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Application of Central Composite Design Based Response Surface Methodology in Parameter Optimization and on Cellulase Production Using Agricultural Waste

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Abstract**—**Response Surface Methodology (RSM) is a powerful and efficient mathematical approach widely applied in the optimization of cultivation process. Cellulase enzyme production by Trichoderma reesei RutC30 using agricultural waste rice straw and banana fiber as carbon source were investigated. In this work, sequential optimization strategy based statistical design was employed to enhance the production of cellulase enzyme through submerged cultivation. A fractional factorial design (2^{6-2}) was applied to elucidate the process parameters that significantly affect cellulase production. Temperature, Substrate concentration, Inducer concentration, pH, inoculum age and agitation speed were identified as important process parameters effecting cellulase enzyme synthesis. The concentration of lignocelluloses and lactose (inducer) in the cultivation medium were found to be most significant factors. The steepest ascent method was used to locate the optimal domain and a Central Composite Design (CCD) was used to estimate the quadratic response surface from which the factor levels for maximum production of cellulase were determined.

Keywords**—**Banana fiber, Cellulase, Optimization, Rice straw

I. INTRODUCTION

WORLD is facing depletion of energy supply and search for *alternative energy* source is inevitable [1]. One of the most immediate and important applications of biomass energy systems could be in the production of ethanol from biomass [2]. Bioethanol would be good substitute to fossil fuels because it considerably lowers the release of carbon dioxide to the atmosphere due to its renewable origin. Furthermore bioethanol is also becoming more interesting for the public as a result of high price of crude oil which puts bioethanol in the same price range as gasoline. A potential raw material for bioethanol production is lignocellulosic material [3]. Alternative lignocellulosic feed stocks include agricultural residues such as rice straw and banana fiber are plentiful in tropical and subtropical regions. Lignocellulosic biomass contains cellulose, hemicellulose, lignin and ash combined structure [4]. Efficient enzymatic degradation of insoluble lignocelluloses often requires a tight interaction between the enzymes and their substrates. In the case of cellulose degradation many cellulases are known to bind to crystalline and amorphous cellulose via cellulose-binding domains (CBDS) which are distinct from the catalytic domains [5].

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The cellulase was demonstrated as one of the key enzyme degrading cellulose. Cellulases are currently sold to the textile industry for cotton softening and denim finishing and to detergent markets for color care, cleaning and anti-redeposition in washing powders. In near future, the cellulase market is expected to increase dramatically if economical conversion of cellulosic biomaterial to ethanol can be demonstrated. The major barrier for this expansion is the current cost of cellulases in biomass saccharification [6].

Factorial designs were introduced in the agricultural area in the 1920s and in the manufacturing and engineering areas and in the manufacturing and engineering thereafter [7, 8]. Theoretical developments, notably the construction of factorial designs were later demonstrated [9]. A full factorial design which includes all possible factor combinations in each of the factors is a powerful tool for understanding complex processes the detailed mechanisms of which are not known and for describing factor interactions in multifactor systems [10]. The optimization of cultivation conditions is an important problem in the development of economically feasible bioprocesses. The optimization of cultivation conditions is an important problem in the development of economically feasible bioprocesses. Combinatorial interactions of process variables with the production of the desired compound are numerous and the optimum processes may be developed using an effective experimental design procedure. Response Surface Methodology (RSM), which is a collection of statistical techniques for designing experiments, building models, evaluating the effects of factors and searching for the optimum conditions, has successfully been used in the optimization of bioprocesses [11, 12, 13, 14]. The objective of this work was to apply Central Composite Design (CCD) based Response Surface Methodology (RSM) to analyze the effects of the process parameters on cellulase production by T.reesei RutC30 and to search for the optimal values for attaining a higher cellulase yield using rice straw and banana fiber.

II. MATERIALS AND METHODS

Trichoderma reesei RutC30 was purchased from ATCC, USA and it was maintained on potato dextrose agar slants for 7days at 30ºC. Rice Straw and banana fiber were collected from near by areas of Chidambaram, India.

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Pretreatment

Pretreatment increases the crystallinity of rice straw while removing lignin and other inhibitors thereby enabling its enzymatic hydrolysis [15]. 100 g of the washed ground cellulosic material was treated separately with 2000 mL of 4% sodium hydroxide solution and autoclaved at 121°C for 30 minutes. Then it was filtered, washed with distilled water and excess alkali present was neutralized with phosphoric acid. Again it was filtered and the residue material was dried at 65°C in a hot air oven to constant weight. To the cellulosic material obtained, the same volume of distilled water was added and heated at 121°C for 30 minutes. The suspension was filtered and the solid material was dried at 65°C to constant weight in a hot air oven [16] and same procedure was repeated for banana fiber.

Cellulase Production

The production medium for the growth of *T.reesei* and production of Cellulase is as follows (g/L): KH_2 PO_4: 2.0, Urea: 0.3, $MgSO_4$.7H2O: 0.3, $CaCl_2.2H_2O$: 0.3 and (mg/L): $FeSO_4.7H_2O$: 5.0, $MnSO_4.H_2O$: 1.6, $ZnSO_4.7H_2O$: 1.4, $CoCl_2.6H_2O$: 2.0. In addition to the one of the carbon substrate, peptone (0.1%) and Tween 80 (polyoxyethylene sorbitan monoleate, 0.1%) were added to the medium to induce cellulase production [17]. Medium was autoclaved for 30 min and seeded with a suspension of T.reesei spores. The initial pH of the medium was adjusted to pH 4 by citrate buffer, and cultured at 28 °C and 220 rpm for eight days.

Analysis and Enzyme Assay

In culture filtrate 20 – 90% ammonium sulfate ($(NH_4)_2SO_4$) was added and precipitated. Precipitates were separated by centrifugation and redissolved in citrate buffer (0.05 M) and centrifuged [17]. Filter paper activity (FPA) was determined according to the method of the International Union of Pure and Applied Chemistry (IUPAC) and expressed as international units (IU). One international unit of cellulase activity is the amount of enzyme that forms 1 μmol glucose (reducing sugars as glucose) per minute during the hydrolysis reaction. Reducing sugar was determined by the dinitrosalicylic acid (DNS) method [18]. Cellulose content of cultures was determined by a shortened procedure of the method of [19]. Ten (10) mL of the total culture was centrifuged (3000 g for 20 min) and the supernatant was carefully removed with a Pasteur pipette. The pellets were suspended in acetic acid-nitric acid reagent (3mL: 150 mL of 80% acetic acid with 15 mL of pure nitric acid) and boiled for 30 minutes in a water bath. After cooling and centrifuging (3000 g for 20 min), the pellets were washed with distilled water (10 mL), and the residual cellulose was dried at 40˚C under reduced pressure until constant weight. The fungal dry biomass was determined by measuring the solid dry weight [20]. By this method, the mycelial weight was calculated from the difference between the total dry weight of the solids (comprising mycelium and residual cellulose). The dry weight of the solids was determined by centrifuging the culture (20 mL; 9600 g for 20 min), washing the pellets three times with water (10 mL), and drying at 40˚C under reduced pressure until constant weight.

Statistical Analysis

The optimization of cultivation conditions was an important problem in the development of economically feasible bioprocesses. Combined interactions of medium parameters for the production of the desired product are large and the optimum process conditions may be developed using an effective experimental design procedure. Response Surface Methodology (RSM), which is a collection of statistical techniques for design of experiments, building models, evaluating the effects of factors and searching for the optimum conditions, has successfully been used in the optimization of bioprocess.

A prior knowledge and understanding of the process and the process variables under investigation are necessary for achieving a more realistic model. A 2^{6-2} Fractional Factorial Designs (FFD) was used to pick factors that influence cellulase production significantly and insignificant ones were eliminated in order to obtain a smaller, more manageable set of factors. In developing the regression equation, the test variables were coded-according to the equation:

$$X_j = (Z_j - Z_{0j}) / \Delta_j \quad (1)$$

Where X_j is the coded value of the independent variable, Z_j is the real value of the independent variable, Z_{0j} is the value of the independent variable on the centre point and Δ_j is the step change value. The linear model observed is expressed as follows:

$$Y = \beta_0 + \sum_{j=1}^{3} \beta_j X_j \quad (2)$$

Where Y is the predicted response, X_j are input variables which influence the response variable Y; β_0 is the intercept; β_j is the j^{th} linear coefficient.

If the mean of the center points exceeds the mean of factorial points, the optimum would be near or with the experimental design space. If the mean of the centre points was less than the mean of the factorial points, the optimum would be outside the experimental design space and the method of the steepest ascent should be applied. The direction of the steepest ascent is parallel to the normal contour line of response curve of the model (Eq. 1) and passes through the center point of FFD. Increment is direct ratio to regression coefficients β_j. Experiments were performed along the steepest ascent path until the response did not increase any more. This point would be near the optimal point and can be used as center point to optimize the medium parameters.

Once critical factors were identified via screening and significant gross curvature was detected in the design space, the central composite design was proceeded obtain a quadratic

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model, consisting of trials plus a star configuration to estimate quadratic effects and central points to estimate the pure process variability and reassess gross curvature, with cellulase production as response. For two factors, the model obtained was expressed as follows:

$$Y=\beta_0+\beta_1X_1+\beta_2X_2+\beta_{11}X_{11}^2+\beta_{22}X_{22}^2+\beta_{12}X_{12} \quad (3)$$

Where Y is the measured response, β_0 is the intercept term, β_1 and β_2 are linear coefficients, β_{12} is the logarithmic coefficient, β_{11} and β_{22} are quadratic coefficients, and X_1 and X_2 were coded independent variables. Low and high factor settings are coded as -1 and 1, the midpoint coded as 0. The factor settings of trails that ran along axes drawn from the middle of the cube through the centers of each face of the tube are coded as -1.414 or 1.414. The SPSS software, version 10.25 was used for regression and graphical analysis of the data obtained by ridge analysis. The MINITTAB software, version 1.4 was used to draw contour plots. The statistical analysis of the model was performed in the form of Analysis of Variance (ANOVA).

III. RESULTS AND DISCUSSION

Optimization of parameters like temperature, initial substrate concentration, inducer concentration, pH and agitator speed were made on cultivation process. Central Composite Design (CCD) experiments using Research surface methodology was proved to be an optimal tool for optimization of medium parameters for cellulase production [30]. In this study, the influence of various process parameters on cellulase production using substrates namely rice straw and sugarcane leaf was investigated.

Cellulase Production using Rice straw Substrate

In FFD, the range and the levels of the variables investigated in this study were given in Table 1 Coded values of factors, design and results of experiment were shown in Table 2 The central values (zero level) chosen for experimental design were temperature 28ºC (X_1), rice straw 50 mg/mL (X_2), lactose 50 mg/mL (X_3), pH 4 (X_4), Inoculum age 5 days (X_5) and agitation 220 rpm (X_6). From primary studies rice straw concentration (X_2) and lactose concentration (X_3) were selected as the most significant factors. Less significant factors namely temperature (X_1), pH (X_4), inoculum age (X_5) and agitation (X_6) were selected for optimization using 2^{6-2} fractional factorial design.

Fractional Factorial Design Rice straw Substrate

The SPSS software, version 10.25 was used for regression analysis of FFD. The factorial analysis of variance in Table 1.3 indicates that the concentration of rice straw (X_2) and lactose (X_3) are most significant factors (p value of < 0.05 was used as a cutoff point for significant differences) affecting cellulase production of *T.reesei* RutC30 and temperature (X_1), pH (X_4), inoculum age (X_5) and agitation (X_6) were found to be less significant parameters. A linear regression equation could be obtained from the regression results of fractional factorial experiment.

$$Y = 21.09 - 0.55\,X_1 + 3.10\,X_2 + 2.24\,X_3 + 0.60\,X_4 + 0.48\,X_5 + 0.82\,X_6 \quad (4)$$

The regression coefficients and determination coefficient (R^2) for the linear regression model of cellulase production were presented in Table 1.3 and 1.4. The model was highly significant ($p<0.01$) and adj.R^2 = 0.555. The significant difference between the mean (7.34 U/mL) of responses at all fractional factorial points and the response (5.8 U/mL) at the center points indicates that the optimal point is outside the experimental design space and the method of steepest ascent should be applied.

Steepest Ascent Path Rice straw Substrate

The direction of the steepest ascent path can be determined by Eq. (4) and the regression results. Since the parameters namely temperature (X_1), pH (X_4), inoculum age (X_5) and agitation (X_6) less significant factors and were fixed at constant value. Rice straw concentration (X_2) and lactose concentration (X_3) were significant factors, and coefficients of X_2 and X_3 are positive, which means that increasing their concentrations has positive effects on the cellulase production. Rice straw was chosen as a standard because its coefficient is higher. One basal increment (D) was defined as the increase of rice straw concentration of 10 mg/mL each time. Experimental design of the steepest ascent and corresponding results are shown in Table 5 After the second step on the path, further experimentation cannot increase the cellulase activity. The highest filter paper activity was achieved in the second step. These results indicate that the concentration of rice straw and lactose of the second step was near optimal. Thus the second step was chosen as the center point to optimize the medium composition.

Central Composite Design Rice straw Substrate

Concentration of rice straw (Z_2=70 mg/mL) and lactose (Z_3 =60 mg/mL) in the second step were chosen as the center point to optimize the medium composition with a central composite design. Table 6 shows the design of this experiment and the results. Regression analysis was performed to fit the response function with the experimental data. The statistical significance of the second–order model equation was checked by an F-test (ANOVA) and the data are shown in Table 7. The regression model for rice straw production was highly significant ($p<0.01$) with a satisfactory value of determination coefficient (R^2=0.861), indicating that 86.1% of the variability in the response could be explained by the second–order model equation given below in Eq.(5)

$$Y = 10.39 - 0.52\,X_2 - 0.28\,X_3 - 1.28\,X_{22}^2 - 1.65\,X_{33}^2 + 0.15\,X_2X_3 \quad (5)$$

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The ANOVA results show that the model is appropriate. The resulting response surfaces in Fig. 1 show the effect of rice straw and lactose concentration on the cellulase production. This result demonstrated that the response surface had a maximum point. The maximum production of cellulase by T. reesei Rut C30 was obtained in the optimized medium when the initial concentration of rice straw and lactose were 40 mg/mL and 54 mg/mL, respectively. The maximum response predicted from the model was 10.50 U/ mL. Repeated experiments were performed to verify the predicted optimum. The results from three replications (i.e. 10.48, 10.50, 10.48 U/mL) were coincident with the predicted values and the model was proven to be adequate. The final optimum values of the parameter predicted by RSM were temperature 28°C, rice straw 40 mg/mL, lactose 54 mg/mL, pH 4.0, inoculum age 5 days and agitation 220 rpm. Compared with the original medium, the cellulase activity increased from 5.8 to 10.50 U/mL.

TABLE I
FACTORS AND CODED VALUES OF FFD OF RICE STRAW

Symbol			Range and levels		
Variables	Real	Coded	-1	0	+1
Temperature (ºC)	Z_1	X_1	26	28	30
Cellulose (mg/mL	Z_2	X_2	75	50	25
Lactose (mg/mL)	Z_3	X_3	25	50	75
pH	Z_4	X_4	3.5	4.0	4.5
Inoculum age (d)	Z_5	X_5	4	5	6
Agitation (rpm)	Z_6	X_6	200	220	240

TABLE II
EXPERIMENTAL DESIGN AND RESULTS OF FFD OF RICE straw

Run	X1	X2	X3	X4	X5	X6	FPA (U/mL)
1	-1	-1	-1	-1	-1	-1	4.86
2	1	-1	-1	-1	1	-1	5.54
3	-1	1	-1	-1	1	1	6.52
4	1	1	-1	-1	-1	1	8.08
5	-1	-1	1	-1	1	1	5.80
6	1	-1	1	-1	-1	1	8.74
7	-1	1	1	-1	-1	-1	10.48
8	1	1	1	-1	1	-1	8.66
9	-1	-1	-1	1	-1	1	6.04
10	1	-1	-1	1	1	1	6.92
11	-1	1	-1	1	1	-1	9.94
12	1	1	-1	1	-1	-1	8.02
13	-1	-1	1	1	1	-1	8.16
14	1	-1	1	1	-1	-1	4.74
15	-1	1	1	1	-1	1	10.52
16	1	1	1	1	1	1	10.54
17	0	0	0	0	0	0	5.80
18	0	0	0	0	0	0	5.78
19	0	0	0	0	0	0	5.80
20	0	0	0	0	0	0	5.80

TABLE III
REGRESSION RESULTS OF FFD OF RICE STRAW

Term	Estimate	Pr>|t|
Intercept	21.085	0.000
X1	-0.552	0.591
X2	3.101	0.008
X3	2.238	0.043
X4	0.602	0.557
X5	0.476	0.642
X6	0.818	0.428

TABLE IV
ANOVA RESULTS FOR CELLULASE PRODUCTION OBTAINED FROM FFD OF RICE STRAW

Regression	DF	Sum of Squares	R^2	F-value	Pr>F
Linear	1	5.2708	0.3294	8.8426	0.0081
Logarithmic	1	5.1165	0.3197	8.4622	0.0094
Quadratic	2	5.3834	0.3364	4.3101	0.0306
Total Model	4	15.7707	0.9855	21.6149	0.0481

TABLE V
RESULTS OF THE ASCENT PATH EXPERIMENT OF RICE STRAW

Run	Z_2	Z_3	FPA (U/mL)
Origin	50	50	5.80
1	60	55	6.87
2	70	60	6.93
3	80	65	6.90
4	90	70	6.74
5	100	75	6.41
6	110	80	6.11
7	120	85	5.92
8	130	90	5.85

TABLE VI
DESIGN AND RESULTS OF CENTRAL COMPOSITION DESIGN (CCD) OF RICE STRAW

Run	X_2	X_3	FPA/(IU/mL)
1	-1	-1	7.92
2	-1	1	7.06
3	1	-1	7.24
4	1	1	6.98
5	-1.41	0	9.20
6	1.41	0	6.82
7	0	-1	7.40
8	0	1.41	8.36
9	0	0	10.50

10	0	0	10.50
11	0	0	10.48
12	0	0	10.50
13	0	0	10.46

TABLE VII
ANOVA RESULTS FOR CELLULASE PRODUCTION OBTAINED FROM CCD OF RICE STRAW

Regression	DF	Sum of Squares	R^2	F-value	Pr>F
Linear	1	0.00320	0.0004	0.0050	0.9446
Logarithmic	1	0.00418	0.0006	0.0066	0.9367
Quadratic	2	0.01752	0.0025	0.01260	0.9875
Total Model	4	0.0249	0.0035	0.0242	2.8688

Cellulase Production using Banana fiber Substrate

In FFD, the range and the levels of the variables investigated in this study were given in Table 8. Coded values of factors, design and results of experiment were shown in Table 9. The central values (zero level) chosen for experimental design were temperature 28ºC (X_1), banana fiber 50 mg/mL (X_2), lactose 50 mg/mL (X_3), pH 4 (X_4), Inoculum age 5 days (X_5) and agitation 220 rpm (X_6). From primary studies banana fiber concentration (X_2) and lactose concentration (X_3) were selected as the most significant factors. Less significant factors namely temperature (X_1), pH (X_4), inoculum age (X_5) and agitation (X_6) were selected for optimization using 2^{6-2} fractional factorial design.

Fractional factorial design Banana fiber Substrate

The SPSS software, version 10.25 was used for regression analysis of FFD. The factorial analysis of variance in Table 2.3 indicates the concentration of banana fiber (X_2) and lactose (X_3) are the most significant factors (p value of <0.05 was used as a cutoff point for significant differences) affecting cellulase production of *T.reesei* RutC30 and temperature (X_1), pH (X_4), inoculum age (X_5) and agitation (X_6) were found to be less significant factors. A linear regression equation could be obtained from the regression results of fractional factorial experiment.

$$Y = 18.50 - 0.92\,X_1 + 2.34\,X_2 + 1.53\,X_3 + 1.38\,X_4 + 0.37\,X_5 + 1.33\,X_6 \quad (6)$$

The regression coefficients and determination coefficient (R^2) for the linear regression model of cellulase production were presented in Table 10 and 12. The model was highly significant ($p < 0.01$) and adj.R^2 = 0.813. The significant difference between the mean (7.75 U/mL) of responses at all fractional factorial points and the response (7.88 U/mL) at the center points indicates that the optimal point is outside the experimental design space and the method of steepest ascent should be applied.

Steepest Ascent Path Banana fiber Substrate

The direction of the steepest ascent path can be determined by Eq. (6) and the regression results. Since the parameters namely temperature (X_1), pH (X_4), inoculum age (X_5) and agitation (X_6) were less significant factors and are fixed at constant value. Banana fiber concentration (X_2) and lactose concentration (X_3) were the most significant factors, and coefficients of X_2 and X_3 are positive, which means that increasing their concentrations has positive effects on the cellulase production. Banana fiber was chosen as a standard because its coefficient is higher. One basal increment (D) is defined as the increase of banana fiber concentration of 10 mg/mL each time. Experimental design of the steepest ascent and corresponding results are shown in Table 12. After the second step on the path, further experimentation cannot increase the cellulase activity. The highest filter paper activity was achieved in the second step. These results indicate that the concentration of banana fiber and lactose of the second step was near optimal. Thus the second step was chosen as the center point to optimize the medium composition.

Central Composite Design Banana fiber Substrate

Concentration of banana fiber (Z_2=70 mg/mL) and lactose (Z_3 =60 mg/mL) in the second step were chosen as the center point to optimize the medium composition with a central composite design. Table 13 shows the design of experiments and the results. Regression analysis was performed to fit the response function with the experimental data. The statistical significance of the second–order model equation was checked by an F-test (ANOVA) and the data are shown in Table 14. The regression model for cellulase production was highly significant since p <0.049 indicating that 95.1% of the variability in the response could be explained by the second–order model equation given below in Eq.(7)

$$Y = 9.07 - 0.12\,X_2 - 0.42\,X_3 + 0.34\,X_{22}^2 + 0.53\,X_{33}^2 + 0.13\,X_2X_3 \quad (7)$$

The ANOVA results show that the model is appropriate. The resulting response surfaces in Fig 2 show the effect of banana fiber and lactose concentration on the cellulase production. This result demonstrated that the response surface had a maximum point. The maximum production of cellulase by T. reesei Rut C30 was obtained in the optimized medium when the initial concentration of banana fiber and lactose were 50 mg/mL and 56 mg/mL, respectively. The maximum response predicted from the model was 8.50 U/ mL. Repeated experiments were performed to verify the predicted optimum. The results from three replications (i.e. 8.52, 8.58, 8.60 U/mL) were coincident with the predicted values and the model was proven to be adequate. The final optimum values of the parameter predicted by RSM were temperature 28°C, banana fiber 50 mg/mL, lactose 56 mg/mL, pH 4.0, inoculum age 5 days and agitation 220 rpm. Compared with the original medium, the cellulase activity increased from 7.28 to 8.56 U/mL.

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TABLE VIII
FACTORS AND CODED VALUES OF FFD OF BANANA FIBER

Symbol			Range and levels		
Variables	Real	Coded	-1	0	+1
Temperature (ºC)	Z_1	X_1	26	28	30
Cellulose (mg/mL	Z_2	X_2	75	50	25
Lactose (mg/mL)	Z_3	X_3	25	50	75
pH	Z_4	X_4	3.5	4.0	4.5
Inoculum age (d)	Z_5	X_5	4	5	6
Agitation (rpm)	Z_6	X_6	200	220	240

TABLE IX
EXPERIMENTAL DESIGN AND RESULTS OF FFD OF BANANA FIBER

Run	X1	X2	X3	X4	X5	X6	FPA (U/mL)
1	-1	-1	-1	-1	-1	-1	7.52
2	1	-1	-1	-1	1	-1	7.94
3	-1	1	-1	-1	1	1	7.06
4	1	1	-1	-1	-1	1	8.20
5	-1	-1	1	-1	1	1	7.50
6	1	-1	1	-1	-1	1	8.04
7	-1	1	1	-1	-1	-1	7.68
8	1	1	1	-1	1	-1	8.24
9	-1	-1	-1	1	-1	1	8.40
10	1	-1	-1	1	1	1	8.04
11	-1	1	-1	1	1	-1	8.92
12	1	1	-1	1	-1	-1	8.34
13	-1	-1	1	1	1	-1	7.18
14	1	-1	1	1	-1	-1	6.46
15	-1	1	1	1	-1	1	6.70
16	1	1	1	1	1	1	6.74
17	0	0	0	0	0	0	7.96
18	0	0	0	0	0	0	7.98
19	0	0	0	0	0	0	8.04
20	0	0	0	0	0	0	8.02

TABLE X
REGRESSION RESULTS OF FFD OF BANANA FIBER

Term	Estimate	Pr>|t|
Intercept	18.501	0.000
X1	-0.922	0.373
X2	2.343	0.036
X3	1.529	0.150
X4	1.379	0.191
X5	0.372	0.716
X6	1.327	0.207

TABLE XII
ANOVA RESULTS FOR CELLULASE PRODUCTION OBTAINED FROM FFD OF BANANA FIBER

Regression	DF	Sum of Squares	R^2	F-value	Pr>F
Linear	1	3.4480	0.2155	4.9445	0.0392
Logarithmic	1	3.2602	0.2037	4.6064	0.0457
Quadratic	2	3.7659	0.2353	2.6164	0.1022
Total Model	4	10.4741	0.6545	12.1673	0.1871

TABLE XIII
RESULTS OF THE STEEPEST ASCENT PATH EXPERIMENT OF BANANA FIBER

Run	Z_2	Z_3	FPA (U/mL)
Origin	50	50	7.84
1	60	55	7.90
2	70	60	7.96
3	80	65	7.92
4	90	70	7.88
5	100	75	7.74
6	110	80	7.68
7	120	85	7.64
8	130	90	7.60

TABLE XIV
DESIGN AND RESULTS OF CENTRAL COMPOSITION DESIGN (CCD) OF BANANA FIBER

Run	X_2	X_3	FPA (U/mL)
1	-1	-1	10.52
2	-1	1	9.66
3	1	-1	10.04
4	1	1	9.70
5	-1.41	0	9.88
6	1.41	0	9.54
7	0	-141	10.22
8	0	1.41	9.34
9	0	0	9.06
10	0	0	9.10
11	0	0	9.04
12	0	0	9.02
13	0	0	9.02

TABLE XV
ANOVA RESULTS FOR CELLULASE PRODUCTION OBTAINED FROM CCD OF BANANA FIBER

Regression	DF	Sum of Squares	R^2	F-value	Pr>F
Linear	1	0.3192	0.0457	0.5276	0.4828
Logarithmic	1	0.3843	0.0551	0.6414	0.4401
Quadratic	2	3.9553	0.5670	6.5490	0.0152
Total Model	4	4.6588	0.6678	7.718	0.9381

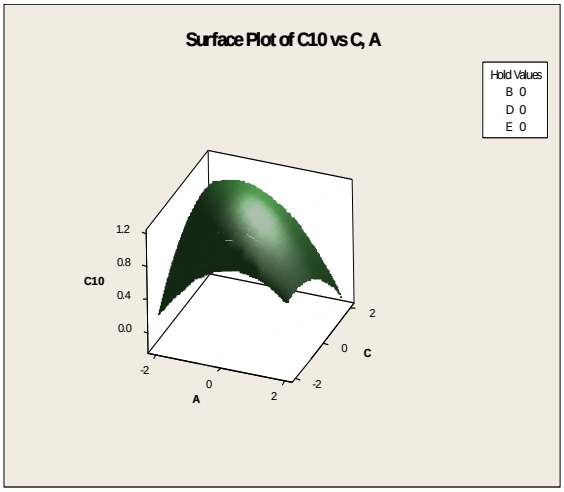


Fig. 1 Three dimensional RSM plot showing the effect of Rice straw concentration and inducer concentration and their mutual effect on the production of cellulase

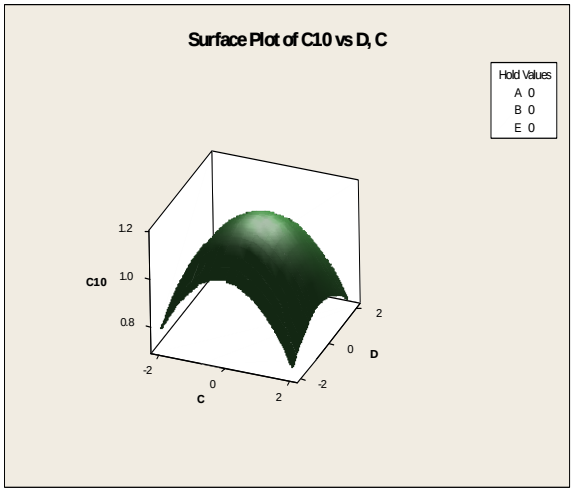


Fig. 2 Three dimensional RSM plot showing the effect of Banana fiber concentration and inducer concentration and their mutual effect on the production of cellulase

IV. CONCLUSION

Response Surface Methodology (RSM) was performed to optimize the process parameters for cellulase production from T.reesei RutC30. A highly significant quadratic polynomial obtained by Central Composite Design (CCD) was very useful for determining the optimal process parameter values of cultivation process that have significant effects on cellulase production. The successful use of lignocellulosic materials as renewable carbon sources is dependent on the development of economically feasible processes for cellulase production.

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