Production of Lovastatin from Aspergillus Terreus MTCC 1782 Under Submerged Fermentation

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***Abstract*:** The lab scale optimization of lovastatin production through submerged fermentation by *Aspergillus terreus* MTCC 1782 has been described. Fermentation experiments for the screening of fermentation media showed that *A. terreus* MTCC 1782 utilized fermentation medium (Medium-2) for production of lovastatin i.e., 258.26 mg/lit in the fermentation broth. The optimized parameters are incubation period 144 hrs., Age of the inoculum 144 hrs. (6 days), inoculum concentration 5 %v/v, incubation temperature 30°C, pH of the medium 6.0, carbon source (2 %v/v glucose), nitrogen source (0.6 %v/v ammonium sulfate) giving an overall yield of 460.29 mg/lit after optimization. The present study indicates that *A. terreus* MTCC 1782 as potential producer of lovastatin under submerged fermentation.

Keywords: Lovastatin, *Aspergillus terreus*, cholesterol, UV Spectrometry, submerged fermentation.

1. INTRODUCTION

Cholesterol is an organic compound, which is produced in humans by a complex metabolism. It acts as a precursor for the synthesis of many steroids, vitamin D and also helps in membrane transport. Increase of cholesterol in humans, leads to Cardiovascular Disorders and finally death. This can be reduced by inhibiting the HMG-CoA reductase, which is an important precursor in formation of mevalonate from Acetyl CoA. Inhibition is done by statin drug, which is produced by many fungi through polyketide pathway.

Lovastatin also known as monacolin k, is an inhibitor of cholesterol biosynthesis and produced as a secondary metabolite by a variety of filamentous fungi such as *Monascus (M.ruber, M.purpureus, M.pilosus, and M.anka), Penicillium (P.citrinum), Paecilomyces viridis, and Aspergillus (A. terreus).* It inhibits 3-hydroxy-3-methyl glutaryl coenzyme A (HMG-CoA) reductase (EC1.1.1.34), an important step in cholesterol bio synthesis and there by lowers the plasma cholesterol levels, Other than reduction of cholesterol, lovastatin is shown to provide various medicinal properties like anti-cancer, bone maturation, multiple sclerosis. It was the first of this class of agents approved for clinical use.

James Gomes *et al.*, (2014) reported that Lovastatin biosynthesis depends on the relative concentrations of dissolved oxygen and the carbon and nitrogen resources. An elucidation of the underlying relationship would facilitate the derivation of a controller for the improvement of lovastatin yield in bioprocesses. To achieve this, batch submerged cultivation experiments of lovastatin production by *Aspergillus flavipus* BICC 5174, using both lactose and glucose as carbon sources, were performed in a 7-L bioreactor and the data used to determine how the relative concentrations of lactose, glucose, glutamine, and oxygen affected lovastatin yield, and a model was developed based on these results and its prediction was validated using an independent set of batch data obtained from a 15-L bioreactor using five statistical measures, including the Willmott index of agreement. A non-linear controller was designed considering that dissolved oxygen and lactose concentrations could be measured online, and using the lactose feed rate and airflow rate as process inputs. Simulation experiments were performed to demonstrate that a practical implementation of the non-linear controller would result in satisfactory outcomes.

Prakash Chaynika and Shivakumar Srividya (2014) reported that Aspergillus terreus MTCC 479 strain screened for lovastatin production in submerged fermentation after 7 days of fermentation, all of them showed positive when screened through bioassay method by the zone of inhibition exhibited by the fungus against *S.cerevisae*. Lovastatin production was further confirmed through the laboratory analytical technique TLC. Colorimetric estimation identified Aspergillus sp. no.76 to be the best producer of lovastatin with a level of 137.5 mg/L. by submerged fermentation.

In the present study, *Aspergillus terreus* MTCC 1782 was used for the production of lovastatin by Submerged fermentation.

2. MATERIALS AND METHODS

2.1 Microorganism used and its culture conditions

The microorganism used is a fungal strain *Aspergillus terreus* MTCC 1782 obtained from Microbial type culture collection (MTCC) Chandigarh, India. The culture was maintained on **potato dextrose agar slants.**

Fig. 1 - *Aspergillus terreus* MTCC 1782

2.2 Potato Dextrose Agar Medium (HIMEDIA): Potato infusion (200ml), Dextrose (20 g/lit), Agar (15 g/lit), pH - 5.6±0.2.
2.2.1 Maintenance conditions: Growth conditions - Aerobic, Temperature - 28^0C, Incubation time - 96 hrs., Subculture - 30 days
2.2.2 Medium composition

Different carbon and nitrogen source-based lovastatin production media were used for submerged fermentation. Containing Trace elements ($Na_2B_4O_7 \cdot 10H_2O$ -100mg, $MnCl_2$ – 50mg, $Na_2MoO_4 \cdot 5H_2O$ - 50mg, $CuSO_4 \cdot 5H_2O$ - 250mg per liter of solution).

2.2.3 Seed medium composition (**Kumar *et al.*, 2000**): Glucose (10 g/lit), Oat meal (10 g/lit), Corn steep liquor (10 g/lit), Polyethylene glycol (0.2 ml/lit), Trace elements (10 ml/lit), pH – 6.0.
2.2.4 Production medium composition
Medium-1 composition (**Monaghan *et al.*, 1981**)**:** Glucose (10 g/lit), Oat meal (10 g/lit), Corn steep liquor (5 g/lit), Tomato paste (40 g/lit), Trace elements (10 ml/lit), pH – 6.0.
Medium -2 composition (Manzoni *et al.*, 1998): Glucose (30 g/lit), Glycerol (70 g/lit), Peptone (8 g/lit), Soyabean meal (30 g/lit), Trace elements (10 ml/lit), pH – 6.4.
2.2.5 Chemicals:
All Chemicals used in this research work are either analytical grade (AR) or gravimetric grade (GR)

- Hydro chloric acid
- Tri fluoro acetic acid
- Ethyl acetate
- Methanol
- Lovastatin
- Tween -80
- Sodium acetate
- Glacial acetic acid
- Monobasic sodium phosphate anhydrous
- Dibasic sodium phosphate anhydrous

2.3 Submerged fermentation:

Submerged fermentation is the cultivation of microorganisms in liquid nutrient broth. Enzymes, antibiotics, organic acids and bioactive compounds are produced using this process. This involves growing carefully selected microorganisms (bacteria and fungi) in closed vessels containing a rich broth of nutrients (the fermentation medium) and a high concentration of oxygen. As the microorganisms break down the nutrients, they release the desired enzymes and compounds into the solution. Fermentation takes place in large vessels(fermenter) with volumes of up to 1,000 cubic meters. The fermentation media sterilizes nutrients based on renewable raw materials like maize, sugars and soya. Most industrial enzymes are secreted by microorganisms into the fermentation medium in order to break down the carbon and nitrogen sources. Batch-fed and continuous fermentation processes are common. In the batch-fed process, sterilized nutrients are added to the fermenter during the growth of the biomass. In the continuous process, sterilized liquid nutrients are fed into the fermenter at the same flow rate as the fermentation broth leaving the system. This will achieve a steady-state production. Parameters like temperature, pH, oxygen consumption and carbon dioxide formation are measured and controlled to optimize the fermentation process. This fermentation technique is best suited for microorganisms that require high moisture.

Advantages:

- Measure of process parameters is easier than with solid state fermentation
- Bacterial and fungal cells are evenly distributed throughout the medium.
- Product purification is easier than with solid state fermentation.

Disadvantages:

- High costs due to the expensive media large reactors are needed and the behavior of the organism cannot be predicted at times.
- There is also a risk of contamination.

2.4 Preparation of reagents

2.4.1 Acetate buffer (0.1mol/lit)

Solution A: 5.8 ml of 0.1 M glacial acetic acid($C_2H_4O_2$) is taken and made up to 1000ml using distilled water

Solution B: 13.6 g/lit of 0.1 M sodium acetate tri hydrate ($CH_3COONa.3H_2O$) is taken

The following table-1 enumerates the volumes of solution A and solution B to be mixed to a total of 100ml in accordance to the required pH.

Volume of solution A (ml)	Volume of solution B (ml)	pH
46.3	3.7	3.6
41.0	9.0	4.0
30.5	19.5	4.4
20.0	30.0	4.8
14.8	35.2	5.0
10.5	39.5	5.2
4.8	45.2	5.6

Table-1: Acetate buffer (Volumes of solutions A and B for required pH)

2.4.2 Phosphate buffer:

Solution (A): 27.8g monobasic sodium phosphate anhydrous dissolved in 1000 ml using distilled water.

Solution (B): 53.65g dibasic sodium phosphate anhydrous dissolved in 1000 ml using distilled water.

The following table enumerates the volumes of solution A and solution B to be mixed to a total of 100ml in accordance to the required pH.

Volume of solution A (ml)	Volume of solution B (ml)	pH
93.5	6.5	5.7
92.0	8.0	5.8
81.5	18.5	6.2
51.0	49.0	6.8
39.0	61.0	7.0
13.0	87.0	7.6
5.3	94.7	8.0

Table-2: Phosphate buffer (Volumes of solutions A and B for required pH)

2.5 Maintenance of culture

The culture was maintained on potato dextrose agar slants, incubated 28°C for 4 days and then slants were stored at 4°C. The organism was sub cultured for every 15 days and used in the subsequent experiments.

2.6 Preparation of Inoculum

The fungal homogenous spore suspension was prepared by dispersing the spores from a 7-day old culture in 0.1% Tween-80 solution with a sterile inoculation loop and transferred into 250 ml Erlenmeyer flasks containing 50 ml seed medium. The flask with medium was inoculated with 3 $x10^7$ spores, held on orbital shaker at 180 rpm for 2 days at 28°C and then was used as inoculum.

2.7 Lovastatin production:

Different carbon and nitrogen source-based lovastatin production media were used for submerged fermentation. 3 ml of spores were inoculated in 250 ml Erlenmeyer flasks, containing 50 ml of the medium-1 and medium-2 and incubated at 28^0C for 10 days.

2.8 Lovastatin extraction:

At the end of 10 days of fermentation, the fermentation broth was acidified to pH 3.0 with 10% 1 N HCl. Then the acidified broth was extracted with equal volume of methanol under shaking condition (180 rpm) at 70°C for 2 hrs. The fungal biomass was separated by filtration using pre-weighed Whatman filter paper. The filtrates were subsequently centrifuged at 3000 g for 10 min and the organic phase was collected.

3 QUANTITATIVE ANALYSIS

3.1 Quantitative analysis of lovastatin

3.1.1 UV spectroscopy

Ultraviolet absorption spectra were obtained with a UV- visible Spectro photometer (shimadzu). A stock solution of lovastatin in methanol was prepared. Wave length scanning between 200- 400nm was performed. A standard curve from 0 to 1000 ug/ml was prepared. All measurements were performed in triplicate. The wave length at which the maximum absorption was observed and which was read the calibration curve was 238 nm. At this wave length there is no interference with methanol.

3.1.2 Procedure for quantitative analysis of lovastatin:

To the 1ml of organic phase 1% trifluoroacetic acid (10 ml) was added for lactonization process. Then the extract was concentrated at 80°C (without vacuum), to this add 10 ml of methanol for qualitative and quantitative estimation by UV Spectrometry. The concentration of lovastatin (µg/ml) present in the sample is obtained by plotting the O.D values on standard graph.

3.1.3 Standard graph:

99.9% pure lovastatin (lactone form) is dissolved in methanol to produce solutions of concentrations 1000µg, 800µg, 600µg, 400µg and 200µg/ml in different test tubes. Each test solution is made up to a volume of 10 ml with methanol and their absorbance is read spectrophotometrically at 238 nm.

Concentration (µg/ml)	O.D at 238 nm
200	0.08
400	0.14
600	0.23
800	0.31
1000	0.45

Table - 3: Standard graph

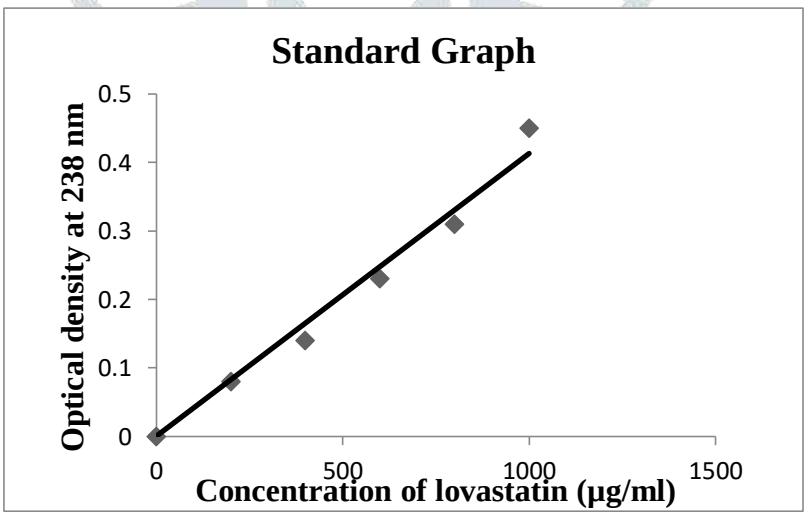

Fig. 2 - Standard Graph

4. OPTIMIZATION PROCESS

4.1 Optimization of process parameters

In order to improve the yield of lovastatin production, various parameters were studied and optimized. They include effect of fermentation time, effect of fermentation temperature, effect of pH, effect of inoculum volume, effect of inoculum age. Impact of carbon and nitrogen (both organic and inorganic) sources on the growth of *Aspergillus terreus* MTCC 1782 were also studied. The protocol adopted for the optimization of process parameters was to evaluate the effect of individual parameter at a time and to incorporate it at the standard level before optimizing the next parameter.

4.1.1 Optimization of fermentation time:

Optimization of fermentation time is carried out by inoculating 3ml of (6% v/v) inoculum (7-day culture) to 250 ml Erlenmeyer flasks, containing 50 ml of the sterilized medium-2 They were incubated for different fermentation times ranging from 24 to 240 hrs. to obtain production profile of lovastatin. The flasks were analyzed for every 24 hrs.

4.1.2 Optimization of age of the inoculum:

Determination of optimum age of inoculum for the production of lovastatin by varying the age of inoculums from 3 to 10 days of 6% v/v inoculums is added in 250 ml Erlenmeyer flasks, containing 50 ml of the sterilized medium-2 and incubated at 28^0C. the flasks were analyzed after144 hrs. (6 days).

4.1.3 Optimization of inoculum concentration:

An experiment was carried out to optimize the size of inoculum for the production of lovastatin ranging from 2 - 8% of inoculum (6-day old culture) is added in 250 ml Erlenmeyer flasks, containing 50 ml of the sterilized medium-2 and incubated at 28^0C. the flasks were analyzed after 144 hrs. (6 days).

4.1.4 Optimization of fermentation temperature

To determine the optimized temperature for lovastatin production, 5% v/v inoculum (6-day culture) is added in 250 ml Erlenmeyer flasks, containing 50 ml of the sterilized medium-2 and incubated at different fermentation temperatures 26,28,30,32,34 and 36^0C.The flasks were analyzed after144 hrs. (6 days)

4.1.5 Optimization of pH:

To determine the optimized pH for lovastatin production, 5% v/v inoculum (6-day culture) is added in 250 ml Erlenmeyer flasks, containing 50 ml of the sterilized medium-2 and pH is maintained with buffer solution (phosphate and acetate) incubated at different pH ranges of 3,4,5,6,7, and 8 at 30^0C, flasks were analyzed after144 hrs. (6 days).

4.1.6 Effect of nutrient source

4.1.6.1 Effect of different carbon sources:

Different organic carbon sources (Maltose, Sucrose, Fructose, Glucose Lactose and Starch) were added to the media at 3% w/v concentration to each flask, and incubation parameters maintained were: Inoculum age - 6-day old culture, Inoculum volume - 5%v/v, Temperature - 30°C, pH - 6.0. Flasks were analyzed after 144 hrs. (6days)

4.1.6.2 Effect of Glucose concentration:

To determine the optimum concentration of Glucose for lovastatin production Different concentrations ranging from 1- 5 %w/v were added to each flask, and incubation parameters maintained were: Inoculum age - 6-day old culture, Inoculum volume - 5%v/v, Temperature - 30°C, pH - 6.0. Flasks were analyzed after 144 hrs. (6days).

4.1.6.3 Effect of different nitrogen sources:

Various organic (peptone, corn step liquor, beef extract and yeast extract) and inorganic (sodium nitrate and ammonium sulfate) nitrogen sources are added to the fermentation media at 0.8% w/v concentration to each flask, and incubation parameters maintained were: Inoculum age - 6-day old culture, Inoculum volume - 5%v/v, Temperature - 30°C, pH - 6.0. Flasks were analyzed after 144 hrs. (6days).

4.1.6.4 Effect of ammonium sulfate concentration

To determine the optimum concentration of ammonium sulfate for lovastatin production Different concentrations ranging from 0.1- 0.8 %w/v were added to each flask, and incubation parameters maintained were: Inoculum age - 6-day old culture, Inoculum volume - 5%v/v, Temperature - 30°C, pH - 6.0. Flasks were analyzed after 144 hrs. (6days).

5. RESULTS AND DISCUSSION

5.1 Screening of media

Two different fermentation media (Medium- 1 and Medium- 2) were screened for the production of lovastatin by *Aspergillus terreus* MTCC 1782 in shake flasks. It was found that fermentation media (Medium- 2) was most suitable for the production of lovastatin. So, the culture medium Medium-2 was selected for further studies.

5.2 Effect of fermentation time:

The effect of incubation period on the lovastatin production by *A. terreus* MTCC 1782 was studied by varying the incubation period of fermentation flasks from 24 to 240 hrs. (1- 10 days). The production of lovastatin after 24 hrs. of incubation was **129.31** mg/lit in the fermentation broth and increased with increasing incubation period reaching to the maximum value of **258.26** mg/lit after **144** hrs. of incubation in the fermentation broth. It declined when further incubation was given to the fungal strain in the shake flasks.

Lopez *et al.*, (2003) reported maximum lovastatin production with *Aspergillus terreus* was obtained on 150 hrs. in their experiments under submerged fermentation.

Incubation time (hrs.)	**Concentration (mg/lit)**
24	129.31
48	133.60
72	146.50
96	165.84
120	200.23
144	**258.26**
168	228.17
192	210.98
216	180.89
240	161.54

Table - 4: Effect of fermentation time

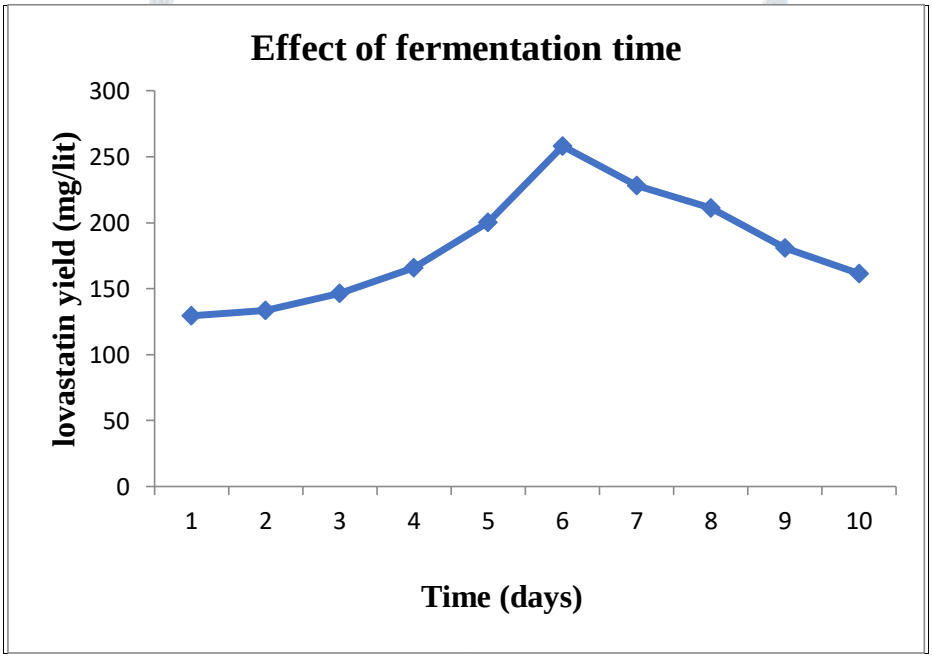

Fig. 3 - Effect of fermentation time

5.3 Effect of inoculum age

The effect of inoculum age on the production of lovastatin by *A. terreus* MTCC 1782 was studied. Different ages of the fungal vegetative inoculum ranging from 72 – 240 hrs. were tested for lovastatin production. Maximum lovastatin production i.e., **269.01** mg/lit in fermentation broth was obtained using inoculum age of **6** days.

Arjumand ahmed *et al*., (2012) reported maximum lovastatin production with 6 days old culture of *Aspergillus terreus* under submerged fermentation.

Inoculum age (Days)	**Concentration (mg/lit)**
3	176.59
4	193.78
5	228.17
6	**269.01**
7	256.11
8	232.47
9	223.87
10	206.68

Table – 5: Effect of inoculum age

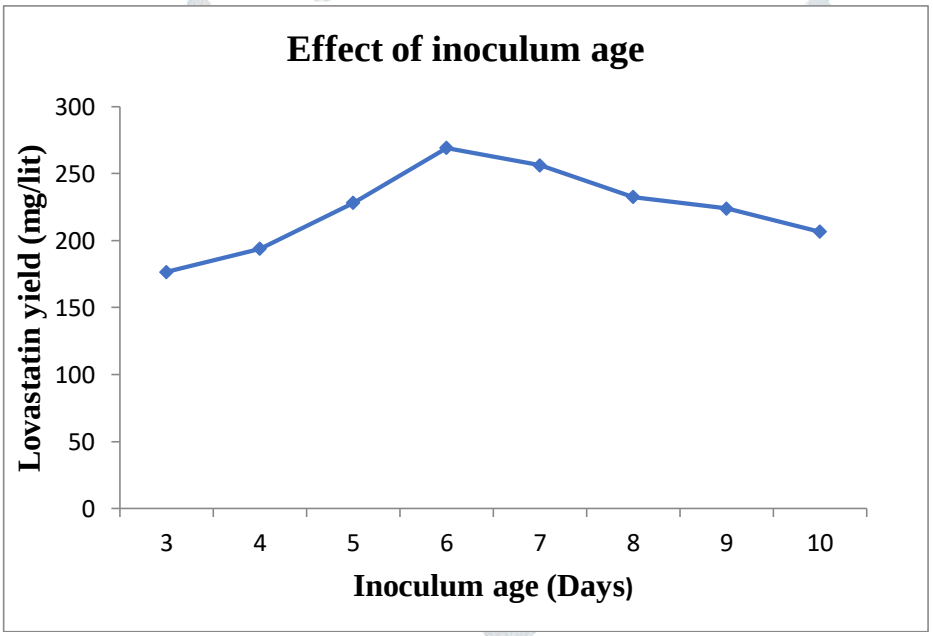

Fig. 4 - Effect of inoculum age

5.4 Effect of inoculum concentration:

An experiment was carried out to optimize the size of inoculum ranging from 2–8% for the lovastatin production. The results showed that maximum lovastatin production was **292.65** mg/lit in the fermentation broth when **5%** (v/v) inoculum was used inoculate the fermentation flasks.

Hajjaj *et al.*, (2001) and Samiee *et al.*, (2003) reported maximum production of lovastatin using 6 %v/v of *Aspergillus terreus* under submerged fermentation.

Inoculum concentration (%v/v)	**Concentration (mg/lit)**
2	189.48
3	213.13
4	232.47
5	**292.65**
6	266.86
7	241.07
8	221.72

Table - 6: Effect of inoculum concentration

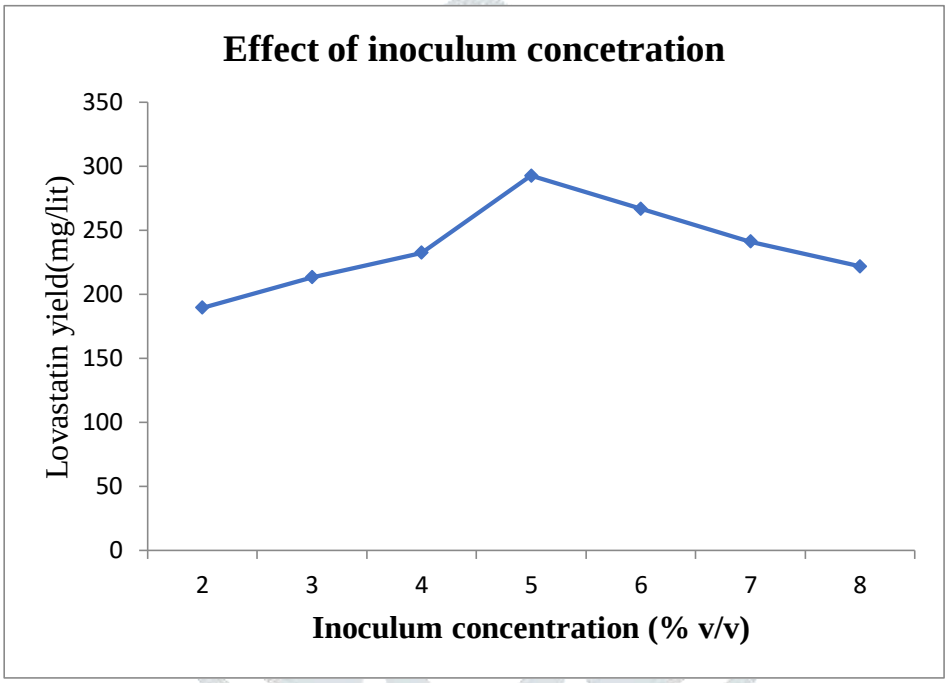

Fig. 5 - Effect of inoculum concentration

5.5 Effect of fermentation temperature:

The effect of different incubation temperatures on the lovastatin production by *A. terreus* MTCC 1782 was studied by culturing the shake flasks in the temperatures ranging from 26 to **36°C,** From the results, it was observed that there was a gradual increase in lovastatin production when the incubation temperature was increased from **26°C** to **30°C**. The maximum lovastatin production, **301.25** mg/lit was observed at **30°C** in the fermentation broth respectively. The lovastatin production was gradually decreased with an increase of temperature from **30°C** to **36°C**.

The maximum production of lovastatin at **30°C** might be due to the fact that this temperature is best for the sporulation, growth and proliferation of mycelial mass for the production of secondary metabolites. **Arjumand ahmed *et al.*, (2012).**

Samiee *et al.*, (2003), and Gupta *et al.*, (2007) reported maximum lovastatin production with *Aspergillus terreus* at a temperature of 28°C in their experiments under submerged fermentation.

Atalla *et al.*, (2008) reported maximum lovastatin production with *Aspergillus terreus at a* temperature of 30°C in their experiments under submerged fermentation

Temperature (°C)	**Concentration (mg/lit)**
26	236.77
28	262.56
30	**301.25**
32	286.20
34	260.41
36	249.66

Table - 7: Effect of fermentation temperature

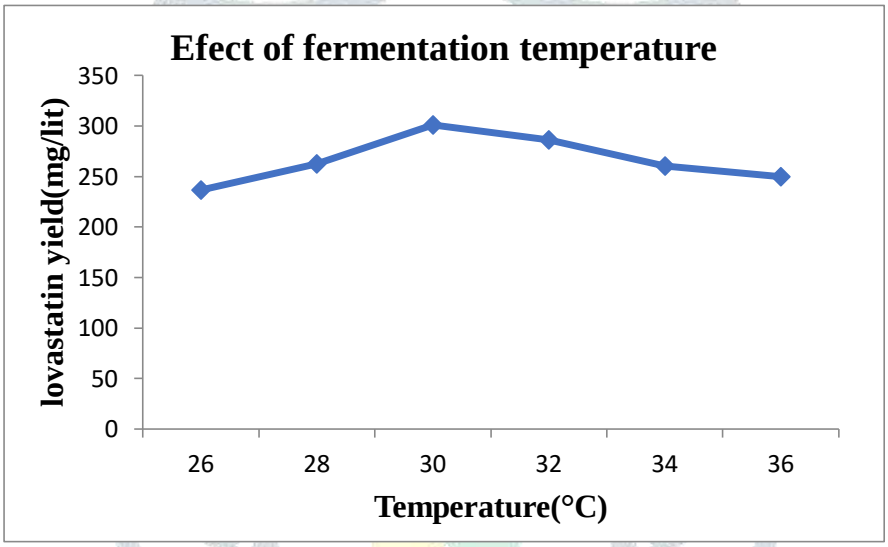

Fig. 6 - Effect of fermentation temperature

5.6 Effect of initial pH:

The effect of initial pH of culture medium on the production of lovastatin was studied by varying the initial pH of culture media from **3.0** to **8.0**. Lowest lovastatin production was observed at pH **3.0** i.e., **236.77** mg/lit in the fermentation broth. The production was started to increase at higher pH values and maximum lovastatin production i.e., **337.78** mg/lit in the fermentation broth was observed at **pH 6.0** and then it was decreased by increasing the initial pH above **6.0.** All the secondary metabolic activities normally occur at some specific pH and variation of pH during the fermentation process drastically affect them It might be due to the fact that at pH 6.0, the permeability of cell membrane is enhanced by metallic ion for maximum production of lovastatin in the fermentation process.

Madan & Thind, (2000) reported maximum lovastatin production with *Aspergillus terreus* at pH 6.0 in their experiments under submerged fermentation.

pH	**Concentration (mg/lit)**
3	236.77
4	256.11
5	284.05
6	**337.78**
7	290.50
8	266.86

Table - 8: Effect of pH

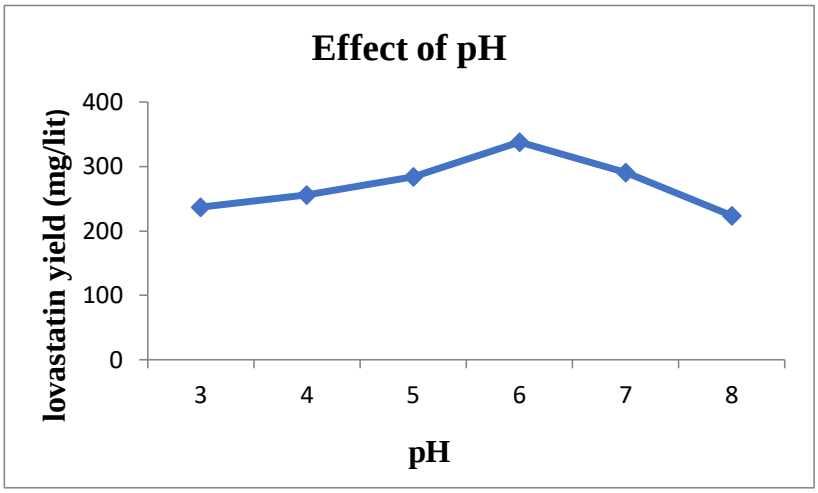

Fig. 7 - Effect of pH

5.7 Screening of carbon source:

Different carbon sources including glucose, maltose, lactose, sucrose, fructose and starch were evaluated for the maximum lovastatin production by *A. terreus* MTCC 1782. The results showed that glucose gave maximum lovastatin production i.e., **342.08** mg/lit in fermentation broth. followed by lactose with a slight difference.

It is due to the fact that it is easily available carbon source and it oxidized very rapidly in the cells thus act as a readily available source of energy. **Hajjaj *et al.*, (2001).**

Hajjaj *et al.*, (2001) also reported significant increase in lovastatin production using glucose as carbon source for *Aspergillus terreus* under submerged fermentation

Carbon source (3 %w/v)	**Lovastatin yield (mg/lit)**
Maltose	277.60
Sucrose	292.65
Fructose	303.40
Glucose	**342.08**
Lactose	333.49
Starch	329.19

Table - 9: Effect of carbon source

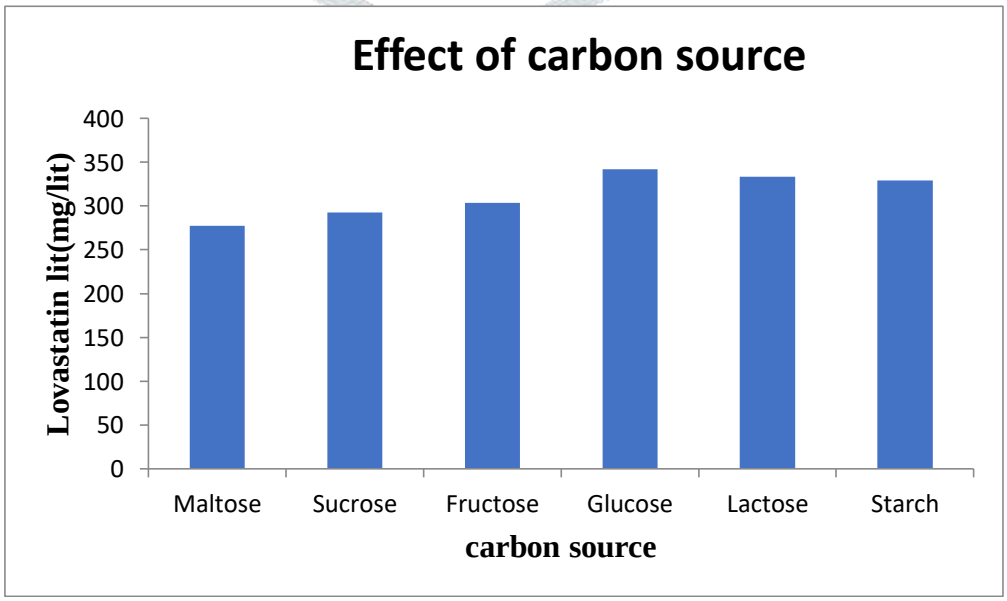

Fig. 8 - Effect of carbon source

5.8 Effect of glucose concentration:

An experiment was carried out to optimize the concentration of **glucose 1-5 (%w/v)** in the culture medium. Yield of lovastatin was increased as the glucose concentration was increased and reached maximum **361.43** mg/lit at **2%** glucose concentration Therefore, **2%** glucose was selected as the most suitable concentration for the production of lovastatin by *A. terreus* MTCC 1782.

Arjumand ahmed *et al.*, (2013) reported maximum lovastatin production yield obtained with 5% w/v of glucose as carbon source by *Aspergillus terreus* in their experiments under submerged fermentation.

Concentration of glucose (%w/v)	Lovastatin yield (mg/lit)
1	290.50
2	**361.43**
3	344.23
4	339.93
5	327.64

Table - 10: Effect of glucose concentration

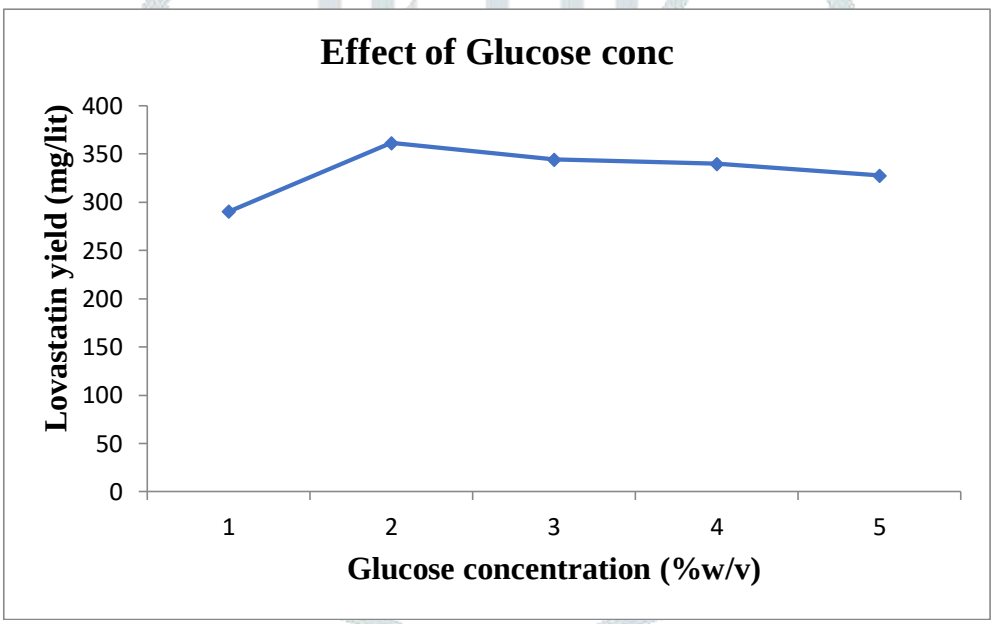

Fig. 9 - Effect of glucose concentration

5.9 Screening of nitrogen source:

Different nitrogen sources i.e. organic and inorganic such as corn steep liquor, peptone, beef extract yeast extract, ammonium sulphate and sodium nitrite were evaluated for the lovastatin production by *A. terreus* MTCC 1782. Of all the sources, **ammonium sulfate** gave maximum lovastatin production i.e., **395.81** mg/lit in the fermentation broth.

This results accordance with **Lopez *et al.*, (2003)** results and reported as significant increase in lovastatin production using ammonium sulfate as inorganic nitrogen source for *Aspergillus terreus* under submerged fermentation.

Nitrogen source (0.8 %w/v)	**Lovastatin yield (mg/lit)**
Peptone	365.72
Corn step liquor	370.02
Ammonium sulfate	**395.81**
Sodium nitrite	378.62
Yeast extract	311.99
Beef extract	288.35

Table – 11: Effect of nitrogen source

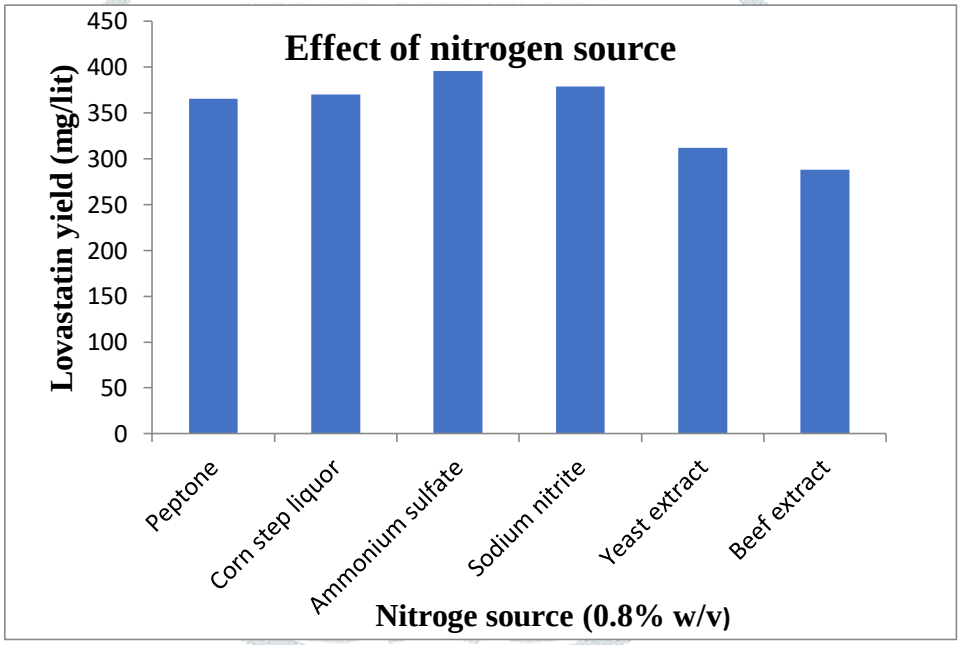

Fig. 10 - Effect of nitrogen source

5.10 Effect of Ammonium sulfate concentration:

The amount of **ammonium sulfate (0.1-0.8%)** was further optimized for the production of lovastatin. and It was noted that ammonium sulfate at **0.6%** concentration gave maximum yield of lovastatin i.e., **460.29 mg/lit** in the fermentation broth.

Arjumand ahmed *et al.*, (2013) reported maximum lovastatin production yield obtained with 0.3 %v/v of ammonium sulfate as nitrogen source by *Aspergillus terreus* in their experiments under submerged fermentation.

Concentration of ammonium sulfate (%w/v)	Lovastatin yield (mg/lit)
0.1	331.20
0.2	354.98
0.3	378.62
0.4	408.71
0.5	428.05
0.6	**460.29**
0.7	421.61
0.8	400.11

Table - 12: Effect of Ammonium sulfate concentration

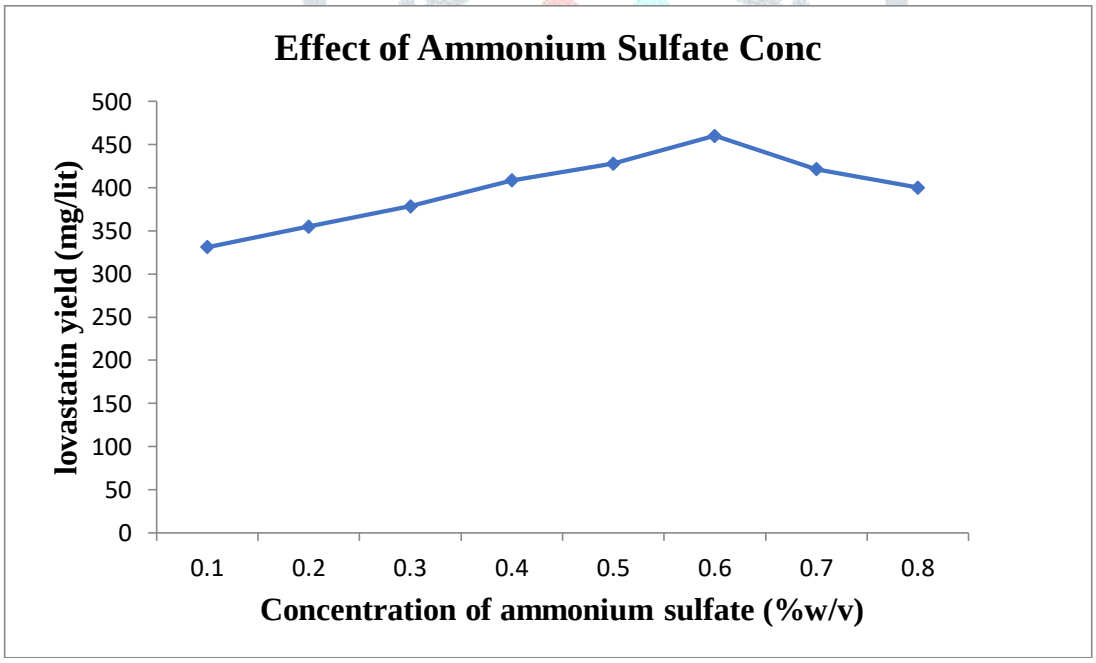

Fig. 11 - Effect of Ammonium sulfate concentration

5.11 Optimized parameters

The lovastatin production by *Aspergillus terrus* MTCC 1782 under Submerged fermentation was carried out under optimized conditions. Which include:

Fermentation time	:	144hrs (6 Days)
Inoculum age	:	6 Day old Culture
Inoculum volume	:	5 (% v/v) spores
Temperature	:	30°C
pH	:	6
Carbon source	:	Glucose
Glucose concentration	:	2 (%w/v)
Nitrogen source	:	Ammonium sulfate
Ammonium sulfate concentration	:	0.6 (%w/v)

Optimization of all these factors resulted in the maximum production of lovastatin. **460.29 mg/lit**

6. SUMMARY AND CONCLUSION

6.1 SUMMARY

- In the present study, the production of lovastatin by *Aspergillus terreus* MTCC 1782 under submerged fermentation has been carried out.
- Medium- 2 gave a high lovastatin production in submerged fermentation.
- The physico-chemical process parameters of fermentation like incubation, temperature, pH, Inoculum volume and Inoculum age were optimized by single step -by-step optimization process. The nutritional supplementation study was carried out using some carbon, nitrogen sources to enrich the production medium and to enhance the lovastatin yield. The optimized conditions and maximum activities obtained are as follows.

- **Fermentation time**
  Maximum lovastatin yield of **258.26 mg/lit** obtained after **6** days of incubation.
- **Inoculum age**
  6-day old culture of Inoculum was observed to be the optimum for high yield of lovastatin **269.01 mg/lit** through submerged fermentation.
- **Inoculum volume**
  Optimum Inoculum concentration of 5% **(v/v)** is observed suitable for the production of lovastatin of **292.65 mg/lit.**
- **Fermentation temperature**
  Maximum lovastatin yield of **301.25 mg/lit** was obtained at **30ºC** on 6 days of incubation
- **Initial pH**
  Maximum lovastatin yield of **337.78mg/lit** was obtained at **pH 6**.
- **Carbon source**
  Addition of Glucose **(2% w/v)** as carbon source showed maximum Lovastatin yield of **361.43 mg/lit.**
- **Nitrogen source**
  Addition of ammonium sulfate **(0.6% w/v)** as nitrogen source showed maximum lovastatin yield of **460.29 mg/lit.**

6.2 CONCLUSION:

The strain initially showed **161.54 mg/lit** of lovastatin production. After optimization of the physical and nutritional conditions, *Aspergillus terreus* MTCC 1782 was capable of producing about 3-fold increased lovastatin i.e., **460.29 mg/lit** in the fermentation broth. The strain holds a promise for scale up production of lovastatin under submerged fermentation after a comprehensive study.

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