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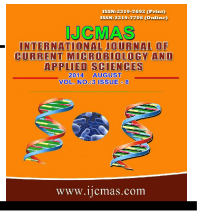

Original Research Article

Production of lipase by Immobilized Cells of *Aspergillus niger*

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Keywords

Lipase, Aspergillus niger, Immobilized cells,

A B S T R A C T

As the requirement of the enzyme lipase is higher, it is widely investigated. In this study, production of lipase was done from waste: wheat bran by using free *Aspergillus niger* and immobilized *Aspergillus niger* cells. The main purpose was to monitor the effect of immobilized cells on lipase activity after several reuses. Lipase activity for free cells was 4.84 mg/100 ml which considerably increased to 5.28 mg/100 ml by the immobilized cells. Cells were washed and reused for four fermentations, and the lipase activity for all was nearly same i.e 5.25mg/100ml.

Introduction

Lipases are well-known as one of the important group of enzymes in international market and have the most influential economy in terms of sales after proteases and amylases [19]. Lipases are also called as triacylglycer-ol acylhydrolases,(EC 3.1.1.3). These are subclasses of the esterases. It catalyzes the hydrolysis reaction of triglycerols to free fatty acids and mono-diglycerols in the oil-fat interface [6, 22].

Lipases have broad variety of industrial applications such as, food industry (improvement of flavor), detergent (hydrolysis of oil and fats), pharmaceutical (synthesis of chiral drugs), paper (control of pitch), medicine (triglyceride measurement), cosmetics (exclusion of lipids), wastewater (decomposition and removal oil), leather (elimination of fat from animal skin) [1,2,8,20].

Lipases occur widely in bacteria, yeasts and fungi [10,11,15]. Fungi are considered as one of the best lipase sources and are widely used in the food industry. *Aspergillus niger* is among the most well known lipase producers. Some of the lipase producing micro-organisms are as follows: *Bacillus subtilis* [10], *Staphylococcus aureus* [11], *Aspergillus oryzae* [15], *Pseudomonas aeruginosa* [9], *Lactobacillus species* [14], *Penicillium species* [16].

Immobilization of cells is the attachment of cells in distinct solid phase. Immobilization is commonly accomplished using a high molecular hydrophilic polymeric gel such as alginate, carrageenan, agarose, etc. In these cases, the cells are immobilized by entrapment in the gel by a drop-forming procedure.

Immobilization of microbial cells in biological processes can occur either as a natural phenomenon or through artificial process [17].

After the first report on application of Immobilized cell in industrial productions, several researchers have attempted whole cell immobilization and its use as an alternative to conventional microbial fermentation. Immobilized cells in production of ethanol, organic acids, amino acids, enzymes, antibiotics, steroids etc. have been extremely studied both in laboratory and pilot scale [14].

As the requirement of lipase is high, production by free *Aspergillus* cells and immobilized cells was carried out by the reuse of beads again and again. The purpose was to study whether the reuse of immobilized cells could affect the lipase activity.

Materials and Methods

Maintenance of culture

Culture of *Aspergillus niger* was maintained on Potato dextrose agar and stored at 4^0C.

Fermentation by *Aspergillus niger*

5 gms of wheat bran was taken as a substrate in 250ml conical flask and moistened with 5 ml of sterilized minimal salt solution (121^0C for 30 min). After cooling, flask was inoculated with the spore suspension of *A.niger* and incubated at 30^0C for 7 days.

After one week, 100ml of D.W was added to the flask and the mixture was shaken for 30 min at room temperature to facilitate the extraction of enzyme from fermented wheat bran. At the end of the extraction, the suspension was squeezed through a double layered muslin cloth and it was centrifuged at 12,000 RPM for 5 min. The clear supernatant obtained was used as extracellular enzyme.

Immobilization of *Aspergillus niger*

For immobilization, 1.5% sterile sodium alginate solution, 1% sterile calcium chloride solution, Spore suspension of *Aspergillus niger* was prepared. Sodium alginate and broth culture with spores were mixed thoroughly. This mixture was extruded through a syringe in a beaker containing 1% calcium chloride solution. Beads were formed as Shown in Fig.I and were allowed to harden for 15-20 minutes then were kept for curing at 4^oC for 24 hours. Curing makes the beads firm so that they do not break the beads thoroughly with physiological saline. Next day, the beads were washed thoroughly with physiological saline and were used for fermentation.

Fermentation by the immobilized cells of *Aspergillus niger*

5 gms of wheat bran was taken as a substrate in 250ml conical flask and moistened with 5 ml of sterilized minimal salt solution (121^0C for 30 min). After cooling, flask was inoculated with the immobilized cells (beads) and was incubated at 30^0C for 7 days.

After one week, enzyme was extracted by centrifugation. The beads were removed by filtration. To check the ability of immobilized cells to produce lipase, these beads were reused four times for fermentation.

For that, after 7 days of incubation, beads were recovered from media, washed in saline and re-inoculated in fermentation media. Lipase activity after fermentation was measured.

Assay of lipase

For the determination of lipase activity, titration method was performed. As lipase is lipolytic enzyme, it hydrolyses fats such as triacylglycerol into free fatty acids and glycerol. This free fatty acid is measured by titration method [18].

One unit of lipase activity was defined as the amount of enzyme which produces 1 µmole fatty acids per minute under the assay condition [5].

Results and Discussion

Lipase was produced by Solid state fermentation by *Aspergillus niger* using wheat bran as a substrate. Immobilization was accomplished using a high molecular hydrophilic polymeric gel such as alginate. The cells were immobilized by entrapment in the gel by a drop-forming procedure. These immobilized cells were used for fermentation process. For determination of lipase activity, the titration method was performed. Amount of free fatty acids was calculated by using following formula:

$$\text{Acid produced (gm/litre)} = \frac{\text{B-A} \times \text{Normality of NaOH} \times \text{Equivalent Weight of butyric acid}}{1000}$$

From the value of acid produced, enzyme activity was calculated.

For Fermentation by *Aspergillus niger* cells, Lipase activity was found to be 4.84 mg/100ml.

When immobilized cells were used lipase activity was slightly increased to 5.28 mg/100ml. Immobilization of cells was found to be effective for lipase production.

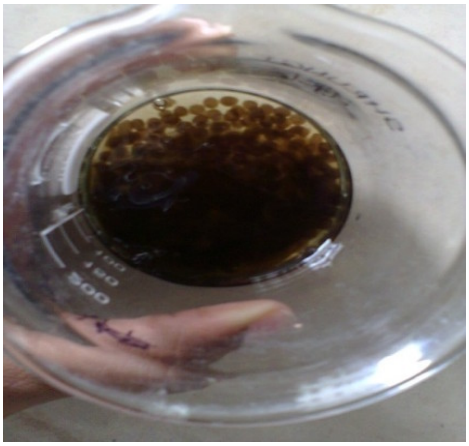
To check the ability of immobilized cells, these cells were washed and used 4 times in fermentation. Enzyme activity for each reuse was calculated and it was noted that the lipase activity for all was found to be nearly same i.e 5.25mg/100ml.

Micro-organisms are the best sources for the production of useful enzymes. Cell immobilization technology is aptly suited to produce extracellular enzymes. There is growing interest in applying cell immobilization techniques for the continuous production of enzymes. Entrapment of microbial cells within the polymeric matrices i.e mostly in alginate gel is preferred by many researchers. [12,13,21].

P. Ellaiah et al[3] used different materials of gel, for immobilization of whole cells of *Aspergillus niger* (ANT 90). Maximum production was observed with 3% alginate.

Several researches have attempted the production of a-amylase by immobilized cells; 24-fold increase in the productivity compared to batch fermentation with free-cells was attained [7].

Fig.I Immobilized cells of *Aspergillus niger*



In the current study, results showed that the productivity obtained by the immobilized cells was considerably higher than the free cells. Also, when these immobilized cells were used 4 times for fermentation, lipase activity was found to be nearly same, thereby showing the stability of immobilized cells even after several reuses.

One can conclude that the use of immobilized whole microbial cells could eliminate the often tedious, time consuming, and expensive steps involved in isolation and purification of intracellular enzymes. It also tends to enhance the stability of the enzyme by retaining its natural catalytic surroundings during immobilization and subsequent continuous operation.

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