International Research Journal of Engineering and Technology (IRJET) e-ISSN: 2395-0056
Volume: 02 Issue: 06 | Sep-2015 www.irjet.net p-ISSN: 2395-0072

Standardization of process parameters for the maximum production of extracellular lipase by bacteria, isolated from indigenous sources

Debadrita Paul[1], Sumit Saha[2], Sougata Pramanick[3], Soham Chattopadhyay[4*]

[1]*Student, Department of Biotechnology, Heritage Institute of Technology Kolkata, West Bengal, India*
[2]*Student, Department of Biotechnology, Heritage Institute of Technology Kolkata, West Bengal, India*
[3]*Student, Department of Biotechnology, Heritage Institute of Technology Kolkata, West Bengal, India*
[4*]***Corresponding Author**: Assistant Professor, Department of Biotechnology, Heritage Institute of Technology Kolkata, West Bengal, India*

Abstract–Lipases (triacylglycerol acylhydrolases, E.C. 3.1.1.3) are hydrolases, which act under aqueous conditions on the carboxyl ester bonds present in triacylglycerols to liberate fatty acids and glycerol. Out Of the different lipase sources, bacterial lipase is of considerable economic importance because of potential industrial applications. Efforts to optimize lipase production have been done in different reports in literature but still this field holds a wide scope of research due to different novel types of lipases which are yet to be found out and utilized scientifically owing to their characterization and optimum production. In the present study,lipase producing bacteria were screened from water samples around industrial areas near Kolkata and partially characterized. They were used to produce lipase by optimizingmedia components and physicochemical parameters. The growthprofile in production mediashowed that lipase was produced followingnon-growth associated pattern with its maximum activity at 37 °C and pH 7. Lipase production was found to be optimum with an unsaturated oil (olive oil, 10 ml/L) as inducing agentas compared to other oils in media. Lipase activity also increased with the addition of non-ionic detergent like Triton-X 100 upto a certain concentration (3 ml/L).The standardized conditions obtained were as follows: Peptone 10 g/L, yeast extract 3 g/L and $MgSO_4$ 0.5 ml/L. The experimental data were validated and 57% increase in lipase production was observedas compared to standard production media described in the literatures.

Key Words:*Lipases, Activity, Production, Media Optimization*.

1. INTRODUCTION

Triacylglycerol lipases (E.C. 3.1.1.3) are hydrolases, which act on the carboxyl ester bonds present in triglycerides under aqueous conditions to liberate fatty acids and glycerol[1]. Lipases have been found in many species of animals, plants, and microorganisms. However, the enzymes from microbial sources are currently receiving more attention [2, 3]. Microorganisms including bacteria, fungi, yeast etc. are considered as preferred sources of extracellular lipases. Due to their bulk production, extracellular bacterial lipases are of considerable commercial importance. Some important lipase-producing bacterial genera include Bacillus, Pseudomonas and Burkholderia [4].

Lipases as a commercial enzyme areused widelyin industry, such as food, detergent, chemical and pharmaceuticals [5, 6, 7]. The availability of lipases with suitable properties are increasing in numbersand new researchare carried out to commercialize biotransformation and synthesis based on lipases [8].

Recently, lipases have beenconsidered as key enzymes for their multidimensional properties, which find usage in a wide range of industrial applications [4]. This is clearly demonstrated by the amount of information reported in the literature ranging from production, purification, and various industrial applications. This however, require greater understanding of the importance of optimization of lipase production both in small scale and large scale.

Bacterial lipases are produced in the production media generally in the presence of lipids [4], stimulated by triglycerides, with the presence of complex nitrogen sources, preferably organic nitrogen sources as peptone and yeast extract [10, 11].In addition to the various chemical constituents of a production medium, physiological parameters such as pH, Temperature and incubation period also play an important role in influencing production by different microorganisms [4]. Furthermore, effects of cations like Ca^{+2} and Mg^{+2} have been reported to enhance the lipase activity for some lipases [3, 12]whereas reports are also available on mild inhibition of activity by calcium ions for some other lipases[13]. The optimization of media components is the primary step towards the maximum production of lipase using different concentration of the substrates used in the production media [14].

Thus, the aim of the present work was to optimize the different media parameters for maximum lipase production, using the single parameter optimization method of determining optimum conditions [15], by a newly isolated gram negative coccus bacterial strain which is not commonly found in bacterial lipase literatures. As for the parameters taken into consideration, medium composition, medium pH and the incubation temperature were examined for optimization of the lipase production.

2. MATERIALS AND METHODS

2.1 Sample Collection

Samples were collected from oil contaminated industrial regions situated in and around Kolkata, West Bengal, India.

2.2 Isolation and Screening of Lipolytic Bacteria

In order to screen the microorganisms with lipolytic activity, the collected water samples were diluted to 10^{-2} dilution and 20 µL of each filtrate was spread on a LB agar plate supplemented with Rhodamine B(0.9%, w/v) and olive oil (1%, v/v) [16]. The plates were incubated at 37 °C for 2 days. Bacterial colonies that showed bright halos under UV light were selected and were sub-cultured in order to attain a pure colony.

2.3 Characterization of the Isolates

To partially characterize the bacterial strain showing lipolytic activity, gram staining was done from smears taken from the pure colony that was isolated by sub-culturing.

2.4 Selection of Production Media

From literature, among different bacterial lipase production media, the composition of the selected production media was as follows (At pH 7) : peptone (10 g/L), Olive oil (10 g/L), Yeast extract (5 g/L), NaCl (1 g/L), Na_2HPO_4 (8.63 g/L), NaH_2PO_4 (6.08 g/L), Autoclaved $MgSO_4$ (1 ml from 500 g/L stock) [17].

2.5 Lipase Activity Assay

Lipase activity was measured by titrimetric method using olive oil as a substrate where an emulsion of olive oil in 2% PVA solution in the ratio of 1:9 was made. 1ml of this emulsion was added to 0.8 ml phosphate buffer (pH 7) and in this mixture, 0.2ml of centrifuged production sample was added. The reaction was incubated for 30 min at 37 °C and then stopped using ethanol: acetone (1:1) mixture and titrated with 0.1N NaOH using phenolphthalein indicator[18]. One unit of lipase activity was defined as the amount of lipase required to liberate 1µmol of fatty acid per minute.

2.6 Growth Characteristics of the bacterial strain in production medium

The bacterial strains were grown in the selected production media at 37 °Cand samples were collected at 3 hour intervals. The $O.D_{600}$ was measured for the collected samples and accordingly the growth curve was obtained as a measure of Log N (N= No. of cells/ml) vs. Time (hours). The same samples were used to measure lipase activity and subsequently a graph was obtained, relating the growth with lipase production, with respect to time.

2.7 Optimization of Production Conditions

2.7.1. Effect of Temperature on Lipase Production

For selection of optimum temperature for lipase production, incubation temperatures 30, 37, 40 and 45 °C were selected with 48 hours incubation period for each temperature variation keeping other media parameters un-altered. The optimumactivity was quantitativelymeasuredat 37 °C.

2.7.2. Effect of Media Components on Lipase production

For selection of optimum activity for lipase production with respect to different media component concentration, the media components were varied as follows: Peptone:2.5–20g/L; Olive oil: 2.5–15 ml/L; Yeast Extract: 3–7g/L; Autoclaved $MgSO_4$: 0.25–1.5 ml (from 500 g/L stock)/L. The components were varied one at a time keeping other parameters constant and lipase activity assay was performed for the optimum activity determination.

2.7.3. Effect of Different oils on Lipase Production

Keeping the other media components unchanged with their standard composition, three different oils, namely, Olive, Sunflower and Coconut oil were used to improve lipase production. Lipase activity was measured and best suitable lipid source was selected for further studies.

2.8 Standardization of physicochemical parameters

2.8.1Effect of Temperature on Lipase Activity

Bacterial lipases generally have an optimum temperature in the range of 30–50 °C[16, 19].For selection of optimum temperature for lipase activity, incubation temperatures ranging from 30°C to 45°C were selected during lipase activity assay keeping other parameters constant.

2.8.2Effect of pH on Lipase Activity

Generally Bacterial lipases have a neutral or alkaline pH optima [20, 21, 22].For selection of optimum pH for lipase activity, Phosphate buffer of varying pH from 6.5 to 8 was selected during lipase activity assay keeping other parameters same.

2.8.3Effect of Triton X on Lipase Activity

For selection of optimum lipase activity with non-ionic detergent Triton X, different volumes of Triton X ranging from 1ml/L to 4ml/L were used in activity assay keeping other parameters same.

2.9 Checking the validity of the optimized conditions for lipase production

The optimized conditioned obtained from experiments were validated by comparing lipase activity in standard production media to that of optimized media.

3. RESULTS AND DISCUSSION

3.1 Isolation and Screening of lipolytic Bacteria

The Bacterial colonies from the collected water sample that showed bright halos in Rhodamine agar plate under UV light were selected and they were sub-cultured in order to attain a pure colony.

3.2 Characterization of the Bacterial strain

It was observed from Gram staining that the lipase producing bacteria are gram negative and coccusshaped (Fig.1). This is not very common in lipase producing strains reported in literatures.

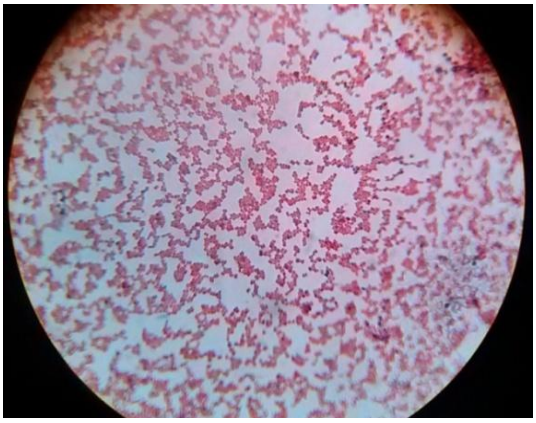

Fig.1: Gram Staining Characteristics of the Bacterial strain

3.3. Correlation between Growth characteristics and lipase activity

From Fig.2, the relation between the growth characteristics of the lipolytic bacterial strain and its lipase production characteristics with respect to time can be described as a non-growth associated production. From the graph we can determine that Lipase production tends to increase at the end of the log phase of the growth curve, similar to the trend in other bacterial lipase literature [4] where the lipase production peaks from the late log phase generally for extracellular bacterial lipase.

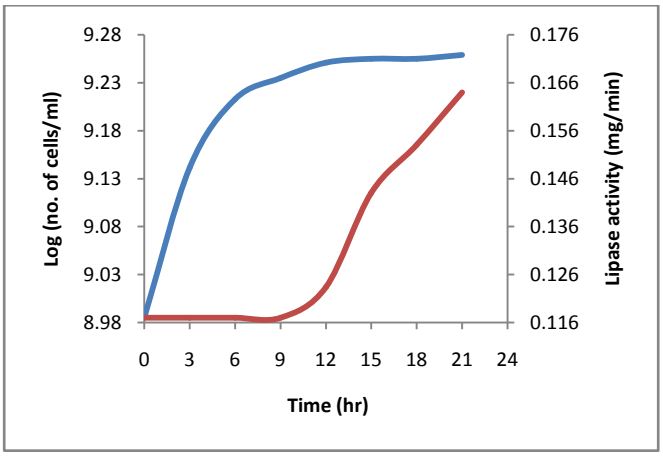

Fig.2: Growth characteristic of the isolated bacterial strain with lipase activity

3.4 Optimization of lipase production

3.4.1. Effect of Temperature on Lipase Production

The result shows that lipase production was optimum at a temperature of 37 °C at 48 hours (Fig.3). The study was hereafter conducted at incubation temperature of 37 °C for 48 hours as an optimized parameter.

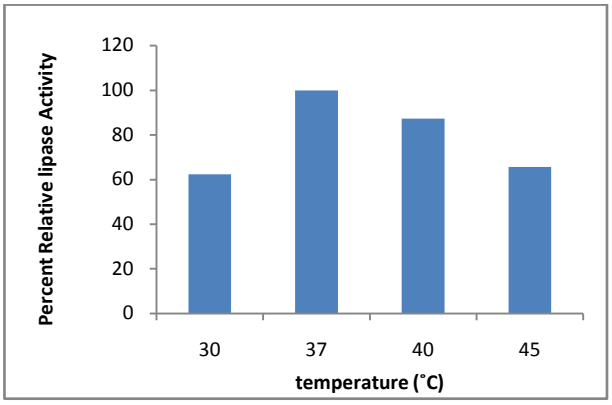

Fig.3: Temperature optimizationfor lipase production

3.4.2. Effect of Media Components on Lipase production

The experiments werecarried out to determine the concentrations of the media components for optimization of lipase production. From Fig.4the olive oil concentration wasdetermine to be approximately10 ml/L as an optimum value. The percent relativelipase activity reduced with further increase in concentration of olive oil. This might be due to the fact that higher amount of olive oil hinders the microbial growth by restricting oxygen transfer and in turn reduces the lipase production.

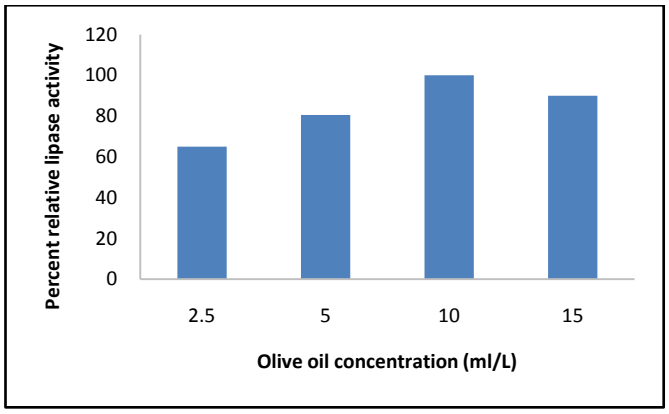

Fig.4: Optimization of lipase production using different olive oilconcentration

Fig.5 shows peptone concentration to be around 10 g/L as an optimized value. From the figureit is observed that, beyond 10 g/L, almost saturated relative lipase activity was found for increasing peptone concentration.

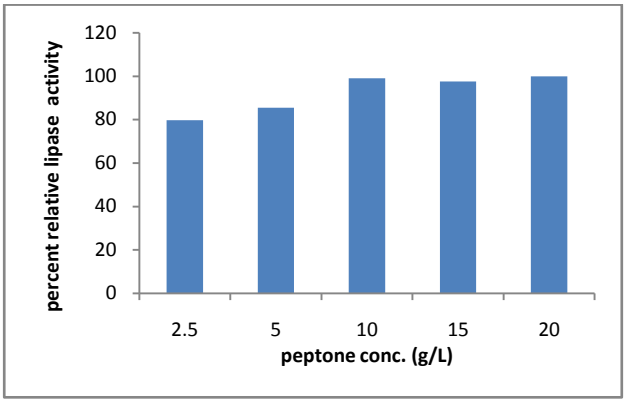

Fig.5: Optimization of lipase production using different peptoneconc.

Fig.6 shows optimum Yeast extract concentration to be around 3g/L and the relative percent lipase activity generally tends to decrease with increase in yeast extract concentration in the media.

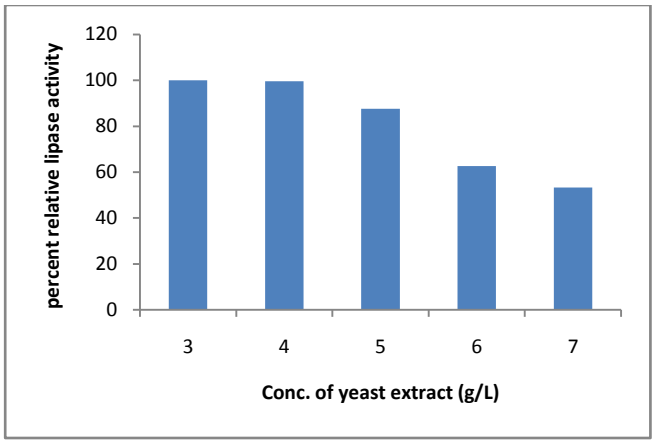

Fig.6: Optimization of lipase production using different yeast extractconc.

Fig.7 shows optimum concentration of $MgSO_4$wasto be around 0.5 ml/L (from 500 g/L stock). Relative percent Lipase activity tends to increase with increase in $MgSO_4$concentrationdue to active site affinity enhancement by divalent Mg^{+2} ions but gradually decreases with increase in $MgSO_4$ concentration. This might bedue to increase in Mg^{+2} ions results in an inhibitory effect on the active site of lipase [3].

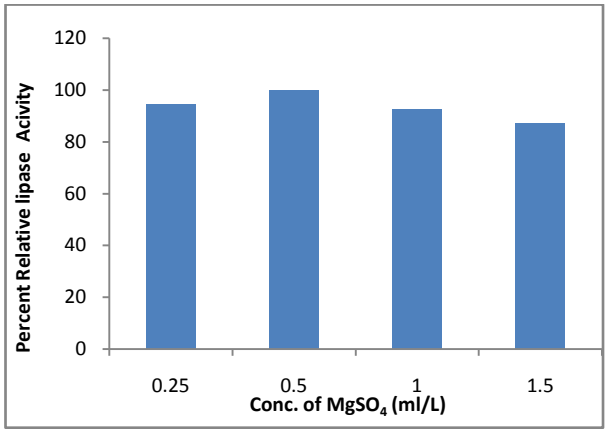

Fig.7: Optimization of lipase production using different $MgSO_4$ concentration

3.4.3. Effect of Different oils on Lipase Production

Fig.8 shows the effect of using different oils as the lipid source in production media with the rest of the media components remaining same. Comparing Lipase production, determined by quantitative Lipase activity assay, the production media using olive oil as lipid source shows maximum lipase production compared to sunflower oil and coconut oil. Use of olive oil for lipase production was also reported earlier [15]. The reason might be due to the influence ofhigher amount of unsaturated fatty acids present in olive oil (70%) as compared to the other oils used.

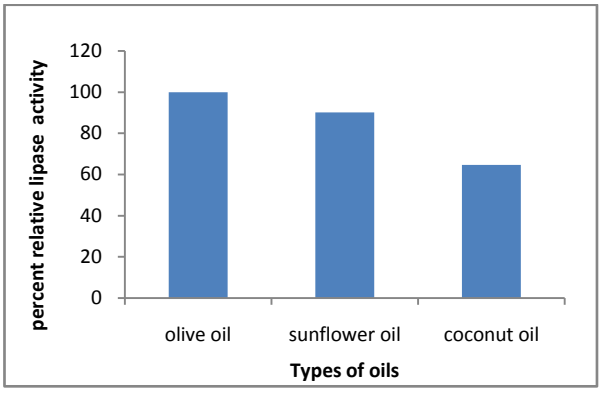

Fig.8: Optimization of lipase production using different oils in production media

3.5 Optimization of Lipase activity

3.5.1.Effect of Temperature on Lipase Activity

The result in Fig. 9 shows that percent relative lipase activity was optimum at 37 °C temperature with other parameters constant. Increase in temperature facilitates reaction by providing kinetic energy whereas temperature beyond optimum value leads to change in active site conformation, leads to lowering the activity. A higher incubation temperature generally tends to denature the enzyme thus resulting in decreased activity.

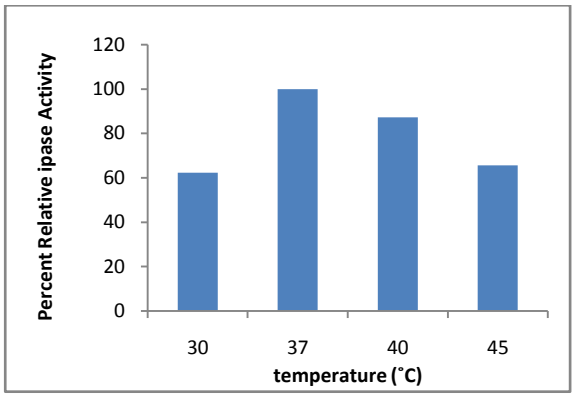

Fig.9: Optimization of lipase activity using different incubation temperatures

3.5.2.Effect of pH on Lipase Activity

Fig.10 shows that percent relative lipase activity was optimum at pH 7 keeping other parameters unchanged. Enzymes binds with substrates by ionic interactions. Change in pH alters the net charges present in protein and hence influence enzyme activity. For the present study, pH 7 was selected for further experiments.

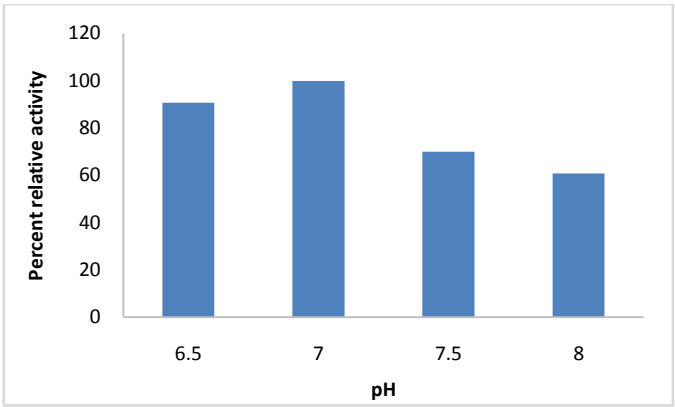

Fig.10: Optimization of lipase activity using different pH

3.5.3.Effect of Triton X on Lipase Activity

Fig.11 shows that increase in percent relative lipase activity was observed with the addition of 150 µl of the Triton X 100 (3 ml/L) into the reaction mixture. As lipase acts on oil-water interface, detergents help in better lipase function by decreasing the surface tension at oil-water interface [23].Further increase in

detergent amount may have led to denaturation of protein, which in turn reduced lipase activity.

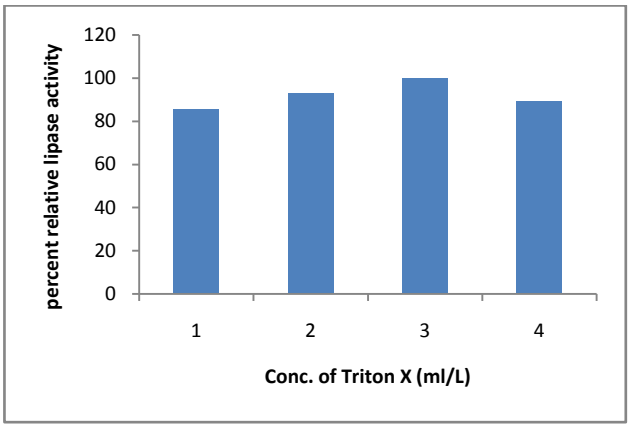

Fig.11: Optimization of lipase activity using different Triton X concentration

3.6 Validation of the optimized conditions for lipase production

Tovalidatedifferent parameters obtained from experiments, lipase production was compared between the optimized media and the standard un-optimized lipase production media selected from literature. Fig.12shows that lipase production had increased by around 57% using the optimized conditions as compared to the media composition mentioned in literatures.

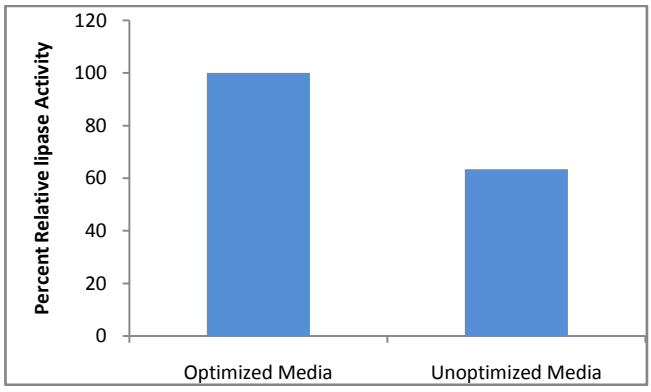

Fig.12: Comparison between optimized and un-optimized lipase production media

4. CONCLUSION

In the present study, lipase producing bacteria was isolated, screened and partially characterized from water sample isolated from indigenous sources. It was observed that lipase production was enhanced in presence of olive oil and a non-ionic detergent like Triton X 100. Physicochemical parameters as well as media components were standardized by single parameter optimization method and it was observed that almost 57% increase in lipase activity was obtained after optimization. Thus the present study provides an optimized media composition with reaction conditions suitable for lipase production.

ACKNOWLEDGEMENT

The authors would like to acknowledgeHeritage Institute of Technology Kolkata for providing the necessary supports for carrying out the present work.

REFERENCES

[1] Nardini M, Dijkstra BW.'Alpha/betahydrolase fold enzymes: the family keeps growing'. Curr Opin Struct Biol., vol 9, no 6, pp 732-737, Dec 1999.

[2] Kempka, A.P., N.L. Lipke, T.L.F. Pinheiro, S. Menoncin, H. Treichel, D.M.G. Freire, M.D. Luccio and D. Oliveira. 'Response surface method to optimize the production and characterization of lipase from Penicillium verrucosum in solid state fermentation'. Biop. Biosys. Eng., vol 31, pp 119-125, 2008.

[3] Sharma, R., Y. Chisti and U. Banerjee. 'Production, purification, characterization and applications of lipases'. Biotechnol Adv., vol 19, pp 627-662, 2001.

[4] Gupta R, Gupta N and Rathi P. 'Bacterial lipases: an overview of production, purification and biochemical properties'.Appl Microbiol Biotechnol.,vol 64, no 6, pp 763-781, Jun 2004.

[5] Jaeger K-E, Dijkstra BW and Reetz MT. 'Bacterial biocatalysts: molecular biology, three-dimensional structures and biotechnological applications of lipases'. Annu Rev Microbiol., vol 53, pp 315-351, 1999.

[6] Jaeger K-E, Ransac S, Dijkstra BW, Colson C, Heuvel M van and Misset O. 'Bacterial lipases'. FEMS Microbiol Rev., vol 15, pp 29-63, 1994.

[7] Pandey A, Benjamin S, Soccol CR, Nigam P, Krieger N and Soccol UT. 'The realm of microbial lipases in biotechnology'. Biotechnol Appl Biochem., vol 29, pp 119, 1999.

[8] Liese A, Seelbach K, Wandrey C and editors. 'Industrial biotransformations'. Weinheim: Wiley-VCH, 2000.

[9] Ghosh P. K., Saxena R. K., Gupta R., Yadav R. P. and Davidson S. 'Microbial lipases: production and applications'. Sci. Progress. Vol 79, no 2, pp 119-157, 1996.

[10] E-Mobarak-Qamsari, R-Kasra-ermanshahi, and Z-Moosavi-nejad. 'Isolation and identification of a novel, lipase-producing bacterium *Pseudomnas aeruginosa* KM110'. Iran J Microbiol. Vol 3, no 2, pp 92-98, Jun 2011.

[11] Pabai F, Kermasha S and Morin A. 'Lipase from Pseudomonas fragi CRDA 323: partial purification, characterization and interesterification of butter fat'. Appl Microbiol Biotechnol.,vol 43, pp 42-51, 1995.
[12] Alvarez FJ and Stella VJ. 'The role of calcium ions and bile salts on the pancreatic lipase-catalyzed hydrolysis of triglyceride emulsions stabilized with lecithin'. Pharmacological Res.,vol 6, pp 449-452, 1989.
[13] Karadzic I, Masui A, Zivkovic LI and Fujiwara N. 'Purification and characterization of an alkaline lipase from *Pseudomonas aeruginosa* isolated from putrid mineral cutting oil as component of metalworking fluid'. J Biosci Bioeng., vol 102, pp 82-89, 2006.
[14] Namita Gupta, Vikram Sahai andRani Gupta. 'Alkaline lipase from a novel strain *Burkholderia multivorans*: Statistical medium optimization and production in a bioreactor'.Process Biochemistry, Vol 42, no 4, pp 518-526, Apr 2007.
[15] M.Hasan-Beikdashti, H. Forootanfar, M.S. Safiarian,A. Ameri, M.H. Ghahremani, M.R. Khoshayand and M.A. Faramarzi. 'Optimization of culture conditions for production of lipase by a newly isolated bacterium *Stenotrophomonas maltophilia'.* Journal of the Taiwan Institute of Chemical engineers, Vol 43, no 5, pp670-677, 2012.
[16] Wang Y, Srivastava KC, Shen GJ and Wang HY. 'Thermostable alkaline lipase from a newly isolated thermophilic Bacillus strain A30-1 (ATCC 53841)'. J Ferment Bioeng.,vol 79, pp 433-438, 1995.
[17] N.Kulkarni and RV.Gadre. 'Production and properties of an alkaline thermophilic lipase from Pseudomonas fluorescens NS2W'. Journal of Industrial Microbiology & Biotechnolog., vol 28, pp 344-348, 2002.
[18] Nobuhiro Watanabe, Yasuhide Ota, Yasuji Minoda and Koichi Yamada. 'Isolation and Identification of Alkaline Lipase Producing Microorganisms, Cultural Conditions and Some Properties of Crude Enzymes'. Agricultural and biological chemistry, vol 41, no 8, pp 63, 1977.
[19] Litthauer D, Ginster A and Skein EVE. 'Pseudomonas luteola lipase: a new member of the 320-residue Pseudomonas lipase family'. Enzyme Microb Technol., vol 30, pp 209-215, 2002.
[20] Dharmsthiti S and Kuhasuntisuk B.'Lipase from Pseudomonas aeruginosa LP602: biochemical properties and application forwastewater treatment'. J Ind Microbiol Biotechnol., vol 21, pp 75-80, 1998.
[21] Lesuisse E, Schanck K and Colson C. 'Purification and preliminary characterization of the extracellular lipase of Bacillus subtilis 168, an extremely basic pH-tolerant enzyme'.Eur J Biochem., vol 216, pp 155-160, 1993.
[22] Sunna A, Hunter L, Hutton CA and Bergquist PL. 'Biochemical characterization of a recombinant thermoalkalophilic lipase and assessment of its substrate enantioselectivity'. Enzyme Microb Technol., vol 31, pp 472-476, 2002.
[23] Martinelle M, Holmquist M and Hult K.'On the interfacial activation of Candida antarctica lipase A and B as compared with Humicola lanuginosa lipase'. Biochim Biophys Acta.,vol 1258, pp 272-276, 1995.