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Optimization of Lipase Activity from *Pseudomonas Fluorescens* Using Response Surface Methodology

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Abstract: To ensure optimal lipase activity from *Pseudomonas fluorescens*, selection of various parameters for an optimized fermentation process is vital. The current study is aimed at the optimization of process parameters of the fermentation based on three basic process parameters during fermentation including pH, fermentation temperature and incubation hours so as to improve lipase activity using response surface methodology (RSM) coupled with central composite design method (CCD). As per the experimental data, correlation between the three investigated parameters with respect to lipase activity of *Pseudomonas fluorescens* was obtained. The results of analysis of variance (ANOVA) proved that the correlation proposed by quadratic regression was adequate for predicting lipase activity of *Pseudomonas fluorescens*. It was concluded that the lipase activity was significantly influenced by fermentation parameters. The fermentation temperature of 30 ºC, pH value of 8.5 and incubation of 30 hours were found to be optimal for optimal enzyme activity.

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Introduction

RSM consolidates statistical procedures towards experimental design, evaluation of effect of variables thereby seeking the optimum process correlation. This is commonly used in optimization of fermentation processes such as in ethanol production and food industry [1-3]. Confined sets of experimental runs which are desired to provide sufficient information for statistically acceptable results, adaptability alongwith investigation of common correlation between various interdependent factors in a process towards the most suitable condition [4]. The current study was based on RSM that is rendered as the major tool for process optimization which can be used to determine the optimum values of a cluster of parameters vital for the process.

Based on the initial studies in our lab with other microbial lipases, *Pseudomonas fluorescens* was chosen for optimization by RSM. RSM optimization studies have been done for maximum production of lipase by taking one variable at a time (OVAT) [5-7]. RSM was used to evaluate the individual as well as combined effect of the screened parameters such as pH, temperature, time of incubation etc. Different variables such as temperature, pH and incubation time of the microorganism were considered. Five code levels were considered in the range of -2 to +2 for all the factors so as to determine the response of different levels. The media components were uniform for all the experiments as best media has been selected for growth, on the basis of results of media optimization. Performance of the experiments was assessed through the lipase activity achieved after every experiment.

Methodology

Submerged fermentation in shake flask culture was carried out in 500 mL Erlenmeyer flasks. Conditions were maintained for each experiment as per the central composite design. The same was inoculated after sterilization of the media at 121 ℃ for 15 minutes. Each experiment was performed in triplicates for minimizing errors. Design of the experiments and statistical analysis was done in this study using central composite design with 3 factors: pH, temperature and incubation hours. The factors and their respective levels for the investigation have been shown in Table 1.

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Table:1 Factors and levels including actual values for the optimization studies

Factors	Code levels				
	-2	**-1**	**0**	**+1**	**+2**
pH	7	7.75	8.5	9.25	10.0
Temperature	30	37.5	45	52.5	60
Incubation hours	12	21	30	39	48

Regression analysis was performed by analysis of the regression equation by statistical analysis using ANOVA, regression coefficient (R^2), F-test (over-all model significance), t-test and P-value (each coefficient significance). The dependence of independent variables on experimental results have been described in Table: 2.

Table: 2 Values of variables and the observed response

Exp no.	pH Factor 1	Temp Factor 2	Hours of incubation Factor 3	Specific activity (U/mg)
1	8.50	45	12	7.90
2	8.50	45	30	10.90
3	7.75	52.50	21	10.62
4	8.50	45	30	11.85
5	8.50	45	30	11.40
6	8.50	60	30	14.38
7	7.00	45	30	10.41
8	8.50	45	30	11.95
9	9.25	37	39	13.76
10	7.75	37	39	14.8
11	8.50	45	30	9.47
12	9.25	37	21	8.56
13	8.50	45	30	11.60
14	9.25	52.50	21	12.04
15	9.25	52.50	39	11
16	8.50	45	48	16.81
17	7.75	37	21	8.91
18	7.75	52.50	39	11.27
19	**8.50**	**30**	**30**	**19.70**
20	10	45	30	13.54

Experiments in triplicate were performed for the above-mentioned combinations of parameters and lipase activity was reported for each combination. The combination of different parameters (pH, temperature and incubation time) is described in Table 2.

Results and Discussion

To optimize the lipase production by *Pseudomonas fluorescens*, the physico-chemical parameters effecting the lipase production were optimized. Optimization of the lipase production was carried out with the help of response surface

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methodology (RSM) to find the effect of interactions among various parameters. Central composite design was employed to investigate the interaction among variables such as pH, temperature and incubation hour described in Table 3.

Table:3 Factors and levels including actual values used in central composite design for the optimization of lipase production by *Pseudomonas fluorescens*

Factors	Code levels				
	-2	**-1**	**0**	**+1**	**+2**
pH	7	7.75	8.5	9.25	10.0
Temperature	30	37.5	45	52.5	60
Incubation hours	12	21	30	39	48

The central composite design resulted in a total of 20 experiments and lipase activity of all the flasks at the desired incubation time was studied as a response. The experimental design with response is shown in Table 2 (last column).

In an attempt to achieve the best fit regression of the model, the regression equation and coefficient of determination (R^2) were determined. The model exhibited a moderate determination of coefficient (R^2 = 0.7657) which explained that 76.57% variables contributed to the lipase production. The results of the analysis of variance (ANOVA) is presented in Table 4, which demonstrates the model is significant owing to a very low probability value [(P_{model} > F) = 0.0284].

Table 4 ANOVA for the selected quadratic model

Source	Degree of freedom	F-value	P>F
Regression	9	3.63	0.0284
Residual error	10		
Total	19		

Values of "Prob > F" less than 0.0500 indicate model terms are significant. The highest lipase activity was observed in **Experiment No. 19**. It was observed that the optimal value for the test variables were pH (8.5), temperature (30 ºC) and h of incubation (30 hr) which are in close to the results of optimization studies by one variable at a time.

The investigations with the *Pseudomonas fluorescens* indicated that the highest activty can be achieved at varied combinations of pH, temperature and incubation time. Further, optimization of media compostion and the model reduction w.r.t. the optimized process conditions is expected to improve the overall lipase production.

Conclusion

The study concluded that the lipase production is greatly affected by the process parameters such as pH, temperature and incubation period. Undoubetdly, the RSM is an effective method for the optimization of any process. The method limits the number of experimental variables and is a less labor intensive method thereby, shorter and less time consuming experments can be designed.

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