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OPTIMIZATION OF THE FERMENTATION PARAMETERS FOR THE PRODUCTION OF ETHANOL FROM AGRICULTURAL WASTE

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Abstract

In this study, design of experiment for response surface methodology was used to analyse and optimise the simultaneous effect of solid substrate loading, broth pH and concentration of acid used for hydrolysis during bioethanol production from corn cob via hydrolysis and fermentation using Saccharomyces cerevisiae. A three-variable central composite design was used to develop a statistical model to describe the relationship between concentration of ethanol produced and the selected independent variables. The fermentation conditions were then optimised using RSM. The model was statistically significant, with a low standard deviation (0.13) and did not show lack of fit (R^2=0.9884). Ethanol production was significant at high levels of solid loading, pH and acid concentration indicating that these variables positively influenced ethanol production. The optimum values of solid substrate loading, pH and acid concentration obtained from RSM were 9.2 g, 5.6 and 1.7 %w/w respectively. Under these conditions, the ethanol concentration was obtained as 6.41 %v/v. The developed model was validated to predict the yield of ethanol during fermentation.The observed results indicate the viability of corn cob as a bioethanol feedstock and corroborate the efficiency of central composite design (CCD) in determining the optimum values of the fermentation parameters for maximum ethanol production.

***Keywords*:** Response surface methodology, Optimisation, corn cobs, Hydrolysis, Central composite design

1.0 INTRODUCTION

In the last few decades, there has been an excessive use and reliance on fossil fuel as the only viable source of energy. Again, increasing prices of petroleum products, and environmental concerns regarding fossil fuel usage have led to increased research into development of sustainable alternative sources of energy [1-3].Because possible crisis of fossil fuel availability at record high prices and the global energy crisis [4],biofuels have been considered as suitable alternatives as they are cleaner than fossil fuels[5].

Biofuel is a type of fuel whose energy is derived from biological carbon fixation. Biofuels include fuels derived from biomass conversion [6]. Bioethanol is a biofuel which is an alcohol made by fermentation, mostly from carbohydrates produced in sugar or starch crops such as corn or sugarcane [6].It has been acknowledged worldwide that agricultural residues are one of the best choices to replace grains for fuel ethanol production, without endangering food security, although many challenges still exist for their commercial conversions, due to their recalcitrance to degradation as well as their unique chemical composition[7]. Lignocelluse materials are mostly agricultural wastes like, wood, wood chips, corn cobs, rice shafts etc., and the bioconversion of lignocellulosic materials to fermentable sugars and then to bioethanol has been considered as cost effective, as this materials are renewable and would otherwise serve as low grade feedstock for animal grazing [8].

Corn cobs is the by-product of the corn crop, it has a woody material, and is sometimes used as a low grade feed stock for grazin animals. It has a typical composition of 45% of cellulose, 30% - 35% of hemicellulose, and 15% of lignin. It is readily available, has a good xarbohydrate composition that has made it considerable as a lignocellulosic feedstock for bioethanol production.The conversion of lignocellulosic materials to bioethanol basically involves three processes; pretreatment,

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hydrolysis and fermentation. Pretreatment involves the initial steps taken to break down lignin, hydrolysis is done mostly by dilute acid or enzymes, it is done to produce fermentable sugars from lignocellulosic materials [9]. The hydrolyzate from this is then fermented using a suitable enzyme, most commonly *Saccharomyces cerevisiae* as it can ferment increased amounts of sugars in the medium when the necessary nutrients and conditions are at optimum levels [10].

Design of experiment (DOE) for response surface methodology (RSM) is an important strategy for optimising multivariable processes. It is quicker method of performing experiments with different variables for optimal result. [11]. And it has been successfully applied to various experiments in optimizing of bioprocesses [12-14].

The objective of this study is to optimise the production of bioethanol from corn cobs via separate hydrolysis and fermentation using *Saccharomyces cerevisiae.* A three variable central composite design was used to study the effect of acid concentration, solid substrate loading and fermentation pH on the concentration of ethanol produced. Response surface methodology was used to obtain the optimum values of these variables as well as the chosen response (ethanol concentration). This was done by generating response surface plots showing the effect of any two variables on ethanol concentration while keeping the third variable constant.

2.0 MATERIALS AND METHODS.

2.1 Feedstock Collection and Pretreatment.

Corn cobs was collected from a waste bin at Uselu market in Benin City, Edo state,Nigeria. Its extraneous materials were removed and then sun dried.Combined pretreatment was performed on the corn cobs. Physical (Size reduction) and Chemical (alkaline) pretreatments was done after milling to particle sizes of 2 mm and less. Alkaline pretreatment was done using 40g of NaOH pellets dissolved in 500ml of water to attain a 2M of NaOH, the prepared concentration was then mixed with the ground corn cobs and allowed to heat at 100^{o}C. the solution was then filtered and the filtrate was washed to removed the high alkalinity and allowed to dry.

2.2 Hydrolysis of Samples

Acid hydrolysis of the corn cobs was carried out in an autoclave using dilute sulphuric acid concentration in the range 0.5-2.0%w/w at a temperature of 100^{o}C for 30 minutes according to the experimental design. The solid loading was varied from 6 to 10 g. At the end of the hydrolysis reaction, the solid residue was separated by the use of whatmann's filter paper. The hydrolysate was stored for further use.

2.3 Culture Media and Fermentation

The hydrolyzates obtained were adjusted pH-wise by the introduction of requisite amounts of base and acid in order to meet the specifications provided by the design software. The fermentation studies were carried out using dry Baker's yeast (*Saccharomyces cerevisiae)* in the hydrolyzates obtained. 10ml of activated yeast was inserted into each run case. Activation of the dry baker's yeast was done by heating up 50g of yeast in 100ml of distilled water for a few minutes. 5g of glucose was added to the broth, following the inclusion of 10ml of activated yeast. The fermentation is carried out 72 hours.

2.4 Experimental Design

A three variable central composite design (CCD) for response surface methodology was used to develop a statistical model for the fermentation process. The ranges of the variables that were optimised (fermentation pH, solid substrate loading and concentration of acid used for hydrolysis) are as shown in Table 1. The CCD is a design that combines the vertices of the hypercube whose coordinates are given by a 2^n factorial design with star points [15]. The star points provide the estimation of curvature of the nonlinear response surface. The experimental design made up of 20 runs was developed using Design Expert® 7.0.0 (Stat-ease, Inc. Minneapolis, USA). The levels of the independent variables as shown in Table 1 were selected based on preliminary experiments. The relation between the coded values and actual values are described as follows:

$$x_i = \frac{X_i - X_0}{\Delta X} \quad (1)$$

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 centre point and ΔX_i is the step change in the actual value of the independent variable.

Table 1: Independent variables and their levels for CCD experimental design

Independent Variable	Symbols	Coded and Actual Levels				
		-α	-1	0	+1	+α
Fermentation pH	X_1	4	4.41	5	5.59	6
Solids loading (g)	X_2	6	6.81	8	9.19	10
Acid concentration (%w/w)	X_3	0.50	0.80	1.25	1.70	2

A second degree polynomial was fitted to the experimental data using the statistical package Design Expert® 7.0.0 (Stat-ease, Inc. Minneapolis, USA) to estimate the response of the dependent variable.The following generalised second order polynomial equation was used to estimate the response of the dependent variable.

$$Y = b_0 + \sum b_i X_i + \sum b_{ii} X_i^2 + \sum \sum b_{ij} X_i X_j + E \quad (2)$$

where Y_i is the predicted response, $X_i$and X_j are the independent variables, b_o is offset term, b_i and b_{ij} are the single and interaction effect coefficients and E is the error term.

The complete experimental design and results consisting of coded levels, actual variables, predicted responses (ethanol yield)are given in Table 2 alongside the experimental data for comparison.

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

Run No.	Factors						Response	
	Coded values			Actual values			Ethanol produced (% v/v)	
	x_1	x_2	x_3	X_1	X_2	X_3	Experimental	Predicted
1	0	0	-α	5.00	8.00	0.50	4.09	4.38
2	-1	+1	+1	4.41	9.19	1.70	3.36	3.62
3	+1	-1	+1	5.59	6.81	1.70	4.03	4.25
4	0	0	0	5.00	8.00	1.25	4.11	4.19
5	-1	-1	-1	4.41	6.81	0.80	5.56	5.89
6	-1	+1	-1	4.41	9.19	0.80	4.05	4.44
7	0	+α	0	5.00	10.00	1.25	4.31	4.67
8	-1	-1	+1	4.41	6.81	1.70	1.23	1.55
9	+1	+1	-1	5.59	9.19	0.80	2.69	2.98
10	0	0	0	5.00	8.00	1.25	3.86	4.19
11	0	0	+α	5.00	8.00	2.00	3.45	3.99
12	+1	-1	-1	5.59	6.81	0.80	3.49	3.90
13	-α	0	0	4.00	8.00	1.25	3.52	3.66
14	0	-α	0	5.00	6.00	1.25	3.5	3.71
15	0	0	0	5.00	8.00	1.25	4.1	4.19
16	0	0	0	5.00	8.00	1.25	3.95	4.19
17	0	0	0	5.00	8.00	1.25	4.03	4.19
18	+1	+1	+1	5.59	9.19	1.70	6.52	6.85
19	0	0	0	5.00	8.00	1.25	3.94	4.19
20	+α	0	0	6.00	8.00	1.25	4.36	4.71

3.0 RESULTS AND DISCUSSIONS

3.1 Modelling Using RSM

The results obtained from the 20 experimental runs carried out according to the Box-Behnken design are summarised in Table 2. The proposed second degree polynomial was fitted to the data presented in Table 2 using multiple linear regressions to determine the optimum conditions for the fermentation of corn cob. By applying multiple regression analysis on the experimental data, the following second degree polynomial was found to represent the relationship between the total reducing sugar produced and acid concentration, pretreatment time and pretreatment temperature adequately. By applying multiple regression analysis on the experimental data, the following second degree polynomial was found to represent the relationship between pH of fermentation, solid substrate loading and concentration of acid used for hydrolysis for the production of bioethanol from corn cobs using *Saccharomyces cerevisiae:*

$$Y = 51.59 - 6.52\,X_1 - 2.76X_2 - 35.48X_3 + 0.19X_1X_2 + 4.42\,X_1X_3 + 1.63X_2X_3 \quad (3)$$

where X_1, X_2 and X_3, represent the pH of fermentation, solid substrate loading and concentration of acid used for hydrolysis respectively. The response, Y is ethanol concentration.

The statistical software package 'Design Expert' has been used for regression analysis of the experimental data and to draw the response surface plot. Analaysis of variance (ANOVA) was used to estimate the statistical characteristics of the model fitting. The results of the ANOVA carried out to determine the fit of the statistical model are presented in Tables 3 and 4.In

order to ensure a good model, a test for significance of the regression model and individual model coefficients was needed to be performed accompanying with the lack-of-fit test. Normally, the significant factors can be ranked based on the F-value or p-value (also named 'Prob. > F' value). The larger the magnitude of the F-value and correspondingly the smaller the 'Prob. > F' value, the more significant is the corresponding coefficient [16].

Table 3: ANOVA for quadratic model

Source	Sum of Squares	Degree of Freedom (DF)	Mean Square	F - Value	p-value Prob > F	Remarks
Model	19.51	6	3.25	184.84	< 0.0001	Significant
X_1	1.14	1	1.14	64.70	< 0.0001	Significant
X_2	0.99	1	0.99	56.13	< 0.0001	Significant
X_3	0.22	1	0.22	12.40	0.0038	Significant
$X_1 X_2$	0.14	1	0.14	8.13	0.0136	Significant
$X_1 X_3$	11.02	1	11.02	626.46	< 0.0001	Significant
$X_2 X_3$	6.00	1	6.00	341.22	< 0.0001	Significant
Residual	0.23	13	0.018			
Lack of Fit	0.18	8	0.023	2.31	0.1856	Not significant
Pure Error	0.049	5	$9.737*10^{-3}$			
Core Total	19.74	19				

In order to ensure a good model, a test for significance of the regression model and individual model coefficients was needed to be performed accompanying with the lack-of-fit test. Normally, the significant factors can be ranked based on the F-value or p-value (also named 'Prob. > F' value). From Table 3,the Model F-value of 184.84 with a low P-value ($p < 0.0001$) showed that the model was significant. Values of "Prob > F" less than 0.0500 indicate model terms are significant. In this case X_1, X_2, X_3, $X_1 X_2$, $X_1 X_3$, $X_2 X_3$ are significant model terms. Values greater than 0.100 indicate the model terms are not significant. The "Lack of Fit F-value" of 2.31 and P-value of 0.1856 implies the Lack of Fit is not significant relative to the pure error. There is 18.56% chance that a "Lack of Fit F-value" this large could occur due to noise. Non-significant lack of fit is good as we want the model to fit.

Table 4: Statistical information for model

Std. Dev.	0.13	R^2	0.9884
Mean	3.91	Adj R^2	0.9831
C.V. %	3.39	Pred R^2	0.9657
PRESS	0.68	Adeq Precision	66.205

"Adeq Precision" measures the signal to noise ratio. A ratio greater than 4 is desirable,therefore the ratio of 66.205 indicates an adequate signal. This model can be used to navigate the design space. The coefficient of determination (R^2) of the model was 0.9884 (Table 4), which indicated that the model adequately represented the real relationship between the variables under consideration. An R^2 value of 0.9884 means that 98.84% of the variability was explained by the model and only 1.16 % was as a result of chance. The "Pred R^2" of 0.9657 is in reasonable agreement with the "Adj R^2" of 0.9831.The coefficient of variation (C.V.) obtained was 3.39 %. The C.V indicates the degree of precision with which the treatments were carried out. A low value of C.V suggest a high reliability of the experiment [11]. Adequate precision value measures the signal to- noise ratio, and a ratio greater than 4 is generally desirable [17].Adequate precision value of 66.205 indicates an adequate signal and suggests that the model can be used to navigate the design space.

The effect of the indepent variables on ethanol yield is shown in the response surface and contour plots presented in Figures 1 and 2. Figure 1 shows the effect of substrate concentration and pH on ethanol concentration together with the contour views, while Figure 2 shows the effect of acid concentration and substrate concentrations on ethanol concentration together with the contour view.

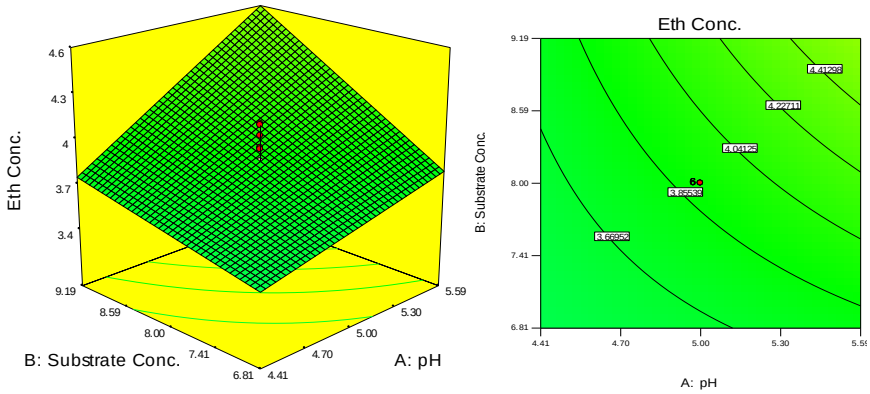

Figure 1: Response surface and contour plots showing the effect of substrate concentration and pH on ethanol concentration

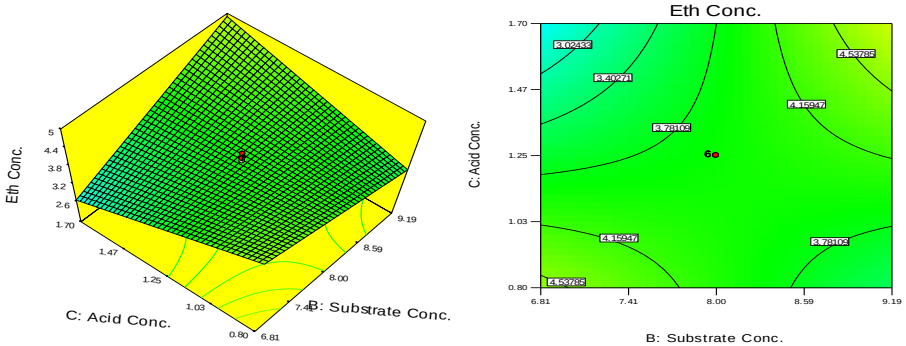

Figure 2: Response surface and contour plots showing the effect of substrate concentration and acid concentration on ethanol concentration

From the plots (Figure 1 and 2), it was easier to understand the interaction between the three factors and also to locate the conditions that give optimum ethanol yield.

The effect of pH and substrate concentration on ethanol concentration is shown on Figure 1. Ethanol production increased with increase in substrate concentration, and the same trend was observed for increase in pH (within range). This trend is similar to what was observed in a related study [18]. Itwas observed that more ethanol was produced as the the pH value was increased. It is suspected that a further increase in substrate concentration would increase ethanol produced, as was observed in the plot above, however the same wouldn't be observed for pH as it would tend towards basicity. This trend in the pH can be because of the fact that yeast and enzymatic cells survive better in non-acidic or mildly acidic medium as was established in arelated study[19].

Figure 2 shows the effect of acid and substrate concentrations on ethanol production with corresponding 3-D and Contour views. An ascending trend is observed from the curve of the 3-D plot thus signifying that with each values increase substrate concentration there is a correspondingly increased value in the ethanol produced. Optimum ethanol yield is observed to be obtained at an acid concentration value of 1.70 % w/w and substrate loading of 9.19 g. It is also noted that low values of acid concentration is beneficial for the production of ethanol. Therefore, optimum ethanol production could be obtained at high substrate concentration and relatively high (within range) pH and acid concentration. In the review of these measures, considerations may be made as regards the fermentation conditions like fermenting time, fermentation media etc. it is predicted that with the passage of time, ethanol yield increases until fermentation can no longer be take place. Following the optimization step, corresponding optimum conditions for optimum values of ethanol production were obtained; pH (5.59), substrate concentration (9.19 g) and acid concentration (1.70 % w/w) yielding a total of 6.52%v/v of ethanol.

It was generally observed that high substrate concentration led to an increase in production of ethanol; this may be due to the fact that increase in sustrate concentration leads to provisionof more cellulose and hemicellulose to be acted upon by the fermentation agent after the hydrolysis step. However, very high acid concentrations should be avoided as it sponsors the generation of that can often inhibit or undesirably affect the performance of biocatalysts in bioprocesses on interfering fermentation ability [20].

4. CONCLUSION

The production of ethanol by a bioprocess using corn cob which is an agricultural waste is a value addition to the corn residue.In this study, optimization of ethanol production by *saccharomyces cerevisiae* using areadily available agricultural waste, corn cob was investigated. The use of corn cobs (cheap and readily available biomass) for the successful production of bioethanol have been achieved. The effect of substrate pH, substrate concentration and acid concentration on the ethanol

yield have been investigated, and finally the optimization of the entire process by the use of Response Surface Methodology (RSM) have been achieved. The optimal conditions for ethanol production are identified as acid concentration of 1.70 w/w%, substrate loadingof 9.19 g and pH of 5.59. Using the the optimized condition, the ethanol production reached a contration of 6.52 % v/v. Additionally, these findings are a potential for developing an eco-friendly process for industries, involving in the processing of lignocellulosic substrate containing waste to confer value added products. It can therefore be concluded that corn cobs can serve as a good feedstock for the production of bioethanol which can be used as a more eco-friendly alternative to fossil fuel.

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