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Research Paper

Computation of interactive effects and optimization of process parameters for alkaline lipase production by mutant strain of *Pseudomonas aeruginosa* using response surface methodology

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Abstract

Alkaline lipase production by mutant strain of *Pseudomonas aeruginosa* MTCC 10,055 was optimized in shake flask batch fermentation using response surface methodology. An empirical model was developed through Box-Behnken experimental design to describe the relationship among tested variables (pH, temperature, castor oil, starch and triton-X-100). The second-order quadratic model determined the optimum conditions as castor oil, 1.77 $mL.L^{-1}$; starch, 15.0 $g.L^{-1}$; triton-X-100, 0.93 $mL.L^{-1}$; incubation temperature, 34.12 °C and pH 8.1 resulting into maximum alkaline lipase production (3142.57 $U.mL^{-1}$). The quadratic model was in satisfactory adjustment with the experimental data as evidenced by a high coefficient of determination (R^2) value (0.9987). The RSM facilitated the analysis and interpretation of experimental data to ascertain the optimum conditions of the variables for the process and recognized the contribution of individual variables to assess the response under optimal conditions. Hence Box-Behnken approach could fruitfully be applied for process optimization.

Key words: alkaline lipase, Box-Behnken design, *Pseudomonas aeruginosa*, response surface methodology.

Introduction

The environment friendly characteristics of enzymes from natural sources lead the industries to reflect on enzymes because they are definitely a sustainable alternative to chemicals in industrial processes (Hasan *et al.*, 2010). So, the microorganisms can be the only source to get sufficient amount of enzyme as they can be cultured in large quantities in a reasonably short period by conventional methods of fermentation and they also provide a copious and regular supply of the desired products (Gupta *et al.*, 2002).

Lipases (triacylglycerile hydrolases EC 3.1.1.3) are industrially important enzymes attributed to their regio-, stereo-, chemo- selective reactions and kinetic resolution of racemates (Nelofer *et al.*, 2011). The application spectrum of lipases in new industries is mounting day by day. Recently, these enzymes have been used for biodiesel production (Adamczak *et al.*, 2009), enantioselective deacetylation (Kumar and Gupta, 2008), cyclic resolution of racemic ibuprofen (Liu et *al*., 2009), production of medium-chain triacylglycerols (Low *et al.*, 2007), and the preparation of diacylglycerol-enriched palm olein (Wang *et al.*, 2009).

Lipases are not only attractive as catalysts for the modification and synthesis of useful compounds as discussed above, but they are also used as functional components of mixtures. For example, a large potential market for lipolytic enzymes is in detergent formulations (Macrae and Hammond, 1985). The prelude conditions in favor of the enzymes to be detergent additive are not only its compatibility and stability against various detergent components but also the production level of the enzymes (Joo and Chang, 2006).

The improvement of industrial fermentation processes centered on designing of fermentation medium since

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its composition can appreciably affect product concentration, yield and volumetric productivity (Kennedy and Krouse, 1999). The classical optimization method (single variable optimization) is not only time-consuming and tedious but also fails to depict the overall effects of the parameters in the process and overlooks the combined interactions between physico-chemical parameters, leading to misinterpretation of results (Abdel-Fattah *et al.*, 2005; Bas and Boyaci, 2007). To overcome this difficulty, Fisher (1926) developed the basic theory of experimental design which proves the superiority of study of more than one factor at a time over only one factor at a time. The Response Surface Methodology (RSM) appraises the interaction between the response(s) and the independent variables (Chen *et al.*, 2002) and defines the effect of the independent variables, alone or in combination. Moreover, this method is a valuable tool to resolve the optimum operating conditions decisive for the scale up of the process and to reduce the number and outlay of experiments (Gopinath *et al.*, 2003).

In the light of above facts, the present study deals with statistical optimization for improving alkaline lipase production from mutant strain of *P. aeruginosa* 10,055 using Box-Behnken design.

Materials and Methods

Microorganism and lipase production

A promising mutant strain of *P. aeruginosa* MTCC 10,055 was developed by chemical mutagenesis in our laboratory (Bisht *et al.*, 2012). The culture was maintained on nutrient agar slants containing 1% (v/v) tributyrin and stored at 4 °C.

The composition of production medium containing ($g.L^{-1}$), $NH_4)_2SO_4$, 1.0; KH_2PO_4, 0.6; $MgSO_4$, 0.4; yeast extract, 0.2; castor oil, 2.0; starch, 20; triton-X-100, 1.0; gum arabic, 5.0; and initial of pH 9.0 was determined after using 'One-variable-at-a-time' approach. Fifty milliliters of the production medium was taken in 250 mL Erlenmeyer flask, inoculated with 0.5% (v/v) inoculum (OD_{610} 1.0) and incubated at 35 °C under shaking (120 rpm) for 28 h. After incubation, the fermenting broth was centrifuged at 12,000 *g* for 10 min at 4 °C and the cell-free supernatant was used for lipase assay.

Enzyme assay

Lipase activity was determined spectrophotometrically as described by Winkler and Stuckman (1979) with slight modifications. The substrate solution containing 10 mL of isopropanol with 30 mg of *p*-nitrophenyl palmitate was mixed with 90 mL of Tris-HCl buffer (50 mM, pH 9.0), containing 0.4% Triton-X 100 and 100 mg of gum arabic. Freshly prepared substrate solution (2.4 mL) was incubated at 37 °C with 25 µL of suitably diluted cell-free supernatant for 15 min. After incubation absorbance was measured at 410 nm by using a spectrophotometer (UV-1601, Shimadzu) against a control with heat inactivated enzyme. One unit of enzyme is defined as the amount of enzyme liberating 1 µg of *p*-nitrophenol.mL.min under the assay conditions.

Study of interactions among the medium components using Box-Behnken design

A response surface methodology using a Box-Behnken design (Box and Behnken, 1960) was adopted to appraise the interactions occurring among the factors *viz.,* pH (A), temperature (B), castor oil (C), starch (D) and triton-X-100 (E). The factors at three different levels (-1, 0, + 1) with minimum, central and maximum values and the treatment schedule for the model is given in Table 1 and Table 2, respectively. Six replicates (run) at the center of the design were used for estimation of the pure error and sum of squares.

Statistical analysis

The average of maximum alkaline lipase activity was taken as the dependent variable (response). A second-order polynomial equation, fitted to the data by multiple regression procedure, resulted in an empirical model which is as under

$$Y = \beta_o + \Sigma\, \beta_n X_n + \Sigma\, \beta_{nn} X_n^2 + \Sigma\, \beta_{nm} X_n X_m \qquad (1)$$

For analysis of design based on five factors following model equation was used,

$$Y = \beta_0 + \beta_1 A + \beta_2 B + \beta_3 C + \beta_4 D + \beta_5 E + \beta_{11} A^2 + \beta_{22} B^2 + \beta_{33} C^2 + \beta_{44} D^2 + \beta_{55} E^2 + \beta_{12} AB + \beta_{13} AC + \beta_{14} AD + \beta_{15} AE + \beta_{23} BC + \beta_{24} BD + \beta_{25} BE + \beta_{34} CD + \beta_{35} CE + \beta_{45} DE + \varepsilon \qquad (2)$$

Where Y is the predicted response for alkaline lipase produced; β_0 is the value of the fitted response at the center point of the design; β_1, β_2, β_3, β_4 and β_5 are the linear coefficients; β_{11}, β_{22}, β_{33}, β_{44} and β_{55} are the quadratic coefficients; β_{12}, β_{13}, β_{14}, β_{15}, β_{23}, β_{24}, β_{25}, β_{34}, β_{35} and β_{45} are the interaction coefficients; while 'ε' is the random error. The software package Design-Expert 8.0.5.2 (Stat Ease, Inc., Minneapolis, USA) was used to obtain the coefficients of Eq. (2) based on the data provided in Table 2. The responses under different combinations as defined by the de-

Table 1 - Levels of five independent variables used in RSM in terms of actual and coded factors.

Variables	Levels		
	-1	0	+ 1
Temperature (°C)	30	35	40
pH	8.5	9.0	9.5
Starch ($g.L^{-1}$)	15	20	25
Castor oil ($mL.\ L^{-1}$)	1.0	2.0	3.0
Triton-X-100 ($mL.L^{-1}$)	0.5	1.0	1.5

Table 2 - Box-Behnken design matrix for RSM studies of five independent variables for alkaline lipase production.

Run	Temperature (°C)	pH	Starch ($g.L^{-1}$)	Castor oil ($mL.L^{-1}$)	Triton-X-100 ($mL.L^{-1}$)	Observed response ($U.mL^{-1}$)	Predicted response ($U.mL^{-1}$)
1	30	8.5	20	2.0	1.0	1810	1800.40
2	40	8.5	20	2.0	1.0	1250	1260.65
3	30	9.5	20	2.0	1.0	1150	1154.27
4	40	9.5	20	2.0	1.0	515	539.52
5	35	9.0	15	1.0	1.0	1995	1987.10
6	35	9.0	25	1.0	1.0	1255	1242.60
7	35	9.0	15	3.0	1.0	1630	1651.48
8	35	9.0	25	3.0	1.0	1096	1112.98
9	35	8.5	20	2.0	0.5	1960	1983.35
10	35	9.5	20	2.0	0.5	1090	1105.73
11	35	8.5	20	2.0	1.5	1640	1663.35
12	35	9.5	20	2.0	1.5	1158	1173.73
13	30	9.0	15	2.0	1.0	1762	1790.25
14	40	9.0	15	2.0	1.0	1155	1164.50
15	30	9.0	25	2.0	1.0	1080	1100.25
16	40	9.0	25	2.0	1.0	570	571.50
17	35	9.0	20	1.0	0.5	1601	1604.27
18	35	9.0	20	3.0	0.5	1340	1330.15
19	35	9.0	20	1.0	1.5	1428	1436.77
20	35	9.0	20	3.0	1.5	1250	1245.65
21	35	8.5	15	2.0	1.0	2903	2836.19
22	35	9.5	15	2.0	1.0	982	957.06
23	35	8.5	25	2.0	1.0	1020	999.19
24	35	9.5	25	2.0	1.0	1490	1511.06
25	30	9.0	20	1.0	1.0	1481	1482.56
26	40	9.0	20	1.0	1.0	965	972.81
27	30	9.0	20	3.0	1.0	1325	1317.44
28	40	9.0	20	3.0	1.0	674	672.69
29	35	9.0	15	2.0	0.5	1760	1776.21
30	35	9.0	25	2.0	0.5	1266	1248.71
31	35	9.0	15	2.0	1.5	1740	1764.21
32	35	9.0	25	2.0	1.5	1018	1008.71
33	30	9.0	20	2.0	0.5	1362	1350.17
34	40	9.0	20	2.0	0.5	920	900.42
35	30	9.0	20	2.0	1.5	1377	1351.67
36	40	9.0	20	2.0	1.5	680	646.92
37	35	8.5	20	1.0	1.0	1970	1993.50
38	35	9.5	20	1.0	1.0	1325	1300.38
39	35	8.5	20	3.0	1.0	1735	1751.38
40	35	9.5	20	3.0	1.0	1109	1077.25
41*	35	9.0	20	2.0	1.0	2350	2362.50
42*	35	9.0	20	2.0	1.0	2375	2362.50
43*	35	9.0	20	2.0	1.0	2350	2362.50
44*	35	9.0	20	2.0	1.0	2375	2362.50
45*	35	9.0	20	2.0	1.0	2350	2362.50
46*	35	9.0	20	2.0	1.0	2375	2362.50

*Centre points.

sign (Table 2) were analyzed using analysis of variance (ANOVA) to estimate the statistical parameters.

Results and Discussion

In recent years, statistical experimental designs have been proved to be an effective tool for optimization of process parameters in biotechnological processes. There are several reports for optimization of culture media, using statistical approaches (Kumari *et al.*, 2009; Faiza *et al.*, 2011; Salihu *et al.*, 2011). In the present investigation RSM was used for the optimization of alkaline lipase production by an improved strain of *P. aeruginosa* after optimization of the medium by "one-variable-at-a-time" approach.

Determination of significant variables by Box-Behnken design

Multiple regression analysis on the experimental data, results in following coefficients for alkaline lipase production (Y), considering, temperature (A), initial pH (B), starch (C), castor oil (D) and triton-X-100 (E) was obtained after the analysis of ANOVA

Alkaline lipase activity (Y $U.mL^{-1}$) = 2362.50 - 288.63 × A - 341.81 × B - 320.75 × C - 116.31 × D - 63.00 × E - 18.75 × AB + 24.25 × AC - 33.75 × AD - 63.75 × AE + 597.75 × BC + 4.75 × BD + 97.00 × BE + 51.50 × CD - 57.00 × CE + 20.75 × DE - 796.52 × A^2 - 377.27 × B^2 - 409.35 × C^2 - 454.60 × D^2 - 503.69 × E^2

The analysis of variance for the quadratic regression model demonstrates the aptness of the model for alkaline lipase production. The computed F-value (938.78) for the present model implies significance of the model (Table 3). There is only a 0.01% chance that a large "model F-value" could occur due to noise. In general, calculated F-values should be several times more than tabulated value, if the model was a good prediction of experimental results and estimated factors effects were real (Dutta *et al.*, 2004). A high F-value and a very low probability (PF = 0.0001) indicated that the present model was in a good prediction of experimental results. R^2, or determination coefficient, is the proportion of variation in the response attributed to the model rather than to random error (Henika, 1972). The R^2

Table 3 - Analysis of variance (ANOVA) for fitted second-order polynomial model as per Box-Behnken design.

Source	Sum of squares	df	Mean square	F value	p-value prob > F	Significance
Model	1.339E + 007	20	6.696E + 005	938.78	< 0.0001	Significant
Temperature (A)	1.333E + 006	1	1.333E + 006	1868.66	< 0.0001	
pH (B)	1.869E + 006	1	1.869E + 006	2620.82	< 0.0001	
Starch (C)	1.646E + 006	1	1.646E + 006	2307.78	< 0.0001	
Castor oil (D)	2.165E + 005	1	2.165E + 005	303.47	< 0.0001	
Triton-X-100 (E)	63504.00	1	63504.00	89.03	< 0.0001	
AB	1406.25	1	1406.25	1.97	0.1726	
AC	2352.25	1	2352.25	3.30	0.0814	
AD	4556.25	1	4556.25	6.39	0.0182	
AE	16256.25	1	16256.25	22.79	< 0.0001	
BC	1.429E + 006	1	1.429E + 006	2003.74	< 0.0001	
BD	90.25	1	90.25	0.13	0.7250	
BE	37636.00	1	37636.00	52.76	< 0.0001	
CD	10609.00	1	10609.00	14.87	0.0007	
CE	12996.00	1	12996.00	18.22	0.0002	
DE	1722.25	1	1722.25	2.41	0.1328	
A^2	5.537E + 006	1	5.537E + 006	7762.74	< 0.0001	
B^2	1.242E + 006	1	1.242E + 006	1741.51	< 0.0001	
C^2	1.462E + 006	1	1.462E + 006	2050.31	< 0.0001	
D^2	1.804E + 006	1	1.804E + 006	2528.64	< 0.0001	
E^2	2.214E + 006	1	2.214E + 006	3104.15	< 0.0001	
Residual	17831.92	25	713.28			
Lack of fit	16894.42	20	844.72	4.51	0.0512	Not significant
Pure error	937.50	5	187.50			
Corr. total	1.341E + 007	45				

Note E + n = 10^n.

value always lies between 0 and 1 and for a good fit of model, R^2 should be at least 0.80 (Joglekar and May, 1987). Similarly, Doddapaneni *et al.* (2007) suggested that closer the value of R^2 to 1.0, the stronger the model and the better its prediction efficiency of the responses. The R^2 (0.9987) for this model implied that 99.87% of the sample variation for lipase activity was attributed to the independent variables, and only about 0.13% of the total variation was not explained by the model. However, its value closer to 1.0 suggested that model represents better correlation between experimental and predicted values.

The "Lack of Fit F-value" of 4.51 demontrate that the Lack of Fit is not significant relative to the pure error. There is a 5.12% chance that a "Lack of Fit F-value" this large could occur due to noise. Non-significant lack of fit is good as we want the model to fit (Table 3). Adequate Precision measures the signal to noise ratio and a ratio greater than 4 is desirable. An adequate precision of 127.274 indicated low signal to noise ratio. The coefficient of variation (CV) is the ratio of the standard error of estimate to the mean value of the observed response, expressed as a percentage. A model can be considered practically reproducible if the CV is not greater than 10% (Joglekar and May, 1987). Here, a relatively lower value of the coefficient of variation (CV = 1.81%) indicates precision and reliability of the conducted experiments (Table 4). On the basis of results obtained from ANOVA it can be concluded that the model is highly significant and sufficient to represent the actual relationship between the response and the significant variables and can be used successfully to navigate the design space.

In our study thirteen model terms were found to be significant and the variables with prevalent effects were the linear terms of A ($p < 0.0001$), B ($p < 0.0001$), C ($p < 0.0001$), D ($p < 0.0001$), E ($p < 0.0001$) and the quadratic terms of A^2 ($p < 0.0001$), B^2 ($p < 0.0001$), C^2 ($p < 0.0001$), D^2 ($p < 0.0001$), E^2 ($p < 0.0001$) followed by interaction effects of AE ($p < 0.0001$), BC ($p < 0.0001$), and BE ($p < 0.0001$). The value of 'p' less than 0.0500 indicates the 'significance' of the model terms. The results indicated that the effect order of the linear terms on the yield of alkaline lipase were as follows, pH (F; 2620.82), starch (F; 2307.78) temperature (F; 1868.66) and castor oil (F; 303.47).

Table 4 - Analysis of variance (ANOVA) for response-surface quadratic fitted model.

Parameter	Value
Standard deviation	26.71
Mean	1478.52
C.V. %	1.81
PRESS	68927.67
R^2	0.9987
Adjusted R^2	0.9976
Predicted R^2	0.9949
Adequate precision	127.274

The second-order model can be plotted as a three-dimensional surface representing the response (lipase production) as a function of the two factors at a time while maintaining other three factors at fixed levels (centre point) to understand both the main and the interaction effects of these two factors. The three dimensional response surfaces obtained after analysis showed different shapes which indicated variation in the combined effect of independent variables on lipase production. Figure 1a illustrates the interaction effect of pH with incubation temperature. According to the plot, the optimal value (1810 $U.mL^{-1}$) lied towards the value at central point of the pH (8.5) and temperature (35 °C). However, further increase in pH and temperature resulted in lowest enzyme production (515 $U.mL^{-1}$) of test variables.

The effect of pH and temperature is shown in Figure 1b. The response curve analysis indicated that maximum enzyme units were produced at temperature 30 °C with 15 $g.L^{-1}$ of starch. Supplementation of starch at elevated level (25 $g.L^{-1}$) and incubation at higher temperature (40 °C) had negative effect on the response. Figure 1c depicts the interaction of temperature and castor oil on lipase production. The observation of interactions of castor oil and temperature indicated that lipase production decreased with increase in temperature (30-40 °C) and castor oil concentration (1.0-3.0 $mL.L^{-1}$).

Figure 1d represents the response for the interaction of triton-X-100 with incubation temperature. Maximum lipase was produced at higher level of triton-X-100 (1.5 $mL.L^{-1}$) at lower temperature (30 °C) in the design space. However, lower concentration of triton-X-100 (0.5 $mL.L^{-1}$) had negative effect on lipase production at higher temperature (40 °C). The interaction effect of pH and starch was remarkable, where both the factors supported maximum lipase production (2903 $U.mL^{-1}$) at -1 coded value *i.e.* 8.5 and 15 $g.L^{-1}$ for pH and starch, respectively (Figure 1e). This accorded a run number of 21, which is considered as the optimal condition of test variables.

Figure 1f depicts the production of lipase with respect to triton-X-100 *vs.* pH. Maximum lipase production with these variables was observed at concentration of 0.5 $mL.L^{-1}$ triton-X-100 at pH 8.5. However, further elevation in pH (9.5) decrease the lipase production at the same concentration of triton-X-100 (0.5 $mL.L^{-1}$).

The results obtained as well as predicted by Box-Behnken design showed that a combination of temperature of 35 °C, pH 8.5, starch (15 $g.L^{-1}$), castor oil (2 $mL.L^{-1}$) and triton-X-100 (1 $mL.L^{-1}$) would favor maximum lipase production (2903 $U.mL^{-1}$).

With an aim to test the desirability of the model, the optimum values of the variables were determined as temperature, 34.12 °C; pH, 8.1; starch, 11.29 $g.L^{-1}$; castor oil,

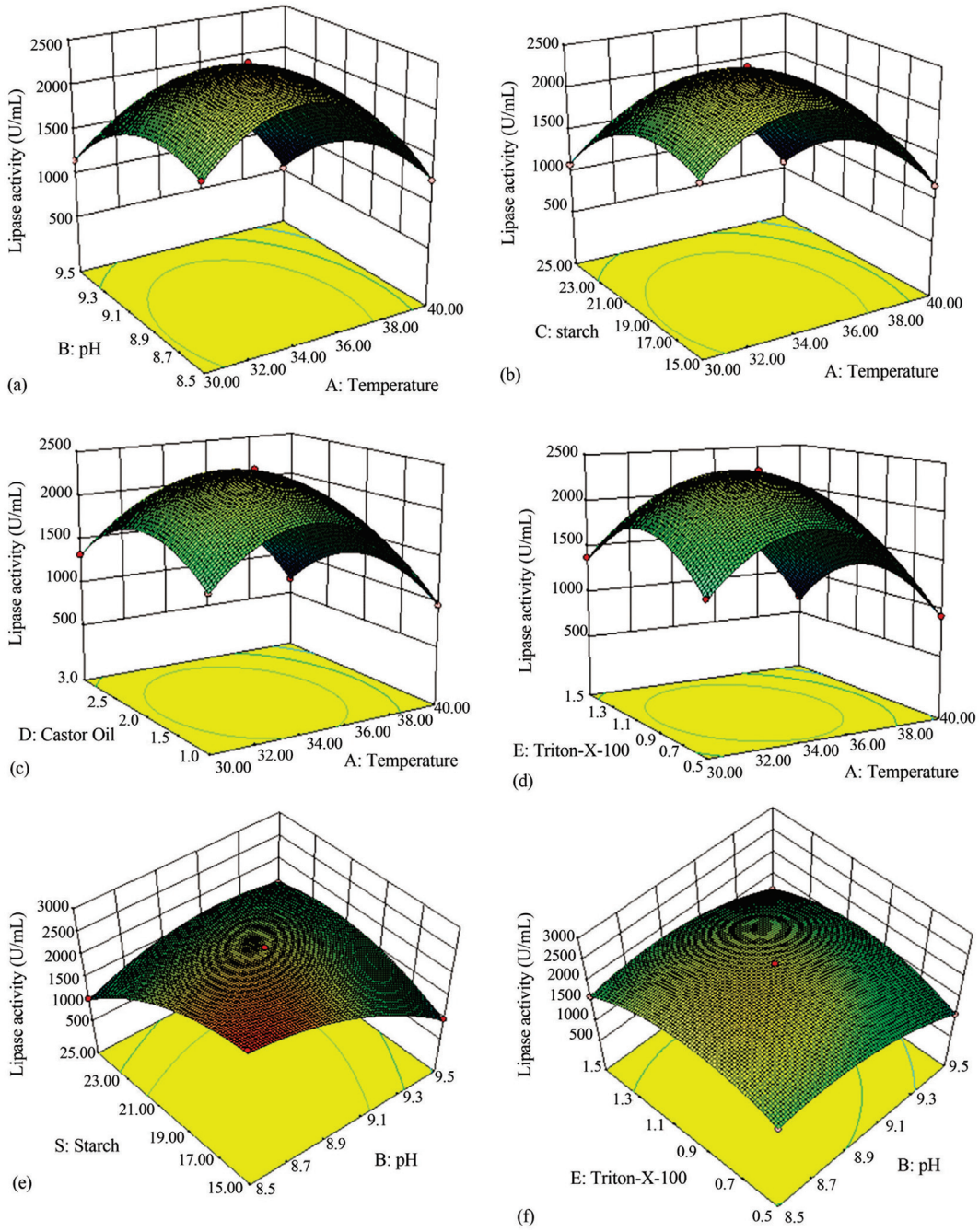

Figure 1 - Response surface plots for alkaline lipase production by *P. aeruginosa* mutant. Each figure illustrates the interaction of two independent while others were kept at their respective centre points. (a) pH and incubation temperature (b) starch and incubation temperature (c) castor oil and incubation temperature (d) triton-X-100 and incubation temperature (e) starch and pH (f) pH and triton-X-100.

1.77 mL.L^{-1} and triton-X-100, 0.93 mL.L^{-1} yielding a maximum lipase production of 3007.25 U.mL^{-1}.

Comparison of observed and predicted response and validation of the model

A regression model could be used to predict future observations on the response Y (alkaline lipase activity) corresponding to particular values of the variables. Figure 2 illustrates the observed lipase activities *vs.* predicted values by the empirical model Eq. (2). A high degree of similarity was observed between the predicted data of the response (2836.19 U.mL^{-1}) from the empirical model and the experimental values (2903 U.mL^{-1}) in the range of the operating variables reflecting the applicability of RSM to optimize the process of enzyme production.

The suitability of the model was validated at shake flask level by additional independent experiments under the optimal conditions predicted by the equation. The

Table 5 - Validation of the response surface model.

S. no.	Temperature (°C)	pH	Starch ($g.L^{-1}$)	Castor oil ($mL.\ L^{-1}$)	Triton-X-100 ($mL.L^{-1}$)	Observed response ($U.mL^{-1}$)	Predicted response ($U.mL^{-1}$)
1*	34.12	8.08	11.29	1.77	0.93	3142.57	3007.25
2	34	7.5	12.5	2.0	1.0	2154.86	2264.35
3	34	8.0	12.0	1.5	1.0	2842.45	2928.31
4	33	7.9	11.75	1.25	0.8	2823.8	2741.96
5	35	8.5	11.5	2.0	1.5	2099.38	2138.68
6	35	8.0	11.0	2.5	0.8	2629.58	2694.11
7	34	8.5	15.0	1.5	0.9	2712.45	2832.39
8	32	8.25	10.5	1.75	0.85	2699.18	2755.47
9	33	8.0	15.0	1.8	1.0	2623.91	2641.23
10	35	8.2	11.5	1.8	0.9	2856.34	2960.77
11	33	8.3	11.25	1.5	0.5	2375.93	2434.61

*Optimum values predicted by the design.

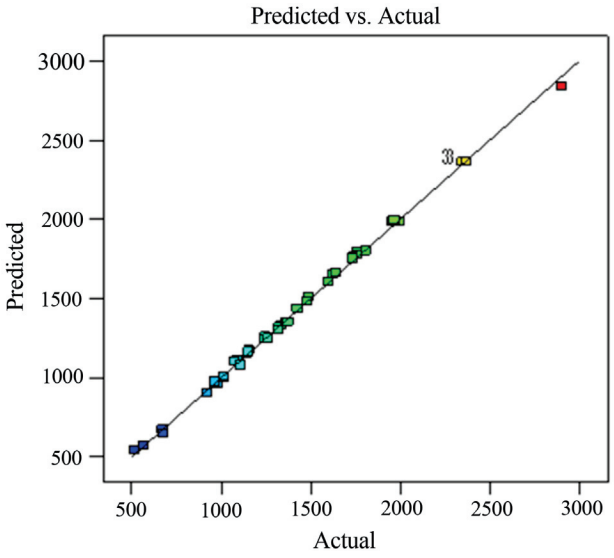

Figure 2 - Observed lipase activity verses predicted lipase activity.

model indicated that the selected levels of pH and starch were limiting as the response is maximum at their lowest values. So within the design space the response surface graphs were unable to illustrate the accurate results. Therefore, further decrease in medium pH along with decrease in starch concentration should be worked out for validation. Table 5 shows the predicted and observed responses of the validation experiments. Under the optimum conditions, obtained by point prediction, the mutant strain was able to produce 3142.57 $U.mL^{-1}$, which is 104.5% of the predicted value. The results showed actual values were closer to the predicted values, supporting the data and the model as valid. However, other tested combinations did not reveal improvement in the response. Thus, by optimizing the fermentation parameters using RSM, the yield of alkaline lipase increased from 2362.5 $U.mL^{-1}$ to 3142.57 $U.mL^{-1}$ by optimizing the fermentation parameters using RSM.

Conclusion

The present study conclusively demonstrates the application of a Box-Behnken design for determination of optimal medium composition for alkaline lipase production by a mutant strain of *P.* aeruginosa. The initial medium pH and supplementation of castor oil had most significant effect on lipase production. The interactions of pH and temperature, pH and castor oil and castor oil and triton-X-100 were found to be crucial on the response. Alkaline lipase production (3142.57 $U.mL^{-1}$) using this optimized medium and culture conditions was about 1.3-fold higher than medium obtained by one-variable-at-a-time approach (2362.5 $U.mL^{-1}$). The model was capable to foresee accurately the lipase activity by altering culture conditions. Application of such approach can be of immense significance for industrial bioprocess.

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References

Abdel-Fattah YR, Saeed HM, Gohar YM, El-Baz MA (2005) Improved production of *Pseudomonas aeruginosa* uricase by optimization of process parameters through statistical experimental designs. Process Biochem 40:1707-1714.

Adamczak M, Bornscheuer UT, Bednarski W (2009) The application of biotechnological methods for the synthesis of biodiesel. Eur J Lipid Sci Technol 111:808-813.

Bas D, Boyaci IH (2007) Modeling and optimization I: Usability of response surface methodology. J Food Eng 78:836-845.

Bisht D, Yadav SK, Darmwal NS (2012) Enhanced production of extracellular alkaline lipase by an improved strain of *Pseudomonas aeruginosa* MTCC 10,055. Am J Appl Sci 9:158-167.

Box GEP, Benhnken DW (1960) Some new three level design for the study of quantitative variable. Technometrics 2:455-475.

Chen CS, Liu KJ, Lou YH, Shieh CJ (2002) Optimization of kojic acid monolaurate synthesis PS from *Pseudomonas cepacia*. J Sci Food Agric 82:601-605.

Doddapaneni KK, Tatineni R, Potumarthi R, Mangamoori LN (2007) Optimization of media constituents through response surface methodology for improved production of alkaline proteases by *Serratia rubidea*. J Chem Technol Biotechnol 82:721-729.

Dutta JR, Dutta PK, Banerjee R (2004) Optimization of culture parameters for extracellular protease production from a newly isolated *Pseudomonas* sp. using response surface and artificial neural network models. Process Biochem 39:2193-2198.

Faiza A, Haq NB, Saima R (2011) Optimization of growth parameters for lipase production by *Ganoderma lucidum* using response surface methodology. Afr J Biotechnol 10:5514-5523.

Fisher RA (1926) The arrangement of field experiments. J Min Agri 33:503-513.

Gopinath SCB, Hilda A, Lakshmi Priya T, Annadurai G, Anbu P (2003) Purification of lipase from *Geotrichum candidum*, conditions optimized for enzyme production using Box-Behnken design. World J Microbiol Biotechnol 19:681-689.

Gupta R, Beg QK, Lorenz P (2002) Bacterial alkaline proteases, molecular approaches and industrial applications. Appl Microbiol Biotechnol 59:15-32.

Hasan F, Shah AA, Javed S, Hameed A (2010) Enzymes used in detergents, lipases. Afr J Biotechnol 9:4836-4844.

Henika RG (1972) Simple and effective system for use with response surface methodology. Cereal Sci Today 17:309-334.

Joglekar AM, May AT (1987) Product excellence through design of experiments. Cereal Foods World 32:857-868.

Joo HS, Chang CS (2006) Production of oxidant and SDS-stable alkaline protease from an alkalophilic *Bacillus clausii* I-52 by submerged fermentation, feasibility as a laundry detergent additive. Enz Microb Technol 38:176-183.

Kennedy M, Krouse D (1999) Strategies for improving fermentation medium performance: A review. J Ind Microbiol Biotechnol 23:456-475.

Kumar SS, Gupta R (2008) An extracellular lipase from *Trichosporon asahii* MSR 54: Medium optimization and enantioselective deacetylation of phenyl ethyl acetate. Process Biochem 43:1054-1060.

Kumari A, Mahapatra P, Banerjee R (2009) Statistical optimization of culture conditions by response surface methodology for synthesis of lipase with *Enterobacter aerogenes*. Brazil Arch Biol Technol 52:1349-1356.

Liu Y, Wang F, Tan T (2009) Cyclic resolution of racemic ibuprofen via coupled efficient lipase and acid-base catalysis. Chirality 21:349-353.

Low CT, Mohammad R, Tan CP, Long K, Ismail R, Lo SK, Lai OM (2007) Lipase-catalyzed production of medium-chain triacylglycerols from palm kernel oil distillate: Optimization using response surface methodology. Eur J Lipid Sci Technol 109:107-119.

Macrae AR, Hammond RC (1985) Present and future applications of lipases. Biotechnol Genetic Eng Rev 3:193-217.

Nelofer R, Ramanan RN, Rahman RNZRA, Basri M, Ariff AB (2011) Sequential optimization of production of a thermostable and organic solvent tolerant lipase by recombinant *Escherichia coli*. Ann Microbiol 61:535-544.

Salihu A, Alam Z, Abdul Karim MI, Salleh HM (2011) Optimization of lipase production by *Candida cylindracea* in palm oil mill effluent based medium using statistical experimental design. J Mol Catal B Enzym 69:66-73.

Wang Y, Zhao M, Ou S, Song K, Han X (2009) Preparation of diacylglycerol-enriched palm olein by phospholipase A1-catalyzed partial hydrolysis. Eur J Lipid Sci Technol 111:652-662.

Winkler UK, Stuckmann M (1979) Glycogen, hyaluronate, and some other polysaccharides greatly enhance the formation of exolipase by *Serratia marcescens*. J Bacteriol 138:663-670.