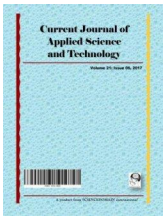

Current Journal of Applied Science and Technology

28(4): 1-14, 2018; Article no.CJAST.42906
ISSN: 2457-1024
(Past name: British Journal of Applied Science & Technology, Past ISSN: 2231-0843, NLM ID: 101664541)

Statistical Modelling of Enzymatic Hydrolysis of Banana Peels for Bioethanol Production

Joseph Tagbo Nwabanne[1] and Charles Bernard Aghadi[1*]

[1]Department of Chemical Engineering, Faculty of Engineering, Nnamdi Azikiwe University, Awka, Nigeria.

Authors' contributions

This work was carried out in collaboration between both authors. Author JTN designed the study, wrote the protocol and wrote the first draft of the manuscript. Author CBA performed the statistical analysis, managed the analyses of the study and managed the literature searches. Both authors read and approved the final manuscript.

Article Information

DOI: 10.9734/CJAST/2018/42906
Editor(s):
(1) Elena Lanchares Sancho, Professor, Department of Mechanical Engineering, University of Zaragoza, Zaragoza, Spain.
Reviewers:
(1) Anju Arora, Indian Agricultural Research Institute, India.
(2) Gisel Chenard Díaz, Federal University of Rio de Janeiro (UFRJ), School of Chemistry (EQ), GREENTEC Laboratory, Brazil.
(3) Yinusa Taiwo Saheed, Federal University Oye-Ekiti, Nigeria.
Complete Peer review History: http://www.sciencedomain.org/review-history/25653

Original Research Article

Received 13th May 2018
Accepted 23rd July 2018
Published 25th July 2018

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

**Corresponding author: E-mail: charles00716@gmail.com;*

8.1% volume weight. This works shows that the 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.

Keywords: Banana peel; enzymatic hydrolysis; fermentation; distillation; optimization.

1. INTRODUCTION

The global production of banana is estimated to be around 96 million tons with approximately a 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].

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

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

2. MATERIALS AND METHODS

2.1 Collection and Preparation of Banana Peel

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

2.2 Composition of Banana Peel

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

2.3 Determination of Proximate Composition

i. The moisture content was determined based on ASTM standard method [11].
ii. The ash content was determined using ASTM standard method [11].
iii. The fibre content was determined according to the standard method of ASTM [12].

iv. The lipid contents of the peels were determined by Soxhlet Fat Extraction method [13].
v. The total protein content was determined by the sum of the total nitrogen using the micro Kjeldahl procedure. The amount of protein obtained was multiplied by the nitrogen content of 6.25 (a constant factor) [14].
vi. The cellulose content is calculated using equation 2.1 [2].

% Cellulose = 100 – (% Protein + % Moisture + % Ash + % Fat + % Fibre) (2.1)

2.4 Enzyme Preparation

2.4.1 Substrate preparation

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

2.4.2 Identification of microorganism (*Aspergillus niger*)

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

2.4.3 Inoculum development

The inoculum was developed using a standard method [16]. Three (3) agar plugs from the Petri dishes were inoculated into one litre Erlenmeyer flask containing 500 ml of sterile fermentation medium. The fermentation medium comprises 20 g/l raw potato starch, 16 g/l peptone, 10 g/l $(NH_4)HPO_4$, 1 g/l NaCl and 1 g/l $MgSO_47H_2O$ in de-ionized water.

2.5 Inoculums Preparation

2.5.1 Hydrolysis inoculums

The inoculum (5%) of the multiplied *A. niger* from a PDA slant was prepared by aseptically transferring 50 g of the pure and screened *A. niger* from the slant to a 1liter volumetric flask. Distilled water autoclaved at 121ºC for 15 minutes was added to make the mark of the flask. The mixture was left for 10 minutes at 150 rpm. The inoculums size was set to have a cell concentration of 1.0×10^8 cells per ml [17].

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

2.5.2 Fermentation inoculums

100 ml of distilled water was heated to 40ºC in a shaker flask and 0.5 % (w/w) of *Saccharomyces cerevisiae* yeast was added to the warm water. The mixture was left at 150 rpm for 10 minutes. The inoculums size was set to have a cell concentration of 5.3×10^7 cells per ml, dilution of the inoculums was done when the concentration of the cells was too high [18].

2.6 Hydrolysis, Fermentation and Distillation Processes

2.6.1 Enzymatic hydrolysis with *Aspergillus niger*

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

2.6.2 Fermentation using *Saccharomyces cerevisiae*

A 250 cm^3 conical flask containing 30cm^3 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 Table 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

3. RESULTS AND DISCUSSION

3.1 Composition Analysis Results

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

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

Std	X_1:temp	X_2:pH	X_3:time	Reducing sugars yield	Std	X_1:temp	X_2:pH	X_3:time	Ethanol yield (BP)
	^{o}C		Day	mg/ml		^{o}C		Day	%
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

The results from the proximate analysis of the BP is presented in Table 2a. From the table, it can be seen that BP contained 6% protein. Feumba et al., [23] reported a close protein content of 10.44%.

The fibre content of the peels was found to be 15.4%. Abubakar et al., [24] also reported a similar fibre content of 14.8%.

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

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

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

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.

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

Residual is the difference between the observed value of the dependent variable and the predicted value. Residual values are used for residual plots (Figs. 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 (Figs. 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.

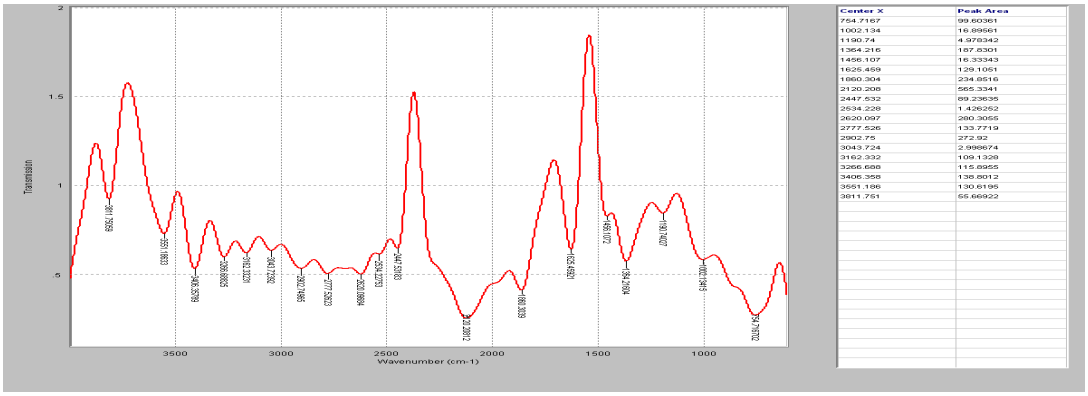

Fig.1. FTIR result of banana peels

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 (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 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 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 (Pred R^2) is used to determine how well a regression model can make predictions. 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 Adj-R^2 and 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.

Figs. 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]. Figs 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 (Figs. 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 (Figs 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_1X_2	0.045	1	0.045	0.259624	0.6214	
X_1X_3	0.052	1	0.053	0.344533	1.0000	
X_2X_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_1X_2	2.42	1	2.42	23.2789	0.0007	
X_1X_3	0.72	1	0.72	6.925953	0.0251	
X_2X_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

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.

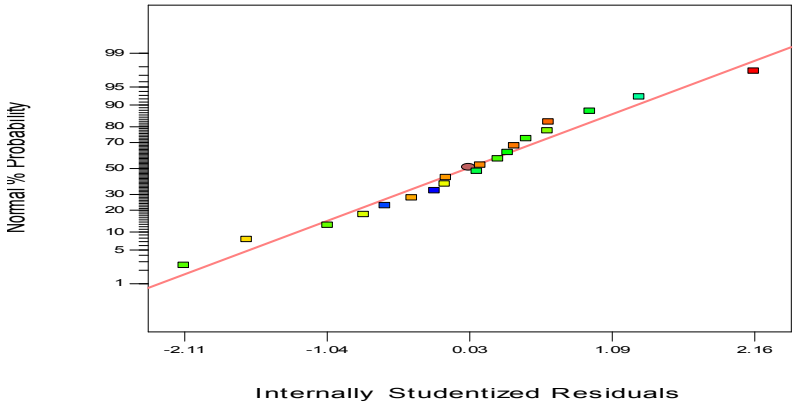

Fig. 2. Normal plots of residuals (hydrolysis process)

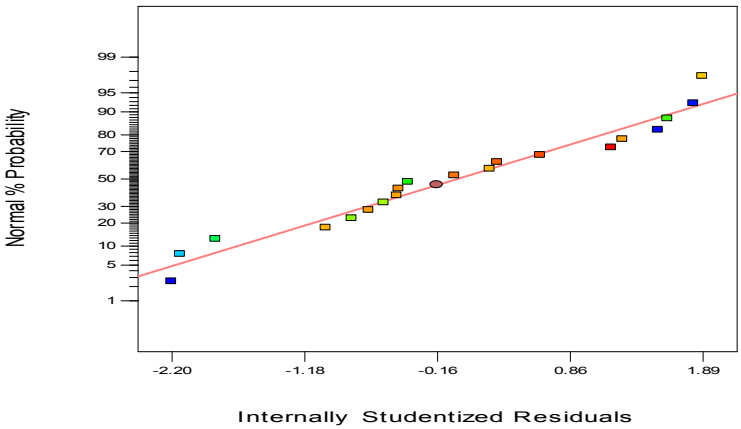

Fig. 3. Normal plots of residuals (fermentation process)

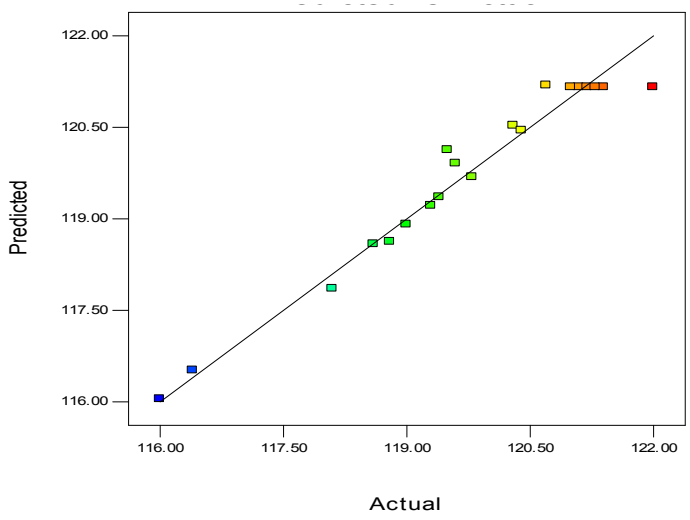

Fig. 4. The plot of predicted vs actual (hydrolysis process)

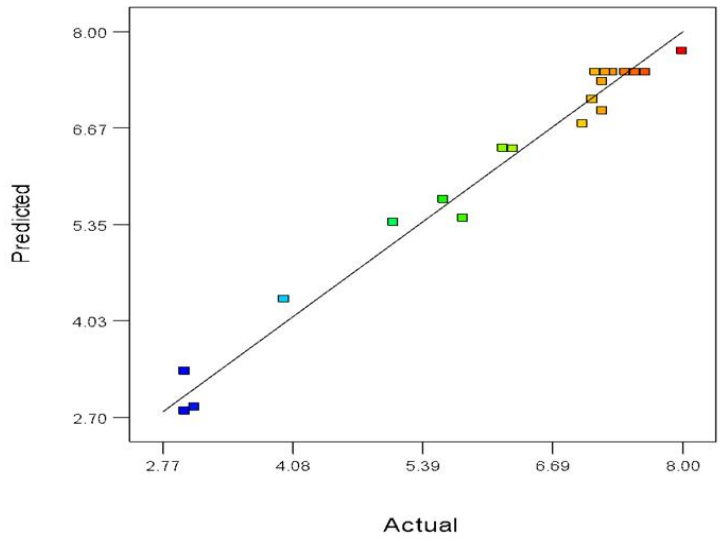

Fig. 5. The plot of predicted vs actual (fermentation process)

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.

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

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

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.

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

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

3.2.3 Three dimensional (3D) response surface plots

The 3-D response surface plots are presented in Figs. 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.

Figs. 6-8 show the 3-D response surface plots for the hydrolysis process. Fig. 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. Fig. 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 (Fig. 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.

Figs. 9-11 show the 3-D surface plots for the fermentation process. Fig. 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. Fig. 10 showed a similar temperature effect but it interaction with time was not significant. The interaction of time with pH was however significant (Fig. 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.

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.

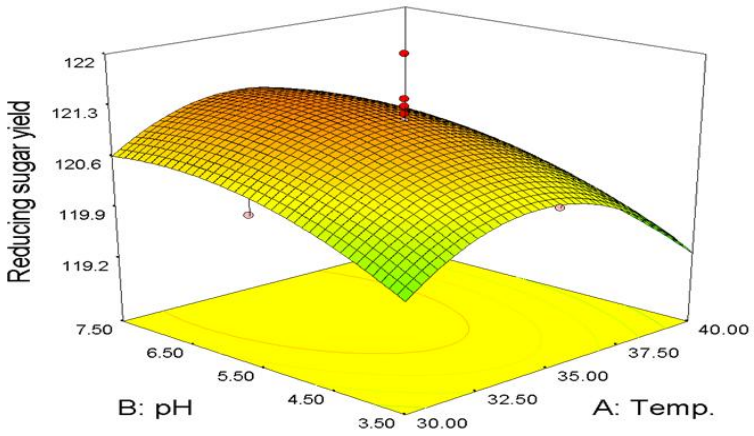

Fig. 6. pH Vs temperature 3D plot for hydrolysis

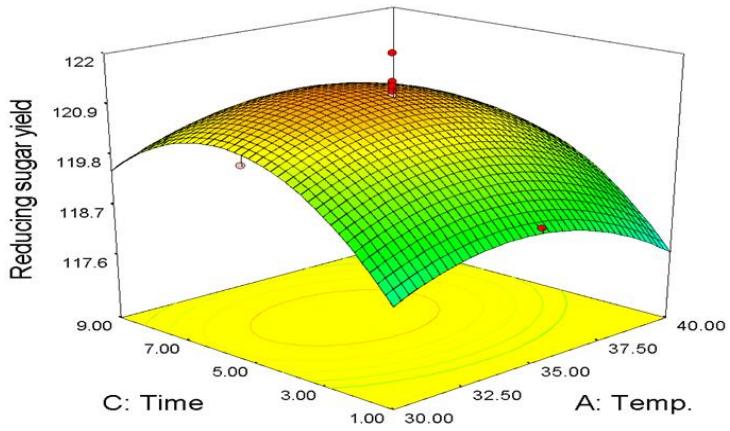

Fig. 7. Time Vs temperature 3D plot for hydrolysis

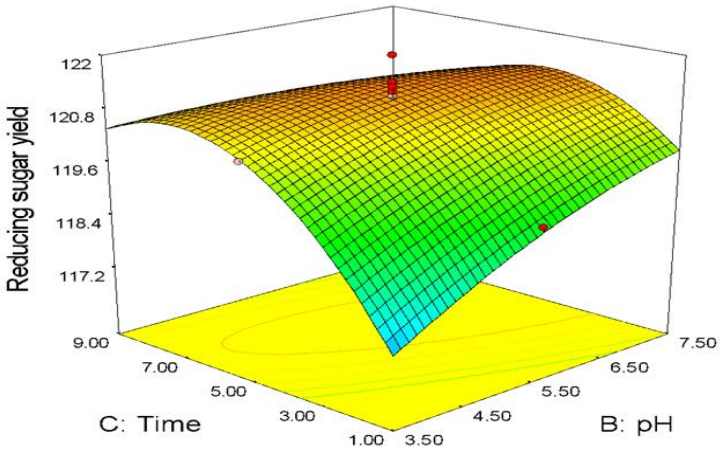

Fig. 8. Time Vs pH 3D plot for hydrolysis

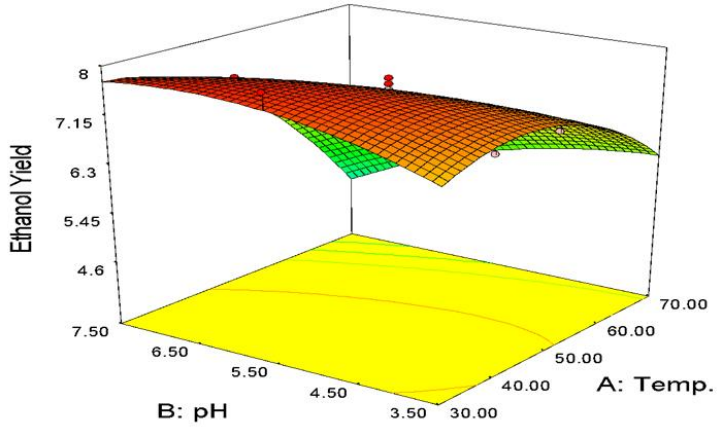

Fig. 9. pH Vs temperature 3D plot for fermentation

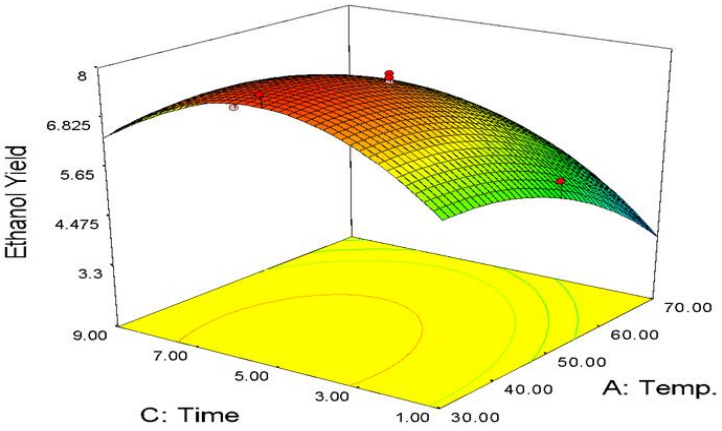

Fig. 10. Time Vs temperature 3D plot for fermentation

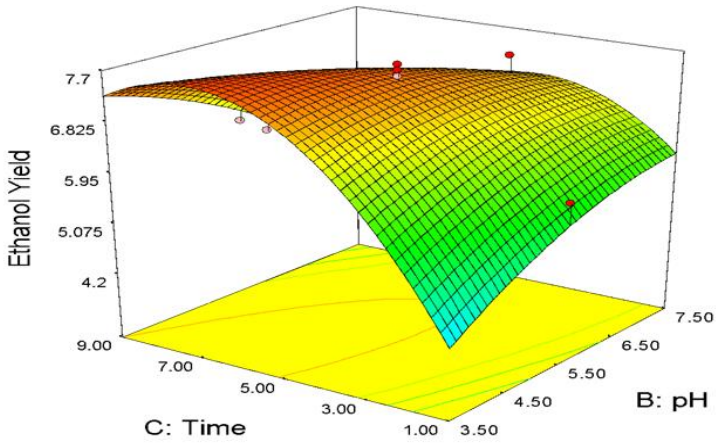

Fig. 11. Time Vs pH 3D plot for fermentation

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

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.

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.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Food and Agriculture Organization of the United Nations. FAO Publications Catalogue, Rome, Italy. 2017. Available:http://www.fao.org/3/b-i6407e.pdf
2. Anhwange BA, Ugye TJ, Nyiaatagher TD. The chemical composition of *Musa*

sapientum (banana) peels. Electronic Journal of Environmental Agricultural and Food Chemistry. 2009;8(1):437-442.

3. Taherzadeh MJ, Karimi K. Acid-based hydrolysis processes for ethanol from lignocellulosic materials: A Review. Bio Resources. 2007;2(1):472-499.
4. Lynd LR. Overview and evaluation of fuel ethanol from cellulosic biomass: technology, economics, the environment, and policy. Annual Reviews, Energy Environment. 1996;21(1):403-465.
5. Ezeonu NC. Alternative sources of petrochemicals from readily available biomass and agro-products in africa: a review. Journal of Petrochemical and Environmental Biotechnology. 2016;7(1): 301-305.
6. Chongkhong Sininart. Response surface optimization of ethanol production from banana peels by organic acid hydrolysis and fermentation. Songklanakarin Journal of Science and Technology. 2017;39(2): 245-252.
7. Barve Apurva, Kishori Tarfe. The efficiency of waste banana peels in bioethanol production, research & reviews. A Journal of Life Sciences. 2017;7(3):28-32.
8. Vaitheki S, Deepa B. A comparative study on the production of bioethanol from individual and mixed fruit wastes. Imperial Journal of Interdisciplinary Research (IJIR). 2016;2(5):652-653.
9. Alula Gebregergs. Optimized ethanol production from banana peel. Thesis submitted to the Department of biological and chemical engineering, Mekelle institute of technology, Ethiopia; 2014.
10. Amenaghawon NA, Osagie EI, Ogbeide SE. Optimization of combined acid and enzymatic hydrolysis of cocoyam starch to produce fermentable hydrolysate. Pertanika Journal of Science and Technology. 2016;24(1):123–136.
11. ASTM D2974-14, standard test methods for moisture, ash, and organic matter of peat and other organic soils. ASTM International. West Conshohocken, PA; 2014.
12. ASTM D629-15, standard test methods for quantitative analysis of textiles. ASTM International. West Conshohocken, PA; 2015.
13. AOAC official method of analysis. Method 945.16. Oil in cereal adjuncts. 18th ed. AOAC International. Gaithersburg, MD; 2005.
14. Kjeldahl J. A new method for the determination of Nitrogen in organic matter. Zeitschrift für Analytische Chemie. 1883;22:366-382.
15. National Committee for Clinical Laboratory Standards (NCCLS). Reference method for broth dilution antifungal susceptibility testing of conidium-forming filamentous fungi. Proposed standard M38-P. National Committee for Clinical Laboratory Standards. Wayne, Pa; 1998.
16. American Association for Clinical Chemistry (AACC) Method 44–15A. Approved Methods of the AACC. St. Paul, MN; 1983.
17. Igbokwe PK, Idogwu CN, Nwabanne JT. Enzymatic hydrolysis and fermentation of plantain peels: Optimization and kinetic studies. Advances in Chemical Engineering and Science. 2016;6:216-235.
18. Ahmad F, Jameel AT, Kamarudin MH, Mel M. Study of growth kinetic and modeling of ethanol production by saccharomyces cerevisiae. African Journal of Biotechnology. 2011;16:18842-18846.
19. Miller GL. Use of dinitrosalicylic acid reagent for determination of reducing sugar. Analytical Chemistry. 1959;31(3): 426-428.
20. Itelima J., Onwuliri F, Onwuliri E, Onyimba I, Oforji S. Bioethanol production from banana, plantain and pineapple peels by simultaneous saccharification and fermentation process. International Journal of Environmental Science and Development. 2013;4(2):213-216.
21. Oyeleke SB, Jibrin NM. Production of bioethanol from guinea cornhusk and millet husk. African Journal of Microbiology Research. 2009;3(4):147-152.
22. Silverstein RM, Bassler GC, Morrill TC. Spectrometric identification of organic compounds. 4th ed. John Wiley and Sons. New York, USA; 1981.
23. Feumba DR, Ashwini RP, Ragu M. Chemical composition of some selected fruit peels. European Journal of Food Science and Technology. 2016;4(4):12-21.
24. Abubakar US, Yusuf KM, Safiyanu I, Abdullahi S, Saidu SR, Abdu GT. Proximate and mineral composition of corn

cob, banana and plantain peels. International Journal of Food Science and Nutrition. 2016;1:25-27.

25. Shrivastava A, Sandagar P, Baja I, Singhal R. Media optimization for the production of U-linolenic acid by cunninghamella echinulata varielegans MTCC 522 using response surface methodology. International Journal of Food Engineering. 2008;4(2):1-32.

26. Draper NR, Smith H. Applied Regression Analysis, 2nd edition, Wiley press NewYork; 1981.

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sciencedomain.org/review-history/25653