



Optimization of Bioethanol Production from Cassava Peels

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ABSTRACT: The bioethanol production from waste is acquiring attraction as a strategy for increasing energy security. This study aims to optimize the production of ethanol from cassava peel using Box Bhenken experimental design. The total carbohydrate content of about 90% in cassava peel was subjected to enzymatic hydrolysis using Alpha-amylase followed by Simultaneous Saccharification and Fermentation (SSF) by *Saccharomyces cerevisiae* for bioethanol production. The production of bioethanol from cassava peels was investigated for 1-4 hours (hydrolysis time), 0.5–1.5mg/L (enzyme loading), and 1-5 days (incubation time). A statistical model was developed and validated to predict the yield of bioethanol after fermentation, and the Response Surface Methodology (RSM) was used to optimize the conditions. The results revealed that the maximum ethanol yield of 1.911% was obtained at the optimum hydrolysis time, enzyme loading, and incubation time (i.e. 2.5 hours, 1 mg/L, and 3 days respectively).

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Cassava (*Manihot esculenta* Crantz) is a shrubby perennial crop in the family of Euphorbiaceae. (Sriroth *et al.*, 2012). Cassava is grown for its edible starchy tuberous root which contains about 94% carbohydrate and 6% containing vitamins and proteins. It is extensively produced as an annual crop in tropical countries such as Nigeria, Brazil, Thailand, etc. (Amenaghawon *et al.*, 2014). World production of cassava root was estimated by FAO (2012) to be 230 million tonnes in 2008 with Nigeria accounting for about 54 million tonnes as the world's largest producer of cassava. Large tons of cassava wastes are discarded annually in Nigeria when cassava is processed into various food products (Ozoegwu *et al.*, 2017). These cassava wastes are removed to obtain good quality food products such as fufu, garri, tapioca macaroni, and pudding and also serve as animal feeds. (Bechhoff *et al.*, 2018). Unfortunately, these wastes often end up in open dumps or drainage systems which threatens both surface water and general human health condition. It is, therefore, necessary to convert these wastes to useful end products rather than allowing them to become a nuisance to the environment. During the processing of the cassava to obtain food, a solid waste material known as cassava peels are generated. (Amenaghawon *et al.*, 2014), which has been (Prado *et al.*, 2005) identified as a high cellulose material which makes it a potential substrate for the production of value-added products such as organic acids, bioethanol, biopolymers, etc. Bioethanol is a principal

fuel that can be used as a petrol substitute for the vehicle. Therefore, this study was initiated to explore the possibility of using cassava peels as a substrate for producing ethanol. Thus this work tends towards investigating the possibility of transforming cassava peels (agricultural waste) to bioethanol, thereby contributing toward alternative energy supply as well as creating employment opportunity. This study is aimed at improving the bioethanol yields from cassava peels through the optimization of its fermentation processes using the Response Surface Methodology (RSM).

MATERIALS AND METHODS

Lignocellulose Feedstock Collection, Preparation, and Pretreatment: Cassava peels were procured from a local cassava processing plant in Ekosodin, Benin City, Edo State, Nigeria. It was sun-dried for three days before further drying in a preheated oven at 110 °C for 6 hours to reduce its moisture content and prevent biodeterioration. The dried cassava peels were milled into small particles to increase their surface area and make the cellulose readily available for hydrolysis. The dried bagasse was stored under dry conditions before use. Pre-treatment of biomass was performed by measuring 40 g of cassava peels which were soaked in 0.2M NaOH. The mixture was hydrothermally pretreated by heating with an autoclave at a temperature of about 120 °C and for

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about 90 mins. The different pretreated cassava peels slurries were obtained and used for further analysis.

Enzymatic Hydrolysis and Fermentation: The pretreated cassava peel slurries were allowed to cool to room temperature before they were neutralized using hydrochloric acid and then, hydrolyzed by alpha-amylase. Enzymatic hydrolysis was conducted at 55 °C at different hydrolysis time (1-4) hours. The buffer for hydrolysis was 0.05M acetate buffer (pH 4.8). After hydrolysis, the hydrolyzed sampled was used for fermentation for ethanol production. The medium was maintained at 30 °C inoculated with 5 g/l of activated dried yeast (*Saccharomyces cerevisiae*) at different enzymes loading in a flask and incubated at room temperature for 1-5 days. The percentage of ethanol yield (M) was determined as shown in equation (1):

$$M = \frac{N}{P} \quad (1)$$

For the percentage of the ethanol measured in the sample (N), a refractometer will be used to determine

the amount (%) of ethanol concentration (P) present in the sample, given in equation (2) (Nutawan *et al.*, 2010):

$$P = R \times 0.5 \quad (2)$$

Where R is the amount of initial sugar concentration in fermentation solution (%)

Experimental Design for Bioethanol Production: A three-variable Box-Behnken Design (BBD) for RSM was used to develop a statistical model for the fermentation process. This is because there was a need to determine the optimum variable combination that could give the desired output with a minimum number of the experiment, without the need for studying all possible combination experimentally. The less number of experimental design points reduced the cost of experiment and optimization processes. The levels of variables optimized are shown in Table 1.

Table 1: Coded and actual levels of the factors for four-factor Box-Behnken Design

Variables	Units	Symbols	Coded and actual levels		
			-1	0	+1
Hydrolysis time	Hours	X ₁	1	2.5	4
Enzymes Loading	mg/L	X ₂	0.5	1.0	1.5
Fermentation Time	Days	X ₃	1	3	5

This design is suitable for exploring quadratic response surfaces and construction of a second-order polynomial model (Amenaghawon *et al.*, 2014), the response of the surface to the various variables was used in optimizing the process using several experimental runs. The number of experimental runs is given by equation (3):

$$N = K^2 + K + C_p \quad (3)$$

Where K is the factor number and c_p replicate the number of the central point. Design Expert® 7.0.0 (Stat-ease, Inc. Minneapolis, USA), statistical software used to develop the experimental design. The coded and actual values of the independent variables were calculated as shown in equation (4):

$$x_i = \frac{X_i - X_o}{\Delta X_i} \quad (4)$$

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 center point and ΔX_i is the step-change in the actual value of the independent variable. The generalised second-order polynomial equation as shown in equation (5), was

adopted as a statistical model to estimate the response of the dependent variable (i.e. ethanol yields are obtained). This is because a linear model is insufficient for a mathematical description with adequate precision. That is, a linear model is prone to showing a high error value.

$$Y_i = b_o + \sum b_i X_i + \sum b_{ij} X_i X_j + \sum b_{ii} X_i^2 + e_i \quad (5)$$

Where Bi is the linear coefficient, Bii is the quadratic coefficient, Bij is the second-order term, Y_i is the predicted response or dependent variable, X_i and X_j are the independent variables, b_o is the offset term, b_i and b_{ij} are the single and interaction effect coefficients and e_i is the experimental error term (Amenaghawon *et al.*, 2013).

RESULTS AND DISCUSSION

Modelling and Optimization of Response: The coded and actual value of the factors X1 (Hydrolysis time, hours.), X2 (Enzymes Loading, mg/L) and X3 (Incubation time, days) as designed by design expert 7.0 and their corresponding responses are shown in Table 2:

Table 2: Three-level factorial Box-Behnken design and experimental responses of the dependent variable Y (Ethanol yield, %), RSM predicated responses

Run No.	Coded values of factors			Actual values of factors			Responses (%)	
	X1	X2	X3	X1	X2	X3	Actual	RSM predicated
1	0	0	0	2.5	1.0	3.0	1.899	1.78
2	1	-1	0	4.0	0.5	3.0	1.764	1.78
3	1	1	0	4.0	1.5	3.0	1.799	1.85
4	0	-1	1	2.5	0.5	5.0	1.701	1.83
5	0	-1	-1	2.5	0.5	1.0	1.399	1.41
6	0	0	0	2.5	1.0	3.0	1.851	1.39
7	0	0	0	2.5	1.0	3.0	1.899	1.73
8	0	1	1	2.5	1.5	5.0	1.799	1.71
9	1	0	-1	4.0	1.0	1.0	1.421	1.41
10	-1	0	-1	1.0	1.0	1.0	1.399	1.45
11	-1	0	1	1.0	1.0	5.0	1.699	1.70
12	-1	1	0	1.0	1.5	3.0	1.867	1.79
13	0	0	0	2.5	1.0	3.0	1.901	1.89
14	0	1	-1	2.5	1.5	1.0	1.45	1.89
15	1	0	1	4.0	1.0	5.0	1.72	1.89
16	0	0	0	2.5	1.0	3.0	1.911	1.89
17	-1	-1	0	1.0	0.5	3.0	1.811	1.89

Table 2 shows the observed value of the experiment. It can be seen that maximum ethanol yield was 1.91% and was observed at hydrolysis time of 2.5 hours, enzyme loading of 1.0 mg/L, and an incubation time of 3 days.

Determination of Appropriate Model: The investigation was carried out on linear, cubic, two-factor interaction, and quadratic models to determine the best model that is statistically significant and

best describes the relationship between the response and the inputs (independent variables). From the model summary statistics shown in Table 3, it is seen that the quadratic model has the maximum predicted and adjusted R² value. Thus, it can be concluded that the quadratic model best describes the relationship between the response and the independent variables.

Table 3: Model summary statistics.

Source	Std. Dev.	R-squared	Adjusted R-squared	Predicted R-squared	Press
Linear	0.17	0.3603	0.2127	-0.0902	0.61
2FI	0.19	0.3615	-0.0216	-1.2462	1.27
Quadratic*	0.030	0.9892	0.9753	0.8838	0.066
Cubic	0.024	0.9961	0.9842		+

Analysis of Variance (ANOVA): Regression analysis was performed to fit the response. The model developed represents ethanol yield (Y) as a function of

hydrolysis time (X₁), enzyme loading (X₂), and incubation time (X₃). The model is given by equation (6) in terms of the actual factors.

$$Y = 074415 + 0.12286X_1 + 0.26005X_2 + 0.48317X_3 - 0.0073X_1X_2 - 0.00833X_1X_3 + 0.011750X_2X_3 - 0.024322X_1^2 - 0.10890X_2^2 - 0.069431X_3^2 \tag{6}$$

The second-order polynomial (equation 6) was used to calculate the predicted responses presented in Table 2. A comparison of the predicted values with the responses obtained from the experiment shows a little deviation which mean that there is high extent of correspondence. The significance of fit for the second-order polynomial for the ethanol yield was investigated by carrying out an analysis of variance (ANOVA) as shown in Tables 4 and 5. The model F value of 71.12 and very low p-value (<0.0001) implies that the investigated process variables significantly influenced the output. Since all the terms in a regression model aren't equally important, the p-value

is used to check the significance of each coefficient. The "lack of fit" F value of 2.329 implies that there was an insignificant lack of fit. The authenticity of the model was assessed through the lack of fit test. The P-values for the lack of fit test was not significant (P>0.05) demonstrating that the model was fit for predicting the ethanol yield. The coefficient of variation (CV) obtained was 1.71% (Table 5). This value indicates the degree of precision with which the treatments were compared (Qi *et al.*, 2009). The relatively low value of CV obtained showed that the treatments were carried out with high precision and reliability (Montgomery, 2005; Mason *et al.*, 1989).

Table 4: Analysis of variance for response surface quadratic model

Source	Sum of Squares	Df	Mean Square	F Value	p-value Prob > F	
Model	0.5577	9	0.062	71.12	< 0.0001	Significant
X ₁	0.0006	1	0.0006	0.744	0.4171	
X ₂	0.0072	1	0.0072	8.263	0.0238	
X ₃	0.1953	1	0.1953	224.1	< 0.0001	
X ₁ X ₂	0.0001	1	0.0001	0.127	0.7325	
X ₁ X ₃	2E-07	1	2E-07	3E-04	0.987	
X ₂ X ₃	0.0006	1	0.0006	0.634	0.4521	
X ₁ ²	0.0126	1	0.0126	14.47	0.0067	
X ₂ ²	0.0031	1	0.0031	3.581	0.1003	
X ₃ ²	0.3248	1	0.3248	372.7	< 0.0001	
Residual	0.0061	7	0.0009			
Lack of Fit	0.0039	3	0.0013	2.329	0.2159	not significant
Pure Error	0.0022	4	0.0006			
Cor Total	0.5638	16				

An Adequate precision value of 21.97 was obtained. Cao *et al.* (2009) reported that adequate precision gives an indication of the signal-to-noise ratio and suggested that a value greater than 4 is generally desired. The value of 21.97 obtained indicates an adequate signal and the model can be used to navigate the design space. The coefficient of determination (R^2) was obtained as 0.989. This indicated that the model satisfactorily represents the relationship between the independent variables (hydrolysis time, enzyme loading, and incubation time) and the response (ethanol yield). The R^2 value indicates that 98.9% of the variability in the response could be explained by the statistical model, while 1.1% could not be accounted for by the independent variables (Amenaghawon *et al.*, 2014). The R^2 value indicates the degree to which the model was able to predict the response. The closer the R^2 value is unity, the better the model can predict the response (Qi *et al.*, 2009). The "Predicated R-Squared" of 0.8838 is in reasonable agreement with the "Adjusted R-Squared" of 0.9753, that is, the difference is less than 0.2.

Table 5: Statistical information for ANOVA

Parameter	Value
Standard deviation	0.03
Mean	1.723
C.V. %	1.71
PRESS	0.07
R-Squared	0.989
Adj R-Squared	0.975
Pred R-Squared	0.884
Adeq Precision	21.97

Parity Plot: The actual and predicted responses shown in Table 2 were plotted to analyze the correlation between them as shown in Figure 1. It is observed from the plot that the data points are distributed near the straight line. This further indicates that the quadratic model could be

employed as a significant model for predicting response over the independent input variables.

Optimization of Ethanol Fermentation of Cassava Peels: 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. To determine the optimal levels of the independent variables affecting the ethanol yield from cassava peels, three-dimensional (3D) response surface and contour plots were constructed according to the regression model. The 3D plots were generated by keeping two factors at their optimum point and varying the other two factors within their experimental ranges. The plots show how hydrolysis time, enzyme loading, incubation time affects the ethanol yield. The contour plot may be rising ridges, saddle point, elliptical or circular plot. A circular or elliptical plot indicates that there exists a significant interaction between the operating parameters (Kanmani *et al.*, 2013).

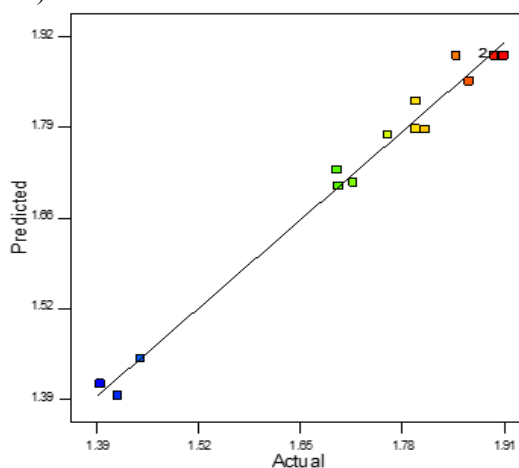


Fig 1: Parity plot of predicted values against actual values of ethanol yield

Analysis of Response Surface Plots. A. Interaction between hydrolysis time, enzymes loading, and ethanol yield: The effect of hydrolysis time and enzyme loading on the ethanol yield is shown in Figure 2. It was observed that an increase in hydrolysis time and also an increase in enzyme loading, increased ethanol yield. The plot showed that the enzyme loading had a significant effect on the ethanol yield within the range of hydrolysis time investigated (1-4 hours). If the enzyme loading is too high, a problem with enzyme accessibility could inhibit the conversion of cellulose. The increasing of viscosity material could also affect to reduce enzyme mobility. The longer the hydrolysis time, the more cellulose is converted into simple glucose so that when a lot of simple glucose fermentation process can be converted by

saccharomyces cerevisiae into bioethanol, but an extension of the hydrolysis time, (> 2.5 hours.) can negatively affect the degree of saccharification, probably due to enzymes denaturation. The maximum ethanol yield was obtained at an enzyme loading of 1.0 mg/L and a time of about 3 hours hydrolysis time. It can be observed that any further increase in time leads to a decrease in the ethanol yield. A similar result was reported by (Betiku and Taiwo, 2015) who reported optimum enzyme loading is a ratio of 1.0 mg/L for the production of ethanol from breadfruit starch hydrolysate. Similarly, (Hajar *et al.*, 2012) reported an optimum enzyme loading of 1.5 mg/L during a hydrolysis time of 2.5 hours to obtain an ethanol yield of 1.93% from pineapple peel extract.

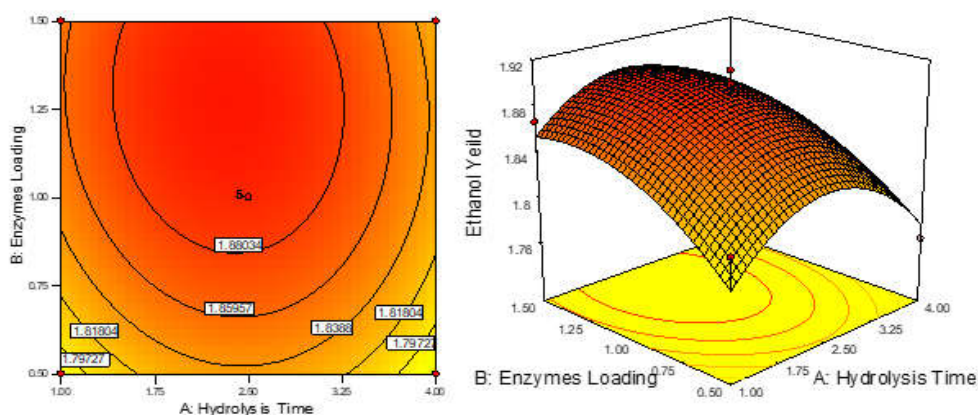


Fig 2: Response surface plot and corresponding contour plot of the simultaneous effect of hydrolysis time and enzyme loading on the ethanol yield.

B. Interaction between hydrolysis time, incubation time, and ethanol yield: Figure 3 shows the interactive effect of hydrolysis time and incubation time. It was observed that an increase in incubation time and hydrolysis time leads to an increase in ethanol yield. The production of ethanol starts after the lag phase of about 1 hour and reached a maximum of about 3 hours. As the fermentation keeps on going longer, the ethanol production became stagnant or even decreased. This might be caused by the formation of inhibitory compounds, such as furfural, acetic acid, and phenolic. Phenolic compounds are produced from partial lignin damage, which could occur during a pretreatment process. This corresponds to the low delignification degree, hence the remains of the pretreatment process caused inhibition. During fermentation, a decrease in ethanol could also be caused by the composition of the substrate, reduction of the enzyme's active sides, and the inefficiency of mass transfer. A similar result was reported in a previous study (Nanssou *et al.*, 2016) who reported an

optimum incubation time of 3 hours was observed for the production of ethanol from plantain peel.

C. Interaction between enzymes loading, incubation time, and ethanol yield: Figure 4 illustrates the effect of enzyme loading and incubation time on ethanol yield. It was observed that an increase in enzyme loading, increase in incubation time leads to an increase in ethanol yield. At low incubation times, the ethanol yield increased slowly with an increase in enzyme loading from 0.5 mg/L to 1.5 mg/L. For the range of enzyme loading investigated, intermediate levels of the time were needed to obtain a high ethanol yield, and any further increase in time resulted in a decrease in ethanol yield. The maximum ethanol yield of about 1.911% was obtained at an incubation time of about 3 days and enzyme loading of about 1.0 mg/L. Shanavas *et al.* (2011) reported the optimum yield of ethanol from cassava starch was 2.0% using an enzyme loading of 1.0 mg/L and a reaction time of 180 mins.

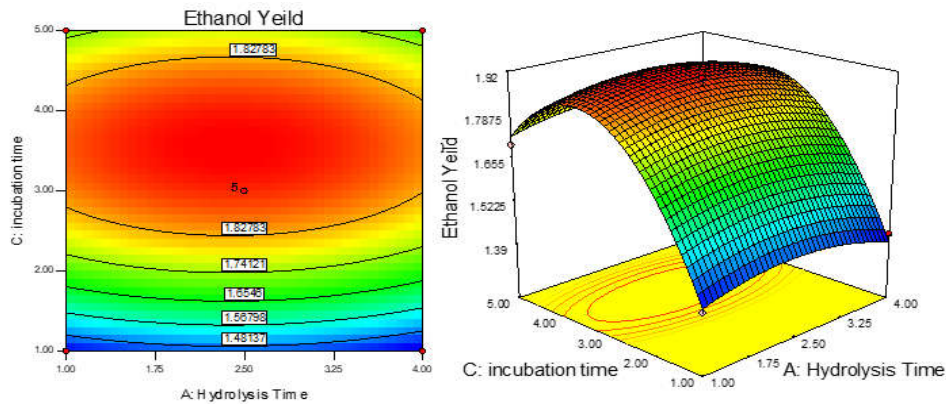


Fig 3: Response surface plot and corresponding contour plot of the simultaneous effect of hydrolysis time and incubation time on the ethanol yield

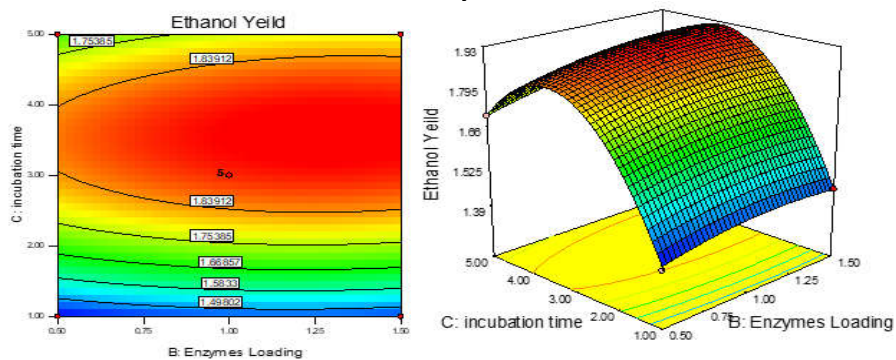


Fig 4: Response surface plot and corresponding contour plot of the simultaneous effect of enzymes loading and incubation time on the ethanol yield

Numerical Optimization: Figure 5 below, shows that hydrolysis time, enzyme loading, and incubation time were optimized based on the model developed by each response. The maximum ethanol yield from cassava

predicted by the model was 1.913%. The optimal hydrolysis time, enzymes loading and incubation time were 2.9 hours, 1.42 mg/L, and 3.58 days respectively.

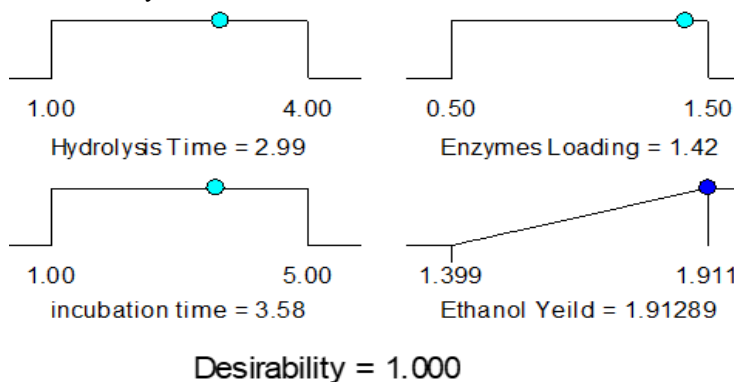


Fig 4: Ramp numerical optimization of response

Validation of Statistical Model: To confirm the validity of the statistical model, three confirmation experimental runs were performed at the chosen optimum fermentation conditions. The result shows that the maximum experimental ethanol yield of 1.911% obtained was close to the predicted value of 1.913%. The excellent correlation between the

predicted and measured values of these experiments shows the validity of the statistical model.

Conclusions: This study investigated the performance of RSM on the modeling and optimization of the three variables (hydrolysis time, enzyme loading, and incubation time) on ethanol production from cassava peels. A significant quadratic model

($p < 0.05$) has been obtained to predict the concentration of ethanol yield as a function of the three input factors using RSM. Further investigation into the use of cassava peels for bioethanol production is required. Also, more agro-waste products are needed to check for their usability for producing value-added products.

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