American Journal of Microbiological Research, 2015, Vol. 3, No. 2, 85-92
Available online at http://pubs.sciepub.com/ajmr/3/2/8

DOI:10.12691/ajmr-3-2-8

Statistical Optimization of Medium Composition for Xylanase Production by Solid State Fermentation Using Agroresidues

Chaturvedi S[1,*], Kohli K U[1], Rajni S[2], Khurana SMP[1]

[1]Amity Institute of Biotechnology, Amity University Haryana Manesar, 122413, India
[2]Amity Institute of Microbiology, Amity University Noida, Uttar Pradesh, India
*Corresponding author: sarikachaturvedi@gmail.com

Received January 13, 2015; Revised April 05, 2015; Accepted April 16, 2015

Abstract Two stage statistical designs were used to optimize xylanase production from a newly isolate *Bacillus licheniformis* under solid state fermentation. Plackett burmann and central composite design in response surface methodology were used to build statistical models to screen out the significant variables and then study the effect of three significant variables on xylanase production. Twelve variables screened initially with Plackett burmann design were substrate concentration, glucose, ammonium sulphate, KH_2PO_4, K_2HPO_4, $FeSO_4$, $MgSO_4$, $MnSO_4$, yeast extract peptone, $CaCl_2$ & NaCl. Further three variables ammonium sulphate, glucose and peptone were selected via central composite design for xylanase production. The maximum xylanase production after optimization was increased 2.38 fold yield over conventional strategy. For glucose, ammonium sulphate and peptone were significantly showing that these were the most significant factors affecting the enzyme production. 99% of total variation was explained by the model elaborated. The determination coefficient (R^2) as shown by analysis of variance(ANOVA) was 0.9974 showing adequate credibility of the model. The properties of the isolated enzyme are adequate for its use industries as pulp and paper industry, textile industry, food processing & wine industry.

Keywords: *Bacillus licheniformis, Solid state fermentation, Plackett Burman design, Central composite design, Response surface methodology*

Cite This Article: Chaturvedi S, Kohli K U, Rajni S, and Khurana SMP, "Statistical Optimization of Medium Composition for Xylanase Production by Solid State Fermentation Using Agroresidues." *American Journal of Microbiological Research*, vol. 3, no. 2 (2015): 85-92. doi: 10.12691/ajmr-3-2-8.

1. Introduction

Xylan is widely distributed in lignocellulosic agricultural wastes including corncob, rice husk, wheat bran, wheat straw since Xylan derivatives are frequently used to induce the production of xylanase by microorganisms, using either soild state or submerged fermentation. Xylanase (1,4-D xylan xylanohydrolase; E.C 3.2.1.8) hydrolyzes the major hemicellulosic polysaccharide xylan and randomly cutting the arabinoxylan backbone produces a wide range of arabinoxylan fragments. The production cost of enzyme is very important for their industrial production. 30-40% of the costs is usually affected by the cost of substrate. Thus, by the utilization of low cost substrates as agricultural wastes in fermentation processes. The profitability can be effective increased.

Production of xylanase by solid-state fermentation (SSF) using various lignocellulosic substrates has been reported previously [1,2]. Solid state fermentation has numerous advantages over submerged fermentation, such as high titres & higher productivity, lower operational, simpler fermentation equipments, less effluent generation, easy process management and capital costs. The utilization of wheat bran as substrate in SSF processes is an alternative avenue and value addition to these residues. On the other hand, high yield xylanase production strains have been screened [3], but the fermentation time was 4~12 d and that was not adequate in meeting industrial needs [3,4,5,6,7].

The biotechnological applications of xylanases has been in the preparation of animal feed, pulp and paper industry, textile industry, food processing, chemical industry, wine industry and waste treatment [8,9,10,11,12]. Xylanase has also important application in industry due to their enormous potential to modify & transform the lignocelluloses & cell wall materials abundant in vegetable biomass. Xylanase are widely distributed occurring both prokaryotes & eukaryotes [13]. Among the prokaroytes, bacteria [14] and cyanobacteria from marine environment produce [15] and in eukaryotes as fungi [16], yeast [17,18] have been reported to synthesize xylanase. Several bacterial species secrete high levels of extra-cellular xylanase [19].

In the present study, the objective was to optimize the best medium for the production of extracellular xylanase by isolated strain of *Bacilus licheniformis* strain in solid

state fermentation (SSF) by employing Placket Burman and Central composite design. In contrast statistically planned experiments effectively advantageous due to reduce the number of experiments for a large number of factors. Among various nonlinear and quadratic optimization techniques available response surface methodology is the most studied and employed techniques for optimization process in the recent years. In that a specific statistical approach e.g. Plackett Burman designs & Central composite designs under RSM which also minimize the error in determining the values for significant parameter [20]. The effect of medium composition for enhanced enzyme yield is evaluated using statistical design experiments.

2. Materials and Method

2.1. Isolation & Screening of the Xylanase Producing Bacteria from Decaying Wood

Colonies developed were assayed by xylanolytic activity on the xylan agar plates. Colonies showing haloes around them were picked up & purified. The strains were stored at 4 ℃ and sub cultured routinely after every three four weeks.

2.2. Preparation of Inoculum

The isolated strains inoculated in yeast extract, peptone, xylan (2:1:0.5%w/v) and incubated at 45 ℃ for 16 h at 160 rpm, and 1% v/v of these cultures were used as Standard inoculums.

2.3. Preparation of Substrates

Oat spelt xylan (Himedia Laboratories Pvt. Ltd., India) was used for enzyme assay. The different substrates as corn cob, rice husk, rice straw, sugarcane bagassae & wheat bran were procured from the local mills. These substrate washed 2-3 times in distilled water. The water was then decanted and substrates were dried in an oven and powdered using mortar and pestle. The powder was then sieved (2-3 mm particle size).

2.4. Solid State Fermentation

The fermentation media (basal salt solution BSS) containing 5 g of wheat bran/corn cob and of BSS %: $MgCl_2.6H_2O$, 6.6; K_2HPO_4, 0.5; KH_2PO_4, 0.5; $(NH_4)_2S0_4$, 2.0 g; pH 6.7 and moisture ratio 1:4 were inoculated with 1% (v/w) of inocula (16 h old). after this solid substrate was suspended in 50 mM glycine-NaOH buffer (pH 9) vortexed thoroughly to extract the xylanase. The flask without the inoculation was used as control and assayed for enzyme activity.

2.5. Enzyme Extraction

Enzyme was extracted with 50 mM glycine-NaOH buffer (pH 9), and vortexes thoroughly to extract the xylanase enzyme and squeezed through a wet muslin cloth. The enzyme extracted was centrifuged at 15000 rpm for 30 min at 4 ℃. The clear supernatant was used in the enzyme assay.

2.5.1. Xylanase Assay

Xylanase activity was measured by incubating 0.5 ml of 0.4% (w/v) oat spelt xylan in 0.02 M phosphate buffer (pH 7.0) and 0.5 ml of suitably diluted enzyme extract at 45 ℃ for 30 min. The release of reducing sugar was measured as xylose by dinitro salicylic acid method [21]. One unit (U) of xylanase is defined as the amount of enzyme that releases 1 µmol xylose/ml/min under the experimental conditions. The experiments were carried out in duplicates.

2.5.2. Cellulase Assay

Cellulase was assayed [22] using sodium nitrate buffer (0.1M, pH 7.0) at 45 ℃. One unit of cellulose is defined as the amount of enzyme that liberates 1 µmol reducing sugar as glucose/ml/ min under assay condition.

2.6. Selection of Substrate

Corn cob, rice husk, rice straw, sugarcane bagassae & wheat bran were used substrate in the basal media in different flasks. One substrate further was selected for SSF which gave the maximum xylanase production. This substrate along with other factors was used for optimization.

2.7. Statistical Design

Statistical analysis was applied for statistical analysis & to investigate the significant factor(s) for the maximum production of enzyme. In the media numerous factors affecting the enzyme production. As Response Surface Methodology consists of a group of empirical techniques used for evaluation of relationship between cluster of controlled experimental factors and measured response. RSM with a complete factorial design was used to evaluate the optimization of variables. A prior knowledge with understanding of the related bioprocesses is necessary for a realistic modeling approach. The statistical software package, Stat ease (Design Expert 9.0.7.1) was used for the analysis of the experimental data, the statistical optimization parameters was performed in two steps.

2.7.1. Plackett-Burman Design

Plackett-Burman design is a two level fractional factorial design. PB was used to determine which factors significantly affect xylanase production that screens out as the main factors affecting a process with least number of experiments. 12 variables (substrate concentration, glucose, ammonium sulphate, KH_2PO_4, K_2HPO_4. $FeSO_4$, $MgSO_4$, $MnSO_4$, yeast extract, peptone, $CaCl_2$ & NaCl) were screened in 20 experimental runs and insignificant ones were eliminated in order to obtain a smaller, manageable set of factors. The low level (-1) and high level (+1) with experimental xylanase activity of each factor are listed in Table 1. F value and P values and the proportion of variance R^2 determined the model were significant at P=5% level.

2.7.2. Central Composite Design

In the second level optimization, central composite design is based on model building by regression for the purpose of process optimization with experimental

designs. Significant factors obtained from PB design, three nutrient components (glucose, $(NH_4)_2SO_4$, and peptone) were selected for the second level optimization employing CCD under RSM. The three independent variables were studied over six different levels (Table 2) and sets of 20 trials (batch experiments) were carried out. All variables were taken at a central coded value of zero. The minimum and maximum ranges of variables investigated are listed in Table 3. Upon the completion of experiments, the average maximum xylanase were taken as the response (Y). A multiple regression analysis of the data was carried out for obtaining an empirical model that relates the response measured to the independent variables. A second order quadratic polynomial model of the form of Equation (1) was used to fit the data:

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_{11} X_1^2 + \beta_{22} X_2^2 + \beta_{33} X_3^2 + \beta_{12} X_1 X_2 + \beta_{13} X_1 X_3 + \beta_{23} X_2 X_3$$

where Y is the measured response for xylanase activity; β_0 is the intercept term; β_1, β_2 and β_3 are the linear coefficients; β_{11}, β_{22} and β_{33} are the quadratic coefficients; and β_{12}, β_{13} and β_{23} are the coded interaction coefficients and X_1, X_2, X_3 coded independent variables respectively. The terms which were not statistically significant were removed from the model and added to the lack of fit.

3. Results and Discussion

Microorganism The extra cellular xylanase was obtained from the newly isolated strain of *Bacillus licheniformis* KJ842626 and its yield dependent on different growth factors.

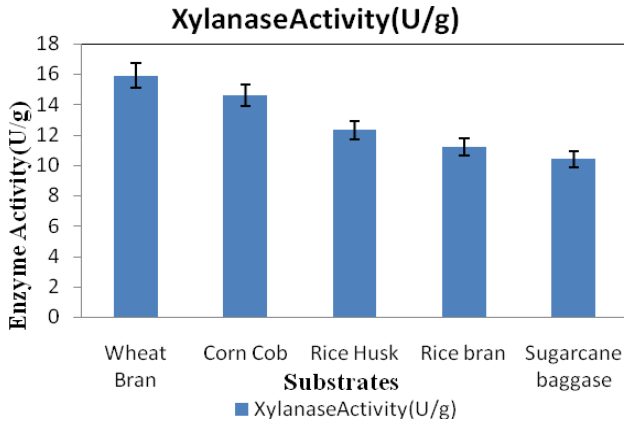

Figure 1. Xylanase production with different substrates (Wheat bran, Corncob, Rice husk, Rice bran, Sugarcane baggase)

Selection of the Solid Substrate Five different agriculture wastes were selected for xylanase production by solid state fermentation (SSF). The maximum enzyme production (15.9 U/g) wheat bran followed by corncob (14.6 U/g) the different wastes as rice husk, rice bran, sugarcane baggase (12.3 11.2, 10.5 U/g) exhibited low enzyme production in the similar conditions (Figure 1). Wheat bran contains 30% cellulose, 27% hemicellulose, 21% lignin, and 8% ash commercial [23]. This explains the better substrate for xylanase production as it is easily available throughout the world, cheap and good source of hemicelluloses. *Bacillus licheniformis* KJ842626 also assayed for cellulase activity. It indicated its cellulase-free xylanase producing nature. Several researchers reported the suitability of wheat bran for xylanase production in SSF [24]. A new thermoalkalophilic species of *Bacillus arseniciselenatis* DSM 15340 also produced xylanase with wheat bran as a substrate under solid state fermentation [25]. Therefore wheat bran was chosen as a substrate for further optimization.

Statistical Optimization of media In this statistical optimization firstly Plackett Burman design used for screening of the significant variables effecting the enzyme production. Second step, central composite design optimized significant variables along with their interaction on enzyme production.

Plackett Burman Design Plackett Burman is a complete factorial design for screening experiments. This is used to identify the significant variables affecting a response with two factor interactions. It was carried out by using 20 run to identify the significant factors for xylanase production. Twelve variables (Substrate concentration, glucose, ammonium sulphate, KH_2PO_4, K_2HPO_4, $FeSO_4$, $MgSO_4$, $MnSO_4$, yeast extract, peptone, $CaCl_2$ & NaCl) were analyzed for their effects on xylanase production. Experimental xylanase yields are given in Table 1.

The xylanase activity varied between 3.48 to 16.5 U/g with in tested conditions as given by PB design. Further the polynomial equation which was used to explain the xylanase production as(Eq 2):

$$Y = +0.45076 + 18.60000A + 132.10000B + 3.47948C + 4.30370E + 1.28283F + 4.10774G + 11.27273H + 0.22222K$$

Where Y is the xylanase activity (U/g), A, B, C, E, F, G, H, K represents substrate concentration, glucose, ammonium sulphate, K_2HPO_4, $FeSO_4$, $MgSO_4$, $MnSO_4$ & peptone. ANOVA showed that the Model F value of 13.45 implies the model is significant with factors ammonium sulphate, glucose & peptone as significant model terms. The p value of model is significant (0.0002) as less that 0.05 is desirable (Pro>F" less than 0.05). Further the p value of B, C, K variables were < 0.0001, 0.0007, 0.0128 respectively were considered to have a significant effect on xylanase production. These three factors were further selected for further studies. The coefficient of determination (R^2) for xylanase activity was calculated as 0.8613, that is close to 1 and it's explain to 86.13% variability of the response. The predicted R^2 value 0.6717 and the adjusted R^2 value are 0.7973. Adeq Precision measures the signal to noise ratio. A ratio greater than 4 is desirable. The ratio of our model is 11.954 which indicated an adequate signal. This model is applicable to the design space. Approximately similar results are obtained [26,27,28].

The pareto graph is important tool for analyzing about all the parameters then to focus on the most significant factors. It uses to separate the less significant factor by identifying that factor having the greatest cumulative effect on the system. It is shown by a series of bars in which impact of factors is represented by heights. In the present analysis, the Student's t values for glucose, ammonium sulphate & peptone were significantly showing that these were the most significant factors affecting the enzyme production (Figure 2). Thus the values of pareto chart & ANOVA table, three most

significant factors were selected for second level optimization by CCD. Other negatively factors were eliminated.

Central composite Design for second level optimization: CCD was carried in order to determine the optimal concentration of crucial factors and their interaction with each other after the PB designing. In this step glucose, ammonium sulphate & peptone were optimized by response surface methodology (RSM). In all 20 runs the xylanase activity varied from 12.15 to 43.12. The results of analysis of variance are represented in Table 3. And the second order polynomial Eq. 2 was used to explain the xylanase production and interaction among the factors (in coded form) as:

$$Y = +15.21308 + 0.88019A + 7.41422B - 0.51563C$$
$$-0.017800AB - 4.53333E - 004AC + 0.037000BC$$
$$-2.35238E - 003A^2 - 1.73581B^2 + 9.17909\ E - 003C^2$$

Where y is the xylanase activity, A ammonium sulphate, B glucose & C peptone respectively.

Table 1. Plackett Burman design with experimental values of xylanase production

Std	Run	A;Substrate Concentration	B:Glucose	C:Ammonium Sulphate	D:KH2PO4	E:K2HPO4	F:FeSO4	G:MgSO4	H:MnSO4	I:Yeast Extract	J:Peptone	K:CaCl2	L:NaCl	Xylanase Activity(U/G)
1	15	0.05	0.05	0.001	0.001	0.3	1	0.3	0.1	0.01	10	0.001	0.5	13.2
2	19	0.01	0.05	1	0.001	0.03	1	0.3	0.1	10	0.01	0.1	0.01	15.9
3	13	0.05	0.01	1	1	0.03	0.01	0.3	0.1	10	10	0.001	0.5	12
4	16	0.05	0.05	0.001	1	0.3	0.01	0.003	0.1	10	10	0.1	0.01	13.9
5	20	0.01	0.05	1	0.001	0.3	1	0.003	0.001	10	10	0.1	0.5	16.5
6	9	0.01	0.01	1	1	0.03	1	0.3	0.001	0.01	10	0.1	0.5	9.87
7	3	0.01	0.01	0.001	1	0.3	0.01	0.3	0.1	0.01	0.01	0.1	0.5	5.74
8	4	0.01	0.01	0.001	0.001	0.3	1	0.003	0.1	10	0.01	0.001	0.5	6.13
9	6	0.05	0.01	0.001	0.001	0.03	1	0.3	0.001	10	10	0.001	0.01	7.56
10	14	0.01	0.05	0.001	0.001	0.03	0.01	0.3	0.1	0.01	10	0.1	0.01	12.6
11	2	0.05	0.01	1	0.001	0.03	0.01	0.003	0.1	10	0.01	0.1	0.5	4.56
12	7	0.01	0.05	0.001	1	0.03	0.01	0.003	0.001	10	10	0.001	0.5	8.12
13	11	0.05	0.01	1	0.001	0.3	0.01	0.003	0.001	0.01	10	0.1	0.01	10.9
14	8	0.05	0.05	0.001	1	0.03	1	0.003	0.001	0.01	0.01	0.1	0.5	8.75
15	17	0.05	0.05	1	0.001	0.3	0.01	0.3	0.001	0.01	0.01	0.001	0.5	14.6
16	18	0.05	0.05	1	1	0.03	1	0.003	0.1	0.01	0.01	0.001	0.01	15.11
17	12	0.01	0.05	1	1	0.3	0.01	0.3	0.001	10	0.01	0.001	0.01	11.5
18	10	0.01	0.01	1	1	0.3	1	0.003	0.1	0.01	10	0.001	0.01	10.2
19	5	0.05	0.01	0.001	1	0.3	1	0.3	0.001	10	0.01	0.1	0.01	6.89
20	1	0.01	0.01	0.001	0.001	0.03	0.01	0.003	0.001	0.01	0.01	0.001	0.01	3.48

Table 2. Analysis of variance (ANOVA) for selected factorial model for production of Xylanase

	Sum of		Mean	F	p-value	
Source	Squares	df	Square	Value	Prob > F	Significant
Model	246.92	6	41.15	13.45	< 0.0001	significant
B-Glucose	139.60	1	139.60	45.64	< 0.0001	significant
C-Ammonium Sulhate	60.41	1	60.41	19.75	0.0007	significant
E-K2Hpo4	6.75	1	6.75	2.21	0.1612	
F-FeSO4	8.06	1	8.06	2.64	0.1284	
G-MgSO4	7.44	1	7.44	2.43	0.1428	
K-Peptone	24.64	1	24.64	8.06	0.0140	significant
Residual	39.76	13	3.06			
Cor Total	286.68	19				
Std Dev 1.75 R Squared 0.8613 Mean 10.37 Adj RSquared 0.7973 C.V% 16.86 Pred RSquared 0.6717 PRESS 94.12 Adeq Precision 12.962						

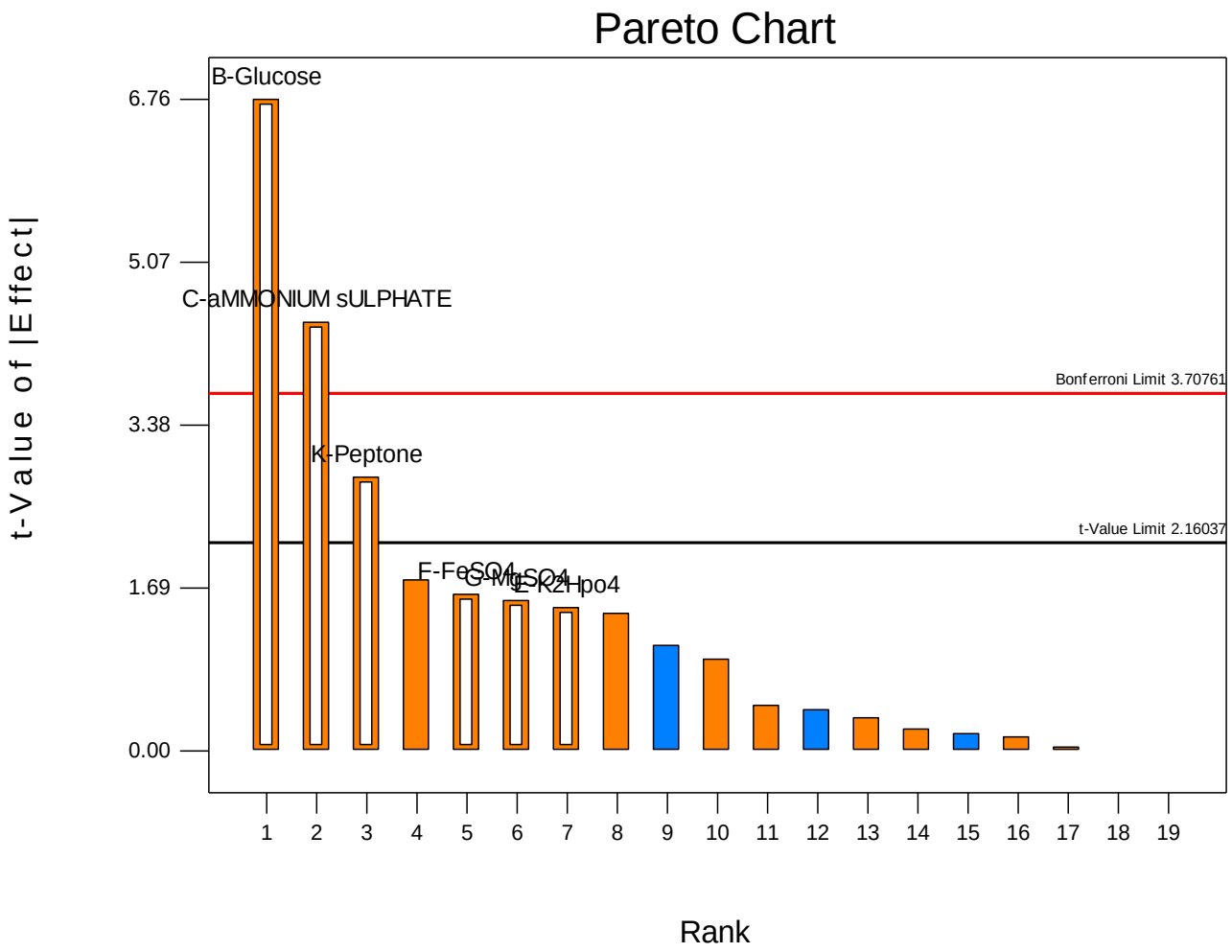

Figure 2. Pareto chart showing the relative effect of various factors on enzyme production

Table 3. ANOVA for Response Surface Quadratic model of Central Composite Design

Source	Sum of squares	Df	Mean square	F value	P value(Prof > F)
Model	505.89	9	56.21	217.07	< 0.0001
A	35.36	1	35.36	136.57	< 0.0001
B	71.76	1	71.76	277.12	< 0.0001
C	50.00	1	50.00	193.09	< 0.0001
AB	57.10	1	57.10	220.49	< 0.0001
AC	0.010	1	0.010	0.040	0.8494
BC	35.43	1	35.43	136.84	< 0.0001
A2	41.53	1	41.53	160.39	< 0.0001
B2	3.92	1	3.92	15.14	0.0115
C2	8.70	1	8.70	33.61	0.0022
Residual	1.29	5	0.26		
Lack of Fit	0.11	1	0.11	0.37	0.5744
Pure Error	1.18	4	0.30		
Cor Total	507.18	14			

Results of analysis variance (ANOVA) of Central composite design

Std Dev 0.51
R Squared 0.9974
Mean 30.84
Adj RSquared 0.9929
C.V%1.65
Pred RSquared 0.9729
PRESS 13.72
Adeq precision 55.308

The ANOVA of model implies the F value 217.07 as the model is significant and a P value(P>F) (< 0.0001). Similarly, P value of 0.0001 was reported in *Bacillus pumulisSV-85S* for xylanase production [29]. The model F Value of A,B,C,AB,AC,BC,A^2,B^2,C^2 are significant. The lack of Fit F value is *0.37* P value (P>F) (< *0.0001*) implies the lack of fit is non significant that give the model is significant.

Determination coefficient R^2 value is 0.9974 indicated that only 1% of the total variations were not explained by this model. This implies that experimental data is quite satisfactory. The adjusted determination coefficient 'Adjusted R^2 0.9929 supported the model is significant. The Predicted R^2 0.9729. Adequate precision is a measure of the signal to noise ratio and a value generally greater than 4 is desirable so the Adequate Precision value 55.308 indicates the adequate signal.

CCD was used for three variables to evaluate their effect on xylanase production. To investigate the

interaction between two factors on xylanase production, RSM was use and three dimensional plots were drawn between two factors keeping third factor at fixed level. The circular shape of the curve indicates no interaction while elliptical shape indicates good variation of two variables. The response surface plots between glucose, ammonium sulphate and peptone are shown in figure 2. The enzyme production in the initial unoptimized conditions was found to be 20.14, while after optimization the maximum production was 48.12 U/g after 48 h when the glucose concentration 17.5 g/l with peptone 5 g/l. This show that carbohydrate and nitrogen source are used for the maximum production of xylanase enzyme.

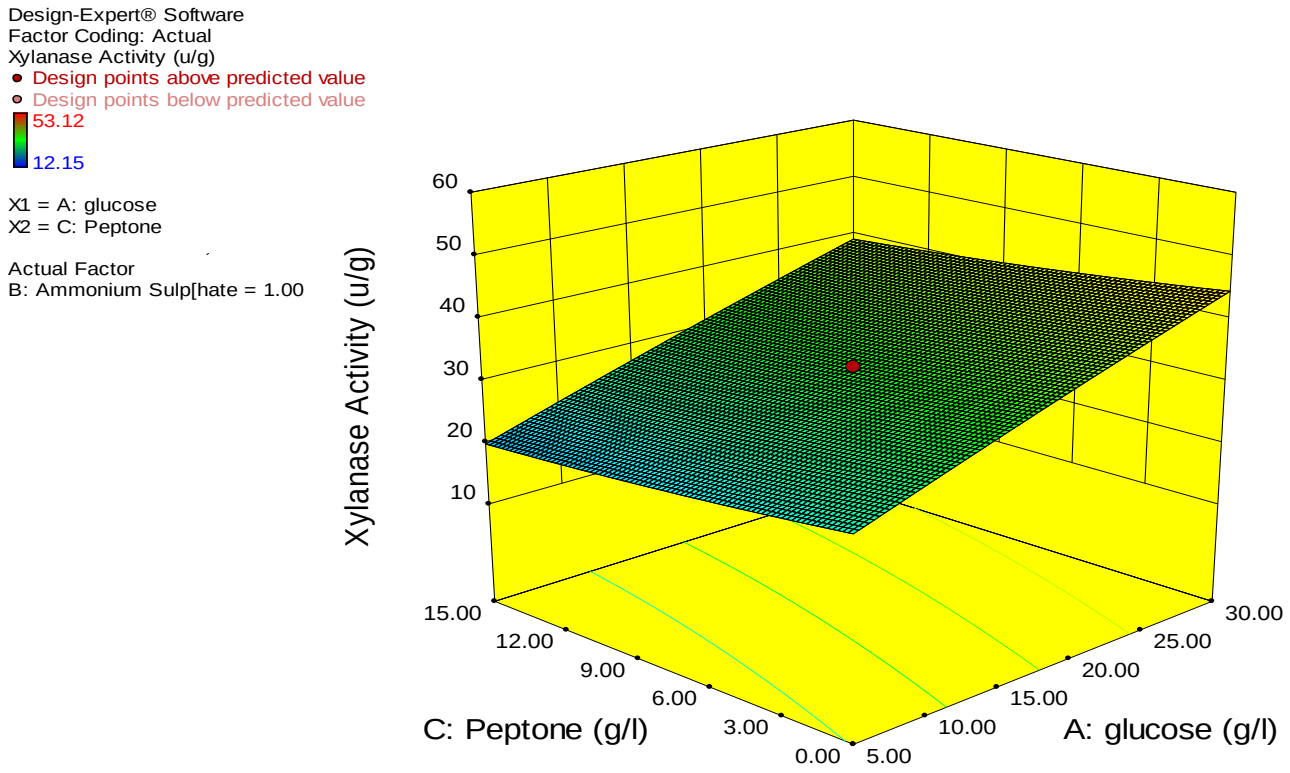

Figure 3. Response Surface plot showing the effect of Glucose & peptone and their mutual interaction on Xylanase activity

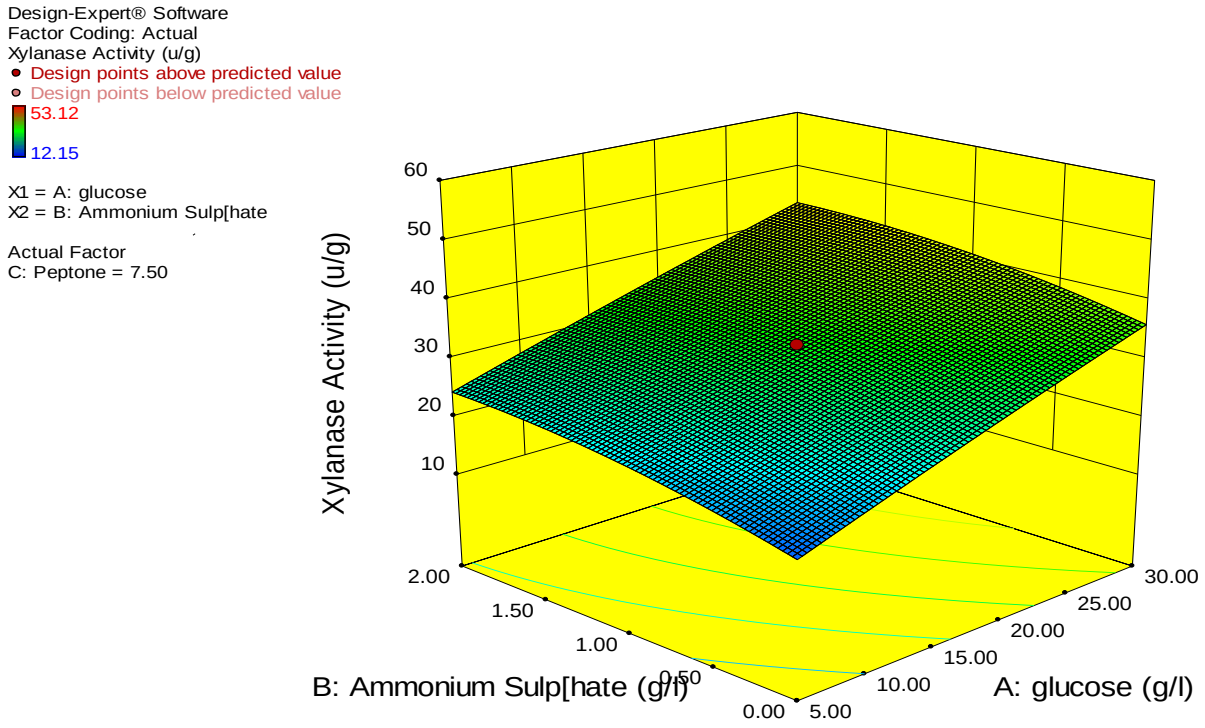

Figure 4. Response Surface plot showing the effect of $(NH_4)_2SO_4$ and glucose and their mutual interaction on Xylanase activity

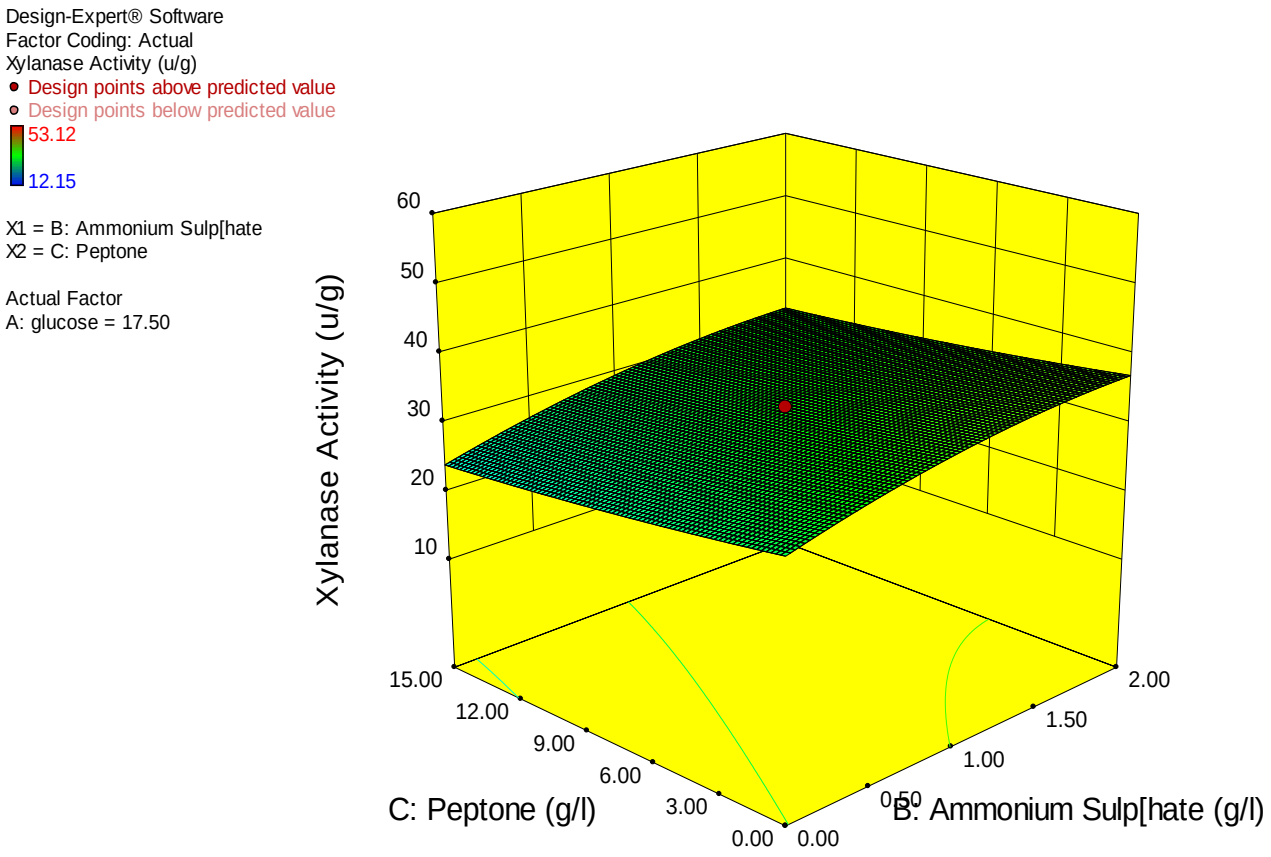

Figure 5. Response Surface plot showing the effect of $(NH_4)_2SO_4$ and peptone and their mutual interaction on Xylanase activity

4. Conclusion

This work demonstrates the statistical steps comprising of Plackett Burman and Central composite factorial design to optimize condition that contribute the maximum enzyme production. Optimization of fermentation process under solid state fermentation through statistical approach could overcome the limitation of classical method. Based on Statistical approach the elaborated model resulted in 2.38 fold increase in xylanase activity over conventional process. Xylanase have applications in various industries as pulp and paper industry, textile industry, food processing & wine industry. Owing to the novel properties of the enzyme, the increase in production after optimization would help in the industrial application of enzyme. Present study also demonstrates the utilization of the agricultural residues for the production of enzymes at low cost.

Acknowledgments

The authors kindly thank the faculty and staff of Amity Institute of Biotechnolgy, Haryana. We acknowledge Ms Shikha Khendelwal for their support in using software.

Competing Interests

The authors declare that they have no conflict of interests.

References

[1] Ghanem, N.B., Yusef, H.H. and Mahrouse, H.K, "Production of *Aspergillus terreus* xylanase in solid-state cultures: application of the Plackett-Burman experimental design to evaluate nutritional requirement," Bioresour Technol, 73 (2). 113-121. June 2000.

[2] Li, Y., Liu, Z.Q., Cui, F.J., Xu, Y.Y. and Zhao, H, "Application of Plackett-Burman experimental design and Doehlert design to evaluate nutritional requirements for xylanase production by *Alternaria mali* ND-16," Appl Microbiol Biotechnol, 77 (2). 285-291. Nov 2007.

[3] Yang, S.Q., Yan, Q.J., Jiang, Z.Q., Li, L.T., Tian, H.M. and Wang, Y.Z, "High-level of xylanase production by the thermophilic *Paecilomyces themophila* J18 on wheat straw in solid-state fermentation," Bioresour Technol, 97 (15). 1794-1800. Oct 2006.

[4] Narang, S, Sahai, V. and Bisaria, V.S, "Optimization of xylanase production by *Melanocarpus albomyces* IIS-68 in solid state fermentation using response surface methodology" J Biosci Bioengg, 91 (4). 425-427. Oct 2001.

[5] Park, Y.S., Kang, S.W., Lee, J.S., Hong, S.I. and Kim, S.W, "Xylanase production in solid state fermentation by *Aspergillus niger* mutant using statistical experimental designs," Appl Microbiol Biotechnol, 58 (6). 761-766. Mar 2002.

[6] Bakri, Y., Jacques, P. and Thonart, P, "Xylanase production by *Penicillium canescens* 10-10c in solid-state fermentation," Appl Biochem Biotechnol, 108 (1-3). 737-774. Spring 2003.

[7] Wu, M., Li, S.C., Yao, J.M., Pan, R.R. and Yu, Z.L, "Mutant of a xylanase-producing strain of *Aspergillus niger* in solid state fermentation by low energy ion implantation" World J of Microbiol. Biotechnol, 21 (6-7). 1045-1049. Oct 2005.

[8] Hang, Y.D. and Woodams, E.E, "Xylanolytic activity of commercial juice processing enzyme preparation" Lett Appl Microbiol, 24. 389-392. May 1997.

[9] Csiszar, E., Losonczi, A., Koczka, B., Szakacs, G. and Pomlenyi, A, "Degradation of lignin-containing materials by xylanase in biopreparation of cotton," Biotechnol Lett 28. 749-753. May 2006.

[10] Battan, B., Sharma, J., Dhiman, S.S and Kuhad, R.C, "Enhanced production of cellulose-free thermostable xylanase by *Bacillus pumilus* ASH and its potential application in paper industry," Enzy Microb Technol, 41. 733-739. Nov 2007.

[11] Dhiman, S.S., Sharma, J. and Battan, B, "Industrial applications and future prospects of microbial xylanases: a review," Bioresour, 3. 1377-1402. Nov 2008.

[12] Dhiman, S.S., Garg, G., Mahajan, R., Garg, N. and Sharma, J, "Single lay out and mixed lay out enzymatic processes for biobleaching of kraft pulp," Bioresour. Technol, 100. 4736-4741. Oct 2009.

[13] Dekker, R.F.H. and Richards, G.N., Hemicellulases: their occurrence, purification, properties and mode of action, Adv Carbohydrate Chem Biochem, 32. 277-352. Feb 1976.

[14] Sunna, A., Moracci, M., Rossi, M. and Antranikian, G, "Glycosyl hydrolases from hyperthermophiles," Extremophiles, 1. 2-13. Feb 1997.

[15] Dekker, R.F.H. "Biodegradation of hemicelluloses, In Biosynthesis and biodegradation of wood components," Higuchi T. (ed) Academic Press, Tokyo. 1985, 505-533.

[16] Kuhad, R.C., Manchanda, M. and Singh, A, "Optimization of xylanase production by a hyperxylanolytic mutant strain of *Fusarium oxysporum*," Process Biochem, 33. 641-647. Aug 1998.

[17] Hrmova, M., Biely, P., Vrsanska, M. and Petrakova, E, "Induction of cellulose and xylanase-degrading enzyme complex in the yeast of *Trichosporon cutaneum*," J Arch Microbiol, 138. 371-376. Aug 1984.

[18] Beg, Q.K., Kapoor, M., Mahajan, L. and Hoondal, G.S, "Microbial xylanases and their industrial applications; a review" J Appl Microbial Biotechnol, 56. 326-338. June 2001.

[19] Polizeli, M.L.T.M., Rizzatti, A.C.S., Monti, R., Terenzi, H.F., Jorge, J.A. and Amorim, D.S, "Xylanases from fungi: Properties and industrial applications," Appl Microbiol and Biotechnol, 67 (5): 577-591. 2005.

[20] Garapati, S.L., Chaganti, S.R., Ravella, S.R., Phil, J.H. and Reddy, S.P, "Enhanced production of xylanase by a newly isolated *Aspergillus terreus* under solid state fermentation using palm industrial waste: A statistical optimization," Biochem Engg J, 48. 51-57. Dec 2009.

[21] Miller, G., Blum, L.R. and Burton, A.I, "Use of dinitosalisalic acid reagent for determination of reducing sugars," Anal Chem, 31. 426-428. 1959.

[22] Mandels, M., Andreotti, R. and Roche, C. "Measurement of saccharifying cellulose," Biotechnol Bioengg Symp, 6. 21-31. 1976.

[23] Gawande, P.V. and Kamat, M.Y, "Production of *Aspergillus* xylanase by lignocellulosic waste fermentation and its application," J of Appl Microbiol, 87 (4): 511-519. Oct 1999.

[24] Heck, J.X., Soares, L.H.D.B., Hertz, P.F. and Ayub, M.A.Z. "Purification and properties of a xylanase produced by *Bacillus circulans* BL53 on solid-state cultivation," Biochem Engg J, 32 (3). 179-184. Dec 2006.

[25] Rajashri, D.K. and Anandrao, R.J, "Isolation, Purification, and Characterization of Xylanase Produced by a New Species of *Bacillus* in Solid State Fermentation," Intl J of Microbiol, 2012. 1-9. Oct 2012.

[26] Garg, G., Kaur, R.M.A. and Sharma, J. Xylanase production using agroresidue in solid state fermentation from *Bacillus pumulis* ASH for biodelignification of wheat straw pulp. Biodegradation, 22. 1143-1154. 2011.

[27] Liu, C., Sun, Z.T., Du, J.H. and Wang, J, "Response surface optimization of fermentation conditions for producing xylanase by *Aspergillus niger* SL-05" J Ind Microbiol Biotechnol, 35. 703-711. July 2008.

[28] Fang, T.J., Liao, B.C. and Lewe, S.C, "Enhanced production of Xylanase by *Aspergillus carneus* M34 in solid state fermentation with agricultural waste using statistical approach," New Biotechnol, 27 (1). 25-32. Feb 2010.

[29] Nagar, S., Gupta, V.K., Kumar, D., Kumar, L. and Kuhad, R.C. "Production and optimization of cellulase free alkali stable xylanase by *Bacillus pumilus* SV-85S in submerged fermentation" J Ind Microbiol Biotechnol, 37. 71-83. Jan 2010.