P-002	**Hybrid sol-gel matrices used for entrapment of *Aspergillus oryzae* PP cells for the production of α-amylase**	

Kabaivanova L.[1#*], Chernev G.[2], Evstatieva Y.[3], Nikolova D.[3], Yordanova M.[3], Ilieva S.[3],
[1] Bulgarian Acad Sc, Sofia, Bulgaria [2] Univ Chem Technol & Metal, Sofia, Bulgaria [3] Sofia Univ "St. Kliment Ohridski", Sofia, Bulgaria
* Supervisor # Contact email: lkabaivanova@yahoo.com

INTRODUCTION AND OBJECTIVES

The critical significance of bioecapsulation is evident from the fact that many commercial bioprocesses rely on immobilized catalysts (Fernandes 2010). Important advantage of the whole-cell immobilization is the enhancement of the synthesis of bioactive substances for a longer period and the multiple use of the obtained biocatalysts. Amylases are among the industrially important hydrolytic enzymes with a significant role in starch degradation to produce syrups and whole grain hydrolysis for alcohol production. They are widely applied in textile, feed and brewing industries (Pandey 2010). Researchers have attempted the production of α-amylase by immobilized cells (Ramakrishna 1993). Passive immobilization of *Aspergillus awamori* spores for subsequent glucoamylase production was reported (Bon and Webb 1989). Finding more efficient biocatalysts can be achieved through involving suitable immobilization techniques. The sol-gel method can be used for synthesizing hybrid biomaterials on the basis of hydrolysis of alkoxide precursors, followed by condensation and polycondensation of the hydroxylated units, which leads to the formation of a porous gel. The application of sol-gel glass to entrap active biomolecules is now well documented (Gill 2001, Jin and Brennan 2002, Livage 2006) Investigations have appeared recently in the field of hybrids materials synthesis and the benefits of the best of the two worlds - inorganic and organic. The biopolymer-containing hybrid materials of silica prepared by sol–gel processes have drawn attention owing to their promising properties and biocompatibility with living matter (Nassif 2002).
The aim of the present study is to synthesize hybrid materials suitable for the entrapment of *Aspergillus oryzae* PP cells and to evaluate the enhancement of α-amylase production during the fermentation process.

EXPERIMENTAL PART

Matrix synthesis

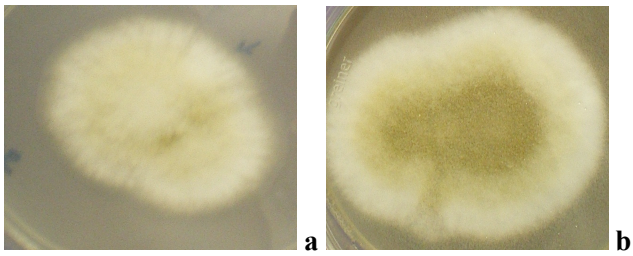
The results on the application of hybrid matrices containing different quantities of polyethylene oxide (PEO) -5 to 20 wt. % and a silicon precursor tetraethylortosilicate (TEOS), purchased by "ABCR" are discussed. A polystep sol-gel procedure is carried out. The samples were prepared at room temperature as films.

Microorganism, media and growth conditions

Spore and pellet suspensions of *Aspergillus oryzae PP* strain (Faculty of Biology, Sofia University, Department of Biotechnology) were immobilized in the hybrid matrices. The investigated strain was grown on Potato-dextrose agar and Sabouro agar for 10 days cultivation at 30°C. Culture medium containing (g/l): NH_4CI-1,0; urea-0,3; KH_2PO_4-2,0; $(NH_4)_2SO_4$-1,4; $MgSO_4.7H_2O$- 0,3; $CaCI_2.2H_2O$-0,4; Glucose-20; maize extract-10 ml. The immobilized preparations were placed in 500 ml conical flasks with 50 ml fermentation medium and put on a rotary shaker at 200 r.p.m.

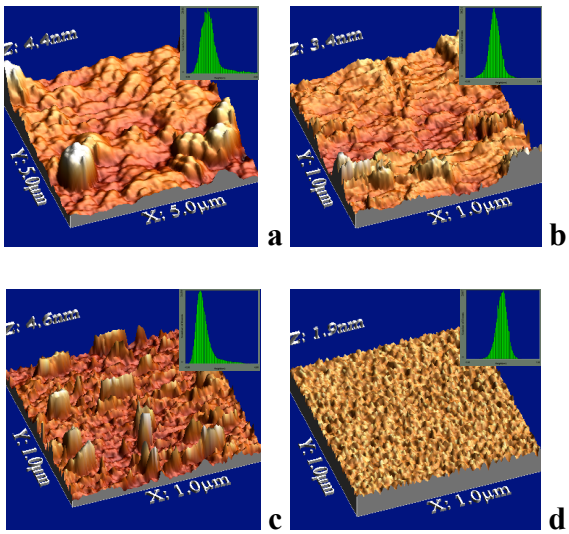
RESULTS AND DISCUSSION

Hybrid matrices were synthesized by the sol-gel method. Before the synthesis, experiments were carried out to prove that PEO is a suitable organic constituent for the matrix. The obtained results showed that the diameter of the single colonies of *Aspergillus oryzae* PP in a medium, containing PEO is bigger than this of the control, showing the favorable effect of this constituent on the growth (Fig. 1). Based on this result the hybrid matrices used for the entrapment of spore material as well as vegetative cells of the investigated strain contained PEO as an organic part.



a **b**

Figure 1 : Single colony diameter of cultivated stain in culture medium (a) and medium containing PEO (b)



a **b** **c** **d**

Figure 2 : AFM images of matrices with different PEO concentration a-5%; b-10%, c-15%, d-20%

From AFM images of hybrid samples the size of particles and their aggregates have been determined and the surface roughness of most samples shows irregularity of quite small height (Fig. 2). The surface of the matrices with 10 and 15 % PEO permits mycelium growth.

The quantity of spore suspension and its concentration influenced the α-amylase activity of entrapped fungal strain. Table 1 represents the enzyme activity when spore suspension of 10^6 spores/ml was included into the volume of the matrix. The α-amylase activity appeared to be 2.41 IU/ml at the 288 h from the beginning of the fermentation process which imposed to increase the concentration of the spores to 10^8 spores/ml. This factor could ensure better growth, development and increase in the biosynthetic capability of the producer of α-amylase (Table 2). Highest enzyme yield (34.32 IU/ml at the 504h of the fermentation process) was reached with 6 ml spore suspension and a concentration of 10^8 spores/ml.

Table 1 : α-amylase activity of spore suspension with concentration 10^6 spores/ml

Fermentation time (h)	α-amylase activity (IU/ml)	
	4 ml	2 ml
196	1,59	0
288	2,41	0
324	2,05	0

Table 2 : α-amylase activity of spore suspension with concentration 10^8 spores/ml

Fermentation time (h)	α-amylase activity (IU/ml)		
	2 ml	4 ml	6 ml
168	0	0	0
336	0	0	1,44
504	1,04	2,83	34,32
624	0,59	2,63	29,54

In the next experiments the material for immobilization was in the form of pellets, cultivated in a medium with soluble starch, lacking in soy flour and bran. Thus obtained vegetative material was entrapped in a hybrid matrix and transferred in a fermentation medium to evaluate its biocatalitic activity (Fig. 2).

The concentration of the organic constituent in the hybrid matrix plays its role over the α-amylase activity which was highest for the matrix with 10% PEO -324.36 IU/ml at the 576 h. At the 456 h a transfer into fresh fermentation medium was carried out.

The enzyme activity of free cells was 233.7 IU/ml at the 48 h. Similar activity was retained for 864h after the beginning of the fermentation process using a hybrid matrix that contained 15% PEO - 223,5 IU/ml. The high value of activity for the cells included in matrices with 10% and 15% PEO is probably due to the adequate pore size regulated by the percent of organic constituent, giving good access of the components of the fermentation medium, namely the starch as an inducer of α-amylase synthesis and to the oxygen, necessary for the strain development. That is why there is no activity when the matrix with 5% organic constituent is included probably due to mass transfer limitations becauce of insufficient pore size.

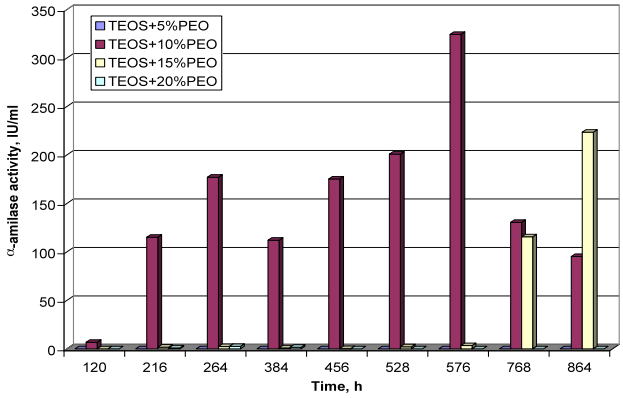


Figure 3 : Biosynthesis of α-amylase by immobilized pellets of *Aspergillus oryzae PP* strain in hybrid matrices with 5, 10, 15 и 20 % PEO

Introduction of 20% organic material did not lead to an increase in the enzyme yield.

CONCLUSIONS

The immobilized in hybrid matrices culture of *Aspergillus oryzae PP* preserved its biosynthetic capability for a long period of time, retaining high level of activity for 864h after the beginning of the fermentation process. Enhanced enzyme production was measured when the immobilized preparations were introduced in fresh cultivation medium. The sol-gel matrix, synthesized on the basis of TEOS and 10% and 15% PEO appeared to be suitable to achieve successful immobilization of the fungal material permitting the substrate and oxygen to penetrate and realize α-amylase production higher than that of the free cells.

REFERENCES

- Fernandes P. (2010) *Enzymes in Food Processing: A Condensed Overview on Strategies for Better Biocatalysts.* Enzyme Research Review Article
- Pandey, A. et al. (2010) *Advances in microbial amylases*. Biotechnol Appl. Biochem. 31 135-152
- Ramakrishna, S. V. et al. (1993) *α-amylase production by free and immobilized Bacillus subtilis.* Appl. Biochem. Biotechnol. 37 275–282
- Bon E. and Webb C. (1989) *Passive immobilization of Aspergillus awamori spores for subsequent glucoamylase production.* Enzyme and Microbial Technology 11, Issue 8 495-499
- Gill I. (2001) *Bio-doped nanocomposite polymers: sol-gel bioencapsulates.* Chem Mater 13 3404-3421
- Jin W. and Brennan JD. (2002) *Properties and application of proteins encapsulated within sol-gel derived materials*. Anal Chim Acta 4611-36
- Livage J. and Coradin T. (2006) *Living Cells in Oxide Glasses* Reviews in Mineralogy and Geochemistry 64 -1 315-332
- Nassif N. et al. (2002) *Living bacteria in silica gels*. Nature Materials 1 42-44