0273-2289/1559-0291 (Online)/07/141/377–390/$30.00

Screening and Optimization of Media Constituents for Enhancing Lipolytic Activity by a Soil Microorganism Using Statistically Designed Experiments

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Received September 4, 2006; Accepted October 3, 2006

Abstract

Soil contaminated with vegetable cooking oil was used in the isolation of a lipase-producing microorganism. The effectiveness of two different statistical design techniques in the screening and optimization of media constituents for enhancing the lipolytic activity of the soil microorganism was determined. The media constituents for lipase production by the isolated soil microorganism were screened using a Plackett-Burman design. Oil, magnesium sulfate, and ferrous sulfate were found to influence lipolytic activity at 24 and 72 h of culture with very high confidence levels. Whereas oil and ferrous sulfate showed a positive effect, magnesium sulfate indicated a negative effect on the lipolytic activity. A central composite design (CCD) followed by response surface methodology was used in optimizing these media constituents for enhancing the lipolytic activity. The regression model obtained for 72 h of lipolytic activity was found to be the best fit, with $R^2 = 0.97$, compared with the other model. An optimum combination at 9.3 mL/L of oil, 0.311 g/L of magnesium sulfate, and 0.007 g/L of ferrous sulfate in the media gave a maximum measured lipolytic activity of 7.1 U/mL at 72 h of culture. This increase in lipolytic activity was found to be 10.25% higher than the maximum experimentally observed value in the CCD.

Index Entries: Lipases; soil microorganism; media optimization; Plackett-Burman design; response surface methodology; central composite design.

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Introduction

Lipases are water-soluble enzymes, which catalyze the hydrolysis of ester bonds. The major substrate includes water-insoluble lipids. Owing to their enantiomeric and regioselective property, lipases are used in a large variety of industrial processes such as fat modification, purification of chiral compounds, and biofuel production *(1)*. The main sources of lipases include plants, animals, and microorganisms. Owing to their multifold properties, easy extraction procedures, and unlimited supply, microbial lipases are considered important from an industrial standpoint *(2,3)*. Among the various sources of known microbial lipases, fungal lipases are studied more than bacterial lipases because fungal lipases are easy and cheaper to extract, highly stable to thermal and pH variations, and substrate specific. The main sources of fungal lipases reported are *Aspergillus niger*, *Candida cylindracea*, *Humicola lanuginosa*, *Mucor miehei*, and *Rhizopus arrhizus*; and the major bacterial lipase producers are *Achromobacter* sp., *Alcaligenes* sp., *Arthrobacter* sp., *Pseudomonas* sp., *Staphylococcus* sp., and *Chromobacterium* sp. *(1)*.

In spite of the abundance of information available on known and well-characterized microbes responsible for lipase production, there is still an increasing interest in finding new sources of lipases for various applications. Because of the potential commercial applications of lipase, the study of lipase pro-duction by microorganisms isolated from different soil sources is an infi-nite, open, and exciting area of research *(4,5)*. Because the amount of lipase produced by microorganisms is usually reflected directly in the lipolytic activity of microbial culture *(6)*, screening and optimization of media con-stituents and fermentation conditions plays an important role in enhancing the lipolytic activity of these microbial isolates.

Conventional screening and optimization techniques involve varying factor levels while maintaining the other factors at an unspecified constant level. By these methods, the combined effect of the factors is generally neglected; moreover, they are time-consuming and require a sufficiently large number of experimental runs. These limitations of a classic method can be eliminated by screening and optimizing all the affecting factors collectively by statistical experimental design and empirical model building using regression analysis. The most commonly used technique for optimization based on statistical methods is known as response surface methodology (RSM) *(7)*. It involves the principle of randomization and replication, which also helps in understanding the effects of individual variables and their interaction on final response *(8)*. To reduce the number of factors to be used in an optimization study, screening of factors is normally performed employing a statistical design such as Plackett-Burman.

Plackett-Burman Design

Plackett-Burman design describes an efficient screening design when only the main effects of the factors are to be studied. Such designs are two-level factorial designs in which the total number of runs is a multiple of 4

(instead of 2^n in the case of full factorial design) *(8)*. These designs do not have a defining relation because interactions are not identically equal to the main effects. However, these designs are very useful for economically detecting large main effects, assuming all interactions are negligible when compared with the few important main effects. Once factors are screened for their effect on response, optimization is then carried out using RSM.

Response Surface Methodology

RSM is a collection of mathematical and statistical techniques useful for developing, improving, and optimizing processes and can be used to evaluate the relative significance of several affecting factors even in the presence of complex interactions. It is applied mostly in cases in which factor response relationship is unknown. Therefore, the first step in RSM involves a regression modeling of the system. Generally, a low-order polynomial function such as a quadratic function is chosen for modeling the system. The goodness of the fit of the quadratic polynomial is expressed in terms of coefficient of determination, R^2 value. To augment experimental data with enough points to fit into a polynomial model, a central composite design (CCD) is employed. This design also helps to reduce the number of experiments and allows extraction of more information about possible interaction effects between factors *(9)*.

To obtain the optimized values of the factors, the regression model equations are differentiated with respect to each factor, equated to zero, and the resulting equations are solved simultaneously. Recently, RSM has been effectively used in optimization of various biotechnological and industrial processes *(7,9,10,11)*. However, the use of such nonconventional statistical techniques in screening followed by optimization of media constituents for enhancing lipolytic activity by a soil microorganism has not yet been reported in the literature.

In the present study, the media constituents used for lipase production by a microorganism isolated from soil sample were screened for their significant main effects using Plackett-Burman design. RSM was then employed using CCD to optimize concentration levels of the influential media constituents for enhancing lipolytic activity by the soil microorganism.

Materials and Methods

Chemicals and Reagents

The substrate used for lipase assay, *p*-nitrophenyl acetate, was obtained from Sigma Aldrich (Germany). All the other chemicals and reagents used were of analytical grade and obtained from HiMedia (Mumbai, India). The refined sunflower oil used was of 99% purity and commercial grade.

Microorganism and Culture Conditions

Soil contaminated with vegetable cooking oil was collected near a food-preparing outlet of North Guwahati, India. A simple and rapid

method described by Ko et al. *(4)* was adopted for isolating a lipase-producing microorganism from this soil sample. For the production of lipase, the soil microorganism was grown in mineral salt medium having the following composition: 0.8 g/L of K_2HPO_4, 0.2 g/L of KH_2PO_4, 0.05 g/L of $CaSO_4 \cdot 2H_2O$, 0.5 g/L of $MgSO_4 \cdot 5H_2O$, 1 g/L of $(NH_4)_2SO_4$, and 0.01 g/L of $FeSO_4$. The mineral salt medium was supplemented with 3 mL/L of sunflower oil as the carbon source and 0.01% Tween-80 as the emulsifier. All experiments were carried out at 30°C in a 250-mL Erlenmeyer flask containing 50 mL of the oil-supplemented mineral salt medium and agitated at 180 rpm.

Plackett-Burman Design for Screening of Media Constituents

A Plackett-Burman design of eight runs with three center-point replicates was used to screen the media constituents for their effects on lipolytic activity by the soil microorganism. All the media constituents, K_2HPO_4, KH_2PO_4, $CaSO_4$, $MgSO_4$, $(NH_4)_2SO_4$, $FeSO_4$, and oil, were chosen as factors in the screening study. A minimum feasible concentration level was used for each constituent, and correspondingly a maximum concentration level was chosen in such a way that theuncoded values represented the levels –1, 0, and 1, respectively, in which 0 is ascribed tothe center-point value. Table 1 provides the range and levels of the factors used and Table 2 the various experimental combinations. Experiments were performed using 0.01% Tween-80 as the emulsifying agent. At the end of 24 and 72 h, the culture was centrifuged at 10,000*g* for 10 min, and the supernatant was subsequently assayed for lipolytic activity. The results obtained in terms of lipolytic activity at 24 and 72 h of culture were analyzed using the statistical software package Minitab 14.1.

Optimization of Media Constituents Using RSM

A CCD *(8)* containing three factors—oil, magnesium sulfate, and ferrous sulfate—was employed in the optimization study for enhancing lipolytic activity of the soil microorganism at 24 and 72 h of culture. Table 3 shows the range and levels of these three factors. Based on the quadratic regression model developed for predicting experimental lipolytic activity at 72 h of culture, RSM was employed for optimizing the media constituents.

Analytical Methods

All kinds of optical measurement were performed with a Varian Carry-50 UV-Visible spectrophotometer. A standard method *(12)* for the assay of lipases was employed using the chromogenic substrate *p*-nitrophenyl acetate *(13)*. The enzyme activity of the culture supernatant was measured with the standard lipase assay method. One milliliter of culture supernatant was incubated with 2 ÊL of 200 m*M* *p*-nitrophenyl acetate solution in dichloromethane and 1 mL of buffer containing 20 m*M* Tris-HCl (pH 8.0), 150 m*M* NaCl, and 0.01% Tween-80. After incubating the mixture for 30 min at room temperature, the enzyme activity of the culture supernatant was measured

Table 1
Experimental Range and Levels of Media Constituents Used in Plackett-Burman Design for Screening

	Range and level		
Independent variable	–1	0	+1
Oil (mL/L)	1	3	5
$MgSO_4$ (g/L)	0.3	0.5	0.7
$FeSO_4$ (g/L)	0.005	0.01	0.15
$(NH_4)_2SO_4$ (g/L)	0.5	1	1.5
$CaSO_4$ (g/L)	0.03	0.05	0.07
KH_2PO_4 (g/L)	0.1	0.2	0.3
K_2HPO_4(g/L)	0.5	0.8	–

Table 2
Plackett-Burman Design for Screening Media Constituents

Oil	$FeSO_4$	$(NH_4)_2SO_4$	$MgSO_4$	$CaSO_4$	KH_2PO_4	K_2HPO_4
+1	–1	–1	+1	–1	+1	+1
+1	+1	–1	–1	+1	–1	+1
+1	+1	+1	–1	–1	+1	–1
–1	+1	+1	+1	–1	–1	+1
+1	–1	+1	+1	+1	–1	–1
–1	+1	–1	+1	+1	+1	–1
–1	–1	+1	–1	+1	+1	+1
–1	–1	–1	–1	–1	–1	–1
0	0	0	0	0	0	0
0	0	0	0	0	0	0
0	0	0	0	0	0	0

Table 3
Experimental Range and Levels of Media Constituents Used in CCD

	Range and level				
Independent variable	–α	–1	0	+1	+α
Oil (mL/L)	0.13	1.5	3.5	5.5	6.86
$MgSO_4$ (g/L)	0.16	0.3	0.5	0.7	0.86
$FeSO_4$ (g/L)	0.0016	0.005	0.01	0.15	0.018

at 410 nm. One unit of lipolytic activity of the culture was defined as 1 nmol of *p*-nitrophenol produced/min of incubation *(14)*.

Results and Discussion

Ko et al. *(4)* showed through their method that a lipolytic microorganism can easily be detected by a clear zone around its colonies in an agar plate containing sunflower oil as the carbon source. Similarly, in our study,

a lipolytic microorganism was detected and used for lipase production. Because the main purpose of this study was to enhance the lipolytic activity of the soil microorganism by screening and optimization of media constituents employing nonconventional design techniques, identification of the microorganism was not attempted. However, a detailed identification and characterization study of the soil microorganism and the lipase produced has been recently undertaken and will be reported in a separate article.

The lipolytic activity by the microorganism was monitored at different time intervals during its growth in lipase production medium, and a maximum variation in the lipolytic activity was observed at 24 and 72 h of culture.

Screening of Media Constituents Using Plackett-Burman Design

All seven media constituents (K_2HPO_4, KH_2PO_4, $CaSO_4$, $MgSO_4$, $[NH_4]_2SO_4$, $FeSO_4$, and oil) were studied for their effect on lipolytic activity by the microorganism. Experiments were performed as per combinations of the factors shown in Table 2. The results in terms of lipolytic activity at 24 and 72 h of culture were analyzed using a statistical software package. Of the factors studied, oil, magnesium sulfate, and ferrous sulfate showed significant main effects on lipolytic activity at both time points of culture. The main effect of oil was found to be large and positive compared with that of the other two factors. Figure 1 shows the effects of all seven factors on lipolytic activity at 72 h of culture. Whereas magnesium sulfate had a negative effect on lipolytic activity, ferrous sulfate showed a slightly positive effect. All the other factors had no significant effect on the response.

The results were analyzed in the form of analysis of variance (ANOVA), which is a statistical technique that subdivides the total variation in a set of data into component parts associated with specific sources of variation for the purpose of testing hypotheses on the parameters of the model *(8)*. Table 4 presents the ANOVA for lipolytic activity at 24 and 72 h of culture.

The mean sum of squares (MS) of the model term is obtained from the ratio of sum of squares (SS) and degrees of freedom (df). The Fisher's *F* value is calculated by dividing the MS owing to the model by the MS owing to error. ANOVA indicates that the main effects of the factors in the model term were highly significant ($p < 0.02$). Table 4 also shows a term for error, the MS value, which indicates that the amount of variation in the response data that is left unexplained by the model is low.

To assess the significance of each individual factor on the lipolytic activity, a student's *t*-test was performed and the results are given in Table 5. Generally a large *t* value and lesser *p* value indicate a high significance of the corresponding model term. In this screening study, oil was found to have a maximum effect on lipolytic activity at both time points of culture, followed by magnesium sulfate. Oil has also been shown to be an important factor in lipase production of other microorganisms such as *Candida rugosa (14)* and *Rhizopus oligosporous (15)*. Ferrous sulfate was found to have a slightly significant effect on the lipolytic activity at 24 h of culture

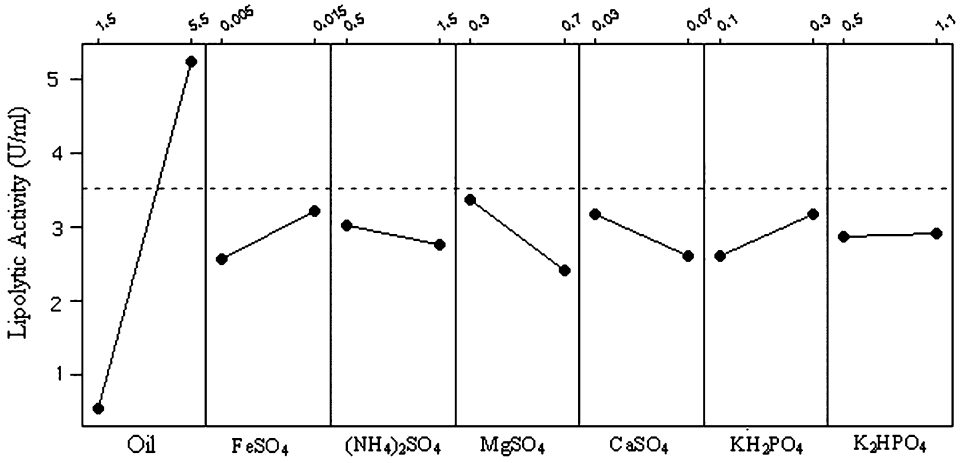

Fig. 1. Main effects plot of all factors on lipolytic activity at 72 h of culture.

Table 4
ANOVA for Lipolytic Activity Obtained in Screening Study[a]

	At 24 h of culture					At 24 h of culture				
Source	df	SS	MS	*F*	*p*	df	SS	MS	*F*	*p*
Main effects	7	1617.72	231.11	53.2	0.019	7	1755.35	250.76	58.82	0.017
Error	2	8.68	4.34			2	8.53	4.26		

[a]df, degrees of freedom; SS, sum of squares; MS, mean sum of squares.

($p = 0.069$), but at 72 h of culture, the value ($p = 0.121$) was slightly higher. All the other factors did not show any significant effect on lipolytic activity. Of the three significant factors, whereas magnesium sulfate showed a nega-tive effect on lipolytic activity, the other two factors (oil and ferrous sulfate) had a positive effect. These findings of the main effects of factors on lipoly-tic activity were also in agreement with those of the main effects plot dis-cussed earlier.

Based on the student's *t*-test and ANOVA, the three factors oil, magnesium sulfate, and ferrous sulfate were chosen for further optimization study.

Optimization of Media Constituents Using RSM

Using the experimental results of CCD, regression model equations were developed for predicting the lipolytic activity at 24 and 72 h of culture and are given in Table 6. These equations depict a quadratic relationship between lipolytic activity, $f(X)$, and the media constituents oil (X_1),

Table 5
Student's *t*-Test for Lipolytic Activity Obtained Using Plackett-Burman Design for Screening

	At 24 h of culture		At 24 h of culture	
Term	*t*	*p*	*t*	*p*
Constant	21.04	0.002	22.45	0.002
Oil	18.14	0.003	19.43	0.003
$MgSO_4$	–4.56	0.045	–3.98	0.058
$FeSO_4$	3.62	0.069	2.61	0.121
$(NH_4)_2SO_4$	–2.65	0.118	–1.04	0.407
$CaSO_4$	–1.21	0.349	–2.33	0.146
KH_2PO_4	–0.04	0.975	2.27	0.152
K_2HPO_4	1.12	0.379	0.27	0.814

Table 6
Quadratic Regression Model Equations Obtained for Lipolytic Activity at 24 and 72 h of Culture in Optimization Study

	Regression model equation	R^2
Lipolytic activity (24 h)	$f(X) = 0.17X_1^2 + 0.14X_2^2 + 0.18X_3^2 - 0.29X_1X_2 - 0.26X_1X_3 + 0.12X_2X_3 - 0.0007X_1 + 0.08X_2 - 0.31X_3 + 0.519$	0.79
Lipolytic activity (72 h)	$f(X) = -0.22X_1^2 - 0.36X_2^2 - 0.68X_3^2 - 0.13X_1X_2 + 0.03X_1X_3 + 0.21X_2X_3 + 1.29X_1 - 0.16X_2 - 0.76X_3 + 4.65$	0.97

magnesium sulfate (X_2), and ferrous sulfate (X_3). Kaushik et al. *(6)* also found that quadratic regression models adequately predicted measured lipolytic activity by *Aspergillus carneus*.

Table 7 shows the CCD along with experimental and model predicted values of lipolytic activity at 24 and 72 h of culture. It is quite evident that whereas the predicted values of lipolytic activity at 72 h of culture closely match the experimentally measured values, those at 24 h of culture show some deviation.

Statistical analysis of the results in the form of ANOVA was done as before. Table 8 presents ANOVA for the lipolytic activity at 24 and 72 h of culture. The Fisher's *F* values of both models owing to regression were found to be high. The large *F* value indicates that most of the variation in the response can be explained by the regression model equation. The associated *p* value is used to judge whether *F* is large enough to indicate statistical significance or not. A *p* value <0.05 generally suggests that the model is considered to be statistically significant at >95% confidence level *(8)*.

Table 7
CCD Showing Experimental and Regression Model Predicted Lipolytic Activity at 24 and 72 h of Culture

			At 24 h (U/mL)		At 72 h (U/mL)	
Oil (mL/L)	$MgSO_4$ (g/L)	$FeSO_4$ (g/L)	Measured	Predicted	Measured	Predicted
1.5	0.3	0.005	1.54	1.32	3.51	3.13
5.5	0.3	0.005	1.39	1.38	6.07	5.90
1.5	0.7	0.005	2.21	1.85	2.70	2.64
5.5	0.7	0.005	0.98	0.73	4.62	4.88
1.5	0.3	0.015	0.03	–0.07	1.47	1.10
5.5	0.3	0.015	1.04	1.04	4.07	4.02
1.5	0.7	0.015	1.29	0.93	1.40	1.46
5.5	0.7	0.015	1.09	0.86	3.59	3.86
0.13	0.5	0.01	0.61	1.00	1.45	1.84
6.86	0.5	0.01	0.89	0.99	6.44	6.19
3.5	0.16	0.01	0.79	0.75	3.37	3.88
3.5	0.83	0.01	0.52	1.05	3.71	3.33
3.5	0.5	0.0016	1.3	1.56	3.86	4.00
3.5	0.5	0.018	0.27	0.50	1.45	1.44
3.5	0.5	0.01	0.54	0.51	4.64	4.65
3.5	0.5	0.01	0.51	0.51	4.71	4.65
3.5	0.5	0.01	0.58	0.51	4.67	4.65
3.5	0.5	0.01	0.47	0.51	4.62	4.65
3.5	0.5	0.01	0.54	0.51	4.64	4.65
3.5	0.5	0.01	0.56	0.51	4.66	4.65

Table 8
ANOVA for Lipolytic Activity Obtained in Optimization Study[a]

	At 24 h of culture					At 72 h of culture				
Source	df	SS	MS	*F*	*p*	df	SS	MS	*F*	*p*
Regression	9	3.81	0.42	4.34	0.016	9	39.92	4.43	39.93	0.001
Linear	3	1.46	0.48	5.00	0.023	3	31.16	10.38	93.50	0.001
Square	3	0.97	0.32	3.33	0.065	3	8.24	2.74	24.75	0.001
Interaction	3	1.37	0.45	4.68	0.027	3	0.51	0.17	1.55	0.263
Error	10	0.97	0.09			10	1.11	0.11		
Total	19	4.79				19	41.03			

[a]df, degrees of freedom; SS, sum of squares; MS, mean sum of squares.

The linear and square terms of the regression model for lipolytic activity at 72 h of culture were found to be highly significant ($p < 0.01$) compared with those of the other model. However, the interaction terms in the regression model for lipolytic activity at 24 h were found to be significant whereas they were not at all significant at 72 h.

Overall, the regression model for lipolytic activity at 72 h was highly significant ($p = 0.001$) compared with the model for lipolytic activity at 24 h ($p = 0.016$). This finding indicates that the second-order polynomial model for lipolytic activity at 72 h was adequate in representing the actual relationship between the response (lipolytic activity) and the variables compared with the other model. This is also reflected in its high value of the coefficient of regression ($R^2 = 0.97$), implying that the model did not explain only about 3% of the sample variation.

To determine the significance of the regression coefficient of the factors, the results were subjected to a student's *t*-test; Table 9 gives the results. The regression coefficients for the linear terms oil (X_1)and $FeSO_4$ (X_3) in the model for lipolytic activity at 72 h were found to be highly significant ($p < 0.002$). However, the *p* value for the term of $MgSO_4$ (X_2) was higher ($p = 0.1$). The significance of all the coefficients of the quadratic terms in the model was found to be higher ($p < 0.05$). As observed earlier in ANOVA for lipolytic activity at 72 h, the regression coefficient terms for interaction between any two factors were not found to have a considerable effect ($p > 0.1$) on the response. With respect to the regression coefficients for lipolytic activity at 24 h, the linear terms for oil and magnesium sulfate were not at all significant ($p > 0.3$) but the term for ferrous sulfate was highly significant ($p = 0.004$). However, the coefficients of quadratic effect of oil and magnesium sulfate showed considerable effect, with *p* values of 0.06 and 0.05, respectively. Among the interaction effects in the model for lipolytic activity at 24 h, the coefficient terms between oil and magnesium sulfate and those between oil and ferrous sulfate also showed high significance ($p < 0.03$). Such an observation in significance of interaction effects between the variables would have been lost, however, if the experiments had been carried out by conventional methods. Ravikumar et al. *(9)* observed similar interaction effects between two independent variables using RSM.

Considering the ANOVA and student's *t*-test, we chose the model for predicting lipolytic activity at 72 h of culture to optimize the media constituents. The model equation for 72 h of lipolytic activity, shown in Table 6, was optimized by partially differentiating the equation and then equating it to zero. Corresponding local maxima were further checked by second-order sufficient condition using a Hessian matrix. The optimized values of the media constituents are given in Table 10. Their combination gave a maximum predicted lipolytic activity of 7.002 U/mL at 72 h of culture.

Figures 2–4 show the response surface contour plots to estimate lipolytic activity at 72 h of culture with respect to the independent variables oil, magnesium sulfate, and ferrous sulfate. These contour plots also show the relative effects of any two variables when the concentration of the remaining variable is held constant at its center-point value.

Table 9
Student's *t*-Test for Lipolytic Activity Obtained in Optimization Study

	At 24 h of culture		At 72 h of culture	
Term	*t*	*p*	*t*	*p*
Constant (*C*)	4.069	0.002	34.37	0.001
Oil (X_1)	–0.008	0.994	14.4	0.002
$MgSO_4$ (X_2)	1.043	0.321	–1.81	0.100
$FeSO_4$ (X_3)	–3.731	0.004	–8.48	0.001
X_1^2	2.065	0.066	–2.56	0.028
X_2^2	1.657	0.128	–4.21	0.002
X_3^2	2.215	0.051	–7.79	0.001
$X_1 * X_2$	–2.691	0.023	–1.12	0.287
$X_1 \times X_3$	2.374	0.039	0.31	0.757
$X_2 \times X_3$	1.085	0.303	1.81	0.102

Table 10
RSM-Optimized Values of Media Constituents for Maximum Lipolytic Activity at 72 h of Culture

	Optimal value	
Media constituent	Coded value	Uncoded value
Oil	3.15	9.3 mL/L
$MgSO_4$	–0.944	0.311 g/L
$FeSO_4$	–0.6118	0.007 g/L

From the nature of the response surface contours, whether elliptical, circular, or saddle point, interaction between the variables may be predicted. The response surface contour plots of mutual interaction between the variables $MgSO_4$ and oil and between $MgSO_4$ and $FeSO_4$ shown in Figs. 2 and 4, respectively, were found to be elliptical and, hence, indicate more significant interactions. However, Fig. 3 shows a circular nature of the response surface contours between the variables oil and $FeSO_4$, indicating lesser significance of their interaction. These findings of interaction effects between the variables were also found to be consistent with the student's *t*-test of regression coefficients for lipolytic activity at 72 h. Figure 5 shows a corresponding three-dimensional (3D) surface contour plot between $MgSO_4$ and $FeSO_4$ on lipolytic activity. Ravikumar et al. *(9)* also studied such interac-tion effects between independent variables using contour plots on dye removal by a novel substrate. From the response surface contour plots, the maximum predicted lipolytic activity can also be obtained from the surface confined in the smallest curve of the contour diagram. The corre-sponding coordinates in the region of the contour diagram gave the opti-mum values of the respective factors.

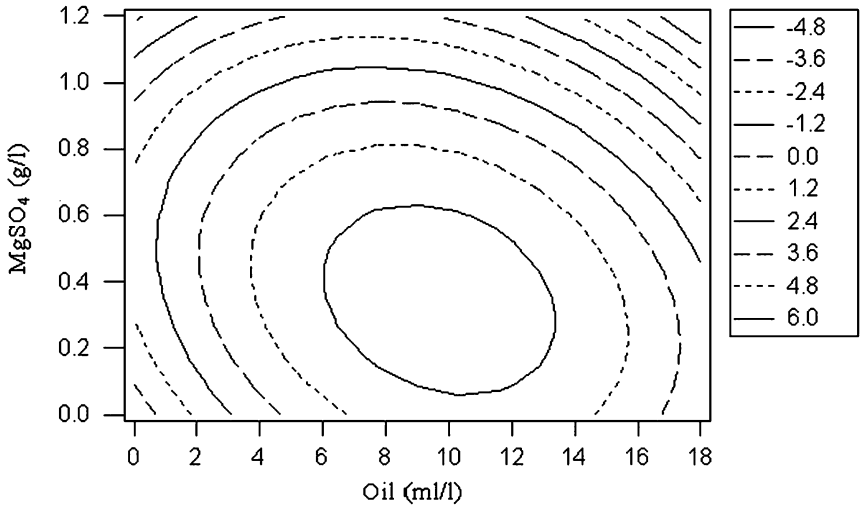

Fig. 2. Response surface contour plot showing relationship between oil and magnesium sulfate on lipolytic activity at 72 h of culture.

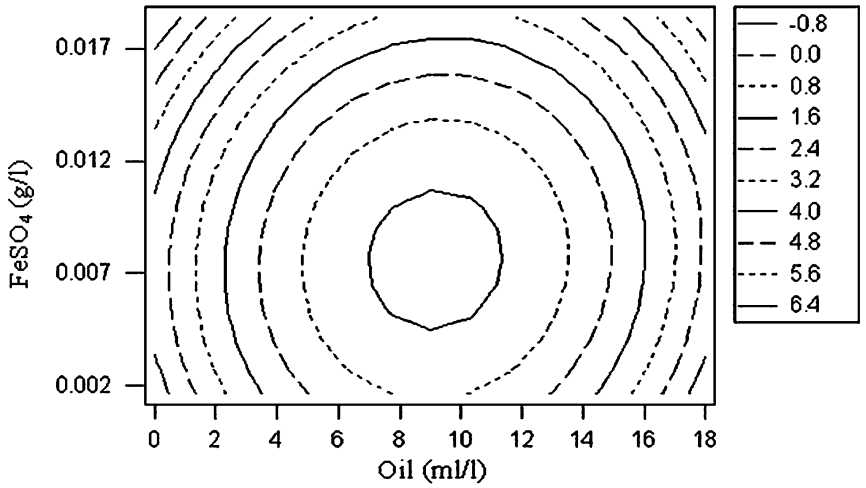

Fig. 3. Response surface contour plot showing relationship between oil and ferrous sulfate on lipolytic activity at 72 h of culture.

The optimum values of the media constituents drawn from the contour diagrams in Figs. 2–4 were in close agreement with those obtained by optimizing the regression model equation for lipolytic activity at 72 h of culture. These optimum values were experimentally verified under batch shake-flask condition, and the corresponding maximum lipolytic activity was found to be 7.1 U/mL, confirming the predicted lipolytic activity value. This value was found to be 10.25% higher than the maximum measured lipolytic activity observed in the CCD of experiments shown in Table 7.

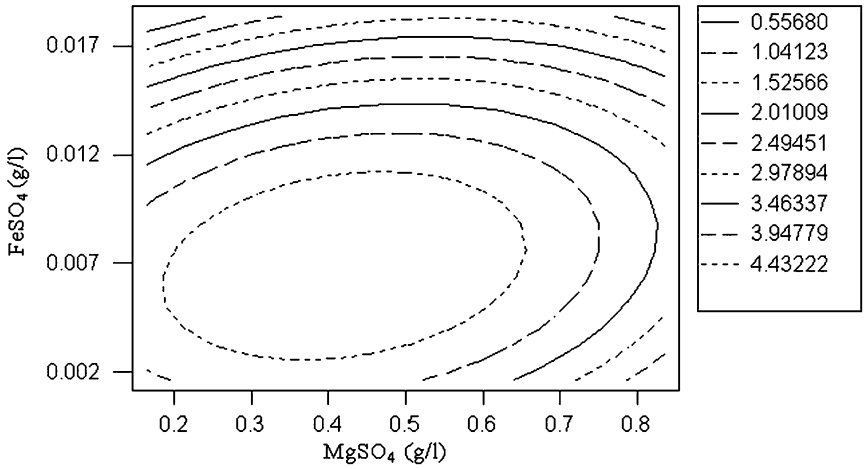

Fig. 4. Response surface contour plot showing relationship between ferrous sulfate and magnesium sulfate on lipolytic activity at 72 h of culture.

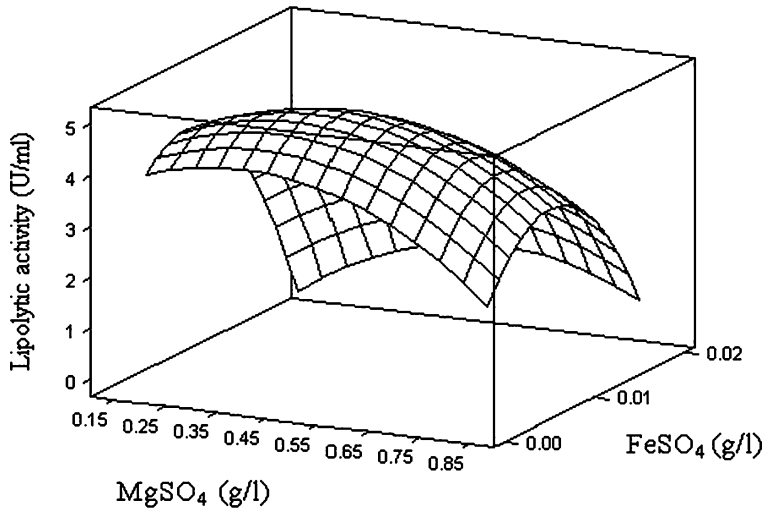

Fig. 5. 3D response surface contour plot showing relationship between ferrous sulfate and magnesium sulfate on lipolytic activity at 72 h of culture.

The results of the study clearly indicate the effectiveness of nonconventional, statistically based design techniques in screening and optimizing media constituents for enhancing the lipolytic activity of the soil microorganism. However, further research on the characterization of the soil microorganism and the lipase produced, which is under way, will be necessary to improve its applicability in bioprocesses.

Conclusion

A microorganism isolated from soil contaminated with vegetable cooking oil was tested for lipase production. Based on Plackett-Burman design for screening media constituents, oil, magnesium sulfate, and ferrous sulfate were found to be the most influential factors affecting lipolytic activity by the soil microorganism at 24 and 72 h of culture. An optimum combination of oil, magnesium sulfate, and ferrous sulfate at the respective levels of 9.3 mL/L, 0.311 g/L, and 0.007 g/L in the media, obtained by employing an RSM optimization technique, resulted in 10.25% enhancement of lipolytic activity by the soil microorganism at 72 h of culture. The use of nonconventional, statistically based design techniques allowed good interpretation of the results obtained and, therefore, proved useful for enhancing the lipolytic activity of the soil microorganism.

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