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Submerged Culture Studies for Lipase Production by *Aspergillus niger* NCIM 584 on Soya Flour

[1]*Maryam N. Hosseinpour,* [1]*Ghasem D. Najafpour,* [2]*Habibollah Younesi and* [1]*Marjan Khorrami*

[1]Faculty of Chemical Engineering, Babol Noshirvani University of Technology, Babol, Iran
[2]Department of Environmental Sciences, College of Natural Resources & Marine Sciences, Tarbiat Modares University, Nour, Iran

Abstract: Among enzymes, lipases were found as lipolytic enzyme with numerous industrial applications. The culture and desired growth conditions for production of extracellular lipase from fungus *Aspergillus niger* strain NCIM 584 was extensively investigated. Enzyme production was carried out in a submerge culture using major nutrients from soya flour as main constituent of the media. The optimum weight percentage of soya flour, glucose and olive oil concentrations on lipase production were defined as 7.5 wt%, 12.5 and 12 g/l, respectively. Combination of nitrogen sources such as yeast extract and peptone were suitable nitrogen sources. The activity of lipase was maximized at pH value of 7. In addition, the optimum growth temperature was observed at 30°C. Also, maximum enzyme activities were observed in presence calcium ions with concentration of 7.5mM.

Key words: Lipase · *Aspergillus niger* · Growth condition · Enzyme activity · Soya flour · Submerged culture

INTRODUCTION

Enzyme is protein which is synthesized as intra and extra cellular compounds. Enzymes energize and catalyze biochemical reactions with high specificity and enhance the reaction rate [1-2]. Lipases are one of the highly commercialized enzymes; have an important role in the world enzyme market. Lipase are found and ranked after proteases and amylases [3].

Lipases are widely used in industrial application such as, detergent industry, pharmaceutical industry, pulp and paper industry, production of biodiesel, dairy and bakery foods, fats and oils [4-6]. Lipases are found in animal, plant and microorganisms. Besides the stability, selectivity and broad substrate specificity microbial lipases are more promising in terms of availabilities and productivities [6-7]. Among the various sources of lipases, fungi is recognized as the best enzyme producer and also used for industrial application [7].

Hydrolysis of lipids, fats such as triglycerides may take place in the emulsion of water and by the means of enzyme known as lipases. The enzyme catalyzed the hydrolysis of triglycerides which acts in an oil–water interface [7-8]. Lipases can catalyze esterification, interesterification, transesterification, acidolysis, alcoholysis and aminolysis reactions in aqueous and non-aqueous media [5, 8].

Aspergillus niger is one of the important microorganisms for lipase production and is suitable for use in food industry. Extensive studies focused on conditions of living organism for production of lipase showed significant variation among various strains [9].

The aim of the present work is to investigate the production of lipase by *A. niger* using soya flour as the main substrate. Also, media composition and activities of the synthesized enzyme were determined.

MATERIALS AND METHODS

Microorganism: In this study, *Aspergillus niger* NCIM 584 was used. The organism was supplied by National collection of Industrial Microorganisms (Chandigarh, India). The bacterial strain was stored and maintained in nutrient agar slants at 4°C.

Culture Conditions: The fermentation medium for lipase production contained: KH_2PO_4, $MgSO_4 \cdot 7H_2O$, KCl, yeast, peptone, glucose, olive oil and with concentration of 2, 0.5, 0.5, 0.25, 0.25, 12.5, 12 g/l, respectively.

Corresponding Author: Ghasem D. Najafpour, Faculty of Chemical Engineering, Babol Noshirvani University of Technology, Babol, Iran. Fax: +981113210975, E-mail: najafpour@nit.ac.ir.

Table 1: Chemical composition and physical properties of soya flour

Composition	Weight %
Moisture	9.00
Ash	5.00
Protein	35.00
Sugar	6.07
Fat	18.00
Particle size	
< 177 µm	21.00
250 µm < for the size of <177 µm	33.00
> 250 µm	46.00

All chemicals and reagents used in experiment were analytical grade supplied by Merck (Darmstadt Germany). Soya flour available in the local market was added to nutrient broth with concentration of 7.5 wt %. The chemical composition and physical properties of soya flour is summarized in Table 1.

The microorganisms were grown in a 100 ml Erlenmeyer flask contained 50 ml fermentation medium. The inoculated media incubated at 180 rpm and 30°C on an incubator-shaker for 4 days. At the end of cultivation period, the mycelium was separated by filtration with Whatman filter paper (no. 41; diameter of 125mm) and then remaining mycelia was removed by centrifugation at 1000 rpm for 5 min. Several parameters affecting on lipase production and activities were investigated. Effects of substrate concentration (glucose and olive oil) and several nitrogen sources (ammonium chloride, peptone, yeast and urea) on lipase production were experimented. The enzyme activities with respect to pH (6-8) and temperature (25-45°C) were also evaluated.

Lipase Assay: Lipase activity was determined by colorimetric method with *p*-nitrophenyl palmitate as substrate. The assay mixture was incubated at 30°C for 5 min and the *p*-nitrophenol released was measured at 410 nm according to method discussed in the literature [10]. Based on definition, one unit (U) of lipase activity was defined as the amount of enzyme liberates one micromole of *p*-nitrophenol per milliliter per minute under the standard assay conditions.

RESULTS AND DISCUSSION

Effect of Soya Flour Concentration: For efficient enzyme production, several carbon sources for production of lipase were evaluated. Among various substrates; soya flour with concentration of 2.5 to 12.5% were experimented. Generally, soya flour is enriched with protein, fat and nutrients. The supplementary nutrients were olive oil, glucose, yeast extract as nitrogen source with concentration of 10, 10, 0.5 g/l, respectively. The media pH was 7 and incubated at 32°C. Fig. 1 shows enzyme relative activities with respect to soya flour (wt %). The enzyme activities were in the range of 75-100%. Maximum enzyme activity was obtained with 7.5% soya flour. Maximum enzyme activity at optimum soya flour concentration was 3252 U/l.

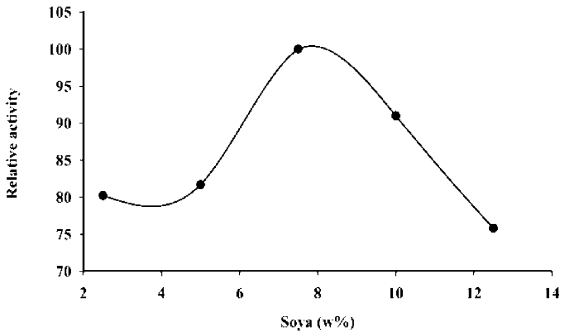

Fig. 1: The influence soya flour concentration of on lipase activity

Effect of Olive Oil Concentration: Soluble organic carbon is essential energy source for the growth of cells. It has been reported that various fats, fatty acids, plant oils, triglycerides, ester-based detergents and other substances were the best inducers in lipase synthesis while microorganisms were able to utilized carbon source as energy [11]. Several olive oil concentrations in the media ranged from 3 to15 g/l were investigated while other media fixed conditions such as soya flour concentration 7.5 (wt %), glucose concentration of 10 g/l, yeast extract 0.5 g/l, pH value of 7 and media temperature was set at 32°C. The best result was observed for concentration of olive oil at 12 g/l and enzyme activity was 4455.84 U/l (Fig. 2). The optimum concentration of olive oil for production of lipase by *Bacillus* sp. was reported at 1 wt % [12]. Adham and Ahmed [9] obtained the best concentration of olive oil for the lipase from *A. niger* NRRL3 in a submerged fermentation culture with about 2 wt %. Contesini *et al.* [13] have demonstrated the best concentration of olive oil by *A. niger* AC-54 in a solid state fermentation with wheat bran as substrate at 1.6 wt %. Falony *et al.* [14] indicated the best concentration of olive oil by *A. niger* J-1 in solid state fermentation with wheat bran as substrate at 1.5 wt %. It was found that presence of fatty acids such as olive oil as carbon source was essential for the growth of *A. niger* and also for the liberation of enzyme.

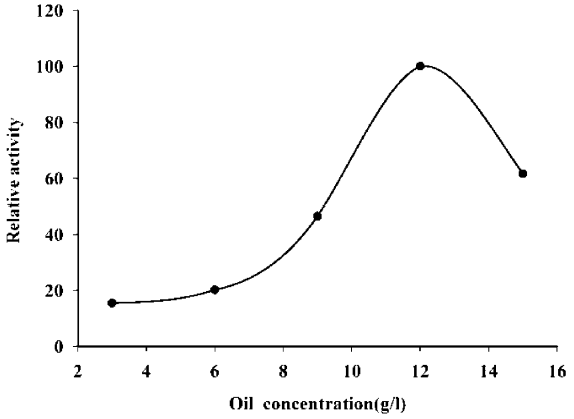

Fig. 2: The influence of olive oil concentration on lipase production

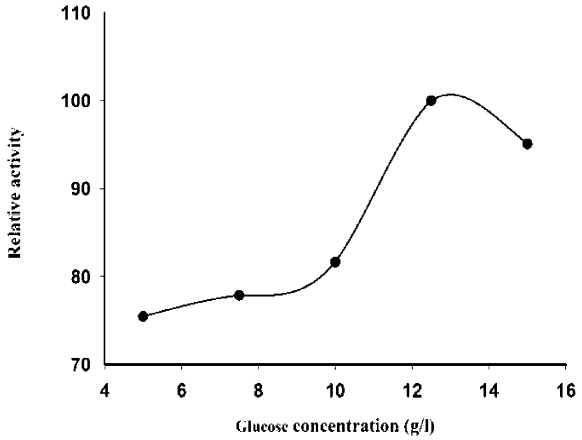

Fig. 3: The influence of substrate concentration on lipase production

Effect of Substrate Concentration on Enzyme Production: Glucose concentration in the media were in the range of 5 to 15 g/l while other media components were: soya flour 7.5 wt %, olive oil 12 g/l, yeast extract as nitrogen source 0.5 g/l, pH 7 and incubation temperature 32°C. Experimental results showed that maximum enzymes were obtained with 12.5g/l glucose concentration (Fig. 3) and enzyme activity at this glucose concentration was 4845.16 U/l. Adham and Ahmed [9] reported that the optimum concentration of glucose for the lipase using *A. niger* NRRL3 in a submerge fermentation was 20g/l Contesini *et al.* [13] also reported that the best concentration of glucose by *A. niger* AC-54 in solid state fermentation with wheat bran as substrate at 4.8 %. In an independent investigation by Falony *et al.* [14] stated that maximum concentration of olive oil by *A. niger* J-1 in solid state fermentation with wheat bran as substrate defined at 1.5 %.

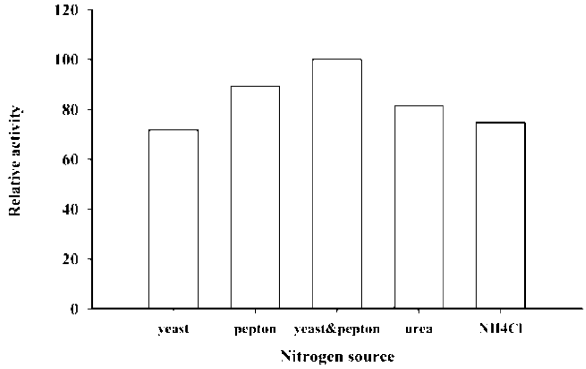

Fig. 4: The influence of nitrogen sources on enzyme activities

Effect of Nitrogen Source: Organic and inorganic nitrogen sources play important role in synthesis of lipase. The effect of various nitrogen sources were experimented in the submerged cultures [12]. The relative activity of enzyme was affected by the variety of nitrogen sources such as yeast extract, peptone and mixture of yeast extract and peptone, urea and ammonium chloride each at fixed concentration of 0.5 g/l (Fig. 4) and optimum lipase activity observed 5289.175 U/l. Other media composition such as soya flour, olive oil, glucose concentration, pH of the media and incubation temperature were 7.5 wt%, 12, 12.5 g/l, 7 and 32°C, respectively. Fig. 4 demonstrates that the mixture of yeast extract and peptone had the maximum lipase activities. It has been reported that, microorganisms provide high yields of lipase when organic nitrogen sources are used [15]. Maximum lipase production was obtained with *Geotrichum–like R59* when the medium contained urea at a concentration of 0.4% as nitrogen source [15]. Tan and his coworkers [16] indentified that in lipase production by *Candida sp.* soybean meal and casein were the best nitrogen sources among all other organic nitrogen sources. Salleh and his research group [17] obtained maximum extracellular lipase production by *Rhizopus oryzae*, when the medium contained peptone as nitrogen source. The obtained results also showed that organic nitrogen source was essential for lipase production.

Effect of Temperature on Lipase Activity: Activity of lipase was determined under standard assay conditions at temperature ranged from 25 to 45°C. Other media conditions were remained constant, as stated above. The maximum lipolytic activity was obtained at 3°C.

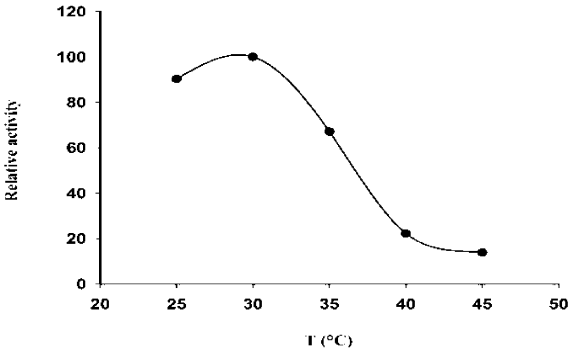

Fig. 5: The influence of temperature on lipase activity

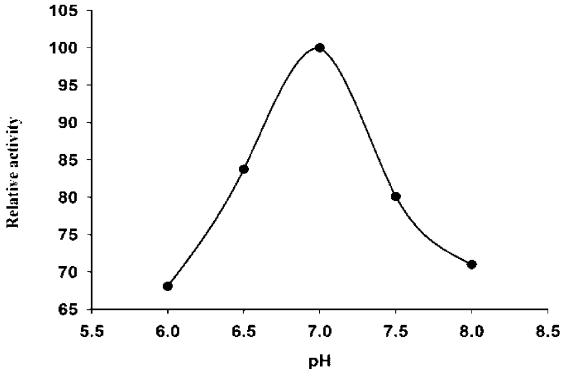

Fig. 6: The influence of pH on lipase activity

The influence of temperature is observed in Fig. 5. The optimal temperature 30°C for lipase activity was reported by Essamri *et al.* [18]. The enzyme was synthesized by *R. oryzae*, which showed maximum activity. Other independent work by Pera *et al.* [19] reported that an optimum temperature for the lipase activity obtained from *A. niger* ATCC MYA-135 at 37°C. The maximum lipase activity for *Candida sp.* was obtained at optimum temperature, 28°X. For any media temperature greater than 33°C, the relative activities of lipase drastically dropped.

Effect of pH on Lipase Activity: Most of enzymes are having great activities at optimum pH near neutral condition. The effect of pH on lipase activity was investigated at various pH ranging from 6 to 8 (other conditions were fixed as stated above). The pH of the reaction mixture was varied by means of buffer phosphate buffer (pH ranged 6 to 8). The enzyme showed that the optimal activity at pH 7.0 and the data demonstrated effect of pH on enzyme activity is shown in Fig. 6. Kamini *et al.* [20] have reported that optimum pH for the lipase from *A. niger,* strain MTCC 2594 was pH 7.0. In addition; Adham and Ahmed [9] obtained optimum

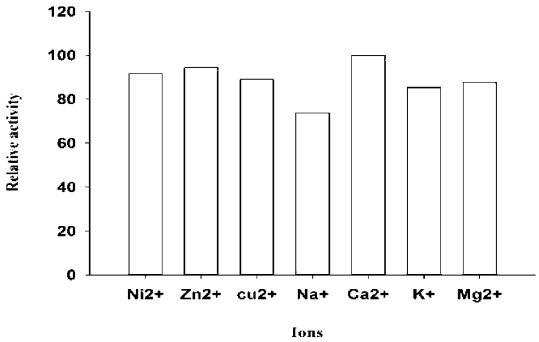

Fig. 7: The influence of trace metal ions on lipase activity

pH for the lipase from *A. niger* NRRL3 at pH near 7.2. Mahadik *et al.* [21] reported that optimum activities of lipase by fungi *A. niger* NCIM 1207 were in the range of between 2.5 and 3.0.

Effects of Trace Metal Ions: The stimulatory role of trace metal ions and chelators in enzyme synthesis and activities were explained in the literature [22]. Several trace metal ions including Ni^{2+}, Zn^{2+}, Na^{+}, Cu^{2+}, K^{+} and Mg^{2+} (chlorides salt) were examined by assaying the enzyme activity after incubation for 1 h at 30°C and pH 7. The concentration of each ion in the medium was very low (7.5 mM). The replicated data were averaged and mean values were reported. It was found that Ca^{2+} was the best ion to stimulate lipase activity (Fig. 7). Also maximum enzyme activities in presence of Ca^{2+} were reported by Adham and Ahmed [9]. Tan *et al.* [16] observed that Mg^{2+} and K^{+} were more beneficial for the biosynthesis of lipase. In addition, Saxena *et al.* [22] have reported that presence of trace metal ion of Mg^{2+} as cofactor was very essential for lipase activity.

CONCLUSION

Lipase was produced by *A. niger* in the submerge fermentation using soya flour as the main carbon source. Other supplementary nutrients such as olive oil and nitrogen sources were required for enzyme production. The best nitrogen sources were combination of peptone and yeast extract for maximum enzyme activities. The maximum enzyme activity and the yield for soya flour as carbon source at optimum condition was 6366 U/l and 76.4 U enzymes per g of soya flour, respectively. The desired pH and suitable temperature were 7 and 30°C, respectively. Among trace metal stimulants, Ca^{2+} was found to be very influential on enzyme activities.

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