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Utilisation of Response Surface Methodology for the Production of Ethanol from Corn Cob

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Abstract:

In this study, simultaneous saccharification and fermentation (SSF) was utilised for the production of bioethanol from corn cob. Box-Behnken Design (BBD) was performed using Statistica v10 software to determine the optimum operating conditions.The parameters being optimized were sulphuric acid concentration, yeast amount and reaction time. From the analysis of variance (ANOVA), the most influential parameter on bioethanol production was the sulphuric acid concentration. The predicted bioethanol conversion was found in good agreement with the experimental value, with coefficient of determination (R^2) as 0.99997 and Adjusted R^2 as 0.99971. The optimum parameters of 1.281 % v/v of sulphuric acid corresponding to 6.575 % wtof yeast and 45.38 hrof reaction time were obtained after analyzing the response (bioethanol conversion).

Keywords — **Bioethanol, Saccharification and Fermentation, ANOVA, Coefficient of Determination (R^2), STATISTICA V.10.**

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I. INTRODUCTION

The current global economy is heavily reliant on fossil fuels such as crude oil, coal, and natural gas. These are utilized in the manufacturing of gasoline, power, and other products[1]. Excessive use of fossil fuels, especially in large cities, has resulted in significant levels of pollution in recent decades. The amount of greenhouse gases in the atmosphere has risen dramatically [2].Global energy consumption has gradually expanded in tandem with the growth of the human population and the rise of industrial affluence. The restricted supply of fossil fuels has an impact on transportation fuel imports. Within the next few years, annual global oil production will begin to decline[3].As a result of the increased need for renewable energy sources, new technologies to generate biofuels have been developed [1,2]. The conversion of biomass into fuel ethanol, which is the cleanest liquid fuel alternative to non-renewable fossil fuels, has received attention [4]. Energy consumption for transportation, heating, and industrial processes is steadily increasing. Oil and natural gas prices continue to rise as demand for the limited global supply of non-renewable energy supplies grows [5].The only liquid fuels fuel that does not add to the greenhouse gas effect are bioethanol and biodiesel [6]. The growing need for bioethanol for a variety of industrial applications, including alternative energy, industrial solvents, cleaning agents, and preservatives, has necessitated expanded production.

Bioethanol can be used as a fuel either on its own or in blend with gasoline (gasohol). It is utilized as a 10% solution in gasoline (E-10) in the United States, and it is blended (24 percent ethanol, 76 percent gasoline) and hydrated in flexible-fuel cars in Brazil [7]. E-15 (15 percent ethanol, 85 percent gasoline) and E-85 (85 percent ethanol E-15 percent gasoline) are two other combinations. Bioethanol can also be used to replace other additives in gasoline, such as octane boosters, and an ethanol–gasoline blend can give high brake power [8]. Other advantages of using bioethanol as a biofuel include the fact that it is completely biodegradable and sulphur-free, and the products of incomplete oxidation are less hazardous than those produced by other alcohols [9].

Sugar crops (sugar cane, sugar beet, sorghum, fruits e.t.c), starchy crops (corn, wheat, and barley), and cellulosic crops (stems, leaves, trunks, branches, and husks) are among the raw materials that can be used for alcoholic fermentations, with the latter requiring pre-treatment to make fermentation possible.

Corn is commonly utilized in the United States and China, whereas sugar cane is more widely used in tropical locations (India, Brazil, and Colombia) [10]. Ligno-cellulosic biomasses, such as forest management residues, agricultural residues, or particular plants, are increasingly being used [11].

Agricultural residues are made up of lignocellulose, making them a desirable feedstock for bioethanol production. Saccharomyces species are commonly used in bio-ethanol fermentation because it ferments glucose to ethanol and is known for its high insensitivity to temperature and substrate concentration, quick fermentation rates, and high ethanol tolerance [11, 12]. Pretreatment and hydrolysis are frequently required in bioethanol fermentation from lignocellulosic materials in order to convert these materials to monomeric sugars before fermentation can begin [13, 14].

II. MATERIALS AND METHODS

Sample Collection and Preparation:

Corn cob was obtained fromKaduna Main Market and was ground to powdered form.

Baker's yeast, *Saccharomyces cerevisae* was purchased from a local retailer in Kaduna State of Nigeria, and cultured on yeast extract agar. 1g of dried yeast sample was measured into 10 ml distilled water in flat bottom conical flask. Bottle was shaken rigorously for even distribution of the cells [15].

Simultaneous Saccharification and Fermentation (SSF):

The batch SSF was performed containing alkaline treated corn cob (10 % w/v) in 250 ml at room temperature for 72 hours. 0.5M Sodium Hydroxide was added for pH adjustment. After the enzymes were added the mixture was left for 1hour for presaccharification at room temperature. Thereafter theinoculum was added at a concentration of 5 g /l ofwet cells. The same procedure was repeated for other experimental runs.

III EXPERIMENTAL DESIGN

Box Behnken Design (BBD) was chosen as the basis to investigate the effect of sulphuric acid concentration, reaction time and catalyst loading on the production of bioethanol from corn cob. Optimization using STATISTICA V.10 ofthe three selected input parameters was considered with bioethanol volume as the response parameter. The coded anduncoded levels of the independent variables are shown in Table 1 whereas the experimental runs can be viewed in Table 2. For statistical analysis, the relationship between the coded and actual (uncoded) variables can be represented by Equation (1).

$$X_i = \frac{Z_i - Z^*}{\Delta Z} \qquad (1)$$

Where Xi = the coded ithvariable, Zi = the actual ithvariable, ΔZ = step change of Z variable, Z* = center point values for the ithvariable, Number of variable, i = 1-3.

Table 1:Coded and UncodedLevel of the Independent Variables

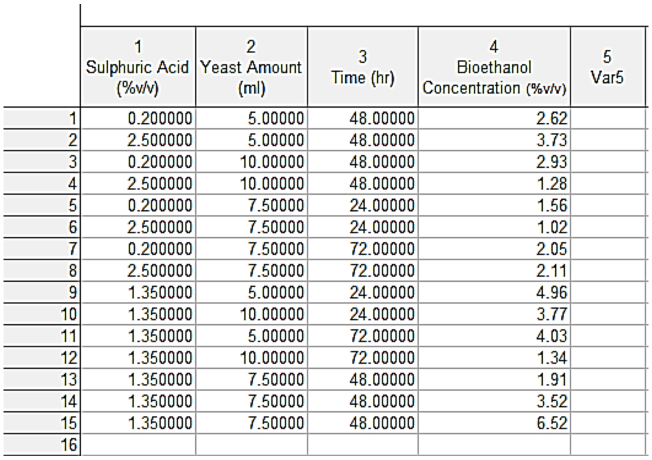
S/No Independent Variables	Coded Levels		
	-1	0	+1
1. X_1: H_2SO_4(%v/v)	0.2	1.35	2.5
2. X_2: Yeast Amount (ml)	5	7.5	10
3. X_3: Time (hr)	24	48	72

By this design, a total of 15 experimental runs were carried out. The center point was replicated three times to evaluate errors. Equation (2) is the polynomial model equation that was used to fit the experimental data obtained during the bioethanol production experiments.

$$Y = \beta_o + \sum_{i=1}^{k} \beta_o X_i + \sum_{i=1}^{k} \beta_{ii} X^2{}_i + \sum_{i<j}^{k} \sum_{j}^{k} \beta \qquad (2)$$

Where Xi are independent variables upon which Y is dependent, β_0 is the offset or constant term, while βi is the ith linear coefficient, βii and βij are the quadratic and interaction coefficients respectively.

TABLE 2: BBD DESIGN MATRIX OF THE EXPERIMENTAL RUNS AND RESPONSE



	1 Sulphuric Acid (%v/v)	2 Yeast Amount (ml)	3 Time (hr)	4 Bioethanol Concentration (%v/v)	5 Var5
1	0.200000	5.00000	48.00000	2.62	
2	2.500000	5.00000	48.00000	3.73	
3	0.200000	10.00000	48.00000	2.93	
4	2.500000	10.00000	48.00000	1.28	
5	0.200000	7.50000	24.00000	1.56	
6	2.500000	7.50000	24.00000	1.02	
7	0.200000	7.50000	72.00000	2.05	
8	2.500000	7.50000	72.00000	2.11	
9	1.350000	5.00000	24.00000	4.96	
10	1.350000	10.00000	24.00000	3.77	
11	1.350000	5.00000	72.00000	4.03	
12	1.350000	10.00000	72.00000	1.34	
13	1.350000	7.50000	48.00000	1.91	
14	1.350000	7.50000	48.00000	3.52	
15	1.350000	7.50000	48.00000	6.52	
16					

IV. RESULTS

The results for the bioethanol volume for each experimental run of the input parameters (i.e., sulphuric acid, time and yeast volume) are shown in Table 2. The results were used to run ANOVA and Multiple Regression Analysis in STATISTICA V10 software. From which the optimum bioethanol volume and the corresponding optimum variables can be predicted. Statistical analysis of the model was performed to evaluate the ANOVA and check the adequacy of the empirical model.

The significance of the linear, quadratic and interactive terms of the process variables were checked by p-tests. The results (as shown in Table 3) showed that quadratic effect of the sulphuric acid is the most significant with least p-value of 0.006370. The significance of the rest of the terms were checked in the same manner.

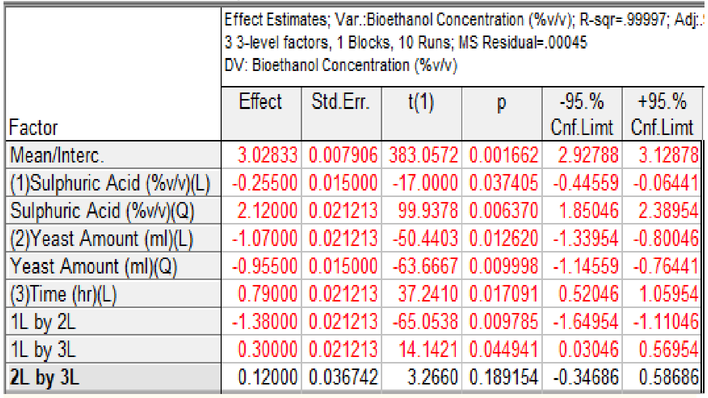
The coefficients of the model equation which are used for the prediction of the optimum bioethanol volume were determined by multiple regression analysis.

V. POLYNOMIAL MODELLING

Equation (3) is the model equation with the coefficient gotten after regression analysis of the response. Thus, let BE be bioethanol volume, while A, B and C represent Sulphuric acid, Yeast amount, and Time respectively.

$$BE = 8.72534 + 5.75643A - 2.23B + 0.00162C - 0.24AB + 0.00543AC + 0.001BC - 1.60302A^2 + 0.15280B^2 \quad (3)$$

TABLE 3: EFFECTS ESTIMATES AND REGRESSION COEFFICIENT OF PREDICTED QUADRATIC POLYNOMIAL MODEL



Effect Estimates; Var.:Bioethanol Concentration (%v/v); R-sqr=.99997; Adj.:
3 3-level factors, 1 Blocks, 10 Runs; MS Residual=.00045
DV: Bioethanol Concentration (%v/v)

Factor	Effect	Std.Err.	t(1)	p	-95.% Cnf.Limt	+95.% Cnf.Limt
Mean/Interc.	3.02833	0.007906	383.0572	0.001662	2.92788	3.12878
(1)Sulphuric Acid (%v/v)(L)	-0.25500	0.015000	-17.0000	0.037405	-0.44559	-0.06441
Sulphuric Acid (%v/v)(Q)	2.12000	0.021213	99.9378	0.006370	1.85046	2.38954
(2)Yeast Amount (ml)(L)	-1.07000	0.021213	-50.4403	0.012620	-1.33954	-0.80046
Yeast Amount (ml)(Q)	-0.95500	0.015000	-63.6667	0.009998	-1.14559	-0.76441
(3)Time (hr)(L)	0.79000	0.021213	37.2410	0.017091	0.52046	1.05954
1L by 2L	-1.38000	0.021213	-65.0538	0.009785	-1.64954	-1.11046
1L by 3L	0.30000	0.021213	14.1421	0.044941	0.03046	0.56954
2L by 3L	0.12000	0.036742	3.2660	0.189154	-0.34686	0.58686

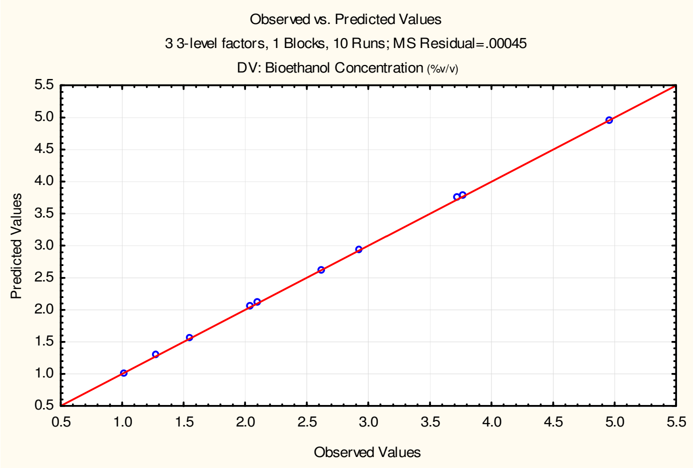


Fig. 1: Actual vs Predicted Values Plot for Bioethanol Production

The value of coefficient of determination (R^2) for the model was 0.99997 and Adjusted R^2 is 0.99971 both indicating the good fitness of the model. Fig. 1 is a presentation of how good the experimental data fits the predicted model. Thus, the predicted model with coefficient of determinant (R^2) of 0.99997 can be used in predicting the bioethanol volumeas the response of the experimental actual values.

VI. RESPONSE SURFACE ANALYSIS AND PARETO CHART

A. *Response Surface Analysis*

The 3D plot shows the effect of interaction among the variables: sulphuric acid concentration with yeast amount, reaction time with sulphuric acid concentration and reaction time withyeast amount on bioethanol volume are presented in Fig. 2, 3 and 4respectively.Fig. 2 is a case of saddle plot, the optimum bioethanol conversion achieved appears to be at high yeast amount and high sulphuric acid volume with prevalence of quadratic effect for both effects being significant.

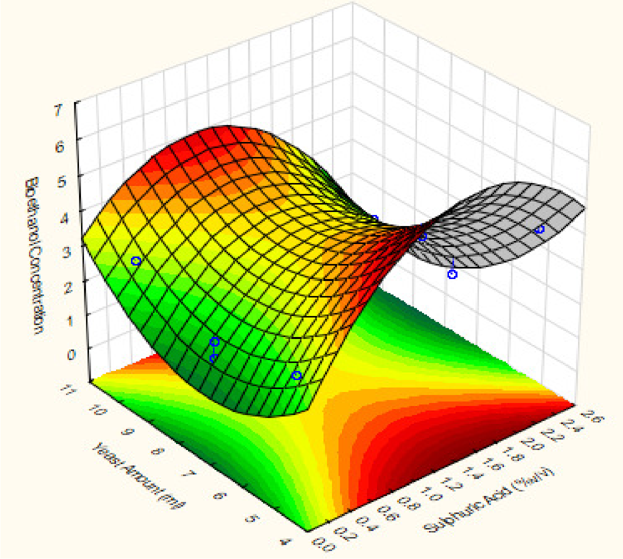


FIG. 2: 3D RESPONSE SURFACE PLOT OF BIOETHANOL CONVERSION AGAINST SULPHURIC ACID AND YEAST AMOUNT

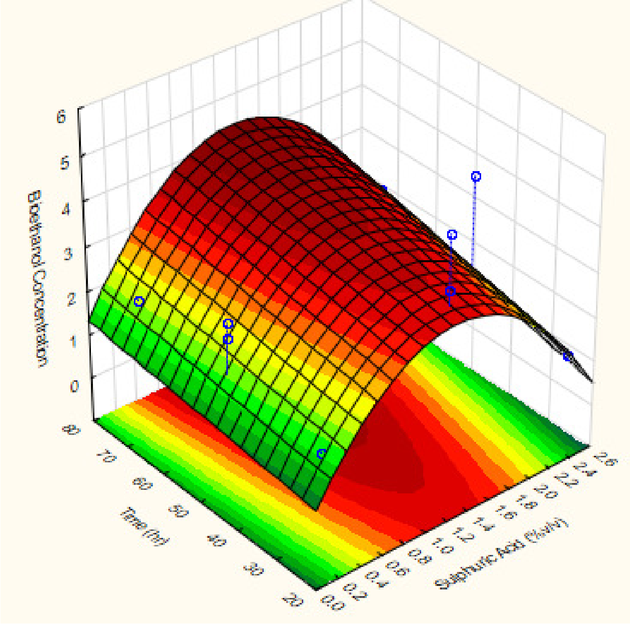


FIG. 3: 3D RESPONSE SURFACE PLOT OF BIOETHANOL CONVERSION AGAINST SULPHURIC ACID AND TIME

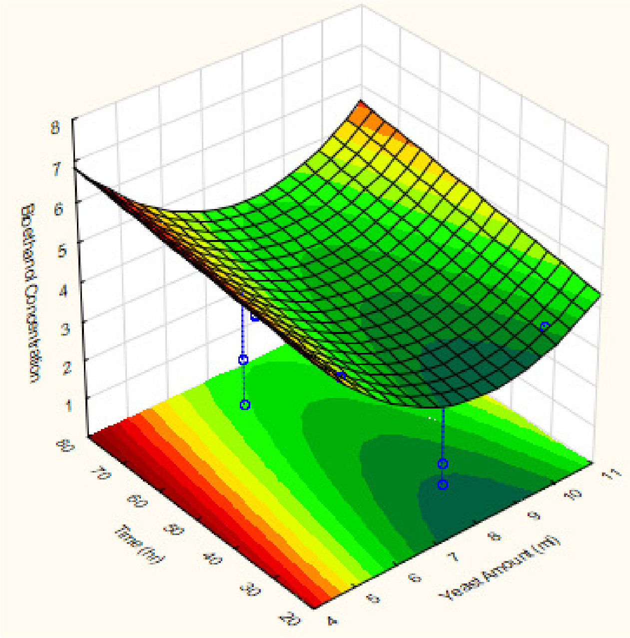


FIG. 4: 3D RESPONSE SURFACE PLOT OF BIOETHANOL CONVERSION AGAINST REACTION TIME AND YEAST AMOUNT

Fig. 3 illustrates the effects of time and sulphuric acid volume on bioethanol conversion increases with an increasing time with the linear term being more significant whereas the sulphuric acid concentration is dominated by the quadratic term which increases with an increase in bioethanol conversion up to 1.4 % v/v before it starts to drop. Fig. 4 indicates the combined effects of yeast and time on the bioethanol conversion. Combine effect of time and yeast amount does not favour an increase in bioethanol conversion.Thus, all the three plots exhibit high significance quadratic effects with quadratic term of sulphuric acid being most significant. This gives a curvature of the response surface and the optimum values to be located near the topmost position of the plots.

B. Pareto Chat

The various effects of the input parameters on the output parameter are further elaborated by Fig. 5. It is obvious that the quadraticeffect of sulphuric acid at confidence level of 95% is the most significant followed by the linear interactive effect of both sulphuric acid and amount of yeast. Also of significance are the quadratic and linear effects of yeast amount, the lineareffect of time and the combine linear effect of both sulphuric acid and time.Thus, the quadratic effect of time and combine linear effect of both yeast amount and time are of no significance on the response (Bioethanol conversion).

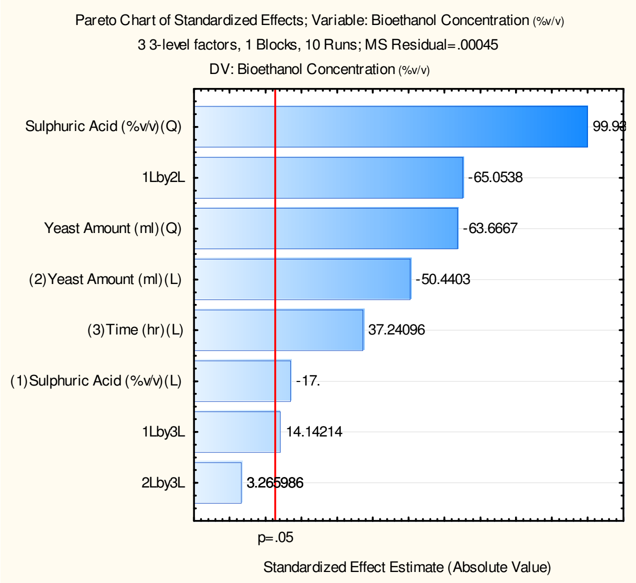


Fig. 5: Pareto Chart of the Standardized Effect for Bioethanol Response

VII. OPTIMIZATION OFBIOETHANOL SYNTHESIS

Multiple regression analysis of the experimental results by STATISTICA V10 software yielded the optimum input values of sulphuric acid concentration(A), yeast amount (B) and reaction time (C) in coded and uncoded terms are presented in Table 4. The uncoded variables were evaluated using Equation (1).

TABLE 4: MULTIPLE REGRESSION SUMMARY OF OPTIMUM INPUT PARAMETERS

Factor	**Coded Parameter**	**UncodedParameter**
Sulphuric Acid (% v/v)	- 0.063	1.281
Catalyst amount (% wt)	- 0.37	6.575
Time (hr)	- 0.109	45.38

The optimum parameter of 1.281 % v/v of sulphuric acid corresponding to 6.575 % wtof yeast and 45.38 hrofreaction time.

VIII. CONCLUSION

The optimization of bioethanol production process from corn cob was made possible by BBD using response surface methodology in 15 experimental runs. A second-order quadratic model capable of predicting the bioethanol yield based on the investigated process variables was developed.

The optimum parameters of 1.281 % v/v of sulphuric acid corresponding to 6.575 % wtof yeast and 45.38 hrof reaction time were obtained after analyzing the response with STATISTICA V10. ANOVA shows that the quadratic effect of sulphuric acid at confidence level of 95% is the most significant followed by the linear interactive effect of both sulphuric acid and amount of yeast. Also of significance are the quadratic and linear effects of yeast amount, the linear effect of time and the combine linear effect of both sulphuric acid and time.Thus, the quadratic effect of time and combine linear effect of both yeast amount and time are of no significance on the response (Bioethanol conversion).

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