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Statistical optimization of Lipase yield using *Aspergillus sydowii* BTSS 1005 a novel isolate identified from a marine sediment at East Coast of Visakhapatnam

Panchagnula Bindiya
Department of Biotechnology, College of Science and Technology, Andhra University, Visakhapatnam-530003, Andhra Pradesh, India.

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ABSTRACT

Lipase, a versatile biocatalyst, has many biotechnological applications. A lipolytic fungal strain BTSS 1005 was isolated from a marine sediment in the eastern coast of Visakhapatnam, after sequencing studies of my previous work, it was identified as a novel strain of Aspergillus sydowii. It was found to produce a thermostable lipase by submerged fermentation during my research and hence was selected for bioprocess development. In the present work, media composition was optimized for the enhancement of lipase activity using statistical techniques. The major ingredients were chosen through Plackett-Burman experimental design and optimum ingredient level was investigated through Central Composite Design, of Response Surface Methodology, for best lipase yield. The activity of lipase produced by submerged fermentation studies, initially determined as 28.64 U/ml before optimization, was predicted 63.94 U/ml by using RSM. This study was useful for paving way for usage of different statistical models in optimization of cultural parameters in industrial productions of bioactive compounds.

Corresponding Author:

Dr. Panchagnula Bindiya
Department of Biotechnology, College of Science and Technology
Andhra University, Visakhapatnam-530003
Andhra Pradesh, India.
Email: bindiya.au@gmail.com

1. INTRODUCTION

Lipases (EC 3.1.1.3, triacylglycerol acyl hydrolases) are multi-purpose biocatalysts that catalyze hydrolysis of triglycerides to glycerol and free fatty acids, as well as synthetic reactions like esterification, transesterification and interesterification in a non-aqueous environment. Their selective mediation of highly productive transformations makes them significant in pharmaceutical, detergent, food and biodiesel industries [1, 2, 3]. Microbial lipases, especially fungal lipases, are favored in industry because they are secreted extracellularly, are stable and have broad substrate specificity. A marine fungal isolate *Aspergillus sydowii* BTSS 1005 was explored for lipase production through submerged fermentation in the present study. Lipase production was enhanced by optimizing the media composition and culturing conditions [4]. The marine fungi have the capacity to tolerate extreme environmental conditions and hence are stable in nature. *Aspergillus sydowii* strain BTSS 1005, which was isolated from a marine sediment was found promising for extracellular lipase synthesis [5]. Nevertheless, the enzyme yields are very sensitive to the nutritional content of the medium, which differs among species and even among strains [6].

Therefore, the optimization of the fermentation medium was critical to achieve maximum lipase productivity. Classical one-variable-at-a-time (OVAT) optimization approaches are time-consuming, particularly if multiple variables have a simultaneous effect on the outcome. Different techniques of statistics like Plackett–Burman design (PBD) and Response Surface Methodology (RSM), enable effective screening and modeling of intricate interactions between media components and process factors [7]. Central Composite Design (CCD), a type of RSM, has been extensively used for identifying optimal levels of contributing factors with minimal experimental runs.

The ideal components of the media vary between different species and even within the strains of some species for any bioactive compound's commercial production, hence the composition of the production medium must be optimized. Industrial lipase production using the isolated fungi by submerged cultivation for maximum yield improvement was the objective of this study. Optimization of media components using traditional methods was time consuming, laborious and costly [8,9,10,11]. Response surface methodology (RSM) remains effective for creating models for problem analysis, and can be applied for optimization of different operating conditions for large scale industrial productions. Statistical optimization methodologies like Plackett–Burman design and Response Surface Methodology (RSM) allow effective determination of relevant factors and optimization of their levels by conducting fewer experiments thereby reducing time of research. [10, 11, 12, 13]. *Aspergillus sydowii*, a filamentous marine fungus, well-known for its robust metabolic capacity and environmental adaptability produces a wide variety of industrially useful enzymes like cellulases, lipases, proteases and xylanases that are highly stable across a broad pH and temperature ranges. Enzymes from *Aspergillus sydowii* are highly valued in biocatalysis, food processing, detergents, pharmaceuticals and bioremediation due to their efficacy, adaptability as substrates and environmental friendliness. *Aspergillus sydowii* BTSS 1005 was studied for lipase extraction as the lipase produced from this novel strain was found to be highly stable in my previous studies. My objective was to enhance lipase yield for commercial industrial production and to reduce the production cost and market the lipase enzyme.

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2. METHOD

2.1. For preparation of inoculum

Aspergillus sydowii BTSS 1005 inoculum was prepared in the form of spore suspension at 2.6 X 10^7 spores / ml from potato dextrose agar medium, a petri dish culture, stored at 28 °C for 7 days. Inoculum level used was 10.0 % (v/v) to induce growth and was incubated for 5 days at 28 °C and 100 rpm on rotary shaker. This inoculum was used for production of lipase using shake flask experiments for optimization studies.

2.2. Shake Flask experiment

Submerged fermentation was conducted with medium composition comprising sucrose at 2 % (w/v), ammonium chloride at 3.5% (w/v), olive oil at 3% (v/v) and Tween 80 at 0.2% (v/v). Incubation temperature of 32 °C, initial pH of 8.0, agitation at 80 rpm, incubation period of 96 h and 10 % (v/v) of inoculum was employed for maximum lipase production by *Aspergillus sydowii* strain BTSS 1005. The optimization experiments were carried out in 250 ml Erlenmeyer flasks containing 45 ml of culture medium. The samples were withdrawn at regular intervals to estimate extracellular lipase activity for 24 h periods till 120 h. Lipase production were investigated by employing all the optimized parameters as per the previous study. All experiments were done in triplicate and average of readings was used for further analysis.

2.3. Lipase activity assay

The activity of lipase was assayed by titrimetric estimations using olive oil as substrate by emulsion method [14]. The units of enzyme activity were considered as the quantity of enzyme that releases 1µmole equivalent fatty acid / ml / min at 30 °C for fixed experimental conditions as one unit. The experiments were conducted thrice and average was reported. Standardization experiments were done with uninoculated medium samples and estimation experiments were repeated the same way as done for test samples.

2.4. Experimental design and statistical analyses

2.4.1. Preliminary experiments

Plackett-Burman design of experiments was used for screening of the medium components. Medium components investigated were sucrose, ammonium chloride, olive oil, Tween 80 and cultural parameters selected were temperature, pH, agitation, time of incubation and volume of inoculum. For carrying out the Plackett-Burman design of experiments, the influence of different concentrations of sucrose, ammonium chloride, olive oil and Tween 80 were investigated at different levels. The results were tabulated and graphs were plotted to choose the minimum and maximum levels of Plackett-Burman design of experiments. The minimum and maximum level were chosen from the results obtained from the shake flask experiments that are shown in Table 1. The experimental variables chosen was shown in Tables 2, 3 shows the range of factors investigated in the P-B design. The Plackett-Burman design matrix was formulated from the chosen levels of the factors. The Plackett–Burman design (PBD) was used for exploring and assessing the impact of multiple factors, and has been applied extensively for many optimization studies [15]-[18]. The variables derived in the initial experiments were analyzed by PBD to detect the variables which greatly impacted the lipase productions. Every independent variable was placed at two levels, -1 (low level) and +1 (high level) [18] They are chosen according to the findings from initial experiments from my previously published research work [5].

2.4.2. Central Composite Design (CCD)

This widely utilized class of design finds much use for best fitting. Typically, the CCD is a 2^k factorial with n_f runs, 2k axial or star runs, and n_C runs at center. It has cube points at unit cube corners star points on axis or off, center at origin. Figure 1, Table 5 shows the central composite design for refining and optimizing parameters selected by PBD.

2.4.3. The Box-Behnken Design

Box and Behnken [19] suggested a few three-level designs for the fitting of response surfaces. Figure 1 depicts Box-Behnken design utilizing three factors. Box–Behnken design (BBD), both spherical and rotational, a major response surface methodology earlier employed in the various optimization studies on fungi

was used in this present study [20,21]. Contour plots for response surfaces were developed to show the accuracy and for better understanding of the interactions.

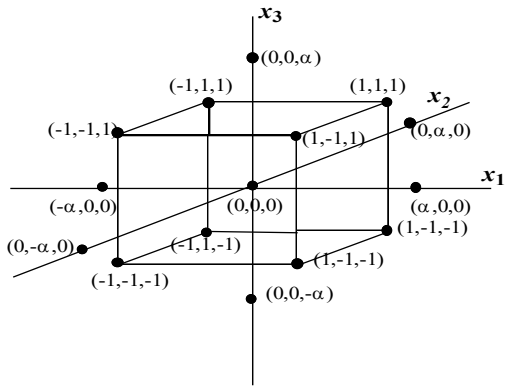
2.4.4. Analysis of Variance table

The Analysis of variance Table, DF = Degree of freedom, 1 represents the degree of freedom of two concentrations (2-1=1). The 8 represent the degree of freedom of total number of replicates (9-1=8). The degree of freedom of residuals or error is obtained by the difference of the total replicates with the four concentrations.

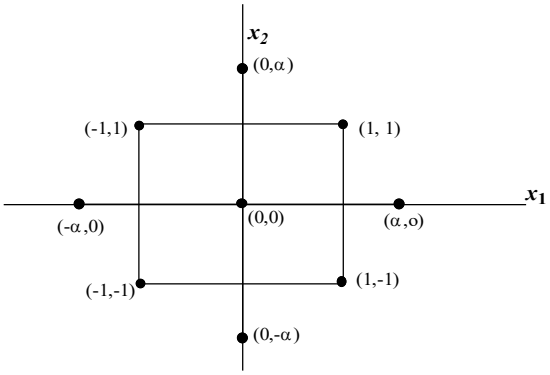
The findings were also presented in p level of significance based on ANOVA table and pareto chart of standardized effects. Table 4, Figure 2 Blue color bar indicates the parameters' significance based on the P value level of significance in the ANOVA table. The red color line indicates the p-level of significance. The parameters with highest significance will cross the red color p-level line. The graph clearly indicates that temperature and Tween 80 were traversing more of the p-level of significance line. R2 value is nearer to one, (0.99 and 0.89396 respectively), validating the accuracy of the experimental results. (Table 4,7 and 8) Figure 3 shows the observed and predicted values for lipase production.

2.4.5. Treatment of data

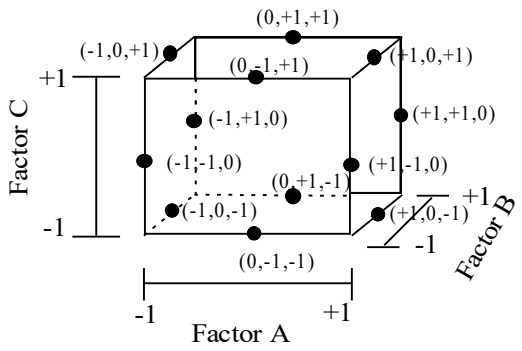
The triplicate culture flasks were represented for each treatment. STATISTICA 7.0 software (StatSoft Inc., USA) was used for analyzing data and to generate the plots.



Runs	A	B
1	0	0
2	1	1
3	-1	1
4	1	-1
5	-1	-1
6	0	α
7	0	-α
8	-α	0
9	α	0
10	0	0



(a) CCD for k = 2, factors x_1 and x_2 is given by



(b) The Central Composite Design for k=3, factors x_1, x_2 and x_3 is given by

Figure 1. Central composite design (3 factors) and Box-Behnken design (three factors)

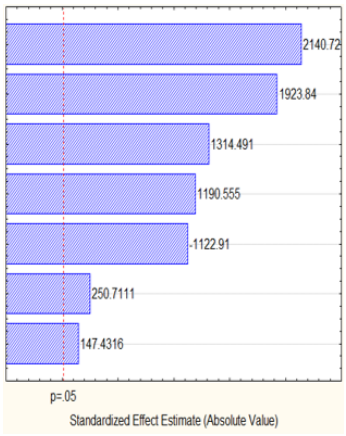


Figure 2. Pereto Chart of standardized effects, showing the most significant value in the production of lipase using *Aspergillus sydowii* BTSS 1005.Top to down: Temperature (°C), Tween 80 (v/v), Olive oil (v/v), Agitation (rpm), pH, Ammonium chloride(w/v), Sucrose(w/v).

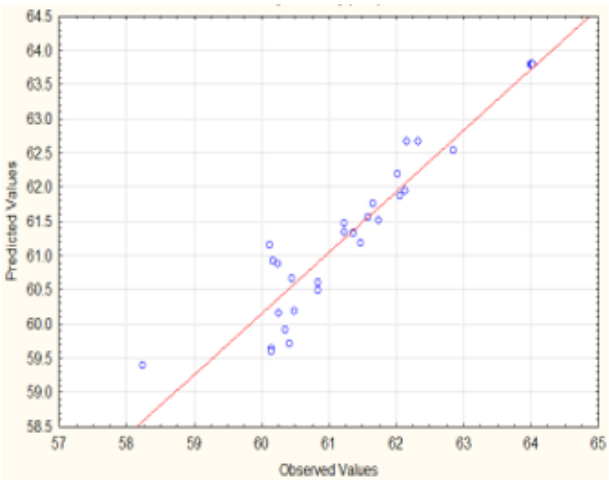


Figure 3. Observed vs predicted values for lipase production using *Aspergillus sydowii* BTSS 1005

Table 1. Independent variables with their experimental ranges and levels: (Lipase activity U/ml)

Parameter	-1	0	+1
Sucrose	46.62	48.618	43.956
Ammonium chloride	53.28	54.612	51.948
Olive oil	58.608	60.606	57.942
Tween 80	60.606	62.604	60.606
Temperature	62.604	63.936	59.94
pH	62.604	63.936	61.938
Agitation	61.938	63.936	62.604
Time of incubation	63.27	63.936	62.604
Volume of inoculum	62.604	63.936	55.944

Table 2: Experimental variables

Sucrose (w/v)	Ammonium chloride (w/v)	Olive oil (v/v)	Tween 80 (v/v)	Temperature (°C)	pH	Agitation (rpm)
1.50%	3%	2.50%	0.1%	30	7.5	70
2%	3.5%	3%	0.2%	32	8	80
2.50%	4%	3.5%	0.3%	34	8.5	90

Table 3. Plackett - Burman Design of experiments: Design Matrix

S. No	Sucrose (w/v)	Ammonium chloride (w/v)	Olive oil (v/v)	Tween 80 (v/v)	Temperature (°C)	pH	Agitation (rpm)	Lipase Activity (U/ml)
1	1.00	2.50	2.00	0.20	32.00	8.00	60.00	62.53
2	2.00	2.50	2.00	0.10	28.00	8.00	80.00	61.21
3	1.00	3.50	2.00	0.10	32.00	7.00	80.00	62.84
4	2.00	3.50	2.00	0.20	28.00	7.00	60.00	62.23
5	1.00	2.50	3.00	0.20	28.00	7.00	80.00	63.25
6	2.00	2.50	3.00	0.10	32.00	7.00	60.00	62.85
7	1.00	3.50	3.00	0.10	28.00	8.00	60.00	61.32
8	2.00	3.50	3.00	0.20	32.00	8.00	80.00	63.936
9	2.00	3.50	3.00	0.20	32.00	8.00	80.00	63.935

Table 4. ANOVA showing medium components and process variables: R-square = 0.99; Adj:1. 7 Factor Screening Design; MS Residual=.0000005 DV: Enzyme Activity (U/ml)

Parameter	SS	df	MS	F	p
(1) Sucrose (w/v)	0.010868	1	0.010868	21736	0.004318
(2) Ammonium chloride (w/v)	0.031428	1	0.031428	62856	0.002539
(3) Olive oil (v/v)	0.863943	1	0.863943	1727885	0.000484
(4) Tween 80 (v/v)	1.850580	1	1.850580	3701160	0.000331
(5) Temperature (°C)	2.291356	1	2.291356	4582712	0.000297
(6) pH	0.630460	1	0.630460	1260920	0.000567
(7) Agitation (rpm)	0.708711	1	0.708711	1417421	0.000535
Error	0.000001	1	0.000001		
Total SS	7.767421	8			

Table 5. Central Composite Design for Refining and Optimizing Parameters Selected by PBD

S.No.	Temperature (°C)	pH	Agitation (rpm)	Tween 80 (v/v)	Olive Oil (v/v)	Enzyme Activity (U/ml)
1	30.00	7.50	70.00	0.10	3.50	60.412
2	30.00	7.50	70.00	0.30	2.50	60.845
3	30.00	7.50	90.00	0.10	2.50	60.145
4	30.00	7.50	90.00	0.30	3.50	60.845
5	30.00	8.50	70.00	0.10	2.50	60.147
6	30.00	8.50	70.00	0.30	3.50	60.485
7	30.00	8.50	90.00	0.10	3.50	60.356
8	30.00	8.50	90.00	0.30	2.50	60.258
9	34.00	7.50	70.00	0.10	2.50	61.48
10	34.00	7.50	70.00	0.30	3.50	61.365
11	34.00	7.50	90.00	0.10	3.50	62.056
12	34.00	7.50	90.00	0.30	2.50	62.025
13	34.00	8.50	70.00	0.10	3.50	61.74
14	34.00	8.50	70.00	0.30	2.50	61.653
15	34.00	8.50	90.00	0.10	2.50	61.584
16	34.00	8.50	90.00	0.30	3.50	61.235
17	28.00	8.00	80.00	0.20	3.00	58.24
18	36.00	8.00	80.00	0.20	3.00	62.85
19	32.00	7.00	80.00	0.20	3.00	60.241
20	32.00	9.00	80.00	0.20	3.00	60.45
21	32.00	8.00	60.00	0.20	3.00	60.175
22	32.00	8.00	100.00	0.20	3.00	61.235
23	32.00	8.00	80.00	0.00	3.00	60.122
24	32.00	8.00	80.00	0.40	3.00	62.125
25	32.00	8.00	80.00	0.20	2.00	62.325
26	32.00	8.00	80.00	0.20	4.00	62.158
27	32.00	8.00	80.00	0.20	3.00	63.986
28	32.00	8.00	80.00	0.20	3.00	64.023
29	32.00	8.00	80.00	0.20	3.00	64.014
30	32.00	8.00	80.00	0.20	3.00	64.025

Table 6. Critical Values for lipase production by *Aspergillus sydowii*

Parameters	Critical
Temperature (°C)	33.10603
pH	7.97903
Agitation (rpm)	81.01217
Tween 80 (v/v)	0.21283
Olive Oil (v/v)	2.95749

Table 7. Estimated Regression Coefficients. R-square=.89396; Adj:.65832, 5 factors, 1 Block, 30 Runs; MS Residual=.6570678 DV: Enzyme Activity (U/ml)

Parameter	Regression	Constants
Mean/Intercept	-391.612	
(1) Temperature (°C) (L)	11.632	X_1
Temperature (°C) (Q)	-0.177	X_1^2
(2) pH (L)	49.576	X_2
pH (Q)	-3.025	X_2^2
(3) Agitation (rpm) (L)	1.120	X_3
Agitation (rpm) (Q)	-0.007	X_3^2
(4) Tween 80 (v/v) (L)	65.302	X_4
Tween 80 (v/v) (Q)	-56.166	X_4^2
(5) Olive Oil (v/v) (L)	8.802	X_5
Olive Oil (v/v) (Q)	-1.129	X_5^2
1L by 2L	0.018	X_1X_2
1L by 3L	0.003	X_1X_3
1L by 4L	-0.611	X_1X_4
1L by 5L	-0.066	X_1X_5
2L by 3L	-0.020	X_2X_3
2L by 4L	-1.479	X_2X_4
2L by 5L	-0.002	X_2X_5
3L by 4L	-0.022	X_3X_4
3L by 5L	0.008	X_3X_5
4L by 5L	-2.574	X_4X_5

Table 8. ANOVA showing critical media components: R-square=.89396; Adj:.65832, 5 factors, 1 Blocks, 30 Runs; MS Residual=.6570678 DV: Enzyme Activity (U/ml)

Parameter	SS	df	MS	F	p
(1) Temperature (°C) (L)	14.82868	1	14.82868	22.56796	0.001043
Temperature (°C) (Q)	13.30245	1	13.30245	20.24518	0.001490
(2) pH (L)	0.07009	1	0.07009	0.10667	0.751430
pH (Q)	15.24751	1	15.24751	23.20539	0.000951
(3) Agitation (rpm)(L)	0.25979	1	0.25979	0.39538	0.545111
Agitation (rpm)(Q)	11.83837	1	11.83837	18.01698	0.002159
(4) Tween 80 (v/v) (L)	0.95880	1	0.95880	1.45921	0.257839
Tween 80 (v/v) (Q)	8.41239	1	8.41239	12.80293	0.005948
(5) Olive Oil (v/v) (L)	0.00002	1	0.00002	0.00003	0.995505
Olive Oil (v/v) (Q)	2.12308	1	2.12308	3.23115	0.105802
1L by 2L	0.00515	1	0.00515	0.00783	0.931406
1L by 3L	0.05605	1	0.05605	0.08530	0.776855
1L by 4L	0.23888	1	0.23888	0.36355	0.561426
1L by 5L	0.06878	1	0.06878	0.10467	0.753687
2L by 3L	0.15230	1	0.15230	0.23178	0.641697
2L by 4L	0.08747	1	0.08747	0.13312	0.723646
2L by 5L	0.00001	1	0.00001	0.00001	0.997846
3L by 4L	0.00753	1	0.00753	0.01145	0.917121
3L by 5L	0.02273	1	0.02273	0.03459	0.856589
4L by 5L	0.26497	1	0.26497	0.40326	0.541218
Error	5.91361	9	0.65707		
Total SS	55.76780	29			

3. RESULTS AND DISCUSSION

3.1. Optimization of Lipase Production

3.1.1. Plackett–Burman design

The preliminary experiments revealed high production of lipase in shaker flasks. (Table 1) After analysis by PBD (Table 3), ammonium chloride and sucrose were discarded and 5 variable central composite design, employed for additional optimizations, for processing variables. (Table 5)

3.1.2. Identification of the main PBD factors

To establish the major medium ingredients and range, Plackett–Burman design was employed. (Table 3) A Pereto Chart of Standardization (Figure 2) was plotted based on the A nova (Table 4), derived from the Plackett Burman Design (Table 3). It is seen that the initial five process variables featured in the Pereto Chart of Standardization i.e. temperature, tween 80, olive oil, agitation and pH play an important role compared to the other two parameters i.e. ammonium chloride and sucrose as per the P-value level of significance at 0.05. The parameters close, distant to the level of significance line P-value are most relevant when compared to the parameters far from it. From the findings from the Pereto chart the first five process parameters were chosen for additional optimization by means of Central Composite Design.

3.1.3. Central Composite Design of Experiments

Contour plots shown (Figures 4-12) are the results from the model equations to represent the relation between factors and quantity of each ingredient used in media. The optimal parameters were found and the critical values were computed by application of CCD. ANOVA was used to test the quadratic regression model. The Fisher's F-test with a low probability value indicated that both models were significant statistically. R2 is the fraction of variability described by the experimental parameters and interactions. As R2 values move from 0.99 to 0.89396, the ability of models to predict response values increases [22]. (Table 4,7,8) Response surface plots were further plotted based on the regression equation. Response surface shapes and contour plots reveal the nature and degree of interactions between various components [23]. The peak position in contour plots (Figures 4–12) indicated that the optimal points were within the design boundaries. Each graph depicts the influence of two variables on lipase production when the third variable was maintained at its optimum level [24]-[25]. The 3-D plot illustrates that interactions between variables had a strong impact on lipase production, suggesting the superiority of the responses [18].

3.1.4. Verification of the model

For the validation of the model, the critical levels of the variables were employed as per the calculation to produce lipase from *Aspergillus sydowii* BTSS 1005. The objective of this work was to determine low-cost and simple media for efficient lipase production using fungus *Aspergillus sydowii* BTSS 1005. The individual and combined effect of the main components of the media was studied through Central Composite Design. The effectiveness of optimization through central composite design was confirmed by submerged fermentation study with the critical parameters and found to yield lipase as per the predicted data. Further studies are currently needed to verify large-scale lipase production by *Aspergillus sydowii* using optimized parameters in media.

The optimal predicted values from the above modeling were shown in Table 6. Further few experiments were done at these optimal points and after implementing the parameters it was 64.823 U/ml when compared with predicted activity of 64.03458 U/ml. The above regression coefficients are used for development of polynomial equation of a second order (Table 7). The value of regression is 0.89396, which is approximately near to one, confirming the accuracy of the prediction. The below equation represents the lipase activity (Y) (U/ml) in terms of the experimental parameters.

$$\begin{aligned} Y = {} & -391.612 + 11.632\,X_1 + 49.576\,X_2 + 1.120\,X_3 + 65.302\,X_4 + 8.802\,X_5 - 0.177\,X_1^2 - 3.0025\,X_2^2 \\ & - 0.007\,X_3^2 - 56.166\,X_4^2 - 1.129\,X_5^2 + 0.018\,X_1X_2 + 0.003\,X_1X_3 - 0.611\,X_1X_4 \\ & - 0.066\,X_1X_5 - 0.020\,X_2X_3 - 1.479\,X_2X_4 - 0.002\,X_2X_5 - 0.022\,X_3X_4 \\ & + 0.008\,X_3X_5 - 2.574\,X_4X_5 \end{aligned}$$

The interaction between pH and temperature on lipase activity was recorded lowest at 8.0 and 28 °C, and at 32 °C was found to be maximum (Figure 4). The enzyme activation energy and denaturation kinetics are co-dependent. The positive effect of temperature enhances at a specific optimal pH. The effect of agitation on temperature with pH at 8.0 and temperature at 32 °C showed maximum lipase activity at 80 rpm. (Figure 5) At an optimal temperature, along with moderate agitation, enhances oxygen transfer, which promotes robust microbial growth and increase enzyme secretion. Variation of lipase activity with respect to the Tween 80 and temperature at 32 °C showed maximum activity at the critical value of 0.20 % (v/v) (Figure 6). At moderate temperature, Tween 80 enhances emulsification and improves substrate dispersion. When compared between olive oil and temperature at 32 °C, lipase activity was more at 3.0 % (v/v) (Figure 7). Specific temperature reduces viscosity, increases droplet mobility and hence improve lipase-olive oil interaction. Combination of agitation and pH led to enhanced lipase activity at 80 rpm and pH 8.0 (Figure 8).

Their combined impact can produce synergistic or antagonistic interactions depending on the *operation range hence needs balancing. The lipase activity enhanced with a combination of 3.0 % (v/v) olive* oil and pH 8.0 (Figure 9). Olive oil forms hydrophobic droplets whose emulsification stability was influenced by pH, as charge interactions at the interface support stable emulsions allowing lipase access to the substrate at a suitable pH. Tween 80 with 0.20 % (v/v) and agitation at 80 rpm enhanced lipase activity (Figure 10) Tween 80 improves lipase activity when agitation supplies adequate mechanical dispersion. The combination of olive oil at 3.0 % (v/v) and agitation at 80 rpm, the enzyme activity was maximum (Figure 11). Inductive effect of olive oil works at its best when agitation range supports cell integrity and metabolism. Based on the graph, we can easily say that the lipase activity was more at 3.0 % (v/v) of olive oil and 0.20 % (v/v) of Tween 80 (Figure 12). The interaction pattern indicates the effectiveness of olive oil as inducer and was highly dependent on the emulsification property of Tween 80 whose compositions was optimized. Out of 9 parameters checked (Table 1) the most important and significant parameters were found by the pareto chart (Figure 2). The more the significance, the more the bar chart will be. The first five have longer bars in the diagram and are important when compared with the remaining parameters. Ammonium chloride and sucrose was excluded based on Figure 2 and remaining experiments were conducted using the first five significant factors. Then the optimized values were found (Table 6).

Among the tested variables, Tween 80 (emulsifier) and olive oil (lipid inducer) showed significant positive effects ($p < 0.05$), indicating their critical role in enhancing enzyme yield. Other variables were found to have less significant influence within the tested range. This preliminary screening effectively eliminated factors having no influence on optimization, demonstrating the efficiency of statistical design. [1] Analysis of variance (ANOVA) showed that the model was highly significant ($p < 0.001$), with an R^2 value of 0.89396, indicating excellent correlation between observed and predicted responses. The lack-of-fit test showed ($p > 0.05$) which confirms this model's adequacy. The increase in lipase activity from 28.64 U/ml to 64 U/ml represents a 2.2-fold improvement, aligning with enhancements reported in similar studies on *Aspergillus sp*. [4, 5, 8] This confirms the utility of RSM in improving biocatalyst yields for industrial applications. Additionally, the role of Tween 80 as both an emulsifier and surfactant may have facilitated increased secretion of extracellular lipase, consistent with reports on fungal lipase physiology. Three-dimensional response surface plots and two-dimensional contour plots help to understand interactions between different parameters and predict optimum concentrations. Results endorse the utility of RSM and CCD to improve lipase production from *Aspergillus sydowii* BTSS 1005.

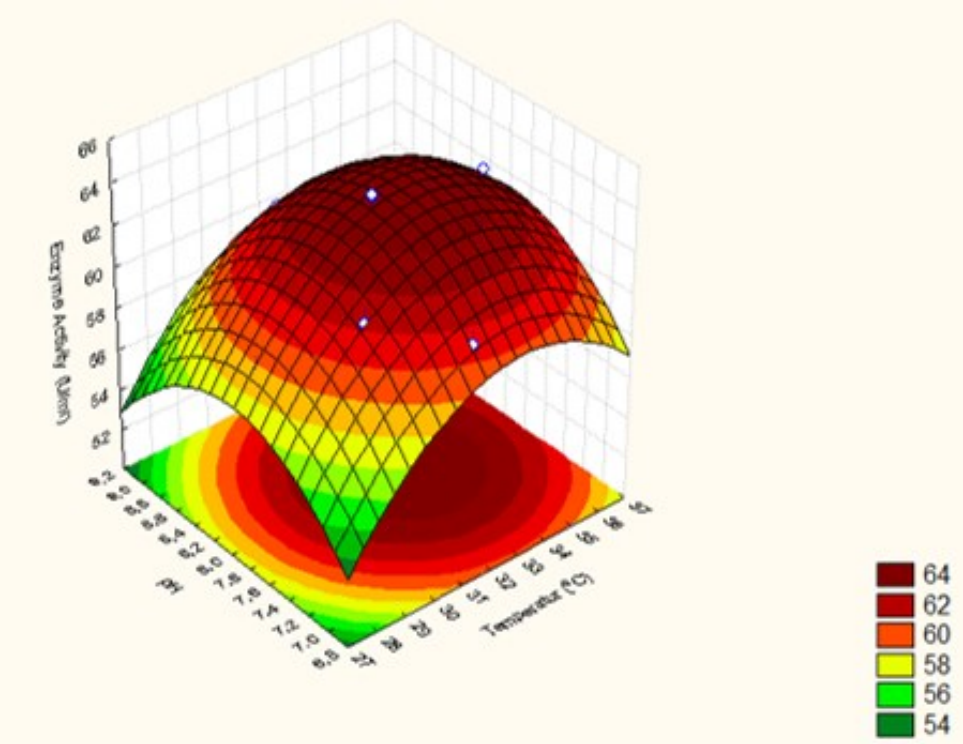


Figure 4. Contour plot depicting interaction effects of pH and temperature on lipase activity

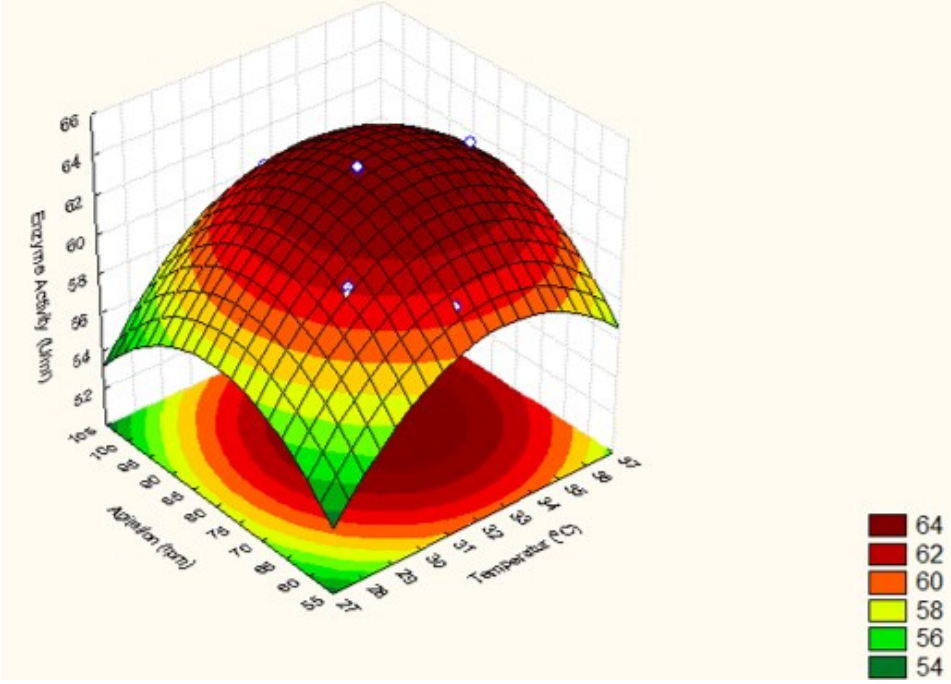


Figure 5. Contour plot depicting interaction effects of agitation and temperature on lipase activity

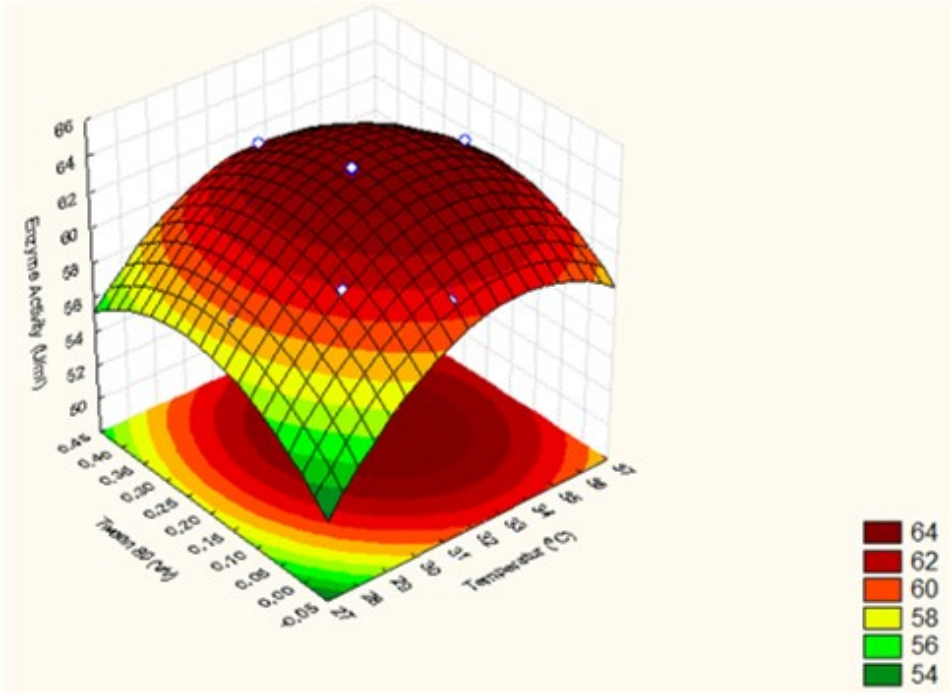


Figure 6. Contour plot depicting interaction effects of Tween 80 and temperature on lipase activity

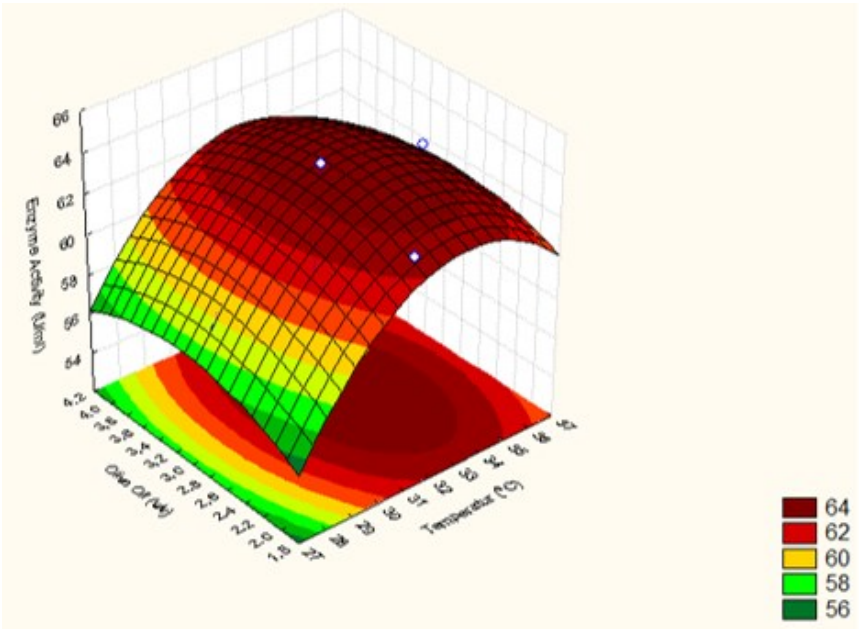


Figure 7 Contour plot depicting interaction effects of olive oil and temperature on lipase activity

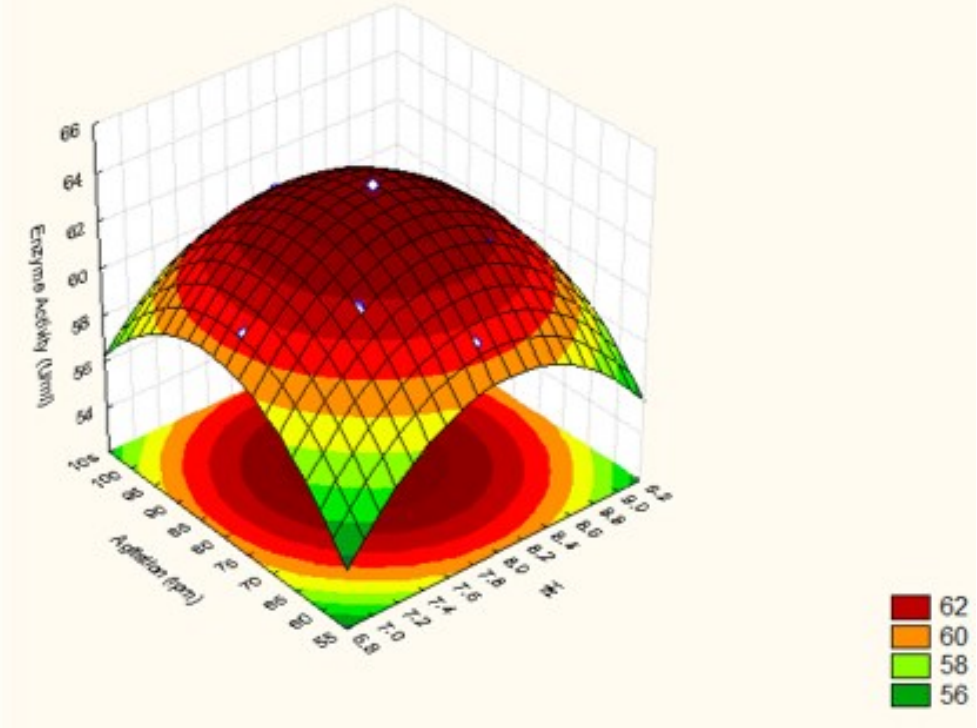


Figure 8. Contour plot depicting interaction effects of agitation and pH on lipase activity

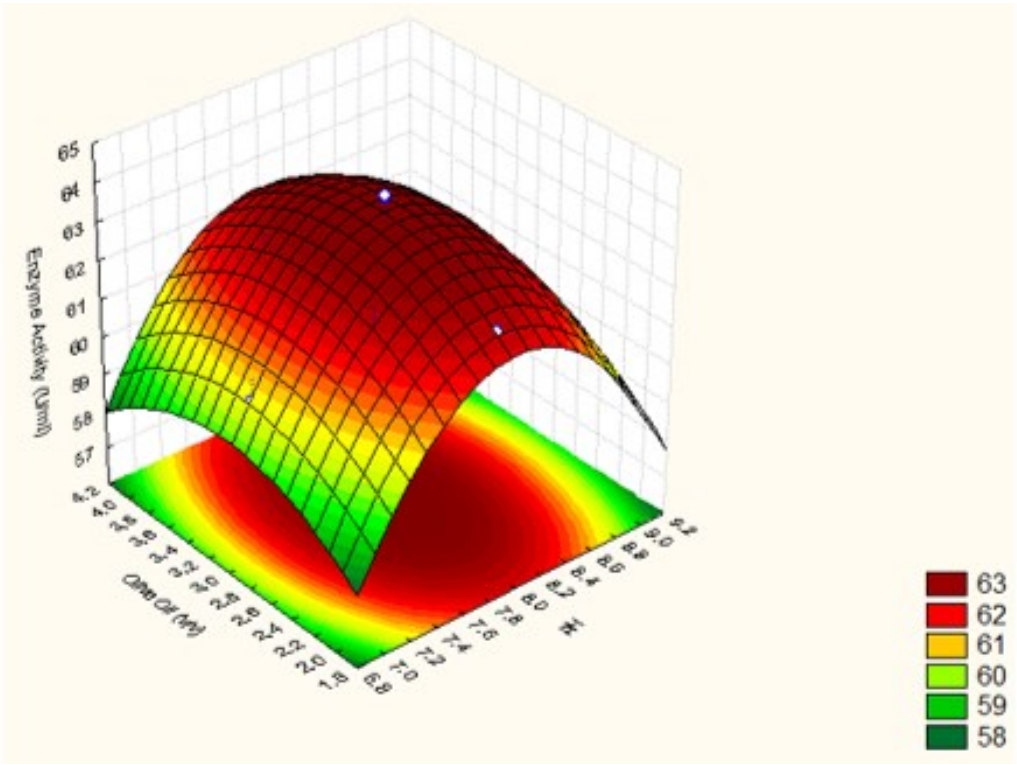


Figure 9. Contour plot depicting interaction effects of olive oil and pH on lipase activity

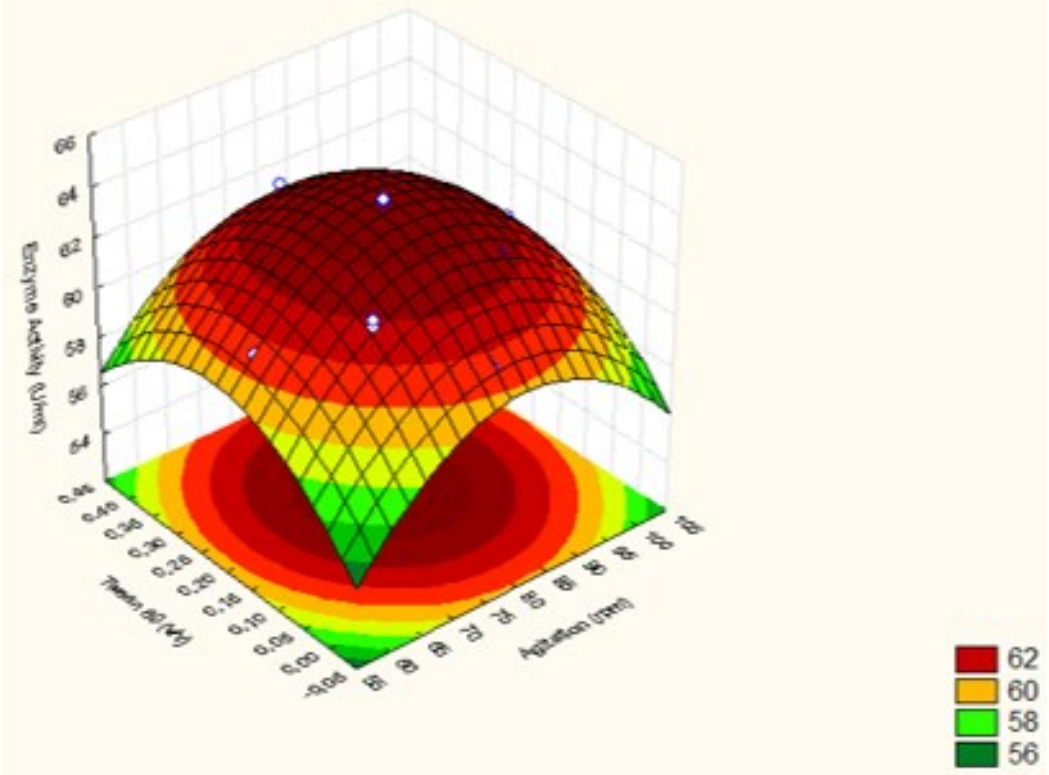


Figure 10. Contour plot depicting interaction effects of Tween 80 and agitation on lipase activity

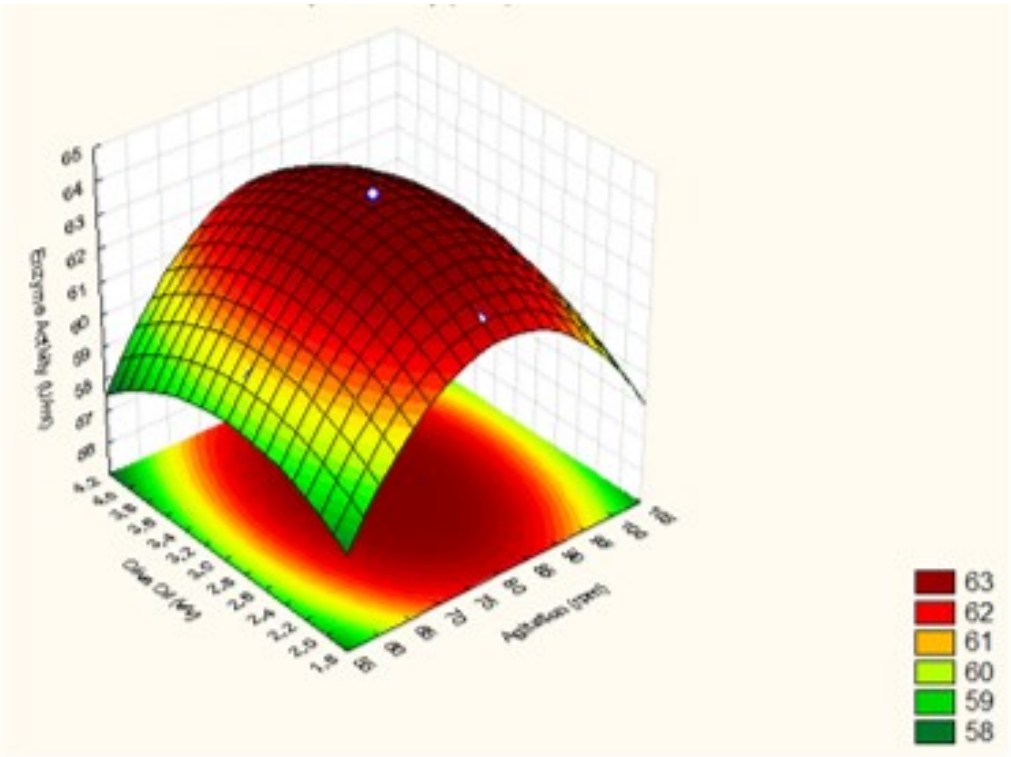


Figure 11. Contour plot depicting interaction effects of olive oil and agitation on lipase activity

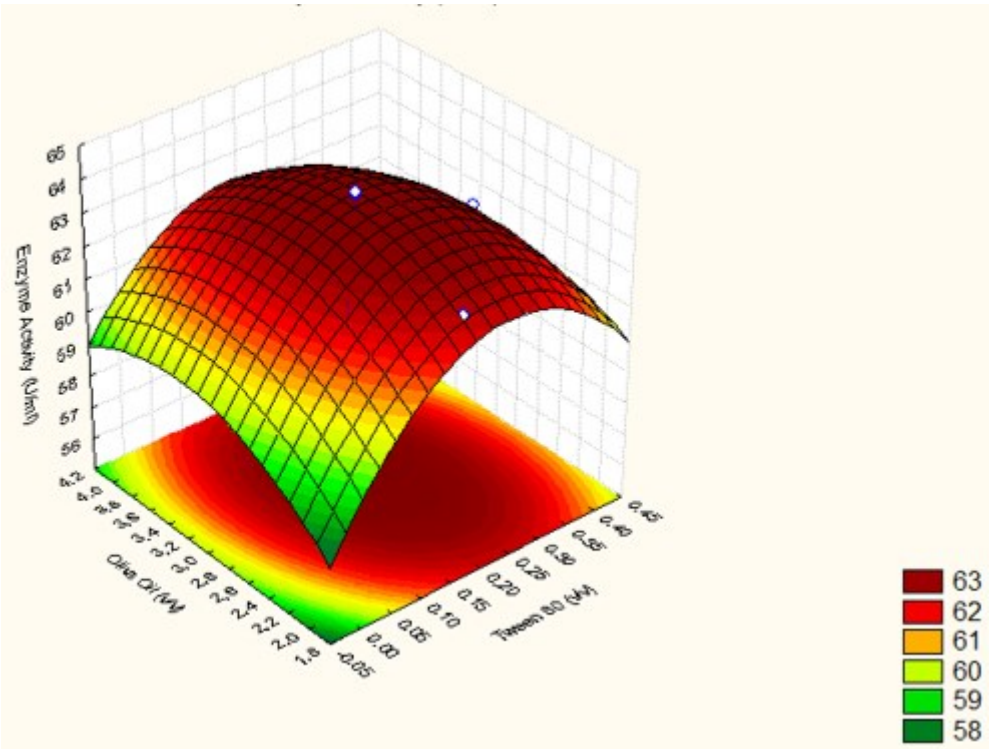


Figure 12. Contour plot depicting interaction effects of olive oil and Tween 80 on lipase activity

4. CONCLUSION

This study successfully demonstrated the use of statistical optimization for enhancing lipase production from marine-derived *Aspergillus sydowii* BTSS 1005. The fermentation studies showed lipase yield at its maximum strength. For further analysis, ammonium chloride and sucrose was removed based on PB design and 5 variable central composite design was used for further optimization of these process variables. Plackett–Burman design determined the most critical components necessary to enhance yield. Contour plots further helped in understanding interaction among the variables and optimal concentration of each component was determined further. With the help of ANOVA, the quadratic regression model was evaluated.

Central Composite Design in the above design of experiments showed the interaction between variables and generated a mathematical equation so that at any pH, any temperature and at any Tween 80 concentration etc. one can find out the lipase activity. The three-dimensional graphs also showed the same information. Central composite design gave the optimized parameters. The results determined by statistical methods like RSM and BBD helped to enhance yield of lipase from *Aspergillus sydowii* BTSS 1005. Bioprocess optimization for industrial scalability of *Aspergillus sydowii* BTSS 1005 as a potential lipase producer was made possible from this study.

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BIOGRAPHY OF AUTHOR

Dr. PANCHAGNULA BINDIYA is Assistant Professor (c) in the Department of Biotechnology at College of Science and Technology, Andhra University Visakhapatnam, Andhra Pradesh, India with Teaching and Research experience of 23 years. She received her M.Sc. Biotechnology, M.Phil. Botany, MBA and Ph.D. Biotechnology from Andhra University, Visakhapatnam. Her research interests include Industrial Biotechnology, Microbiology, Bioremediation and Bioinformatics. She authorized eleven research papers, including seven international, two national publications and two book chapters, in reputed scientific journals. She can be contacted at email: bindiya.au@gmail.com

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