International Journal of Engineering, Science and Mathematics
Vol. 7 Special Issue 4(1), April 2018,
ISSN: 2320-0294 Impact Factor: 6.765
Journal Homepage: http://www.ijmra.us, Email: editorijmie@gmail.com
Double-Blind Peer Reviewed Refereed Open Access International Journal - Included in the International Serial Directories Indexed & Listed at: Ulrich's Periodicals Directory ©, U.S.A., Open J-Gage as well as in Cabell's Directories of Publishing Opportunities, U.S.A

Immobilization of Lipase from *Rhizopus arrhizus* by Entrapment in Calcium Alginate Beads

Malabika Chakraborty

Abstract

An extracellular lipase producing mutant stain of *Rhizopus arrhizus* isolated and purified was immobilised in Ca-alginate beads by Gel entrapment process. The alginate beads were prepared as sodium alginate in crude enzyme extract and $CaCl_2$ to increase its reusability and stability. In the presence of Ca^{+2} cations, the enzyme-alginate suspension was crosslinked to form beads at room temperature. The effects of reaction parameters such as Na-alginate and $CaCl_2$ concentrations were optimized and the corresponding bead sizes were measured for the alginate maximum production of lipase by immobilized enzyme. The optimum concentration of Na-alginate and $CaCl_2$ were found to be 6% (w/v) and 0.4M respectively. The maximum production of immobilized lipase was observed with bead size 1.5mm. After immobilization, 60-% lipase activity was retained. The entrapped *lipase* was stable over a wide range of temperature (30^0c – 60^0c) and pH (5-9.5). The Ca-alginate entrapped fungal lipase can be used in the industry.

Keywords:

Immobilization
Lipase
Rhizopus arrhizus
Entrapment
Calcium

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1. Introduction

Lipases (triaclglycerol acyl hydrolases E.C.3.1.1.3) are an important group of enzymes which catalyse the hydrolysis of triglycerides to release long chain free fatty acids and glycerol [1]. Lipases can act at an oil-water interface and also catalyse the interesterification and transesterification reactions [2] .Due to the versatile nature of lipase, it can be used in food industry, flavor making, pharmaceuticals, biodetergents and recently in cosmatics and perfumery [3].

The occurance of lipase is found in many animals and plants as well as in microorganisms. Lipases from vaious microbial sources has been investigated and reported to be a unique catalyst for commercial lipase production including *Rhizopus arrhizus*, *Aspergillus niger*, *Candida rugosa*, *Mucor* and *Penicillium* [4-7].Microbial lipases are of immense important in industries for their bulk production in shorter time, broad selection of sources and stability as well as ease of manipulation. Fungal lipases are mainly obtained by submerged fermentation in which the production of lipase is greatly influenced by temperature and pH [8]. Presently focus has been shifted towards reusability of the enzyme as well as thermal stability, several attempts has been made to immobilize the enzyme [9,10]. The immobilized enzymes are more stable than free enzyme and can act at different pH ranges and shows thermal stability over a wide range [11]. Entrapment is one of the immobilization techniques, in which the enzymes are physically confined in a certain defined region of space with

retention of catalytic properties [12]. Alginates are reported to be the most frequently used polymer due to their nontoxic and mild gelling properties [7,13].The use of alginate is inexpensive and in this method , the size of beads and the amount of entrapped enzyme can be controlled by adjusting the concentration of sodium alginate and calcium chloride [6,14]. The immobilized enzyme can easily be recovered and recycled from the reaction mixture.
The present study exploits the idea of producing immobilized lipase followed by optimization of parameters for higher lipase activity, as well as optimum pH and thermal stability of the immobilized lipase for future use in industry.

2. Method

Microorganism and cultural conditions: A lipase producing strain of *Rhizopus arrhizus* , grown on Potato Dextrose Agar medium was cultivated in a basal fermentation medium consisted of: Glucose -2.0%,Urea -0.7%,K_2HPO_4-0.3%,KCl-0.05%,$MgSO_4.7H_2O$-0.05%,$ZnSO_4.7H_2O$-1µg/ml,$CoCl_2.6H_2O$-3µg/ml,Castor Oil-1%, pH-3.5.
Lipase estimation : The produced lipase was assayed according to the method described by Earle Renshaw and San Clemente [15] based on titration of free fatty acids liberated from castor oil by the catalytic action of lipase.One unit of lipase activity was expressed as µ mole of free fatty acids liberated per ml per minute.
Protein estimation : Protein estimation was done by Lowry method [16].
Immobilization :The enzyme was immobilized in calcium alginate beads by gel entrapment method. The alginate beads were prepared as 3% sodium alginate in enzyme exteact and 0.1M calcium chloride.
Effect of sodium alginate and calcium chloride concentration on the immobilized lipase activity: The cross linking of sodium alginate with calcium ions leads to the formation of immobilized beads depends on the concentrations of both the constituents.Sodium alginate concentration was optimized by preparing beads using 3%-8%(w/v) and calcium chloride concentration using 0.1M-0.5M. The activity of the entrapped lipase were estimated.
Effect of temperature and thermal stability:The effect of temperature has been determined by carrying out enzymic reactions at different temperatures ranging from 20^0-60 ^{0}C. Thermal stability was checked by pre-incubating the immobilized beads at different temperatures from 20^0-60^0Cfor 1 hour and corresponding lipase activities has been estimated.
Effect of pH and pH stability: The effect of pH has been determined by carrying out enzymic reactions at different pH ranging from 5.0-9.5.The pH stability of the immobbilized enzy me was demonstrated by pre-incubating the immobilized beads at different pHbuffers ranging from 5.0-9.5for 1 hour.The corresponding lipase activities were measured.
Storage: The immobilized Ca-alginate beads were stored at 4^0Cfor future use.
All the experiments were carried out in triplicate sets and data obtained were correlated significantly.

3. Results and Analysis

3.1 Immobilization of lipase: It has been observed that the lipase activity was restored after immobilization.
3.2 Effect of sodium alginate and calcium chloride concentrations in Ca-alginate beads:The Ca-alginate beads showed maximum lipase activity at 6% sodium alginate concentration(fig.1) amd 0.4 M calcium chloride (fig.2).
3.3 Effect of temperature and thermal stability: Immobilized Ca-alginate beads showed maximum lipolytic activity at 40^0C (fig.3) and showed thermal stability from 30^0-

60^0C.Compared to higher thermal stability at 40^0C, this immobilized lipase retained 60% of its activity at 50^0C and 40% at 60^0C(fig.4).

3.4 Effect of pH and pH stability: The immobilized enzyme showed its optimum lipolytic activity at pH 8.5(fig.5). The enzyme was found to be fairly stable at pH range 5.0-9.5(fig.6).

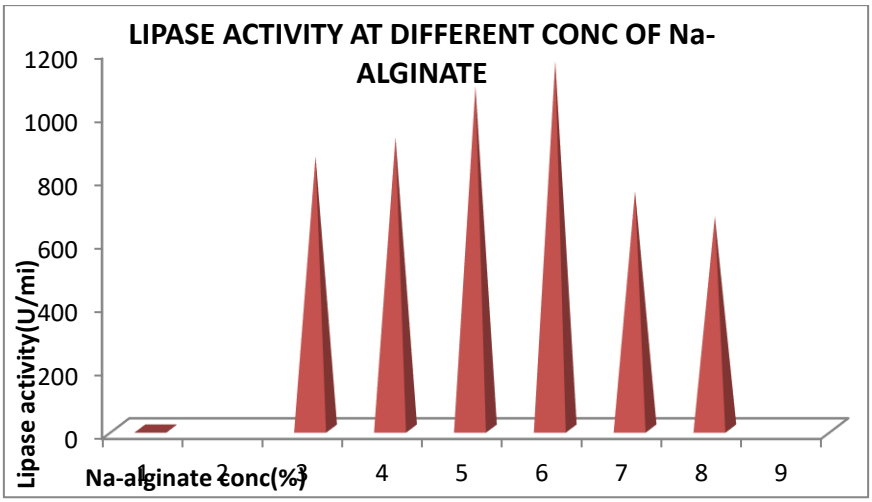

Fig.1

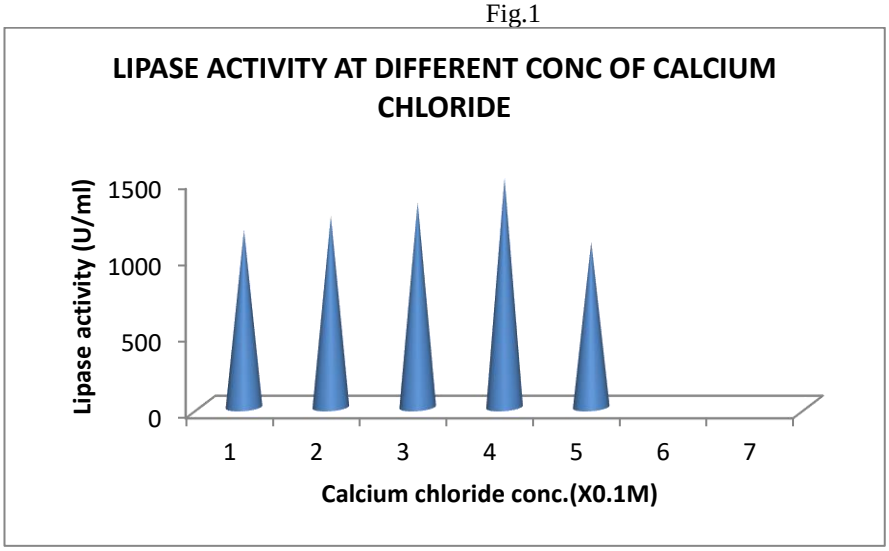

Fig.2

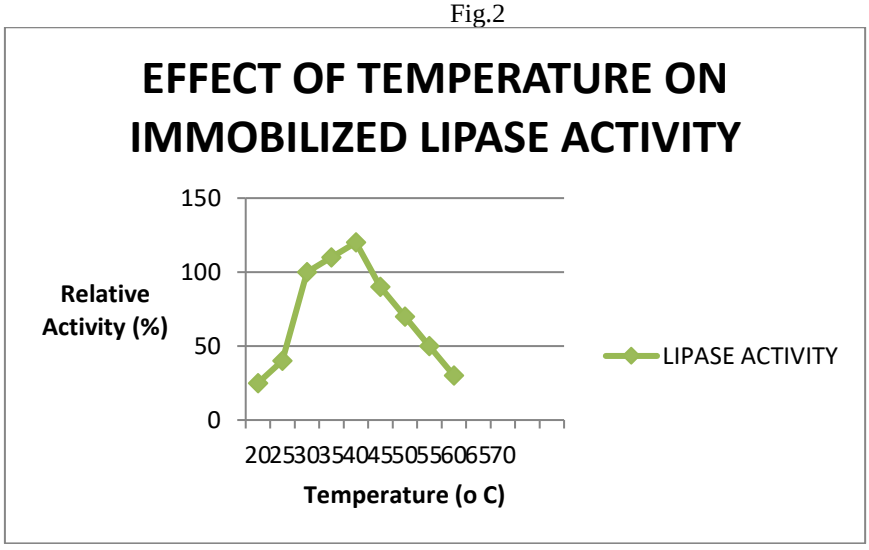

Fig.3

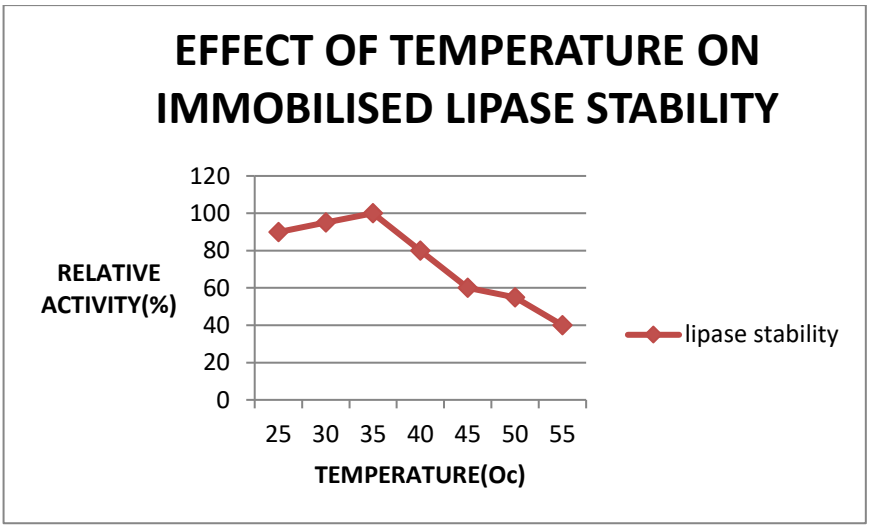

Fig.4

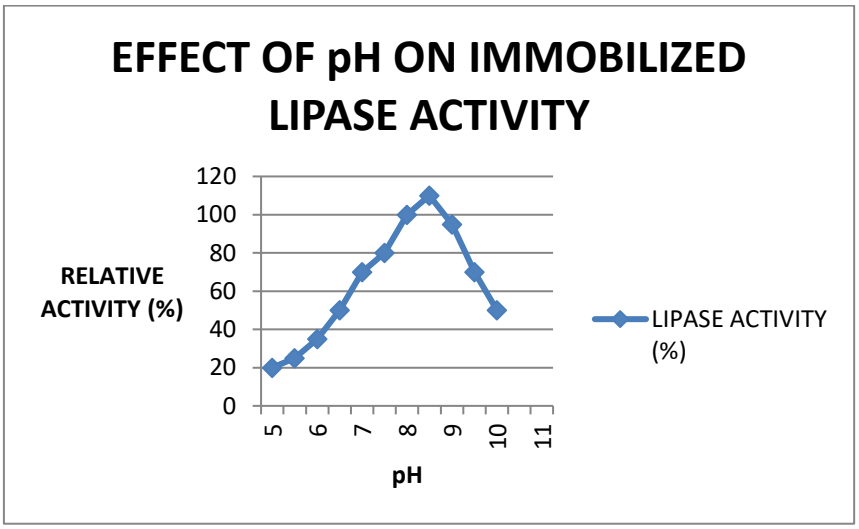

Fig.5

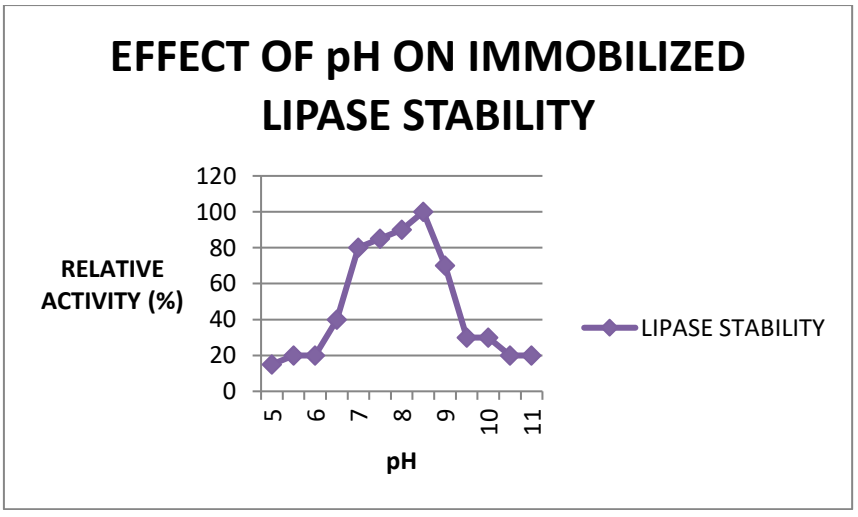

Fig.6

44.Conclusion

Lipase entrapped inside the Ca-alginate beads showed optimum temperature at 40^0C and optimum pH at 8.5 . It could be effectively used in industries as it has several advantages over free enzyme, including a higher thermal stability and wide working range of pH.

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