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Lipase Production in Solid State Fermentation Using *Aspergillus niger*: Response Surface Methodology

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ABSTRACT

Among enzymes, lipases have been widely investigated because of their numerous industrial applications. In this study, optimization of lipase production by *Aspergillus niger* in solid state fermentation from rice bran as solid substrate was investigated. The optimal conditions were obtained with the aid of central composite design (CCD) under response surface methodology (RSM). In the analysis of 20 experimental runs, effects of three variables such as olive oil concentration, glucose concentration and humidity ratio on lipase activity were evaluated. The interaction between lipase activity and independent paired parameters were illustrated by three-dimensional diagrams while other factors were set at zero level. The optimum values of oil concentration, glucose concentration and humidity ratio were 1.78, 2.54 and 1.95% (g/g_{dss}), respectively and the enzyme activity for optimum conditions was121.53 (U/g_{dss}). Also, the effect of temperature and pH on the relative lipase activity in enzyme reaction was evaluated. The lipase produced was highly active at 30.3°C and pH value of 6.87.

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1. INTRODUCTION

Enzymes are protein catalysts that enhance the rate of biochemical reactions [1]. Lipases are well-known as one of the important group of enzymes in international market and have the most influential economy in terms of sales after proteases and amylases [2]. Lipases (triacylglycer- ol acylhydrolases, EC 3.1.1.3) catalyze the hydrolysis reaction of triacylglycerols to free fatty acids and mono- diglycerols in the oil-fat interface [3-4].

Lipases have broad variety of industrial applications such as food industry (improvement of flavor), detergent (hydrolysis of oil and fats), pharmaceutical (synthesis of chiral drug), paper (control of pitch), medicine (triglycerides measurement), cosmetics (exclusion of lipids), wastewater (decomposition and removal oil), leather (elimination of fat from animal skin) [5-8]. Uses of lipase in food industries are generally regarded as safe (GRAS) by the United States Food and Drug Administration (US-FDA) [9-10].

In literature review, several substrates with various organisms are used for lipase production. Table 1 summarized the feedstock sources and microorganism used in lipase synthesis. Lipases were produced by microorganisms, animals and plants. The enzymes which are originated from microorganisms are preferable choice for industrial applications, because they are more stable even in organic solvents with low costs. Generally, bacteria, yeast and fungi are three main sources of lipase synthesis. About 75% of industrial enzymes markets were captured by lipase for the purpose of hydrolysis. In recent reported literature about 90% of produced lipases were obtained from microbial sources [4, 11-14]. Among the fungi, *Aspergillus niger* is known as one of the best extracellular enzyme producers.

Solid state fermentation (SSF) and submerged fermentation (SmF) are considered as two fermentation techniques for enzyme production [15]. The solid state fermentation system involves growth and metabolism of moist solid substrate in absence of free water [16]. SSF has many preferences to SmF for microbial enzyme production including: high yield and productivities, decreased capital and operating costs, facilitated fermentation media, superior oxygen distribution, fewer operational troubles, simpler equipment and control system and opportunity of using biomass and

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TABLE 1. Lipase production by different microorganisms and various feed stock sources

Feed stock	Fermentation	Microorganism	Lipase activity	Reference
Whey and soybean meal	SmF	*Aeromonas sobria LP004*	419.84 (Units/ml)	[25]
Wheat bran	SSF	*Rhizopus oligosporous*	48.0 (U/g s)	[15]
Wheat bran	SSF	*Aspergillus niger*	9.14(IU/g dss)	[17]
Wheat bran	SSF	*Aspergillus niger*	630 (IU/g DSS)	[18]
Ground-nut oil refinery residue	SmF	*Penicillium citrinum*	898 (U/l)	[26]

argoindustrial residues as main substrate [17-19]. Due to low humidity requirements in SSF system, fungi have greater productivity toward other microorganisms. Various solid substrates for lipase production are reported in the literatures including: gingelly oil cake, babassu oil cake, soybean meal, wheat barn, polymeric resin and mixture of rice, corn flour, wheat flour, wheat bran, rice bran and soybean powder [2, 18, 20-26]. Rice bran is a rich source of nutrients and known as an agricultural waste in our country. Rice bran is found in abundance in north of Iran. it can be used as inexpensive substrates for production of valuable enzyme.

Process conditions and medium composition are considered as two important parameters that affect on lipase activity and production in solid state fermentation. The classical optimization method for the medium and culture condition involves varying one parameter at a time while keeping the others at constant level. This method is inappropriate for optimization and has various disadvantages since the effects of interaction among variables are neglected. In addition, as the process is time consuming, determination of the optimum levels would be expensive and necessitates a number of experiments [27-30]. Central composite design (CCD) and response surface methodology (RSM) are useful design tools for optimization of experiments that indicate correlations between variables and responses [31-33].

The aim of the present research was to investigate the optimum fermentation conditions for lipase production using *Aspergillus niger* (National Collection of Industrial Microorganisms) 584 in solid state fermentation with rice barn. The effects of three parameters on enzyme production were examined with central composed design.

In addition, the influence of temperature and pH on the relative lipase activity in the enzyme reaction was investigated.

2. EXPERIMENTALTAL

In this study, *Aspergillus niger* NCIM 584 was used. The organism was supplied by National Collection of Industrial Microorganisms (Chandigarh, India). The Fungus strain was stored and maintained in nutrient agar slants at 4°C. Morphological features of *Aspergillus niger* NCIM 584 with fibrous and cylindrical symmetric morphology of mycelia are presented in Figure 1.

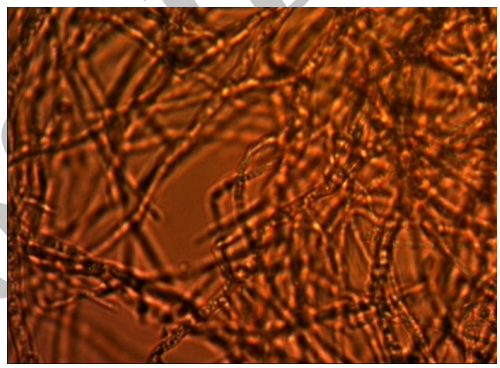

Figure 1. Morphology of *A. niger* NCIM 584

The fermentation medium for lipase production contained: KH_2PO_4, $MgSO_4 \cdot 7H_2O$, KCl and yeast extract with concentration of 2, 0.5, 0.5 and 0.5 g/l, respectively. Glucose and olive oil concentrations were design variables and changed according to experimental design (Table 2). All chemicals and reagents used in experiments were analytical grade supplied by Merck (Darmstadt, Germany). Rice bran was used as the solid substrate that was locally supplied from north of Iran. The chemical composition and physical properties of rice bran is summarized in Table 3.

The microorganism was grown in a 100 ml Erlenmeyer flask which contained 50 ml of the fermentation medium. The seed culture was cultivated as inoculum. The inoculated media were incubated at 180 rpm and 30°C in an incubator-shaker for 2 days. The prepared seed culture in form of mycelium was added to solid substrate.

A 5 gram of rice bran which was initially sterilized and dried was placed in a Petri dish with diameter of 7.8 cm. The substrate was moistened with defined volume of 2 days old fungous culture and then incubated in a desiccator for the period of 4 days. The bottom of

desiccator was filled with distilled water. The desired moisture content within the desiccator was provided by means of a spurger which was connected to an external air pump**.**

At the end of fermentation period, the mold substrate was added to a 100-ml Erlenmeyer containing 50 ml of the aqueous solution of NaCl (1%) and triton X-100 (1%). The resulting mixture was then agitated in an incubator shaker at 30°C and 180 rpm for 2 hours [18]. The biomass was separated by filtration, using Whatman filter paper (no. 41; diameter of 125mm); for the enzyme extraction from the fermented rice barn and then the remaining mycelia was removed by centrifuge at 1000g for 5 minutes.

TABLE 2. Experimental ranges and levels of the independent variables

Variables	Symbol	Range and levels				
		-2	-1	0	1	2
Olive oil concentration (g/g_{dss}) %	X_1	1	1.5	2	2.5	3
Glucose concentration (g/g_{dss}) %	X_2	1.5	2	2.5	3	3.5
Humidity ratio	X_3	1	1.5	2	2.5	3

TABLE 3. Chemical composition and physical properties of rice bran

Composition	Weight %
Moisture	8
Ash	4
Protein	7
Sugar	9.77
Acidic number	0.036
Particle size	
< 250 µm	23
600 µm <particles <250 µm	39
> 600 µm	38

Several factors influence the stability of enzyme in enzyme reaction. Lipase activity measured at various temperatures and pHs. The optimum temperature and pH for lipase stability in enzyme reaction by central composite design with two parameters and 13 experiments were determined.

The hydrolytic activity of lipase was determined by spectrophotometric method with *p*-nitrophenyl palmitate as chromatic substrate. The assay mixture was incubated for 5 min and the *p*-nitrophenol released was measured at 410 nm according to the method discussed in literature [34-35]. Based on definition, one unit (U) of lipase activity was defined as the amount of enzyme liberates one micromole of *p*-nitrophenol per minute under the standard assay conditions.

For lipase production in solid state fermentation, culture conditions were optimized by means of central composite design (CCD) under response surface methodology (RSM). The RSM include statistical and mathematical techniques useful for developing, improving and optimizing processes. Also, the RSM appraises correlation between controlled experimental parameters and measured responses according to route for one or more selected criteria [36-37]. Optimization studies were carried out by studying the effect of three variables including olive oil, glucose concentrations and humidity ratio (The volume of liquid/The weight of solid substrate). The chosen independent variables used in this study were coded according to Eq. (1):

$$x_i = \frac{X_i - X_0}{\Delta X_i} \quad (1)$$

where x_i is the dimensionless coded value of the ith independent variable, X_0 is the value of X_i at the center point and Δx is the step change value. The behavior of the system is explained by the following empirical second-order polynomial model Eq. (2):

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

where Y is the predicted response, X_i, X_j, . . ., X_k are the input variables, which affect the response Y, X^2_i ,X^2_j , . . . , X^2_k the square effects, X_iX_j, X_iX_k and X_jX_k the interaction effects, β_0 is the intercept term, β_i (i=1, 2, . . ., k) the linear effect, β_{ii} (i=1, 2, . . ., k) the squared effect, β_{ij} (i=1, 2, . . ., k; j=1, 2,. . ., k) the interaction effect and ε a random error [37-38].

Design Expert 7.01 (Stat-Ease Inc., Minneapolis, MN, USA) software was used for the regression and graphical examination of the experimental results. The optimum values of the variables were acquired by solving the regression equation. Also, the analyzing response surface plots indicated effects of the parameters on lipase production. Each of the parameters was coded at five levels: (−α, −1, 0, +1 and +α; α=2) [37].

3. RESULTS AND DISCUSION

In solid state fermentation, the range and level of three parameters (olive oil concentration, glucose concentration and humidity ratio) were determined based on the reported data in literature for lipase production [17-18, 25]. The experiments designed using central composite design under response surface methodology to optimize lipase production and investigate interaction between variables. The range of independent variables used in this study in terms of actual and coded values is summarized in Table 2.

Maximum enzyme activity obtained with 20 experiments for three factorial designs (2^3) at five level and six star points and six replicated points. Actual value and statically predicted lipase activity for experiments are shown in Table 4. Mathematical model (Eq. 3) represents relationships between lipolytic activity (Y) and oil concentration (X_1), glucose concentration (X_2) and humidity ratio (X_3) in coded units.

$$Y = +119.75 - 7.64X_1 + 1.78X_2 + 0.21X_3 + 2.54X_1X_2 + 3.39X_1X_3 - 1.82X_2X_3 - 8.65X_1^2 - 5.0X_2^2 - 7.37X_3^2 \quad (3)$$

TABLE 4. Experimental design based on central composite design (CCD) used in this study.

Run No.	Coded values			Enzyme activity (U/g $_{dss}$)	
	X_1	X_2	X_3	Actual	Predicted
1	-1	-1	-1	109.33	108.49
2	1	-1	-1	79.42	81.36
3	-1	1	-1	110.23	110.61
4	1	1	-1	93.88	93.62
5	-1	-1	1	104.45	105.75
6	1	-1	1	91.52	92.19
7	-1	1	1	101.49	100.60
8	1	1	1	95.31	97.19
9	-2	0	0	99.87	100.42
10	2	0	0	71.46	69.87
11	0	-2	0	97.20	96.19
12	0	2	0	103.34	103.31
13	0	0	-2	89.94	89.85
14	0	0	2	91.64	90.68
15	0	0	0	121.64	119.75
16	0	0	0	121.95	119.75
17	0	0	0	118.32	119.75
18	0	0	0	117.22	119.75
19	0	0	0	120.84	119.75
20	0	0	0	119.58	119.75

Variables X_1X_2, X_1X_3 and X_2X_3 are interaction effect of oil concentration-glucose concentration, glucose concentration-humidity ratio and oil concentration-humidity ratio, respectively. Whereas X_1, X_2 and X_3 express the values of the main effect of variables including oil concentration, glucose concentration and humidity ratio, respectively. The adequacy of the second-order response surface model fitted in the form of analysis of variance (ANOVA) and the importance of the coefficients are observed in Table 5. The significance of each coefficient was determined by F-values (variation of data about mean value) and P-values (probability). It was observed from Table 4 that F-values and P-values were high and very low, respectively. The results showed that the model is in good prediction of experimental result. Low P-values of linear and quadratic terms for olive oil concentration, glucose concentration showed high linear and quadratic effects of these parameters on response factor. Humidity ratio had high quadratic effect, but the P-value (0.6635) for the linear term of this variable was high; that indicated insignificant linear effect. In addition, values of interaction effect of variables were significant.

The ANOVA for response surface quadratic model is given in Table 6. The F-value (129.68) and low P-values ($P < 0.0001$) indicate that the regression model were valid. The lack of fitness of more than 0.05 demonstrated significance of model for lipase production. The correctness of the model was also ensured by the multiple correlation coefficients (R^2). The R^2 Value varied from 0 to 1 and when R^2 value is quite close to 1, the predicted value is relatively close to actual value. This means the model definitely predicted the actual value and the response was excellent The multiple correlation coefficient (R^2) was 0.9915; even less than one percent (only 0.85%) of total variation cannot be expressed by the regression model. The predicted multiple correlation coefficient (Pred.- R^2 = 0.963) was in reasonable agreement with the adjusted multiple correlation coefficient (Adj. R^2 = 0.984). Also, the coefficient of variance (C.V. =1.79 %) was low, which indicates significant precision and reliability of the experimental data. Adequate precision, measures the signal to noise ratio. A ratio greater than 4 is demanded.

TABLE 5. Regression analysis using the 2^3 factorial central composite design.

Model term	Coefficient estimate	Standard error	F-value	P-value
Intercept	119.75	0.74		
X_1	-7.64	0.46	274.54	< 0.0001
X_2	1.78	0.46	14.90	0.0032
X_3	0.21	0.46	0.20	0.6635
X_1X_2	2.54	0.65	15.16	0.0030
X_1X_3	3.39	0.65	27.10	0.0004
X_2X_3	-1.82	0.65	7.77	0.0192
X_1^2	-8.65	0.37	553.70	< 0.0001
X_2^2	-5.00	0.37	184.98	< 0.0001
X_3^2	-7.37	0.37	401.86	< 0.0001

Adequate precision was 38.263, indicating that the model could be used to navigate the design space.

The interaction of the lipase activity and independent variables were demonstrated by three-dimensional response surface diagram. In each 3D curve, effect of two factors on enzym activity are shown, maintaining other variable constant at level zero. Figure 2 indicates the relationship between olive oil concentration and glucose concentration on lipolytic activity. The low P-value (0.0030) shows significant interactions between variables. Lipase activities increased with an increase of olive oil concentration from 1 to 1.8 % (g/g_{dss}) as well as with media glucose concentrations ranging from 1.5 to 3.5 % (g/g_{dss}). Also enzyme activity was significantly reduced at concentrations higher than 1.8 % (g/g_{dss}). Maximum lipase activity was obtained at 2.5 % (g/g_{dss}) of glucose concentrations. Use of glucose was compared with oils as fatty acids were assimilated faster than carbohydrates in the cells, but presence of fatty carbon source is necessary, since olive oil was used as carbon sources and inducers of microorganism for lipase production. Increasing the olive oil and glucose concentration in the media over the optimum value may impose an inhibitor effect on lipase production that was most probably due to catabolite repression. Nevertheless, disincentive effect of olive oil concentrations was more than glucose concentrations. In solid state fermentation lipase production, optimum condition for *A. niger* was also found by Falony et al. .[17]; the maximum enzyme activity occurred when the concentrations of olive oil and glucose were 1.5 and 1.5 % (g/g dss), respectively.

Experiments were performed to investigate the influence of olive oil concentration and humidity ratio on lipolytic activity, in the range of 1 to 3 % (g/g_{dss}) and 1 to 3, respectively, (Figure 3). The P-value was low (0.0004), that indicates significant interaction of independent variables. The optimum concentration of olive oil was defined at 1.76 % (g/g_{dss}) . Enzyme activity has increased with an increase in humidity ratio ranging from 1 to 1.96. When the ratio of humidity was higher than optimum level of the moisture, the lipase activity decreased. This means, the water content has influenced on the physical properties of the solid substrate. Increasingthe humidity ratio above the optimum value led to a significant decrease in lipase activity. That was probably due to decrease in substrate porosity, reduced oxygen transfer and also changes occurred in the texture of solid particles. In addition, for the values lower than the optimized level, the humidity ratio has decreased the solubility of the solid substrate, lowered the degree of swelling and produced a high water tension [31].

The interaction of glucose concentration and humidity ratio on enzyme activities are shown in Figure 4. Optimum lipase activity was observed in mid-values of both variables. The P-value (0.0192) was low, which depicted the good interaction of the two variables. The maximal lipase activity was obtained at optimum glucose concentration and humidity ratio of 2.58 % (g/g_{dss}) and 2.01, respectively. Falony et al. [17, 31] have demonstrated that the best moisture value for *A. niger* in a solid state fermentation with wheat bran as substrate was 65 % (g/g_{dss}). Also, Mahadik et al. [18] have reported that the best ratio of water content to wheat bran was 2.5 for the lipase produced from *A. niger* NCIM 1207.

The best conditions for lipase production obtained were at olive oil concentration of 1.78 % (g/g_{dss}) , glucose concentration of 2.54 % (g/g_{dss}) and humidity ratio of 1.95. Lipase activity obtained at optimal conditions and at a desirability value of 0.982 was 121.53 (U/g_{dss}). The obtained value was in the range of reported data for lipase production in literature.

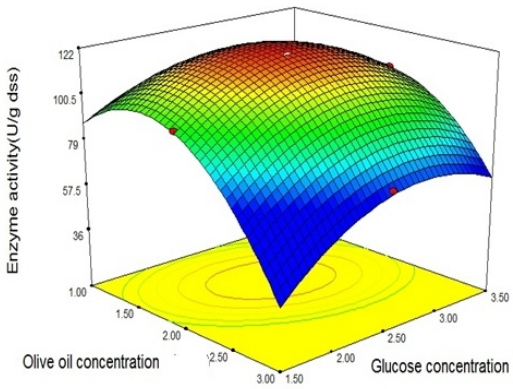

Figure 2. The effect of olive oil and glucose concentration on lipase activity (other variable was set at zero level), showing the effect of olive oil and glucose concentrations and their interaction effects on lipase production

TABLE 6. Analysis of variance (ANOVA) for response surface quadratic model

Source	Sum of squares	Degrees of freedom	Mean square	F-value	Probability (P) > F
Model	3967.30	9	440.81	129.68	<0.0001
Lack of fit	16.11	5	3.22	0.9	0.5443
Residual	33.99	10	3.40		
Pure error	17.88	5	3.58		
Total	4001.30	19			

3. 1. Effect of Temperature and pH on Lipase Activity In enzymatic reaction, various factors such as pH and reaction temperature may affect on enzyme activity. Optimization of temperature and pH in lipolytic

reaction were investigated by central composite deign. The ranges and level of variables are shown in Table 7. The results of CCD experiments for studying the interaction effect of two independent variables on enzyme relative activity are summarized in Table 8. The correlation between lipase activity (Y_1) as a function of temperature (X_1) and pH (X_2) are represented in following regression:

$$Y = 94.12 - 11.77X_1 + 2.18X_2 + 3.19X_1X_2 - 5.95X_1^2 - 2.17X_2^2 \quad (4)$$

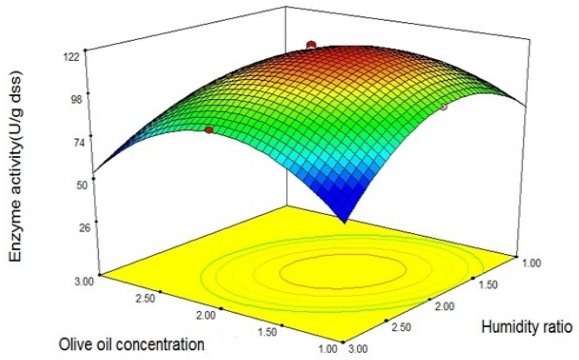

Figure 3. The effect of olive oil concentration and humidity ratio on lipase activity (other variable was set at zero level), showing the effect of olive oil concentration and humidity ratio and their interaction effects on lipase production

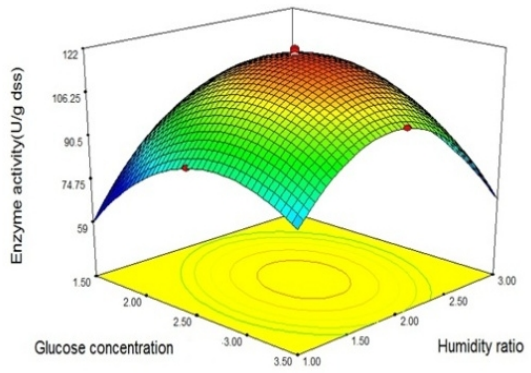

Figure 4. The effect of glucose concentration and humidity ratio on lipase activity (other variable was set at zero level), showing the effect of glucose concentration and humidity ratio and their interaction effects on lipase production.

TABLE 7. Experimental ranges and levels of the variables for optimization of enzymatic reaction

Variables	Symbol	Range and levels				
		-2	-1	0	1	2
Temperature ˚C	X_1	25	30	35	40	45
pH	X_2	6	6.5	7	7.5	8

TABLE 8. Experimental design based on CCD for optimization of enzymatic reaction

Run No.	Coded values		Relative enzyme activity	
	X_1	X_2	Actual	Predicted
1	1	1	77.534	79.60
2	-1	1	97.82	96.77
3	1	-1	66.949	68.85
4	-1	-1	100	98.78
5	-2	0	92.511	93.86
6	2	0	48.538	46.76
7	0	-2	81.213	81.08
8	0	2	90.112	89.82
9	0	0	91.827	94.12
10	0	0	93.597	94.12
11	0	0	96.361	94.12
12	0	0	92.958	94.12
13	0	0	96.732	94.12

TABLE 9. Regression analysis using CCD for optimization of enzymatic reaction

Model term	Coefficient estimate	Standard error	F-value	P-value
Intercept	94.12	0.92		
X_1	-11.77	0.64	339.64	<0.0001
X_2	2.18	0.64	11.64	0.0112
$X_1 X_2$	3.19	1.11	8.32	0.0235
X_1^2	-5.95	0.46	165.82	< 0.0001
X_2^2	-2.17	0.46	22.01	0.0022

ANOVA for response surface quadratic model

Source	Sum of squares	Degrees of freedom	Mean square	F-value	Probability (P) > F
Model	2579.16	5	515.83	105.32	<0.0001
Residual	34.28	7	4.90		
Pure error	18.57	4	4.64		
Lack of fit	15.71	3	5.24	1.13	0.4377
Total	3504.90	12			

The significance of regression coefficients are given in Table 9. The F-value (105.33) with low P-value (P<0.0001) proves the high significance of the model. The multiple correlation coefficient was 0.9869 which indicates the model accuracy. The predicted multiple correlation coefficient (0.9775) was in reasonable agreement with the adjusted multiple correlation coefficient (0.9298).

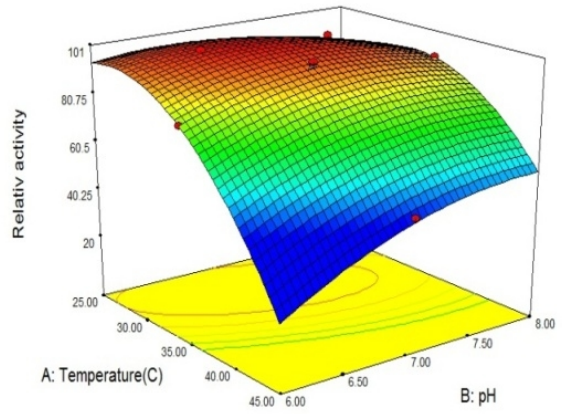

Figure 5. The effect of temperature and pH on relative lipase activity, showing the effect of temperature and pH and their interaction effects on lipase activity in enzymatic reaction

Figure 5 demonstrates the interaction between temperature and pH on the relative lipase activity on enzyme reaction, in the range of 25 to 45°C and 6 to 8, respectively. The low P-value (0.023) indicates significant interaction between variables. From Figure 5, the relative enzyme activity increased with an increase in temperature from 25 to 30.3°C, and when the temperature was greater than 30.3°C, the relative lipase activity was reduced. Pera et al. [10] reported that an optimum temperature for the lipase activity obtained from *A. niger* ATCC MYA-135 was 37°C. The maximum lipase activity for *Rhizopus oryzae* was obtained at optimum temperature, of 30°C [39].

Optimum pH for enzymatic reaction was 6.87. Generally, most of enzymes have great activities at optimum pH near neutral condition. Kamini et al. [25] have reported that the best pH for the lipase obtained from *A. niger*, strain MTCC 2594 was 7. In addition, Adham and Ahmed [40] have obtained optimum pH for the lipase from *A. niger* NRRL3, the pH value was reported to be near 7.2.

4. CONCLUSION

Lipase was produced by *A. niger* in solid state fermentation using rice bran as the solid substrate. Application of response surface methodology (RSM) under central composite design for determination of the optimal culture conditions for lipase production was successfully demonstrated. The concentrations of olive oil, glucose and humidity ratio had considerable influence on lipase activities and the optimum value of these variables were 1.78, 2.54 % (g/g_{dss}) and 1.95, respectively. Maximum lipase activity at optimum conditions predicted by RSM was 121.53 U/g and the obtained value in actual experiment was 119.74 (U/g_{dss}). Also, the highest performance of the produced lipase was obtained at 30.3ºC and pH value of 6.87.

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Lipase Production in Solid State Fermentation Using *Aspergillus niger*: Response Surface Method

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چکیده

آنزیم لیپاز در میان آنزیم‌ها کاربرد صنعتی متنوعی دارد. در این تحقیق از اسپاریجیلوس نیجر در تخمیر حالت جامد با استفاده سوبسترا (بستر) سبوس برنج برای تولید بهینه لیپاز استفاده گردید. شرایط بهینه با استفاده از نرم افزار به‌روش پاسخ سطح حاصل گردید. در آنالیز داده ها از ۲۰ آزمایش تجربی تاثیر سه متغیر شامل غلظت روغن زیتون، گلوکز و رطوبت بر فعالیت آنریم مورد بررسی قرار گرفت. اندرکنش فعالیت آنزیمی و تاثیر آن بر دو پارامتر دیگر مستقل به‌صورت نمودار سه بعدی در حالی‌که سایر فاکتورهای در سطح صفر قرار داشتند ترسیم گردید. غلظت بهینه برای روغن زیتون، گلوکز و رطوبت نسبی به ترتیب۱/۷۸ ، ٪ ((g/g_{dss}) ۲/۵٤ و ۱/۹۵ می‌باشد. تاثیر دما و pH بر فعالیت نسبی لیپاز مورد ارزیابی قرار گرفت. آنزیم لیپاز تولید شده در دمای ۳۰/۳ و ٦/۸۷ به حداکثر فعالیت خود رسید.

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