STUDIES CONCERNING INFLUENCE OF THE INOCULUM UPON AMYLOGLUCOSIDASE'S BIOSYNTHESIS OF ASPERGILLUS NIGER

***Adriana DABIJA[1], Cristina-Gabriela CONSTANTINESCU (POP) [1], Amelia BUCULEI[1]**

[1]Faculty of Food Engineering, Stefan cel Mare University, adriana.dabija@fia.usv.ro
*Corresponding author
Received 12 December 2011, accepted 15 February 2012

Abstract: *Amyloglucosidase is used in food industry in the saccharification processes of starch raw materials. In the alcohol and beer industry amyloglucosidase is used to obtain glucose syrup and in the baking industry, to obtain crystallized sugar etc. This enzyme can be prepared by biosynthesis from culture molds of genus Aspergillus, Rhizopus or yeasts of genus Endomycopsis and catalyzes splitting of bonds α-1, 4, α-1,6, α-1,3 glucosidic from starch, dextrin, glycogen, maltose, with the successive release of glucose molecules at a rate dependent on the degree of polymerization and structure of substrate.*
This paper researched the influence of vegetative and sporiferous inoculum on the accumulation dynamics of amyloglucosidase, under the conditions of cultivating selected mould of genus Aspergillus.
Following the experiments performed to obtain amyloglucosidase under the conditions of cultivating the genus Aspergillus mold (AC-6), it is recommended the use for insemination basic nutrient medium of the vegetative inoculum at a rate of 10% for which it is achieved amyloglucosidase activity 10 times higher than using at insemination spore suspensions.

Keywords: *mould cultures, spore suspensions, vegetative inoculum*

1. Introduction

The amyloglucosidase (α1,4 glucohydrolase, glucoamylase, glucogenase, γ-amylase) is biosynthesis produced by the mould cultures like genus *Aspergillus*, *Rhizopus,* or yeasts *Endomycopsis fibuliger*, *Endomycopsis bispora* [1, 2]. The structures of multiple forms of *Aspergillus niger* glucoamylase have been shown to be closely related. It is known that *Aspergillus niger* is industrially useful in view of their use in the production citric acid and enzymes such as glucosidase, glucoamylase etc. Production processes have been thoroughly optimized by improvements in process technology and by strain breeding. Classical improved strains have been obtained using random mutagenesis, recombination and genetic modification based on transformation of protoplasts [3, 4, 5, 6].
Amyloglucosidase (AMG) (1,4-α-D glucan glucanohydrolase, EC 3.2.1.3.) is an industrially important biocatalyst. AMG is a multi-domain, exo-acting enzyme that catalyzes the hydrolysis of starch and related substrates from the non-reducing ends mainly by cleaving α-1,4- glycosidic linkages and a few α-1,6 linkages, but at a very slow pace [7].
Also, the amyloglucosidase catalyses the scission of glucosidal links α-1,4, α-1,6, α-1,3 in starch, dextrin, glycogen, maltose producing glucose molecules successively disengaging at a rate dependent of

polymerization's degree and substratum's structure on. The bond α-1,4 is faster hydrolyzed than the α-1.6, while maltotriose and maltose are faster hydrolyzed than the oligosaccharides. The enzyme is widely used in baking and the production of ethanol, molasses, and crystalline glucose. The enzyme has been immobilized using different methods including alginate entrapment.

The micromycetes can produce multiple shapes of enzyme, which is differentiating among them by weight molar, kinetics constant, substratum specification, extra-cellular enzymes which concentration depend on medium's composition, growing conditions, microbic agent's nature. The amylo glucosidase, beside α- and β-amylase, has a lot of industrial applications in saccharifying processes of starchy raw, in alcohol industry and breweries, in obtaining glucose syrups and crystallized glucose, in bakery industry. For instance, amyloglucosidase is used when the brewers want to obtain beer having low carbohydrates content (diet beer). In bakery field, for the higher glucose contents of dough the amyloglucosidase is used too, because that provides for a better yeast multiplication and encourages the obtaining of bread with good-looking crust and soft core.

The optimum conditions for the enzyme activity are: temperature - 55÷60°C and pH - 4.5÷5.5. The enzyme is inactived by heating to 80°C on 5 minutes or to 75°C on 40 minutes long. The enzyme activity is canceled using the procedure of exchanger ions' crossing or the treating with active carbon [1].

In this present work we have observed the influence of vegetative sporiferous inoculum upon the accumulation dynamics of amyloglucosidase under growing's conditions for a selected mould belonging to the *Aspergillus* genus.

2. Experimental

For the obtaining of sporiferous inoculum, the spores from a growing on inclined malt must with agar (MMA) have been carried away by sterile physiological serum, and the determination of spores number was performed using the chamber Thoma method. For the obtaining of vegetative inoculum, spores suspensions have been inseminated on the medium having the following composition [g/%]:

- Maize flour 5
- Yeast extract 0.25
- pH medium (HCl addition) 5.5

The inseminated medium has been maintained under stirring condition at 25°C for 48 hours long having as effect the spores' germination and growth of vegetative hyfa.

For the experiments, it has been used different quantities of vegetative or sporiferous inoculum, which was inseminated on basic medium having the composition [g/%] as following:

- Maize flour 15
- Malt flour 0.4
- pH medium (HCl addition) 5.5

The growing was made under stirring conditions at 25÷28°C for 96 hours long. For the fermented medium, which contains extra-cellular amyloglucosidase, it was determined values for the pH, refractometric degree and amyloglucosidase activity.

An amyloglucosidase activity unit means the glucose quantity [mg] obtained for 1 cm^3 enzyme filtrate at 60°C in one hour long from starch soluble 4% solution having pH=4.2.

3. Results and Discussion

The insertion of $5 \cdot 10^7 \div 20 \cdot 10^7$ spores/cm^3 suspension on basic medium and growing medium under same conditions and the analysis of enzyme filtrate after that, gave

the opportunity to show the synthesized results in table 1.

Table 1.
The influence of sporiferous inoculum upon amyloglucosidase's activity

Variant	Spores no./cm^3 mediu m	Enzyme filtrate's characteristics			
		pH	Refractometric degree	Enzyme activity [AGU/cm^3 enzyme filtrate]	Relative activity % optimum variant
1	$5 \cdot 10^7$	3.8	12.8	54.4	100.0
2	$10 \cdot 10^7$	4.0	12.5	41.6	76.4
3	$15 \cdot 10^7$	3.5	12.2	28.2	52.9
4	$20 \cdot 10^7$	4.5	12.0	24.0	44.1

Among the four used alternatives for the experiments, only the first one ($5 \cdot 10^7$ spores/cm^3) shows a maximum enzyme activity, than, gradually, the activity cuts down so that the doubling of spores number for inoculum leads a reduction by 23% of enzyme activity, while for the four times growth of spores number leads to the decrease of enzyme activity with 66% (fig.1).

Through the determination of extract using refractometric way it can see when the inoculated spores number start growing in medium the soluble substances' consumption, of medium become more intensive. This fact it not become favorably for amyloglucosidase's elaboration.

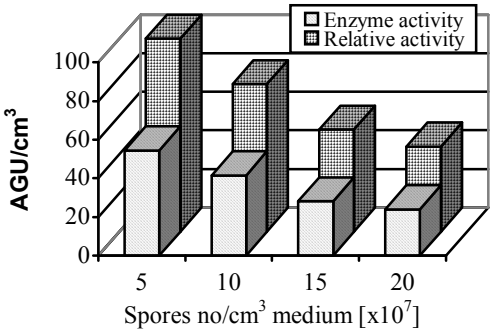

Fig.1. The influence of sporiferous inoculum upon amyloglucosidase's activity and relative activity.

For the study of vegetative inoculum's influence it has been used 4 variants having the variation of inoculum quantity between 5÷30% given basic medium. The results for analysis of enzyme filtrate are shown in table 2.

Table 2.
The influence of vegetative inoculum upon the amyloglucosidase's activity

Variant	Inoculum quantity [%]	Enzyme filtrate's characteristics		
		pH	Enzyme activity [AGU/cm^3 enzyme filtrate]	Relative activity [% optimum variant]
1	5	3.0	363	67
2	10	2.8	541	100
3	20	2.5	521	96
4	30	2.5	482	89

Using the vegetative inoculum in quantity of 10%, it was obtain maximum amyloglucosidase's activity by ten times more than the sporiferous inoculum's using.

The doubling of inoculum quantity (variant 3) it was not lead to the enzyme activity's growth. Through the exaggerated growth of inoculum quantity (variant 4), the enzyme activity decreases with 11% (fig.2).

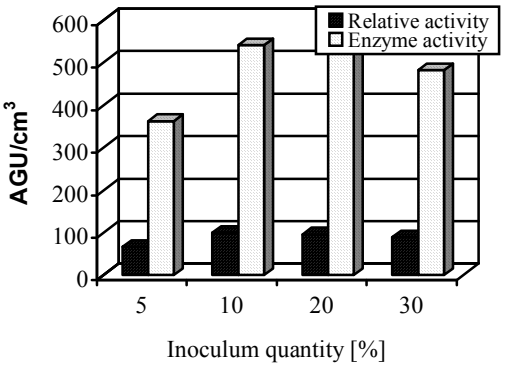

Fig.2. The influence of vegetative inoculum upon the amyloglucosidase' activity and relative activity

The lower values of pH obtained for the vegetative inoculum is owing to the metabolically activity of it, which is more intense than spores activity because the last

one needs additionally adaptation and germination periods when its own enzyme activity could have a common evolution.

4. Conclusion

For the amyloglucosidase's obtaining under conditions of selected mould growing which belongs to *Aspergillus* genus, it is recommended, for the insemination of basic nutritive medium, to use vegetative inoculum in 10% proportion having as effect an amilogluosidase's activity of 10 times more than the utilization for insemination of spores' suspensions.

5. References

[1]. BANU C., *et al., Biotechnology of Food Industry*, Tehnica, Bucharest, (2000)
[2]. BRIGS D.E., *et al., Malting and Brewing Science,* Chapman et Hall, London, (1995)
[3]. GIESEN R., *Fungal starter cultures for fermented foods: molecular aspects*, Trends in Food Science et Technology, 8, 251-254, (1993)
[4]. DAN V., *Food Microbiology*, Alma, Galati, (2001)
[5]. ROCKTOTPAL, K., *et al., Magnetically recyclable, antimicrobial and catalytically enhanced polymer-assisted green monosystem immbolized Aspergillus niger amyloglucosidase*, Applied Microbiology and Biotechnology, 6, 1983-1992, (2010)
[6]. SARMA USHA, *Fascinating Potential of Aspergilli.* Indian Journal of Chemical Biochemistry, vol.25, 4, 331-334, (2010)
[7]. SVENSSON, B., et al., *Characterization of two form of glucoamilase from Aspergillus niger*, Carlsberg Research Communications, 1, 55-69, (1992)