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A REVIEW ON OPTIMIZATION OF GROWTH PARAMETERS FOR ENHANCED FUNGAL LIPASE PRODUCTION

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Abstract:

Lipases catalyze hydrolysis of fat/lipid into their components. Microbial lipases are preferred than the animal and plant lipases because of their stability, specificity and unique properties. Microbial lipases are excreted by a variety of bacteria, fungi and yeasts. Lipases are used in various industries (pharmaceutical, food, detergent, oil, paper etc). At present the demand of industries for lipases are very higher, therefore we have to increase the production of enzyme form indigenous microbial strains in order to fulfill the various applications in industries. Normally microbial strain produces little amount of lipases to meet the cellular requirements but for the industrial purposes, the amount of lipases can be enhanced by the optimization technique. Different parameters (carbon sources, nitrogen sources, pH, temperature and incubation period) can be optimized in order to increase productivity of lipase. Lipase production is normally stimulated in presence of lipidic carbon sources (oil) into the culture media, which indicates the inducible nature of microbial lipases. By keeping the above points in mind, the present review is focused on optimization of culture medium constituents and growth conditions for increased growth of lipolytic fungi and productivity of extracellular lipase.

Keywords: *Lipases, microbial strains, optimization technique, lipolytic fungi, pH, temperature, carbon source*

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INTRODUCTION:

Lipases belonging to the class Hydrolases catalyze hydrolysis of insoluble triacylglycerols to generate glycerol and monoacylglycerols, diacylglycerols and free fatty acids [1]. Lipases are manufactured by a variety of living organisms (bacteria, fungi, plants and animal). But microbial lipases are more useful for industries than other sources of lipases [2]. For improved production of enzyme from a variety of fungal organisms, optimization studies are of most significance. Production of lipase depends on the type of fungal strains, constituents of the culture medium, culture conditions, temperature, pH and the type of nitrogen and carbon sources [3]. As the quantity of enzymes produced by parent strains is low, thus excess production of the enzymes requires optimization of culture medium. The optimization technique is the process in which different medium ingredients and cultivation conditions are optimized to support the highest growth of particular microbial strain in order to increase the production of metabolites for biotechnological applications. The microorganisms produce a variety of important products but the quantity produced is useful for them only, for that reason excess production of metabolites hardly occurs. Hence, it is required to optimize the culture conditions constantly for overproduction of metabolites and to make the fermentation procedure inexpensive [4]. The main advantages of culture medium optimization are as follows: (i) decrease in overall cost of fermentation process; (ii) raise in the productivity and (iii) formulation of production medium.

Optimization of culture conditions for lipase production:

The majority of the microbial lipases are extracellular in nature and their production is significantly affected by composition of medium, sources of carbon and nitrogen besides physic-chemical factors such as dissolved oxygen, pH and temperature. Generally, high productivity can be obtained by optimization of culture medium. Submerged fermentation (SmF) is a method of choice for the production of microbial lipases but solid state fermentation (SSF) can be used also [2, 5].

Impact of carbon sources on lipase production:

Carbon source has always been documented as the main factor for the expression of lipase activity due to inducible nature of lipases. Lipase production is generally stimulated by lipids [6]. Lipases are normally produced when the medium is supplemented with lipid such as oil or any other inducer, such as fatty acids, Tween-20, Tween-80, hydrolysable esters, glycerol, triacylglycerols and bile salts. Lipidic carbon sources are essential for getting a high amount of lipase [5, 7].

Ghasemi *et al.* [8] studied lipase production by *A. niger* in Kilka fish oil (4.59 U ml^{-1}) and olive oil (5.46 U ml^{-1}). Cihangir and Sarikaya [9] reported that the medium containing olive oil as source of carbon exhibited both the highest production of lipase and also the highest biomass by *Aspergillus* sp. when compared to other media with different carbon sources. Bindiya and Ramana [4] investigated the effect of various non lipidic sources of carbon (galactose, glucose, xylose, fructose, lactose, maltose, sucrose and mannitol) and lipidic carbon sources (coconut oil, palm oil, cucumber oil, olive oil, mustard oil, sunflower oil and neem oil) on production of lipase by *A. sydowii*. Among the lipidic carbon sources, maximum activity (55 U ml^{-1}) of lipase was obtained in the presence of olive oil (1% v/v). On the other hand, among the non lipidic carbon sources highest lipase activity (40Uml^{-1}) was achieved with sucrose (1% w/v) and lowest activity (18 U ml^{-1}) with fructose (1% w/v). Further, the enzyme production was investigated under different concentrations of sucrose (0.50 to 3.0% w/v). Sucrose at 2% concentration exhibited maximum activity of lipase (49 U ml^{-1}).

Lima *et al.* [10] investigated impact of various lipidic carbon sources (soyabaen oil, sunflower oil and olive oil, 1% v/v) on the efficiency of lipase production by *P. aurantiogriseum*. Highest lipase activity (25 U ml^{-1}) was achieved using olive oil. Lipid source as well as its concentration both significantly influences the microbial lipase production [11, 12]. Therefore, the influence of various concentrations of olive oil was investigated on the production of lipase and maximum production was achieved at a concentration of 3% v/v. Sumathy *et al.* [13] investigated the effect of various concentrations (0.5% and 1.0% v/v) of lipidic inducers (olive oil, gingely oil and mustard oil) on efficiency of lipase activity by three different fungi, *A. niger*, *R. oryzae* and *F. oxysporum*. Amongst all, highest activity (6 U ml^{-1}) was achieved by *A. niger* with olive oil (1% v/v). Costa *et al.* [14] reported highest activity of lipase (12.50 U ml^{-1}) by *Penicillium wortmanii* using olive oil (5% v/v). Nwuche and Ogbonna [15] reported production of lipase by twelve fungal isolates belonging to genera *Trichoderma*, *Aspergillus*, *Mucor* and *Penicillium* using SmF in the presence of palm oil as source of carbon. *Tichoderma* sp. (8.24 U ml^{-1}) and *Aspergillus* sp. (7.54 U ml^{-1}) were the maximum lipase producers, while the least activity (5.72 U ml^{-1}) was obtained by *Mucor* sp. Shukla and Desai [16] investigated influence of various oils on the activity of lipase by *Pseudomonas* sp. isolated from samples of oil mills. Olive oil was the excellent inducer of lipase activity (2.6 U ml^{-1} min^{-1}), followed by coconut oil (2.3 U ml^{-1} min^{-1}),

sunflower oil (2.2 U ml^{-1} min^{-1}), ground nut oil (1.6 U ml^{-1} min^{-1}) and mustard oil (1.30 U ml^{-1} min^{-1}). Bhavani *et al.* [17] tested extracellular lipase production by eight lipolytic bacterial isolates in liquid media and reported that bacterial isolate named B1 exhibited the highest lipase activity of 14 U ml^{-1} at 30 ºC after 48 h of incubation.

Ghosh *et al.* [18] previously reported that activity of lipase from *R. nigricans* was significantly increased by the addition of glucose and triglycerides into the medium which reveals that beside glucose, an inducer is also required for lipase production. Falony *et al.* [19] reported activity of lipase by *A. niger* using SmF in the presence of various carbon sources (sunflower oil, olive oil, coconut oil, glycerol and starch). The lipase activity was 0.53 U ml^{-1} with olive oil (2% v/v) but it was increased to 0.99 U ml^{-1} when glucose (2% w/v) and olive oil (2% v/v) were added into the medium. Sarkar and Laha [20] reported production of lipase by *A. niger* using SmF in the presence of various concentrations (0.5-2.0%) of two carbon sources olive oil and glucose together. Maximum activity of lipase (1.46 U ml^{-1}) was observed when olive oil (2% v/v) and glucose (2% w/v) were added into the medium. Similar results were also obtained by Sharma *et al.* [21], where mixture of glucose and olive oil supported highest production of lipase by a wild and a mutant strain of *A. niger*.

Beside olive oil, several investigators [22, 23, 24] reported Tween 80 as the excellent carbon source for production of lipase. Similarly, Bussamara *et al.* [25] reported increase in the efficiency of lipase activity up to 150% from the yeast *Pseudozyma hubeiensis* HB85A in presence of Tween-80 into the medium. Iftikhar *et al.* [24] reported that among all the concentrations of Tween-80 (0.2% to 1.0% v/v), maximum lipase activity was achieved by both the parent (5.68 ± 0.01 U ml^{-1}) and mutant (22.62 ± 0.01 U ml^{-1}) strain of *R. oligosporus* IIB-63 at 0.6% concentration as it gave optimum quantity of Tween-80 for the lipase production. However, Lianghua and Liming [26] reported that Tween-80 decreased the production of lipase by *B. coagulans* as compared with the other sources of carbon. Another investigator [27] studied the impact of glycerol and Tween-20 on lipase production by three different *Aspergillus* sp. Highest lipase activity (5.6 U ml^{-1}) was achieved from *A. niger* followed by *A. aculeatus* (5.3 U ml^{-1}) and *A. paraciticus* (4.5 U ml^{-1}) using Tween-20 as source of carbon.

Impact of nitrogen sources on lipase production:

Cihangir and Sarikaya [9] reported optimum lipase activity (14.83 U ml^{-1}) by novel isolate of *Aspergillus* sp. when medium was supplemented with peptone (1% w/v) followed by yeast extract (12.76 U ml^{-1}), $(NH_4)_2SO_4$ (8.83 U ml^{-1}), NH_4NO_3 (7.50 U ml^{-1}), soyabean meal (6.87 U ml^{-1}) and urea (5.48 U ml^{-1}). Lowest activity in urea may be attributed to its toxicity to the cells. Lipase activity was also influenced by mixture of organic nitrogen sources as described by Ulker *et al.* [28] where maximum lipase activity by *T. harzianum* was achieved in a medium containing glucose and peptone as source of carbon and nitrogen, respectively, while minimum activity was obtained with glucose and yeast extract medium. However, Pimentel *et al.* [29] previously reported highest activity of lipase (409 U ml^{-1}) by *P. citrinum* when yeast extract (0.5% w/v) was supplemented to the medium as nitrogen source. Naz and Jadhav [27] also reported maximum lipase activity (6.8 U ml^{-1}) by *A. aculeatus* using yeast extract as source of nitrogen. Similarly, lipase activity was enhanced by *Ophiostoma piceae* when $(NH_4)_2SO_4$ and peptone were added into production medium [30].

Recently, Aruna and Khan [31] reported that maximum activity of lipase (47.49 U ml^{-1} min^{-1}) was obtained by *Staphylococcus pasteuri* SNA59 when yeast extract was the source of nitrogen into the fermentation broth. Veerapagu *et al.* [5] reported that among ten various organic sources of nitrogen, proteose peptone (168.7 U ml^{-1}) and peptone (132.5 U ml^{-1}) increased the productivity of lipase by *P. gessardii*, whereas production of lipase was very little with soy peptone, casein and soyabean meal.

Lima *et al.* [10] investigated the influence of various nitrogen sources (yeast extract, peptone, meat peptone, casein, $(NH_4)_2SO_4$ and KNO_3) on the efficiency of lipase production by *P. aurantiogriseum*; $(NH_4)_2SO_4$ being the most efficient one with 13 U ml^{-1} lipase activity. Bindiya and Ramana [4] used various nitrogen sources (1% w/v) to investigate their influence on production of lipase by *A. sydowii*. The nitrogen sources used in the study were $NaNO_3$, KNO_3, NH_4NO_3, NH_4Cl, $(NH_4)_2SO_4$, urea, beef extract, malt extract, yeast extract, tryptone and peptone. Highest activity (49 U ml^{-1}) was observed using NH_4Cl. Further, enzyme production was studied under different concentrations of NH_4Cl (0.50 to 5.0% w/v) and the optimum concentration was found to be at 3.5% w/v. Similarly, NH_4Cl was reported as the excellent source of nitrogen for optimum lipase activity by *Candida cylindracea* [32]. Salihu *et al.* [33] reported optimum activity of lipase by *A. niger* when medium was fortified with Tween-80 (1% v/v), $(NH_4)_2SO_4$ (0.35% w/v) and Na_2HPO_4 (0.40% w/v). Papaparaskevas *et al.* [34] reported that inorganic nitrogen source, $(NH_4)_2SO_4$ stimulated lipase production by the yeast *Rhodotorula glutinis*. Abdel-Fattah and Hammad [35] reported highest lipase production by *A. niger* and *A. terreus* when the medium was supplemented with KNO_3.

Impact of temperature on lipase production:

Mahmoud *et al.* [36] investigated impact of temperatures on production of lipase by incubating the cultures of *A. terreus* at various temperatures viz. 10 ºC, 20 ºC, 30 ºC and 45 ºC. Highest activity of lipase was obtained at 45 ºC (15 U ml^{-1}), which was followed by 30 ºC (12 U ml^{-1}), 20 ºC (9.5 U ml^{-1}) and 10 ºC (3.0 U ml^{-1}). Similarly, Mukhtar *et al.* [37] studied the impact of various incubation temperatures ranging from 25 to 55 ºC on the productivity of lipase by *A. niger*. Highest production was achieved at 30 ºC, followed by 35 ºC, 40 ºC, 45 ºC, 25 ºC, 50 ºC and 55 ºC. Sumathy *et al.* [13] reported that cultures of *A. niger* were incubated at 25 ºC, 30 ºC and 37 ºC, followed by estimation of activity of lipase after 48 h, 72 h, 96 h and 120 h. Highest activity of lipase was achieved in the culture incubated at 30 ºC after 96 h of incubation. Bindiya and Ramana [4] investigated lipase production by *A. sydowii* at various incubation temperatures. Highest activity (64 U ml^{-1}) was obtained at 32 °C, followed by 30 °C, 28 °C, 26 °C, 24 °C, 22 °C, 20 °C and 34 °C, respectively. Reshma and Shanmugam [38] reported that highest activity of alkaline lipase was obtained when the culture of *A. brasiliensis* was incubated at 27 °C with pH of 7.0. The majority of lipolytic microrganisms are mesophilic in nature (growing at moderate temperature range from 25 to 40 °C) [39].

Lima *et al.* [10] investigated production of lipase by *P. aurantiogriseum* under different incubation temperatures (26-32 ºC). Lipase production was highest at 29 ºC. Another investigator [40] studied optimization of lipase production by *P. citrinum.* Highest production of lipase was achieved at pH 7.0 and temperature of 22 ºC. Falony *et al.* [19] reported that lipase production by *A. niger* was highest at 40 ºC. Thakur *et al.* [41] recently investigated lipase production by *P. stutzeri* MTCC5618 at different incubation temperatures (25ºC,30 ºC and 37 ºC) and maximum activity was reported at 30 ºC with 1.34 fold with respect to control. Shukla and Desai [16] reported highest activity of lipase (2.6 Uml^{-1} min^{-1}) by *Pseudomonas* sp. at 30 ºC. An optimum temperature of 30 ºC has been documented by Pagori *et al.* [42] for the activity of *R. chinensis* lipase. However, Naz and Jadhav [27] reported an optimum temperature of 55 ºC for the growth and lipase production (6.5 U ml^{-1}) by *A. niger* followed by 45 ºC (5.6 U ml^{-1}), 35 ºC (3.5 U ml^{-1}) and 25 ºC (4.9 U ml^{-1}).

Impact of intial pH on lipase production:

Potent lipolytic fungi such as *Aspergillus* sp., *Geotrichum* sp., *Rhizopus* sp., *Rhizomucor* sp., *Mucor* sp. and *Penicillium* sp. are able to grow and secret extracellular lipases within the pH range of 6.0 to 8.0 [43]. Rai *et al.* [44] studied lipase production by *A. niger* at different pH ranging from 4.0-9.0. Highest activity of lipase (2.4 U ml^{-1}) was obtained using organic nitrogen source at pH 7.0 after 144 h of incubation. Abdel-Fattah and Hammad [35] studied the effect of various pH (2.0-8.0) on production of lipase by *A. niger* and *A. terreus*. Highest production was achieved at pH 6.0 and it decreased above pH 6.0 in this experiment. Bindiya and Ramana [4] investigated activity of lipase by *A. sydowii* at different pH of fermentation broth. pH 8.0 was the best for high yield of lipase, followed by pH 7.5, 8.5, 7.0, 9.0, 6.5 and 6.0. Similarly, Mahmoud *et al.* [36] investigated influence of various pH on production of lipase by growing *A. terreus* at various pH ranging from 2.0-12.0. An optimum pH of 8.0 was found for highest lipase activity (15 U ml^{-1}). *A. terreus* demonstrated the lipase activity of 12 U ml^{-1} at pH 12.0, while the activity was not found at pH 2.0, which indicates that alkaline conditions promotes growth and lipase production by the fungus.

Activity of extracellular lipase was increased by NCIM 1207 strain of *A. niger* when cultivated at pH 2.5 [45]. Similarly, Naz and Jadhav [27] reported increased production of lipase (62.7 U ml^{-1}) by *A. niger* when cultivated at pH 5.0, followed by pH 4.0 (54.6 U ml^{-1}), pH 6.0 (11.8 U ml^{-1}), pH 7.0 (5.6 U ml^{-1}), pH 8.0 (5.1 U ml^{-1}) and pH 9.0 (4.5 U ml^{-1}). Selvamohan *et al.* [46] reported highest activity of lipase by *B. amyloliquefaciens* at pH 9.0 at 48 h of incubation. Bhosale *et al.* [47] recently reported pH 9.0 for highest lipase activity by thermo-alkalophilic *Bacillus* sp. 8C after investigating the influence of various initial pH of fermentation broth on lipolytic efficiency. Aruna and Khan [31] observed maximum lipase production by *Staphylococcus pasteuri* SNA59 when pH of the fermentation broth was adjusted to 10.0. Geon-Ho *et al.* [48] noticed optimum production of lipase from *Yarrowia lipolytica* under alkaline conditions at pH 9.0. Highest activity of lipase at pH 9.0 suggests that organism requires slightly alkaline pH for its metabolic processes and for the production of lipase [49]. However, Shukla and Desai [16] reported highest lipase activity (1.9 U ml^{-1} min^{-1}) by *Pseudomonas* sp. at pH 7.5. The bacterium was able to produce high quantity of lipase within the alkaline pH range (7.5-9.0) than in the acidic pH range (5.0-6.5).

Impact of incubation period on lipase production:

The impact of incubation time on production of lipase by different microorganisms has been studied by many workers. An optimum activity of lipase by *Fusarium solani* was reported by Maia *et al.* [50] at 25 ºC after 72 h of incubation. The high yield of lipase was also reported by the fungus *P. simplicissimum* and *Vibrio fischeri* at same incubation period of 3 days by Gutarra *et al.* [39] and

Ranjitha *et al.* [51], respectively. Recently, Mukhtar *et al.* [37] investigated lipase production by *A. niger* at various incubation periods ranging from 24-120 h. Highest activity of lipase (5.0 U ml^{-1}) was achieved at 72 h which gradually declined and reached to 2.0 U ml^{-1} at 120 h due to exhaustion of nutrients and accumulation of toxic metabolic waste products. Another investigator [27] reported maximum activity of lipase by *A. paraciticus* (35.5 U ml^{-1}), *A. aculeatus* (15.0 U ml^{-1}) and *A. niger* (14.2 U ml^{-1}) after 2 days of incubation. Thereafter, declining trend in lipase activity was observed with the rise of incubation period beyond 72 h.

There are studies which showed the best lipase activity at 4-5 days of incubation. Costa and Peralta [14] found the highest activity of lipase (16.66U ml^{-1}) at 4 days of incubation by a novel strain of *P. wortmanii* in SmF, followed by decrease in activity with the increase of incubation period. Similarly, Bindiya and Ramana [4] reported that highest enzyme production (64 U ml^{-1}) by *A. sydowii* was achieved at 96 h of incubation. The activity increased from 48 h to 96 h of incubation, thereafter declined and reached to minimum at 168 h of incubation. The highest increase in 5 days cultures of *A. niger* and *A. terreus* have been reported by Abdel-Fattah and Hammad [35], thereafter the gradual decline was observed up to 192 h of incubation.

Higher incubation periods have also been documented occasionally by many investigators such as Cihangir and Sarikaya [9] found lipase activity by novel isolate of *Aspergillus* sp. over a 10 days period and Ulker *et al.* [28] reported optimum lipase activity (0.24 U ml^{-1}) by *T. harzianum* in a 168 h culture of the organism. In an another report, Shu *et al.* [52] showed the increase of activity in lipase by *Antrodia cinnamomea* lipase using glucose (3% w/v) and olive oil (0.01% v/v) in aerated bioreactor after 18 days of fermentation.

CONCLUSION:

Lipases are very important enzymes among the other hydrolytic enzymes for industrial applications. Lipases are efficiently synthesized by microorganisms but its amount is low. Its amount can be increased by optimization of culture conditions (carbon and nitrogen sources) and growth conditions (pH, temperature and incubation period). Optimized culture media and conditions support the growth of the microbes and productivity of enzymes.

REFERENCES:

1.Das A, Shivakumar S, Bhattacharya S, Shakya S, Swathi SS. Purification and characterization of a surfactant-compatible lipase from *Aspergillus tamarii* JGIF06 exhibiting energy-efficient removal of oil stains from polycotton fabric. 3 Biotech, 2016; 6(2): 1-8.

2.Sharma R, Yusuf C, Banerjee UC. Production, purification, chracterization, and applications of lipases. Biotech Adv, 2001; 19(8): 627-662.

3.Thakur S. Lipases, its sources, Properties and Applications: A Review. Int J Sci Eng Res, 2012; 3(7): 1-29.

4.Bindiya P, Ramana T. Optimization of lipase production from an indigenously isolated marine *Aspergillus sydowii* of Bay of Bengal. J Biochem Technol, 2012; 3(5): 503-211.

5.Veerapagu M, Narayanan AS, Ponmurugan K, Jeya KR. Screening, selection, identification, production and optimization of bacterial lipase from oil spilled soil. Asian J Pharm Clin Res, 2013; 6(3): 62-67.

6.Sivakumar N. Production of Microbial Lipase Enzyme-Review. Int J Adv Res Biol Sci, 2014; 1(1): 01-12.

7.Gupta R, Gupta N, Rathi P. Bacterial lipases: An overview of production, purification and biochemical properties. Appl Microbiol Biotechnol, 2004; 64(6): 763-781.

8.Ghasemi M, Farahbakhsh A, Farahbakhsh A, Safari AA. Using Submerge Fermentation Method to Production of Extracellular Lipase by *Aspergillus niger*. International Journal of Biological, Biomolecular, Agricultural, Food Biotechnol Eng, 2014; 8(9): 1020-1023.

9.Cihangir N, Sarikaya E. Investigation of lipase production by a new isolated of *Aspergillus* sp. World J Microbiol Biotechnol, 2004; 20(2): 193-197.

10.Lima VMG, Krieger N, Sarquis MIM, Mitchell DA, Ramos LP, Fontana JD. Effect of nitrogen and carbon sources on lipase production by *Penicillium aurantiogriseum*. Food Technol Biotechnol, 2003; 41(2): 105-110.

11.Maia MMD, Heasley A, Morais MMCD, Melo EHM, Morais MA, Ledingham WM, Lima JL. Effect of culture conditions on lipase production by *Fusarium solani* in batch fermentation. Bioresour Technol, 2001; 76(1): 23-27.

12.Silva WOB, Mitidieri S, Schrank A, Vainstein MH. Production and extraction of an extracellular lipase from the entomopathogenic fungus *Metarhizium anisopliae*. Process Biochem, 2005; 40(1): 321-326.

13.Sumathy R, Vijayalakshmi M, Deecaraman M. Studies on Lipase production from fungal strains by different inducers at varied concentrations - A comparative study. Int J Environ Sci, 2012; 3(3): 1072-1078.

14.Costa MA, Peralta RM. Production of lipase by soil fungi and partial characterization of lipase from a selected strain (*Penicillium wortmanii*). J Basic Microbiol, 1999; 39(1): 11-15.

15.Nwuche CO, Ogbonna JC. Isolation of Lipase Producing Fungi from Palm Oil Mill Effluent (POME) Dump Sites at Nsukka. Braz Arch Biol Technol, 2011; 54(1): 113-116.
16.Shukla BN, Desai PV. Isolation, Characterization and Optimization of Lipase Producing *Pseudomonas* sp. from Oil Contaminated Sites. Int J Curr Microbiol & Appl Sci, 2016; 5(5): 902-909.
17.Bhavani M, Chowdary GV, David M, Archana G. Screening, Isolation and Biochemical Characterization of Novel Lipase Producing Bacteria from Soil Samples. Int J Biol Eng, 2012; 2(2): 18-22.
18.Ghosh PK, Saxena RK, Gupta R, Yadav RP, Davidson S. Microbial lipases: Production and applications. Sci Prog, 1996; 79(2): 119-157.
19.Falony G, Armas JC, Mendoza JCD, Hernandez JLM. Production of Extracellular Lipase from *Aspergillus niger* by Solid-State Fermentation. Food Technol Biotechnol, 2006; 44(2): 235-240.
20.Sarkar D, Laha S. Optimization of extracellular lipase enzyme production from *Aspergillus niger* by submerged and solid state fermentation process. Int J Pharma Bio Sci, 2013; 4(4): 978-985.
21.Sharma AK, Sharma V, Saxena J, Kuila A. Comparative studies of enhanced lipase production from wild and mutagenic strain of *Aspergillus niger* LPF-5. Afr J Biotech. 2016; 15(41): 2292-2300.
22.Dai D, Xia L. Enhanced production of *Penicillium expansum* PED-03 lipase through control of culture conditions and application of the crude enzyme in kinetic resolution of racemic Allethrolone. Biotechnol Prog, 2005; 21(4): 1165-1168.
23.Singh S, Kaur G, Chakraborti AK, Jain RK, Banerjee UC. Study of the experimental conditions for the lipase production by a newly isolated strain of *Pseudomonas aeruginosa* for the enantioselective hydrolysis of (±)-methyl trans-3 (4-methoxyphenyl) glycidate. Bioprocess Biosyst Eng, 2006; 28(5): 341-348.
24.Iftikhar T, Niaz M, Haq IU. Comparative studies on the lipolytic potential of wild and mutant strains of *Rhizopus oligosporous* var. *microsporous* IIB-63 isolated from lipid rich habitats. Pak J Bot, 2010; 42(6): 4285-4298.
25.Bussamara R, Fuentefria AM, de Oliveira E, Broetto L, Simcikova M, Valente A, Vainstein MH. Isolation of a lipase-secreting yeast for enzyme production in a pilot-plant scale batch fermentation. Bioresour Technol, 2010; 101(1): 268-275.
26.Lianghua T, Liming X. Purification and partial characterization of a lipase from *Bacillus coagulans* ZJU318. Appl Biochem Biotechnol, 2005; 125(2): 139-146.
27.Naz S, Jadhav SK. Studies of the Estimation of Lipase Production Capability of Some Fungal Species and their Application in Oil Spillage Degradation. Int J Sci Res, 2013; 4(1): 2154-2159.
28.Ulker S, Ozel A, Colak A, Karaoglu SA. Isolation, production, and characterization of an extracellular lipase from *Trichoderma harzianum* isolated from soil. Turk J Biol, 2011; 35(5): 543-550.
29.Pimentel MC, Krieger N, Coelho LC, Fontana JO, Melo EH, Ledingham WM, Lima Filho JL. Lipase from a Brazilian strain of *Penicillium citrinum*. Appl Biochem Biotechnol, 1994; 49(1): 59-74.
30.Gao Y, Breuil C. Extracellular lipase production by a sapwood-staining fungus *Ophiostoma piceae*. World J Microbiol Biotechnol, 1995; 11(6): 638-642.
31.Aruna K, Khan K. Optimization studies on production and activity of lipase obtained from *Staphylococcus pasteuri* SNA59 isolated from spoilt skin lotion. Int J Curr Microbiol Appl Sci, 2014; 3(5): 326-347.
32.Brozzoli V, Crognale S, Sampedro I, Federici F, D'Annibale A, Petruccioli M. Assessment of olive-mill wastewater as a growth medium for lipase production by *Candida cylindracea* in bench-top reactor. Bioresour Technol, 2009; 100(13): 3395-3402.
33.Salihu A, Bala M, Alam MZ. Lipase production by *Aspergillus niger* using sheanut cake: An optimization study. J Taibah Univ Sci, 2015; DOI: 10.1016/j.jtusci.2015.02.011.
34.Papaparaskevas D, Christakopoulos P, Kekos D, Macris BJ. Optimizing production of extracellular lipase from *Rhodotorula glutinis*. Biotechnol Lett, 1992; 14(5): 397-402.
35.Abdel-Fattah GM, Hammad I. Production of Lipase by Certain Soil Fungi, Optimization of Cultural Conditions and Genetic Characterization of Lipolytic Strains of *Aspergilli* Using Protein Patterns and Random Amplified Polymorphic DNA (RAPD). Online J Biol Sci, 2002; 2(10): 639-644.
36.Mahmoud GA, Koutb MMM, Morsy FM, Bagy MMK. Characterization of lipase enzyme produced by hydrocarbons utilizing fungus *Aspergillus terreus*. Eur J Biol Res, 2015; 5(3): 70-77.
37.Mukhtar H, Hanif M, Rehman AU, Nawaz A, Haq IU. Studies on the Lipase Production by *Aspergillus niger* through Solid State Fermentation. Pak J Bot, 2015; 47: 351-354.
38.Reshma CH, Shanmugam P. Isolation and Characterization of the Lipase from *Aspergillus brasiliensis*. Int J Biotechnol Bioeng Res, 2013; 4(5): 481-486.
39.Gutarra MLE, Godoy MG, Maugeri F, Rodrigues MI, Freire DMG, Castilho LR. Production of an acidic and thermostable lipase of the mesophilic fungus *Penicillium simplicissimum* by solid-state fermentation. Bioresour Technol, 2009; 100(21): 5249-5254.

40.Maliszewska I, Mastalerz P. Production and some properties of lipase from *Penicillium citrinum*. Enzyme Microb Technol, 1992; 14(3): 190-193.
41.Thakur V, Tewari R, Sharma R. Evaluation of Production Parameters for Maximum Lipase Production by *Pseudomonas stutzeri* MTCC 5618 and Scale-Up in Bioreactor. Chin J Biol, 2014; 2014: 1-14.
42.Pagori N, Cheikhyoussef A, Xu Y, Wang D. Production and biochemical characterization of extracellular lipase from *Rhizopus chinensis* CCTCC M201021. Biotechnol, 7 2008; (4):710-717.
43.Treichel H, De Oliveira D, Mazutti MA, Di Luccio M, Oliveira VJ. A Review on Microbial Lipases Production. Food Bioprocess Tech, 2010; 3(2): 182-196.
44.Rai B, Shrestha A, Sharma S, Joshi J. Screening, Optimization and Process Scale up for Pilot Scale Production of Lipase by *Aspergillus niger*. Biomed Biotechnol, 2014; 2(3): 54-59.
45.Mhetras NC, Bastawde KB, Gokhale DV. Purification and characterization of acidic lipase from *Aspergillus niger* NCIM 1207. Bioresour Technol, 2009; 100(3): 1486-1490.
46.Selvamohan T, Ramadas V, Sathya TA. Optimization of Lipase Enzyme Activity Produced By *Bacillus amyloliquefaciens* Isolated From Rock Lobster *Panlirus homarus*. Int J Mod Eng Res, 2012; 2(6): 4231-4234.
47.Bhosale H J, Uzm SZ, Bismile PC. Optimization of Lipase Production by Thermo-Alkalophilic *Bacillus* sp. 8C. Res J Microbiol, 2015; 10(11): 523-532.
48.Geon-Ho L, Bae J, Suh M, Kim I, Hou CT, Kim H. New finding and optimal production of a novel extracellular alkaline lipase from *Yarrowia lipolytica* NRRL Y-2178. J Microbiol Biotechnol, 2007; 17(6): 1054-1057.
49.Kiran GS, Shanmughapriya S, Jayalakshmi J, Selvin J, Gandhimathi R, Sivaramakrishnan S, Arunkumar M, Thangavelu T, Natarajaseenivasan K. Optimization of extracellular psychrophilic alkaline lipase produced by marine *Pseudomonas* sp. (MSI057). Bioprocess Biosyst Eng, 2008; 31(5): 483-492.
50.Maia MDMD, Morais MMCD, Morais MAD, Melo EHM, Filho JLDL. Production of extracellular lipase by the phytopathogenic fungus *Fusarium solani* FS1. *Revista de Microbiologia*, 1999; 30(4): 304-309.
51.Ranjitha P, Karthy ES, Mohankumar A. Purification and Characterization of the Lipase from Marine *Vibrio fischeri.* Int J Biol, 2009; 1(2): 48-56.
52.Shu CH, Xu CJ, Lin GC. Purification and partial characterization of a lipase from *Antrodia cinnamomea*. Process Biochem, 2006; 41(3): 734-738.