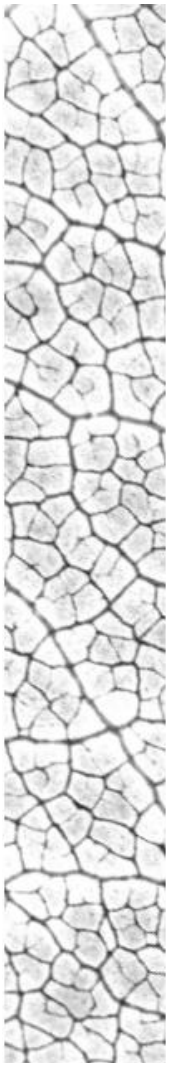

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Cell immobilization for efficient enzymes production

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Abstract
Cells catalysis is efficient methodology that has been extensively applied in various biological processes. However, industrial strains are vulnerable to environmental change, leading to poor stability and productivity. In this regards, large potentialities are embedded in immobilized cells. In particular, the immobilization techniques are of great significance in improving the catalytic performance of natural biocatalysts. Effective method of enzyme production by immobilization of microbial cells on solid career in submerged conditions has been developed. It was determined that design of proposed equipment gives the opportunity to increase enzymatic activity of immobilized cells compared to free cells by several times. A cultivation of *Aspergillus oryzae M* has been carried out for 49 days by immobilization of fungal cells in submerged conditions of growth. Enzymatic activity was enhanced significantly after 6 days of cultivation of immobilized cells and keeps the same value for 49 days of fungal cultivation. The alpha-amylase activity has been increased to 696 U/ml.

Keywords: immobilization, alpha-amylase, cultivation, productivity, *Aspergillus oryzae*

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INTRODUCTION

Much research activity over the past decades has focused on the enzymes structure, diversity of enzymes producing microorganisms, and mechanisms of substrates hydrolysis; biochemical characteristics related to enzyme activity and stability, development of novel recombinant and mutant microbial strains along with different optimization strategies leading to expansive production and yield of enzymes, etc. (Abdal et al. 2020, Hafsan et al. 2020, Zhang et al. 2019). However, there have been relatively few studies have used living cells in immobilized systems in spite of the fact that the major advantage of immobilized cells in contrast to immobilized enzymes or free cells is the reduction of bioprocessing cost. This comes from the repeated and continuous use of biocatalyst, the maintenance of a high cell density, higher metabolic activity, better productivity, absence of foreign substances, and controlled process of enzyme-genesisability of various enzymes simultaneous production, etc (Garde et al. 1981, Yu et al. 2019, Oliveira et al. 2019, Morikawa, 2006, Bayat et al. 2015)

Despite the fact that among microorganisms that produce amylases there are bacteria, fungi, yeast and actinomycetes, in the recent period micromycets got wide application. However, great drawback of industrial strains is their low activity, though the chief requirement to enzymes is their high catalyst activity which is directly connected with the activity of the microorganism that produces this enzyme (Kun et al. 2019, Zhang et al. 2019). The ability to control the formation of enzymes through the regulation of their biosynthesis and the selection of appropriate nutrient media and cultivation conditions allows not only to increase the yield of enzymes, but also to obtain enzymes with certain properties. In this regards, large potentialities are embedded in immobilized cells (Żur et al. 2020, Dobreva et al. 1998, Chen et al. 2014; Villegas, 2016). The proposed in this study enzyme production technology based on immobilization of microbial cells on a carrier will allow obtaining active isolate with increased enzymatic activity. Cultivation of microbial cells in the immobilized state was accomplished according to proposed method in submerged growth conditions (Blieva R. 2016). A novel immobilization technique is developed by using the cheapest and most easily available material with high adsorption surface as an

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immobilizing matrix for absorption of stores of immobilized cells, which has been for the first time used for immobilization of fungal cells. Design of proposed equipment gives the opportunity to increase the activity of immobilized cells culture filtrate comparing to free cells (Blieva et al. 2019). Amylases are among the most important industrial enzymes (Sales et al. 2020, Rani et al. 2013, Sindhu et al. 2017). In the present work alpha-amylase production using immobilized *Aspergillus oryzae M* was studied.

MATERIALS AND METHODS

Microorganism

Aspergillus oryzae M fungal strain (own collection) was used in this study. *A. oryzae M* was maintained on Potato Dextrose Agar medium (PDA). For inoculum preparation, 25 ml of sterile distilled water was added to the 5-day-old culture grown on PDA plates and scraped aseptically with inoculating loop. This suspension, having spore concentration of $1.3x10^7$ cells/ml, was used as inoculums for the fungal cultivation.

Alpha-amylase Enzyme Assay

The alpha-amylase activity was assayed by spectrohotometric measurement of a starch-iodine complex. The reaction mixture (15 mL) consisted of 10 mL of 1% (w/v) soluble starch and 0,5 mL appropriately diluted enzyme source in 25 mL of distilled water. After incubation at 30^0C temperature for 10 min the reaction was stopped by addition of iodine solution with 0.2mol/dm^3 HCl. Then the enzymatic hydrolysis of starch was determined on spectrophotometer at 670nm. One unit of the α-amylase activity was defined as the amount of enzyme that hydrolyses 1 g of starch per minute in 30^0C, pH 4.

Enzyme Production

For inoculums preparation, 25 ml of sterile distilled water was added to the 5-day-old culture grown on PDA plate and scraped aseptically with inoculating loop. This suspension with spore concentration of $1.3 \cdot 10^7$ cells/ml, was used as inoculums for the fungal cultivation. Submerged fermentation was carried out in 750 ml Erlenmeyer flasks with round - shaped carrier for fungal immobilization by taking 100mL of mineral salt medium (%): NH_4NO_3 – 0,5; KH_2PO_4 – 0,1; $MgSO_4$ – 0,05; KCL – 0.05; $FeSO_4$ – 0.001; maltose – 1,0; starch – 1,0. They were incubated at 30°C on a rotary shaker (180 rpm) for 49 days. The immobilization

Medium was exchanged at 3-day intervals. The experiments were carried out in triplicates. The results were expressed as mean ± standard deviation using Excel 2010.

RESULTS AND DISCUSSION

Cultivation of *A. oryzae M* was carried out for 10 days to do comparative analysis of alpha-amylase production

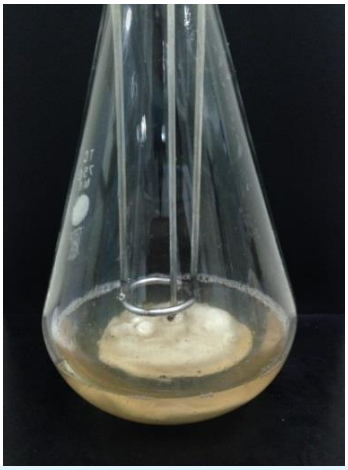
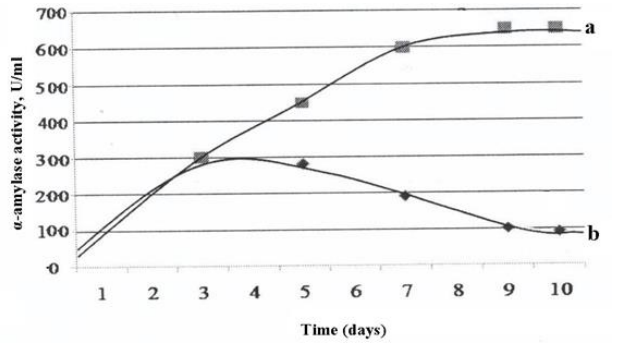


Fig. 1. Immobilization of *A. oryzae M*



a - free cells; b – immobilized cells

Fig. 2. Dynamics of α-amylase production in *A. oryzae M*

in free cells (FC) and immobilized cells (IC). Morphology of immobilized *A. oryzae M* on carrier presented on **Fig. 1**. *A. oryzae M* was immobilized perfectly twisting around carrier.

A comparative analysis of α-amylase production in free cells (FC) and immobilized cells (IC) showed that immobilization procedure has significant effect both on growth and enzyme biosynthesis. Stationary phase of enzymes biosynthesis has been increased from 2.5 to 3 times. At the same time, during cultivation of FC cells autolysis is observed, which leads to a gradual fall of the enzymatic activity of the culture filtrate **(Fig. 2).**

Moreover, it was determined that design of proposed equipment gives the opportunity to increase enzymatic activity of immobilized cells compared to free cells. At the second stage of experiments cultivation of *A. awamori M* in immobilization state was carried out for 42 days (**Fig. 3**). Every 3^d day alpha-amylase activity was assayed.

As shown on **Fig. 3** enzymatic activity was enhanced significantly after 6 days of cultivation of immobilized cells and keeps the same value for 49 days of fungal cultivation. The immobilized system showed a significant stability of the enzyme biosynthesis. The maximum of enzyme synthesis was in 9, 18, 27, 36 and 45 days of fungal cultivation. Enzyme activity ranged from 312 to

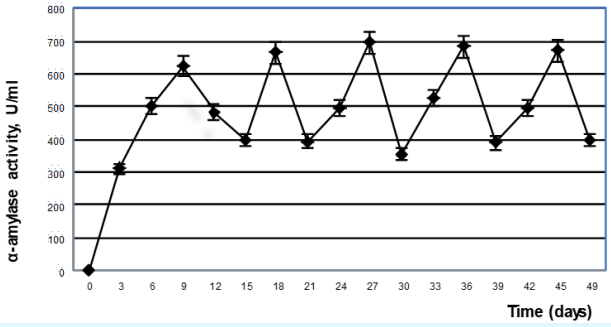


Fig. 3. α-amylase production by immobilized *A. oryzae M*

696 U/ml. It was found that presented method of fungal cultivation in the immobilized state allows continuous enzyme production up to 49 days and creates the opportunity to obtain enzymes repeatedly in every 2-3 days. In addition, activation and stabilization of enzyme-producing ability of the immobilized fungal cells was observed, which is maintained throughout the whole period of fungal cultivation.

CONCLUSION

It is obvious that proposed method of fungal immobilization is simple and accessible. Such equipment construction also allows giving a raise in culture productivity; the repeated and continuous use of biocatalyst. Also, new devices and equipment give the way to improve quality of filtrates (to make them more clear) and exclude time-consuming processes of recharging fermentative vials, that require manual removing of microbial cells. Enzymatic activity was enhanced significantly after 6 days of cultivation of immobilized cells and keeps the same value for 49 days of fungal cultivation. Such method prolongs producers' cultivation period to 49 days and more and create the opportunity to obtain enzyme repeatedly in every 2-3 days of cultivation. Enzymatic activity was enhanced significantly after 6 days of cultivation of immobilized cells and keeps the same value for 49 days of fungal cultivation. The alpha-amylase activity increased to 696 U/ml.

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