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ENHANCEMENT OF XYLANASE PRODUCTION BY SOL-GEL IMMOBILIZATION OF *ASPERGILLUS AWAMORI* K-1

M. YORDANOVA[1*], Y. EVSTATIEVA[1], G. CHERNEV[2], S. ILIEVA[1], R. DENKOVA[1] and D. NIKOLOVA[1]
[1] Sofia University "St. Kliment Ohridski", Department of Biotechnology, Faculty of Biology, Sofia, Bulgaria
[2] UCTM, Department of Silicate Technology, Faculty of Metallurgy and Material Sciences, Sofia, Bulgaria

Abstract

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Technological potential of *Aspergillus awamori* species is of particular interest in the investigation of new methods for increasing the cell viability and xylanase activity. The purpose of the present investigation was to study the immobilization of *Aspergillus awamori* K-1 cells for higher xylanase production using sol-gel encapsulation in hybrid matrices composed of tetraethylorthosilicate as an inorganic precursor, 5% xylan and 5, 10 or 15% calcium alginate as organic compounds. The enzyme synthesis of immobilized cells under batch fermentation conditions were compared with xylanase activity of free cell culture used as control. According to the results, culture immobilized in hybrid matrix composed of tetraethylorthosilicate and 5% calcium alginate was found to display xylanase activity of 28,88 IU.ml^{-1} higher than the control enzyme activity of 22,04 IU.ml^{-1}. In contrast to the free cell culture, immobilized cells retain their viability and biosynthetic capabilities during continuous fermentation processes.

Key words: Aspergillus, xylanase, immobilization, sol-gel hybrids, alginate

Introduction

Microbial xylanases (1,4-β−D-xylan xylanohydrolase, EC 3.2.1.8) are the preferred catalysts for xylan hydrolysis due to their high specificity, mild reaction conditions, negligible substrate loss and side product generation (Squina et al., 2009). Industrial degradation of xylan is accomplished predominantly by the genera *Aspergillus* known as efficient producers of extracellular xylanolytic enzymes in high levels (MacCabe et al., 1996; Ruanglek et al, 2007). Among the multitude of methods for enhancement of enzyme production, sol–gel encapsulation has proven to be a particularly easy and effective way to immobilize whole cells in order to obtain high thermal and long-term operational stability (Taylor, 2004). The use of some organic polymers, such as calcium alginate in sol-gel inorganic-organic materials provides formation of hybrid matrices offering enhancement of cell viability and good substrate diffusion in biocomposites (MacCabe et al., 1996). In this context, we report here the xylanase production of the fungal strain *Aspergillus awamori K-1* immobilized in hybrid matrices composed of tetraethylorthosilicate (TEOS) as an inorganic precursor, 5% xylan and 5, 10 or 15% calcium alginate as organic compounds. The enzyme synthesis of the immobilized cells was investigated up to 744th h during batch fermentation process and compared with xylanase activity of free cell culture.

Materials and Methods

Microorganism and fermentation conditions

The fungal strain *Aspergillus awamori* K-1 was obtained from strain collection of Department of Biotechnology, Faculty of Biology, Sofia University "St. Kliment Ohridski" (Bulgaria) and used in the present study. The cultures were maintained on potato dextrose agar (Difco) at 28–30°C in order to obtain dense sporulation.

**E-mail: maria_jord@mail.bg*

Mycelium cultures were prepared by transferring 10% (v/v) of spore suspension into 100 ml of growth medium in 500-ml shake flasks. After submerged cultivation at 28–30°C and 250 rpm, the mycelia were used for free fermentation process and sol-gel immobilization in hybrid matrices.

Xylanase production by free and immobilized mycelium cultures was carried out at 28–30°C and 250 rpm in 500-ml shake flasks containing 50 ml of fermentation medium. Samples for analysis of xylanase activity were collected periodically up to 744 h of the fermentation process.

Immobilization technique

Sol -gel transparent silica hybrid matrices were synthesized at room temperature. The hybrid materials were prepared by substituting part of the inorganic precursor TEOS with 5% starch and 5, 10 or 15 wt% calcium alginate. To obtain one sample, the cell immobilization was carried out using 10 ml mycelium culture. The time of gelatinization depended on the concentration of organic compounds.

Xylanase assay

Xylanase activity was determined by measuring the amount of reducing sugars released by hydrolysis of birchwood xylan (Sigma) through a colorimetric assay, based on the Somogyi–Nelson method with xylose as a standard (Shomogyi, 1951). One unit of xylanase activity was defined as the amount of enzyme required to liberate 1 μmol of xylose per min at 40°C, pH – 4.0.

Results and Discussion

According to the results, xylanase activity of 28.88 IU.ml^{-1} was obtained from culture immobilized in sol-gel matrix composed of TEOS, 5% xylan and 5% calcium alginate at 168th h of the fermentation process (Figure 1A).

Expression of xylanase activity by this culture is almost 1.5–fold higher compared to the enzyme activity of 22.04 IU.ml^{-1} reported in free cell cultures at 72th h. Culture immobilized in hybrid matrix containing 10% calcium alginate showed xylanase activity of 26.92 IU.ml^{-1} at 240th h of the cultivation and also higher than the control (Figure 1B).

Biosynthetic capabilities of *Aspergillus awamori* K-1 cells immobilized in hybrid matrix composed of TEOS, 5% xylan and 15% calcium alginate were also investigated in the present study. Xylanase activity obtained exhibited 22.79 IU.ml^{-1} at 288th h of the cultivation and was almost equal to the enzyme activity of the free mycelium (Figure 1C)

Based on the results of this study, calcium alginate has a positive effect on the productivity of immobilized *Asper-*

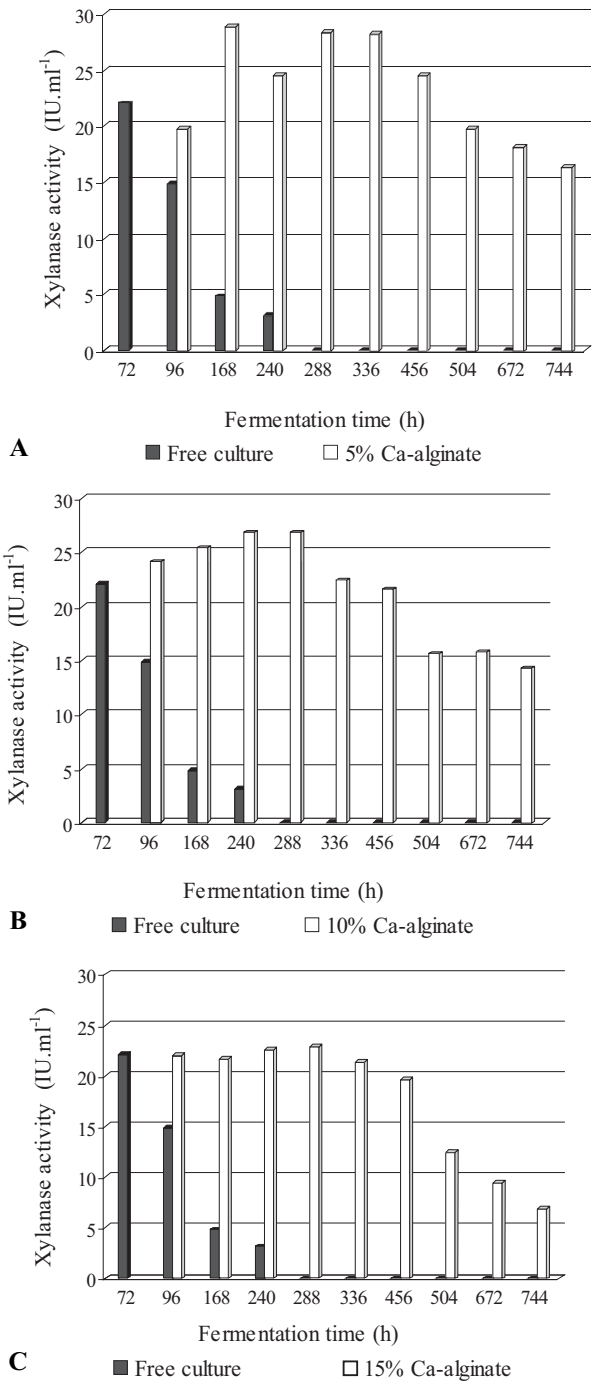

Fig. 1. Xylanase production by free and immobilized cultures of *Aspergillus awamori* K-1 in sol-gel hybrid matrices composed of TEOS, 5% xylan and different concentration of calcium alginate: A – 5% calcium alginate; B – 10% calcium alginate; C – 15% calcium alginate

gillus awamori K-1 regardless of its amount in the hybrid biocomposites. Immobilized cultures also retained their biosynthetic capabilities during long-term fermentation process as opposed to the free mycelium. The sol-gel matrices used in this research provided high porosity for better diffusion of nutrients and product. Therefore, this analysis determines hybrid matrices composed of TEOS, 5% xylan and 5, 10 and 15% calcium alginate as suitable for sol-gel immobilization of *Aspergillus awamori* K-1 in order increase xylanase production.

Conclusions

In summary, the fungal strain *Aspergillus awamori* K-1 was successfully immobilized in hybrid matrices composed of TEOS, 5% xylan and 5, 10 or 15% calcium alginate. The immobilized cultures were able to increase the capacity of xylanase production and retain their biosynthetic capabilities during continuous cultivation compared with free cell culture. The use of calcium alginate as an organic compound in the matrix formation also solves the diffusion problems in the interior of the support. Consequently sol-gel encapsulation could be considered as a promising technique for immobilization of *Aspergillus awamori* K-1 in hybrid matrices to produce enzymes of industrial importance, such as xylanase.

Acknowledgments

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