Optimization of process variables for enhanced production of extracellular lipase by *Pleurotus ostreatus* IBL-02 in solid-state fermentation

Saima Rehman[1], Haq Nawaz Bhatti[1], Muhammad Bilal[2]* and Muhammad Asgher[2]
[1]Environmental & Material Chemistry Laboratory, Department of Chemistry, University of Agriculture, Faisalabad, Pakistan
[2]Industrial Biotechnology Laboratory, Department of Biochemistry, University of Agriculture, Faisalabad, Pakistan

Abstract: In the present study, *Pleurotus ostreatus* IBL-02, a white rot basidiomycete was exploited for lipase production in solid-state fermentation (SSF). Different agro-industrial wastes such as canola-oilseed cake, cotton-oilseed cake, linseed-oil cake, sesame-oilseed cake, rice bran and wheat bran were screened for fermentative production of the lipolytic enzyme. The enzyme profile of *P. ostreatus* showed the highest activity of lipase on canola oil seed cake as a substrate under SSF conditions. Various physiological factors such as incubation time, humidity level, culture pH, incubation temperature and supplementation of carbon and nitrogen sources were optimized to induce the lipase synthesis capability of *P. ostreatus* at an optimal level. Optimum lipase activity (3256 U/gram dry substrate) was measured in the solid fermentation medium using moisture level, 50.0%; pH, 4.0; temperature, 30 °C and olive oil, 2.0% after 72 h of incubation period with glucose and urea as carbon and nitrogen supplements, respectively. Glucose supplementation significantly stimulated the lipase production, while nitrogen addition did not exert any significant effect on lipase yield. Overall, under optimized bioprocess conditions, the enzyme activity was improved up to 1.6 folds with respect to the original enzyme activities. The current findings indicate that culture conditions have great influence on the lipase production potential of *P. ostreatus* for commercial purpose.

Keywords: *Pleurotus ostreatus*, lipase, solid-state fermentation, optimization, process parameters.

INTRODUCTION

Lipases (triacylglycerol hydrolases, E.C. 3.1.1.3) are a class of serine hydrolases that catalyze the hydrolysis of triacylglycerol (TAG) to glycerol and free fatty acids (FFA) (Kumar *et al*., 2005). Remarkable activity and stability features in non-aqueous environments make lipases also to catalyze several unnatural reactions such as hydrolysis, inter-esterification, esterification, alcoholysis, acidolysis and aminolysis with high enantioselectivity (Ghaly *et al*., 2010). Further, the inimitable distinctiveness including substrate specificity, regio and stereospecificity, chiral selectivity and gentle conditions to carry out a diversity of biochemical conversions (Sakinc *et al*., 2007) have made them attractive for the production of a broad range of natural products, pharmaceuticals, fine chemicals, food constituents and bio-lubricants (Gupta *et al*., 2003; Amin *et al*., 2011; Rehman *et al*., 2011). Other applications include bioremediation of fat containing waste effluents, synthesis of nutraceuticals, cosmetics, detergent formulations, perfumery, as biosensor in diagnostics (Brust *et al*., 2011), treatment of malignant tumors and detection of target DNA sequences (Pinijsuwan *et al*., 2011), bio-surfactants, and paper manufacture (Park *et al.,* 2005; Fang *et al*., 2006; Gupta *et al.,* 2007; Grbavcic *et al.,* 2007; Franken *et al.,* 2009). Literature survey revealed that 1000 tons of lipases are required annually only in the detergent industry as an additive (Hassan *et al*., 2006).

Lipases are ever-present biocatalysts of significant physiological consequence and industrial perspective (Sharmaa *et al*., 2001), and usually, do not require the cofactors. Although, they can be produced in plants, animals, and microorganism, but lipases from microbial source such as bacteria (Gupta *et al*., 2004), yeast (Vakhlu and Kour, 2006) and fungi (Fernandez-Lafuente, 2010) are of particular interest essentially due to their high yield and easy extraction/recovery. These microorganisms have the advent of genetic manipulation for the synthesis of novel compounds with potential commercial aspects (Laxman *et al*., 2005). Both solid-state and submerged (SmF) fermentations have been employed for the synthesis of invaluable arrays of industrially relevant enzymes (Munir *et al*., 2015). However, SSF is privileged over SmF due to its simplicity, low capital investment, least catabolic repression, feedback inhibition, minimal wastewater output, easy product retrieval and good-quality production (Holker *et al*., 2004). Filamentous fungi are particularly suitable for SSF because of their hyphal mode of fungal growth, greater resistance to low water activity and high osmotic pressure conditions, which makes them competitive over other natural micro-flora for bioconversion of solid substrates (Asgher *et al*., 2016; Sethi *et al*., 2016).

The escalating demand for extracellular enzymes with desirable properties has necessitated the search on

**Corresponding author:* e-mail: bilaluaf@hotmail.com

enzymes from new sources (Cihangir and Sarikaya, 2004). The medium constituents, culturing conditions, incubation period, pH, temperature and the kind of carbon and/or nitrogen sources strongly instigate the production of microbial lipases (Rehman *et al*., 2011). Any divergences from the specified parameters will consequence in an undesirable product. Therefore, optimization of these physicochemical parameters is an important aspect to achieve a high yield of enzymes for industrial applications. In addition, optimization abets in downplaying the magnitude of unexploited constituents at the end of fermentation. Furthermore, there is no defined medium for the preeminent throughput of alkaline serine hydrolase from diverse microbial sources. Each microorganism or its strain has its own nutritional requirement for utmost enzyme output (Bilal *et al*., 2015; Rehman *et al*., 2016). The present study aimed to investigate the utilization of locally available agro-industrial waste as a fermentative feedstuff for the biosynthesis of lipase by *P. ostreatus* using SSF technology. The optimization of process parameters for hyperproduction of lipase was also the focus of present study.

MATERIALS AND METHODS

Procurement and preparation of substrates

Different agro-industrial wastes including canola oilseed cake, sesame oilseed cake, linseed oil cake, cotton oilseed cake, rice bran and wheat bran were procured from the local market of Faisalabad, Pakistan. The substrates were dried and grounded in an electric mill to obtain homogenous particle size by using Octagon Sieve (OCT-Digital 4527-01) and finally stored in airtight jars to avoid humidity.

Strains and inocula development

Fungal strains namely, *Alternaria solani* (DQ 209285), *Fusarium solani* (AB 777258) and *Pleurotus sajukaju* (O 13509) were obtained from the fungal culture collection of the Department of Plant Pathology, University of Agriculture; Faisalabad (UAF). Whereas, *Pleurotus ostreatus* IBL-03 (ATCC 32784) and *Ganoderma lucidum* IBL-05 (Q 494675) were available in the culture stock of Industrial Biotechnology Laboratory (IBL), Department of Biochemistry, UAF. The fungal cultures were maintained on potato dextrose agar (PDA) slants for 3-5 days at pH 4.5 and 28 °C and preserved at 4 °C. Seed cultures were prepared by inoculating a loop-full of respective fungus from the slants to 250-mL Erlenmeyer flasks containing 100 mL Kirk′s basal medium consisting of (g/L): KH_2PO_4, 5.0; NH_4NO_3, 2.0; $(NH_4)_2SO_4$, 4.0; $MgSO_4.7H_2O$, 0.2; tri-sodium citrate, 2.5; peptone, 2.0 and yeast extract 0.1, followed by shaking (120 rpm) at 30°C. After obtaining adequate homogenous spore counting (1×10^8 spores/mL), this medium was used as inoculum (Yasmeen *et al*., 2013).

Optimization of culture conditions for enzyme production

Various physicochemical parameters namely, cultivation time period (24, 48, 72, 96, 120 h), moisture content (40, 50, 60, 70, 80 and 90%), incubation pH (3.0, 4.0, 5.0, 60, 7.0 and 8.0), reaction temperature (25, 30, 35, 40 and 45 °C), olive oil concentration (0, 1.0, 2.0, 3.0, 4.0 and 5.0%) and different carbon and nitrogen sources were studied and optimized to achieve maximum lipase activity. Classical statistical methodology, by changing one factor at a time, and keeping others at optimum levels was adopted for optimization studies.

Time course

Standard inocula (1×10^8 spores/mL, 2.0% v/v) were aseptically inoculated in each 250-mL Erlenmeyer flask containing 100 mL of fermentation medium. The inoculated flasks were placed in a temperature-regulated incubator at 30±0.2°C for up to 120 h. Sterile samples were withdrawn periodically after 24, 48, 72, 96, and 120 h of incubation and analyzed for lipase activity as mentioned before.

Medium pH

The pH of the media was adjusted at different levels (3.0-8.0) using the different pH buffers. The production flasks containing 100 mL of fermenting medium were subjected to fermentation at 30±0.2°C for the optimum time determined before, and lipase activity was monitored.

Fermentation temperature

The inoculated flasks were incubated at different temperatures (25, 30, 35, 40 and 45°C) for optimal incubation time. At the end of incubation period, the enzyme activity was measured by standard assay protocol.

Effect of different carbon sources

For optimization of carbon sources of the fermentation medium, different carbon sources; glucose, maltose, fructose, and lactose were separately added as a sole carbon source and submitted to fermentation using optimized conditions. After the stipulated incubation time, the enzyme activity in the culture supernatant was analyzed.

Effect of different nitrogen sources

Fermentation medium was separately supplemented with nitrogen sources i.e., ammonium nitrate, $(NH_4)_2SO_4$, yeast extract, urea and peptone as a nitrogen source and analyzed for enzyme activity.

Lipase production and recovery

The SSF was carried out in triplicate production flasks containing 10 g of substrate moistened with water (50% w/v) and sterilized at 121°C for 15 min. The sterilized and cooled fermentation medium was inoculated aseptically with a 2.0 mL homogeneous spore suspension (1×10^8 spores/mL) and subjected to fermentation at 30±0.2°C for 72 h under static conditions with occasional observation. After designated fermentation time, the fermented mesh was harvested for a crude enzyme with 100-mL of distilled water. The extracts in the flasks were shaken (150 rpm for 30 min), filtered and supernatants were analyzed for lipase activity (Rehman *et al*., 2011).

Enzyme assay method

Lipase activity was assayed as precisely reported by Amin *et al*. (2011). For this, 0.1 mL of enzyme solution was thoroughly mixed with 0.9mL of a solution containing: 3 mg para-nitrophenol palmitate phosphate (*p*-NPP) dissolved in 1mL of propane-2-ol diluted in 9 mL of the Tris-HCl (50m*M*, pH 8.0) containing 40 mg Triton X-100 and 10mg of Arabic gum. The resultant mixture was incubated at 37°C for 10 min followed by spectrophotometric analysis of para-nitrophenol (*p*-NP) at 410 nm. "One unit of lipase activity was defined as the quantity of enzyme releasing 1μmol of *p*-NP/ min under standard assay conditions".

STATISTICAL ANALYSIS

All the investigations and enzyme assay were repeated at least three times and data were statistically analyzed through analysis of variance (ANOVA) and regression analysis. The ±sign and the corresponding error bars designate a standard deviation of the mean. All analyses were made using statistical software package SPSS version 21.

RESULTS

Different fungal strains, as well as agro-residues, were screened for enhanced production of the lipolytic enzyme in SSF. Results showed that the highest lipase production titer was recorded in *P. ostreatus* culture followed by *G. lucidum*, *F. solani*, *P. sajukaju* and *A. solani* (fig. 1). On the other hand, various agro-industrial wastes were utilized as a sole carbon source for lipase production. It was observed that *P. ostreatus* considerably produced the enzyme in all substrates used in the culture media, but highest production was recorded in the medium containing canola oilseed cake followed linseed oil cake, rice bran, sesame oilseed cake, cotton oilseed cake, and wheat bran (Fig. 2). This enzyme yield was much better than previously reported results; 630 U/gds (Mahadik *et al*., 2002), 26.4 U/gds (Gutarra *et al*., 2005), 384 U/gds (Mala *et al*., 2007), 976 U/gds (Mahanta *et al*., 2008) and 44.8 U/gds (Godoy *et al*., 2009) by *Penicillium simplicissimum*, *Aspergillus niger* NCIM 1207, *A. niger* MTCC 2594, *Pseudomonas aeruginosa* and *P. simplicissimum*, respectively.

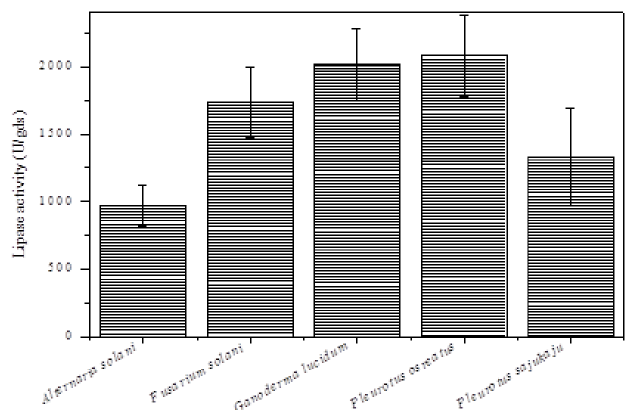

Fig. 1: Screening of different fungal strains for lipase production in the solid-state fermentation of canola oilseed cake.

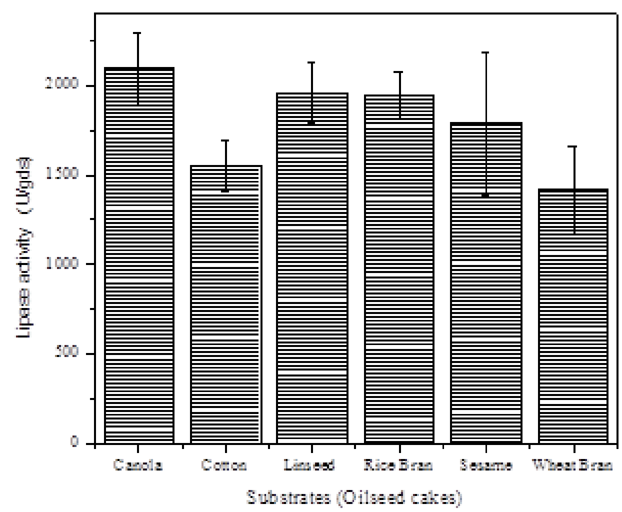

Fig. 2: Effect of different substrates on lipase activity.

Fermentation parameters optimization

Fermentation time course

For optimization of fermentation time course, the inoculated flasks were subjected to fermentation for different time intervals (ranging from 24 to 120 h) and responses are displayed in fig. 3. Results revealed that incubation period significantly (P<0.005) influenced the lipase productivity, and peaked enzyme titer (2097.33 U/gds) was accomplished at 72 h. Beyond the optimum cultivation time, the enzyme activity was reduced that may be due to depletion of medium nutrients or enzyme denaturation.

Effect of initial moisture level

An adequate humidity level is crucial for microbial growth during fermentation. Prior to fermentation, the substrates were moistened with different volumes of Kirk's basal nutrient medium (40 to 90%) to decipher the effect of moisture during the SSF. It was observed that moisture level exerted significant (P<0.03) effect on lipase productivity by *P. ostreatus* and lipase activity

(2142.67 U/gds) was peaked at 50% initial humidity level (fig. 4).

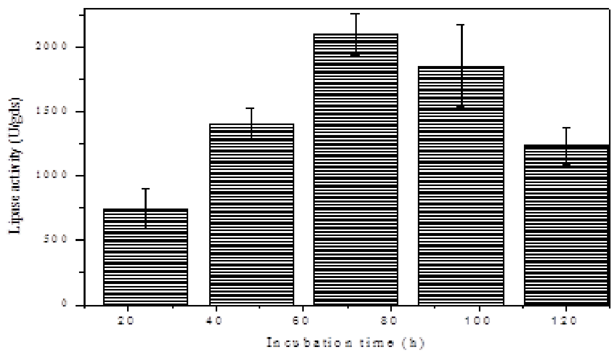

Fig. 3: Effect of incubation time on lipase activity.

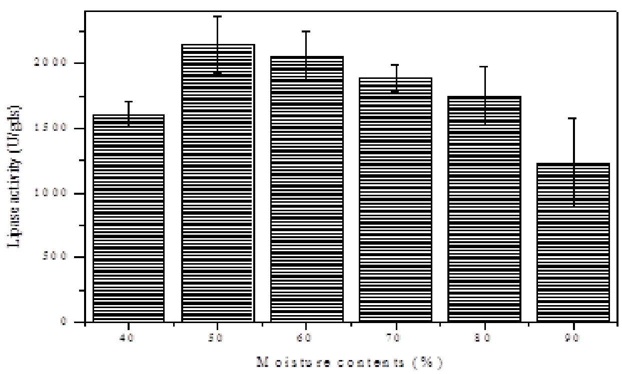

Fig. 4: Effect of initial moisture content on the lipase activity.

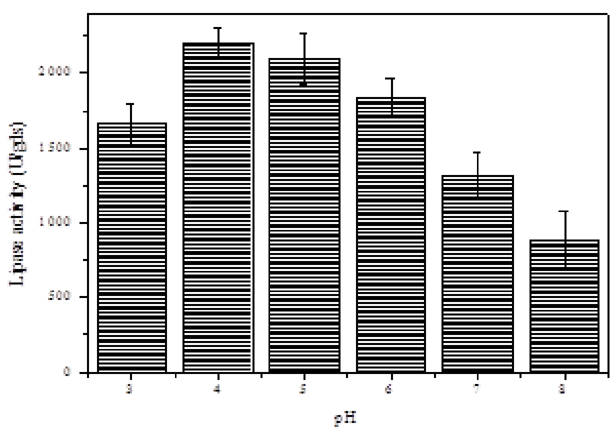

Fig. 5: Effects of different pH levels on lipase activity.

pH value

In order to determine the effect of pH on lipase production by *P. ostreatus,* the fermentation medium was adjusted to different pH levels (3.0 to 8.0) and enzyme profile was assessed. The *P. ostreatus* presented potential capability to produce lipolytic enzyme at slightly acidic pH of 4.0 (fig. 5). A significant inhibition of lipase activity was observed beyond the optimal pH values.

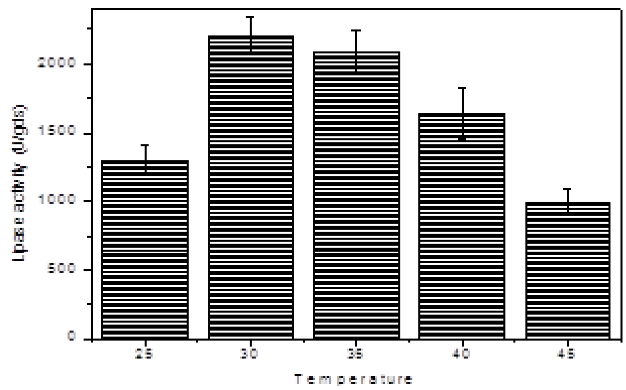

Fig. 6: Effects of different temperatures on lipase activity.

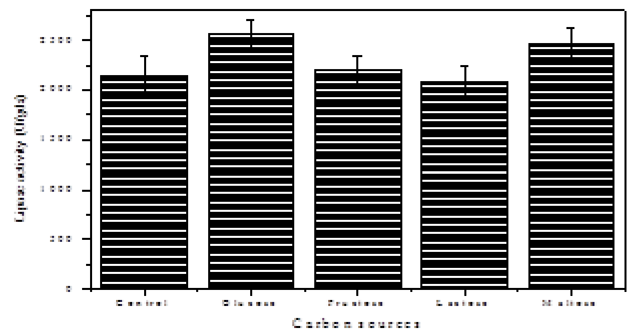

Fig. 7: Effect of additional carbon source on lipase activity

Effect of incubation temperature

Varying temperature points (ranging from 25 to 45 °C) were used to investigate the optimum temperature for improved production of lipase by *P. ostreatus* and results are illustrated in Fig. 5. Enzymatic yield of the fungus was observed to be progressively improved up to a certain temperature but afar this temperature hasty decline of lipase activity was pragmatic. Noticeably, the peaked lipase activity was recorded at 30 °C.

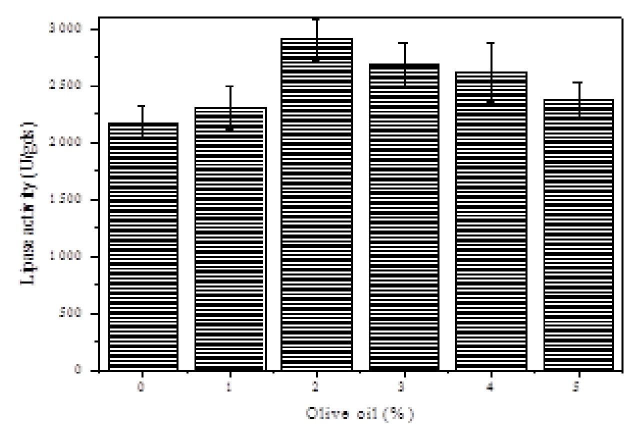

Fig. 8: Effect of olive oil on lipase activity

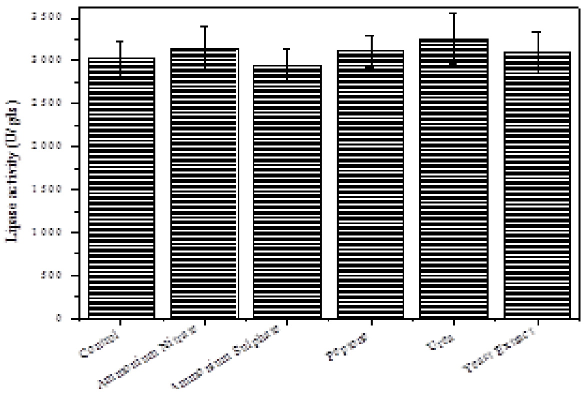

Fig. 9: Effect of supplemented nitrogen on lipase activity

Nutritional parameters

Effect of carbon sources

Various carbon sources (glucose, fructose, lactose, and maltose) were used as additives in canola oil cake at a final concentration of 2.0% to assess their potential stimulatory and/or inhibitory effect on lipase yield under optimized conditions. Results in fig. 6 indicate that the supplemented carbon significantly (P<0.05) exerted enzyme promoting effect. Maximal lipase activity (2564 U/gds) was recorded when glucose was supplemented as a carbon source, and the lower lipase production was observed at other tested carbon sources.

Influence of olive oil concentration

Olive oil is a well-known lipase inducer of the lipase production by many bacterial and fungal strains (Rehman *et al*., 2011). The olive oil concentrations (ranging from 0.0 to 5.0%) were used in the growth medium to evaluate their effectiveness as lipase inducer. It was observed that olive oil had a significant influence on the lipase yield. The enzyme extract contained highest lipase production (2906.67 U/gds) when 2.0% concentration of olive oil was used (fig. 8). The findings were similar to previous reports, where olive oil was observed to be a most influential factor for lipase production (Fadiloglu and Erkmen, 2002; Rajendran *et al*., 2008). Moreover, Azeredo *et al*. (2007) highlighted that olive oil supplemented babassu cake with a C: N ratio of 13: 3 yielded the best enzyme production by *P. restrictum*.

Effect of additional nitrogen sources

Various organic and inorganic nitrogen sources were used in growth medium to examine the influence of nitrogen on lipase activity. Supplemental nitrogen did not affect significantly (P<1.01) on the lipase production by *P. ostreatus* and enzyme activity was maximum in the presence of urea (3256 U/gds).

DISCUSSION

Fungi represent a wide-array of filamentous basidiomycetous organisms with remarkable genetic repertoire for the production of industrially important enzymes and other secondary metabolites (Munir *et al*., 2015). Though, filamentous fungi including *Pleurotus* species have been widely investigated for ligninolytic enzyme production in the past few decades (Yasmeen *et al*., 2013; Bilal *et al*., 2015; Munir *et al*., 2015; Asgher *et al*., 2016; Bilal *et al*., 2016); however, no report is documented on the production of lipolytic enzymes by *P. ostreatus* in a solid-state system. In the present study, different fungal strains, as well as agro-residues, were screened for hyper-production of the lipolytic enzymes under SSF technology. The strain with superior lipase producing aptitude was recorded to be *P. ostreatus* followed by *G. lucidum*, *F. solani*, *P. sajukaju* and *A. solani*.

Fermentation medium plays a pivotal role in enzyme production. Several investigators have reported that optimized medium formulation is prerequisite for the enhanced enzyme production. Therefore, different experiments were carried out to optimize different parameters such as fermentation time, moisture level, pH, temperature, and supplementation of carbon and nitrogen sources. Fermentation duration significantly (P<0.005) influenced the lipase productivity, and the maximum enzyme titer was achieved at 72 h. Exceeding cultivation time beyond the optimum level, the enzyme activity was reduced that may be due to depletion of medium nutrients or enzyme deactivation and/or denaturation caused by the interaction with other components in the medium (Munir *et al*., 2015). The results were in agreement with Gutarra *et al*. (2009), who reported the maximum *Aspergillus niger* and *Pencillium simplicissimum* lipase activities after 72 h. Contrarily, Mahadik *et al*. (2002) recorded optimum lipase production from *A. niger* after 120 h of cultivation period. Genetic variations among microbial strains as well as nature and composition of the substrates have been correlated with variable expression of enzymes after different fermentation durations (Giardina *et al*., 2000; Yasmeen *et al*., 2013).

Adequate humidity level is essential for microbial growth during fermentation. Prior to fermentation, the substrates were moistened with different volumes of Kirk's basal nutrient medium to decipher the effect of moisture during the SSF. Notably, the moisture level exerted significant (P<0.03) effect on lipase productivity by *P. ostreatus* and lipase activity (2142.67 U/gds) was maximized at 50% initial humidity level (fig. 4). Moisture content exhibits great influence on the physical properties of the substrates, and an elevated moisture level reduces the substrate permeability, modify substrate particle structure and decrease gas volume and exchange, that ultimately, leading to poor oxygen transfer and diffusion (Sun and Xu, 2008; Rehman *et al*., 2011). Lower moisture contents reduce the solubility of nutrients contained in the solid substrate, resulting in improper swelling and a higher water tension (Mahanta *et al*., 2008; Amin *et al*., 2008).

Lower enzyme production beyond optimal moisture levels might be attributed to the fact that low diffusion of nutrients and metabolites occur in lower moisture contents whereas compaction of the substrate occurs at higher moisture contents. In previous studies, 60.0% and 71.0% initial moisture contents were suggested to be optimal for hyper-production of lipases by *A. niger* using gingelly oilseed cake and wheat bran, respectively (Mahadik *et al*., 2002). Gutarra and coworkers (2005) noted the optimum enzyme production from babassu cake by *P. simplicissimum* at 70.0% moisture level. The same humidity level was also recorded optimal (Sun and Xu, 2008) for lipase production by *Rhizopus chinensis*.

The pH of the growth medium is considered to be most significant bioprocess parameter, which clouts straightly the fermentation performance and thus, enzyme production during SSF. The growth and enzyme production potential of each micro-organism requires a unique optimum pH as well as a pH range. Culture pH strappingly effects the transportation of several nutrients across the cell membrane and a lot of enzymatic reactions/processes. Divergences in pH fluctuate fluidities of various nutrients and/or inducers, acid-base balance and augmentation of elements among biotic and abiotic phase (Mishra *et al*., 2012). The pH profile revealed that *P. ostreatus* produced maximum lipolytic enzyme at a slightly acidic pH (pH 4.0). A significant inhibition of lipase activity was recorded beyond the optimal pH values. Comparable results have been reported by Lin *et al*. (2006) that pH 5.5 was found to be optimal for lipase enzyme production by *Antrodia cinnamomea*. Nevertheless, the present detected optimum pH values were lower than *Candida rugosa* (pH 7.0), *Pseudomonas aeruginosa* (pH 7.0), *Rhizopus glutinis* (pH 8.0) and marine bacterial lipase (pH 10.0) (Dimitris *et al*., 1992; Camargo de Morais *et al*., 1998; Bhatti *et al*., 2007; Rehman *et al*., 2011).

Incubation temperature is another one of the fastidious factors influencing the fermentation process. Deviations in optimum temperature affect both microorganism specific growth rate as well as enzyme profile. The reduction in enzyme activity at higher temperature might be attributed to thermal denaturation of an enzyme involved in the metabolic pathways, which may result in less product synthesis (Mahanta *et al*., 2008). Besides, protein breakdown, enzyme inactivation could be contributing factors resulting in reduced lipase activity at elevated temperatures. Similar results have been documented previously for *Fusarium oxysporum* (28°C) and *Rhizopus glutinis* (30°C), respectively (Dimitris *et al*., 1992; Rifaat *et al*., 2010). Conversely, the temperature optima results were significantly different from other lipase-producing strains i.e., *Penicillium citrinium* (22°C), *Colletotrichum gloeosporioides* (25°C) and *Rhizopus arrhizus* (26.5°C) (Maliszewska and Mastalerz, 1992; Yang *et al*., 2000; Balaji and Ebenezer, 2008).

Factors like carbon or nitrogen sources and their engrossments have always been of enormous attraction to the industrialists and scientific community for the cut-price media formulation (Asgher *et al*, 2016). It is also well-known that the expenses of growth medium constitute 30-40% of the production cost of commercial bio-catalysts. Therefore, it is of immense significance to optimizing the process conditions for cost-benefit enzyme production. Micro-organisms possess the ability to utilize organic, inorganic and agricultural by-product forms of nitrogen which are necessary to manufacture amino-acids, nucleic acids, proteins and other cell-wall constituents. Both the nature and quantity of available carbon sources influence lipolytic enzymes production by different fungal strains. Falony *et al*. (2006) utilized different carbon sources for lipase enzyme production and noticed wheat bran in combination with glucose a good carbon source for enzyme production by *A. niger*. Irshad and Asgher, (2011) suggested glucose as best carbon additives for WRF strains, including *P. ostreatus*, *S. commune*, and *T. versicolor*. In contrast, Mahanta *et al*. (2008) documented that supplementation of maltose in the growth media led to elevated lipase production by *P. aeruginosa* and *C. rugosa*, respectively. Glucose ($C_6H_{12}O_6$) is extravagantly used in bioprocesses and many studies have revealed that it causes the reduction in proteolytic enzyme synthesis due to catabolic repression (Raju *et al*., 2013). Further, the easily oxidizable nature of glucose in contrast to other substrates makes it more favorable for growth and enzyme production.

Nitrogen sources are the secondary energy source for microorganism and play a pivotal role in the fugal growth and other metabolic activities (Irshad and Asgher, 2011). In the present study, however, the addition of the nitrogen did not affect significantly (P<1.01) on the lipase production by *P. ostreatus*. Similar to our study, no significant effect of nitrogen was observed on lipolytic enzyme production by Kamini *et al*. (1998) and Gutarra *et al*. (2005). On the other hand, substantial improvement in lipase activity was noted by Glu and Erkmen, (2002) in the fermented culture of *C. rugosa* with additionally yeast extract and peptone as nitrogen sources. Likewise, Mahanta and coworkers, (2008) also recorded improvement in enzyme titer by *P. aeruginosa* using peptone as additives.

CONCLUSIONS

In conclusion, *P. ostreatus* displayed considerable prospective for the production of industrially relevant lipase in SSF of canola oil seed cake with utmost 3256 U/gds activities. Optimization of different physico-chemical and nutritional factors significantly (up to 1.6 times) enhanced the enzyme activity. The promisingly high yield of lipase suggests an opportunity to use *P. ostreatus* for commercial scale production of lipase utilizing canola seed cake as fermentative substrate.

REFERENCES

Amin F, Bhatti HN and Rehman S (2011). Optimization of growth parameters for lipase production by *Ganoderma lucidum* using response surface methodology. *Afr. J. Biotechnol.,* **10**(28): 5514-5523.

Amin M, Bhatti HN and Perveen F (2008). Production, Partial Purification and Thermal Characterization of *β*-Amylase from *Fusarium solani* in Solid-State Fermentation. *J. Chem. Soc. Pak.,* **30**: 480-485.

Azeredo LAI, PM Gomes, G Sant Anna, LR Castilho and DG Freire (2007). Production and regulation of lipase activity from *Penicillium restrictum* in submerged and solid-state fermentations. *Current Microbiol.,* **54**: 361-365.

BK Sethi, PK Nanda and S Sahoo (2016). Characterization of biotechnologically relevant extra cellular lipase produced by *Aspergillus terreus* NCFT 4269.10. *Braz. J. Microbiol.,* **47**(1): 143-149.

Balaji V and P Ebenezer (2008). Optimization of extracellular lipase production in *Colletotrichum gloeosporiodes* by solid state fermentation. *Ind. J. Sci. Technol.,* **1**: 1-8.

Bhatti HN, Rashid MH, Nawaz R, Asgher M, Perveen R and Jabbar A (2007). Optimization of media for enhanced glucoamylase production in solid state fermentation by *Fusarium solani*. *Food Technol. Biotechnol.,* **45**(1): 51-56.

Bilal M, Asgher M, Ramzan M (2015). Purification and biochemical characterization of extracellular manganese per oxidase from *Ganoderma lucidum* IBL-05 and its application. *Sci. Res. Enz. Essays,* **10**: 456-464.

Brust B, Lecoufle M and Tuaillon E *et al* (2011). *Mycobacterium tuberculosis* lipolytic enzymes as potential biomarkers for the diagnosis of active tuberculosis. *PLoS ONE*. **6**: 25078.

by the edible basidiomycete *Antrodia cinnamomea* in submerged culture. *Enzyme Microb. Technol.,* **39**: 98-102.

Camargo de Morais MM, Morais JMA, Melo EHM, Lima-Filho JL (1998). Production of extracellular lipase by a *Candida rugosa* strain isolated in Pernambuco. *Bras. Rev. Microb.*, **29**: 134-137.

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

Dimitris P, C Paul, K Dimitris and JM Basil 1992. Optimizing production of extra cellular lipase from *Rhodotorula glutinis*. *Biotechnol. Lett.,* **14**: 397-402.

Fadiloglu S and Erkmen O (2002). Effects of carbon and nitrogen sources on lipase production by *Candida rugosa*. *Turk. J. Eng. Environm. Sci.,* **26**: 249-254.

Falony G, JC Armas, JCD Mendoza and JLM Hernandez (2006). Production of extracellular lipase from *Aspergillus niger* by solid-state fermentation. *Food Technol. Biotechnol.,* **44**(2): 235-240.

Fang Y, Lu Z and Lv F *et al* (2006). A newly isolated organic solventtolerant *Staphylococcus saprophyticus* M36 produced organicsolvent-stable lipase. *Curr. Microbiol*., **53**: 510-515.

Fernandez-Lafuente R (2010). Lipase from *Thermomyces lanuginosus*: Uses and prospects as an industrial biocatalyst. *J. Mol. Catal. B: Enzym.,* **62**: 197-212.

Franken LPG, Marcon NS, Treichel H, Oliveira D, Freire DMG and Dariva C (2010). Effect of treatment with compressed propane on lipases hydrolytic activity. *Food Bioproc. Technol.,* **3**: 511-520.

Ghaly AE, D Dave, MS Brooks and S Budge (2010). Production of biodiesel by enzymatic trans-esterification: *Review. Am. J. Biochem. Biotechnol.,* **6**: 54-76.

Giardina P, Palmieri G, Fontanella B, Rivieccio V and Sannia G (2000). Manganese peroxidase is enzymes produced by *Pleurotus ostreatus* grown on wood sawdust. *Arch. Biochem. Biophys.,* **376**: 171-179.

Glu SF and E Erkman (2002). Effects of carbon and nitrogen sources on lipase production by *Candida rugosa. Turkish J. Eng. Env. Sci.,* **26**: 249-254.

Godoy MG, Gutarra MLE, Maciel FM, Felix SP, Bevilaqua JV, Machado OLT and Freire DMG (2009). Use of a low-cost methodology for biodetoxification of castor bean waste and lipase production. *Enzyme Microb. Technol.,* **44**(5): 317-322.

Grbavcic SZ, Dimitrijevic-Brankovic SI, Bezbradica DI, Siler-Marinkovic SS, Knezevic ZD (2007). Effect of fermentation conditions on lipase production by *Candida utilis*. *J. Serb. Chem. Soci.,* **72**(8-9): 757-765.

Gupta N, Shai V and Gupta R (2007). Alkaline lipase from a novel strain *Burkholderia multivorans*: Statistical medium optimization and production in a bioreactor. *Process Biochem.,* **42**(2): 518-526.

Gupta R, Gupta N and Rathi P (2004). Bacterial lipases: An overview of production, purification and biochemical properties. *App. Microbio. Biotechnol.,* **64**: 763-781.

Gupta R, Rathi P, Gupta N and Bradoo S (2003). Lipase assays forconventional and molecular screening: an overview. *Biotechnol. Appl. Biochem.,* **37**: 63-71.

Gutarra MLE (2003). Produção de lipase porfermentação no estado sólido: seleção de fungos produtores e estudo das condições de cultivo. MSc Thesis, Departamento de Bioquímica, IQ/UFRJ, Rio de Janeiro/RJ, Brazil.

Gutarra MLE, Cavalcanti EDC, Casnlo LR, Freire DMG and Santanna GL (2005). Lipase production by solid-state fermentation: Cultivation conditions and operation of tray and Packed-Bed Bioreactors. *App. Biochem. Biotechnol.,* **121-124**: 105-116.

Gutarra MLE, Godoy MG, Maugeri F, Rodrigues MI, Freiro DM and Castilho LR (2009). Production of acidic and thermostable lipases of mesophilic fungus *Penicillium simplicissimum* by solid-state fermentation. *Bioresour. Technol*., **100**: 5249-5254.

Hasan F, AA Shah and A Hameed (2006). Industrial applications of microbial lipases. *Enzyme Microb. Technol.*, **39**: 235-251.

Holker U, Hofer M and Lenz J (2004). Biotechnology advantages of laboratory-scale solid-state fermentation with fungi. *App. Microbio. Biotechnol.*, **64**: 175-186.

Irshad M and Asgher M (2011). Production and optimization of ligninolytic enzymes by white-rot fungus *Schizophyllum commune* IBL-06 in solid-state medium banana stalks. *Afr. J. Biotechnol.*, **10**: 18234-18242.

Kamini NR, T Fujii, T Kurosu and H Iefuji (1998). Lipase production from *Aspergillus niger,* by solid-state fermentation using gingelly oil cake. *Process Biochem.*, **33**(5): 505-511.

Laxman RS, Sonawane AP, More SV, Rao BS, Rele MV, Jogdand VV, Deshpande V and Rao MB (2005). Optimization and scale up of production of alkaline protease from *Conidiobolus coronatus*. *Process Biochem.*, **40**(9): 3152-3158.

Lin ES, CC Wang and S Sung (2006). Cultivating conditions influence lipase production lipase production. *Enzyme Microb. Technol.*, **44**: 317-322.

Mahadik ND, US Puntambekar KB Bastawde JM Khire and DV Gokhale (2002). Production of acidic lipase by *Aspergillus niger* in solid state fermentation. *Process Biochem.*, **38**: 715-721.

Mahanta N, Gupta A and Khare SK (2008). Production of protease and lipase by solvent tolerant *Pseudomonas aeruginosa* PseA in solid-state fermentation using *Jatropha curcas* seed cake as substrate. *Bioresour. Technol.*, **99**: 1729-1735.

Mala JGS, NG Edwinoliver, NR Kamini and R Puvanakrishnan (2007). Mixed substrate solid state fermentation for production and extraction of lipase from *Aspergillus niger* MTCC 2594. *J. Gen. Appl. Microbiol.*, **53**: 247-253.

Maliszewska I, P Mastalerz (1992). Production and some properties of lipase from *Penicillium citrinum*. *Enzyme Microb. Technol.*, **14**:190-193.

Mishra S, Kocher GS, Sagar P and Savitha P (2012). Production of alkaline protease by adsorbed cells of *Bacillus circulans* MTCC 7906 under batch conditions. *Int. J. Microbiol. Res.*, **3**: 104-108.

Asgher, M., Abdul Wahab, Muhammad Bilal, Hafiz Muhammad Nasir Iqbal. Lignocellulose degradation and production of lignin modifying enzymes by *Schizophyllum commune* IBL-06 in solid-state fermentation. *Biocatal. Agric. Biotechnol.*, **6**: 195-201.

Munir N, Asgher M, Tahir IM, Riaz M, Bilal M and Shah SMA (2015). Utilization of agro-wastes for production of ligninolytic enzymes in liquid state fermentation by *P. chrysosporium*-IBL-03.*IJCBS7*, pp.9-14.

Park H, Lee K, Chi Y and Jeong S (2005). Effects of methanol on the catalytic properties of porcine pancreatic lipase. *J. Microb. Biotech.*, **15**(2): 296-301.

Pinijsuwan S, Shipovskov S, Surareungchai W, Ferapontova EE and Gothelf KV (2011). Development of a lipase-based optical assay for detection of DNA. *Org. Biomol. Chem.*, **9**: 6352-6356.

Rajendran A, Palanisamy A and Thangavelu V (2008). Evaluation of medium components by plackett-burman statistical design for lipase production by *Candida rugosa* and kinetic modeling. *Chin. J. Biotech.*, **24**(3): 436-444.

Raju EVN and Divakar G (2013). Screening and isolation of fibrinolytic protease producing mesophilic bacteria from slaughter houses in Bangalore. *International Journal of Pharmaceutical Sciences and Research,* **4**(9): 3625.

Rifaat HM, AA Mahalawy, HA El-Menofy and SA Donia (2010). Production, optimization and partial purification of lipase from *Fusarium oxysporum. J. Appl. Sci. Environment. Sanit.*, **5**(1): 39-53.

Rohit Sharmaa, Yusuf Chistib, Uttam Ch and Banerjee (2001). Production, purification, characterization and applications of lipases. *Biotechnology Advances,* **19**: 627-662.

Saima Rehman, Haq Nawaz Bhatti, Ijaz Ahmad Bhatti and Muhammad Asgher (2011). Optimization of process parameters for enhanced production of lipase by *Penicillium notatum* using agricultural wastes. *Afr. J. Biotechnol.*, **10**(84): 19580-19589.

Sakinc T, Kleine B and Gatermann SG (2007). Biochemical characterization of the surface associated lipase of *Staphylococcus saprophyticus*. *FEMS Microbiol. Lett.*, **274**: 335-341.

Satyendra Kumar, Khyodano Kikon, Ashutosh Upadhyay, Shamsher S. Kanwar, Reena Gupta. Production, puriWcation, and characterization of lipase from thermophilic and alkaliphilic *Bacillus coagulans* BTS-3. *Protein Expression and Purification*, **41**: 38-44.

Shaheen I, Bhatti HN and Ashraf T (2008). Production, purification and thermal characterization of invertase from a newly isolated *Fusarium* sp. under solid-state fermentation. *Int. J. Food Sci. Technol.*, **43**: 1152-1158.

Sun SY and Xu Y (2008). Solid-state fermentation for whole-cell synthetic lipase production from *Rhizopus chinensis* and identification of the functional enzyme. *Proces. Biochem.*, **43**: 219-224.

Vakhlu J and Kour A (2006). Yeast lipases: Enzyme purification, biochemical properties and gene cloning. *Electron. J. Biotech.*, **9**: 1-17.

Yang X, B Wang, F Cui and T Tan (2005). Production of lipase by repeated batch fermentation with immobilized *Rhizopus arrhizus*. *Process Biochem.*, **40**: 2095-2103.

Yasmeen Q, Asgher M, Sheikh MA and Nawaz H (2013). Optimization of ligninolytic enzymes production through response surface methodology. *Bio. Res.*, **8**: 944-949.