Annuaire de l'Université de Sofia "St. Kliment Ohridski"
Faculte de Biologie
2015, volume 100, livre 4, pp. 14-20
First National Conference of Biotechnology, Sofia 2014

ZEOLITES AS POTENTIAL MATRICES FOR IMMOBILIZATION OF *ASPEGILLUS ORYZAE* PP

MARIYA ABAEVA*, YANA EVSTATIEVA, VALENTIN SAVOV, SVETLA ILIEVA, DILYANA NIKOLOVA

Department of Biotechnology, Faculty of Biology, Sofia University "St. Kliment Ohridski", Sofia, Bulgaria
**Corresponding author: maria_jord@mail.bg*

Keywords: immobilization; *Aspergillus oryzae*; zeolites, α-amylase activity

Abstract: *Aspergillus oryzae* was used predominantly for commercial production of the enzyme α-amylase by batch fermentation technique. The use of this enzyme in many industrial fields determines the development of new methods for enhancement of the productivity of the fungal species.

Adsorption on a solid support material is the most general, easiest to perform and oldest protocol of physical immobilization methods, used to increase cell viability.

The present investigation describes the use of different types of zeolites for immobilization of fungal cells of the fungal strain *Aspergillus oryzae* PP and the production of the enzyme a-amylase. Spore suspension was immobilized in situ by natural adsorption, i.e. through direct contact between cells and three types of zeolites: natural and modified with Cu^{2+} and Fe^{2+} at the beginning of the cultivation processes. α-amylase production of immobilized cultures was investigated up to 744th h during batch fermentation and compared with enzyme activity of free cell culture. Immobilized cultures demonstrated a successful adsorption onto all types of zeolites tested in the present study. α-amylase production of 1050,72 SKB U/ml at 216th h of the fermentation process by immobilized cultures on zeolite modified with Cu^{2+} was detected. Immobilized cultures of all the types of zeolites are able to retain their biosynthetic capabilities up to 744th h due to the solidity and the porosity of the investigated zeolite particles.

INTRODUCTION

Aspergillus oryzae species are particularly interesting because they are easy to cultivate and widely used in traditional fermentation industries (Porfiri et al., 2012). They are also known to be good producers of α-amylases used in a number of processes such as bread making, brewing, starch processing, pharmacy, textile,

detergency and paper manufactures (Kara et al., 2005; Sahnoun et al., 2012). In this context, many scientists have directed their attention on the development of new methods for enhancement of enzyme stability and cell productivity. The advantages of the use of immobilized microorganisms and enzymes have a special relevance in the area of many industries (Mateo et al., 2007). Adsorption of cells onto a solid support surface is probably the mildest of cell immobilization techniques. In its simplest form it is also one of the cheapest methods and therefore of particular use for enhancement of product yield in industrial processes (Tampion et al., 1987). The advantage of this method is the minor influence on the conformation of the biocatalyst. There is no need for utilization of chemicals which could cause a damage to the microbial cells, so the catalytic activity could be preserved (Xing et al., 2000; Vasconcellos et al., 2012). Recently, the use of zeolites as support for immobilization has been a subject of growing interest due to the potential properties of this class of materials. Zeolites are microporous materials that present an important role in several technological areas, mainly due to their adsorption ability and presence of active acid centres (Knezevic et al., 1998; Calgaroto et al., 2011; Mitchell et al., 2011). Suh et al (1994) reported enhancement of the rate of cyanide degradation by immobilized cells of *Pseudomonas fluorescens* NCIB 11764 on zeolite. Cultures of *Lactobacillus rhamnosus* ATCC 7469 were immobilized onto zeolite indicated as an efficient carrier for the production of lactic acid on liquid distillery stillage (Djukic-Vukovic et al, 2013). Savov et al. (2009) immobilized cells of the fungal strains *Trichoderma viride* and *Trichoderma harzianum* onto zeolite modified with Fe ions and detected a positive effect on bioremediation processes. *Trichoderma harzianum* cell cultures were also immobilized onto zeolites modified with Cu, Zn and Mn (Angelova et al., 2009) to improve enzyme production.

The aim of the present study is to investigate the α-amylase production by immobilized spore suspension of the fungal strain *Aspergillus oryzae* PP onto three types of zeolites - natural and modified with Cu^{2+} and Fe^{2+}. α-amylase biosynthesis by the immobilized cells was investigated up to 744th h during batch fermentation processes and compared with α-amylase activity of free cell cultures used as control.

MATERIALS AND METHODS

Aspergillus oryzae PP was provided by the Department of Biotechnology, Faculty of Biology, Sofia University "St. Kliment Ohridski", Bulgaria. Cultures of the investigated strain were maintained on Sabouraud agar at 28^{o}C in order to obtain dense sporulation. Four different zeolites – natural, modified with microelements, Cu and Fe ions were provided by NIPRORUDA Company, Bulgaria and also used for immobilization of the investigated strain.

1. Culture conditions and immobilization technique

A Mandels' liquid medium with 6% (w/v) starch and 1% (w/v) wheat bran was inoculated with 20% (w/v) spore suspension. 2,5% (w/v) of natural or modified zeolite were also added to the medium. Cells were cultured under shaking (250 rpm) at 28°C for 24 h in order to obtain adsorption on the zeolite carrier.

2. α-amylase production

A fermentation medium of Chapek with 5% (w/v) starch was added to the immobilized cultures. α-amylase production by free and immobilized cultures was carried out on a rotary shaker at 250 rpm and 28°C. Samples were collected for analysis of α-amylase activity up to 744th h of the fermentation process.

3. α- amylase assay

α-amylase activity was determined spectrophotometrically by the method of Sandstedt, Kneen and Blish using 1% starch solution as a substrate (Cereal Chem. 16, 172, Technical Bulletin No. 1024). One unit of α-amylase activity was defined as the amount of the enzyme which catalyses the hydrolysis of 1 g soluble starch for 1h at 40°C, pH – 4,7.

RESULTS AND DISCUSSION

Cell immobilization is often used to improve the performance of a traditional continuous fermentation process and lead to retention of cell viability and productivity.

The aim of this set of experiments is to investigate the production of α-amylase by the fungal strain *Aspergillus oryzae* PP using different types of zeolites for cell immobilization. It was found that the strength of interactions between the microbial cells and particles of carriers depends on the specific properties of zeolites. Three types of zeolites - natural and modified with Cu^{2+} or Fe^{2+} were used in order to determine their ability to absorb the fungal cells. Zeolites were added to the liquid medium and inoculated with a spore suspension of the investigated strain. The submerged cultivation was carried out for 24 hours to adsorb *Aspergillus oryzae* PP spores on natural or zeolite, modified with metal ions. α-amylase production by free and immobilized cultures was obtained in a liquid medium containing starch as an inducer up to 744th h of the fermentation process.

According to the results, spore suspension of the fungal strain *Aspergillus oryzae* PP was successfully absorbed on all the three types of zeolites used in the present study. α-amylase production of 634,58 SKB U/ml was detected at 264th h of the cultivation by the culture immobilized on natural zeolite. This activity shown on Fig. 1 represents 26% of maximum α-amylase activity of 2439,07 SKB U/ml reported for free mycelium culture used as control.

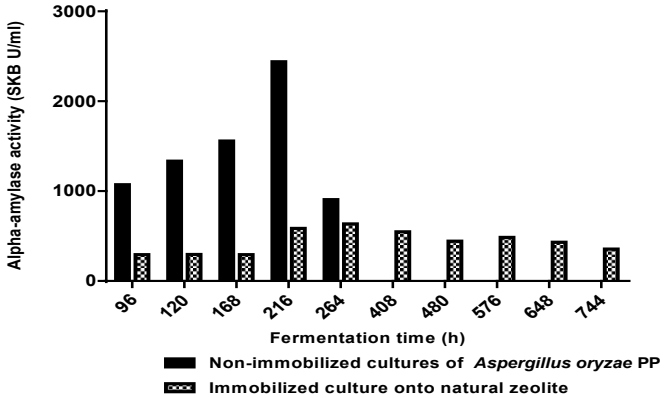

Figure 1. Variation of α-amylase activity of *Aspergillus oryzae* PP free and immobilized cultures on natural zeolite.

Biosynthetic capabilities of *Aspergillus oryzae* PP culture immobilized on modified zeolite containing Fe were also investigated in this study and presented on Fig. 2. α-amylase activity obtained exhibited 875,10 SKB U/ml at 216th h of the fermentation process by the culture adsorbed on zeolite modified with Fe. This activity detected represents 35% of the enzyme production value of 2439,07 SKB U/ml obtained from the free mycelium culture at 264th h of the control cultivation process.

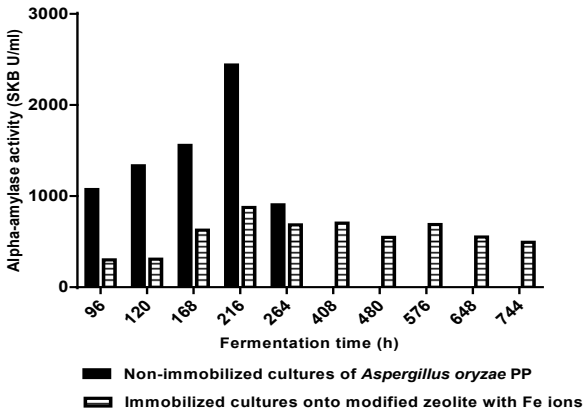

Figure 2. Biosynthesis of α-amylase by free and immobilized cultures onto zeolite modified with Fe^{2+}.

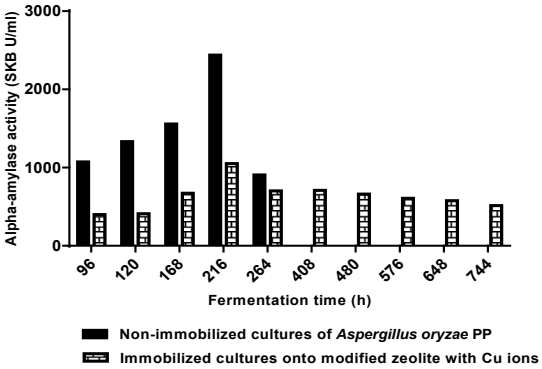

Figure 3. α-amylase activity of free and immobilized cultures onto zeolite modified with Cu.

Aspergillus oryzae PP culture, immobilized on Cu^{2+}-modified zeolite demonstrated the maximum α-amylase activity of 1050,72 SKB U/ml at 216th h of the fermentation process compared to the other types of carriers. This value presented on Fig. 3 appears to be 43% of enzyme activity reported for free cell cultures.

According to the results α-amylase activities of immobilized cultures onto three types of zeolites are lower compared with the production values of free cell cultures but retain their biosynthetic capabilities for long-term fermentation processes. Retention of α-amylase production from immobilized cultures up to 744th h of the fermentation process is probably due to the excellent adsorption on the surface and the sufficient pore size of the carrier. Highest amylase production was detected during the cultivation of immobilized cultures of *Aspergillus oryzae* PP onto zeolite modified with Cu^{2+} compared with the activities of the other adsorbed cultures. This is probably due to the positive effect of Cu^{2+} on growth behaviour and metabolism of the investigated strain. Zeolite particles modified with Cu^{2+} also offers an appropriate surface for adsorption of spores. Similar results were reported by Knezevic et al. (1998). Immobilized *Candida cylindrica* lipase on zeolite type Y demonstrated 35% of enzyme activity during hydrolysis of palm oil compared with free enzyme used as control. Further work is required for the enhancement of α-amylase production of immobilized cultures on zeolites.

CONCLUSIONS

In summary, spore cultures of the α-amylase producing strain *Aspergillus oryzae* PP were successfully immobilized on three types of zeolites –

natural and modified with Cu^{2+} and Fe^{2+}. The use of zeolite modified with Cu^{2+} provides a considerable positive effect on enzyme activity compared with other carriers. According to the results α-amylase activity of this immobilized culture represents 43% of maximum enzyme activity detected during the cultivation of free cell culture. In spite of the lower α-amylase production compared with control, all immobilized cultures were capable to retain their biosynthetic activity during long-term fermentation processes. This analysis determines zeolites as efficient carriers for adsorption of fungal cells, but additional experiments are required in order to increase the α-amylase production.

Acknowledgments: This work was supported by grant DMU 03/12 of National Science Fund, Bulgaria.

REFERENCES

1. Angelova G., Chakalov K., Popova T, Savov V 2009. Influence of Fe, Cu, Mn, Zn, Co Chelators on the Biotransformation of Humic Substances of Lignite, *Biotechnology & Biotechnological Equipment*, 23 (sup1), pp.796-800
2. Calgaroto C, Scherer RP, Calgaroto S, Oliveira JV, de Oliveira D, Pergher SBC. 2011. Immobilization of porcine pancreatic lipase in zeolite MCM 22 with different Si/Al ratios. *Applied Catalysis A*, 394: 101–104
3. Djukic-Vukovic AP, Ljiljana V. Mojovic LV, Jokic BM, Nikolic SB, Pejin JD. 2013. Lactic acid production on liquid distillery stillage by *Lactobacillus rhamnosus* immobilized onto zeolite. *Bioresource Technology*, 135: 454–458
4. Kara A, Osman B, Yavuz H, Besirli N, Denizli A. 2005. Immobilization of a-amylase on Cu^{2+} chelated poly(ethylene glycol dimethacrylate-n-vinyl imidazole) matrix via adsorption. *Reactive & Functional Polymers*, 62: 61–68
5. Knezevic Z, Mojovic L, Adnadjevic B. 1998. Palm oil hydrolysis by lipase from *Candida cylindrica* immobilized on zeolite type Y. *Enzyme Microb. Technol.*, 22: 275-280
6. Mateo C, Palomo JM, Fernandez-Lorente G, Guisan JM, Fernandez-Lafuente R. 2007. Improvement of enzyme activity, stability and selectivity via immobilization techniques. *Enzyme and Microbial Technology*, 42: 1451–1463
7. Mitchell S, Pérez-Ramírez J. 2011. Mesoporous zeolites as enzyme carriers: Synthesis, characterization and application in biocatalysis. *Catalysis Today*, 168: 28–37
8. Porfiri MC, Farruggia BM, Romanini D. 2012. Bioseparation of α-amylase by forming insoluble complexes with polyacrylate from a culture of *Aspergillus oryzae* grown in agricultural wastes. *Separation and Purification Technology*, 92: 11–16
9. Sahnoun M, Bejar S, Sayari A, Triki MA, Kriaa M, Kammoun R. 2012. Production, purification and characterization of two α-amylase isoforms from a newly isolated *Aspergillus oryzae* strain S2. *Process Biochemistry*; 47: 18–25
10. Sandstedt, Kneen, Blish. 1939. Cereal Chem. 16, 172 and Technical Bulletin No. 1024, U.S. Department of Agriculture.

11. Savov V, Chakalov K, Angelova G, Popova T, Hristova R, 2009. Study of immobilized microbial composition on the reclamation process of heavy metal polluted soils., XI Anniversary scientific conference, *Biotechnol. & Biotechnol.* EQ. 23/2009/SE
12. Suh YJ, Park JM, Yang JW. 1994. Biodegradation of cyanide compounds by *Pseudomonas fluorescens* immobilized on zeolite *Enzyme Microb*. Technol., 16: 529-533
13. Tampion J, Tampion MD, 1987. Immobilized cells: Principles and applications, Cambridge University Press.
14. Vasconcellos A, Paula AS, Filho RAL, Farias LA, Gomes E, Aranda DAG, Nery JG. 2012. Synergistic effect in the catalytic activity of lipase *Rhizomucor miehei* immobilized on zeolites for the production of biodiesel. *Microporous and Mesoporous Materials*; 163: 343–355
15. Xing GW, Li XW, Tian GL, Ye YH. 2000. Enzymatic peptide synthesis in organic solvent with different zeolites as immobilization matrixes. *Tetrahedron*; 56: 3517-3522