Lovastatin Production from Cunninghamella blakesleeana under Solid State Fermentation

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Research

Keywords: Cunninghamella blakesleeana, RSM, Lovastatin, Optimization

Posted Date: May 5th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-455135/v1

Abstract

Our earlier paper had established the fact that new soil fungi known as *Cunninghamella blakesleeana* is potent enough to produce lovastatin significantly. At present, there are no reports on the media optimization for the lovastatin production. Hence, the objective is to optimize the fermentation conditions for lovastatin production by *Cunninghamella blakesleeana* under Solid State fermentation (SSF) condition through screening the critical factors by one factor at a time and then, optimize the factors selected from screening using statistical approaches. SSF was carried using the pure culture of *Cunninghamella blakesleeana* KP780148.1 with wheat bran as substrate. Initial screening was performed for physical parameters, carbon sources and nitrogen sources and then optimized the selected parameters through PBD and BBD. Screening result indicated the optimum values of the analysed parameter for the maximal production of lovastatin by *Cunninghamella blakesleeana* were selected. Out of the nine factors $MgSO_4$, $(NH_4)_2SO_4$, pH and Incubation period were found to influence the lovastatin production significantly after PBD. The optimal levels of these variables and the effect of their mutual interactions on lovastatin production were determined using BBD surface design. The optimum medium composition was found to be MgSO4(0.2 g/L), $(NH_4)_2$ SO_4 (12.5 g/L), pH (6) and Incubation period (7 days). Experimental studies showed a yield of 7.39 mg/g at the above optimized conditions which were observed to be very nearby to the predicted value and hence the model was successfully validated.

Hence, this is the first report on the optimization of critical parameters for lovastatin production by Cunninghamella *blakesleeana*.

Introduction

Hypercholesterolemia is a regular metabolic disorder associated with cardiovascular morbidity and mortality (Csonka et al. 2015). In cholesterol biosynthesis, enzyme hydroxyl methyl glutaryl coenzyme A (HMG-CoA) reductase converts the substrate HMG-CoA to mevalonate. Inhibition of HMG-CoA reductase during cholesterol biosynthesis causes the accumulation of HMG-CoA which was further metabolized to simple compound and none of the lipophilic intermediates were observed. Lovastatin ($C_{24}H_{36}O_5$), a fungal secondary metabolite is a potent antihypercholesterolemic drug which acts as a competitive inhibitor of HMG-CoA reductase (Saleem et al. 2013). Lovastatin was reported to be an effective antitumor agent and plays a vital role in regulation of many physiological responses (Newman et al. 1994; Daborah et al. 1992; Alberts et al. 1980). Hence, it had many clinical applications and possesses more advantages when compared to other available statins. Production of lovastatin through fermentation was more economic than chemical synthesis. Lovastatin, which is an intracellular product, is accrued from the vegetative part of fungi during the polyketide metabolic pathway (Azeem et al. 2018). Filamentous fungi such as *Aspergillus terreus*, *Penicillium* sp., *Monascus ruber* and *Monascus purpureus* have been reported for lovastatin production. Commercial production of lovastatin was commonly performed through *A. terreus* batch fermentation (Manzoni et al. 1999; Szakacs et al. 1998; Kumar et al. 2000; Hajjaj et al. 2001; Maia et al. 2001; Sayyad et al. 2007) and it was typically carried out at 28°C with

a broad pH range from 5.8 to 6.3 (Kumar et al. 2000). Usually deposited growth of *A. terreus* gives high yield than filamentous expansion due to oxygen impediment of viscosity growth (Hajjaj et al. 2001). Fermentation production of lovastatin depends on the media composition in which carbon and nitrogen sources play a crucial role because these nutrients were directly linked with the product formation (Szakacs et al. 1998; Kumar et al. 2000; Hajjaj et al. 2001). Hence, designing an ideal culture condition was a prerequisite and crucial in the production of any metabolites (Embuscado et al. 1994).

A statistical experimental design grants an ordered and proficient design to accomplish the targets. At present, Plackett–Burman design (PBD) and Response Surface Methodology (RSM) which is a central composite method (Suwannarat et al. 2019) widely employed to determine the critical factors quickly from multi-variables (Bae and Shoda, 2005; Usha et al. 2011; Vohra and Satyanarayana, 2002; Baskar and Renganathan, 2009; Vuddaraju et al. 2010; Ramon-Portugal et al. 1997). RSM is a compilation of statistical techniques applied for experimental designs, generating models, determining the influence of factors and detecting the factors of optimum conditions for advantageous responses (Vohra and Satyanarayana, 2002). Statistical experimental design minimizes the error in evaluating the influence of parameters and in parallel it exhibits the parameter variations in a synchronous, ordered and competent way. RSM is a successful optimizing tool which takes the estimated data through a proper experimental design to determine and solve the multivariable parameters (Bae and Shoda, 2005). Mathematical equation was built up to define the joint effect of the applied or test variable in the response. The numerical analysis occupies a predominant role in building up a normal plan for biochemical process optimization (Reynders et al. 1996; Plackett and Burman, 1946; Pandey et al. 2001).

Our previous paper had shown the fact that new soil fungi known as *Cunninghamella blakesleeana* KP780148.1 was a potent producer of lovastatin and till now no work was reported on the optimization of media components for lovastatin production under Solid State fermentation (SSF). Hence, the objective of the current study was to optimize the fermentation conditions for lovastatin production by *Cunninghamella blakesleeana* KP780148.1 under Solid State fermentation (SSF). Since it was difficult to obtain an accurate model for biochemical process such as lovastatin production and the complete laboratory screening will be expensive, we screened using the method critical factors by one factor at a time and then optimizing the factors selected from screening using statistical approaches of Plackett-Burman (PBD) and Box-Behnken Design (BBD).

Materials And Methods

Preparation of seed culture

In Potato Dextrose Agar (PDA) slants, pure culture of *Cunninghamella blakesleeana* KP780148.1 was maintained, which was regularly sub-cultured for every two weeks.
To prepare spore suspension, 0.1% of sterilized Tween-80 was added to a well-sporulated slant and the surface of the slant was scratched with inoculation needle followed by agitation in cyclomixer which helps to suspend the spores evenly. Haemocytometer was used to measure the concentration of spore

suspension and the desired spore level of 1×10^8 spores/mL was achieved by adjusting it with media (Alexopoulos, 1996; Kavitha et al. 2012)

Solid state fermentation (SSF)

The solid substrate wheat bran was dried at 60°C and stored. The wheat bran (10g) was transferred to a Petri plate which was moistened with distilled water containing $MgSO_4.7H_2O$ (0.15 g/L), $(NH_4)_2HPO_4$ (0.25 g/L), NaCl (1 g/L) and then the Petri plates were autoclaved at 121°C for 30 min. After cooling, the solid substrate was inoculated with fungal spore suspension (10%, v/w). The final moisture content of media was adjusted to 60% using sterile distilled water. Then the media was mixed well with sterile stainless-steel spatula and incubated for 7 days at 28°C in humidity-controlled incubator. The fully-grown cultures in Petri plates were harvested and analysed for lovastatin content (Kavitha et al. 2012; Valera et al. 2005)

Lovastatin extraction and estimation

Fermented material was dried at 40°C for 24 h and crushed to powder.
The powdered material (2g) was extracted with 10 mL of ethyl acetate by shaking at
180 rpm for 2 h followed by filtration through Whatmann No. 1 filter paper. To the extract, equal volume of 1% trifluoroacetic acid was added and incubated for 10 min (lactonization of hydroxyl acid form of lovastatin) (Kamath et al. 2015). The filtrate was collected in vials and preserved at 4°C for further analysis. Lovastatin in the clear extract was estimated by high performance liquid chromatography (HPLC) (Shimadzu LC 8A) using a C18 column. A mixture of acetonitrile and water (acidified with 1.1% phosphoric acid) (70:30 v/v) were used as mobile phase. The mobile phase flow rate was maintaine data 1.5 mL/min and lovastatin was detected at 238 nm (Kavitha et al. 2012; Samiee et al. 2003).

Optimization of fermentation conditions for lovastatin production

Initially, the method of one factor at a time was adopted for screening of optimum condition of critical factors such as physical parameters, carbon sources and nitrogen sources then the factors selected from initial screening were optimized using PDB and BBD for optimal production of lovastatin.

One factor at a time

Effect of moisture content

Different moisture contents ranging from 50%, 60%, 70%, 80% and 90% (adjusted with distilled water) was used in the media to understand the influence of water availability, substrate swelling and oxygen diffusion on lovastatin production. The moisture content suitable for maximum lovastatin production was maintained for subsequent setups (Kavitha et al. 2012).

Effect of inoculum size

Different inoculum size ranging from 1% to 15% v/v were used to understand their influence on lovastatin production. A spore suspension of $l \times 10^8$ spores/mL obtained from 4-7 days old culture grown on PDA was used (Naik et al. 2012).

Effect of incubation period

Incubation periods ranging from 1 to 10 days were planned to understand their influence on lovastatin production. The fermentation was performed at different incubation periods while maintaining the other conditions at optimum level. The optimum incubation period achieved was fixed for subsequent experiments (Kavitha et al. 2012).

Effect of initial pH

Different initial media pH of 4, 5, 6, 7 and 8 (adjusted with 1 N HCl or 1 N NaOH) were used to understand their influence on lovastatin production while other parameters were maintained at their optimum level (Kavitha et al. 2012).

Effect of carbon sources

Different carbon sources such as glucose, fructose, sucrose, lactose and maltose with a concentration of 1% w/v (1g) each was used to understand their influence on lovastatin production (Naik et al. 2012).

Effect of nitrogen sources

Different inorganic nitrogen sources such as ammonium carbonate, ammonium sulphate, ammonium chloride and ammonium nitrate was used to understand their influence on optimal lovastatin production (Naik et al. 2012).

Statistical analysis

All the experiments were performed in triplicate to validate the results. Statistical analysis was performed using the latest statistical tool SPSS version 10. The mean and their corresponding standard deviation of the reading were obtained from the test replicates. The efficiency of critical factors was compared with their respective sources by employing independent sample t-test and paired sample t-test. A ‘p’ value of less than 0.0001 was considered as significant.

Statistical model for optimization of process variables for lovastatin production

Screening of nutritional and physical parameters using Plackett- Burman (PB) design

PBD, a first order model having a set of 12 runs of experiment was applied to evaluate the relative significance of 9 factors that influenced lovastatin production in SSF. The 9 independent variables considered for the study incorporated lactose (g/L), $(NH_4)_2\ SO_4$ (g/L), $MgSO_4$ (g/L), $(NH_4)_2\ HPO_4$ (g/L), NaCl (g/L), pH, Moisture Content (%), incubation Periods (days) and inoculum size (%) apart from the 2

unassigned variables called dummy variables. in which $(NH_4)_2$ HPO_4 and NaCl (g/L) were supplemented in the medium during substrate preparation. These variables used in this analysis were numerical factors and subsequently evaluated at two broadly spaced levels assigned as -1 (low level) and +1 (high level) (Table 1). Statistical software package MINITAB 15.0 software (Minitab Ltd., Coventry CV32TE, UK) was used to build a design matrix and the experimental design used was shown in Table 2 (Kavitha et al. 2014).

The complete set of experiment would be performed in triplicate according to designed matrix using the equation.

$Y = \beta_0 + \sum \beta i\, xi$. ……Eqn.1

Where, Y was the response (lovastatin production), β_0 was the model intercept, βi was variable estimates and xi were independent variables. The variables whose confidence level was higher than 95% had significant influence on lovastatin production.

Optimization of significant variables using Box-Behnken design (BBD)

The lovastatin production affected by range of significant factors and the interrelationship between them was optimized by BBD. Based on PBD results,
4 parameters such as $(NH_4)_2$ SO_4 (g/L), $MgSO_4$ (g/L), pH and incubation periods were chosen and the selected parameters were made into 27 runs. Every independent variable was analyzed at three different levels like low, medium and high which was coded as -1, 0, and +1 respectively. The various levels of nutrients were summarized in Table 4 and the experimental design used was shown in Table 5 (Kavitha et al. 2014).

Experiment validation

The mathematical model generated by implementation of RSM was validated by performing the laboratory experiment.

Results

For any metabolite production optimization of media components and culture conditions was a tedious process as it involves analysis of multiple parameters. Hence in the present study, screening for important parameters by one factor at a time were attempted first then we optimized the selected factors along with $(NH_4)_2$ HPO_4 and NaCl (added during solid substrate preparation) by PB design and BB design (Box and Hunter, 1957; Lewis et al. 1998).

One factor at a time

Among the several factors important for microbial growth and metabolite production under SSF, physical parameters like moisture content, pH, inoculum size and incubation period were the critical factors which

would determine the biomass and product formation in fermentation process (Pandey et al. 2001). First the physical parameters were individually analyzed followed by carbon sources (glucose, fructose, sucrose, lactose and maltose) and nitrogen sources (ammonium carbonate, ammonium sulphate, ammonium chloride and ammonium nitrate) through one factor at a time.

Effect of moisture content

The observed moisture content data revealed that the maximum lovastatin production occurred with increase in substrate moisture content up to 60% then it started declining (Fig. 1). Half of the maximum production was observed at 50% of the moisture content and the highest level of lovastatin production was found to be 3 mg/g of DWS (dry weight substrate) by *Cunninghamella blakesleeana* KP780148.1 at 60%. Further increase in the moisture content resulted in sharp decline of lovastatin production to 0.2 mg/g, 0.77 mg/g and 0.54 mg/g at 70%, 80% and 90% respectively.

Effect of inoculum size

A wide range of inoculum size from 1% to 15% v/v which correspond to 1×10^6 to 15×10^6 spores /mL were analyzed for maximum lovastatin production. For our convenience, the inoculum volume was divided into three levels as low (1 to 5 $\times10^6$ spores/mL
(1% to 5% v/v)), medium (6 to 1 $\times10^7$ spores/mL (6% to 10% v/v)) and high
(11 to 15×10^6 spores/mL (11% to 15% v/v)). The results revealed that the medium and high inoculum size yielded the maximum lovastatin production of 19.65 mg/g of DWS and 16.45 mg/g DWS respectively. But the medium inoculum size was found to be significantly different between low and high ($p<0.0001$) individually as shown in
Fig. 2. In general, this result suggested that the inoculum size optimization was mandatory particularly in SSF as less concentration of spores would result in inadequate biomass in contrast to medium and high spore levels. When the individual spore concentration was analyzed 1×10^7 spores/mL (10% v/v) was found to be the optimal inoculum size for maximum lovastatin yield of 5.4 mg/g of DWS (Fig. 3) when compared to higher inoculum size.

Effect of incubation period

Fermentation flasks were incubated for different duration ranging from 1 to 10 days to determine the optimum incubation period. Lovastatin production was analyzed from the second day of incubation. The production of lovastatin was found to be increased linearly up to 3 days and then non-linearly attaining a peak at 7^{th} day with maximal yield of 4.35 mg/g of DWS as shown in Fig. 4. Experiment with a day interval revealed that both the steady and non-steady increase in lovastatin production occurs equally, which indicates that the increased production of lovastatin was non-linear and it also confirms that 7 days of incubation was sufficient for optimal production of lovastatin. The lovastatin production declined almost linearly with half of the maximal production on the 10^{th} day. The percentage of increase and

decrease in lovastatin production was calculated from maximum lovastatin yield with respect to incubation period (Fig. 4).

Effect of initial pH

The pH of the substrate medium possesses a powerful influence on lovastatin production. The effect of initial pH of the substrate medium on lovastatin production was depicted in the Fig. 5. Optimal lovastatin production was documented to be 3.72 mg/g of DWS at pH 6.0. The increased production of lovastatin was observed at a pH 4, 5 and 6 and then it decreased. The percentage of increase and decrease in lovastatin production was calculated from maximum lovastatin yield with respect to different pH range. Before and after the critical pH of 6, both increase and decrease in lovastatin production occurred in a linear fashion. Simultaneously it confirmed that the pH of 6 was optimum for the maximum production of lovastatin by *C. blakesleeana* KP780148.1. Compared to percentage of increased yield of lovastatin per pH, the initial declined production at pH 7 (more rapid to 41%) was possibly due to its denaturation action on the microbial transport cell membrane which was essential for the growth and product synthesis. It was also found that most of the fungal species were active at pH ranged from 3.5 to 7.0 and the decreased pH prevents microbial contamination.

Effect of different carbon sources

To decide the impact of different carbon sources on lovastatin production, 1% w/v of distinctive carbon sources (fructose, sucrose, lactose, maltose and glucose) were supplemented to the solid-state fermentation medium individually. The lovastatin production was not same with different carbon sources and lactose was identified to be the most favorable carbon source for lovastatin production by *C. blakesleeana* KP780148.1 followed by glucose, maltose and sucrose with a yield of 5.17 mg/g, 4.24 mg/g, 3.5 mg/g and 1.52 mg/g of DWS respectively (Fig. 6). On the other hand, fructose was found to be unfavorable for lovastatin production. When compared to lactose, glucose was observed to lack lovastatin production by 18% followed by maltose (32%), sucrose (71%) and fructose (87%). This clearly established the fact that lactose was an excellent carbon source inducer for lovastatin production followed by glucose.

Effect of different nitrogen sources

To study the influence of different nitrogen sources on lovastatin production by *C. blakesleeana* KP780148.1, 1% w/v concentration of ammonium chloride, ammonium nitrate, ammonium carbonate, ammonium sulphate and urea was added to the solid-state fermentation medium. Fig. 7 indicated that lovastatin production was invariably affected by different types of nitrogen sources. Among the different nitrogen sources tested, the best order of lovastatin production was obtained with ammonium sulphate followed by ammonium carbonate and urea with the yield of 5.52 mg/g, 2.4 mg/g and 1.7 mg/g of DWS respectively. Rest of the nitrogen sources produced negligible quantity of lovastatin. When the percentage of maximal lovastatin production by ammonium sulphate was compared with rest of the nitrogen sources, it was found that ammonium carbonate; urea, ammonium

chloride and ammonium nitrate resulted in nearly reduction in yield percentage by 69%, 97% and 98% respectively. The statistical significance was also high ($p<0.0001$) as indicated in the Fig. 7. Hence, the excellent nitrogen source for lovastatin production by *C.blakesleeana* KP780148.1 was found to be ammonium sulphate.

Screening for optimal physical parameters and media components by PBD

From the initial screening results through one factor at a time, parameters such as moisture content, inoculum size, incubation period, pH, lactose, ammonium sulphate along with $(NH_4)_2HPO_4$ and NaCl were selected for subsequent optimization using PBD. PBD was performed to optimize the components of medium for lovastatin production by *C. blakesleeana* KP780148.1. The independent variables of media components with their corresponding minimum and maximum concentration were applied in PBD optimization study (Table 1). 9 selected variables were preferred for PBD with a set of 12 study runs. The design matrix chosen to study the effect of significant variables on lovastatin production and their respective responses were shown in Table 2. The result revealed the maximum lovastatin concentration to be 7.08 mg/g in run 2. However, the outcome of selected variable modeling experiment through PBD showed that only 4 out of 9 parameters significantly affected the lovastatin production. The remaining 5 parameters were unselected owing to their non-significance ($p>0.05$) based on their response under evaluation at the confidence level chosen for the study.

Table1. Plackett–Burman design and media components for lovastatin production by *Cunninghamella blakesleeana*KP780148.1

Variables	Medium components(units)	Low value(-)	High value(+)
A	Lactose (g/L)	5	20
B	$(NH_4)_2SO_4$ (g/L)	5	20
C	$MgSO_4$ (g/L)	0.1	0.3
D	$(NH_4)_2HPO_4$ (g/L)	0.2	0.5
E	NaCl (g/L)	0.5	2.5
F	pH	5	7
G	Moisture Content (%)	50	70
H	Incubation Periods (days)	6	8
J	Inoculum Size (%)	5	15

Table2. Plackett–Burman experimental design with 12 runs with corresponding lovastatin production

Run	A	B	C	D	E	F	G	H	J	Lovastatin(mg/g)
1	+	-	+	-	-	-	+	+	+	6.02
2	+	+	-	+	-	-	-	+	+	7.08
3	-	+	+	-	+	-	-	-	+	6.74
4	+	-	+	+	-	+	-	-	-	3.55
5	+	+	-	+	+	-	+	-	-	6.65
6	+	+	+	-	+	+	-	+	-	5.8
7	-	+	+	+	-	+	+	-	+	5.25
8	-	-	+	+	+	-	+	+	-	5.65
9	-	-	-	+	+	+	-	+	+	6.65
10	+	-	-	-	+	+	+	-	+	5.74
11	-	+	-	-	-	+	+	+	-	6.6
12	-	-	-	-	-	-	-	-	-	5.75

The normal plot of standardized effect of significant parameters revealed the degree and trend of their significant effects (Fig. 8). Lactose, $(NH_4)_2HPO_4$, NaCl, moisture content and inoculum size were considered to have insignificant influence on lovastatin production since it was placed closest to the response line. The result showed that $MgSO_4$ and $(NH_4)_2SO_4$ possess the maximum significant effect on lovastatin production as its effects were placed farthest to the left and right of the response line respectively. Remaining other parameters which showed significant enrichment effect on lovastatin production was pH and incubation period. Among the test variables, normal plot analysis revealed that $MgSO_4$, $(NH_4)_2$ SO_4, pH and incubation period were found to play a significant role in lovastatin production which was also validated by pareto plot of the standardized effect. The primary effects plot was valuable in identifying the lovastatin production at the intermediate level of various parameters of independent variables as shown in Fig. 9. The main effect plot of significant response variable and confirmed the results that were demonstrated in Fig. 8. MgSO4, $(NH_4)_2$ SO_4, pH and incubation period were the parameters which showed good fitted means model in lovastatin production.

The main effect plot was used in conjunction with analysis of variance (ANOVA) to examine the mean difference of selected factors in lovastatin production. The parameters such as $MgSO_4$, $(NH_4)_2SO_4$, pH and incubation period showed the fitted means in
Fig. 9. The statistical analysis by ANOVA were displayed in Table 3 and it ensured that they had the most significant ($p<0.05$) enrichment effect on lovastatin production as supported by its F-value. The linear regression coefficient of determination and R^2 of
98 % indicated that the model was significant. The model revealed that both the parameters $MgSO_4$ and

$(NH_4)_2SO_4$ had the largest coefficient which once again confirmed the fact that they exhibit strong enhancement effect on lovastatin formation apart from pH and incubation period.

Table3. Analysis of variance (ANOVA) of regression model from Plackett-Burman design for significant parameters to lovastatin formation

S. No.	Source	DF	Adj.SS	Adj.MS	F-Value	p-Value
1	Model	9	9.63	1.07	17.58	0.055
2	Linear	9	9.63	1.07	17.58	0.055
3	$(NH4)_2SO^4$	1	1.89	1.89	31.01	0.031
4	$MgSO^4$	1	2.48	2.48	40.80	0.024
5	pH	1	1.54	1.54	25.31	0.037
6	Incubation period	1	1.41	1.41	23.23	0.040
Error	2	0.12	0.06			
Total	11	9.75				

Model Summary: S, 0.246745; R^2, 98.75%; adjusted R^2, 93.13%; predicted R^2, 55.06%; P<0.05, 5% significance level. DF, degrees of freedom; SS, sum of squares; MS, mean sum of squares.

Optimization of significant variables using BBD for lovastatin production using

1. ***C. blakesleeana*** **1**

The influence of different significant parameters on lovastatin production was studied using RSM. Optimization of significant parameters was performed by maintaining the other parameters at constant level. These physical and chemical parameters mostly affect the fungal growth and secondary metabolite production. 2^3 full factorials of BBD and RSM were applied to determine the optimal condition of each evaluated significant variable.
To identify the optimum levels of different significant parameters influencing the lovastatin production, solid state fermentation was carried out in conical flasks with optimized nutrients at optimized physical conditions. Both individual and interactive effects of these variables were also studied by conducting the fermentation run at randomly selected factors and at different levels of all three factors.

The end response was calculated in terms of lovastatin production. A total of 27 experiments was carried out to optimize the significant parameters such as $MgSO_4$ (g/L), $(NH_4)_2$ SO_4 (g/L), pH and incubation periods. These parameters were evaluated at three coded levels namely -1, 0 and +1. The optimum levels of selected significant variables were obtained by solving the regression equation using Design Expert software version 9.0 and by analyzing the response surface and contour plots. Table 4 represented the

coded values and the variables level. The experimental and predicted values were also presented along with the BB experimental design (Table 5). The result data revealed the highest lovastatin concentration of 7.74 mg/g in a run trail from 25-27 as shown in Table 5. These results suggested that parameters such $MgSO_4$ of 0.2 g/L, $(NH_4)_2$ SO_4 of 12.5 g/L, pH of 6 and incubation period of 7 days were the optimum conditions for maximum lovastatin production. Table 4 represented the parameters and their corresponding
X variable. Multiple regression analysis of BBD gave the following quadratic polynomial equation for lovastatin production as shown in Eqn. 2.

$$Y == 7.7433 - 0.1392\ X_1 + 0.1150\ X_2 - 0.0667\ X_3 + 0.0592\ X_4 - 17.29\ X_1^{\ 2} - 0.2092\ X_2^{\ 2} - 0.2342\ X_3^{\ 2} - 0.1904\ X_4^{\ 2} + 0.0250\ X_1\ X_2 - 0.0150\ X_2\ X_3 - 0.0875\ X_1\ X_4 - 0.1225\ X_2\ X_3 + 0.1625\ X_2\ X_4 - 0.0775\ X_3\ X_4.$$

…….Eqn.2

Where, *Y* was the yield of lovastatin in mg/g.

Where, X_1 represents the $MgSO_4$, X_2 denotes $(NH_4)_2SO_4$, X_3 represents pH and X_4 denotes incubation period.

Table4. Experimental ranges and the levels ofindependent variables for *Cunninghamella blakesleeana* KP780148.1

S. No.	Parameters	Variables	Levels		
-1	**0**	**+1**			
1	$MgSO_4$	X_1	0.1	0.2	0.3
2	$(NH_4)_2SO_4$	X_2	5	12.5	20
3	pH	X_3	5	6.5	7
4	Incubation Periods	X_4	6	7	8

Middle vale: high+low/2

Table5. Box–Behnken matrix of three variables in coded units along with the observed responses for *Cunninghamella blakesleeana* KP780148.1

Trails	$MgSO_4$ (g/L)	$(NH_4)_2SO_4$ (g/L)	pH	Incubation Periods (days)	Lovastatin production (mg/g)	Predicted Lovastatin production (mg/g)	Residual
1	0.1	5	6	7	7.52	7.410417	0.109583
2	0.3	5	6	7	7.12	7.082083	0.037917
3	0.1	20	6	7	7.62	7.590417	0.029583
4	0.3	20	6	7	7.32	7.362083	-0.04208
5	0.2	12.5	5	6	7.31	7.24875	0.06125
6	0.2	12.5	7	6	7.28	7.270417	0.009583
7	0.2	12.5	5	8	7.58	7.522083	0.057917
8	0.2	12.5	7	8	7.24	7.23375	0.00625
9	0.1	12.5	6	6	7.31	7.3725	-0.0625
10	0.3	12.5	6	6	7.25	7.269167	-0.01917
11	0.1	12.5	6	8	7.62	7.665833	-0.04583
12	0.3	12.5	6	8	7.21	7.2125	-0.0025
13	0.2	5	5	7	7.08	7.129167	-0.04917
14	0.2	20	5	7	7.52	7.604167	-0.08417
15	0.2	5	7	7	7.26	7.240833	0.019167
16	0.2	20	7	7	7.21	7.225833	-0.01583
17	0.1	12.5	5	7	7.52	7.527083	-0.00708
18	0.3	12.5	5	7	7.3	7.27875	0.02125
19	0.1	12.5	7	7	7.4	7.42375	-0.02375
20	0.3	12.5	7	7	7.12	7.115417	0.004583
21	0.2	5	6	6	7.28	7.332083	-0.05208
22	0.2	20	6	6	7.3	7.237083	0.062917
23	0.2	5	6	8	7.06	7.125417	-0.06542
24	0.2	20	6	8	7.73	7.680417	0.049583
25	0.2	12.5	6	7	7.76	7.743333	0.016667
26	0.2	12.5	6	7	7.69	7.743333	-0.05333
27	0.2	12.5	6	7	7.78	7.743333	0.036667

The variance analysis of quadratic regression model established a highly significant model as evident from very low probability value [(P model > F) = 0.0001] through Fisher's F-test. The strength of interaction between each of the independent variable was found to be significant through student's t-test and p-values. Smaller the
p value and larger the t-value indicates the higher significance of corresponding coefficient. Both squared effect and interactive effect were also observed to be significant as the p was less than 0.05 for lovastatin production as shown in Table 6.
The coefficient of determination (R2) would reflect the goodness of fit in RSM based model. Analysis value of coefficient determination (R2 = 95.18%) indicated that only 4.82% of the total variations was not explained by the model for lovastatin.

Table 6. Analysis of variance for lovastatin production by *Cunninghamella blakesleeana*KP780148.1 in SSF

Source	DF	Adj. SS	Adj. MS	F-Value	P-Value
Model	14	1.16048	0.082892	16.92	0.000*
Regression Linear	4	0.48645	0.121613	24.82	0.000*
C	1	0.23241	0.232408	47.44	0.000*
B	1	0.15870	0.158700	32.39	0.000*
F	1	0.05333	0.053333	10.89	0.006*
H	1	0.04201	0.042008	8.57	0.013*
Square	4	0.45033	0.112583	22.98	0.000*
C X C	1	0.15947	0.159468	32.55	0.000*
B X B	1	0.23334	0.233337	47.63	0.000*
F X F	1	0.29245	0.292448	59.69	0.000*
H X H	1	0.19338	0.193379	39.47	0.000*
Interaction	6	0.22370	0.037283	7.61	0.002*
C X B	1	0.00250	0.002500	0.51	0.489
C X F	1	0.00090	0.000900	0.18	0.676
C X H	1	0.03063	0.030625	6.25	0.028*
B X H	1	0.06002	0.060025	12.25	0.004*
B X H	1	0.10563	0.105625	21.56	0.001*
F X H	1	0.02403	0.024025	4.90	0.047*
Error	12	0.05879	0.004899		
Lack-of-Fit	10	0.05432	0.005432	2.43	0.326
Pure Error	2	0.00447	0.002233		
Total	26	1.21927			

Contour and surface plots for lovastatin (Y1) and test variables (Y2) concentrations

Biomass response plots were made with the vertical axis representing lovastatin concentration (Y1) and two horizontal axis (Y2) representing the most significant two-way interaction of significant parameters obtained from PBD and BBD ($MgSO_4$ (0.2 g/L), $(NH_4)_2SO_4$ (12.5 g/L), pH (6) and incubation period (7 days). The plots led to identify the maximum lovastatin production from various combinations of significant factors to validate their optimum levels. This model provided the best fitted surface response for the lovastatin production as shown in Fig. 10-16. These pictures lucidly established the fact that

lovastatin production was sensitive to minor alterations of test variables. The shape of the contour plot determined the significance of mutual interactions (elliptical or circular). An elliptical and circular property of contour plot showed the interactions between respective variables that were significant and negligible correspondingly. This graphical representation provided a method to visualize the relation between the response and experimental levels of each variable, and the type of interactions between test variables (Rahulan et al. 2009). The optimum value of each variable was located based on the hump in the three-dimensional plot or from the central point of corresponding contour plot. Three-dimensional plots and their respective contour plots provided a flexible and convenient graphical representation to understand the inter-relationship between two variables and keeping the rest of two factors constant at a time. By observing the plot, the optimal fermentation conditions for maximum lovastatin production was observed to be 0.2 g/L of $MgSO_4$, 12.5 g/L of $(NH_4)_2SO_4$, 6-pH and 7 days of incubation period which yielded approximately 7.74 mg/g of lovastatin.

Experiment validation

The experiment was conducted to validate the optimum combination of significant studied parameters, predicted by polynomial model. The optimal values were identified to be $MgSO_4$ = 0.2 g/L; $(NH_4)_2SO_4$ = 12.5 g/L, pH = 6 and incubation
period = 7 days. The model forecasted a maximum response or yield of 7.74 mg/g of lovastatin. Experimental studies showed a yield of 7.39 mg/g after the optimized conditions were observed to be very nearby to the predicted value and hence the model was successfully validated. This statistical approach can be applied for any non-standardized or non-optimized media to produce a desired bioprocess product and hence, it would be a gold viable option.

Discussion

SSF has been continuously investigated for commercial production of potent metabolites and wheat bran was commonly used as a complete solid substrate for the growth and metabolite production by microorganism which lack readily soluble sugars (Stevenson et al. 2012; Szakacs et al. 1998) demonstrated the superiority of SSF over submerged fermentation for lovastatin production and they also showed the use of solid substrates like wheat bran and sweet sorghum pulp. The objective of the current study was to optimize the physical parameters like moisture content, pH, inoculum size and incubation period and nutrients such as carbon and nitrogen sources for maximum production of lovastatin by *C. blakesleeana* KP780148.1 where wheat bran was employed as a standardized substrate for SSF (Ferron et al. (2005) also applied lactose as a carbon source and reported the positive impact on lovastatin production by *A. terreus* which was also observed by other researchers similarly as an enhancer effect of lactose on lovastatin production by several fungal strains (Lai et al. 2007; Osman et al. 2011).

The reason behind the lowest production of lovastatin with moisture content below 60% could be because of the heat generation during metabolic activity, poor solubility of nutrients in the solid substrate

and lesser substrate swelling (Panda et al. 2010). Inadequate oxygen availability due to extreme replacement of air by water in void space might be the reason for lesser production of lovastatin when moisture content was above 60%. In the present study 60% (v/w) of moisture content for maximum lovastatin production by *C. blakesleeana* KP780148.1 was in par with *A. terreus* and *A. flavipes* under SSF (Krishna, 1999; Wei et al. 2007). Moreover, (Kamath et al. 2015) had also demonstrated the maximum production of lovastatin 3.5 mg/g of DWS at 60% moisture content in *A. terreus* (KM017693) which was similar to our result.

(Kamath et al. 2015) had studied the optimum inoculum size in *A. terreus* (KM017963) and identified it to be 10^8 spores/mL with the yield of 3.6 mg/g of DWS but in our study it was observed to be 10^7 spores/mL with the yield of 5.4 mg/g of DWS

by *C.blakesleeana* KP780148.1. A recent study in 2012 (Prabhakar et al. 2012) on

A. terreus KLVB28mu21 reported the optimum condition of pH as 5.5, moisture content as 65% and an inoculum size as 1×10^8 spores/mL whereas this study had a negligible variation in all the parameters. The observed variation in the parameters may be due to the metabolic rate of the organism. The present study correlated with the findings observed by (Lopez et al. 2003; Szakacs et al. 1998) with the optimum yield of lovastatin.

In the present study, the result showed that the maximum yield of lovastatin was 4.35 mg/mg of DWS at 7th day in a non-steady state and thereafter, declining phase of yield continued till 10th day which may be due to the onset of organisms death phase subsequent to exhaustion of nutrients and toxin accumulation which was in contrast with results of Pie-Lian and co-workers 2007 (Wei et al. 2007). Similarly, (Osman et al. 2011) tested the efficiency of *A. terreus* strain incubation period and concluded that 8 days of incubation period was sufficient to produce maximal quantity of 45.89 µg/ml lovastatin. In support to the present results, there were numerous literatures that had reported that the broad range of incubation period ranging from 6 to 9 days were optimum for production of lovastatin by many fungi (Endo, 1979; Moore et al. 1985; Gunde-Cimerman et al. 1993; Shindia, 1997). To be more specific, (Samiee et al. 2003) concluded that 7 days of incubation period for *A. terreus* was sufficient for the maximal yield of 55mg per liter lovastatin. Also, (Lopez et al. 2003) observed the optimal yield on the 7th day followed by which the production diminished which was in par with the present results. In contrast, (Elsoud, 2016) observed a maximum yield of 0.87 mg/mL at 10th day of incubation in case of *A. fumigatus.* A report by (Atalla et al. 2008) in *A. terreus* J9 using Dox-rice medium concluded that incubation duration of 4 days was sufficient to cause an optimum

production of lovastatin (932.15 mg/L). The exponential growth of mycelium occurs during trophase resulting in absence or little secondary metabolite product but when the organism enters idiophase, secondary metabolites were produced till lysis (Borrow et al. 1961).

This study demonstrated the potential of *C. blakesleeana* KP780148.1 to

yield maximum lovastatin of 3.72mg/g DWS at pH 6.0 for the first time. Similarly,

(Attala et al. 2008) showed that *A.terreus* could synthesis mevinolin of 96.22 mg/L at pH-6.5. Kamath *et al* in 2015 investigated *A. terreus* (KM017963) under SSF and concluded that the optimum pH was 6 for the maximum yield of lovastatin (3.60mg/g of DWS) which was in accordance with the present report. Other studies (Valera et al. 2005; Chanakya, 2011) had shown that in case of *A. fischeri* and *A. flavipes*, a pH of 5 was sufficient to produce maximum lovastatin. (Elsoud, 2016) determined the range of pH in *Aspergillus fumigates* to be 5.5-6 with the optimum production of lovastatin (0.228 mg/mL) which nearly coincided with present finding. Also, (Shinda et al. 1997) had observed that the pH range from 5.5 to 6 was sufficient for the maximum production as similar to (Samiee et al. 2003) whereas (Lopez et al. 2003) studied the effect of pH on *A. terreus* ATCC 20542 and identified the optimum to be 6.5. (Osman et al. 2011) reported a broad range of pH from 7 to 9 for the optimum production of lovastatin (66.69 µg/mL) with a maximum peak at a pH of 8 in case of *A. terreus* in contrast.

In this study, 5 carbon sources (2 monosaccharides and 3 disaccharides) were evaluated for the potent production of lovastatin and found that the lactose was highly inducible (5.17mg/g of DWS) by *C. blakesleeana* KP780148.1 which was similar to a study reported by (Chanakya, 2011) in *A. fischeri*. In contrast, (Kamath et al. 2015) suggested that lactose showed a negative effect in *A. terreus* when added to a solid substrate for lovastatin production and reported that disaccharides did not showed any positive action on lovastatin production. But in similar, he had observed the positive effect with glucose which coincided with this present report as it was a monosaccharide which gets rapidly metabolized by various fungal strain. (Miyake et al. 2006) had demonstrated glucose as a repressor for lovastatin production in *Monascus pilosus* in contrast to Julio (Alarcon et al. 2003) work on *Pleurotus ostreatus* and this study.

In accordance with this present report, (Lopez et al. 2003) had shown that lactose was an excellent inducer of lovastatin with maximum titers on *A. terreus* ATCC 20542 and *Aspergillus terreus var. aureus* (MUCL 38997).

(Kamath et al. 2015) found that the nitrogen sources such as ammonium molybdate, ammonium oxalate, ammonium sulphate, ammonium nitrate, yeast extract, malt extract and peptone (1%, 2%, 3%, 4% and 5% w/v) did not possess any effect on the productivity of lovastatin in *A. terreus* (KM017963) strain and concluded that nitrogen sources would induce biomass growth alone whereas the present result showed that ammonium sulphate induced a yield of 5.52 mg/g of DWS. Most of the investigators had employed organic nitrogen source like peptone for their study and proved the maximum lovastatin production in *Monascus ruber* (Chang et al. 2002; Chang et al. 2002) and *Monascus pilosus* (Miyake et al. 2006). Likewise, some of the investigators had used yeast extract as a nitrogen source for lovastatin production by *A. terreus* (Lai et al. 2003). Few of the authors had reported the usage of corn steep liquor in *Aspergillus terreus var. aureus* (MUCL 38997) (Chang et al. 2002). But in the present investigation, the potential impact of organic nitrogen sources in lovastatin production was not analysed which might be the limitation of this study. Mevinolin production by *A. terreus* was established by (Atalla et al. 2008) using ammonium sulphate in the medium similar to this study.

(Hajjaj et al. 2001) concluded that urea and ammonium nitrate was a poor yielder of lovastatin which coincided with current result.

(Kavitha et al. 2014) had optimized the nutritional components statistically for lovastatin formation in SSF using PBD and BBD and thereby, obtained a lovastatin concentration of 1.20 mg/g. Further the validation was done experimentally by *A. terreus* MTCC 1782 which showed a concentration of 1.19 mg/g which was very close to statistical result. This research work had highlighted the importance of statistical approach similar to our study. (Wasko et al. 2010) had optimized the media components for the biomass formation of *Lactobacillus rhamnosus* OXY and had demonstrated the importance of PBD and RSM similar to our study.

Conclusion

This study demonstrated that maximum lovastatin could be produced by applying SSF and it was proved to be an excellent alternative for submerged fermentation.
Also the current study high-lighted the importance of statistical approaches employed for optimizing the critical factors necessary for maximum lovastatin production by *C. blakesleeana* KP780148.1. This is the first study on the optimization of critical parameters for lovastatin production by *C. blakesleeana* KP780148.1 and this study also recommended the routine use of statistical approaches for optimization of any non-defined media components and conditions since it was more economic and convenient.

Declarations

Supplementary Information

No additional files included

Acknowledgement

The authors thank the DST FIST (SR/FST/LS-I/2017/10) supported Department of Microbial Biotechnology, Bharathiar University, Coimbatore, Tamil Nadu, India, for providing the common instrumentation facility.

Author's contribution

JB, TM, VK, KJ and DE conceived and wrote the manuscript. AJ supervised the whole research and revised the manuscript. All authors read and approved the final manuscript for publication.

Funding

Not applicable

Availability of data and materials

All data obtained or analyzed during this study are included in this article and available from the corresponding author.

Declaration

Ethics approval and consent to participate

Not applicable.

Consent for publication

All the authors have read and approved the manuscript before the submission.

Competing interests

The authors declare that they have no competing interests.

Abbreviations

SSF: Solid State fermentation; $MgSO_4$: magnesium sulfate; $(NH_4)_2SO_4$: Ammonium Sulphate; RSM: response surface methodology; HMG-CoA: hydroxyl methyl glutaryl coenzyme A; PBD: Plackett–Burman design; BBD: Box-Behnken Design; PDA: Potato Dextrose Agar; NaCl: sodium chloride; (NH4)2HPO4: Diammonium Phosphate; HPLC: high performance liquid chromatography; ANOVA: Analysis of variance; DF: degrees of freedom; SS: sum of squares; MS: mean sum of squares; ATCC: American Type Culture Collection.

References

Alarcon J, Aguila S, Arancibia-Avila P, Fuentes O, Zamorano-Ponce E, Hernandez M (2003) Production and purification of statins from Pleurotus ostreatus (Basidiomycetes) strains. Zeitschrift für Naturforschung C 58(1-2), 62-64

Alberts AW, Chen J, Kuron G, Hunt V, Huff J, Hoffman C, Patchett A (1980) Mevinolin: a highly potent competitive inhibitor of hydroxymethylglutaryl- coenzyme A reductase and a cholesterol-lowering agent. Proceessdings of the National Academy of Sciences 77(7), 3957-3961

Alexopoulos CJ, Mims CN, Blackwell M (1996) Introductory Mycology. John Willey & Sons Inc. New York: USA Google Scholar.

Atalla MM, Hamed ER, El-Shami AR (2008) Optimization of a culture medium for increased mevinolin production by Aspergillus terreus strain. Malaysian Journal of Microbiology 4(2), 6-10

Azeem M, Saleem Y, Hussain Z, Zahoor S, Javed MM (2018) Optimization of culture conditions for the production of lovastatin by Aspergillus terreus in submerged fermentation. Pharmaceutical Chemistry

Journal 52(3), 284-289

Bae S, Shoda M (2005) Statistical optimization of culture conditions for bacterial cellulose production using Box-Behnken design. Biotechnology and bioengineering 90(1), 20-28

Baskar G, Renganathan S (2009) Statistical screening of process variables for the production of L-asparaginase from cornflour by Aspergillus terreus MTCC 1782 in submerged fermentation. Indian Journal of Science and Technology 2(5), 45-48

Borrow A, Jefferys EG, Kessell RHJ, Lloyd EC, Lloyd PB, Nixon IS (1961) The metabolism of Gibberella fujikuroi in stirred culture. Canadian Journal of Microbiology 7(2), 227-276

Box GE, Hunter JS (1957) Multi-factor experimental designs for exploring response surfaces. The Annals of Mathematical Statistics 195-241

Chanakya P (2011) Solid state fermentation for the production of lovastatin by Aspergillus fischeri. Research Journal of Pharmaceutical Science and Biotechnology 1(1)

Chang YN, Huang JC, Lee CC, Shih L, Tzeng YM (2002) Use of response surface methodology to optimize culture medium for production of lovastatin by Monascus ruber. Enzyme and Microbial Technology 30(7), 889-894

Chang YN, Lin YC, Lee CC, Liu BL, Tzeng YM (2002) Effect of rice-glycerol complex medium on the production of lovastatin byMonascus ruber. Folia microbiologica, 47(6), 677-684.

Csonka C, Sarkozy M, Pipicz M, Dux L, Csont T (2015) Modulation of hypercholesterolemia- induced oxidative/nitrative stress in the heart. Oxidative medicine and cellular longevity 2016

Daborah RA, Lein J, Conder MJ, Tewalt GL (1992) Enzymatic deacylation of simvastatin. British Patent GB2255974

Elsoud MA (2016) Optimization of lovastatin production under sub-merged fermentation and its effect on cholesterol blood level in rats. Journal of Innovations in Pharmaceuticals and Biological Sciences 33-42

Embuscado ME, Marks JS, BeMiller JN (1994) Bacterial cellulose II Optimization of cellulose production by Acetobacter xylinum through response surface methodology. Food hydrocolloids 8(5), 419-430

Endo A, Monacolin K (1979) a new hypocholesterolemic agent produced by a Monascus species. The Journal of antibiotics 32(8), 852-854

Ferron MV, Lopez JC, Perez JS, Sevilla JF, Chisti Y (2005) Rapid screening of Aspergillus terreus mutants for overproduction of lovastatin. World Journal of Microbiology and Biotechnology 21(2), 123-125

Gunde-Cimerman N, Friedrich J, Cimerman A, Benicki N (1993) Screening fungi for the production of an inhibitor of HMG CoA reductase Production of mevinolin by the fungi of the genus Pleurotus. FEMS

microbiology letters, 111(2-3), 203-206

Hajjaj H, Niederberger P, Duboc P (2001) Lovastatin biosynthesis by Aspergillus terreus in a chemically defined medium. Applied and environmental microbiology 67(6), 2596-2602

Kamath PV, Dwarakanath BS, Chaudhary A, Janakiraman S Optimization of culture conditions for maximal lovastatin production by Aspergillus terreus (KM017963) under solid state fermentation. HAYATI Journal of Biosciences 2015; 22(4): 174-180

Kavitha V, Janani B, Angayarkanni J (2014) Statistical Optimization of Nutritional Composition for Lovastatin Production in Solid State Fermentation. International Journal of Biotechnology Photon 113, 399-408

Kavitha V, Janani B, Angayarkanni J (2012) Optimization of process parameters for lovastatin production from red gram bran by solid state fermentation. Int J Sci Res 3, 1413-1418

Krishna C (1999) Production of bacterial cellulases by solid state bioprocessing of banana wastes. Bioresource technology 69(3), 231-239

Kumar M S, Jana S K, Senthil V, Shashanka V, Kumar S V, Sadhukhan A K (2000) Repeated fed-batch process for improving lovastatin production. Process Biochemistry 36(4), 363-368

Lai LST, Hung CS, Lo CC (2007) Effects of lactose and glucose on production of itaconic acid and lovastatin by Aspergillus terreus ATCC 20542. Journal of bioscience and bioengineering 104(1), 9-13

Lai LST, Pan CC, Tzeng BK (2003) The influence of medium design on lovastatin production and pellet formation with a high-producing mutant of Aspergillus terreus in submerged cultures. Process Biochemistry 38(9), 1317-1326

Lewis GA, Mathieu D, Phan-Tan-Luu R (1998) Pharmaceutical experimental design CRC Press

Lopez JC, Perez JS, Sevilla JF, Fernandez FA, Grima EM, Chisti Y (2003) Production of lovastatin by Aspergillus terreus: effects of the C: N ratio and the principal nutrients on growth and metabolite production. Enzyme and Microbial Technology 33(2), 270-277

Maia MMD, Heasley A, De Morais MC, Melo EHM, Morais MA, Ledingham WM, Lima Filho JL (2001) Effect of culture conditions on lipase production by Fusarium solani in batch fermentation. Bioresource Technology 76(1), 23-27

Manzoni M, Bergomi S, Rollini M, Cavazzoni V (1999) Production of statins by filamentous fungi. Biotechnology letters 21(3), 253-257

Miyake T, Uchitomi K, Zhang MY, Kono I, Nozaki N, Sammoto H, Inagaki K (2006) Effects of the principal nutrients on lovastatin production by Monascus pilosus. Bioscience, biotechnology and

biochemistry 70(5), 1154-1159

Moore RN, Bigam G, Chan JK, Hogg AM, Nakashima TT, Vederas JC (1985) Biosynthesis of the hypocholesterolemic agent mevinolin by Aspergillus terreus and Determination of the origin of carbon, hydrogen, and oxygen atoms by carbon-13 NMR and mass spectrometry. Journal of the American Chemical Society 107(12), 3694-3701

Naik A, Lele S (2012) Solid state fermentation of pomegranate seed for lovastatin production A bioprocessing approach. Advances in Bioscience and Biotechnology 3(05), 643

Newman A, Clutterbuck RD, Powles RL, Millar JL (1994) Selective inhibition of primary acute myeloid leukaemia cell growth by lovastatin. Leukemia 8(2), 274-280

Osman ME, Khattab O H, Zaghlol GM, El-Hameed RA (2011) Screening for the production of cholesterol lowering drugs (lovastatin) by some fungi. Aust J Basic Appl Sci 5(6), 698-703

Osman ME, Khattab OH, Zaghlol GM, El-Hameed RA (2011) Optimization of some physical and chemical factors for lovastatin productivity by local strain of Aspergillus terreus. Australian Journal of Basic and Applied Sciences 5(6), 718-732

Panda BP, Javed S, Ali M (2010) Optimization of fermentation parameters for higher lovastatin production in red mold rice through co-culture of Monascus purpureus and Monascus ruber. Food and bioprocess technology 3(3), 373-378

Pandey A, Soccol CR, Rodriguez-Leon JA, Nigam P (2001) Production of organic acids by solid state fermentation. Solid state fermentation in Biotechnology-Fundamentals and applications. Asitech Publishers N. Delhi 113-126

Plackett RL, Burman JP (1946) The design of optimum multifactorial experiments. Biometrika 33(4), 305-325

Prabhakar M, Lingappa K, BABU V, Amena S, Vishalakshi N (2012) Characterization of physical factors for optimum lovastatin production by Aspergillus terreus KLVB28mu21 under solid state fermentation. Journal of Recent Advances in Applied Sciences 27

Rahulan R, Nampoothiri KM, Szakacs G, Nagy V, Pandey A (2009) Statistical optimization of l-leucine amino peptidase production from Streptomyces gedanensis IFO 13427 under submerged fermentation using response surface methodology. Biochemical Engineering Journal 43(1), 64-71

Ramon-Portugal F, Delia-Dupuy ML, Pingaud H, Riba JP (1997) Kinetic study and mathematical modelling of the growth of S. cerevisiae 522D in presence of K2 killer protein. Journal of chemical technology and biotechnology 68(2), 195-201

Reynders MB, Rawlings DE, Harrison STL (1996) Studies on the growth, modelling and pigment production by the yeast Phaffia rhodozyma during fed-batch cultivation. Biotechnology letters 18(6), 649-654

Saleem F, Ambreen A, Saleem Y, Naz S, Ahmad A, Syed, Q (2013) Production and optimization of lovastatin by solid state fermentation using Aspergillus terreus. Global J Sci Res 1(2), 33e41

Samiee SM, Moazami N, Haghighi S, Aziz Mohseni F, Mirdamadi S, Bakhtiari MR (2003) Screening of lovastatin production by filamentous fungi. Iranian Biomedical Journal 7(1), 29-33

Sayyad SA, Panda BP, Javed S, Ali M (2007) Optimization of nutrient parameters for lovastatin production by Monascus purpureus MTCC 369 under submerged fermentation using response surface methodology. Applied Microbiology and Biotechnology 73(5), 1054-1058

Shindia AA (1997) Mevinolin production by some fungi. Folia microbiologica 42(5), 477-480

Stevenson L, Phillips F, O'sullivan K, Walton J (2012) Wheat bran its composition and benefits to health, a European perspective. International journal of food sciences and nutrition 63(8), 1001-1013

Suwannarat S, Iewkittayakorn J, Sukpondma Y, Rukachaisirikul V, Phongpaichit S, Chotigeat, W (2019) Optimization of the production of lovastatin from Aspergillus sclerotiorum PSU-RSPG178 under static liquid culture using response surface methodology. Sains Malays 48(1), 93-102

Szakacs G, Morovjan G, Tengerdy RP (1998) Production of lovastatin by a wild strain of Aspergillus terreus. Biotechnology Letters 20(4), 411-415

Usha RM, Rastogi NK, Appaiah KA (2011) Statistical optimization of medium composition for bacterial cellulose production by Gluconacetobacter hansenii UAC09 using coffee cherry husk extract-an agro-industry waste. Journal of microbiology and biotechnology 21(7), 739-745

Valera HR, Gomes J, Lakshmi S, Gururaja R, Suryanarayan S, Kumar D (2005) Lovastatin production by solid state fermentation using Aspergillus flavipes. Enzyme Microb. Technol.37, 521–526

Vohra A, Satyanarayana T (2002) Statistical optimization of the medium components by response surface methodology to enhance phytase production by Pichia anomala. Process Biochemistry 37(9), 999-1004

Vuddaraju SP, Nikku MY, Chaduvula AIR, Dasari VRRK, Donthireddy SRR (2010) Application of statistical experimental designs for the optimization of medium constituents for the production of L-asparaginase by Serratia marcescens. J Microbial Biochem Technol 2, 89-94

Wasko A, Kordowska-Wiater M, Podlesny M, Polak-Berecka M, Targonski Z, Kubik-Komar A (2010) The plackett-burman design in optimization of media components for biomass production of Lactobacillus rhamnosus OXY. Acta Biologica Hungarica 61(3), 344-355

Wei PL, Xu ZN, Cen PL (2007) Lovastatin production by Aspergillus terreus in solid-state fermentation. Journal of Zhejiang University-Science A 8(9), 1521-1526

Figures

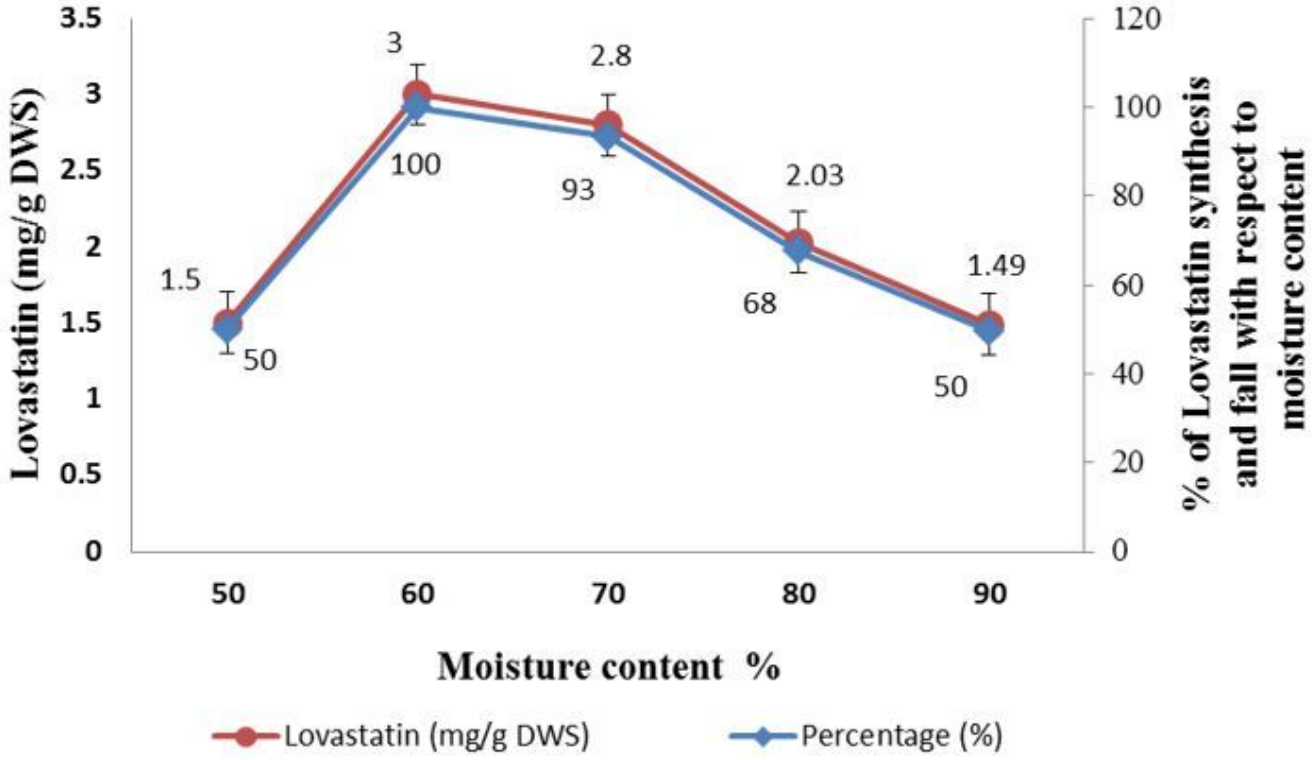

Figure 1

Effect of moisture content (%) on lovastatin production in CunninghamellablakesleeanaKP780148.1 along with percentage of increase and decrease with respect to solid substrate moisture content.DWS = Dry Weight Substance.

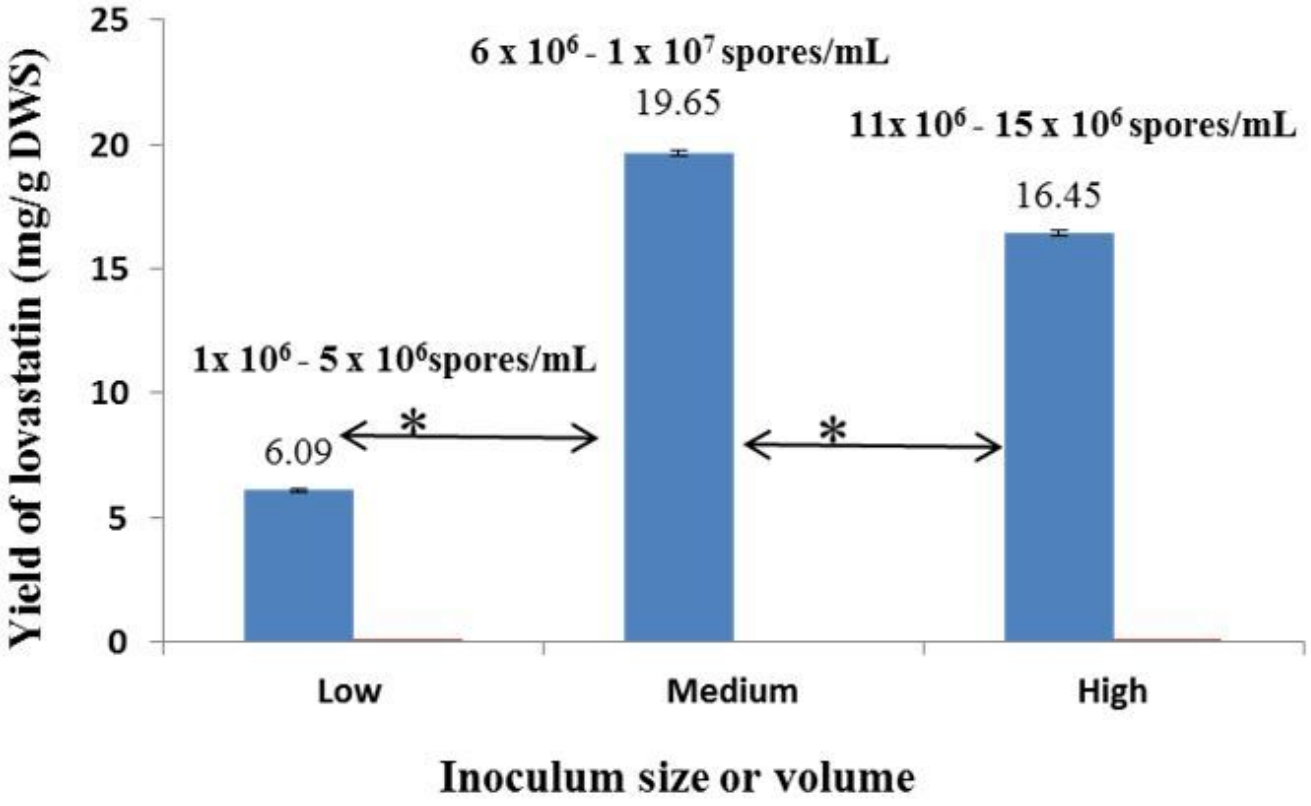

Figure 2

Effect of initial inoculum size range on the production of lovastatin fromCunninghamellablakesleeanaKP780148.1.Low represents 1% to 5% v/v,

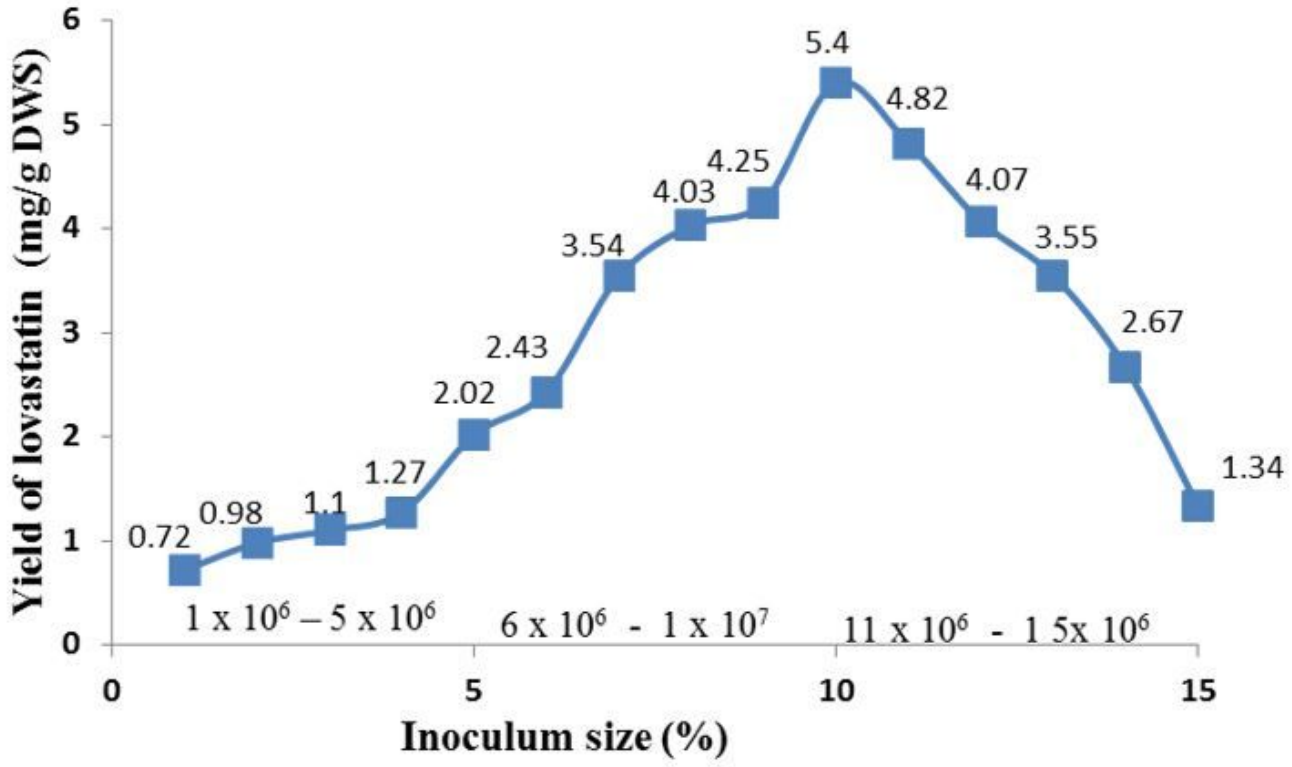

Figure 3

Influence of inoculum size (%) on the production of lovastatin from CunninghamellablakesleeanaKP780148.1.The graph depicts the corresponding spores/mL of the inoculum size (%) with a peak at 10% containing 1x107spores/mL and also representing both progressive elevation and decline in the production of lovastatin.

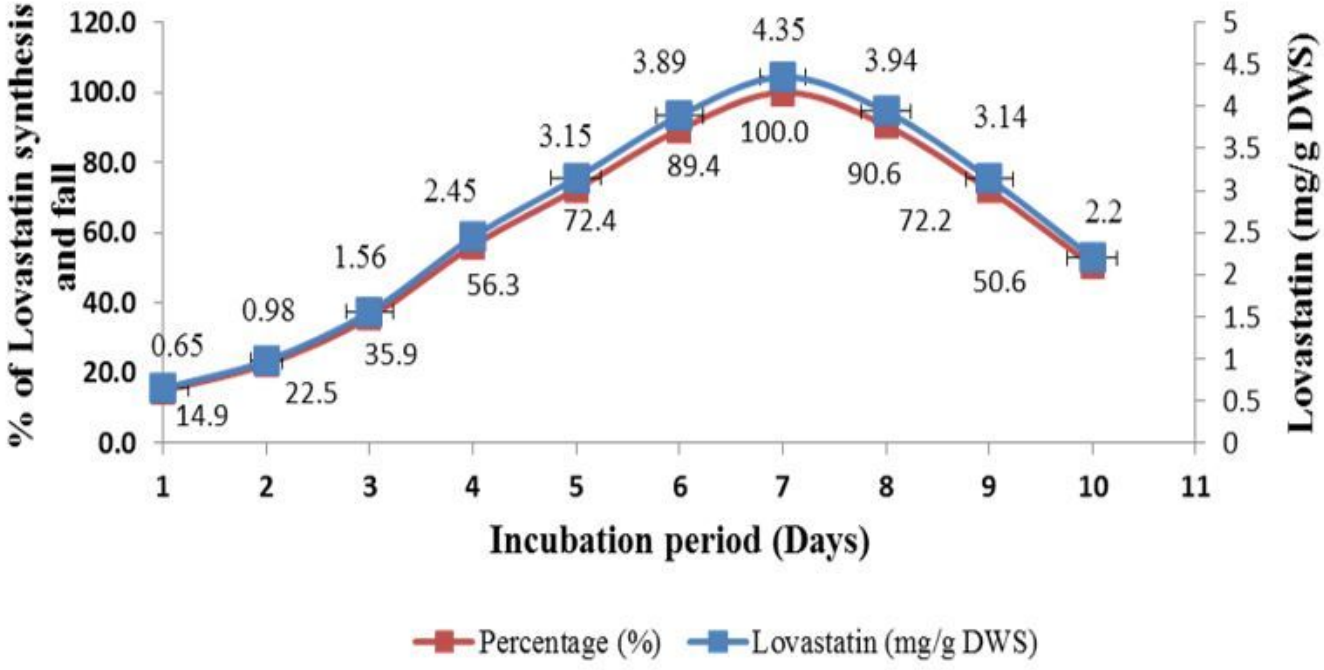

Figure 4

Effect of incubation on the production of lovastatin along with the percentage of increase and decrease. Both linear and non-linear progression of lovastatin production occurs in the graph reaching a peak at 7th day of incubation period and further decline happens in linear fashion.

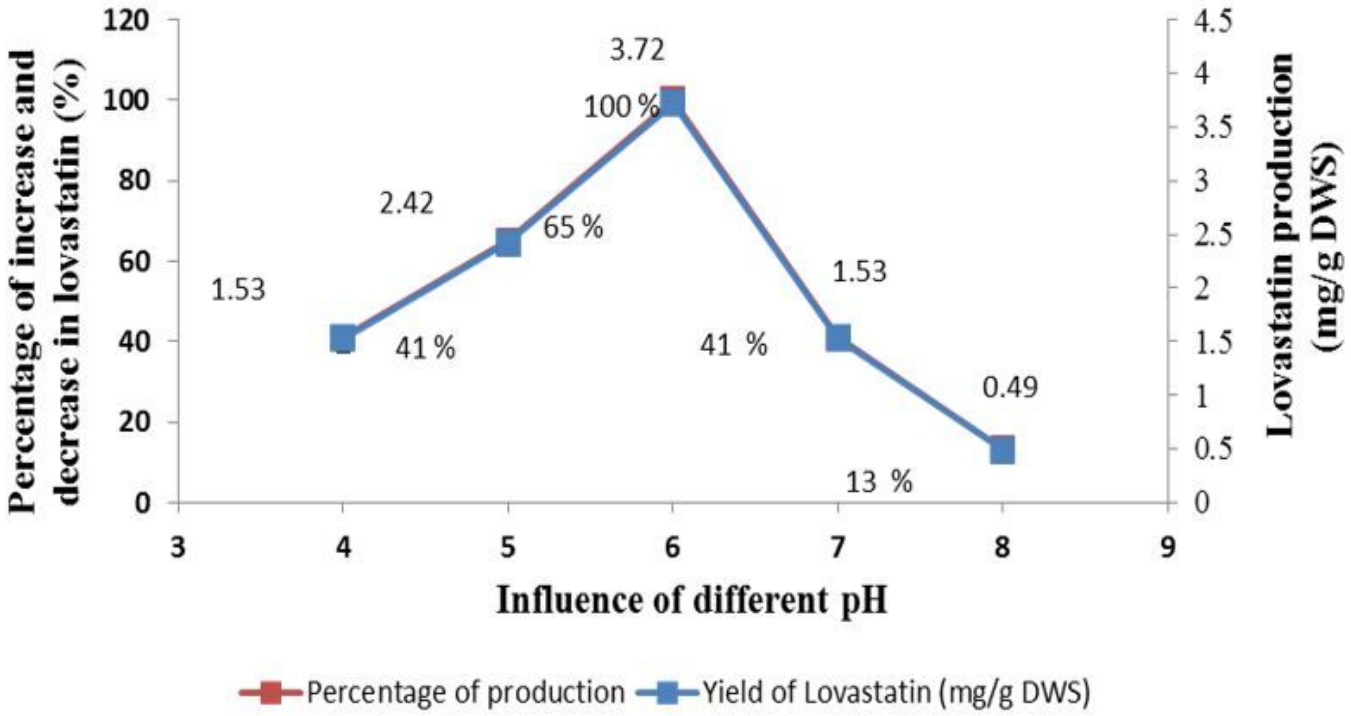

Figure 5

Effect of pH on the production of lovastatin along with the percentage of increase and decrease. Linear progressive production of lovastatin was observed till pH 6.0 and thereafter a linear sharp decline to half of the maximum production at pH 8.0.

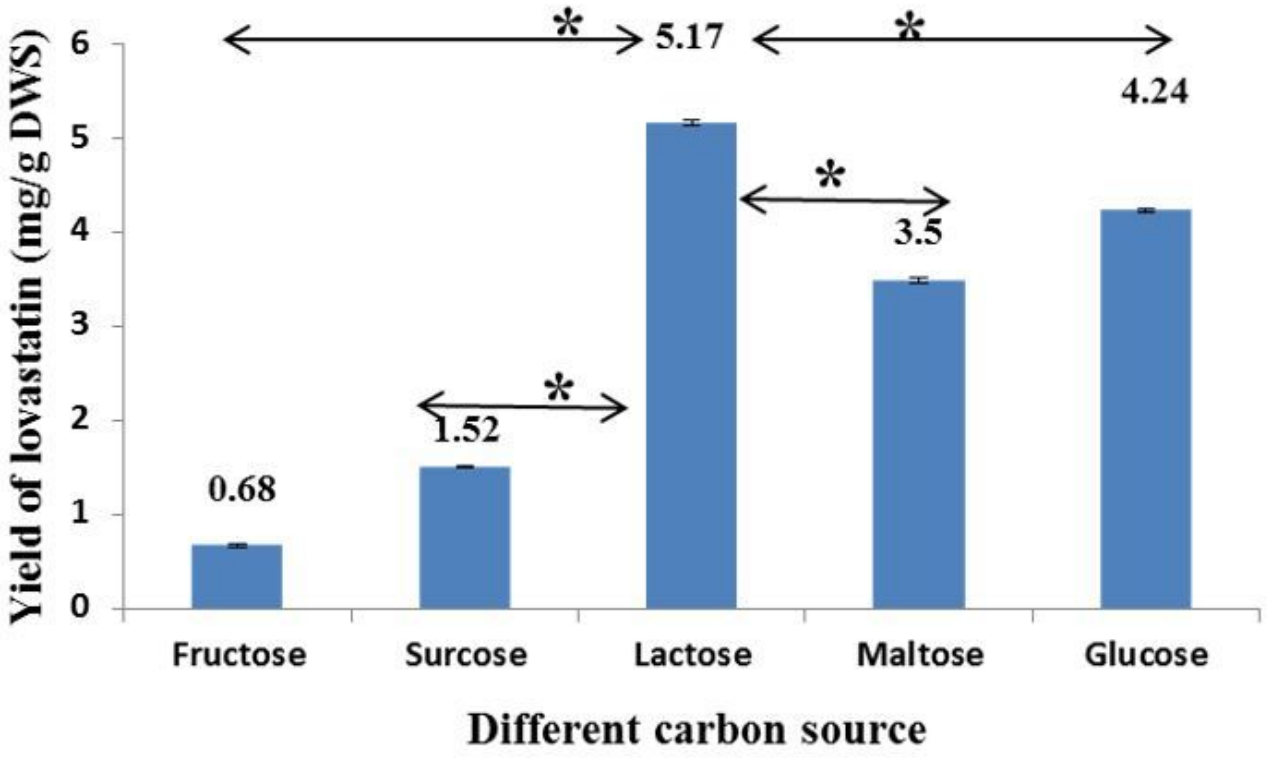

Figure 6

Effect of different carbon sources on the production of lovastatin. The graph respresents the statistical siginifcance of lactose with respective carbon source through double arrow line with a * representing to the corresponding p value of less than 0.0001.

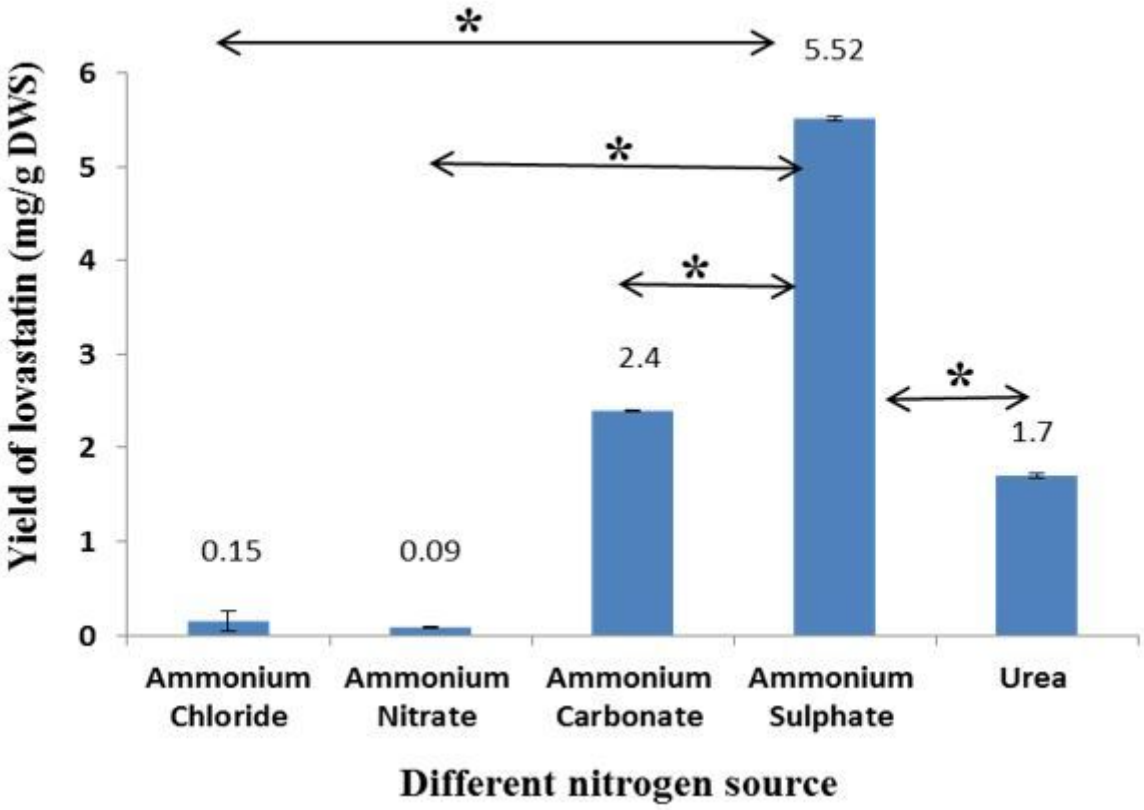

Figure 7

Effect of various nitrogen sources studied on the production of lovastatin fromCunninghamellablakesleeanaKP780148.1. The graph respresents the statistical siginifcance of ammonium sulphate with respective nitrogen source through double arrow line with a * representing to the corresponding p value of less than 0.0001.

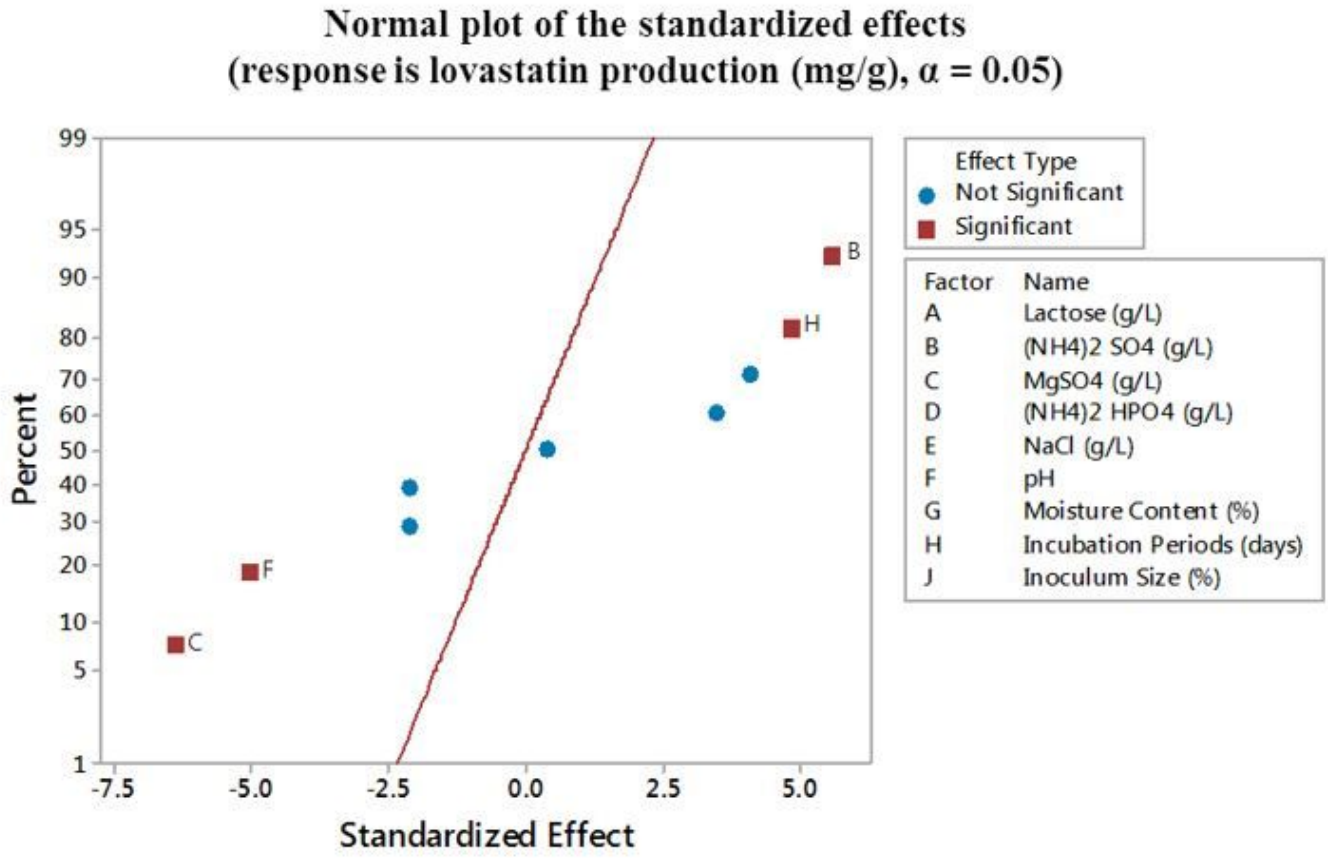

Figure 8

Normal plot of standardized effects of selected parameters of a Plackett-Burman design for lovastatin production.The plot reveals both the significant and non significant parameters.Red boxes refers the significant parameters and blue circle refers to insignificant prameters.

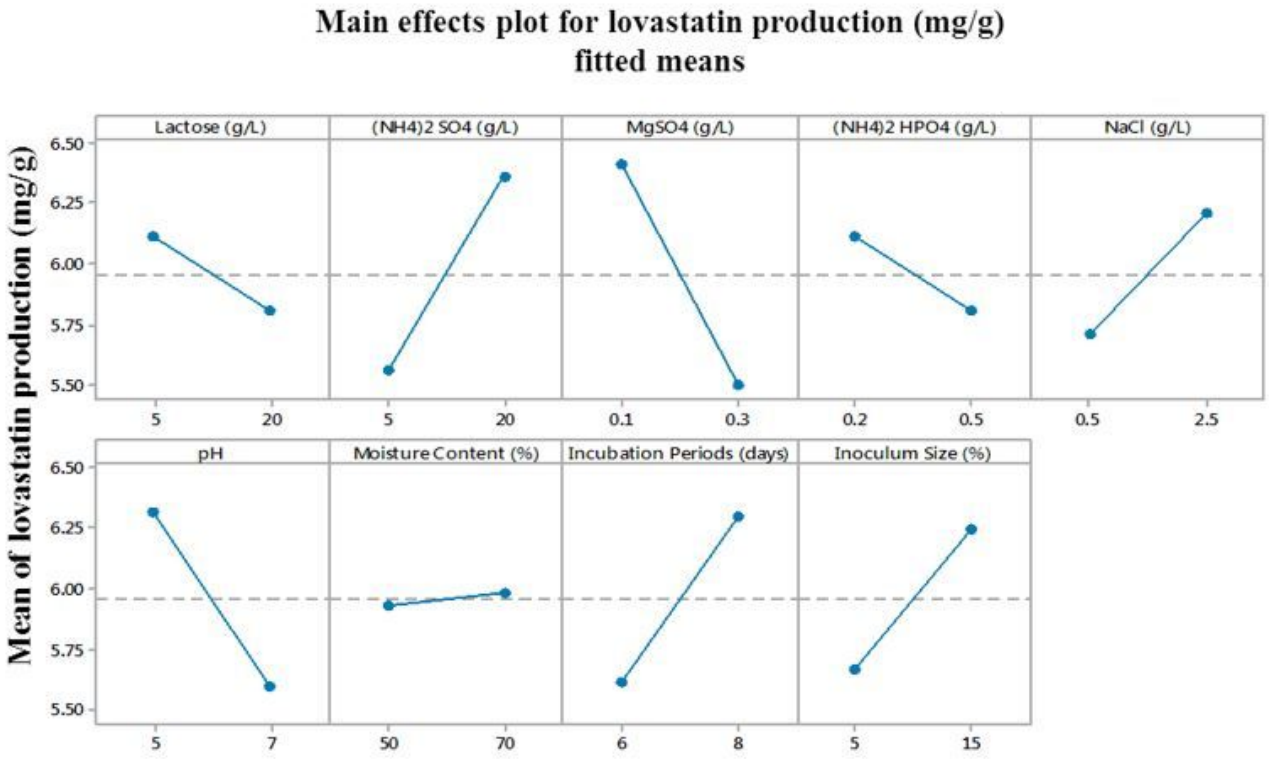

Figure 9

The main effect plots of contributions of selected parameters to lovastatin production by CunninghamellablakesleeanaKP780148.1.

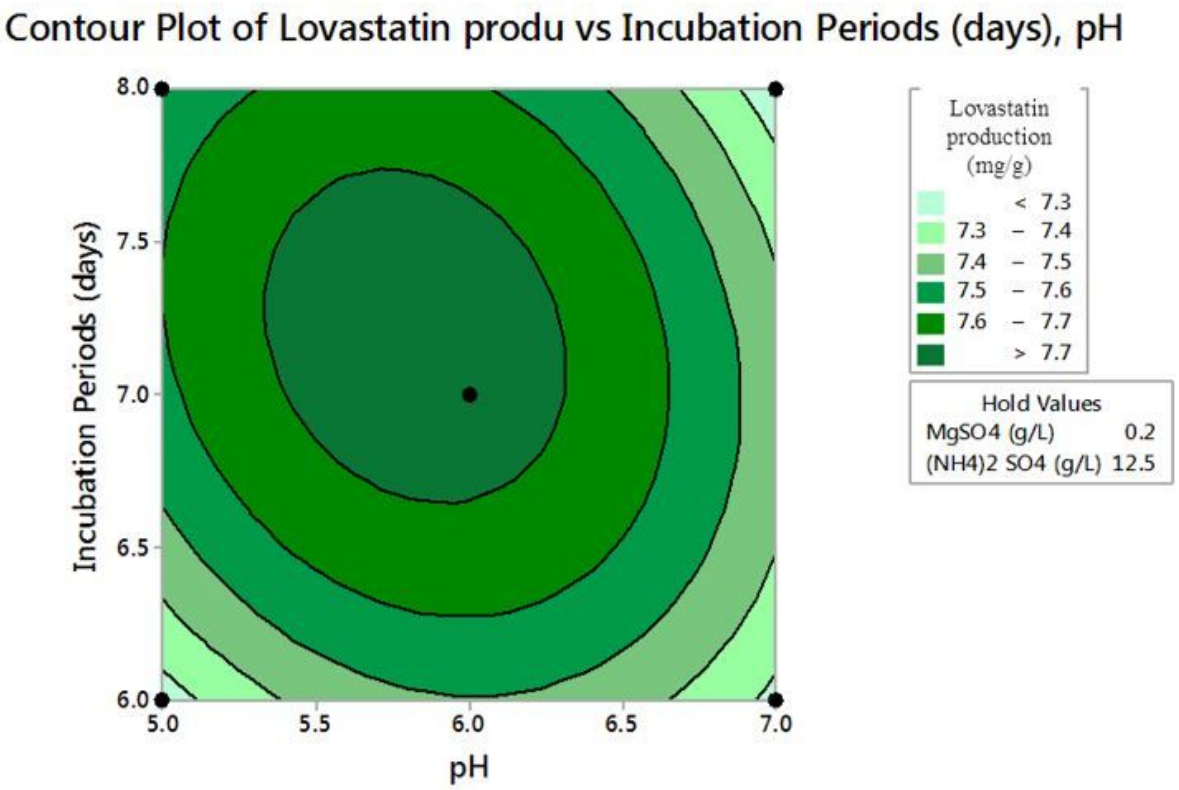

Figure 10

Contour plot of two-way interactions of independent variables (pH vs incubation period) for maximal lovastatin production.

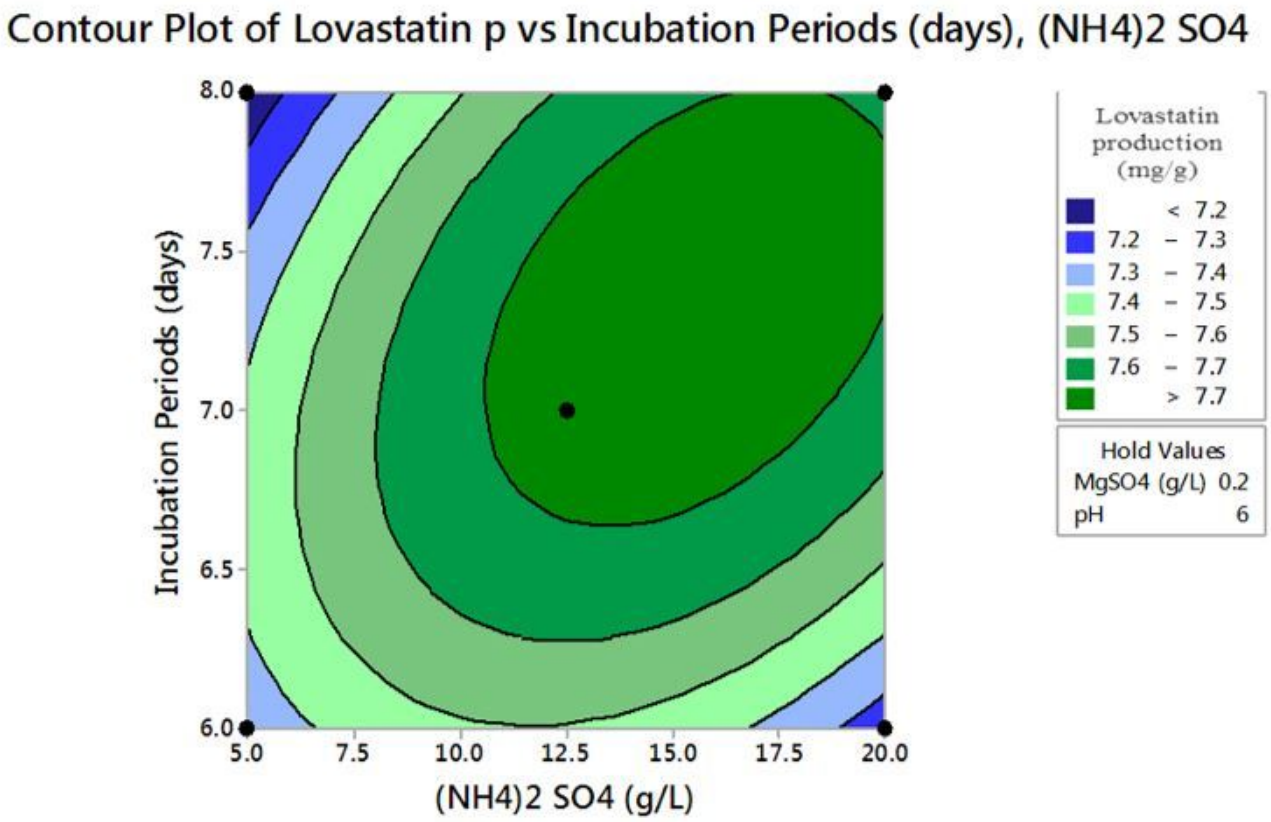

Figure 11

Contour plot of two-way interactions of independent variables (incubation periods and (NH4)2SO4) for maximal lovastatin production.

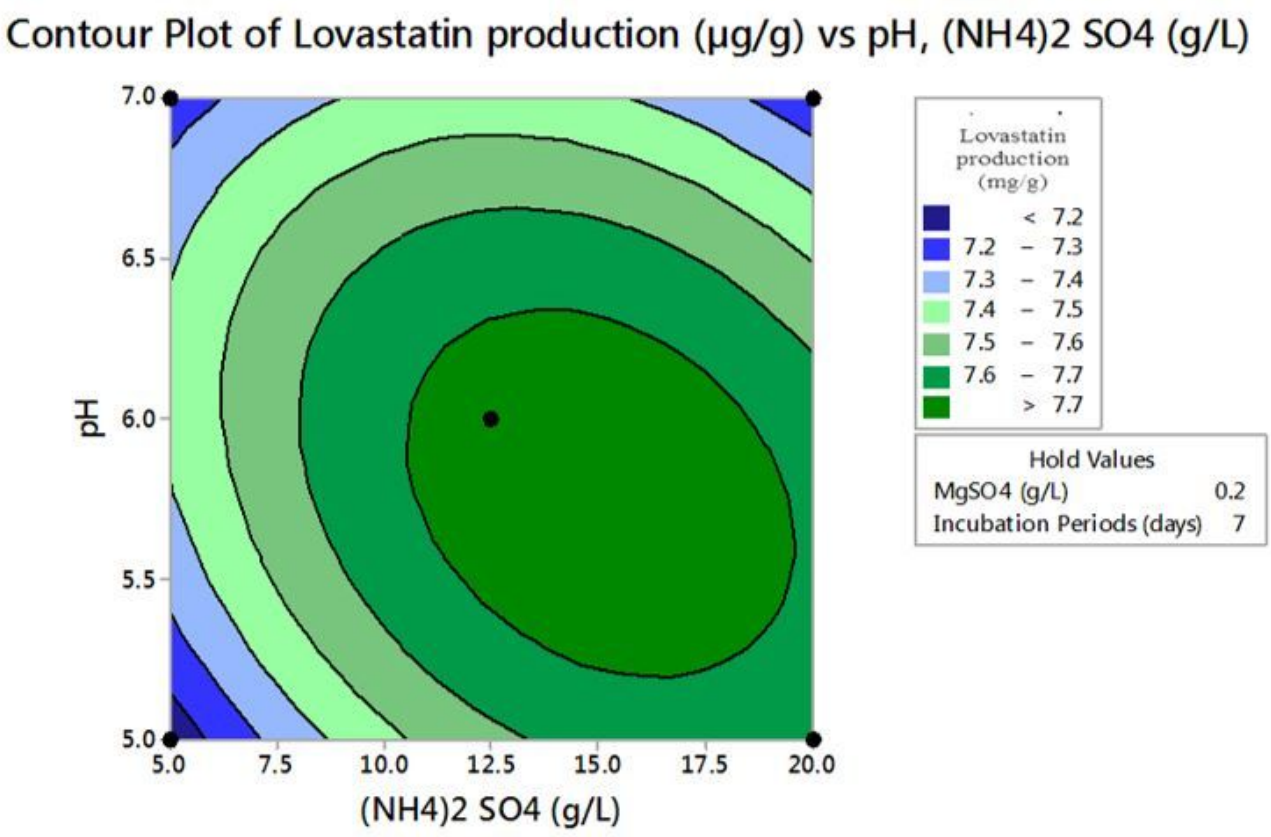

Figure 12

Contour plot of two-way interactions of independent variables (pH and (NH4)2SO4) for maximal lovastatin production.

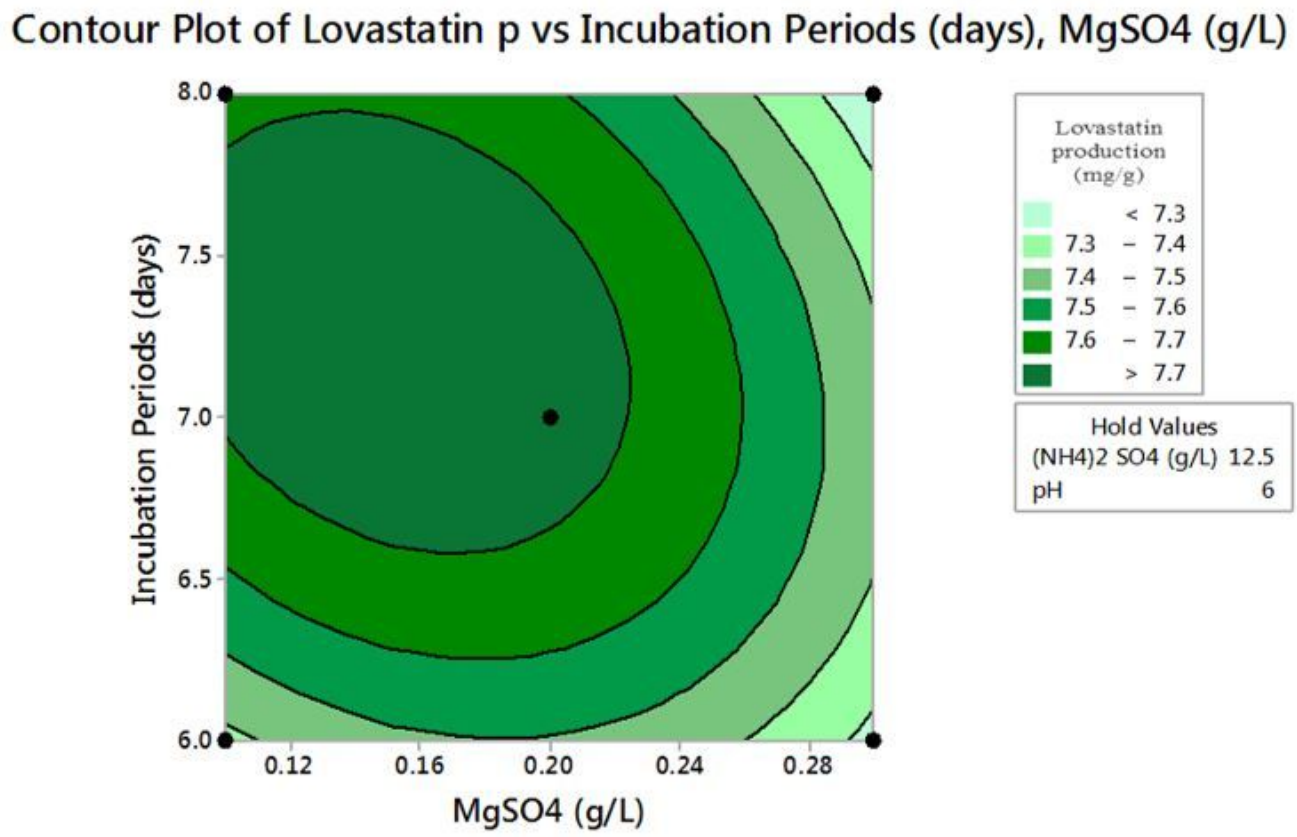

Figure 13

Contour plot of two-way interactions of independent variables (incubation periods and MgSO4) for maximal lovastatin production.

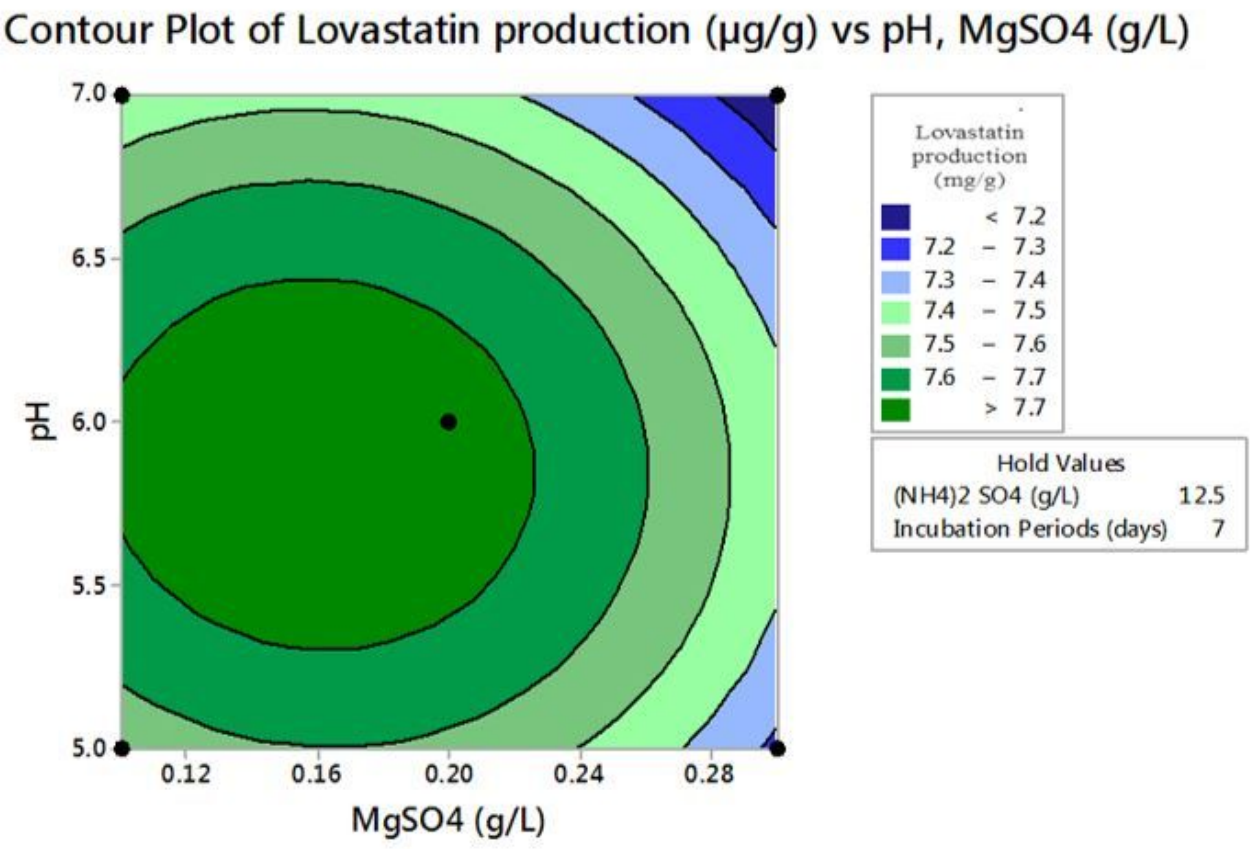

Figure 14

Contour plot of two-way interactions of independent variables (pH and MgSO4) for maximal lovastatin production.

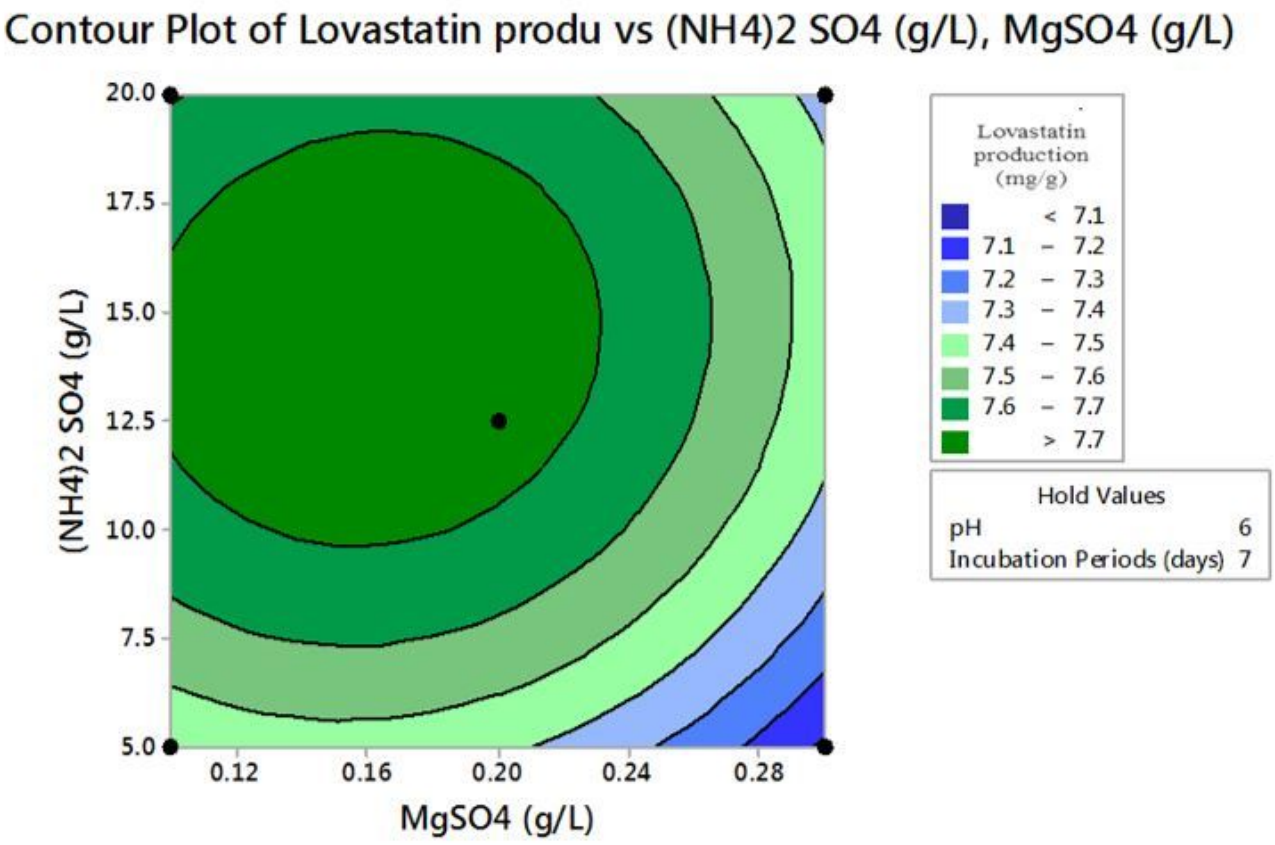

Figure 15

Contour plot of two-way interactions of independent variables (MgSO4 and (NH4)2SO4) for maximal lovastatin production.

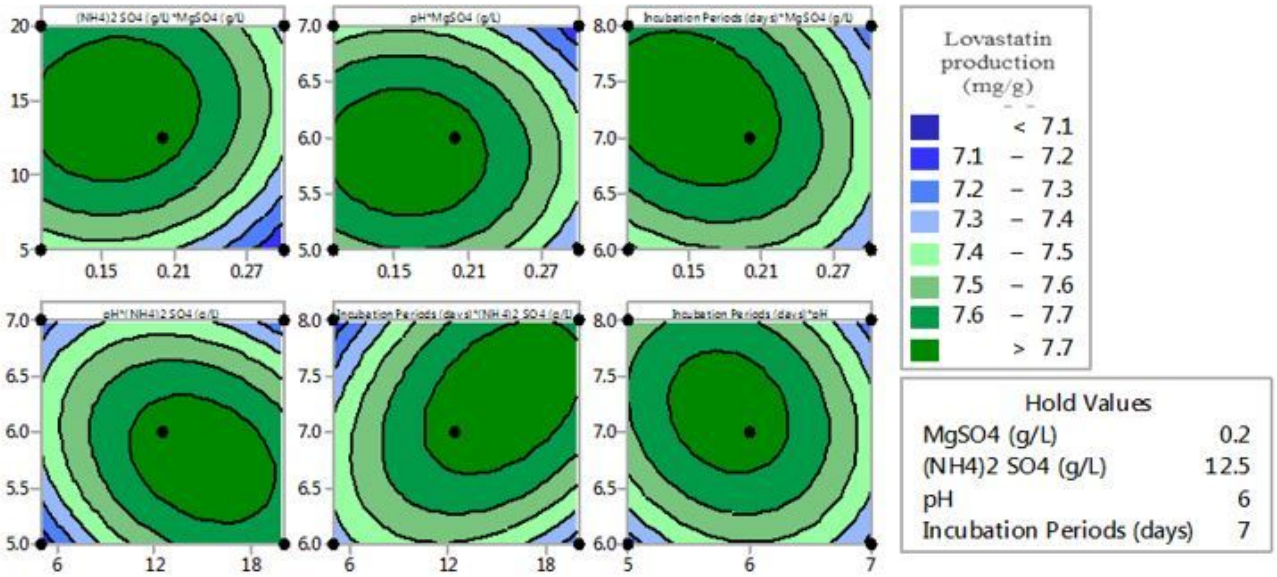

Figure 16

The overall Contour plots of two-way interactions of all the independent variables for maximal lovastatin production.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- GraphicalAbstract.docx