World Applied Sciences Journal 16 (7): 1034-1039, 2012
ISSN 1818-4952
© IDOSI Publications, 2012

Solid State Fermentation of Agricultural Residues for Lipase Production in a Tray-Bioreactor

[1]Samaneh Mohseni, [1]Ghasem D. Najafpour, [1]Zahra Vaseghi and [2,3]Soleiman Mahjoub

[1]Faculty of Chemical Engineering, Biotechnology Research Lab,
Noshirvani University of Technology, Babol, Iran
[2]Department of Biochemistry and Biophysics, Faculty of Medicine,
[3]Fatemeh Zahra Infertility and Reproductive Health Research Center,
Babol University of Medical Sciences, Babol, Iran

Abstract: The novel tray-bioreactor was designed and constructed to produce lipases with high activities. Fermentation was conducted in this bioreactor in order to produce lipase from agricultural products/residues by *Aspergillus niger*. Regarding this issue, several agricultural products and residues including rice bran, sugarcane baggase, wheat bran, barley bran and corn meal were employed. Among them rice bran led to maximum lipase production under similar condition. Furthermore the influence of process parameters including fermentation duration for lipase production, temperature and humidity of the cabinet were investigated. Analysis showed that, the best result for enzyme production was obtained after 96 hours of incubation, at cabin temperature of 35°C and cabin humidity of 90%. The maximum produced enzyme activity under desired conditions was142.732 U/gds.

Key words: Lipase · *Aspergillus niger* · Tray Bioreactor · Rice bran · Solid state fermentation

INTRODUCTION

Lipases which belong to enzyme category are able to catalyze the hydrolysis of triacylglycerols to produce mono, diacylglycerols, glycerol and free fatty acids. Moreover, lipases are able to catalyze esterification, interesterification and transesterification reactions in non-aqueous media [1, 2]. Microbial lipases are known as biocatalysts with special characteristics like actions under mild conditions, stability in organic solvents, high substrate specificity and selectivity [3]. Lipases occur commonly in bacteria, yeasts and fungi [3-5]. However, fungi are recognized as best enzyme producers in industrial applications. Moreover, *A. niger* is among the most famous lipase producers [6, 7]. Due to lipases applications in industry, food additives, esters synthesis, detergents, wastewater treatment, pharmaceutical areas and leather, research around it became world spread [8, 9]. Nowadays because of high demand for enzyme production, considerable progress occurred in this area, but high cost of lipase production still remains the obstacle problem in large-scale industrial applications of enzyme-catalyzed processes [3]. In this case, consumption of agro industrial residues as substrates for lipase production would lead to less final costs in comparison to usage of other substrates [10]. Solid state fermentation (SSF) and submerged fermentation (SmF) processes are effective techniques for mass production of enzymes. Solid state fermentation process has gained high popularities as the growing microorganisms dominated on moistened solid substrates especially on agro-industrial residues [11-13]. Because of mentioned reasons, solid state fermentation has much less contaminations in solid substrate due to absence of free water, little energy consumption, less limiting factors for growth, no liquid waste and little amount of water requirements for the growing microorganisms, is more easier than submerge liquid fermentation process [14]. Although, many literatures on SSF refers to fungal systems; but, there are a few reports on lipase production in SSF by *A. niger* till to date [15, 16].

Corresponding Author: *Dr. Ghasem D. Najafpour*, Faculty of Chemical Engineering,
Biotechnology Research Lab, Noshirvani University of Technology, Babol, Iran.
Soleiman Mahjoub, Department of Biochemistry and Biophysics, Faculty of Medicine,
Babol University of Medical Sciences, Babol, Iran.

Several types of bioreactors including tray, packed bed, stirred bed, rotating drum and fluidized bed bioreactors are employed for enzymes production in large scale [17-20]. The static tray bioreactor offers some advantages over other configurations due to the simplicity of operation [20, 21]. In ancient time, handling SSF in large scale due to difficulties in monitoring process parameters was a great concern [22]. The temperature and moisture content of the bioreactor play major role on fermentation performance. Control of the process parameters at their optimum values would improve the lipase production efficiency compared to the uncontrolled condition [8].

In this study, a noble tray bioreactor containing all controlling units designed and constructed for lipase production. Different substrates were employed in order to study their effects on lipase production. In addition, influence of process parameters including the duration of a semi-batch experiment, temperature and humidity of the cabinet were investigated for high yield of lipase production.

MATERIALS AND METHODS

Materials: All chemical compounds including Yeast extract, peptone, K_2HPO_4, KH_2PO_4, KCl, $MgSO_4.7H_2O$ and glucose were analytical grades and were purchased from Merck (Darmstadt, Germany) except olive oil which was food grade and was provided from local market; while para-nitrophenyl palmitate was supplied from sigma-Aldrich. Rice bran, sugarcane bagasse, wheat bran, barely bran and corn meal were kindly donated from mill factory, Babolsar, Iran.

Microorganism and Growth Media: The fungal strain *A. niger* NCIM 584 which was employed in the present study was supplied from Chandigarh, India. The strain was cultivated on complex agar medium at 30°C for 24 hours and was kept at 4°C. *A. niger* was grown in a medium containing (g/l) yeast extract (0.25), peptone (0.25), KH_2PO_4 (1), KCl (0.5), $MgSO_4.7H_2O$ (0.5), glucose (12.5) and olive oil (12) for the incubation period of 72 hours.

Solid Substrate Preparation: In order to evaluate influence of different substrates on lipase production, some agricultural residues and by products including rice bran, sugarcane baggase, wheat bran, corn meal and barely bran were employed in our experimental tests. These agricultural residues/by products were sieved to achieve uniform particles size in the range of 0.18-2 mm. Moreover all substrates were autoclaved at 121°C for 20 minutes. After mentioned primary preparations substrates should be inoculated with the propagated fungal solution. The substrate which resulted in maximum lipase activities was selected for further studies.

Tray Bioreactor Set up: The noble and effective batch tray bioreactor (45 × 35 ×55 cm) was designed and fabricated. A plexiglass bioreactor contained 3 trays (35 × 25 ×5 cm) having all controlling units for humidity and temperature. Trays were located vertically inside the chamber with equal space (18.3cm). A top tray and the middle one were covered with a linen cloth which acted as heat transfer area or cooling surface and were filled with rice bran as solid substrate. The bottom tray was filled with nutritious solution. An external circulating peristaltic pump (ETATRON, Italy) was supplied to provide sufficient complimentary nutrients and moisture uniformly distributed on the top tray by an injection nozzle at a constant flow rate. At the same time, a humidity controller (DS FOX, accuracy ±2%, Korea) was applied to provide the required moisture inside the bioreactor. On the other hand a heating element connected to a temperature controller (SAMWON ENG, accuracy ±0.2%+1 digit, Korea) was installed in the chamber. In order to monitor the variations of temperature and humidity inside the bioreactor, two probes were placed approximately in the middle of the bioreactor chamber. In addition, four circulating small fans (PIONEER, PC fan 12 volts, 0.18 Amperes, 1.68 Watts, Korea) were placed inside the cabin beside trays 1 and 2 as air circulator created uniform ambient with sustainable conditions.

These parameters were adjusted for the optimal cell growth and certain enzyme activities. The bioreactor temperature and relative humidity were well controlled for maximum enzyme and biomass production. Cabin was totally sterilized by oxidizing chemical. Furthermore, in order to prevent microbial contamination media and raw materials were autoclaved at 121°C for 20 minutes. The schematic diagram of tray bioreactor with auxiliary equipments is shown in Figure 1.

SSF and Sample Preparation: A 5 gram of dried autoclaved substrate was filled in paper bags which were placed on the top and middle trays. Fungous seed culture was cultivated in an Erlenmeyer flask for 72 hours and spread out on rice bran. The bottom tray was filled completely with nutritious solution. In order to provide appropriate moisture content within the bioreactor the

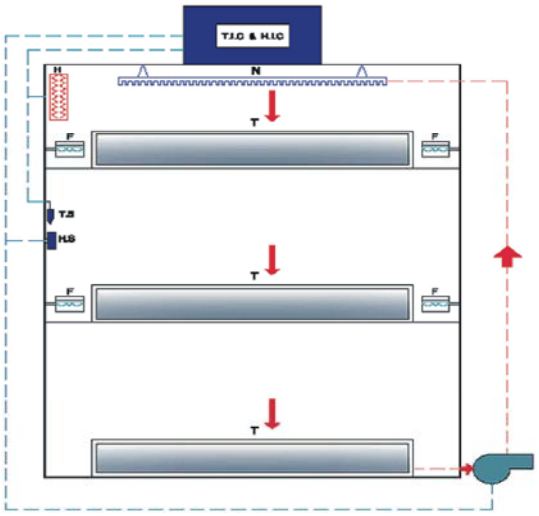

Fig. 1: Schematic diagram of the tray bioreactor set up; H: Heater, T: Tray, F.: Fan, P.: Pump, N: Nozzle T.S.: Temperature Sensor, H.S.: Humidity Sensor, T.I.C.: Temperature Indicator and Controller, H.I.C.: Humidity Indicator and Controller

nutritious solution containing (g/l) yeast extract (0.25), peptone (0.25), KH_2PO_4 (1), KCl (0.5), $MgSO_4.7H_2O$ (0.5) and olive oil (4) was circulated and distributed over the surface of the top tray. After penetrating in solid substrates of the top tray, nutritious solution leached out from the small holes of top tray to the middle one. In the middle tray the remaining solution was leached into bottom tray. Media circulation continued by the pump till the humidity controller reached the set point.

Sampling and Lipase Extraction from the Fermented Solid: Samples of fermented solid were taken from cabinet in every 24 hours for enzyme activity evaluation. Since enzyme should be extracted from the liquid media, fermented solid residue was transferred to a liquid phase; NaCl (1%), Triton X-100 (1%) solution in a100 ml of distilled water in Erlenmeyer flask was used to reach the mentioned enzyme. The solution was kept in rotary shaker for 2 hours at 30°C and 180 rpm. Solid substrate was then filtered with Whatman filter paper (No. 41; diameter of 125mm) from the enzymatic solution by means of a vacuum pump (PLATINUM, USA).

Lipase Activity Assay: Colorimetric method with *p*-nitrophenyl palmitate as substrate was used to determine Lipase activity. The assay mixture was incubated at 50°C for 30 min and the *p*-nitrophenol released was measured at 410 nm in Spectrophotometer (Unico, USA).

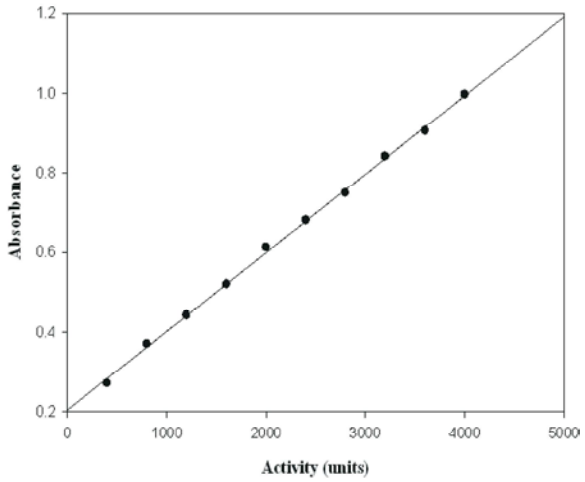

Fig. 2: UV calibration curve for lipase activity at wavelength 410nm

The method was based on the work presented by Mahadik *et al.* [3] in which one unit (U) of lipase activity was defined as the amount of enzyme that liberates one micromole of *p*-nitrophenol per minute under the standard assay conditions. UV calibration curve for lipase activity determination is shown in Figure 2.

Estimation of Total Protein: The crude enzyme solution was centrifuged (Hermle, Type: Z293 M-2, Germany) at 6000g for 5 minute; and the supernatant was used for protein measurement. The protein content of the prepared sample was measured according to Lowry *et al.* [23] using bovine serum albumin (Sigma Chemicals, USA) as standard.

RESULTS AND DISCUSSION

Effect of Different Substrates on Lipase Production: Among the different agricultural residues or agro by-products tested for enzyme production by *A. niger* under similar conditions, maximum lipase activity occurred on rice bran (Figure 3). Rice bran provides all required carbon, nitrogen, contained sugar and proteins for microorganisms' growth which led to lipase production. It is worth noting that, surprisingly rice bran is not only the cheapest residue among selected substrates but also it is the most supportive substrate for lipase production in comparison to other residues/by products used in our study. Further optimization studies were carried out using rice bran as the substrate.

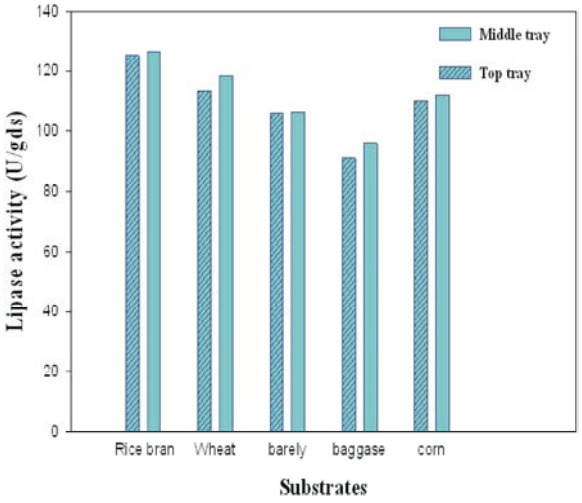

Fig. 3: Effect of different substrates on lipase production by *A. niger*

Time Course of Lipase Production: The duration of a batch experiment which is one of the dominant factors in lipase production should be taken into consideration. Regarding this issue, samples were analyzed for every 24 hours for determination of lipase activities. Activity of the enzyme was evaluated at the fermentation temperatures of 30, 35 and 40°C (Figure 4). In all experimental runs, maximum enzyme activity achieved after 96 hours of inoculation. Mahadik *et al.* [3] has achieved highly active lipase by *A. niger* strain after 120 hours of incubation. Contesini *et al.* [24] reported that the optimal time for *A. niger* lipase production was 96 hours. It should be noted that, incubation time beyond this period did not lead to higher enzyme activity due to the lack of nutritious substances required for the microorganism's growth [6]. The obtained results also showed that as the incubation temperature rose from 25 to 35°C, the lipase activity appreciably increased.

Effect of Bioreactor Temperature: The effect of temperature on lipase production was also investigated. The temperature of incubating chamber was varied from 25 to 45°C with an increment of 5°C. Lipase activity was assayed under several incubating temperatures. The crude enzyme showed more activity at 35°C. It was observed that at lower or higher than 35°C led to production of lipases with much low activities (Figure 5). This means; there was an optimum temperature for maximum lapse production. Olama *et al.* [25] has achieved maximum yields for lipase production at incubating temperature of 30°C using *A. niger*. Gulati *et al.* [26] has

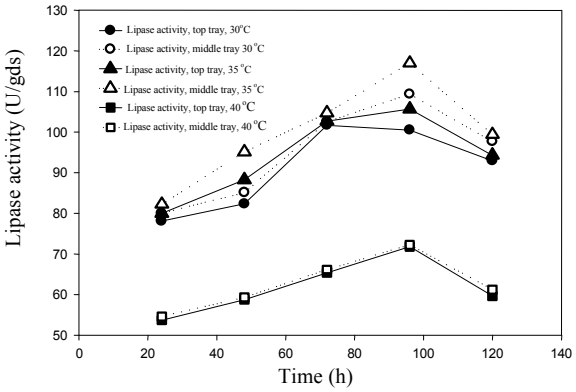

Fig. 4: Time course of lipase production by *A. niger* at 30, 35 and 40°C (U lipase/ gds)

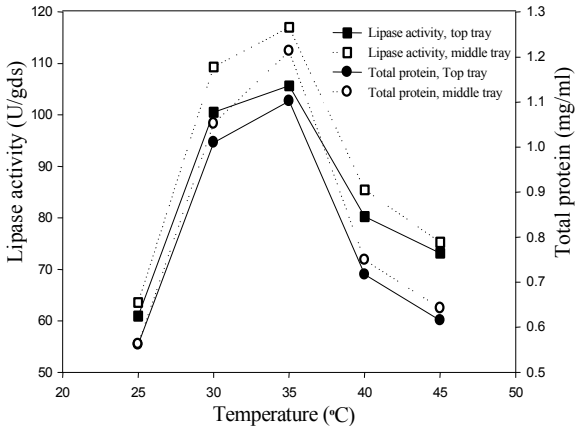

Fig. 5: Effect of cabin temperature on lipase activity produced by *A. niger*

showed that using *A. niger*; maximum activities of lipases occurred at 37°C. The enzymatic solutions at different incubating temperatures were analyzed in order to estimate total protein values. Results are also shown in Figure 4. In fact total protein was measured in all the experiments for more reliability of the obtained data as it was observed in most cases; total protein of the enzymatic solution with higher activities was more than that with lower activities. Also it was found that total protein and lipase activity of the middle tray was higher than that of the top tray except in a few cases. Undoubtedly, in the middle tray lipases were produced not only by consuming nutrition in both substrate and culture medium which was speared over the substrate but also some amount of lipases which were produced on top tray was leached by circulating nutritious solution to the middle tray. Thus, the substrate located in the middle tray was contained more lipases and also total protein content of middle tray was higher than the top tray. Moreover, it

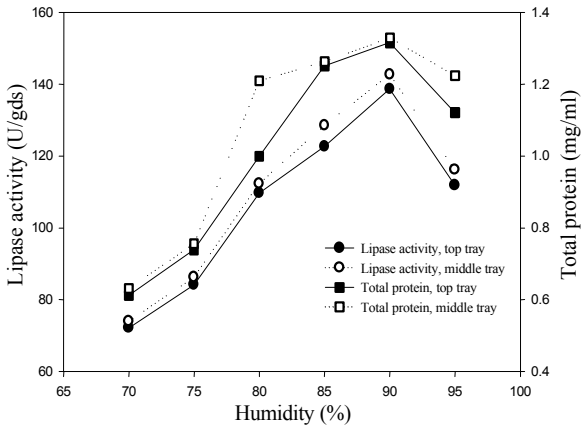

Fig. 6: Effect of cabin humidity on lipase activity produced by *A. niger*

should be noticed that the bottom tray achieved only a little amount of enzymes which were leached from the middle tray. In this regard, the amount of lipase in this tray was the least in compare to top tray and the middle one. Accordingly, lipases produced in the tray which placed at the bottom of the bioreactor did not show significant changes in enzyme activities as compared to those produced in the top and middle trays; so they were disregarded for analysis.

Effect of Bioreactor Humidity: The influence of bioreactor humidity was also investigated by adjusting the humidity of the cabin between 70-95 % with an increment of 5%. Maximum yields for lipase was obtained at humidity of 90% (Figure 6). In order to provide the desired humidity, the humidity controller was set at desired point. In this sense, in support of providing significant humidity the pump started working which led to nutritious solution circulation. The liquid circulation inside the bioreactor increased the humidity of the substrate and consequently created a medium with high moisture content which adversely affected the lipase activity [8].

CONCLUSION

In the present research a noble and effective batch tray bioreactor which was designed and fabricated in our research group was conducted for lipase production by *A. niger*. Influence of various substrates for lipase production was investigated. The satisfactory results were obtained when rice bran was used as the solid substrate with enzyme activity of 126.099 U/gds. Moreover, the influences of process parameters including duration of fermentation, bioreactor temperature and bioreactor humidity were also investigated. The bioreactor optimum operating conditions were obtained after 96 hour of incubation time at optimum cabin temperature of 35°C and cabin humidity of 90%. Maximum lipase activity in the top tray under optimal conditions for process time, temperature and humidity of the bioreactor were 105.652, 105.792 and 138.651U/gds, respectively. Similarly for middle tray, the maximum enzyme activities for the same 3 sets of variables were 116.985, 116.905 and 142.732 U/gds, respectively.

ACKNOWLEDGMENTS

The authors greatly appreciate the financial support and facilities provided by Biotechnology Research Center, Babol, Noshirvani University of Technology, Iran.

REFERENCES

1. Toscano, L., V. Gochev, G. Montero and M. Stoytcheva, 2011. Enhanced Production Of Extracellular Lipase By Novel Mutant Strain Of Aspergillus Niger, Biotechnology and Biotechnological Equipment., pp: 2243-2247.
2. Mahanama, R., D. Boyce, A. Berenjian, F. Dehghani and J. Kavanagh, 2012. The Effect of Enzymatic Pre-treatment on Solid Substrate Fermentation of Vitamin K2. J. American Sci., 8(1): 648-654.
3. Mahadik, N.D., U.S. Puntambekar, K.B. Bastawde, J.M. Khire and D.V. Gokhale, 2002. Production of acidic lipase by *Aspergillus niger* in solid state fermentation. Process Biochemistry, 38(5): 715-721.
4. Ul-Haq, I., S. Idrees and M.I. Rajoka, 2002. Production of lipases by *Rhizopus oligosporous* by solid-state fermentation. Process Biochemistry, 37(6): 637-641.
5. Gordillo, M., A. Sanz, A. Sanchez, F. Valero, J. Montesinos, J. Lafuente and C. Sola, 1998. Enhancement of *Candida rugosa* lipase production by using different control fed-batch operational strategies. Biotechnology and Bioengineering, 60(2): 156-168.
6. Prathumpai, W., S.J. Flitter, M. McIntyre and J. Nielsen, 2004. Lipase production by recombinant strains of *Aspergillus niger* expressing a lipase-encoding gene from Thermomyces lanuginosus. Applied Microbiology and Biotechnol., 65(6): 714-719.

7. Fu, X., X. Zhu, K. Gao and J. Duan, 1995. Oil and fat hydrolysis with lipase from *Aspergillus sp*. J. the American Oil Chemists' Society, 72(5): 527-531.
8. Vaseghi, Z., G.D. Najafpour, S. Mohseni, S. Mahjoub and M.N. Hosseinpour, 2012. Lipase Production in Tray-Bioreactor via Solid State Fermentation under Desired Growth Conditions, Iranica J. Energy and Environment, 3(1): 76-82.
9. Vargas, G.D.L.P., H. Treichel, D. De Oliveira, S.C. Beneti, D.M.G. Freire and M. Di Luccio, 2008. Optimization of lipase production by Penicillium simplicissimum in soybean meal. J. Chemical Technology and Biotechnol., 83(1): 47-54.
10. Pandey, A., C.R. Soccol, J. Rodriguez-Leon and P. Singh-Nee Nigam, Solid State Fermentation in Biotechnology: Fundamentals and Applications" Reference Book. 2001: Asiatech Publishers, Inc.
11. Damaso, M.C.T., M.A. Passianoto, S.C. Freitas, D.M.G. Freire, R.C.A. Lago and S. Couri, 2008. Utilization of agroindustrial residues for lipase production by solid-state fermentation. Brazilian J. Microbiol., 39(4): 676-681.
12. Singhania, R.R., A.K. Patel, C.R. Soccol and A. Pandey, 2009. Recent advances in solid-state fermentation. Biochemical Engineering J., 44(1): 13-18.
13. Stuart, D., D. Mitchell, M. Johns and J. Litster, 1999. Solid-state fermentation in rotating drum bioreactors: Operating variables affect performance through their effects on transport phenomena. Biotechnology and Bioengineering, 63(4): 383-391.
14. Mandviwala, T. and J. Khire, 2000. Production of high activity thermostable phytase from thermotolerant *Aspergillus niger* in solid state fermentation. J. Industrial Microbiology and Biotechnol., 24(4): 237-243.
15. Balaji, V. and P. Ebenezer, 2008. Optimization of extracellular lipase production in *Colletotrichum gloeosporioides* by solid state fermentation. Indian J. Science and Technol., 1: 1-8.
16. Kamini, N., J. Mala and R. Puvanakrishnan, 1998. Lipase production from *Aspergillus niger* by solid-state fermentation using gingelly oil cake. Process Biochemistry, 33(5): 505-511.
17. Ali, H.K.Q. and M. Zulkali, 2011. Design Aspects of Bioreactors for Solid-state Fermentation: A Review. Tic., 43: 44.
18. Mitchell, D.A., N. Krieger, D.M. Stuart and A. Pandey, 2000. New developments in solid-state fermentation: II. Rational approaches to the design, operation and scale-up of bioreactors. Process Biochemistry, 35(10): 1211-1225.
19. Mitchell, D.A., A. Pandey, P. Sangsurasak and N. Krieger, 1999. Scale-up strategies for packed-bed bioreactors for solid-state fermentation. Process Biochemistry, 35(1): 167-178.
20. Rodriguez Couto, S. and M.A. Sanroman, 2005. Application of solid-state fermentation to ligninolytic enzyme production. Biochemical Engineering J., 22(3): 211-219.
21. Pandey, A., P. Selvakumar, C.R. Soccol and Singh - Nee Nigam, Poonam, 1999. Solid state fermentation for the production of industrial enzymes. Current Sci., 77(1): 149-162.
22. Jou, R.Y. and C.T. Lo, 2011. Heat and Mass Transfer Measurements for Tray-Fermented Fungal Products. International J. Thermophysics, pp: 1-14.
23. Lowry, O.H., N.J. Rosebrough, A.L. Farr and R.J. Randall, 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem., 193(1): 265-275.
24. Contesini, F.J., V.C.F. da Silva, R.F. Maciel, R.J. De Lima, F.F.C. Barros and P. De O. Carvalho, 2009. Response surface analysis for the production of an enantioselective lipase from *Aspergillus niger* by solid-state fermentation. The J. Microbiol., 47(5): 563-571.
25. Olama, Z. and A. El-Sabaeny, 1993. Lipase production by *Aspergillus niger* under various growth conditions using solid state fermentation. Microbiologia Madrid, 9: 134-134.
26. Gulati, R., R. Saxena, R. Gupta, R. Yadav and W. Davidson, 1999. Parametric optimisation of *Aspergillusterreus* lipase production and its potential in ester synthesis. Process Biochemistry, 35(5): 459-464.