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ADHERENCE OF *ASPERGILLUS AWAMORI* K-1 AND *TRICHODERMA VIRIDE* SL-45 ON LOOFA SPONGE FOR PRODUCTION OF HYDROLASES

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Abstract

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In nature, filamentous fungi of the genus *Aspergillus* and *Trichoderma* are able to secrete a wide range of different enzymes into their environment. In recent years, one of the most important large-scale biotechnological applications of these fungi is the use as producers of xylanases and cellulases for pulp and paper industry. The cost of the enzymatic hydrolysis by microbial cells is one of the main factors limiting the economic feasibility of biotechnological manufacturers. In this context, the adaptation of cell immobilization techniques to an industrial scale and the use of cheap carriers would simplify the fermentation processes. Loofa (*Luffa cylindrical*) sponge appears to be an excellent carrier because it is biodegradable, does not require the use of chemical products and the immobilization method is very simple. In comparison with other carriers used for immobilization by cell adhesion, the density of loofa sponge is very low while the porosity and specific pore volume are very high. Furthermore, loofa sponge is stable over the whole range of pH and can be autoclaved many times without any visible change in the shape, structure and texture.

The purpose of the present investigation was to study the hydrolase activities immobilized cells of *Aspergillus awamori* K-1 and *Trichoderma viride* SL-45 on loofa sponge. Xylanase and cellulase production by immobilized cultures were evaluated during batch fermentation processes and compared with enzyme activities of free cell cultures. Immobilization technique used in the present study resulted in complete adherence of the cells and also provides higher hydrolase production.

Key words: Aspergillus, *Trichoderma,* xylanase, cellulose, loofa, immobilization

Introduction

Microbial xylanases are the preferred catalysts for xylan hydrolysis due to their high specificity, mild reaction conditions, negligible substrate loss, and side product generation. Lignocellulosic biomass has been projected to be one of the main resources for economically attractive biotechnological applications, and enzymatic hydrolysis is the most potent alternative process for the saccharification of its polymers. Commercial cellulases produced by species of the fungus *Trichoderma* have long been available for cereal foods, brewing, and fruit and vegetable processing and have also been widely evaluated and applied for bioethanol production processes. Cellulase is an enzyme complex capable of hydrolyzing cellulose into glucose molecules and xylanases degrade xylan, the main carbohydrate present in some hemicelluloses, into xylose (Sarkar et al., 2012; Perez et al., 2002). Industrial bioconversion of plant biomass is accomplished predominantly by the genera *Aspergillus* and *Trichoderma* known as efficient producers of extracellular hydrolases in high levels. On batch fermentation, filamentous fungi form strands of interlocking hyphae resulting in very viscous solutions, which lead to poor oxygen mass transfer. In this context, cell immobilization techniques using biological materials such as cellulose sponges have many advantages over suspended cell systems. It has been reported that loofa (*Luffa cylindrica*) sponge is an excellent carrier for immobilization of microorganisms. Luffa sponge is one of such commercially viable and environmen-

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tally acceptable biological material derived from fruit of *Luffa cylindrica* (LC) plant and having recycling capability and triggered biodegradability. The simplicity of the immobilization technique, the strong binding and the low cost of the loofa sponge can help to find future applications for whole cells immobilization (Amin and Mohamed, 2008; Kar et al., 2012).

The purpose of the present investigation was to study the hydrolase activities of *Aspergillus awamori* K-1 and *Trichoderma viride* SL-45 cells immobilized on loofa sponge. Xylanase and cellulase production by immobilized cultures were evaluated during batch fermentation processes and compared with enzyme activities of free cell cultures.

Materials and Methods

Microorganisms

The fungal strains *Aspergillus awamori* K-1 and *Trichoderma viride* SL-45 was obtained from the microbial 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 for four days.

Immobilization technique and fermentation conditions

The immobilization technique was carried out with 0.6 g loofa particles cut into 1 cm x 1 cm x 0.5 cm squares (Kar et al., 2012). The loofa particles were washed in distilled water prior to being sterilized in 500-ml conical flasks containing 100 cm^3 of Mandels medium (Mandels and Andreotii, 1976). The loofa particles were inoculated with spore suspensions of strain *Aspergillus awamori* K-1 and *Trichoderma viride* SL-45 in concentration of 10^7 spores/ml. Immobilized mycelium cultures were obtained during batch cultivation on rotary shaker at 28–30°C and 250 rpm for 24 hours.

Xylanase production was investigated during batch fermentation processes in 50 ml Czhapek fermentation medium up to 744th h at 28–30°C and 250 rpm. Immobilized cultures of *Trichoderma viride* SL-45 on loofa sponge were cultivated for production of cellulase up to 1032nd h on rotary shaker at 28–30°C and 250 rpm. Samples were collected periodically for determination of hydrolase activities.

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.

Cellulase assay

Endo-1,4-β-glucanase activity was detected on sodium carboxy-methyl cellulose (Na-CMC) as substrate according to Wood and Bhat (1978). Reaction mixtures, containing 0.5 ml 1% solution of Na-CMC in 0.05 M Sodium-acetate buffer (pH 4.8) and 0.5 ml enzyme solution, were incubated at 50°C for 30 min.

Results and Discussion

Xylanase activity of immobilized cultures of the fungal strain *Aspergillus awamori* K-1 was determined during batch fermentation process and compared with enzyme production of free cells (Figure 1). According to the results, xylanase activity of 29.43 IU.cm^{-3} was obtained from cultures immobilized in loofa dried fruit, at 696th h of the cultivation. Expression of xylanase activity by immobilized *Aspergillus awamori* K-1 is comparable to the enzyme activity of 29.18 IU.cm^{-3} detected at 168th h during the free cell cultivation.

Immobilized cultures of the fungal strain *Trichoderma viride* SL-45 on loofa sponge particles were also investigated for production of cellulase during batch fermentation process. Based on the natural organic structure of the loofa sponge, cellulase activity of the researched strain were determined during cultivation in basal fermentation medium containing microcrystalline cellulose and in modified medium without this substrate (Figure 2A and 2B).

From the results obtained cellulase activity of 21.86 IU.cm^{-3} were detected at 936th h during fermentation process in liquid medium containing microcrystalline cellulose as an inducing substrate. Expression of cellulose activity by immobilized culture on loofa sponge was found to be approximately 3-fold higher than the cellulase of 7.42 IU.cm^{-3} observed in free culture at 72nd h of the control cultivation (Figure 2A).

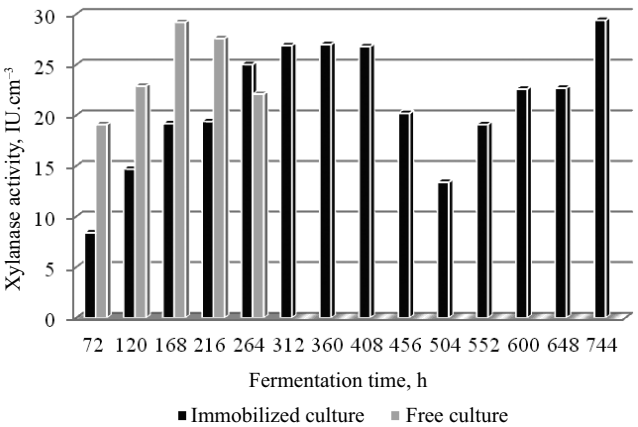

Fig. 1. Xylanase activity of free and immobilized *Aspergillus awamori* K-1 cells on loofa sponge

Biosynthetic capabilities of *Trichoderma viride* SL-45 immobilized cells on loofa were also investigated during cultivation in a medium without microcrystalline cellulose. Cellulase activity of 0.9 IU.cm^{-3} was obtained at 120th h of the fermentation process (Figure 2B). According to these results loofa sponge can be used as an inert matrix for immobilization of cellulase producing fungi and it is not utilized by the researched strain as an inducing substrate for biosynthesis of cellulose-degrading enzymes.

Based on the results of this study, loofa sponge has a positive effect on the productivity of immobilized strains *Aspergillus awamori* K-1 and *Trichoderma viride* SL-45. Xylanase activity of *Aspergillus awamori* K-1 immobilized cells is comparable with enzyme production of free cells but can be used repeatedly as a biocatalyst for production of this enzyme of interest.

Loofa sponge as a carrier used in this research provided high porosity for better diffusion of nutrients and product. This support is applicable as an inert support for immobilization of cellulase producing strain *Trichoderma viride* SL-45. The use of adsorption technique for immobilization this cellulase producing strain also provides higher levels of enzyme yield.

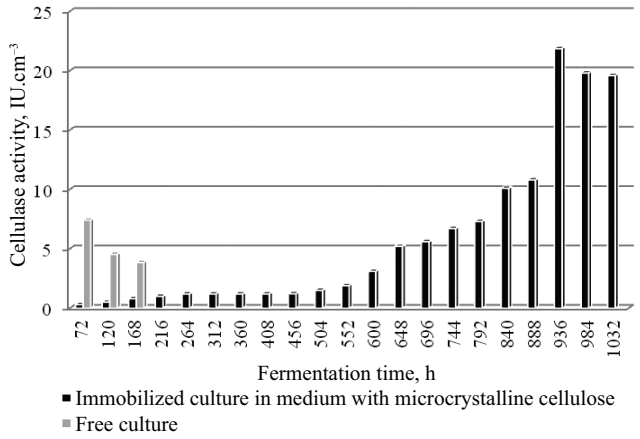

Fig. 2A. Cellulase activity of free and immobilized *Trichoderma viride* SL-45 cells on loofa sponge cultivated in medium with microcrystalline cellulose

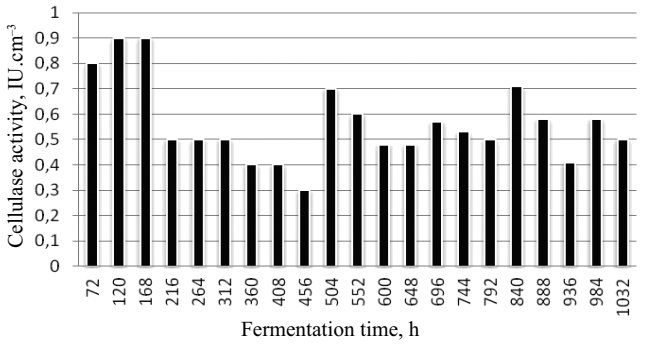

Fig. 2B. Variation of cellulase activity of immobilized *Trichoderma viride* SL-45 cells on loofa sponge cultivated in medium without microcrystalline cellulose

Immobilized cultures of the investigated strains retained their biosynthetic capabilities during fermentation process as opposed to the free cultures. Therefore, this study determines loofa dried fruit as suitable matrix for immobilization of *Aspergillus awamori* K-1 and *Trichoderma viride* SL-45 in order to increase xylanase and cellulase production.

Conclusion

In summary, the fungal strain *Aspergillus awamori* K-1 were successfully immobilized on loofa sponge particles. It was found that this method of immobilization was simple and had no detrimental effects on the growth or activity of the cells. The use of loofa sponge as an inducing substrate in comparison with microcrystalline cellulose for enhancement of cellulase activity was also investigated. The enzyme activities of immobilized *Aspergillus awamori K-1 cells* exhibited are comparable with freely suspended cells.

Immobilized cultures of *Trichoderma viride Sl-45* demonstrated higher cellulase production than free cells. Cultures had also retained their viability and enzyme biosynthetic activities during the fermentation processes. The porosity, low cost and retention of cell viability determined Luffa cylindrical sponge as a suitable matrix for immobilization of *Aspergillus awamori K-1* and *Trichoderma viride Sl-45* cells producing technologically important hydrolase enzymes.

Acknowledgment

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