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# Regulation of the synthesis of cellulase and macerase enzymes by the immobilized association of *Aspergillus awamori 1-8* and *Aspergillus niger 355*

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**ABSTRACT**
The regulation of the synthesis of cellulase and macerase enzymes by nutrient medium composition and cultivation conditions of the association of micromycetes Aspergillus awamori 1-8 and Aspergillus niger 355 was studied. Optimal carbon and nitrogen sources, as well as the absence of catabolite repression in the enzymes production have been established. The level of biosynthesis of enzymes in Aspergillus awamori 1-8 and Aspergillus niger 355 depends not only on the nutrient medium composition, but also on the method of their cultivation. Immobilization of enzymes producers on a carrier and their cultivation in a filamentous-spongy structure allows to obtain the target product for long period of time.
**Keywords:** Aspergillus fungi, selection, immobilized cells, continuous cultivation

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## I. INTRODUCTION

The study of regulation of the synthesis of extracellular microbial enzymes is of great interest, since this is one of the aspects of the problem of general cell metabolism [1-3]. The establishment of physiological pathways controlling the biosynthesis of cellulase and macerase enzymes by the association of micromycetes - *Aspergillus awamori 1-8* and *Aspergillus niger 355* and the selection of appropriate cultivation conditions will allow to increase their biosynthesis.

To establish the physiological needs of the enzyme producers, we used deep cultivation of the free cells of the enzymes producers for controlled enzyme formation created, by establishing the optimum requirements of the producers in the main elements of the nutrient medium. An important factor for increasing enzymes biosynthesis has a growth structure of the producer and cultivation in an immobilized or free state [4-5]. The establishment of physiological pathways controlling the biosynthesis of cellulase and macerase enzymes in the association of micromycetes and the selection of appropriate cultivation conditions will allow to increase their biosynthesis[6-8].

Our research task consisted of: 1) study the effect of nutrient medium composition and condition cultivated on the biosynthesis of cellulase and macerase enzymes; 2) increasing the biosynthesis of the enzymes under investigation by growing their producers in a filamentous-spongy structure and immobilized state. Identification of the advantages of the developed method of cultivation in comparison with traditional periodic cultivation.

## II. MATERIALS AND METHODS

The association of *Aspergillus niger 355* and *Aspergillus awamori 1-8* was used as a producer of a complex of cellulase and macerase enzymes. Cultivation of micromycetes was carried out by immobilized cells under deep growth conditions according to the procedure developed by Blieva RK. With the use of a laboratory apparatus (Patent RK No. 27164, 2013) [9]. Cultivation of the association of micromycetes was carried out for 45 days.

To determine the need for individual components of the nutrient medium, the Capeka medium consisting originally of (%) (NH4) 2SO4 - 0.9, sucrose - 2.0 was used; KH2PO4 - 0.1; MgSO4: 0.05; KCl-0.05; FeSO4 0.001. When studying the potassium in sources of carbon nutrition, mono-, di- and polysaccharides were used, as well as pectin in a concentration of 0.5; 1; 1.5; 2%. Cultivation of producers was carried out on a shaker under periodic growth conditions at 280° C.

The activity of macerase enzymes was determined by viscometric method. As a substrate, 1% solution of highly esterified apple pectin was used. Determination of the decrease in the viscosity of substrates was carried out in a dry viscometer immersed in a water bath at a temperature of 40 ° C. The reaction mixture consisted of 5 ml of substrate,

0.5 ml of 0.1 M acetate buffer pH 4.6 and 0.5 ml culture liquid. Determination of viscosity reduction was carried out at intervals of 2 minutes.

A 1% solution of highly esterified apple pectin was prepared as follows: a sample of pure pectin 2.5 g was taken, which was poured into a 300 ml conical flask with continuous stirring with a magnetic stir bar, where approximately 130 ml of distilled water were pre-poured. The solution was stirred for 4 hours on a stirrer at room temperature. At the end of this time, a solution of ammonia was added to the solution with stirring to adjust the pH to 4.0. Then, the volume of the solution was adjusted to 250 ml with distilled water, thoroughly mixed and filtered through two layers of gauze on a Buchner funnel. The pectin solution was prepared at least 4 hours before the analysis. For a unit of macerase activity, the amount of enzyme that catalyzes the hydrolysis of 1% of apple pectin with decreasing the viscosity of the solution in 1 minute is taken. At 35-400 C.

Determination of the activity of cellulase enzymes was carried out in accordance with GOST 31662-2012. The method is based on the quantitative determination of reducing sugars formed by the action of the enzymes of the cellulolytic complex on the sodium salt of carboxymethyl cellulose (Na-CMC).

## III. RESULTS AND DISCUSSION

Experimental studies were started with testing of carbohydrate ration as the only carbon source in the medium to assess their ability to provide biosynthesis of the enzymes studied by an association of *Aspergillus awamori 1-8* and *Aspergillus niger 355* . Chapec's medium with ammonium sulfate was used as a basis and began by studying The influence of various carbohydrates on the formation of the enzymes being studied. We tested the possibility of synthesizing initially macerase enzymes, as enzymes that determine the decay of plant tissue of plants, in one of the constituent associations - *Aspergillus awamori 1-8.* The assimilation of the tested carbohydrates was monitored, their ability to provide synthesis of the studied enzymes and among them on a specific substrate - pectin was tested (Table 1).

**Table 1**: Influence of various carbon sources on the growth and formation of macerase enzymes by *Aspergillus awamori 1-8*

| Carbon sources in the medium (2% each and inductor 2%) | PH final | Macerivating activity of the culture liquid (culture liquid),% of substrate degradation per 1 minute |
|---|---|---|
| Pectin | 3,4 | 16,52 |
| Glucose | 2,4 | 6,07 |
| Sucrose | 2,3 | 5,18 |
| Maltose | 2,7 | 3,181 |
| Glucose + pectin | 2,2 | 49,86 |
| Sucrose + pectin | 2,3 | 42,07 |
| Maltose + pectin | 2,6 | 33,04 |

This series of experiments showed that macerase enzymes are formed in large quantities in a medium in which a specific source of carbon, polysaccharide-pectin, was taken as the carbon source. Other sources of carbon - mono- and di-sugars gave activity 3-4 times less than on pectin. For another culture that is part of the association - *Aspergillus niger 355* , an optimal source of carbon is established in the medium, which is a mixture of sucrose with pectin.

The best source of carbon was a mixture of glucose with pectin for *Aspergillus awamori 1-8* and for *Aspergillus niger 355* - sucrose with pectin. In order to establish the optimal amount of pectin to be assigned to the medium, an experiment with glucose and sucrose was performed with the addition of various concentrations of both carbohydrates (1% and 2%) and pectin (0.5%, 1%, 1.5% 2 %) (table 2).

**Table 2**: Influence of carbon sources on the macerase enzymes biosynthesis by the association of micromycetes - *Aspergillus awamori 1-8* and *Aspergillus niger 355*

| Carbon sources in the medium | PH | Macerase activity,% of substrate degradation per 1 minute |
|---|---|---|
| Glucose 2% + pectin 0.5% | 2,3 | 43,7 |
| Glucose 2% + pectin 1% | 2,3 | 54,27 |
| Glucose 2% + pectin 1,5% | 2,4 | 68,43 |
| Glucose 2% + pectin 2% | 2,4 | 65,76 |
| Glucose 1% + pectin 1% | 2,4 | 88,69 |
| Sucrose 2% + pectin 0.5% | 2,3 | 26,81 |
| Sucrose 2% + pectin 1% | 2,3 | 40,24 |
| Sucrose 2% + pectin 2% | 2,3 | 71,92 |
| Sucrose 1% + pectin 1% | 2,4 | 89,36 |

In a series of experiments it was established that macerase enzymes biosynthesis with 1% glucose 1% pectin, as well as sucrose 1% with pectin 1% was high. The maximum macerase activity was

89.36% per minute. This is 1.5-2 times higher than at other concentrations of carbohydrates and pectin.

Optimal of mineral and organic nitrogen sources were determined in association of micromycetes. The best source of mineral nitrogen was ammonium phosphate and organic peptone where sucrose and pectin were used as a carbon source (Figure 1).

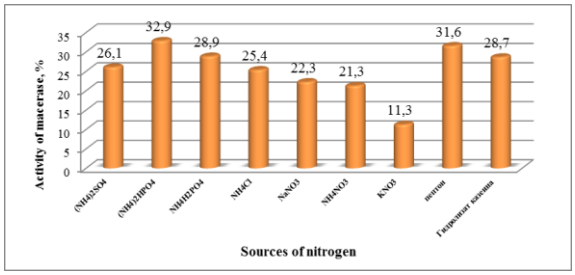


**Figure 1:** Influence of various nitrogen sources on the biosynthesis of macerase enzymes by the association of micromycetes *Aspergillus niger 355* and *Aspergillus awamori 1-8*

With periodic cultivation, micromycetes grow in the form of pellets that are difficult to access nutrients and oxygen. To increase the contact of the culture with the medium, we began to grow the producer of enzymes in the filamentous-spongy structure and in the immobilized state according to the method developed by Blieva (Patent No. 27164, 2013) [9]. Immobilization of mycelial microorganisms on the substrate creates conditions for the continuous and prolonged growth of producers with the multiple production of the target products.

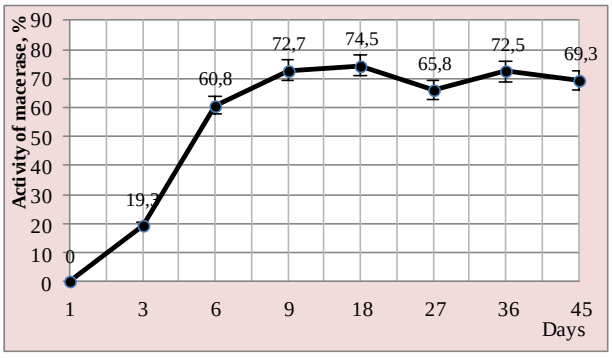


**Figure 2:** Dynamics of macerase enzymes biosynthesis during prolonged cultivation of the association *Aspergillus niger 355* and *Aspergillus awamori 1-8*

In periodic conditions desired product can be obtained only once. With the periodic cultivation of the association *Aspergillus awamori 1-8* and *Aspergillus niger 355* , the maximum production of enzymes is observed on day 3 with a gradual decrease in activity of the culture liquid due to culture lysis on the 5-6 days. With the continuous cultivation of immobilized producers, the target enzymes were obtained continuously and repeatedly (Figures 2, 3).

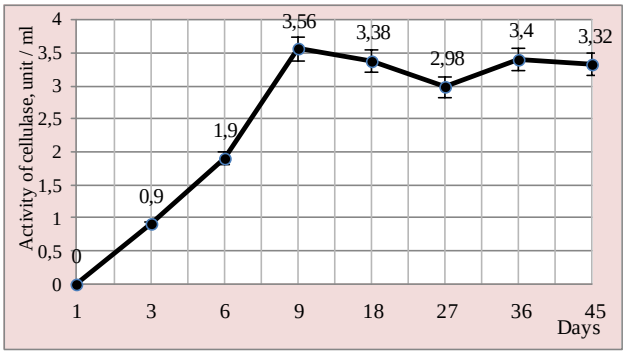


**Figure 3:** Dynamics of the formation of the cellulase enzymes with prolonged cultivation of the association *Aspergillus niger 355* and *Aspergillus awamori 1-8*

Thus, it has been established that the process of biosynthesis of cellulase and macerase enzymes in the immobilized association of *Aspergillus awamori 1-8* and *Aspergillus niger 355* can be carried out continuously on the optimal nutrient medium. The conditions for obtaining enzymes are created in high enzymatic level for a long period of time (up to 45 days). By regulation of the composition of the nutrient medium, it is possible to significantly increase the biosynthesis of cellulase and macerase enzymes in the selected association of *Aspergillus awamori 1-8* and *Aspergillus niger 355*. Cultivation of producers in a filamentous-spongy immobilized structure, in contrast to free pellets, increases the productivity of the culture and allows the product to be produced repeatedly.

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