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NATURAL AND MODIFIED ZEOLITES AS MATRICES FOR THE IMMOBILIZATION OF *TRICHODERMA VIRIDE* SL-45

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Abstract

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The cellulase produced by *Trichoderma* strains is regarded as the most suitable agent for hydrolysis of cellulose materials. *Trichoderma viride* is one of the best known producers of cellulolytic enzymes, synthesizing technologically significant quantities of the complete set of extracellular cellulases for the degradation of crystalline cellulose.

The meaning of the concept of "modification" of mineral systems is that important minerals (bentonites, natural zeolites, vermiculite, kaolinite) acquire new and useful practical properties after definitive treatment (chemical, tribochemical, thermal, ion exchange). Immobilization of microbial cells and enzymes is defined as the physical closing or localizing of intact cells or enzymes in a specific region of space, while the desired catalytic activity is retained. Characteristic for the immobilized bioobjects is that they can be used repeatedly and continuously. Many microorganisms have the ability to attach to different types of natural surfaces to ensure greater proximity with their substrate.

Natural zeolites are a group of minerals formed by deformation of volcanic ash in an alkaline environment. They have porous crystalline structure formed by interconnected channels in three different directions. The presence of free molecules of the alkali metals and water determine their unique properties – ion exchange capacity with a high selectivity and high ability for adsorption of liquids and gases. For the purpose of this experiment natural and modified zeolites are used. Four variants of immobilization of the studied strain are constructed by the method of adsorption with the using of natural and modified zeolites. A comparison is made between the classical medium of Mandels and a modified medium. Due to the inclusion of microelements in the matrix (modified zeolite) a correction of the salt composition of the classical medium is made.

Key words: Trichoderma; cellulase activity; immobilization; zeolite

Introduction

Microbiological technologies are based on the use of microorganisms which produce useful products and therefore, the aim is to increase their yield. *Trichoderma* is a *genus* of *fungi* that is of scientific interest as they secrete a range of enzymes, including cellulase. Cellulosolytic enzymes are involved in enzymatic hydrolysis of cellulose, one of the most abundantly occurring organic materials that can be converted to products with significant commercial interest. Bioconversion of cellulose to monomeric sugars has been intensively studied in the recent years to produce bioethanol and bio-based products, food and animal feeds, many valuable chemicals (Haapala et al., 1995; Adsul et al., 2007; Jäger et al., 2010; Adsul et al., 2011). A good strategy to improve a low stability of free enzymatic preparations with cellulase activity could be immobilization in/on inorganic porous supports (Paljevac et al., 2007; Takimoto et al., 2008; Dragomirescu et al., 2010).

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Scientific studies show that natural zeolites increase the enzymatic activity of soil, but the small opening of their pores prevents absorption of biomolecules. (Conception-Rosabal et al., 1997)

Immobilization of microorganisms on suitable materials is one of the modern methods in biotechnology. Binding cells of microorganisms has many advantages over the use of free cells, and to immobilized enzyme. In each immobilization procedure, the strive is to maintain the biosynthetic activity of strains at a maximum level. The purpose of this study was to investigate the effect of immobilization of strain *Trichoderma viride SL- 45* on natural and modified zeolites as inorganic matrices.

Materials and Methods

Microorganism and fermentation conditions

The object of this study is strain *Trichoderma viride* SL-45, being kindly provided from the microbiological collection of the *Department* of *Biotechnology,* Faculty of *Biology, Sofia University "St. Kliment Ohridski".*

The culture was maintained on potato dextrose agar at 28°C for four days. The inoculums were cultivated in 500 cm^3 flasks which contained 100 ml Mandels mineral salt medium (Mandels et al., 1974) with added 20% glucose and 1% maize extract at 28°C with constant shaking (250 rpm) for 24 hours. The fermentation mixture (50 ml) consisted of Mandels mineral salt medium with added 2% microcrystaline cellulose Micricel® and 1% wheat bran. Fermentation process for free and immobilized cells cultures was held at 28°C with constant shaking 250 rpm and the endo-1,4-β-glucanase (Cx) activity was measured at every 24 hours (Bara M.T. et al., 2003, Cohen-Kupiec et al., 1999)

Cellulase activity

The endo-1,4-β-glucanase activity was detected on sodium carboxy-methyl cellulose (Na-CMC) as a substrate according to Wood and Bhat (Wood and Bhat, 1988). The reaction mixture 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 was incubated at 50°C for 30 min.

Immobilization of strain Trichoderma viride Sl-45 in inorganic matrices (zeolites)

Natural and modified zeolites are used for the purpose of this investigation: Natural zeolites (25 g/l); Fe^{2+}-modified zeolites (25 g/l); Cu^{2+} -modified zeolites (25 g/l); natural and modified zeolites mixture (Fe^{2+} and Cu^{2+}) (20:5 g/l). Thus established variants were appropriately recalculated and added in Erlenmeyer flasks of 500 ml containing 50 ml of fermentation medium without wheat bran (Angelova et al., 2009). The flasks were inoculated with 5 ml of spore suspension. In order to adsorb the relevant spore culture, the flasks were incubated for 24 hours. For the subsequent fermentation was used flasks inoculated with 10 ml mycelium culture.

Fermentation process for free and immobilized cells cultures was held at 28°C with constant shaking 250 rpm and samples for analysis were periodically collected.

Using the adsorption method in natural and modified zeolites, four variants of strain immobilization were developed.

- Variant 1. Immobilization of spore suspension from strain *Trichoderma veride* SL-45 in classic Mandels' medium and nature zeolite.
- Variant 2. Immobilization of spore suspension from strain *Trichoderma veride* SL-45 in classic Mandels' medium and modified with iron ions zeolite.
- Variant 3. Immobilization of spore suspension from strain *Trichoderma veride* SL-45 in classic Mandels' medium and modified with copper ions zeolite.
- Variant 4. Immobilization of spore suspension from strain *Trichoderma veride* SL-45 in classic Mandels' medium and a mixture of natural and modified zeolites.

Results and Discussion

The cellulase activity was preserved and increased during the batch fermentation process (up to 840-th hours) and on the base of that we confirm the positive effect of the method of immobilization of the genus *Trichoderma* compared to other authors (Duff, 1988; Atttalla and Salleh, 2010).

The obtain result at Figure 1 shows cellulase activity of the free culture. The experiments revealed that the highest cellulase activity of the free strain *Trichoderma viride* SL-45 was 8.98 [U/ml] at the 120th hour.

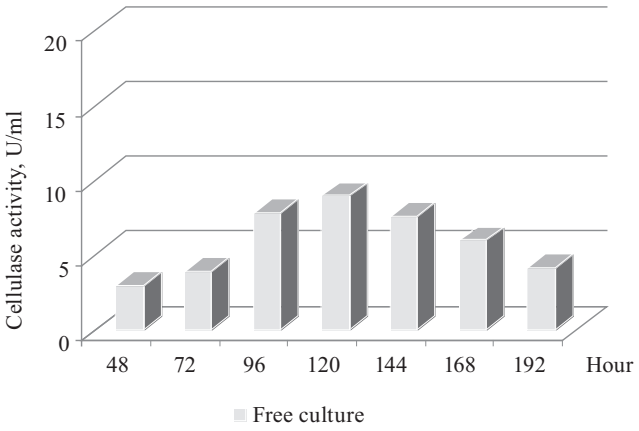

Fig. 1. Cellulase activity of free culture of *Trichoderma viride* SL-45

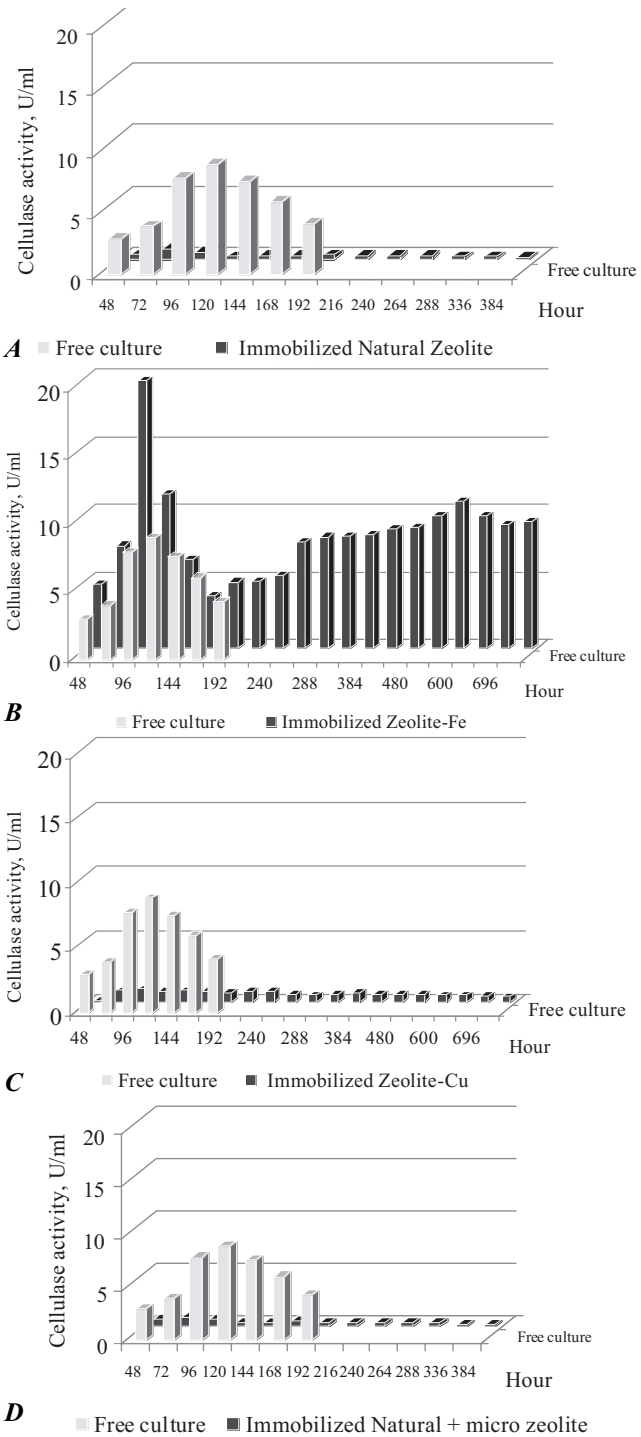

Fig. 2. Cellulase activity of free culture and immobilized *Trichoderma viride* SL-45 cultivated in Mandels medium with: A) natural zeolites; B) iron ions modified zeolite; C) copper ions modified zeolite and D) mixture of natural and modified zeolite

The presented results at Figure 2 A showed very low values of cellulase activity for Variant 1 (Immobilization of spore suspension from strain *Trichoderma veride* SL-45 on classic Mandels' medium and nature zeolite) at the 120^{-th} hours (0.19 [U/ml]), compared to the free culture (8.98 [U / ml]).

The results of Figure 2 B shows that the cellulase activity for Variant 2 (Immobilization of spore suspension from strain *Trichoderma veride* SL-45 on classic Mandels' medium and modified with iron ions zeolites) at the 120^{-th} hours (11.4 [U/ml]) this value was more than 1.5 times higher than that of the free culture.

The results presented in the Figure 2 C shows that Variant 3 (Immobilization of spore suspension from strain *Trichoderma veride* SL-45 on classic Mandels' medium and modified with copper ions zeolite) has lower enzyme activity. Comparing them with those of the free culture, they were 9 times lower.

The presented results for Variant 4 (Immobilization of spore suspension from strain *Trichoderma veride* SL-45 on classic Mandels' medium and a mixture of natural and modified zeolites) shows very low levels of cellulase activity, approximately 34-times lower than these of the free culture (Figure 2D).

Conclusions

The optimal result of the immobilization of the selected strain *Trichoderma viride* SL-45 was established at Variant 2 (Immobilization of spore suspension from strain *Trichoderma veride* SL-45 on classic Mandels' medium and modified with iron ions zeolite.). For all other newly developed variants, very low or no cellulase activity was reported. Based on these data it can be concluded that the natural Fe-modified zeolites proved suitable adsorbent carrier for strain *Trichoderma viride* SL- 45.

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