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# Production Of Xylanase Enzyme By Aspergillus terreus Under SSF Using Response Surface Methodology

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**Abstract:** Production of Xylanase enzyme by *Aspergillus terreus (*MTCC- 1782) in SSF was investigated. Wheat bran obtained from the local market was used as the source of carbon for the organism. The effects of twelve medium components were investigated using Plackett-Burman design and the components namely- Peptone, Yeast Extract and $Cacl_2.2H_2O$ were found to be significant on the production of xylanase. Their respective concentrations were also optimized using central composite design of response surface methodology to obtain the maximum enzyme yield. Under the optimum conditions the maximum xylanase activity of 415.03 IU/gds of was obtained.
**Keywords:** Xylanase, *Aspergillus terreus*, solid state fermentation.

## Introduction

Microbial enzymes are a fast growing field in biotechnology. The global market of industrial enzymes was closed to a billion dollars in 1990 and crossed the $2.0 billion mark in 2005 [1]. The market has been estimated at $3.3 billion in 2010 and is expected to reach $4.4 billion by 2015. Endo-1,4-β-xylanase (EC 3.2.1.8) is the main enzyme in the xylanolytic complex and it acts on the main xylan chain to form xylose, xylobiose and other xylooligosaccharides (XOS). Xylanases have applications in various industries, such as the prebleaching of kraft pulps, the improvement of bread quality, the clarification of fruit juices and xylitol production [2,3]. More recently, Xylooligosaccharides (XOS) produced from xylan-containing lignocellulosic materials have been described as an emerging prebiotic that could be incorporated into many food products [4]. A recent application of xylanase is in the production of biofuels. It is estimated that the total energy content of global xylan and cellulose waste is equivalent to almost 640 billion tons of oil [5]. Xylanase have potential application in food, feed, paper, pulp, textile industries [6], bread making and beer production[7] .

Filamentous fungi are industrially important producers of this enzyme due to extracellular release of xylanases, higher yield compared to yeast and bacteria[8]**.** Solid state fermentation (SSF) was chosen for the present research because it has been reported to be of more grated productivity than that of submerged fermentation [9, 10]. Economically, SSF offers many advantages including superior volumetric productivity, use of inexpensive substrates when compared with submerged fermentation [11]. The production cost and the yields of enzymes are considered the major problems in commercial exploitation [12]. The use of purified xylan as a substrate to induce xylanase synthesis increases the cost of enzyme production. Therefore, for commercial applications, there have been attempts to develop a bioprocess to produce xylanase in high quantities from simple and inexpensive substrates[13]. The use of agro-industrial residues, such as wheat bran, corncobs,

sugarcane bagasse and similar substrates, to achieve better xylanase activity allows enzyme production costs to be cut, thereby improving the industrial xylan hydrolysis process both technically and economically [14]. The production of xylanase and other enzymes by fungi is known to be depending on the composition of growth medium and fermentation conditions. Therefore reducing the costs of enzyme production is by optimizing the fermentation medium and the process is the goal of basic research for industrial applications[15]. Experimental design methodology is a strategy to use smaller number of experiments and to avoid unnecessary experiments [16]. Response Surface Methodology (RSM) is an effective statistical technique, which provides an investigative approach towards optimization. In addition, it is a collection of mathematical and statistical techniques used in significance of several affecting factors in an optimum manner, even in the presence of complex interactions [17]. The main reason for implementing RSM is to determine the optimum operational conditions for the process or to determine a region that satisfies the operating specifications [18].

This paper reports the use of low cost agro-industrial residue wheat bran as carbon source for the production of xylanase by *Aspergillus terreus*. The medium optimization was carried out by three steps (1) screening the most significant medium components influencing xylanase production by using Plackett-Burman design (2) Optimization of the most significant medium components by applying central composite design (CCD) of response surface methodology (RSM) and (3) Experimental validation of the model.

## Materials And Methods

### Microorganism And Culture Media

*Aspergillus Terreus (MTCC No - 1782)* used in this study was purchased from the Microbial Type Culture Collection and Gene Bank, Institute of Microbial Technology (IMTECH) Chandigarh, India. The stock culture was maintained on agar slants at $5^0$C. The culture was maintained on Czapek Dox Medium and subcultured at an interval of three months. The Czapek Dox medium composition comprises of : *Czapek Concentrate, 10.0 ml; $K_2HPO_4$ 1.0g, Yeast extract, 5.0 g; Sucrose, 30.0 g; Agar, 15.0 g; Distilled water, 1.0 L. *Czapek concentrate: $NaNO_3$, 30.0g; Kcl, 5.0g; $MgSO_4.7H_2O$, 5.0g; $FeSO_4.7H_2O$, 0.1g; and Distilled water, 100.0 ml.

### Substrate Preparation

Agro industrial residue, wheat bran was collected from the local market and oven-dried slowly at $70^0$C for 48 hours and ground to 40 mesh particle size and stored till further use.

### Solid State Fermentation (SSF)

Fermentation was carried out in Erlenmeyer flasks (250 ml) with 10 g of wheat bran, 0.1% (v/v) of Tween-80, supplemented with nutrients concentrations defined by the experimental design. The initial moisture content was adjusted [19] to 80%. Each flask was covered with hydrophobic cotton and autoclaved at $121^0$C for 20 min. After cooling, each flask was inoculated with 2 ml of the spore suspension containing $1x10^6$ spores/ml prepared from 6 day old slants of the culture grown at $30^0$ C and the inoculated flasks were incubated at $30^0$C in an incubator. During preliminary screening process, the experiments are carried out for 6 days and it was found that the maximum Xylanase production occurs at the $4^{th}$ day. Hence experiments are carried out for 4 days. After fermentation, the flasks were removed and contents mixed with 50.0 ml of 0.05 M Na-citrate buffer (pH 5.3) were agitated for 30 minutes at 200 rpm in an orbital shaker at $30^0$C. The enzyme was extracted by squeezing fermented bran through a wet cotton cloth. The sample was centrifuged at 15, 000 rpm for 20 min. The supernatant was filtered through Whatman No. 1 filter paper and the clear filtrate was used for determination of enzyme activity.

### Enzyme Assay

Xylanase activity was determined by mixing 0.5 ml of 1% (w/v) oat spelt xylan (prepared in 0.05M Na-citrate buffer, pH 5.3) with 0.5 ml of suitably diluted enzyme and the mixture was incubated at 50°C for 30 min (Bailey method) [20]. The reaction was stopped by the addition of 3 ml of 3, 5-dinitrosalicylic acid (DNS) and the contents were boiled for 30 min. After cooling, the absorbance was read at 540 nm. The amount of reducing sugar liberated was quantified[21] using D-xylose as standard. One International unit (IU) of xylanase activity was defined as the amount of enzyme that liberates 1 µmol of xylose equivalents per minute under the assay conditions. Xylanase production was expressed as units IU/g of dry substrate (IU/gds).

Carboxy methyl cellulase activity was assayed by adding 0.5 ml of appropriately diluted enzyme to 0.5 ml of 1 % (w/v) of carboxymethyl cellulose (CMC) in 0.05M Na-citrate buffer, pH 5.3 and incubating at $50^0$C for 30 min. The amount of reducing sugars released during the reaction was measured using the DNS method [21] and D-glucose was used as the standard. One International unit of cellulase activity was defined as the amount of enzyme that liberated 1 µmol of glucose equivalent under the assay conditions.

**Screening Of Nutrients Using Plackett-Burman Design**

Placket-Burman design is an effective and efficient technique for the optimization of medium components and can be used to select the significant factors and to eliminate the insignificant one in order to obtain more manageable and smaller set of factors[22]. Based on the Plackett–Burman design, each factor was examined at two levels, low (−1) and high (+1). To determine which variable significantly affect xylanase production by *Aspergillus Terreus*, Plackett-Burman design using statistical software package MINITAB (Release 15.1, PA, USA), was used. For screening purpose a total of twelve medium components (Table-1) were tested for their significance in 20 experimental runs (Table-2) and insignificant ones were eliminated. Table 1 illustrates the factors under investigation as well as the levels of each factor used in the experimental design. Table 2 shows the design matrix. Significant nutrient components viz. Peptone, Yeast Extract and $Cacl_2.2H_2O$ which increases the xylanase production was identified.

**Optimization Of Screened Components Using CCD**

In order to enhance the production of xylanase, central composite design of Response Surface Methodology (RSM) was employed to optimize the three most significant factors, identified by the Plackett–Burman design. RSM is useful for small number of variables (up to five) but is impractical for large number of variables, due to high number of experimental runs required [23]. CCD was used to obtain a quadratic model, consisting of factorial trails and star points to estimate quadratic effects and central points to estimate the pure process variability with xylanase production. The statistical model was obtained using the Central Composite Design (CCD) with three independent variables [Peptone, Yeast Extract, $Cacl_2.2H_2O$]. Each factor in this design was studied at three different levels (Table 4) and a set of 20 experiments was carried out. All the variables were taken at a central coded value considered as zero. The minimum and maximum ranges of variables were used. The full experimental plan with respect to their values in coded form is shown in Table 5. All the experiments were carried out in triplicates and the average value was taken as the response.

**Statistical Analysis And Modeling**

This method is suitable for fitting a quadratic surface and it helps to optimize the effective parameters with minimum number of experiments as well as to analyze the interaction between the parameters. In order to determine the existence of a relationship between the factors and response variables, the collected data were analyzed in a statistical manner, using regression. A multiple regression analysis of the data was carried out for obtaining an empirical model that relates the response measured to the independent variables. The quadratic regression models are one of the most widely used in practice. They allow description of the object in a comparatively wide area of the input variables change [24].

A second order polynomial equation is,

$$Y = \beta_0 + \sum_{i=1}^{k} \beta_i X_i + \sum_{i=1}^{k} \beta_{ii} X_i^2 + \sum_{i=1,i<j}^{k-1} \sum_{j=2}^{k} \beta_{ij} X_i X_j \qquad (1)$$

Where Y is the measured response, $\beta_0$ is the intercept term, $\beta_i$ are linear coefficients, $\beta_{ii}$ are quadratic coefficient, $\beta_{ij}$ are interaction coefficient and $X_i$ and Xj are coded independent variables. The following equation was used for coding the actual experimental values of the factors in the range of (−1 to +1):

$$x_i = \frac{X_i - X_0}{\Delta X_i} \qquad (2)$$

atistical analysis of the data was performed by design package Design-Expert software (Version 8.0.7.1, Stat-Ease, Inc., Minneapolis, USA) to evaluate the analysis of variance (ANOVA), to determine the significance of each term in the equations fitted and to estimate the goodness of fit in each case.

**Table 1: Nutrient screening using a Plackett Burman design**

| Variables | | Levels mg/10 g dry substrate | |
|---|---|---|---|
| **Nutrient code** | **Nutrient** | **Low (-1)** | **High (+1)** |
| A | $K_2HPO_4$ | 20.0 | 100.0 |
| B | $Cacl_2.2H_2O$ | 20.0 | 30.0 |
| C | $CuSO_4$ | 2.0 | 8.0 |
| D | $FeSO_4.7H_2O$ | 5.0 | 20.0 |
| E | $KH_2PO_4$ | 100.0 | 300.0 |
| F | $MnSO4.7H_2O$ | 50.0 | 150.0 |
| G | Urea | 100.0 | 300.0 |
| H | $MgSO_4.7H_2O$ | 3.0 | 12.0 |
| J | Yeast Extract | 20.0 | 50.0 |
| K | $NH_4NO_3$ | 50.0 | 100.0 |
| L | Peptone | 100.0 | 200.0 |
| M | $ZnSO_4.7H_2O$ | 100.0 | 500.0 |

**Table 2: Plackett–Burman experimental design matrix for screening of important variables for Xylanase production**

| Run No | A | B | C | D | E | F | G | H | J | K | L | M | Xylanase Activity (IU/gds) | CMCellulase Activity (IU/gds) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 1 | 1 | 1 | -1 | -1 | 1 | 1 | -1 | 1 | 1 | -1 | -1 | 141.21 | 51.43 |
| 2 | -1 | 1 | 1 | -1 | -1 | -1 | -1 | 1 | -1 | 1 | -1 | 1 | 253.34 | 44.10 |
| 3 | 1 | -1 | 1 | 1 | 1 | 1 | -1 | -1 | 1 | 1 | -1 | 1 | 161.87 | 46.62 |
| 4 | 1 | 1 | -1 | 1 | 1 | -1 | -1 | -1 | -1 | 1 | -1 | 1 | 279.09 | 44.11 |
| 5 | -1 | -1 | 1 | -1 | 1 | -1 | 1 | 1 | 1 | 1 | -1 | -1 | 159.07 | 30.21 |
| 6 | 1 | -1 | -1 | -1 | -1 | 1 | -1 | 1 | -1 | 1 | 1 | 1 | 344.65 | 38.51 |
| 7 | -1 | 1 | -1 | 1 | -1 | 1 | 1 | 1 | 1 | -1 | -1 | 1 | 180.78 | 44.67 |
| 8 | -1 | 1 | -1 | 1 | 1 | 1 | 1 | -1 | -1 | 1 | 1 | -1 | 260.43 | 53.72 |
| 9 | -1 | 1 | 1 | 1 | 1 | -1 | -1 | 1 | 1 | -1 | 1 | 1 | 231.23 | 50.82 |
| 10 | 1 | -1 | 1 | 1 | -1 | -1 | -1 | -1 | 1 | -1 | 1 | -1 | 230.32 | 66.99 |
| 11 | 1 | -1 | 1 | -1 | 1 | 1 | 1 | 1 | -1 | -1 | 1 | 1 | 290.34 | 58.60 |
| 12 | -1 | 1 | 1 | -1 | 1 | 1 | -1 | -1 | -1 | -1 | 1 | -1 | 305.67 | 45.45 |
| 13 | 1 | -1 | -1 | 1 | 1 | -1 | 1 | 1 | -1 | -1 | -1 | -1 | 155.67 | 45.34 |
| 14 | -1 | -1 | -1 | 1 | -1 | 1 | -1 | 1 | 1 | 1 | 1 | -1 | 218.90 | 44.70 |
| 15 | -1 | -1 | 1 | 1 | -1 | 1 | 1 | -1 | -1 | -1 | -1 | 1 | 149.09 | 35.38 |
| 16 | 1 | 1 | -1 | -1 | 1 | 1 | -1 | 1 | 1 | -1 | -1 | -1 | 250.65 | 31.73 |
| 17 | 1 | 1 | 1 | 1 | -1 | -1 | 1 | 1 | -1 | 1 | 1 | -1 | 344.56 | 35.25 |
| 18 | 1 | 1 | -1 | -1 | -1 | -1 | 1 | -1 | 1 | -1 | 1 | 1 | 296.67 | 49 .40 |
| 19 | -1 | -1 | -1 | -1 | 1 | -1 | 1 | -1 | 1 | 1 | 1 | 1 | 210.09 | 30.27 |
| 20 | -1 | -1 | -1 | -1 | -1 | -1 | -1 | -1 | -1 | -1 | -1 | -1 | 210.59 | 49.21 |

**Table 3: Ranges of the independent variables used in RSM**

| Variables | | Levels (mg/10g dry substrate) | | | | |
|---|---|---|---|---|---|---|
| | **Code** | **-1.68** | **-1** | **0** | **1** | **1.68** |
| Peptone | A | 66.0 | 100.0 | 150.0 | 200.0 | 234.0 |
| Yeast Extract | B | 9.8 | 20.0 | 35.0 | 50.0 | 60.2 |
| $Cacl_2$ | C | 16.6 | 20.0 | 25.0 | 30.0 | 33.4 |

**Table 4: Central composite design (CCD) of factors in coded levels with Enzyme activity as response**

| Run. No | Coded Values | | | Xylanase Activity (IU/gds) | | Carboxy methyl Cellulase Activity (IU/gds) |
|---|---|---|---|---|---|---|
| | A | B | C | Exp. | Pred. | |
| 1 | 1 | -1 | 1 | 405.00 | 406.34 | 115.32 |
| 2 | -1 | -1 | 1 | 375.56 | 372.12 | 98.54 |
| 3 | 0 | 0 | 0 | 413.67 | 413.47 | 134.87 |
| 4 | -1 | 1 | 1 | 318.25 | 310.79 | 110.42 |
| 5 | 0 | 0 | 0 | 413.67 | 413.47 | 126.71 |
| 6 | 1 | 1 | -1 | 379.12 | 379.01 | 95.34 |
| 7 | -1.68 | 0 | 0 | 347.56 | 354.39 | 102.74 |
| 8 | 0 | 0 | 1.68 | 365.45 | 372.36 | 94.56 |
| 9 | 1 | -1 | -1 | 338.14 | 342.05 | 98.51 |
| 10 | -1 | 1 | -1 | 398.00 | 393.11 | 100.46 |
| 11 | 0 | 0 | 0 | 413.34 | 413.47 | 120.01 |
| 12 | -1 | -1 | -1 | 378.34 | 379.75 | 64.05 |
| 13 | 1.68 | 0 | 0 | 373.15 | 371.31 | 82.19 |
| 14 | 0 | 0 | 0 | 413.67 | 413.47 | 89.26 |
| 15 | 0 | 0 | 0 | 413.67 | 413.47 | 97.67 |
| 16 | 0 | 0 | -1.68 | 389.45 | 387.53 | 66.43 |
| 17 | 0 | 1.68 | 0 | 352.87 | 361.50 | 83.76 |
| 18 | 0 | -1.68 | 0 | 385.63 | 381.99 | 65.36 |
| 19 | 1 | 1 | 0 | 373.56 | 368.60 | 98.32 |
| 20 | 0 | 0 | 1 | 413.67 | 413.47 | 101.54 |

**Table 5: Analysis of Variance (ANOVA) for response surface quadratic model for the production of Xylanase**

| Source | Coefficient factor | Sum of squares | DF | F | P > F | |
|---|---|---|---|---|---|---|
| Model | 413.47 | 15011.6 | 9 | 51.39 | < 0.0001 | Significant |
| *A-peptone* | 5.03 | 345.66 | 1 | 10.65 | 0.0085 | |
| *B-Yeast Extract* | -6.09 | 506.94 | 1 | 15.62 | 0.0027 | |
| *C-*$CaCl_2.2H_2O$ | -4.51 | 277.79 | 1 | 8.56 | 0.0152 | |
| *AB* | 5.90 | 278.36 | 1 | 8.58 | 0.0151 | |
| *AC* | 17.98 | 2585.88 | 1 | 79.67 | < 0.0001 | Significant |
| *BC* | -18.67 | 2789.67 | 1 | 85.95 | < 0.0001 | Significant |
| $A^2$ | -17.89 | 4614.81 | 1 | 142.18 | < 0.0001 | Significant |
| $B^2$ | -14.75 | 3135.31 | 1 | 96.6 | < 0.0001 | Significant |
| $C^2$ | -11.85 | 2023.93 | 1 | 62.36 | < 0.0001 | Significant |
| Residual | | 324.57 | 10 | | | |
| Lack of Fit | | 324.48 | 5 | 3575.53 | 0.0001 | |
| Pure Error | | 0.091 | 5 | | | |
| Cor Total | | 15336.2 | | | | |

Std. Dev.- 5.70; $R^2$ – 0.9788; Mean – 383.09; Adj $R^2$ – 0.9598; C.V – 1.49%;
Pred $R^2$ – 0.8392; Adeq Precision – 25.489

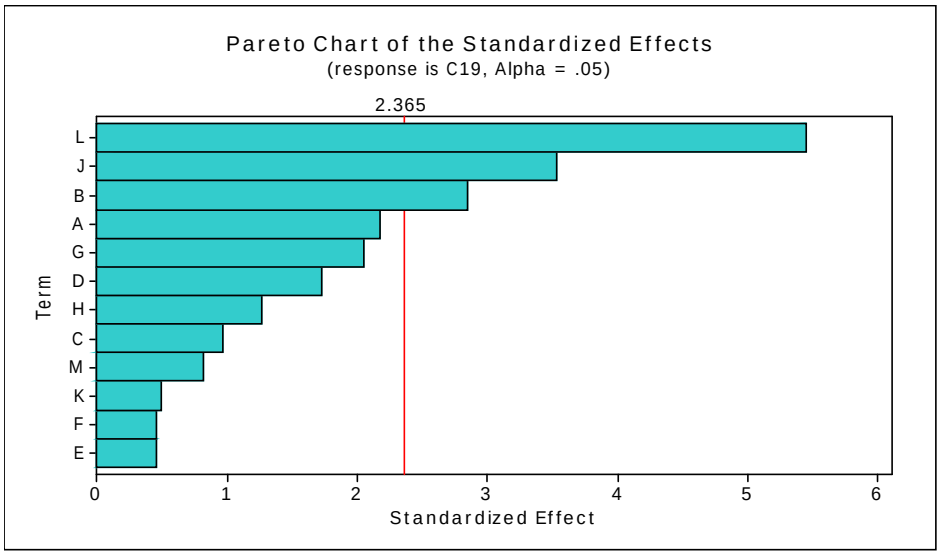


**Fig. 1. Pareto chart showing the effect of media components on Xylanase activity**

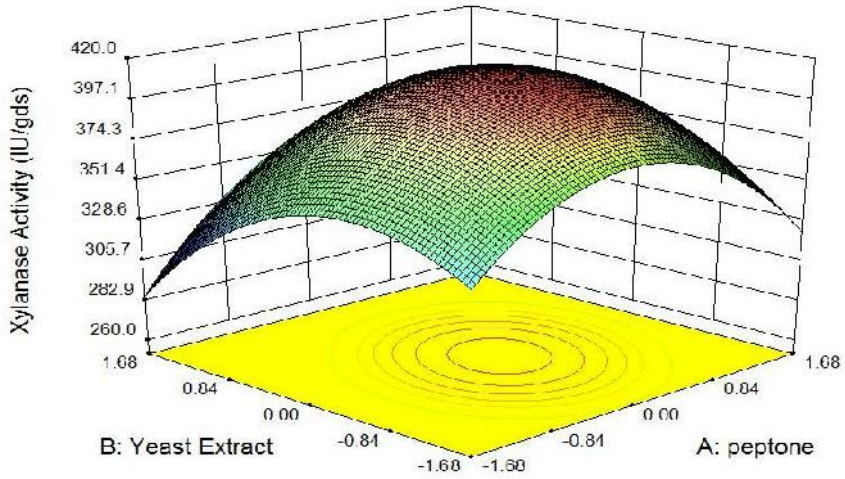


**Fig. 2. Contour plot and Three-dimensional Surface plot showing the interactive effect of Peptone and Yeast Extract on Xylanase activity**

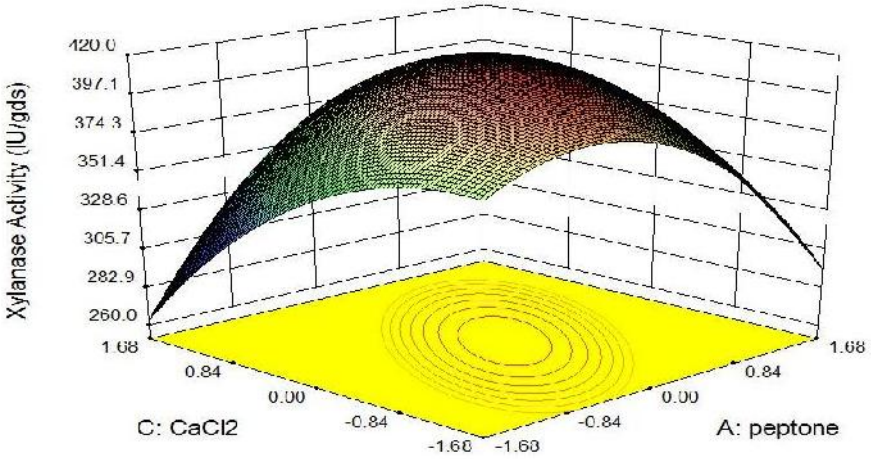


**Fig. 3. Contour plot and Three-dimensional Surface plot showing the interactive effect of Peptone and $Cacl_2$ on Xylanase activity**

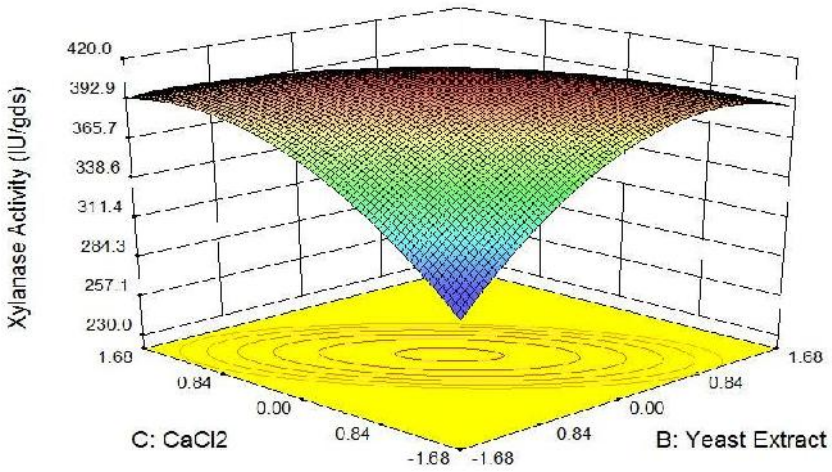


**Fig. 4. Contour plot and Three-dimensional Surface plot showing the interactive effect of Yeast Extract and $Cacl_2$ on Xylanase activity**

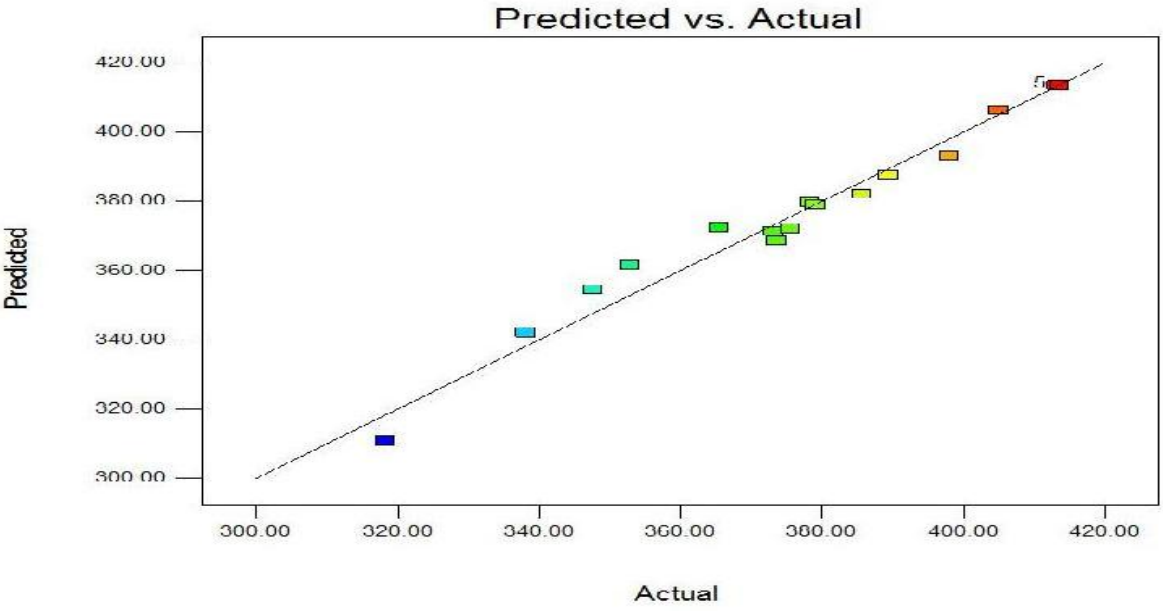


**Fig .5. Predicted Response Vs Actual Value**

## Results And Discussion

The twenty run design given in Table 2 indicates a wide variation in the production of xylanase from 141.21 to 344.65 IU/gds, which reflects the importance of medium optimization to attain a better yield. From the Pareto chart shown in Fig 1, it was found that three components namely: Peptone, Yeast Extract, $Cacl_2.2H_2O$, had positive effect on Xylanase activity. Yeast extract was added to assist with spore germination and initial growth [25]. All the insignificant variables from the Plackett-Burman design were neglected and the optimal concentration of the significant variables was further analyzed using central composite design (CCD) of RSM. For this study, $2^3$ full factorial central composite design with sixteen star points, eight axial points and six replicates at the centre points were employed to fit the second order polynomial model which indicated that 20 experiments were required for this procedure. The coded and actual values of the significant variables are shown in Table 4.

The predicted and observed responses along with design matrix are presented in Table-4, and the results were analyzed by ANOVA. The following second order polynomial equation describing the correlation between xylanase and the four variables was obtained:

$$Y = 413.47 + 5.03A - 6.09B - 4.51C + 5.90AB + 17.98AC - 18.67BC - 17.89A^2 - 14.75B^2 - 11.85C^2 \quad (3)$$

Where, Y is the Xylanase activity (IU/gds), A, B and C are Peptone, Yeast Extract, $Cacl_2.2H_2O$ respectively.

ANOVA for the response surface is shown in Table 5. The model *F* value of 51.39implies the model is significant. There is only a 0.01% chance that a "Model *F* value" this large could occur due to noise. Values of "prob > *F*" less than 0.05 indicate model terms are significant. In this case, the coefficients A, B, $A^2$, $B^2$, $C^2$, AC and BC were found to be highly significant. Values greater than 0.1 indicates model terms are not significant. The fit of the model was checked by the coefficient of determination $R^2$ for xylanase activity was calculated as 0.9788, which is very close to 1, indicating that 97.88% of variability in the response could be explained by the model. Normally, a regression model having an $R^2$ value higher than 0.90 is considered to have a very high correlation [26]. The predicted $R^2$ value of 0.8392was in reasonable agreement with the adjusted $R^2$ value of 0.9598. Usually higher the value of C.V, lower the reliability of the experiment is [27]. Here, a lower value of C.V (1.49%) indicated a better precision and reliability of the experiments. An adequate precision value greater than 4 is desirable. The adequate precision value of 25.489 indicates an adequate signal and suggests that the model can be to navigate the design space. In the present work, all the linear, interactive effects and square effects of *A*, B and C were significant for Xylanase production. The above model can be used to predict the Xylanase production within the limits of the experimental factors.

To study the interaction effects of variables on xylanase production Contour plot & Three-dimensional surface graphs were generated for pair wise combinations of the three factors, with the third & fourth variable in each case fixed at its central (0) level for Xylanase production. The three dimensional curves of the calculated response (Xylanase production) and contour plots from the interactions between the variables are shown in Figures.2 to 4. A circular contour plot indicates that the interactions between the corresponding variables are negligible, while an elliptical contour plot indicates that the interactions between them are significant. Fig.2 shows the dependency of Xylanase activity on Peptone and Yeast extract . As can be seen from Figure 2, an increase in Peptone resulted in an increase in xylanase activity to about 0.015765 g/gds and thereafter Xylanase activity decreased with further increase in Peptone. The same trend was observed in Fig 3. Peptone was the best-utilized nitrogen source for xylanase production among the organic nitrogen sources [28]. Increase in Yeast Extract resulted in increase of Xylanase activity up to 0.003169 g/gds. Yeast extracts contain a lot of vitamins, minerals and amino acids, which are usually used as growth stimulants or growth factors for microbes. This is evident from Fig. 2 and 4. Fig.3 and 4 shows the dependency of Xylanase activity on $Cacl_2.2H_2O$. Xylanase activity was greatly stimulated by Ca2+. It is implied that a few metal ions especially Ca2+ is required for maintaining the structure stability [29]. The effect of $Cacl_2.2H_2O$ on Xylanase observed was similar to other variables. The maximum xylanase activity was observed at 0.002543 g/gds of $Cacl_2.2H_2O$.

The maximum predicted yield is indicated by the confined surface in the plot and the optimum values were obtained by solving the second order polynomial equation [30]. The second degree polynomial equation was maximized by a constraint search procedure using the MINITAB software to obtain the optimal levels of the independent variables and the predicted maximum xylanase activity. The optimum conditions are: Peptone – 0.015765 g/gds ; yeast Extract= 0.003169 g/gds; $Cacl_2.2H_2O$ = 0.002543 g/gds. The predicted values from the regression equation closely agreed with that obtained from experimental values. Along with nutrient optimized xylanase production, very poor carboxy methyl cellulase activity was detected in all the 30 experimental runs**.** Fig.5 shows that the experimental xylanase activity values agree well with the predicted response values.

### Experimental Validation Of The Model

Validation of the experimental model was tested by carrying out the batch experiments under optimal operation conditions. Three repeated experiments were performed, and the results are compared. The xylanase activity 415.03 IU/gds obtained from experiments was very close to the actual response 415.48 IU/gds, predicted by the regression model, which proved the validity of the model.

## Conclusion

The results showed the potential use of solid state fermentation for the production of Xylanase by *Aspergillus terreus* using central composite design of Response Surface Methodology. This study also shows that an agro-industrial waste wheat bran constitutes a cheaper carbon source for the production of Xylanase. In conclusion, a higher xylanase activity of 415.03 IU/gds was obtained with the optimized medium comprising of Peptone – 0.015765 g/gds**;** Yeast Extract= 0.003169 g/gds; $Cacl_2.2H_2O$ = 0.002543 g/gds.

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