© 2015 IJSRSET | Volume 1 | Issue 4 | Print ISSN : 2395-1990 | Online ISSN : 2394-4099
Themed Section: Science and Technology

# Studies on Immobilized Spores of *Aspergillus Fumigatus*

**Ratnasri P.V, Hemalatha K. P. J**
Department of Microbiology, Andhra University, Visakhapatnam-530 003, Andhra Pradesh, India

## ABSTRACT

In this present study, the effect of immobilized spores of Aspergillus fumigatus (MTCC NO. 1399) on amylase production was investigated on four different matrices like agar agar, agarose, sodium alginate and k-Carrageenan. Amylase activity was found to be increased by 11.4% with sodium alginate at 72hrs of incubation when compared with the free spores. The 3rd repeated cycle of immobilized spores of A.fumigatus showed (283±34U/ml) amylase activity and increase percent of amylase activity was 45% when compared to initial amylase activity. The amylase production was decreased for further usage. .

**Keywords:** Aspergillus Fumigatus, Immobilization of Spores, Sodium Alginate, Repeated Cycles, Amylase Activity.

## I. INTRODUCTION

Immobilization can be defined as the fixation of the biocatalysts (microorganisms, enzymes and organelles) to insoluble solid supports. The cell immobilization offers enhanced fermentation productivity, feasibility of continuous processing, cell stability and lower costs of recovery recycling and downstream processing [1,2]. Cell immobilization protects cells against shear force. Immobilization of microbial cells has received increasing attention in the past few years, and immobilized cells have been used for the production of organic acids, amino acids, antibiotics, enzymes, alcohol, and other compounds [3,4,5]. Compared to many unicellular microbes, filamentous cultures present special challenges in the optimization and scale-up because of the varying morphological forms [6].

The use of fungus cells in a immobilized form looks very attractive since it allows to shorten the period of accumulation of cell biomass with high metabolic activity and to prolong the period of cell application in the biotechnological processes [7,8,9,10]. Immobilized cell cultures may be efficient biocatalysts than free cells. The most obvious benefit of the immobilization technique is the capability of continuous cycling which provides a mean for using them in continuous cultures maintaining high cell population to achieve fast reaction rates [11].

The enzyme production by fungi is commonly performed by solid-state fermentation, submerged fermentation or surface-adhesion fermentation [12,13,14,15]. However, biotechnological processes with immobilized growing fungi cells, including those for extracellular enzyme production, seem to be more favorable than traditional fermentation methods since immobilization enables repetitive and continuous use of the microbial cells [14].

## II. METHODS AND MATERIAL

### 1.1 Microorganism

Amylase producing fungal strain of *Aspergillus fumigatus* (MTCC NO. 1399) was isolated [16]. It was maintained on potato dextrose agar plates at 4°C and was sub cultured for every 2 weeks.

### 1.2 Estimation of amylase activity

One milliliter of culture extract (enzyme) was pipetted into test tube, and 1.0 ml of 1% soluble starch in citrate phosphate buffer pH 6.5 was added and incubated in water bath at $40^0$C for 30mins. Then treated with DNS (Dinitro salicylic acid) and the reaction was stopped by boiling for 5mins, and cooled to room temperature and

20ml of distilled water was added and color intensity was measured at 540nm. One unit of amylase activity was defined as the amount of enzyme that releases 1µmol of maltose per minute under the assay conditions. Activity of the enzyme is expressed in units per mg protein.

### 1.3 Immobilization of fungal spores of *Aspergillus fumigatus*

Immobilization increases the resistance of the microorganism against destroying factors, stability and catalytic activity of enzymes [17]. The materials used in immobilization of fungal spores were agar-agar, agarose, sodium alginate, K- carrageenan and algal extract (Caulerpa).

The immobilized fungal spores of *Aspergillus fumigatus* were incubated under optimized conditions to increase the amylase production with basal media containing 2% finger millet, 1% $NH_4NO_3$, 0.14% $KH_2PO_4$, 0.5% KCl, 0.01% $MgSO_4.7H_2O$, 0.001% $FeSO_4.7H_2O$, at 72h and at $35^0$C, with 200rpm in orbital shaking incubator.

### 1.4 Agar- Agar

Two grams of Agar – Agar was dissolved in 100ml distilled water and was sterilized in autoclave for 15min at 121 lb. The media was cooled to $45^0$C and 1ml of (2%) of *Aspergillus fumigatus* spores were added to the 10ml of medium separately and mixed thoroughly for homogenous distribution of cells. Then they were poured in sterile petridish and allowed to solidify. After solidification the agar - agar was cut with sterile knife to get 1cm width and length blocks.

Ten grams of immobilized spores of *Aspergillus fumigatus* were inoculated with 100ml of sterile optimized production media and incubate the conical flasks under optimized conditions. After completion of incubation period the supernatant was collected by filtering through Whatman filter paper under sterile conditions. The supernatant was used for the assay of amylase by DNS method.

Increased (%) of amylase activity of immobilized spores was calculated as follows

$$\textbf{Increased } (\%) \textbf{ of enzyme activity} = \frac{(C_I - C_0)}{C_0} \times 100$$

Whereas $C_I$ is the enzyme activity of immobilized spores, $C_0$ is the enzyme activity of free spores.

### 1.5 Agarose

The fungal spores were immobilized on agarose following the Nilsson method [18] method. Two grams of Agarose was dissolved in 100ml saline water and heated to $55^0$C for 2-3mins. It was cooled to $45^0$C and 1ml of spores ($1\times10^7$) *Aspergillus fumigatus* were added to the 10ml of media separately and mix thoroughly for homogenous distribution of cells. Then they were poured in sterile petridish and allowed to solidify. After solidification the agarose was cut with sterile knife to get 1cm width and length blocks, store at $4^0$C and wash thoroughly with distilled water.

### 1.6 Sodium alginate

Immobilization of fungal spores in Ca-alginate was done by the method of Vassileva [19]. Three grams of sodium alginate solution was prepared in 100ml distilled water and autoclaved for 15mins at 121 lb. Then it was cooled to $40^0$C and 1ml of ($1\times10^7$) of *Aspergillus fumigatus* spores were added to the 10ml of media separately, this mixture extruded drop wise through a sterile syringe (syringe size 5ml and 0.8 mm diameter) into a gently stirred 0.5M calcium chloride solution. Beads of 2- 3 mm diameter were allowed to harden for 20 min at $4^0$C. Later the beads were washed thoroughly with distilled water and stored for further experiments.

### 1.7 K-carrageenan

Immobilization of fungal spores on K-carrageenan was performed by following the method of Wada [20]. Three grams of K-carrageenan was dissolved in 1% NaCl solution and was heated to $60^0$C. Then it was cooled to $40^0$C and 1ml of ($1\times10^7$) of *Aspergillus fumigatus* spores were added to the 10ml of media separately. This mixture was extruded drop wise into a freshly prepared 2% KCl solution and placed it in refrigerator for curing. Later these beads were thoroughly washed with sterile distilled water and preserved in freshly prepared 2% KCl solution for further usage.

### 1.8 Repeated Use of Immobilized spores

In order to test the reuse of immobilized spores for production efficiency, the beads were assayed several times for the hydrolysis of soluble starch. After each production cycle, enzyme assay was performed. The

beads were washed thoroughly with distilled water and stored at $4^0$C.

## III. RESULTS AND DISCUSSION

### 2.1 Amylase activity in immobilized spores of *Aspergillus fumigatus*

Fungal spores of *A.fumigatus* immobilized on 4 different immobilization media (agar-agar, agarose, sodium alginate and K- carrageenan) at different incubation periods from 18 to 90hrs. Immobilized spores of *A.fumigatus* showed the maximum amylase activity (264.93±35U/ml) with sodium alginate, followed by (226.34±54U/ml) with agar agar, (194.35±21U/ml) with K-carrageenan and (174.53±24U/ml) with agarose at 72hrs of incubation period. The minimum amylase activity (103.75±27U/ml) of *A.fumigatus* immobilized spores was observed with K-carrageenan followed by (106.84±14U/ml) with agarose, (116.84±0.9U/ml) with sodium alginate and (118.34±14U/ml) with agar agar at 18hrs of incubation period under optimized conditions. The results were depicted in Figures 1 (a) to 1 (d). The Table 1.1 showed the increased (%) of amylase activity by the immobilized spores of *A.fumigatus*.

The morphological development of *Aspergillus niger* cells on immobilization with the calcium alginate and K-carrageenan [21]. The effect of alginate concentration on enzyme production by *A. niger* and reported maximum production by 3% alginate beads though 4% alginate beads gave more stable beads [9]. By the use of immobilized microbial cells we can overcome some of the problems associated with survival, stability, efficacy, storage, transportation and ease to application [22]. However, more field studies are needed to optimize the effect of the introduced microbial inoculants and their interaction with indigenous microbiota in long term perspective Different materials of gel, for immobilization of whole cells of *Aspergillus niger* (ANT 90), maximum production was observed with 3% alginate [8].

### 2.2 Amylase activity on repeated cycles of immobilized spores

The repeated cycles of immobilized spores of *A.fumigatus*, on sodium alginate showed the maximum (283 ±34U/ml) enzyme activity at 3rd cycle. The results were depicted in Figure 1(e). After completion of 3rd cycle onwards the enzyme activity was decreased and the beads were disintegrated. Percentage of increase in immobilized spore efficiency in repeated cycles on sodium alginate for *A.fumigatus* was 45%. The production of citric acid by immobilization of *Aspergillus niger* cells on Agarose by using the soy as media under repeated batch fermentations. Maximum citric acid (27g/L) was obtained on 10th day of repeated batch fermentation [23].

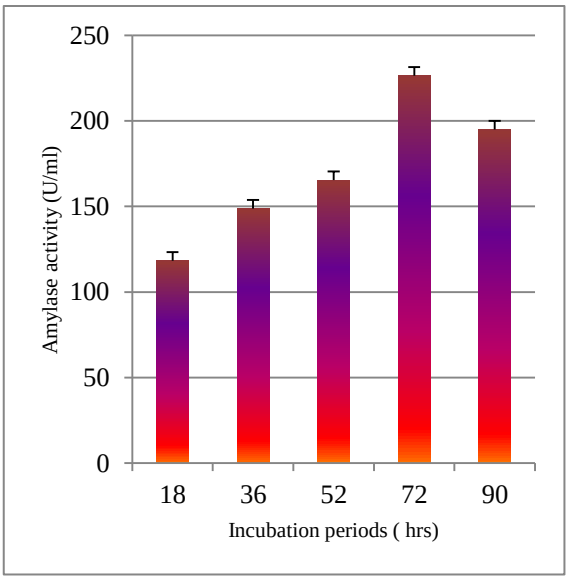


**FIGURE 1(a)**

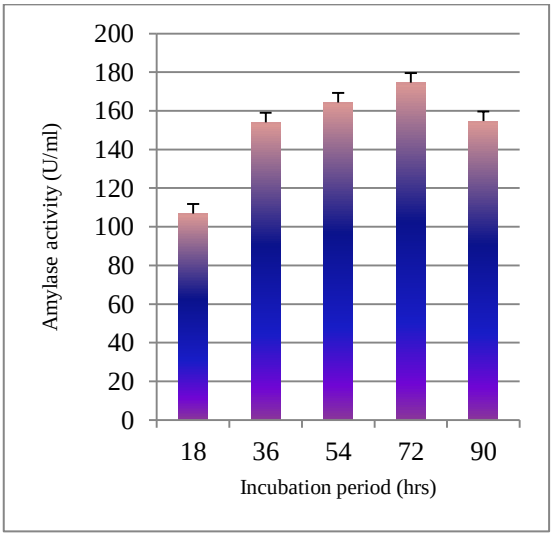


**FIGURE 1(b)**

Figure 1(a) Amylase activity in immobilized spores of *A.fumigatus* on agar-agar and 1(b) on agarose

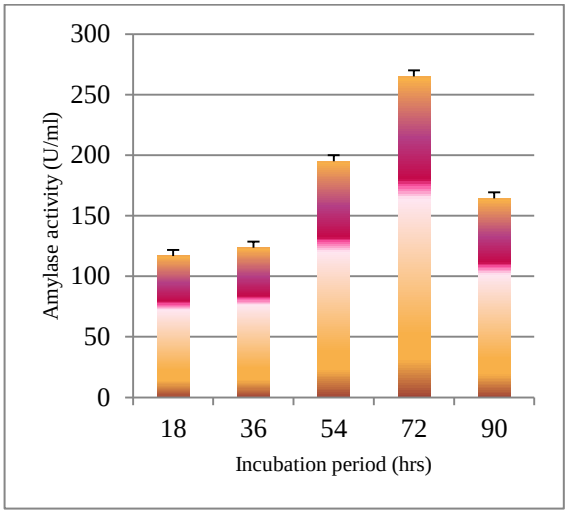


**FIGURE 1(c)**

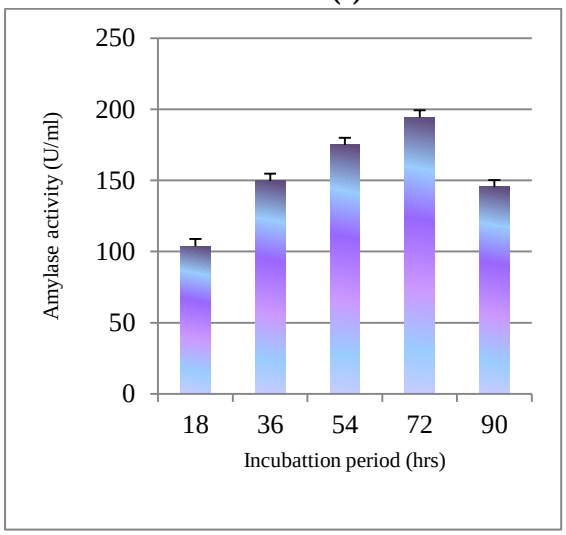


**FIGURE 1(d)**

Figure 1(c) Amylase activity in immobilized spores of *A.fumigatus* on sodium alginate and Figure 1(d) on K-carrageenan

Each value is an average of three parallel replicates. Y error bars indicate the standard error from mean value. The values vary significantly at $p \leq 0.05$.

**Table 1.1 Amylase activity in immobilized spores of *Aspergillus fumigatus* at optimal incubation period**

| **Immobilization media** | Incubation period (hrs) | Amylase activity of normal spores (U/ml) | Amylase Activity of Immobilized spores (U/ml) | Increased (%) amylase activity |
|---|---|---|---|---|
| **Agar-agar** | 72 | 124.23±47 | 226.34±54 | 81.5 |
| **Agarose** | 72 | 106.15±52 | 174.53±24 | 63.3 |
| **Sodium alginate** | 72 | 123.05±31 | 264.93±35 | 114.4 |
| **K-carrageenan** | 72 | 103.34±64 | 194.35±21 | 11 |

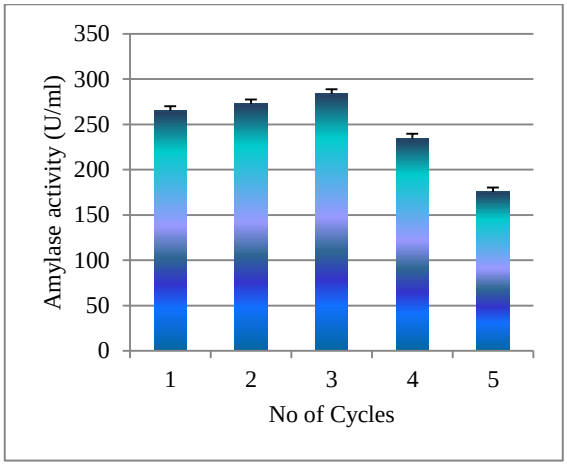


**FIGURE 1(e)**

Figure 1(e) Amylase activity on repeated cycles of immobilized spores of *A.fumigatus* on sodium alginate

## IV. CONCLUSION

The results indicated that the sodium alginate was the best source for immobilization of *Aspergillus fumigatus* spores when compared with other matrices. Immobilization of spores of *Aspergillus fumigsatus* showed the maximum amylase activity (264±35U/ml) with sodium alginate. The repeated cycles of immobilized spores of *A.fumigatus* on sodium alginate showed the maximum enzyme activity (283±34U/ml) at 3rd cycle. Percentage of increase in immobilization of spore efficiency in repeated cycles on sodium alginate for *A.fumigatus* was found to be 45%. The major applications of using this immobilization technique was reusability, large scale production in the cost effective manner.

## V. REFERENCES


**[1]** Margaritis A and Merchant FJA (1984). Advances in ethanol production using immobilized cell systems. Crit. Rev. Biotechnol., 2: 339–393.

**[2]** Stewart GG and Russel I (1986). One hundred years of yeast research and development in the brewing industry. J. Inst Brew., 92: 537–558.

**[3]** Shah ND, Chattoo BB, Kothari BRM and Hegde PVM (1993). Starch hydrolysis 45: 104-109.

**[4]** Hemachander C, Bose N, Puvanakrishnan R (2001) Whole cell immobilization of Ralstonia pickettii for lipase production. Process Biochem., 36: 629–633.

**[5]** Sun Y and Furusaki S (1990). Continuous production of acetic acid from Acetobacter aceti in a fluidized bed bioreactor. J. Ferment Bioeng., 69: 102-110.

**[6]** Wang Bao-e and HU Yong-you (2007). Comparison of four supports for adsorption of reactive dyes by immobilized Aspergillus fumigatus beads. Journal of Environmental Sciences. 19: 451–457.

**[7]** Angelova MB (2000). Comparison of antioxidant enzyme biosynthesis by free and immobilized Aspergillus niger cells. Enz. Microb. Technol., 26, 544-549.

**[8]** Ellaiah P (2004). Production of lipase by immobilized cells of Aspergillus niger. Process Biochem., 39,525-528.

**[9]** Nighojkar S (2006). Production of polygalacturonase by immobilized cells of Aspergillus niger using orange peel as inducer. Process Biochem .,.41,1136-1140.

**[10]** Efremenko EN (2006). L(+)-lactic acid production using PVA-cryogel entrapped Rhizopus oryzae fungus cells. J. C hem. Technol. Biotech., 81, 519-522.

**[11]** Koshcheyenko KA, Turkina MV and Skryabin GK (1983). Immobilization of living microbial cells and their application for steroid transformation. Enzyme and Microbial Technology. 5, 14-21.

**[12]** MacCabe AP, Orejas M, Tamayo EN, Villanueva A and Ramón D (2002) Improving extracellular production of food-use enzymes from Aspergillus nidulans: Review. J. Biotechnol., 96, 43-54.

**[13]** Elisashvili V, Penninckx M, Kachlishvili E, Tsiklauri N, Metreveli (2008). Lentinus edodes and Pleurotus species ligno cellulolytic enzymes activity in submerged and solid state fermentation of lignocellulosic wastes of different composition. J. Biores. Technol., 99, 457-462.

**[14]** Correa MG, Ludeña Y, Ramage G and Villena GK (2012). Recent advances on filamentous fungal biofilms for industrial uses. Appl. Biochem. Biotechnol., 167, 1235 - 1253.

**[15]** Selwal MK and Selwal KK (2012). High-level tannase production by Penicillium atramentosum KM using agro residues under submerged fermentation. Ann. Microbiol., 62, 139-148.

**[16]** Ratnasri PV, Lakshmi BKM, Ambica devi K and Hemalatha KPJ (2014a). Isolation,characterization of Aspergillus fumigatus and optimization of cultural conditions for amylase production. International journal of research in engineering and technology. 3(2): 457-463.

**[17]** Francis Borgio (2011). Immobilization of microbial (wild strains) amylase on Alginate matrix and Coconut fiber for enhanced activity. American Journal of Biochemistry and molecular biology. 1(3): 255-264.

**[18]** Nilson K, Birnbaum S and Flygare S (1983). A general method for the immobilization of cells with preserved viability. Eur J Appl Microbiol Biotechnol., 17: 319-326.

**[19]** Vassileva M, Azcon R, Barea J and Vassilev N (1998). Application of an encapsulated filamentous fungus in solubilization of inorganic phosphate. J. Biotechnol., 63, 67–72.

**[20]** Wada M, Kato J and Chibata I (1979). Immobilization of cells. Eur. J Appl Microbiol Biotechnol., 8: 241-252

**[21]** Eikmeirer H, Westmeiier and Rehm HJ (1984). Morphological development of Aspergillus niger immobilized in Ca- alginate and K-carrageenan. Appl. Microbiol. Biotechnol., 19: 53-57

**[22]** Van Elsas JD and Heijnen CE (1990). Methods for the introduction of bacteria into soil: a review. J. Biol Fertil Soils. 10: 127–133.

**[23]** Khare SK, Jha K and Gandhi AP (2004). Use of agarose- entrapped Aspergillus niger cells for production of citric acid from soy whey. Appl. Microbiol. Biotechnol., 41: 571-573.