International Journal Of

Recent Scientific Research

ISSN: 0976-3031

Volume: 7(4) April -2016

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THE OFFICIAL PUBLICATION OF
INTERNATIONAL JOURNAL OF RECENT SCIENTIFIC RESEARCH (IJRSR)
http://www.recentscientific.com/ recentscientific@gmail.com

ISSN: 0976-3031

Available Online at http://www.recentscientific.com

International Journal of Recent Scientific Research
Vol. 7, Issue, 4, pp. 10678-10685, April, 2016

International Journal of Recent Scientific Research

Research Article

APPLICATION OF STATISTICAL DESIGNS FOR THE OPTIMIZATION OF MEDIUM CONSTITUENTS FOR THE PRODUCTION OF PTERIN DEAMINASE FROM ASPERGILLUS TERRUS JQ 436691

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ARTICLE INFO

Article History:

Received 05th January, 2016
Received in revised form 21st February, 2016
Accepted 06th March, 2016
Published online 28th April, 2016

Keywords:

Optimization, Pterin deaminase, Media constituents, Wheat bran, PBD, RSM, SSF,

ABSTRACT

An experiment was conducted to study the effect of medium constituents for maximization of pterin deaminase production by indigenous fungal isolate *Aspergillus terrus* JQ 436691 under solid state fermentation process wheat bran as the substrate. In Plackett–Burman design (PBD), the pterin deaminase production was increased from 50.2 to 102.20 IU/ml. From pareto chart, the wheat bran, sucrose, yeast extract and urea were identified as significant constituents which influences highly for pterin deaminase production and these variables were subsequently optimized using a Box- Behnken design. The maximum experimental pterin deaminase production of 138.20 IU/ ml was very close to the predicted value 141.29 IU/ml and protein content of 137.10 µg/ml using substrate 10g wheat bran, 2.00 % (w/v) sucrose, 0.03 % (w/v) yeast extract and 0.15% (w/v) urea by Box- Behnken design (RSM). The significant interaction was existed between the amount of substrate wheat bran x sucrose, wheat bran x urea, wheat bran x yeast extract and sucrose x yeast extract for pterin deaminase production since the contour lines were elliptical in nature.

INTRODUCTION

Enzymes are preferred from microbial sources due to their economic production, consistency, ease of process modification and purification. They are more stable than corresponding enzymes derived from plants or animals (Savitri and Azmi, 2003). The enzyme pterin deaminase is widely distributed in both prokaryotes and eukaryotes. This enzyme is identified to be involved in the regulation of pteridines in these organisms (Remold, 1983), also exhibits antitumor and antioxidant activities **(**Kusakabe *et al*., 1979). In a fermentation process, the solid substrate supplies the nutrients to the microbial culture but also serves as a base for the cells (Pandey *et al.*, 1999). There is an increasing trend towards the utilization of solid state fermentation technique to produce several enzymes. It is reported that the filamentous fungi are the best adapted to SSF due to their biochemical and enzymological properties. Screening and evaluation of nutritional requirements are important step for bioprocess development in microorganisms. The statistical approaches are helpful to enhance enzyme yield and reduce the cost of production, thereby making the fermentation process economical and cost effective (Karur and Satyanarayana, 2005).

The traditional method of optimization of parameters involves optimizing one parameter at a time. This is not only time-consuming, but also leads to an incomplete understanding of interaction between the factors (Elibol, 2004**)**. In the optimization of media compounds, Plackett- Burman (1946) designs are used to select the constituents that influence a system. However, they do not give an interactive effect for each constituent (Jalbani *et al*., 2006; Youssef and Berekaa, 2009) and further optimization is needed. Response surface Methodology

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(Box-Behnken, 1960) is widely used to evaluate and understand the interactions between different physiological and nutritional parameters (Charyulu and Gnanamani, 2010**)**. The principle source of pterin deaminase is reported only in few species of *Aspergillus* and the production strategy has not been developed so far. The production of a new microbial enzyme using appropriate selection procedures is essential for cancer therapy. Hence, the present study reports the application of Plackett- Burman and Box- Behnken designs to optimize pterin deaminase production from isolated soil fungal strain *Aspergillus terreus* JQ 436691.

MATERIALS AND METHODS

Microorganism and inoculum

The newly isolated fungal culture of *Aspergillus terreus* JQ436691 was maintained on SDA agar slants, stored at 4ºC and sub cultured for every two weeks. For preparing a spore suspension, to a well sporulated slant 4 d old, sterilized 0.1% Tween 20 solution was added. The concentration of the spore suspension was measured using a haemocytometer and adjusted to $1x10^6$ spores /ml by diluting it suitably.

Solid state fermentation

Agricultural residues wheat bran was used as substrates for solid state fermentation. These residues were crushed using a commercial waring type blender and were dried at 50ºC for 1 h. Solid substrate was accurately weighed to 10 g in 250 ml Erlenmeyer flask and autoclaved at 121ºC for 40 min. After cooling, the substrate was inoculated with 2 ml of spore suspension. Moisture content of media was adjusted by adding distilled water. Media was thoroughly mixed and incubated at 30ºC, in humidity controlled incubator for 4 d.

Extraction of pterin deaminase from solid substrate

Fermented material was extracted by adding 50 ml of sterile distilled water and the mixtures were shaken at 250 rpm and 4ºC in a rotary shaker for 30 min. The extracts were then filtered using whatmann filter paper (No.1) and were used for enzyme assays and determination of total protein content.

Pterin deaminase assay

The assay for measuring pterin deaminase activity was adopted from the method described (Mashburn *et al.*, 1964). In this method, 340 µl of folic acid (0.5M), 40 µl of 50 mm Tris-HCl buffer (pH 8.6) were added and kept for 5 min incubation. Then 50 µl of enzyme was added and incubated for 10 min and the reaction was arrested using 20 µl trichloroacetic acid. Then, the mixture was centrifuged at 8000 rpm for 5 min. A blank with boiled enzyme was set. The supernatant, made up to 500 µl of distilled water and 500 µl of Nesslers reagent were added and incubated for 10 min. The ammonia released was estimated spectrophotometrically at absorbance of 480 nm. Ammonia standard was also simultaneously run using ammonium sulphate.

Protein estimation

Protein content was determined according to the method (Lowry *et al.*, 1951). The standard stock bovine serum albumin (BSA), at a concentration of 1000 µg/ml was prepared. To each test tube 1 ml of folins cocatteau reagent was added and incubates for 30 min. The absorbance measured at 660 nm using a double beam UV – visible spectrophotometer (model SL 164, Elico, Hyderabad, India), and the protein content was determined.

Experimental design and Optimization

Plackett -Burman design

The purpose of the first optimization step involved to identify which constituents of the medium have a significant effect on pterin deaminase production. Twelve experiments were obtained for 11 constituents representing, wheat bran (X_1), sucrose (X_2), yeast extract (X_3), urea (X_4), ammonium chloride (X_5), zinc sulphate (X_6), manganese sulphate (X_7), ferrous sulphate (X_8), potassium di-hydrogen phosphate (X_9), peptone (X_{10}) and ammonium nitrate (X_{11}). Each constituent represented at two levels, upper (high **(+1)** and lower (-1**)** levels of the range covered by each variable and the response shows a 11-run Plackett - Burman experimental design.

Factor	Variable code	Low level (-1)	High level (+1)
Wheat bran (g)	X_1	8.00	10.00
Sucrose % (w/v)	X_2	1.00	2.00
Yeast extract % (w/v)	X_3	0.02	0.04
Urea % (w/v)	X_4	0.10	0.20
Ammonium chloride % (w/v)	X_5	0.01	0.03
Zinc sulphate % (w/v)	X_6	0.01	0.03
Manganese sulphate % (w/v)	X_7	0.01	0.02
Ferrous sulphate % (w/v)	X_8	0.01	0.03
Potassium di-hydrogen phosphate % (w/v)	X_9	0.01	0.02
Peptone % (w/v)	X_{10}	0.50	1.00
Ammonium nitrate % (w/v)	X_{11}	0.02	0.03

The contribution of constituent towards the yield of the enzymes was determined based on the t-value (main effect) obtained from the experimental results. The effect (main effect) of constituent is calculated as follows:

t -value or main effect of constituent X = (average of sum of the enzyme activities where the constituent is '+') - (average of sum of the enzyme activities where the constituent is '-`).

The constituents are ranked based on their t-values. The constituent highest t-value is considered to be the best and ranked one. Pareto chart displays t-value of the effects and determines magnitude and importance of constituents. Two different t-limit plotted based on Bonferroni corrected 't ' and standard 't.' Effects above t-value are possibly significant and should be added if they are not likely to be significant.

Box- Behnken design

In the second phase of medium formulation, Box- Behnken (1960) design was employed mainly to study the interactive effects of the four variables obtained significantly from Plackett- Burman design. In this model, the significant independent constituents *viz.*, wheat bran (X_1), sucrose (X_2), Yeast extract (X_3) and urea (X_4) were included and each factor was examined at three different levels, low (-), high (+) and central or basal (0).

Factor	Variable Code	Level		
		-1 (Low)	0 (Basal)	+1 (High)
Wheat bran (g)	X_1	8.00	9.00	10.00
Sucrose % (w/v)	X_2	1.00	1.50	2.00
Yeast extract % (w/v)	X_3	0.02	0.03	0.04
Urea % (w/v)	X_4	0.10	0.15	0.20

Twenty nine combinations were fitted to the following second order polynomial mode. Although response surface analysis is practical for up to four independent variables, the following quadratic polynomial model represents the relationship fitted for three variables.

$$Y= b_0 + b_1X_1 + b_2 X_2 + b_3X_3 + b_{12}X_1X_2 + b_{13}X_1X_3 + b_{23}X_2X_3 + b_{11}X_1^2 + b_{22} X_2^2 + b_{33} X_3^2$$

Where, Y is the dependent variable (pterin deaminase production); X_1, X_2, X_3 and X_4 are the independent variables; b_0 is the regression coefficient at centre point; b_1, b_2 and b_3 are linear coefficients; b_{12} b_{13} and b_{23} are second order interaction coefficients; and b_{11} and b_{23} are quadratic coefficients. Based on the calculated values of the coefficients, the optimum concentrations were predicted using statistical software design. For coefficient of determination, the quality of fit of the polynomial model equation expressed as R^2. For testing goodness of fit of regression equation, multiple correlation R and determination coefficients R^2 were used. The experimental model was evaluated statistically using standard deviation, ANOVA, coefficient of determination and residuals. A probability level of $p< 0.01$ was considered for the model to be statistically significant. Validation of experiment was repeated three times under the identified optimal conditions in order to confirm the mathematical model to be statistically significant.

RESULTS AND DISCUSSION

Identification of medium constituents using Plackett-Burman design

The utilization of agro-industrial residues for developing any fermentation process is of critical importance because medium composition significantly affects product concentration, yield and productivity of enzyme.

Till last decade there have been only stray reports for the selection of suitable substrate under SSF with statistical optimization for pterin deaminase production from fungal isolates. A total of eleven medium components were screened in twelve experimental runs and the corresponding Placket-Burman experimental design matrix for pterin deaminase production were shown in table1. The observed pterin deaminase production varied from 50.20 IU/ml to 102.20 IU/ml, reflecting the importance of medium optimization to obtain higher yields.

In table 2 represents the effect of each variable along with the mean squares, F-values and p- values. The values of "Prob>F" less than 0.0500 for the four variables *viz.*, wheat bran (X_1), sucrose (X_2), Yeast extract (X_3) and urea (X_4) indicates that model terms were significant. In general, larger the magnitude of 't' and smaller the 'P' values, the more significant is the corresponding coefficient terms (Myers and Montgomery, 2002). The Model F-value of 117002.57 implied that model was significant. Adequate precision which measures the signal to noise ratio was 1044.636. 'Predicted $R^{2'}$ of 0.9999 was in reasonable agreement with 'adjusted R^{2}' of 1.000. The same constituents were confirmed from the pareto chart (Fig 1) which was also used to show the effect of all constituents on pterin deaminase production. The selected variables showed that wheat bran (A), sucrose (B), yeast extract (C) and urea (D) (Threshold of t value = 3.18245) crossed the critical value and was considered as significantly influencing medium components for pterin deaminase production by *Aspergillus terreus* JQ436691. These significant variables were further optimized involving Box-Behnken design. The other nutrients showed negative effect with threshold 't' value< 3.18245.

Optimization of the selected medium constituents using Box-Behnken design

Based on the results of the Plackett- Burman design, wheat bran, sucrose, yeast extract and urea were chosen as the independent input variables and pterin deaminase activity was used as the dependent output variable. An experimental design of 29 runs containing 6 central points was estimated according to Box-Behnken response surface design for four selected parameters.

Table 1 Plackett -Burman design and pterin deaminase activity

Run	Factors											*	**	***
	X_1	X_2	X_3	X_4	X_5	X_6	X_7	X_8	X_9	X_{10}	X_{11}			
1	8.00	1.00	0.02	0.20	0.03	0.03	0.01	0.03	0.02	0.50	0.03	80.24	79.98	79.89
2	10.0	1.00	0.02	0.10	0.03	0.03	0.02	0.01	0.02	1.00	0.02	50.98	50.96	50.91
3	10.0	2.00	0.02	0.20	0.03	0.01	0.02	0.01	0.01	0.50	0.03	50.20	50.18	49.97
4	10.0	1.00	0.04	0.10	0.01	0.01	0.02	0.03	0.02	0.50	0.03	52.72	52.70	51.89
5	8.00	2.00	0.02	0.10	0.01	0.03	0.02	0.03	0.01	1.00	0.03	68.42	68.24	69.31
6	8.00	1.00	0.04	0.20	0.03	0.01	0.02	0.03	0.01	1.00	0.02	64.22	63.98	65.21
7	10.00	2.00	0.04	0.10	0.03	0.03	0.01	0.03	0.01	0.50	0.02	69.18	69.14	68.14
8	8.00	1.00	0.02	0.10	0.01	0.01	0.01	0.01	0.01	0.50	0.02	94.24	93.99	92.98
9	8.00	2.00	0.04	0.20	0.01	0.03	0.02	0.01	0.02	0.50	0.02	73.42	72.98	71.96
10	8.00	2.00	0.04	0.10	0.03	0.01	0.01	0.01	0.02	1.00	0.03	102.20	101.98	101.93
11	10.00	2.00	0.02	0.20	0.01	0.01	0.01	0.03	0.02	1.00	0.02	65.72	65.68	66.61
12	10.00	1.00	0.04	0.20	0.01	0.03	0.01	0.01	0.01	1.00	0.03	51.72	50.98	50.66

*Actual enzyme activity (IU/ml), ** Predicted enzyme activity (Iu/ml), *** Protein estimation µg/ml
X_1 Wheat, X_2 Sucrose, X_3 Yeast extract, X_4 Urea, X_5 Ammonium Chloride, X_6 Zinc sulphate, X_7 Manganese sulphate, X_8 Ferrous sulphate, X9 Potassium dihydrogen phosphate, X_{10} Peptone, X_{11}Ammonium nitrate

Table 2 ANOVA for quadratic model for pterin deaminase activity

Source	Sum of squares	df	Mean square	F value	P value Prob> F
Model	3159.07	10	315.91	1.170E+005	<0.0023*
X_1 - Wheat bran	9.61	1	9.61	3560.11	<0.0107
X_2 - Sucrose	1.24	1	1.24	459.86	<0.0297
X_3 - Yeast extract	39.10	1	39.10	14480.11	<0.0053
X_4 - Urea	1023.42	1	1023.42	3.790E+005	<0.0001
X_5 - Ammonium chloride	125.07	1	125.07	46320.60	0.0625
X_6 - Zinc sulphate	958.73	1	958.73	3.551E+005	0.0586
X_7 - Manganese sulphate	62.47	1	62.47	23137.79	0.0668
X_8 - Ferrous sulphate	301.80	1	301.80	1.118E+005	0.0589
X_9 -Potassium dihydrogen phosphate	11.80	1	11.80	4370.68	0.0678
X -Peptone	625.83	1	625.83	2.318E+005	0.0669
X_{11}-Ammonium nitrate	525.63	1	525.63	0.93	0.3886
X_{12}-Residual	2.700E-003	1	2.700E-003		
Cor Total	5871.48	11			

**Model is Significant: Std.Dev.0.052; R-Squared1.0000; Mean68.61; AdjR-Squared 1.0000; C.V. % 0.076; Pred R-Squared 0.9999; PRESS0.039; Adeq Precision1044.636.

Table 3 Box-Behnken experimental design for optimization of 4 nutrients (each at three levels)

Run	Factor 1 wheat bran (X_1)	Factor 2 sucrose (X_2)	Factor 3 yeast extract (X_3)	Factor 4 urea (X_4)	Actual enzyme activity (IU/ml)	Predicted enzyme activity (IU/ml)	Protein estimation µg/ml
1	9	1.50	0.02	0.10	88.43	85.37	88.01
2	8	1.50	0.04	0.15	60.46	64.22	61.11
3	9	1.50	0.03	0.15	94.10	89.52	93.00
4	9	1.50	0.02	0.20	86.68	85.09	86.10
5	9	1.50	0.03	0.15	89.10	89.52	88.01
6	9	1.50	0.04	0.10	88.64	89.67	88.32
7	9	1.00	0.03	0.10	80.24	79.82	80.12
8	9	1.50	0.03	0.15	87.42	89.52	86.32
9	10	1.50	0.03	0.20	132.22	131.87	131.12
10	9	2.00	0.04	0.15	102.10	95.38	101.81
11	10	1.50	0.03	0.10	122.43	123.36	121.12
12	9	2.00	0.03	0.10	96.64	99.83	95.43
13	10	2.00	0.03	0.15	138.20	141.29	137.10
14	9	1.50	0.03	0.15	88.65	89.52	89.61
15	9	1.00	0.02	0.15	65.10	69.85	65.01
16	10	1.50	0.02	0.15	126.46	125.23	125.12
17	9	1.00	0.03	0.20	79.64	78.98	78.55
18	8	1.50	0.03	0.10	67.80	66.18	66.51
19	9	1.00	0.04	0.15	76.65	77.55	75.12
20	8	2.00	0.03	0.15	62.10	62.46	62.90
21	9	1.50	0.04	0.20	87.43	89.99	86.31
22	9	2.00	0.02	0.15	96.68	93.81	98.12
23	9	1.50	0.03	0.15	88.34	87.22	89.33
24	8	1.00	0.03	0.15	58.42	54.76	59.12
25	9	2.00	0.03	0.20	97.82	100.77	96.01
26	8	1.50	0.03	0.20	60.68	57.78	58.12
27	8	1.50	0.02	0.15	44.28	48.33	43.40
28	10	1.50	0.04	0.15	120.12	118.60	119.01
29	10	1.00	0.03	0.15	108.12	107.20	106.50

Analysis of variance (ANOVA) yielded the following equation representing the mathematical model relating the production of pterin deaminase levels as a function of wheat bran (A), sucrose (B), yeast extract (C) and urea (D).

Final equation in terms of coded factors

Pterin deaminase activity = +89.52 +32.82 * A+10.45 * B+2.31 * C + 0.024 * D+ 6.60 * A * B-5.63 * A * C+ 4.23 * A * D -1.53 * B * C + 0.44 * B * D+0.13 * C * D + 3.43 * A2 - 1.52* B2-3.85* C2 +1.85 * D2

Final equation in terms of actual factors

Pterin deaminase activity= +133.47400 -44.47450* Wheat bran -73.14300* Sucrose+ 8030.51667 * Yeast extract-1016.99667*Urea+ 13.20000 * Wheat bran* Sucrose -563.00000* Wheat bran * Yeast extract +84.55000*Wheat bran* Urea-306.50000 * Sucrose *Yeast extract +17.80000 * Sucrose * Urea+ 270.00000 * Yeast extract * Urea + 3.42775 *Wheat bran2 - 6.07900*Sucrose2 -38547.50000*Yeast extract2+739.10000*urea2

The table 3 showed the predicted responses of Box-Behnken design on the basis of polynomial equation. The

regression equation was assessed statistically for analysis of variance (ANOVA) and the results are predicted in table 4. For testing goodness of fit of regression equation, multiple correlation R and determination coefficients R^2 were evaluated.

response surface and contour plots of medium constituents for the optimization of pterin deaminase production. The contour and 3D surface plots are generally used to represent the interaction effects between the process variables (Bas and Boyacá, 2007).

Table 4ANOVA for response surface quadratic model

Source	Sumof squares	Degree of freedom	Mean squares	Coefficient estimate	F value	P-value Prob>F
Model	14939.36	14	1067.10	89.52	69.74	<0.0001*
A-Wheat bran	12923.86	1	12923.86	32.82	844.64	<0.0001
B –Sucrose	1309.80	1	1309.80	10.45	85.60	<0.0001
C-Yeast extract	64.26	1	64.26	2.31	4.20	0.0596
D-Urea	7.008 E-003	1	7.008 E-003	0.024	4.580 E-004	0.9832
AB	174.24	1	174.24	6.60	11.39	<0.0045
AC	126.79	1	126.79	-5.63	8.29	<0.0121
AD	71.49	1	71.49	4.23	4.67	<0.0485
BC	9.39	1	9.39	-1.53	0.61	0.4464
BD	0.79	1	0.79	0.44	0.05	0.8233
CD	0.07	1	0.07	0.13	4.98	0.9459
A^2	76.21	1	76.21	3.43	0.98	<0.0425
B^2	14.98	1	14.98	-1.52	6.30	0.3392
C^2	96.38	1	96.38	-3.85	1.45	<0.0250
D^2	22.15	1	22.15	1.85	2.69	0.2489
Residual	214.21	14	15.30			
LackofFit	186.50	10	18.65			0.1762**
PureError	27.71	4	6.93			
CorTotal	15153.57	28				

Std. Dev=3.91, R^2=0.9859, AdjR^2=0.9717, PredR^2=0.9263, Adeq Precision=33.044, C.V.%=4.37, Mean= 89.48, Press=1117.36* Model terms significant** Lack of fit not significant

The R^2 value provides a measure of variability in the observed response values which can be explained by the experimental factors and their interactions. For a good statistical model, R^2 should be close to one (Chauhan and Gupta, 2004). In this case, the value of determination coefficients (R^2=0.9263) indicates that 98% of variability in dependent variables (pterin deaminase activity IU/ml) could be explained by this model. In addition, the value of the adjusted determination coefficient (Adj $R^{2'}$ =0.9717) was also very high to advocate for a high significance of the model. Generally, calculated F value should be higher than tabulated value, if the model is a good prediction of experimental results (Cladera- Olivera *et al.*, 2004).

Also, a high F-value and very low probability (P>F= 0.0001) indicated that the present model was in a good prediction of experimental results. The F-value of model (69.74) implied that the model was significant. There is only a 0.01% chance that a "Model F- value" large could occur due to noise (Hamasaveni *et al.*, 2001). The P-values suggests that coefficients for linear and quadratic were mostly significant on wheat bran and sucrose (P<0.0001), while among the interactive effects, wheat bran x sucrose, wheat bran x yeast extract and wheat bran x urea showed significant effects than the other factors. Values of "Prob>F" less than 0.0500 indicates that model terms was significant. The p-values are used as a tool to check the significance of each coefficient and interaction among the coefficients (Liu *et al.*, 2010). Adequate of precision value (33.044), which measured the signal to noise ratio, indicated an adequate signal. A ratio >4.0 was desirable (Chauhan and Gupta, 2004). A very low value of coefficient of variation (C.V= 4.37) showed that the experiment conducted were precise and reliable (Khuri and Cornell, 1993). Figures 2-7 shows the

The fitted response for the regression model was plotted in figures 2-7. The 3D surface plots explained the pair-wise interaction of the four factors affecting the pterin deaminase production. The three dimensional response surface plot between the amount of substrate wheat bran (10.0 g.) and the sucrose content (2.0 % w/v) showed the maximum enzyme activity of 138.2 IU/ml (fig 2). The contour plot (fig 2) showed elliptical nature which indicates that the interactions between the factors *viz.*, wheat bran and sucrose were significant.

The figure 3 represents the combined effect of wheat bran and yeast extract and maximum activity of 120.12 IU/ml was noted at the substrate amount (10g) and yeast extract (0.04 % w/v). The interaction between the substrate wheat bran (10g) and urea (0.2% w/v) resulted in pterin deaminase activity of 132.2 IU/ml (fig 4). The figure 5 showed the response for the interaction of sucrose (2.0%w/v) and yeast extract (0.04 % w/v.) with maximum enzyme activity of 102.1 IU/ml. Therefore, the interaction between the wheat bran (10g.) x sucrose (2.0 % w/v) concentrations, while other variables were kept constant, the maximum production of pterin deaminase activity (138.2 IU/ml) was achieved followed by 132.2 IU/ml in wheat bran (10g) x urea (0.2% w/v.) , 120.12 IU/ml in wheat bran (10g) x yeast extract (0.04% w/v) and 102.1 IU/ ml in sucrose (2.0 %) x yeast extract (0.045 w/v) respectively. There is strong interaction between the variables is expected if the contour lines are elliptical in shape (Priya *et al.*, 2011).

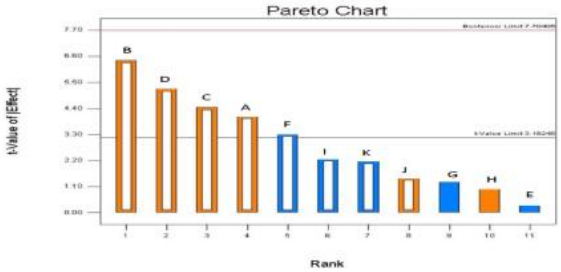

Fig.1: Pareto response for pterin deaminase activity of Plackett Burman Design Value of" Prob >F"" less than 0.0500 indicate model terms are significant. In this study 1.wheat bran (A), 2. Sucrose (B), 3.yeast extract (C) and 4.urea (D) are significant model terms and 5.Ammoniumchloride (E), 6.zinc sulphate (F), 7.manganese sulphate (G), 8.ferrous sulphate(H), 9. Potassium di hydrogen phosphate (I), 10.Peptone (J) and 11.Ammonium nitrate (K) are non significant (Negative effect).

In our study, strong interaction existed between the amount of substrate wheat bran x sucrose (fig2), substrate wheat bran x urea (fig 4), substrate wheat bran x yeast extract (fig 3) and sucrose x yeast extract (fig 5) for pterin deaminase production since the contour lines were elliptical in nature. The response plot between the sucrose content (2.0 % w/v) and urea (0.2% w/v) demonstrated that the activity was 97.82 IU/ml (fig 6).

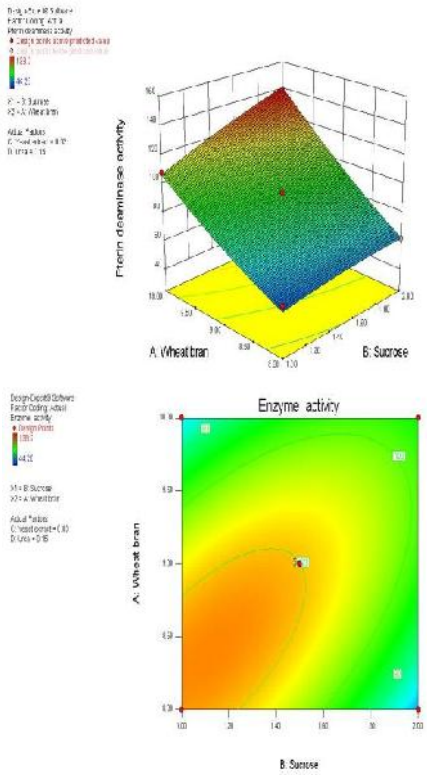

Fig 2 Response surface plot (upper) and its contour plot (lower) of pterin deaminase activity by interaction between wheat bran and sucrose

The graph between yeast extract content (0.04 % w/v) and urea (0.2 % w/v) (fig 7) highlighted a maximum activity of 87.43 IU/ml for the high level of urea content. The interaction between sucrose x urea (fig 6) and yeast extract x urea (fig7) were not significant for pterin deaminase production.

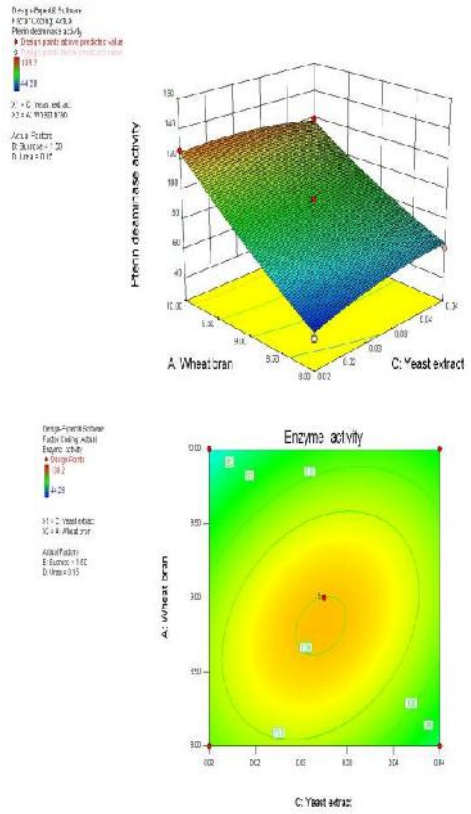

Fig 3. Response surface plot (upper) and its contour plot (lower) of pterin deaminase activityby interaction between wheatbran and yeast extract

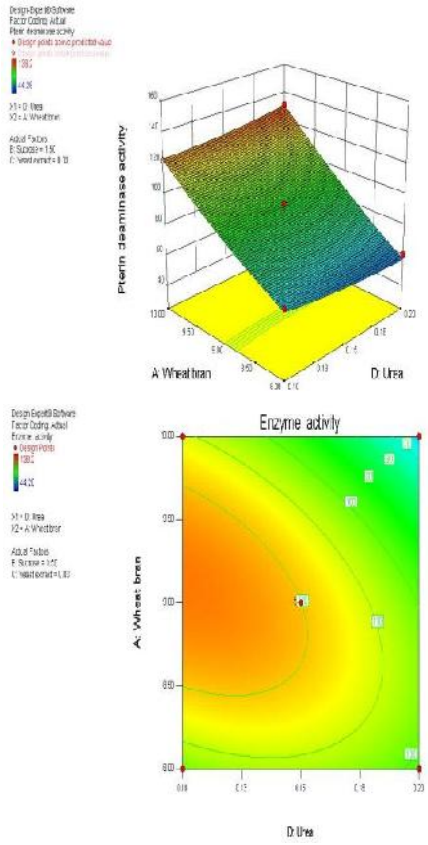

Fig 4 Response surface plot (upper) and its contour plot (lower) of pterin deaminase activity by interaction between wheat bran and urea

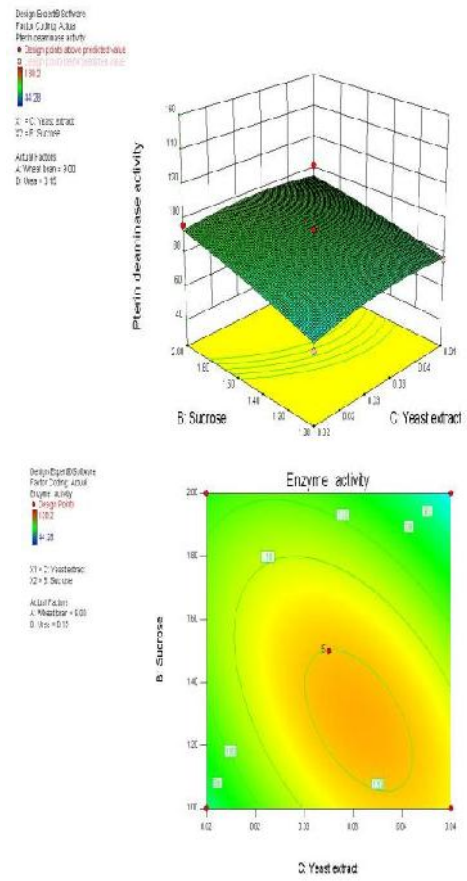

Fig 5 Response surface plot (upper) and its contour plot (lower) of pterin deaminase activity by interaction between sucrose and yeast extract

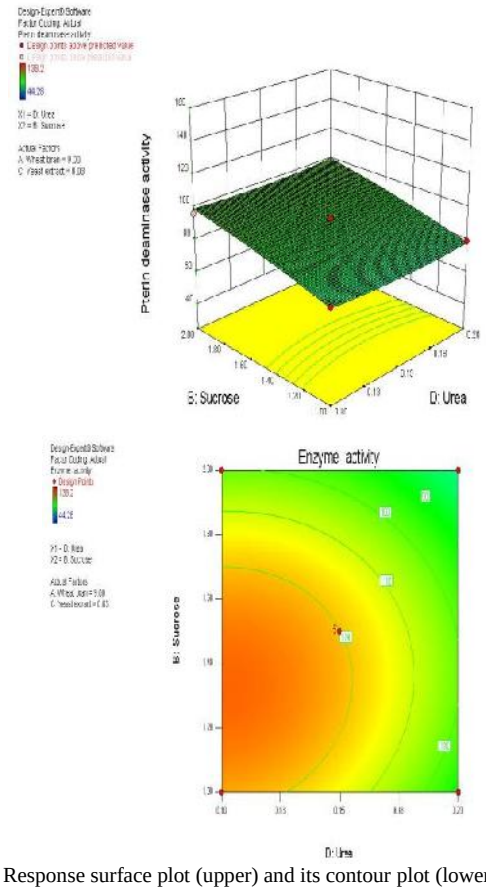

Fig 6 Response surface plot (upper) and its contour plot (lower) of pterin Deaminaseactivity by interaction between sucrose and urea

The maximum experimental pterin deaminase production of 138.2 IU/ml was very close to the predicted value 141.29 IU/ml and protein content of 137.10 µg / ml by Box-behnken design with 10 g wheat bran, 2.00 % (w/v) sucrose, 0.03 % (w/v) yeast extract and 0.15% (w/v) urea. Thus, under optimized conditions, the pterin deaminase yield increased from 42.5 IU/ml in conventional optimization trial to 138.2 IU/ml using RSM. The strain can be used for the production of pterin deaminase enzyme that could be of industrial value.

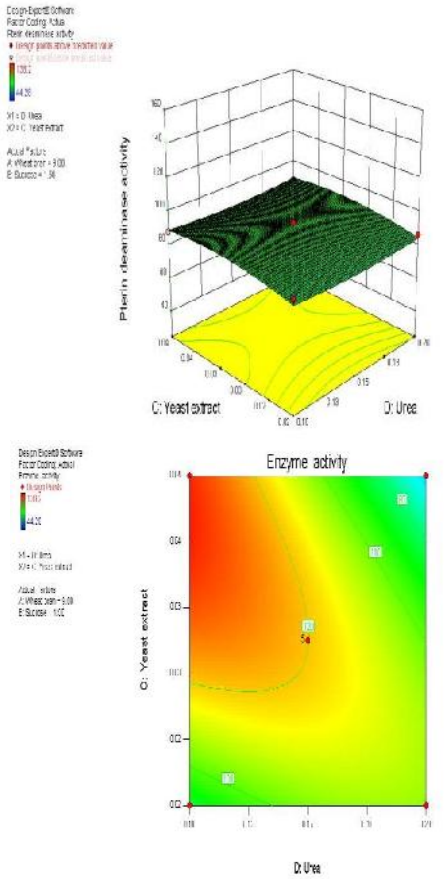

Fig 7 Response surface plot (upper) and its contour plot (lower) of pterin deaminase activity by interaction between yeast extract and urea

CONCLUSION

The indigenous isolate *Aspergillus terrus* JQ 436691was found to be an excellent pterin deaminase enzyme producer using SSF. As far as known, there are no reports of pterin deaminase production from *Aspergillus terreus* for optimization of medium constituents by statistical designs. Four variables: wheat bran, sucrose, yeast extract and urea were identified as significant by Plackett Burman design for pterin deaminase production. These variables were further optimized involving Box-Behnken design. The maximum experimental pterin deaminase production of 138.2 IU/ml was very close to the predicted value 141.29 IU/ml and protein content of 137.10 µg / ml by Box-behnken design with 10 g wheat bran, 2.00 % (w/v) sucrose, 0.03 % (w/v) yeast extract and 0.15% (w/v) urea. A strong interaction was existed between the amount of substrate wheat bran x sucrose, wheat bran x urea, wheat bran x yeast extract and sucrose x yeast extract for pterin deaminase production from the contour plots. The methodology as a whole proved to be adequate for the design and optimization for

obtaining a therapeutically valuable product like pterin deaminase from fungal isolate.

Acknowledgement

The authors gratefully acknowledge the Department of Microbial Biotechnology**,** Bharathiyar University, Coimbatore, Tamil Nadu, India for providing the necessary facilities for successful completion of this research work.

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How to cite this article:

Vijayarani Nallathambi *et al*.2016, Application of Statistical Designs for the Optimization of Medium Constituents for the Production of pterin Deaminase From Aspergillus Terrus Jq 436691. *Int J Recent Sci Res.* 7(4), pp. 10678-10685.

TSSN 0976-3031