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Original Article

Optimization of novel halophilic lipase production by *Fusarium solani* strain NFCCL 4084 using palm oil mill effluent

Kiptoo Geoffry, Rajeshwara N. Achur*

Department of Biochemistry, Kuvempu University, Shankaraghatta, 577451 Shimoga, Karnataka, India



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ABSTRACT

Among different sources of lipases, fungal lipases have continued to attract a wide range of applications. Further, halophilic lipases are highly desirable for biodiesel production due to the need to mitigate environmental pollution caused as result of extensive use of fossil fuels. However, currently, the high production cost limits the industrial application of lipases. In order to address this issue, we have attempted to optimize lipase production by *Fusarium solani* NFCCL 4084 and using palm oil mill effluent (POME) based medium. The production was optimized using a combinatory approach of Plackett-Burman (PB) design, one factor at a time (OFAT) design and face centred central composite design (FCCCD). The variables (malt extract, $(\text{NH}_4)_2\text{SO}_4$, CaCl_2 , MgSO_4 , olive oil, peptone, K_2HPO_4 , NaNO_3 , Tween-80, POME and pH) were analyzed using PB design and the variables with positive contrast coefficient were found to be K_2HPO_4 , NaNO_3 , Tween-80, POME and pH. The significant variables selected were further analyzed for possible optimum range by using OFAT approach and the findings revealed that K_2HPO_4 , NaNO_3 , and Tween-80 as the most significant medium components, and thus were further optimized by using FCCCD. The optimum medium yielded a lipase with an activity of 7.8 U/ml, a significant 3.2-fold increase compared to un-optimized medium. The present findings revealed that POME is an alternative and suitable substrate for halophilic lipase production at low cost. Also, it is clearly evident that the combinatory approach employed here proved to be very effective in producing high activity halophilic lipases, in general.

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1. Introduction

Industrially, biocatalyst enzymes have continued to gain much attention due to their utility in different and diverse areas of applications. However, the increasing production cost has been hampering the development of new applications and proper production technologies [1]. There has been an upsurge in the enzyme market over the years to the tune of billions of dollars and is expected even to increase considerably in the near future [2]. These industrially important enzymes include amylases, cellulases, proteases and lipases which belong to hydrolyzing group and have been found to be suitable for various applications [3–5]. Among these enzymes, lipases (triacylglycerol acylhydrolase: E.C.3.1.1.3) catalyse the hydrolysis of ester bonds of triacylglycerol to diacylglycerol, monoacylglycerol, glycerol and free fatty acids at lipid–water interface, an exquisite phenomenon that makes it unique

and very attractive [6]. Further, lipases also catalyze a variety of biotransformation synthetic reactions such as esterification, inter-esterification, and trans-esterification reactions under micro aqueous as well as non-aqueous media conditions [7–9]. Owing to the ability to catalyze a wide range of reactions, lipases have attracted attention towards novel applications such as in the biodiesel production [5], detergents [10], food industries [11], pharmaceutical industry [12], cosmetics [13] and as biosensors [14]. Lipases are ubiquitous and are produced by plants, animals and microorganisms [15–17]. Among these sources, microbial lipases are widely preferred due to the high productivity efficiency and their ease of genetic manipulation [18]. Further, a lot of interest has been focussed on fungal lipases due to their greater stability and substrate specificity [19]. Among these fungal sources, *Fusarium solani* has been found to be important among potential lipase producers [20,21]. Industrial lipase production by submerged fermentation (SmF) is being used routinely because of the advantages associated, such as the ease in controlling the variables and enzyme isolation. Further, recent developments have seen the possibility of utilizing low cost agro-industrial waste as carbon source [22,23]. However, production factors such as

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* Corresponding author.

E-mail addresses: geoffrykiptoo@yahoo.com (K. Geoffry), rajachur@gmail.com, anr@kuvempu.ac.in (R.N. Achur).

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nutrient concentration, pH, strain, agitation as well as presence and concentration of inducers can affect the productivity of these biocatalysts which inadvertently heighten the production cost, a factor that has continued to be an obstacle and limits the use of lipase to its fullest potential [24]. Therefore, research that focuses to use isolated microorganisms from different environments as well as agro-industrial residues based media composition are urgently needed in achieving high value lipase at low cost, which is a recipe to overcome the impending industrial challenges [25]. A few studies recently have utilized industrial waste such olive oil waste water and dairy effluent for lipase production by *Fusarium solani* [26,27]. However, a lot of industrial waste remain unexplored and therefore, remains an interesting realm to be studied. In this direction, our present study employed a coordinated approach [28] to optimize the novel halophilic lipase production from *Fusarium solani* NFCCI 4084 isolated from Arabian Sea using palm oil mill effluent based medium. By employing statistical - experimental design method; Plackett–Burman (PB) design to screen out and select variables effective in enhancing lipase production, one factor-at-a-time (OFAT) approach to achieve possible optimum level of effective key variables and face centred central composite design (FCCCD), we have optimized and established the relationship among the most influential variables. To our knowledge, this novel approach of simultaneous optimization using statistical design has not been performed before on this halophile, *Fusarium solani*.

2. Materials and methods

2.1. Chemicals

p-Nitrophenyl palmitate (p-NPP) was obtained from Sigma Chemical Co. USA, Tween-80 and all other analytical grade chemicals and reagents were obtained from HiMedia, Mumbai, India.

2.2. Fungal strains and sample collection

The marine fungal strain *Fusarium solani* NFCCI 4084 was isolated from Arabian Sea water collected from the shores of Udupi, Karnataka state, India. The screening for a novel marine lipase with dual ability to catalyse hydrolytic and synthetic activity was initially performed using plate method. The crude lipase was further screened for hydrolytic activity as well as synthetic activity (esterification and transesterification reactions) and the culture was maintained on Potato Dextrose Agar (PDA) [29]. The palm oil mill effluent (POME) was obtained from a palm plant near Kuvempu University, Shankaraghatta, India, by collecting in a clean container and stored at 4 °C until used.

2.3. Preparation of lipase production medium and fungal inoculums

POME based lipase production medium containing different components at varied concentrations was prepared and their effects towards lipase production were assessed under submerged fermentation (SmF). The pH of medium was adjusted according to values predefined by statistical experimental design by using 1 M HCl or 1 M NaOH. Further, it was autoclaved at 121 °C and 15 psi pressure for 20 min. *Fusarium solani* NFCCI 4084 was inoculated in potato dextrose agar (PDA) petri dish plate and incubated at 28 °C for six days till mature spores developed. Six mm diameter disc from the plate was taken as inoculum and added to 30 ml production medium in 100 ml Erlenmeyer flasks and were incubated at 28 °C for 5 days under agitation at 130 rpm as described by Colla et al. [25]. The culture filtrate obtained was considered as crude extracellular lipase and was used for lipase activity assays. All

the experiments were performed in replicates and their standard deviations calculated.

2.4. Lipase activity determination

The lipase activity of culture filtrate was assayed as previously described by Krieger et al. [30] using para-nitrophenol palmitate (p-NPP) as substrate. Briefly, 20 µL of culture filtrate was added to 380 µL of the substrate solution consisting of one part of solution A (3.0 mM p-NPP in 2-propanol) and nine parts of solution B (100 mM Tri-HCl buffer, pH 8.0, 0.4% Triton X-100 and 0.1% gum Arabic), which was freshly prepared before use. The reaction was stopped by boiling for 10 min, followed by centrifugation at 8000g for 10 min. The release of para nitrophenol (p-NP) was measured using EnSpire Multimode Plate Reader at 410 nm against a blank containing only buffer. One unit of enzyme activity is defined as the amount of enzyme that released 1 µmole p-NP per minute. The calculation of lipase activity in units was done by using the standard curve.

2.5. Optimization of lipase production using statistical experimental design

The physicochemical variables play a significant role in influencing lipase yield as well as production cost [31]. Thus, use of different supplements can contribute to best medium combinations which yield cost-effective and high value lipase [32]. The essential variables for lipase production by *Fusarium solani* NFCCI 4084 under SmF were statistically optimized using combinatory approach. Initially, by using Plackett–Burman design (PBD), secondly by one factor at a time (OFAT) approach and finally, by face centred central composite design (FCCCD). Statistical software package Design-Expert 10 (Stat Ease Inc., Minneapolis, USA) was employed for experimental design as well as result analysis.

2.5.1. Screening of key variables using Plackett–Burman design

The use of Plackett–Burman design (PBD), a two-level factorial design to screen physicochemical variables that greatly influences the activity of lipase in production medium is very important. The design is widely employed due to its relevance associated with and the advantage is that it allows the selection of variables that have only considerable positive effect from a larger number of variables to obtain a sizeable cost-effective and manageable variable [33]. In the present study, we aimed to identify the relative effect of 11 variables, selected as per the available literature, on lipase production by using PB design which generated a set of 12 experimental trials (1–12) [33]. The variables assessed were peptone (A), sodium nitrate (B), di-potassium hydrogen phosphate (C), magnesium sulphate (D), malt extract (E), Olive oil (F), ammonium sulphate (G) calcium chloride (H), POME (I), pH (J) and Tween-80 (K). The investigated variables under two widely spaced designated levels as –1 (low level) and +1 (high level), respectively, are shown in Table 1. The responses (lipase activity) of the experimental design were analyzed according to first order polynomial equation:

$$Y = \beta_0 + \sum \beta_i X_i \quad (1)$$

where Y is the response (lipase activity U/ml), β_0 is the model intercepts, β_i is the linear coefficient, and X_i is the level of the independent variable. The design indicated how each factor influence the production process. However, the design model does not describe interaction among variables and therefore serves only as a valuable initial tool to screen the effect of variables and also to select essential variables for further optimization [34]. The effect of individual variable was identified based on the main effect (contrast

Table 1

List of variables used in Plackett–Burman design showing low and high levels of each variable.

Variables	–(low level)	+(high level)
Peptone	0.40	2.00
NaNO ₃	0.20	0.60
K ₂ HPO ₄	0.40	1.20
MgSO ₄	0.04	0.12
Malt extract	0.50	1.00
Olive oil	1.00	3.00
(NH ₄) ₂ SO ₄	0.30	0.60
CaCl ₂	0.02	0.06
POME	0.30	1.20
pH	5.50	8.50
Tween-80	0.05	0.40

coefficient) equation (E), which is calculated by the average of difference between the measurements obtained at high (+1) and low level (–1) as follows:

$$E = \frac{(\text{Total response at high level} - \text{total response at low level}) / \text{No. of trials}}{2} \quad (2)$$

2.5.2. One factor at a time experimental

After PB design which is designed to determine the effect of each variable screened, the variables that exhibited positive effect were subjected to one-factor-at-a-time (OFAT) design approach [35]. However, this depicts net effect of various medium components on lipase activity and that it focuses on single factor variable which does not consider the significance of interactions between various variables and thus there may not be a substantial increase in lipase yield [36,37]. It is therefore, imperative to estimate the possible optimum range of the lipase production variables in POME-based medium. Based on PB design, the variables viz peptone, sodium nitrate, di-potassium hydrogen phosphate, olive oil, pH and Tween 80 in POME based medium were identified to influence lipase production significantly. Due to their positive contribution, the possible optimum levels were examined at different concentrations by using OFAT approach. The concentration ranges determined were 0.2–1.0% (w/v) sodium nitrate; 0.2–1.0% (w/v) di-potassium hydrogen phosphate; 0.25–1.25% (w/v) POME; 0.05–0.25% (w/v) Tween-80 and pH was 7.0–9.0.

2.5.3. Optimization of POME-based medium by FCCCD

The optimization production of lipase by utilizing POME-based medium was achieved by using FCCCD under response surface methodology (RSM) [38,39]. The design was used to determine the interaction as well as to optimize three independent quantita-

tive variables viz; sodium nitrate (X₁), Tween-80 (X₂) and di-potassium hydrogen phosphate (X₃) which were identified to exhibit most significant positive effect following PB and OFAT screening. Whereas, the other two significant variables (pH and POME) were kept constant at their optimum levels as designed by OFAT approach. The experimental design conducted in the present study, as shown in Table 3, included 20 set of combination trials, with each variable being examined at three different levels, low (–1), medium (0) and high (+1). The correlation of dependent (lipase yield) and independent variables can be explained by second order polynomial model Eq:

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_{12} X_1 X_2 + \beta_{13} X_1 X_3 + \beta_{23} X_2 X_3 + \beta_{11} X_1^2 + \beta_{22} X_2^2 + \beta_{33} X_3^2 \quad (3)$$

where Y is the dependent variable (lipase activity); X₁, X₂ and X₃ are independent variables; sodium nitrate (X₁), Tween 80 (X₂) and di-potassium hydrogen phosphate (X₃); β_0 is an intercept term; β_1 , β_2 and β_3 are linear coefficients; β_{12} , β_{13} and β_{23} are the interaction coefficients; and β_{11} , β_{22} and β_{33} are the quadratic coefficients. The responses (lipase activities) data of experimental levels of each were analysed using analysis of variance (ANOVA). The model analysis performed included correlation coefficient (R) and coefficient of determination (R²) which measures regression model goodness of-fit and Fisher's F-test to determine the overall significance of the model. The responses fitted using polynomial model were further expressed in the form of contour and three dimensional response curves to understand the influence of the variables individually as well as in combinations in determining the lipase activity. Further, the model also determines the optimal level of variables for achieving maximal production of lipase.

2.6. Experimental design validation

The designed FCCCD model used to optimize lipase production in relation to three variables was validated. The experimental run combination predicted according to Design Expert point prediction feature was conducted for six times and the obtained lipase activity results were analysed and determined whether it is within the prediction interval (PI) range.

3. Results

3.1. Screening of key variables using Plackett–Burman (PB) design

A total of 12 experimental runs for lipase production were performed using PB design (Table 2). As shown in Table 2, eleven components of POME based production medium were screened at two

Table 2

Plackett–Burman experimental design for the evaluation of 11 components with the actual values for lipase production by *Fusarium solani* NFCCCL 4084 and the design response (lipase activity). The variables as well as their concentration levels were chosen on the basis of literature reports on lipase production.

Run #	A %w/v	B %w/v	C %w/v	D %w/v	E %w/v	F %w/v	G %w/v	H %w/v	J %w/v	K pH	L %w/v	Lipase activity (U/ml) ^a
1	2.00	0.20	0.40	0.04	1.00	1.00	0.60	0.06	0.30	8.50	0.40	0.72 ± 0.0164
2	0.40	0.60	1.20	0.12	0.50	1.00	0.30	0.06	0.30	8.50	0.40	2.36 ± 0.0439
3	0.40	0.20	1.20	0.04	1.00	3.00	0.30	0.06	1.20	8.50	0.05	0.65 ± 0.0137
4	2.00	0.20	1.20	0.12	1.00	1.00	0.30	0.02	1.20	5.50	0.40	1.08 ± 0.0879
5	0.40	0.60	1.20	0.04	1.00	3.00	0.60	0.02	0.30	5.50	0.40	1.62 ± 0.0302
6	2.00	0.60	0.40	0.04	0.50	3.00	0.30	0.06	1.20	5.50	0.40	1.50 ± 0.1895
7	2.00	0.60	0.40	0.12	1.00	3.00	0.30	0.02	0.30	8.50	0.05	0.55 ± 0.0082
8	0.40	0.20	0.40	0.12	0.50	3.00	0.60	0.02	1.20	8.50	0.40	1.67 ± 0.0576
9	2.00	0.20	1.20	0.12	0.50	3.00	0.60	0.06	0.30	5.50	0.05	0.40 ± 0.0521
10	0.40	0.60	0.40	0.12	1.00	1.00	0.60	0.06	1.20	5.50	0.05	0.28 ± 0.0027
11	2.00	0.60	1.20	0.04	0.50	1.00	0.60	0.02	1.20	8.50	0.05	2.25 ± 0.0357
12	0.40	0.20	0.40	0.04	0.50	1.00	0.30	0.02	0.30	5.50	0.05	1.06 ± 0.0659

A, Peptone; B, NaNO₃; C, K₂HPO₄; D, MgSO₄; E, Malt extract; F, Olive oil; G, (NH₄)₂SO₄; H, CaCl₂; I, POME; J, pH; K, Tween-80.

^a Mean ± standard deviation.

Table 3

Experimental design using FCCCD of three independent variables with their actual values and six centre points showing the experimental and predicted response.

Run #	A %V/V	B %w/v	C %w/v	Lipase activity U/ml	
				Experimental	Predicted
1	1.20	0.80	1.00	6.00	6.53
2	0.60	0.40	0.50	6.52	6.39
3	1.80	0.40	0.50	4.55	4.83
4	1.20	1.47	1.00	6.21	6.14
5	1.20	0.80	1.00	7.00	6.53
6	1.80	0.40	1.50	5.70	5.69
7	1.20	0.80	1.00	6.52	6.53
8	1.20	0.80	1.00	6.38	6.53
9	1.20	0.80	1.00	6.52	6.53
10	2.20	0.80	1.00	6.58	6.35
11	1.20	0.80	0.15	6.30	6.22
12	0.60	0.40	1.50	7.34	7.52
13	0.60	1.20	0.50	6.19	6.33
14	0.60	1.20	1.50	7.36	7.21
15	1.80	1.20	0.50	6.69	6.64
16	0.19	0.80	1.00	7.60	7.63
17	1.20	0.80	1.00	6.70	6.53
18	1.80	1.20	1.50	6.99	7.25
19	1.20	0.80	1.80	7.80	7.69
20	1.20	0.12	1.00	5.02	4.88

A: Tween-80; B: K₂HPO₄; C: NaNO₃.

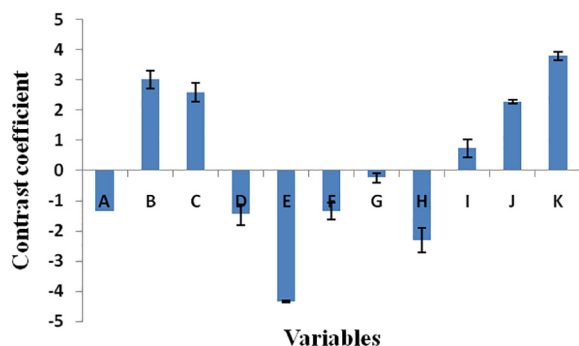


Fig. 1. Contrast coefficient of variables for lipase production by *Fusarium solani* NFCCCL 4084 as determined using Plackett–Burman experimental results (A, Peptone; B, NaNO₃; C, K₂HPO₄; D, MgSO₄; E, Malt extract; F, Olive oil; G, (NH₄)₂SO₄; H, CaCl₂; I, POME; J, pH and K, Tween-80).

levels each. The results of these experiments indicate that the activity of lipase produced from *Fusarium solani* ranges from 0.28 to 2.36 U/ml. Among these, the experimental run #2 indicated highest lipase activity, whereas the experimental run #10 showed least activity. As shown in Fig. 1, the analysis of contrast coefficient of individual component indicates that various components influence the lipase production differently. The variables influencing the lipase production negatively can be ordered as malt extract > (NH₄)₂SO₄ > CaCl₂ > MgSO₄ > olive oil > peptone, whereas, positive effect on lipase production was found to be K₂HPO₄ > NaNO₃ > Tween-80 > POME which includes pH also. Since PB design does assess the concentration of variables, the OFAT approach was further performed on the selected variables to determine the effect of concentration of these variables on lipase production.

3.2. One factor at a time experimental (OFAT) approach

By applying OFAT approach, NaNO₃ was used for inorganic nitrogen supplementation at different concentrations. Highest activity of 3.65 U/ml was shown at 0.8%, w/v concentration of NaNO₃ (Fig. 2). The effect of K₂HPO₄ was assessed at varied concentrations (0.2–1.0% w/v) and it was also found to influence lipase

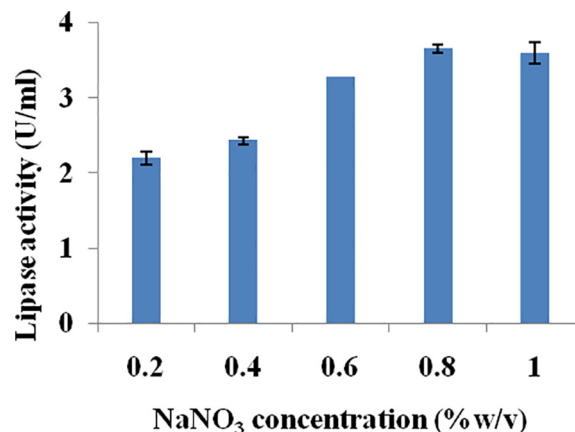


Fig. 2. Effect of different concentrations of NaNO₃ (0.2–1.0%, w/v) on *Fusarium solani* NFCCCL 4084 lipase activity.

production; the maximal activity of 4.06 U/ml was recorded at 0.6% (w/v) (Fig. 3). As depicted in Fig. 4, the utilization of Tween-80 as a carbon source as well as surfactant exhibited a profound effect on lipase production, the highest lipase activity being at 0.1% (v/v). The inductive effect of POME utilized as base medium was assessed at various concentrations and the maximum lipase activity was found to be at 1.0% (w/v). The pH of medium also significantly influences the biosynthesis of extracellular lipase and at pH 8.5, the highest lipase activity of 3.70 U/ml was exhibited

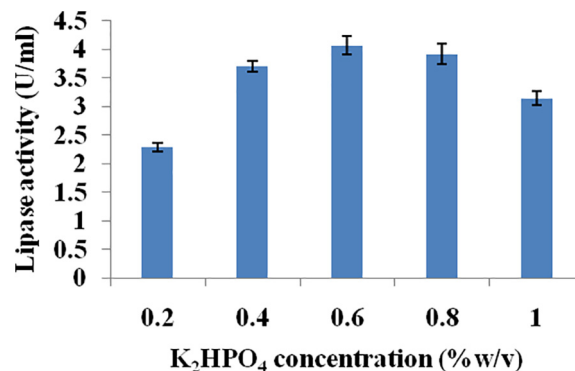


Fig. 3. Effect of different concentrations of K₂HPO₄ (0.2–1.0 %w/v) on *Fusarium solani* NFCCCL 4084 lipase activity.

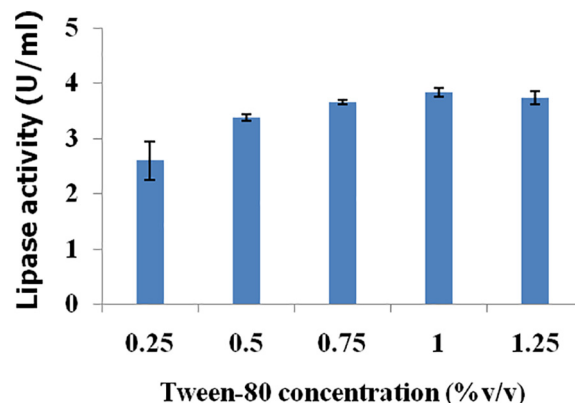


Fig. 4. Effect of different concentrations of Tween-80 (0.25–1.25 %v/v) on *Fusarium solani* NFCCCL 4084 lipase activity.

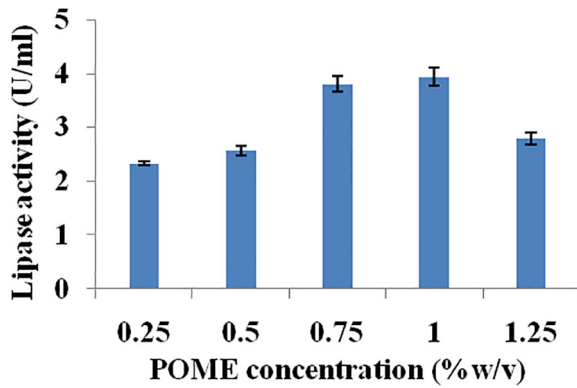


Fig. 5. Effect of different concentrations of POME (0.25–1.25 %, w/v) on *Fusarium solani* NFCCCL 4084 lipase activity.

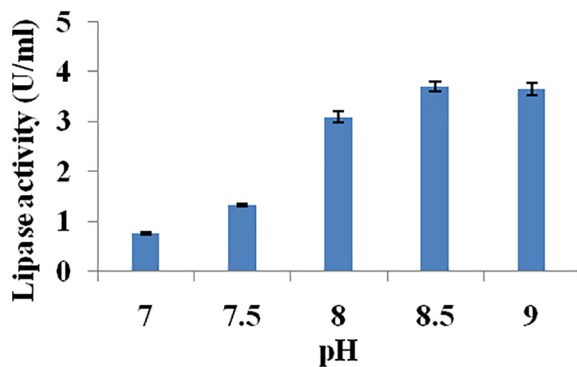


Fig. 6. The effect of pH on *Fusarium solani* NFCCCL 4084 lipase activity.

(Fig. 6). Based on these findings by OFAT approach, a significant 1.7-fold increase in lipase activity was achieved as compared to un-optimized medium and thus Tween-80, K_2HPO_4 and $NaNO_3$ were selected for further optimization using FCCCD, while the other significant variables were fixed at their optimum levels.

3.3. Optimization of POME-based medium by FCCCD

The face centred central composite design (FCCCD) was employed to optimize variables viz; K_2HPO_4 , $NaNO_3$ and Tween-80 that are identified to influence lipase activity after PB and OFAT experiments. The design layout presents 20 corresponding experimental combinations with both predicted and experimental lipase

activity and each variable was examined at three levels (Table 3). It is evident from the results that the highest lipase activity of 7.80 (U/ml) produced by *Fusarium solani* was recorded in run #19 whereas the least activity of 4.83 (U/ml) was recorded in run #3. The relationship between the lipase activity and the selected variables was expressed by the second order polynomial equation:

$$Y (\text{Lipase activity, U/ml}) = +6.53 - 0.38A + 0.37B + 0.44C + 0.16A^2 - 0.36B^2 + 0.15C^2 + 0.47AB - 0.068AC - 0.061BC \quad (4)$$

where the lipase activity is the response (Y), while A, B and C represent the concentrations of $NaNO_3$, K_2HPO_4 and Tween-80, respectively. The analysis of variance (ANOVA) of the designed model, as shown in Table 4, has a F value of 14.05 with a p-value of < 0.0001. The quadratic model indicated a non-significant lack of fit (p-value of 0.6949). Further, the coefficient of determination (R^2) and adjusted R^2 values were found to be 0.926 and 0.860, respectively. The adequate precision value of 13.603 and coefficient of variation (CV) value of 4.57 was also obtained.

The two and three dimensional surface curves, as shown in Fig. 7a–c, were plotted based on the function of concentration of two variables while keeping the other at their optimum level. These are used to establish the interaction among the selected variables and further to determine their optimum concentration for maximum lipase production in POME based medium. The plot curves exhibited the level of interaction, with most significant interaction being shown between Tween-80 and K_2HPO_4 with a p-value of 0.0012 (Fig. 7b). By using this design model, the lipase activity of 7.80 (U/ml), a significant 3.2-fold increase was successfully achieved as compared to un-optimized medium.

3.4. Validation of the experimental model

The point prediction at the two-sided selected factor levels as shown in Table 5 validates prediction of the experimental response surface model. The results obtained indicates comparison of the average of six confirmation samples with the predicted interval and reveals that the average observation of 6.93 U/ml from the confirmation experiment is within the confirmation node's prediction interval of 6.70 U/ml and 7.47 U/ml.

4. Discussion

Based on the experimental result analysis, five of the variables among the examined eleven were selected because of their positive impact on lipase production, whereas six variables that

Table 4
Analysis of variance of surface quadratic model for lipase production.

Source	Sum of Squares	df	Mean Square	F Value	p-value ^a Prob > F	Remarks
Model	11.150	9	1.240	14.05	0.0001	Significant
A-Tween-80	1.970	1	1.970	22.38	0.0008	
B- K_2HPO_4	1.920	1	1.920	21.71	0.0009	
C- $NaNO_3$	2.600	1	2.600	29.46	0.0003	
AB	1.750	1	1.750	19.87	0.0012	
AC	0.037	1	0.037	0.420	0.5326	
BC	0.030	1	0.030	0.340	0.5719	
A ²	0.390	1	0.390	4.420	0.0619	
B ²	1.840	1	1.840	20.88	0.0010	
C ²	0.330	1	0.330	3.770	0.0809	
Residual	0.880	10	0.088			
Lack of Fit	0.340	5	0.067	0.620	0.6949	Not significant
Pure Error	0.55	5	0.11			
Cor Total	12.04	19				

$R^2 = 0.92$, Adj $R^2 = 0.86$, C.V. % = 4.57, Adeq Precision 13.603, Mean = 6.50.

^a Significant at probability $p < 0.05$ was considered to be significant.

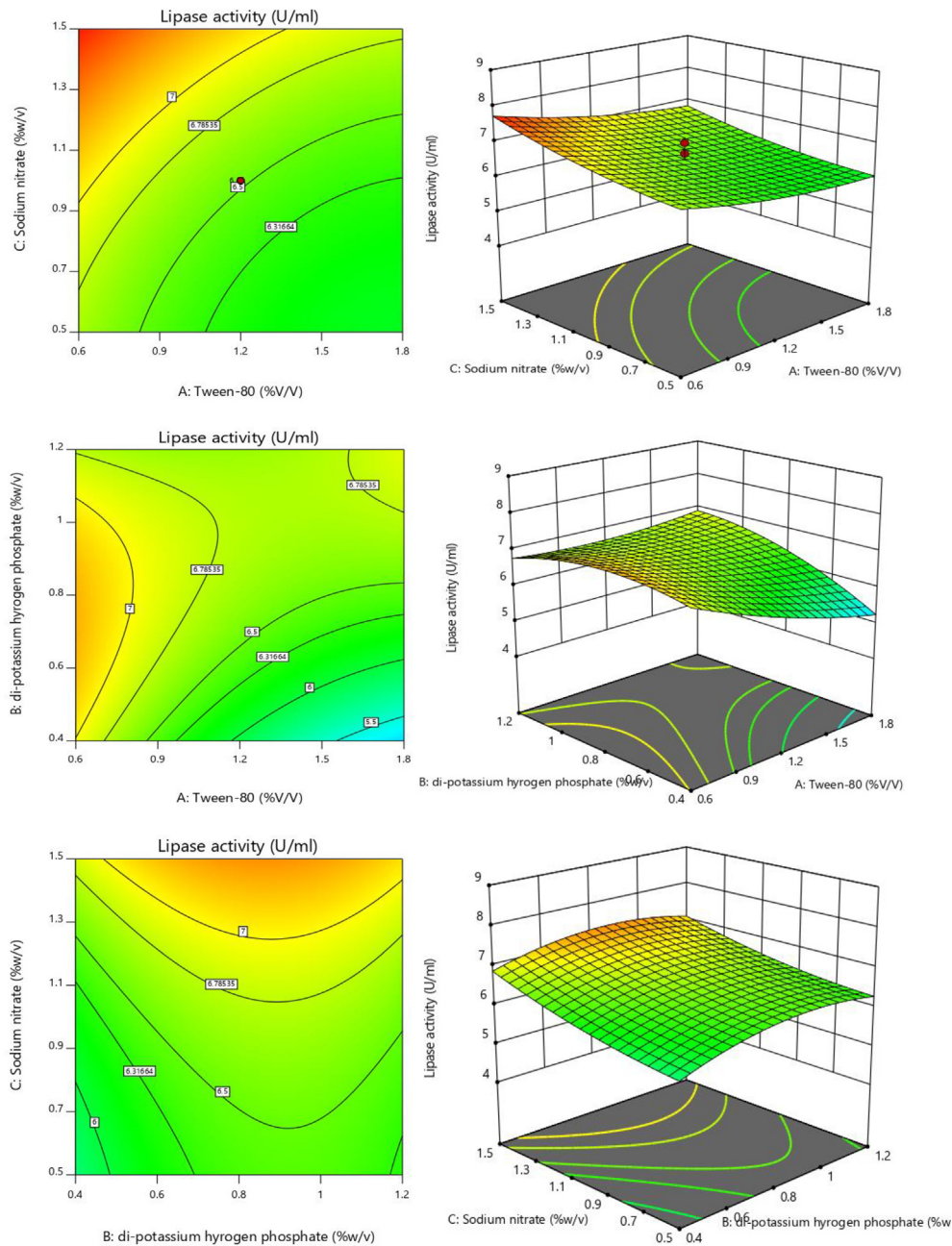


Fig. 7. Response surface graphs depicting the interaction between (a) Sodium nitrate and Tween-80 (b) di-potassium hydrogen phosphate and Tween-80 (c) Sodium nitrate and di-potassium hydrogen phosphate towards halophilic lipase production by *Fusarium solani* NFCCCL 4084.

Table 5

The confirmation report on the prediction and validation of the experimental response surface model.

Factor	Confidence = 95%		n = 6		
	Name	Level	Low level	High level	Actual coding
A	Tween-80	0.90	0.60	1.80	0.90
B	K ₂ HPO ₄	0.72	0.40	1.20	0.72
C	NaNO ₃	1.32	0.50	1.50	1.32

exhibited negative effect were exempted (Table 2). Although, there are other designs for identifying and selecting significant physico-chemical variables for lipase production [40], the present finding affirms the importance of using PB design to screen different vari-

ables to achieve selected significant variables for further stages of optimization [41,25]. The difference between the highest and the least lipase activity was found to be 2.08 U/ml (Table 2). The data clearly indicates the variation effect of medium composition as well as concentrations of variables on lipase activity. The contrast coefficient, which is the average of the difference between the measurements obtained at high (+1) and low (-1) level is as shown in Fig. 1. Further, from these results, it can be noted that malt extract, among variables screened by using PB design, was identified to greatly affect lipase production negatively. Similar results were obtained by Kumar and Gupta [42] who reported that malt extract used for lipase production by *Trichosporon asahii* varies with the type of lipase inducer used and it inhibited lipase production when Tween-80 was used as an inducer.

The assimilation of $MgSO_4$, as previously established, enhanced fungal lipase production [43,44]. This enhancement is associated to the regulatory role it plays by increasing adenosine triphosphate metabolism and nucleic acid synthesis [45,46]. However, these results are in contrary to our findings which showed to affect lipase production negatively. Further, the high magnesium found to be present in POME could be sufficient for lipase synthesis [47].

Further, previous studies by Mukhtar et al. [48] and El-Batal et al. [49] indicated that olive oil exhibits good inductive effect, whereas the present findings showed repressive effect. However, our results are in accordance with studies by Malilas et al. [50], who reported the lipase production by eight fungal strains using olive oil as an inducer in SmF, which showed no noticeable lipase activity from *Fusarium solani* after seven days of incubation. Further, the negative effect of olive oil could indicate that the presence of oleic acid that is essential for lipase production may have been substituted by oleic acid contained in Tween-80.

Based on the PB result analysis of various variables of POME based medium in SmF, the variables found to be having positive effect on lipase production such as Tween-80, K_2HPO_4 , $NaNO_3$, pH and POME were selected for further optimization using OFAT design to identify the optimal level of variables. This is because PB design only reveals the influence of each variable and does not specify the exact quantity of the selected variables. Our selection of agro-industrial POME as a base medium to enhance lipase production is due to the cost-effects that is associated and to address the concerns to conserve the environment. The high lipase activity recorded in the present study could be attributed to the presence of several inorganic minerals present in POME that have shown efficient fungal growth and secretion of extracellular lipase [47]. In contrast, it should be noted that the observed negative effect on lipase production shown by the presence of some inorganic variables ($(NH_4)_2SO_4$, $CaCl_2$, and $MgSO_4$) were in fact found to enhance alkaline lipase production by *Fusarium solani* isolated from entrails of fish [51].

The variation in medium components as well as their concentrations has been found to influence fungal lipases production greatly [16,25]. Therefore, in the present study, we aimed to estimate and establish the required concentrations of selected variables. A significantly high lipase activity of 3.93 (U/ml) recorded with POME could be due to the presence of high content of saturated fatty acids [16]. This is in accordance with the studies of Salihu et al. [52], where POME upon addition to other production components was identified to greatly enhance the lipase production by *Penicillium citrinum*. Our results also reveal that the increase in concentration beyond optimal level leads to a decrease in lipase activity (Fig. 5). This repressive effect therefore, could be linked to increased mineral contents present in POME which are required in small quantities only [47]. Further, it could also be due to increased palm oil concentration which can interfere with the rate of oxygen diffusion and aeration affecting fungal growth negatively and in turn lipase production [16].

The observed significant enhancement in lipase production by *Fusarium solani*, when supplemented with $NaNO_3$, is in accordance with the similar trend reported earlier by Salihu et al. [52]. It has been identified that $NaNO_3$, among components analysed, influence the lipase production by *Penicillium citrinum* (ATCC 42799) positively. This could be explained by the fact that inorganic nitrogen sources are more easily utilised [25]. However, this finding is in contrast to a study where $NaNO_3$ was found to exhibit lowest level of lipase activity among the estimated nitrogen sources [16].

It is evident from the present findings that a significant enhancement of lipase production was exhibited when K_2HPO_4 was selected which is the only mineral salt used. These results are in agreement with the studies of Kumar et al. [53] where

K_2HPO_4 was found to be responsible for maximum lipase production (5.59 IU/ml) by *Bacillus pumilus*.

The enhanced lipase production recorded upon Tween-80 supplementation as carbon source is due to the fact that it can fulfil the dual role as a surfactant as well as inducer for extracellular lipase and this could also be associated to the presence of oleic acid. These results are in accordance with the findings of Salihu et al. [54], where enhanced lipase yield was found while using Tween-80 as carbon source.

The pH of the medium is very critical for lipase production particularly in SmF [5]. The variation in pH is directly associated with the change in lipase activity and this could be explained based on the fact that biological processes require optimum physiological pH [55]. Therefore, the high extracellular lipase secretion recorded over a pH range of 7.0–9.0 may have promoted *Fusarium solani* cell membrane permeability to secrete extracellular lipase efficiently. Further, as expected, the produced lipase is alkaline which it is in agreement with the observations made by Raza et al. [56] also.

In the present study, the overall enhancement of activity shown by *Fusarium solani* lipase using POME-based medium reveals that FCCCD is an important approach to achieve optimum medium composition with minimal supplementation at limited experimental runs to better understand the relationship among the examined variables [54]. The variations exhibited in experimental runs corroborate the demonstration of second order polynomial equation in which lipase production by *Fusarium solani* has shown dependence on the selected medium components.

Based on ANOVA (p -value), the results showed that the model is significant and that there is only 0.01% chance for the model (F -value) to occur as a result of noise. The significance of the model being less than 0.05 ($prob > F$), the three linear terms A (Tween-80), B (K_2HPO_4) and C ($NaNO_3$), one interaction term AB and one quadratic term B were statistically found to be highly significant. The lack of fit, which is obtained through the comparison of residual error to the pure error was found to be significant and therefore, it demonstrates that experimental responses observed effectively fit with the present designed model.

The coefficient of determination (R^2) obtained by ANOVA is used to determine the efficiency of the model and that if the value is closer to 1, then there is a better correlation between the predicted and observed values and the model is regarded as fit. Thus, the results obtained here indicated a value of 0.92 (R^2) as revealed by the model and thus we can conclude that the model is adequate and fit [57]. The adequate precision measures the signal to noise ratio and the value of >4 by a model is required because it will indicate the desirability of the model. Therefore, the high value of adequate precision (13.60) recorded here indicate that the model is desirable and can be used to navigate the design space. The coefficient of variation (CV) indicates the precision and reliability of the experimental responses and thus low CV value (4.57) obtained here indicated a good precision of the experiment.

The analysis of response surface curve generated demonstrates the synergic interaction among the three selected variables. It can be noted that Tween-80 and K_2HPO_4 plot showed the most saddle nature as compared to other interactions plots. This finding affirms the strong inductive influence of Tween-80 on lipase production and it is in agreement with high lipase activity (104 U/ml) which was achieved through synergistic effect of Tween-80 with other factors [42].

In determining the reliability of the designed model, a confirmation sample was obtained by the average of six series of trials at a single combination factor setting which is often one of the solutions recommended by numerical optimization. The analysis of obtained results confirmed that the model is suitable for predicting the results with high correlation.

From our findings, it is important to note that the optimization using FCCCD resulted in a significant increase in lipase production by using an optimized POME based medium containing 0.8 % K_2HPO_4 , 1.84 % $NaNO_3$, 1.2 % Tween-80, and 1.0 % POME at pH 8.5 compared to un-optimized culture medium. This is in accordance with other studies who have employed the statistical experimental design which also resulted in an increase in lipase activity [28,25].

5. Conclusions

In summary, by using combinatory approach of PB design, OFAT design and FCCCD to optimize halophilic lipase production by *Fusarium solani* NCFCL 4084, we have achieved high lipase activity with limited supplementation of POME based production medium. The use of agro-industrial POME is the best alternative substrate for lipase production due to its cost effectiveness, reliability and availability. It is well established that the industrial applicability and adaptability of lipase is largely influenced by the production process. Thus, it is very important to produce a suitable and highly effective lipase at a low cost. From our studies, it is clearly evident that the combinatory approach employed here proved to be very effective in producing halophilic lipases. Further, it is worth mentioning that the low cost production of halophilic extracellular lipase by *Fusarium solani* has not been attempted before by using this combinatorial approach.

Conflict of interest

The authors declare that they have no conflict of interest.

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