

Improved Cellulase Production using Sorghum Biomass as Carbon Source by *Aspergillus sp.* using Plackett-Burman and Response Surface Methodology under SmF Conditions

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Abstract

Introduction: Combinational optimization techniques were applied to enhance Endo (CMCase) and Exo glucanase (FPase) production from a newly isolated *Aspergillus sp.* under submerged fermentation (SmF) conditions. In order to enhance the cellulase production, a set of statistical techniques were used to optimize fermentation parameters. The statistical techniques of Plackett-Burman (PB), Response Surface Methodology (RSM) and Artificial Neural Networks (ANN) were used sequentially for optimization strategy. Statistical analysis of PB design showed that the Mandel's media constituents of Peptone, MgSO₄, Urea and Sorghum Biomass showed a substantial positive effect on enzyme production. The PB optimum media formulation increased CMCase and FPase activity from 1.2 IU/ml to 2.52 IU/ml and 0.95 IU/ml to 3.0 IU/ml respectively. The Response Surface Methodology (RSM) was adopted to accomplish the best process conditions among the three selected variables (Peptone; MgSO₄ and Sorghum Biomass) for ameliorating CMCase and FPase activity from optimized media of PB design. At optimum levels of parameters, CMCase and FPase activities were 3.52 IU/ml and 5.56 IU/ml respectively. Artificial Neural Network (ANN) was used to compare the results obtained from the CCD (Central Composite Design) of RSM. A Back Propagation Neural Network tool with three inputs and single output neuron with 15 hidden layers, 3-15-1 topology was used to fit the data and was fitted in good acquiescent with the RSM results ($R^2 = 0.99$).

Keywords: *Aspergillus sp.*, Sorghum Biomass, Cellulase, Submerged Fermentation, RSM, ANN.

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INTRODUCTION

Bioethanol production from lignocellulosic biomass has had more attention during recent years due to its vast availability and generation of value added products. As compared to commercialize first generation bioethanol, biomass derived second generation bioethanol not only

replace fossil fuels, but also gives added value to agri and forest residues (Sweeney and Xu, 2012). The strategy employed currently in bioethanol production from lignocellulosic biomass is a sequential process of pre-treatment of biomass to remove lignin and hemicellulose followed by enzymatic hydrolysis of residual biomass to simple sugars and subsequent conversion to ethanol (Sudha and Swamy, 1997). Enzymatic hydrolysis of cellulosic biomass is promising option for breakdown of lignocellulosics to simple sugars. The use of cellulase enzymes in the conversion of biomass to ethanol or the fermentation products is not economical due to the high biomass conversion cost (Sukumaran *et al.*, 2005). In order to make bioethanol production more economical, cost of the cellulase enzyme used in the hydrolysis process should be decreased by screening efficient cellulolytic producers. Cellulases are a group of enzymes

viz: endoglucanases (carboxymethylcellulase, E.C. 3.2.1.4), exoglucanases (avicelase, E.C. 3.2.1.91), and cellobiases (β -glucosidase, E.C. 3.2.1.21), act synergistically for complete hydrolysis of cellulose to glucose (Lynd *et al.*, 2002). In addition to the three major groups of cellulolytic enzymes, there are a number of other enzymes that attack hemicelluloses viz. xylanase, β -xylosidase, galactomannase, glucomannase, acetylesterase (Veeresh Juturu and Jin Chuan Wu, 2012). These enzymes work together to assault cellulose and hemicellulose to fermentable sugars. Many bacterial and fungal species can produce cellulolytic enzymes; the fungal enzymes are usually preferred because they are extracellular, adaptive and usually secreted in large quantities (Nwodo *et al.*, 2005). Among fungi *Trichoderma* and *Aspergillus* have been reported to be the best cellulase producers and enzyme from these organisms are made available commercially (Makut *et al.*, 2010). The present work focuses on cellulolytic enzyme production by a fungal strain, isolated in our laboratory from soil samples collected near festering vegetables. Enzyme production from any microbial culture is a function of fermentation parameters and components of the medium which are the key factors influencing the production. The present study was carried out to optimize the fermentation parameters statistically for maximal CMCase and FPase production from an isolated *Aspergillus sp.* The optimization technique of PB design was used in the present study to know the effect of each media constituent on enzyme production. It has a limitation that this method is not able to identify the combined or interactive effect of the components of the fermentation process. This limitation can be overcome by applying statistical experimental designs with the second - order model. Initial screening of all production media components was done by PB design to identify the most significant component of the response variables (CMCase and FPase). This is followed by a Central Composite Design (CCD) for identification of optimum values of the significant components identified earlier for enhanced production of enzymes. Artificial Neural Network (ANN) was applied for simulation of experimental data obtained from central composite design (CCD).

MATERIALS AND METHODS

Isolation of fungal cultures

The soil samples collected at festering vegetables near Rythu Bazaar, Hyderabad was brought to the laboratory in sterile polyethylene bags. The soil sample (1 gm) was dissolved in 10 ml of potato dextrose broth and ten dilutions were prepared with sterile PD broth. Each dilution was spread on to sterile potato dextrose agar plates and incubated for 4 – 7 days at 30 °C. After seven days of incubation, fungal colonies were isolated and

further purified by sub-culturing on the potato dextrose agar plate.

Screening and Identification of cellulase producing fungi

Isolated fungal cultures were screened for cellulolytic activity quantitatively by inoculating each of these cultures in production media by using the sorghum biomass (sorghum stalks of < 2mm size) as a carbon source (Mandels and Reese, 1957). Fungal inoculum was prepared by inoculating a loopful of the fungal spores into sterilized potato dextrose broth (30°C, 24hr). The inoculum of 10 % (v/v) was inoculated into a conical flask containing 100 ml of autoclaved production media (Mandel's Media) and was incubated in a shaker at 120 rpm (30°C) for 6 days. Two milliliters aliquot of the sample was collected from the fermentation medium for every day till 6 days to check the enzyme activity. The aliquots were centrifuged at 8000 rpm for 15 min at 4°C; the supernatants were checked for CMCase and FPase activities. Isolated cellulolytic fungi showing highest activity was identified based on colony morphology and their sporulating structure. A wet mount of culture was prepared by suspending in to a few drops of Lacto phenol cotton blue solution and observed under light microscope.

Effect of different lignocellulosic biomass in cellulase enzyme production

Among the tested organisms, the strain showing highest activity was used for enzyme production with various agri residues of rice straw, sorghum stalks (Two different varieties named as Sorghum Biomass-1 and Sorghum Biomass-2, based on their Lignocellulosic content) and sugar cane bagasse.

Plackett-Burman method

Eleven media components were screened for optimal production of CMCase and FPase enzymes by employing a two level fraction factorial Plackett-Burman design. This method is very useful and widely applied for screening the major components of production media (Bari *et al.*, 2009). The independent variables selected for this study were (g/100 ml): Peptone - 0.2, Urea -0.03, ZnSO₄-1.4 mg, MgSO₄-0.03, FeSO₄-0.05, KH₂PO₄-0.02, CaCl₂-0.01, and Sorghum Biomass-2, Ammonium Sulfate -0.14. For Plackett-Burman analysis, each media component at two levels of concentrations was selected. The total number of experiments with combination of different components and their levels were 12 as given in Table 1. The experiment was conducted in 250 ml conical flask with 100 ml sterile Mandel's medium. Three trial runs have been taken to assess the reproducibility of results. After 72 hours of incubation the culture broth was collected and centrifuged at 8,000 rpm for 15 min at 4 °C and the supernatant containing the crude enzyme was checked for CMCase and FPase activity. The main effect

of each variable was calculated as the difference between the average of measurements made at the high value (+) and at the low value (-) (Rashid *et al.*, 2009). Plackett-Burman experimental design is based on the first order model

$$Y = \beta_0 + \sum \beta_i X_i$$

Where Y is the response variable (CMCase and FPase activity), β_0 is the model intercept and β_i is the variable estimates. The factors that have a confidence level above 95% are considered as the most significant factors that affect enzyme production.

Response Surface Methodology (RSM)

Medium components showing a positive effect on both enzyme production from Plackett-Burman design were selected and their concentration was optimized by using Response Surface Methodology (Design-Expert version 7.1.6 (Stat-Ease, Inc.), software). The parameters considered in the RSM experiments were Sorghum Biomass, MgSO_4 and Peptone (Table 3). The 2^3 Central Composite Rotatable Design (CCRD) with three independent variables at two levels, six star (axial) points and five central points (total 20 runs) was adopted to find linear, quadratic and interaction effects of independent process variables on experimental responses. A second order polynomial model was fitted to each set of experimental data to predict optimal reaction conditions by following equation:

$$Y = b_0 + \sum b_i x_i + \sum b_{ii} x_i^2 + \sum b_{ij} x_i x_j$$

Where Y is the value of the response and X is the coded value of factors, i and j are the linear and the quadratic value of coefficients. The statistical significance of regression coefficients and effects was checked by analysis of variance (ANOVA).

Artificial Neural Network (ANN)

A feed forward neural network (multilayer perception) with back propagation algorithm is widely applied for prediction (Rummelhat *et al.*, 1986). Multilayer perceptron consisting of three layers input, hidden and output layer, which are connected by neurons. Information in a BNN (Backpropagation Neural Network) is stored as weights, which are the connections between neurons in successive layers and as bias values (Mullai and Rene, 2008). Each neuron in the hidden and output layers first calculates the weighted sum of its inputs and passes the result through a transfer function to produce an estimate as output that corresponds to the input data set. The result is compared to the corresponding desired values and the error is back-propagated through the network to adjust the connection weights according to the learning rule. This procedure is repeated iteratively, until the predetermined target RMSE is reached. The type of transfer function employed affects the neural network learning rate and is instrumental in its

performance (Ajdari *et al.*, 2013). The hyperbolic tangent was used as the transfer function for the input and hidden layer nodes and linear activation function was used as the output layer activation function. The algorithm used to train the ANN in this study was Levenberg-Marquandt (LM) algorithm. The networks with few hundred weights, the LM algorithm is best suitable with fastest convergence with lower mean square error than other algorithms in many cases (Demuth and Beale, 2005). The number of neurons in hidden layer for each model varies and it is determined by trial and error. Trials have been done for model by changing the number of hidden neurons in order to find the best structure. The best number of nodes was selected from the model that gave the best performance with highest correlation coefficient (R -value). Data generated from CCRD was used for modeling in the ANN by Matlab 7.4 software.

Validation of optimized Conditions

To test reliability and estimation capabilities of employed techniques the predicted response obtained from RSM and ANN were compared with the experimental Values (Table 3). The Coefficient of determination (R^2) and Absolute Average deviation (AAD) of RSM and ANN were evaluated and compared. AAD and R^2 were calculated by the following formula

$$AAD = \left\{ \left(\sum_{i=1}^p ((y_{i,exp} - y_{i,cal}) / y_{i,exp}) / p \right) \right\} \times 100$$

$$R^2 = 1 - \frac{\sum_{i=1-n} (Model\ prediction_i - Experimental\ Value_i)^2}{\sum_{i=1-n} (Average\ Experimental\ Value - Experimental\ Value_i)^2}$$

Where $Y_{i,exp}$ and $Y_{i,cal}$ are the experimental and calculated responses, 'p' is the number of the experimental run and 'n' is no. of experimental data. R^2 is a measure of the amount of the reduction in the variability of response obtained. It must be close to 1 and AAD between the predicted and observed data must be as small as possible (Bas and Boyasi, 2007).

Analytical Methods

CMCase activity was measured by the method of T.K. Ghose, 1987, where determination of the amount of reducing sugars liberated from 1% carboxymethylcellulose (CMC) (100 mM citrate buffer, pH 4.5) by the action of enzyme. Enzyme substrate mixture was incubated at 50 °C for 15min and the reaction was stopped by the addition of DNSA solution (Miller, 1959). One unit of CMCase activity was defined as the amount of enzyme that released 1 μ mole of glucose per minute under given set of conditions. Similarly FPase activity was measured by using the same procedure as mentioned above by using Whatman No.1 filter paper (50 mg) as substrate.

RESULTS AND DISCUSSION

Isolation of the cellulase producing fungi

Among the four different fungal cultures isolated, the culture showing better activity was selected for further screening. The morphological studies and microscopic analysis of the selected fungal strain showed that the species belongs to the genus of *Aspergillus* (Figure 1). From figure 2 it is evident that both CMCase and FPase activities were high when sorghum biomass 2 present in the production medium as compared to other biomass and the same was used through the study. The highest activities of CMCase and FPase enzyme were 1 IU/ml of CMCase and 1.1 IU/ml at 72hr of fermentation period 50°C.

Palckett-Burman Method (PB)

The PB experiment was conducted in 12 runs to evaluate the significant effect of eleven media components on the production of both enzymes using *Aspergillus sp.*. Table 1 shows PB experimental design and enzyme activities. The main effect of each constituent on the CMCase and FPase production was calculated as the difference between the average measurement calculated at the higher (+) and lower (-) levels of the constituent (Figure 3). The positive value indicates that the high concentration of this variable is near optimum and a negative value indicates that the low concentration of this variable is near optimum (Pal and Khanum, 2011). The obtained data showed a range of positive main effect values, indicating that the presence of high levels of Peptone, Urea, MgSO₄ and Biomass has a positive effect on production of both enzymes. On the other hand, the presence of MnSO₄, ZnSO₄, FeSO₄, and CaCl₂ showed negative effect on CMCase and FPase production. The fermentation parameters, which were showing negative effect on enzyme production were not taken into account, while three factors showing significant positive effect were selected for next optimization strategy.

Response Surface Methodology

The three different influential factors for enzyme production viz; Sorghum Biomass, Magnesium sulfate, Peptone were selected for RSM analysis. Minimum and maximum concentrations of each selected parameter were fixed based on preliminary experimental results and literature review (where optimum enzyme production was noticed). Experimental design presented in Table 2 was prepared using the above selected parameter levels according to fractional factorial central composite design. It was noticed that the enzyme production values vary depending on the experimental conditions (Table 2). The results clearly indicated the influence of the selected fermentation factors of CMCase and FPase production, where minimum and maximum enzyme activities were noticed as 1.098 to 4.718 IU/ml and 3.620 to 6.29 IU/ml.

Which depicts the best possible optimal conditions obtained for CMCase and FPase after performing response surface methodology. Figures 4 and 5 describe the response obtained by designing contour and surface plots. The response was explained with quadratic regression model and expressed by a second order polynomial equation. The coefficients of the equation, analysis of variance (ANOVA) were shown in the Table 3.

Where,

Final Equation in Terms of Coded Factors

$$Y_1 = 2.614827 + 0.291427 * A - 0.14388 * B + 0.818998 * C + 0.0275 * A * B + 0.2025 * A * C + 0.26 * B * C - 0.257454 * A^2 - 0.02362 * B^2 - 0.0908 * C^2$$

$$Y_2 = 5.496711 + 0.050757 * A - 0.12704 * B - 0.23262 * C - 0.045 * A * B - 0.1625 * A * C + 0.2675 * B * C - 0.70445 * A^2 - 0.24129 * B^2 + 0.094581 * C^2$$

Where Y₁, Y₂ are response terms of CMCase and FPase. A, B and C are response

variables for Peptone, Biomass and MgSO₄. The optimized media composition was Presented in the Table 4. The optimum activities of the CMCase and FPase were 3.52 IU/ml and 5.56 IU/ml. An isoresponse surface plot on behalf of the effect of peptone and biomass was observed for CMCase production (Figures 4a). An increase in the concentration of peptone up to 0.3 g and at low levels of biomass 0.5g had showed improved CMCase Production. The graphical illustrations in Figure 4b reveal that the FPase activity was optimum at its centre point & followed a shallow surface. Increase in peptone and biomass concentration increased FPase activity from 2.2 IU/ml to 5.33 IU/ml at center point and then starts decreasing as the concentration of the variables increases. Figure 5a depicts that at higher concentration of peptone and MgSO₄, highest CMCase activity of 3.69 IU/ml was noticed. Figure 5b illustrates the interaction behavior of the peptone and MgSO₄. FPase activity (5.64 IU/ml) was more at low concentrations of MgSO₄ and at the peptone concentration of 0.2 g. These plots showed a highest activity at center point and decreased at extreme operating conditions. The more non-elliptical nature of the contour plots describes that there is no mutual interaction between the tested variables (Mullai and Fathima, 2010). As the MgSO₄ concentration is increased, an increase in CMCase activity was noticed at biomass concentration of 1.25% (Figure 6a). Further, the surface plot (Figure 6b) showing interactions between biomass and MgSO₄ showed a rather complex behavior in comparison to those explained earlier. Low levels of MgSO₄ and biomass concentration increased the FPase activity and decreased further.

Artificial Neural Networks

The Central Composite Design experimental data was divided into training and testing sets. For training, among a total of 20 experimental runs 17 runs (approx. 70%) were selected and remaining 3 runs (approx. 20%; the data shown in bold letters in Table 2) were used for testing. Various topologies (from 1 to 20 hidden neurons) were examined using the LM algorithm. The optimum topology was selected based on the minimum error of testing (R). After repeated trials, it was found that a network with 15 hidden neurons produced the best performance and a network topology of 3-15-1 was used. The results for training and testing data were summarized and presented in Table 2 for both CMCase and FPase. The scatter diagram of predicted values *versus* actual values was also shown in Figure 7. It shows that the model prediction fits well with the experimental observations. Minor variation in the prediction may be due to the inherent variability of the biological system

(Singh *et al.*, 2008). The goodness of fit was determined by the coefficient R^2 , which describes the extent of variance in the modeled variables (Table 5). The developed model was used to predict the enzyme production at optimum conditions as shown in Table 4.

Comparison of RSM and ANN Models

The estimation capabilities of RSM and ANN were also examined in this study. The predicted responses, obtained from RSM and ANN, were compared with the actual values (Experimental Value). The root mean square error (RMSE), coefficient of determination (R^2) and absolute average deviation (AAD) were used together to compare the RSM and ANN for CMCase and FPase. The actual and predicted values for the RSM and ANN design were presented in Table 2. The comparative values of RMSE, R^2 and AAD were given in Table 5. The root mean squared error (RMSE) for the design matrix by the RSM and ANN for CMCase and FPase is 0.035 and 0.46211 and 0.046 and 0.544; the coefficient of determination for both RSM and ANN (R^2) is 0.999.

Table 1: Plackett Burman Design and the response variable (CMCase and FPase)

A- Peptone	B- Urea	C- MnSO ₄	D- ZnSO ₄	E- MgSO ₄	F- FeSO ₄	G- CoCl ₂	H- KH ₂ PO ₄	J- CaCl ₂	K- Biomass	L- Ammonium Sulphate	CMCase (IU/ml)	FPase (IU/ml)
0.2	0.03	0	0	0	0.05	0	0.02	0.01	1	0.14	1.108	1.664
0.2	0.03	0	1.4	0.03	0.05	0	0	0	2	0	2.555	3.724
0.2	0	0	0	0.03	0	2	0.02	0	2	0.14	2.277	3.58
0.1	0.03	1.6	0	0.03	0.05	2	0	0	1	0.14	1.055	1.9038
0.1	0	0	1.4	0	0.05	2	0	0.01	2	0.14	0.499	0.6716
0.2	0	1.6	1.4	0.03	0	0	0	0.01	1	0.14	1.087	3.335
0.1	0	1.6	0	0.03	0.05	0	0.02	0.01	2	0	0.049	1.997
0.1	0	0	0	0	0	0	0	0	1	0	1.4178	0.4829
0.1	0.03	1.6	1.4	0	0	0	0.02	0	2	0.14	0.3052	2.6642
0.2	0	1.6	1.4	0	0.05	2	0.02	0	1	0	0.9155	1.2261
0.1	0.03	0	1.4	0.03	0	2	0.02	0.01	1	0	1.7326	3.4913
0.2	0.03	1.6	0	0	0	2	0	0.01	2	0	1.6605	1.9038

Table 2: RSM design and experimental and predicted values by RSM and ANN

Media Components			CMCase Activity (IU/ml)			FPase Activity (IU/ml)		
Peptone	Biomass	MgSO ₄	Experimental	Predicted by RSM	ANN	Experimental	Predicted by RSM	Predicted By ANN
0.3	0.5	0.01	2.42	2.404178	2.42	5.52	5.530961	5.52
0.2	1.25	0.02	2.58	2.614827	2.615	5.43	5.496711	5.474
0.031821	1.25	0.02	2.9	2.852898	2.9	3.4	3.418864	3.4
0.1	0.5	0.01	2.24	2.281324	2.24	5.02	5.014448	5.02
0.2	1.25	0.003182	0.98	0.980634	2.5476	6.21	6.155437	4.2701
0.2	2.511345	0.02	2.27	2.306037	2.27	4.65	4.600568	4.65
0.2	-0.01134	0.02	2.82	2.789999	2.82	4.98	5.027884	4.98
0.3	2	0.01	1.68	1.651412	1.68	4.6	4.651878	4.6
0.2	1.25	0.02	2.72	2.614827	2.615	5.44	5.496711	5.474
0.2	1.25	0.02	2.61	2.614827	2.615	5.61	5.496711	5.474
0.2	1.25	0.036818	3.73	3.735403	3.73	5.32	5.373015	5.32
0.1	2	0.01	1.42	1.418558	2.615	4.28	4.315364	4.28
0.2	1.25	0.02	2.59	2.614827	1.98	5.46	5.496711	5.474
0.2	1.25	0.02	2.61	2.614827	2.615	5.51	5.496711	5.474

0.368179	1.25	0.02	3.78	3.833139	3.78	3.61	3.589588	3.61
0.1	0.5	0.03	2.97	2.994319	2.97	4.39	4.339217	4.39
0.1	2	0.03	3.16	3.171553	3.16	4.72	4.710133	6.1823
0.2	1.25	0.02	2.58	2.614827	2.615	5.53	5.496711	5.474
0.3	0.5	0.03	3.93	3.927173	3.93	4.24	4.20573	4.24
0.3	2	0.03	4.26	4.214408	3.3181	4.39	4.396647	4.39

Table 3: ANOVA Results for the CCRD for FPase and CMCase. For FPase,R-Squared-0.995712, Adj R-Squared-0.991852, Pred R-Squared-0.981824; For CMCase:R-Squared-0.99787, Adj R-Squared - 0.995953Pred R-Squared-0.990634

	CMCase			FPase		
	Sum of Squares	F-Value	p-value Prob > F	Sum of Squares	F-Value	p-value Prob > F
Model	12.65217	520.4797	< 0.0001	9.848877	257.9867	< 0.0001
A-Peptone	1.159873	429.4297	< 0.0001	0.035183	8.294496	0.0164
B-Biomass	0.282728	104.6766	< 0.0001	0.220416	51.96335	< 0.0001
C-Mgso4	9.160431	3391.543	< 0.0001	0.738972	174.2131	< 0.0001
AB	0.00605	2.239942	0.1654	0.0162	3.819162	0.0792
AC	0.32805	121.4567	< 0.0001	0.21125	49.80234	< 0.0001
BC	0.5408	200.2249	< 0.0001	0.57245	134.9555	< 0.0001
A^2	0.95522	353.6593	< 0.0001	7.151595	1685.994	< 0.0001
B^2	0.00804	2.976892	0.1152	0.839071	197.8117	< 0.0001
C^2	0.118805	43.98601	< 0.0001	0.128917	30.39224	0.0003

Table 4: Optimum Response predicted by RSM and ANN and the experimental values

Peptone	Biomass	MgSO4	CMCase			FPase		
			Experimental	RSM	ANN	Experimental	RSM	ANN
0.22	1.57	0.03	3.52	3.525401	3.8297	5.56	5.301973	5.0927

Table 5: Comparison of RSM and ANN

	ANN			RSM		
	RMSE	R ²	%AAD	RMSE	R ²	%AAD
CMCase	0.46211	0.978	18.309	0.035	0.999	0.046
FPase	0.544	0.997	0.1328	0.046	0.999	0.04

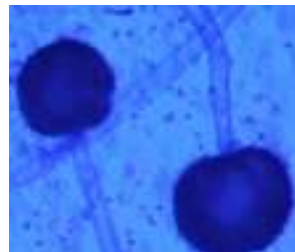


Figure 1: Structure of *Aspergillus sp.* under microscope

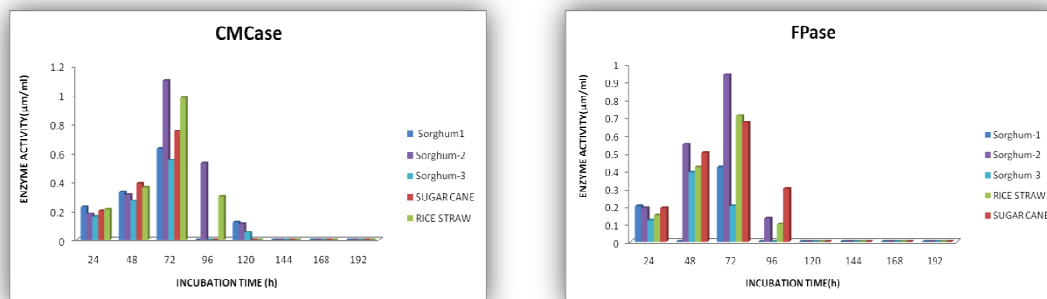


Figure 2: CMCase and FPase activities on different biomass

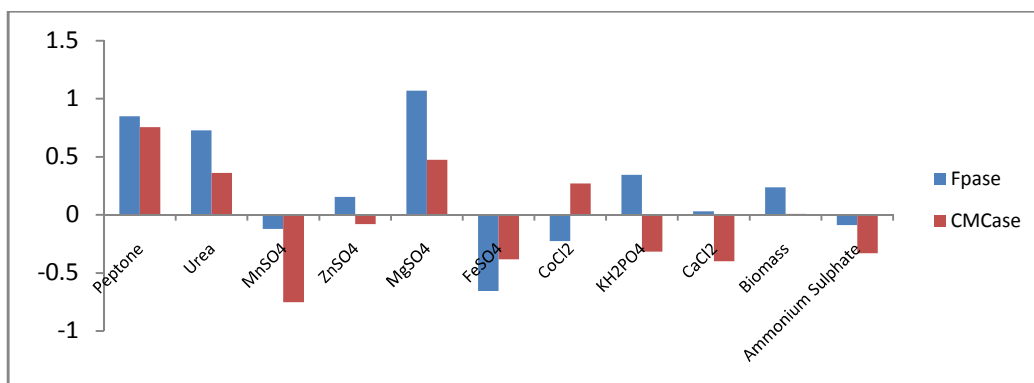


Figure 3: Main effects of the medium constituents on CMCase and FPase production from Plackett Burman experimental results

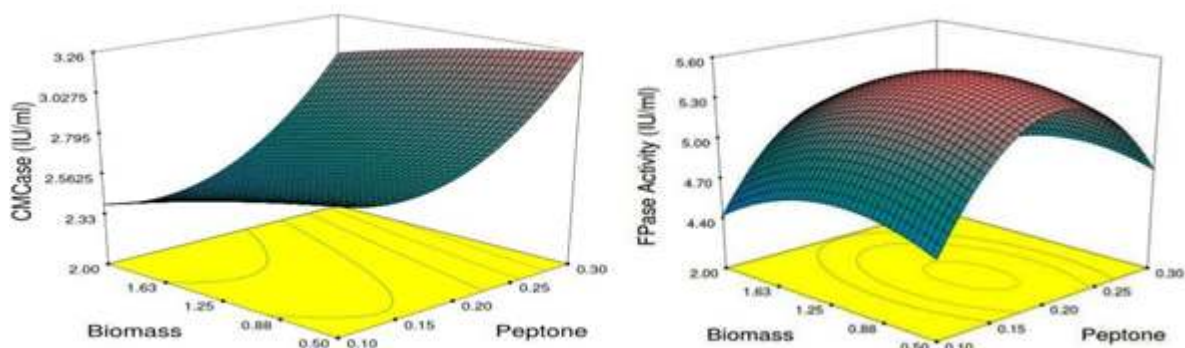


Figure 4a & 4b: Response surface plots of CMCase and FPase for Peptone and Biomass at MgSO_4 concentration of 0.2grams

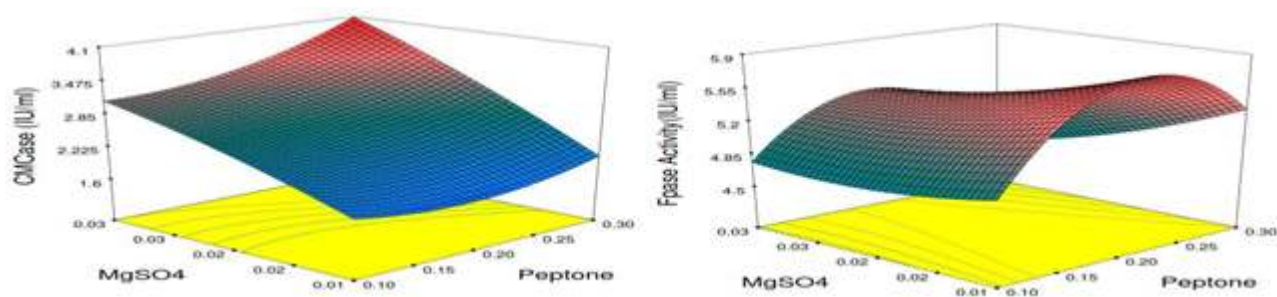


Figure 5a & 5b: Response surface plots of CMCase and FPase for Peptone and MgSO_4 at biomass concentration of 1.25 gram

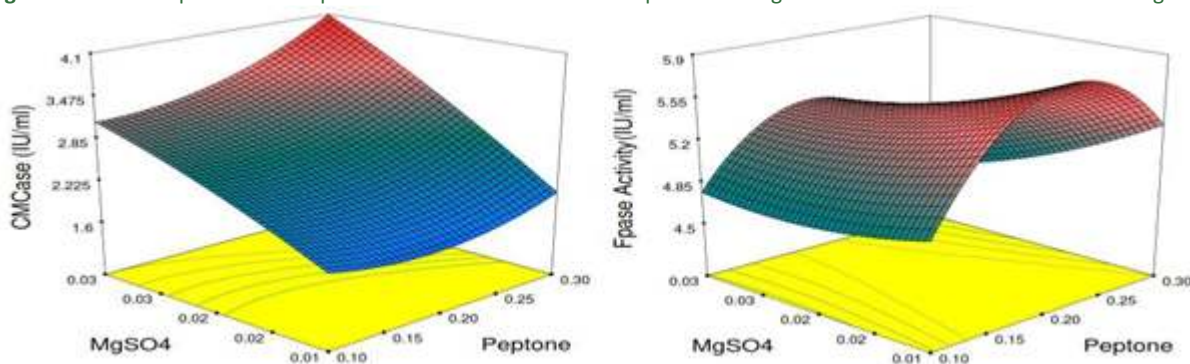


Figure 5a & 5b: Response surface plots of CMCase and FPase for Peptone and MgSO_4 at biomass concentration of 1.25 gram

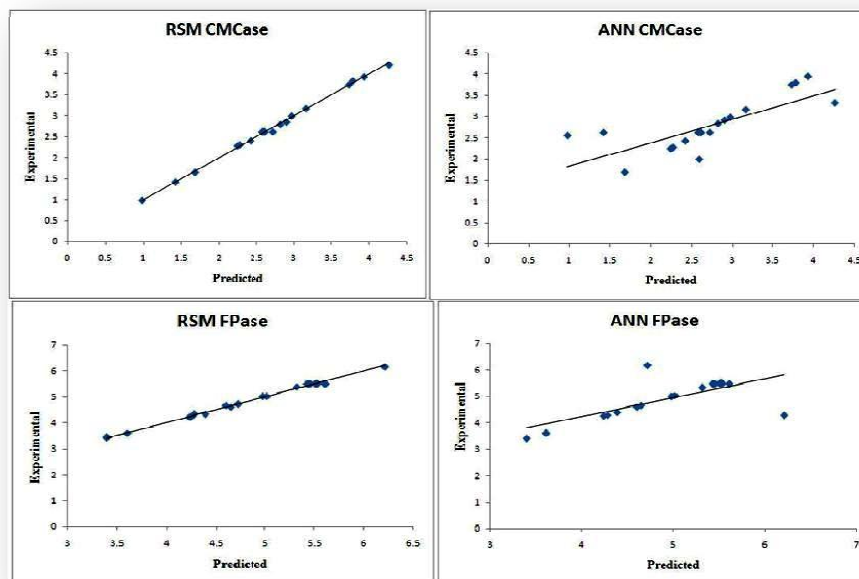


Figure 7: The scatter plot of RSM and ANN model predicted values versus actual values for CCRD design matrix for CMCase and FPase

CONCLUSION

In the present study, eleven constituents of the Mandel's media were studied by experimental designs of Plackett-Burman, RSM and Back Propagation Neural Networks. PB analysis showed that the factors Peptone, Urea, MgSO_4 and Sorghum biomass were influencing the CMCase and FPase production positively. The media components which showed a positive effect on production were selected and a 2^3 full factorial central composite design were applied to study the combined effects of the nutrients. The optimal concentration obtained from RSM was Sorghum biomass (1.57 %), Peptone (0.22%), and MgSO_4 (0.03%). The PB and CCD optimum media formulation increased CMCase and FPase activity from 1.2 IU/ml and 0.95 IU/ml to 3.52 IU/ml and 5.56 IU/ml respectively. The performance of the CCRD (Central Composite Rotatable Design) design with Back Propagation ANN in the estimation of fermentation parameters (Peptone, MgSO_4 and Sorghum biomass) for CMCase and FPase production from *Aspergillus sp.* was studied. Both models provide quality predictions for the three independent variables in terms CMCase and FPase production with ANN showing more accuracy in estimation. The superiority of ANN over RSM would make the estimation technique a very helpful tool which is well suited for modeling the fermentation process.

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