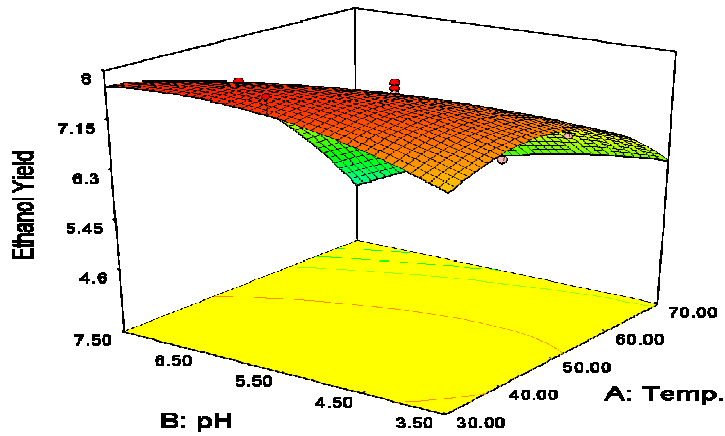


Fig.9: pH Vs temperature 3D plot for fermentation

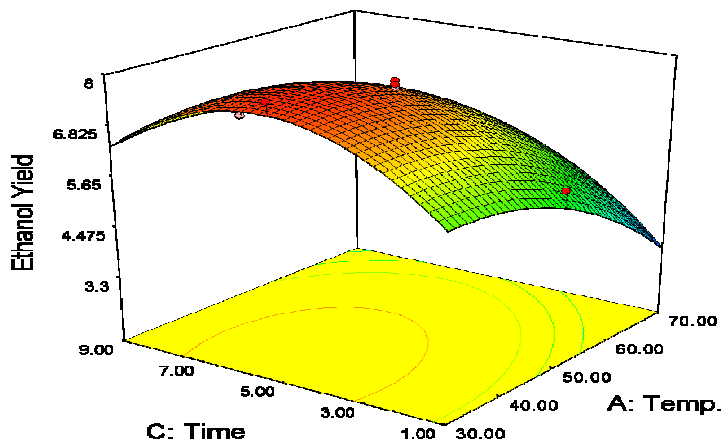


Fig.10: Time Vs temperature 3D plot for fermentation

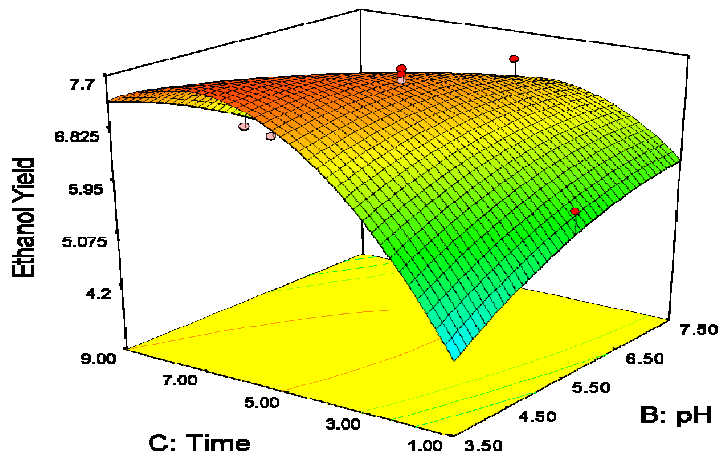


Fig.11: Time Vs pH 3D plot for fermentation

3.2.4 Verification of the Optimum Numerical Solution for Hydrolysis

The determination of the optimum levels of the hydrolysis and fermentation processes factors for maximizing the reducing sugars and ethanol yield is one of the primary objectives of this optimization

study. A combination of the experimental and predicted optimum values for the processes are presented in Table 6 and 7. The experimental values obtained were closely related to the predicted results obtained at the optimum conditions. This confirms the significance of the models developed.

The results of this study conform closely to those obtained in literature. 9.2% ethanol yield was obtained by Chongkhon [6] this implies that there is a high yield in a shorter time when acid hydrolysis is used. Co-cultures of *Aspergillus niger* and *Saccharomyces cerevisiae* yielded a lower volume of 6.34% [7] which means that separate hydrolysis and fermentation is more effective in bioethanol production from lignocellulosic materials. The use of hot water for pre-treatment provided a lower yield of 5.3% [8] when compared with the 8.1% obtained in this study implies that enzyme hydrolysis is more effective than the use of hot water.

Table 6: The predicted and experimental validated result of the hydrolysis process

Factors	Temp (°C)	pH	Time (day)	Predicted Reducing Sugar(mg/ml)	Experimental Validated Result (mg/ml)	Desirability (%)
Optimum	34	6.5	5	121.3	122	.88

Table 7: The predicted and experimental validated result of the fermentation process

Factors	Temp (°C)	pH	Time (day)	Predicted Ethanol Yield (%)	Experimental Validated The result (%)	Desirability (%)
Optimum	34	6	5	7.8	8.1	.97

4. CONCLUSION

The optimisation and modelling of enzymatic hydrolysis and fermentation of banana peel for ethanol production were successfully carried out, and the following conclusions were drawn from the work:

- The characterisation of the sample showed the presence of cellulose and OH functional groups for alcohol and phenols. This makes them quite suitable raw materials for bioethanol production.
- *Aspergillus niger* isolated from rotten cassava is effective in the hydrolysis of lignocellulosic materials to release reducing sugars.
- The yield of reducing sugar and ethanol from hydrolysis and fermentation processes respectively is dependent on temperature, time, and pH.

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