DYNAMICS OF CELLULASIC ACTIVITY IN MIXED CULTURES OF FUNGI BY USING PHYSICAL AND CHEMICAL PRETREATMENTS OF THE SUBSTRATE

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Abstract: The authors present the results concerning the physical and chemical (acid and alkaline) pretreatments on cellulasic activity (endoglucanase - E.C. 3.2.1.4., exocellobiohydrolase - E.C. 3.2.1.91., □-glucosidase - E.C. 3.2.1.21) by using mixed cultures of *Chaetomium globosum* and *Trichoderma viride*. It was observed that evolution of cellulases activity depends by the type of pretreatment, by the substrate utilized and by the culture age.

INTRODUCTION

It is generally known that cellulose is resistant to enzymic hydrolysis due to its high crystalline structure and lignin contents which block cellulolytic enzymes resulting in a slow and incomplete hydrolysis. For this reasons it is recommended the application of the pretreatments for cellulose materials, as well as the usage of species with high cellulolytic efficiency (R. Scriban, 1985).

In the continuation of our extensive research started several years ago, in which cellulolytic fungus monocultures were used, other studies concerning the stimulation of cellulolytic activity by using mixed fungus cultures has been performed. Scientific literature shows that biosynthesis of cellulasic enzymes are stimulated in mixed cultures.

Thus, Tofan Clemansa et al. (1988) showed that by using of mixed cultures of filamentous fungi (*Trichoderma viride*, *Aspergilus niger*) and yeast (*Saccharomyces cerevisiae* and *Candida robusta*) the endoglucanase and β-glucosidase activity is increased (compared to the values obtained in monocultures).

Cr. Simionescu et al. (1988) studied the biodegradation of the vegetal materials under the influence of mixed cultures of *Trichoderma viride*, *Trichoderma lignorum*, *Aspergillus niger*, *Chaetomium elatum*, *Candida utilis*, *Saccharomyces ellipsoideus*.

Tamas Juhasz et al. (2003) also noticed an enhancement of β-glucosidase activity in mixed cultures of *Aspergillus niger* BKMF 1305 and *Trichoderma viride* RUT C30 using waste paper as a natural carbon source.

The pretreatments are used in order to destroy the crystalline structure of cellulose as much as possible and also to expand the surface area and modify the polymerization level, which was also confirmed by other authors (Tofan and Segal, 1992).

This paper discusses the results concerning the physical and chemical (acid and alkaline) pretreatments on cellulasic activity: endoglucanase - E.C. 3.2.1.4., exocellobiohydrolase - E.C. 3.2.1.91., β-glucosidase - E.C. 3.2.1.21 in a new combination of mixed cultures (*Trichoderma viride* and *Chaetomium globosum*).

MATERIALS AND METHODS

The research was effectued with *Chaetomium globosum* and *Trichoderma viride* from the Biological Research Institute of Iasi collection. For these studies, the species were cultivated on Czápek-Dox medium with the following formula: $NaNO_3$ – 3g, H_2PO_4 – 1g, $MgSO_4 \times 7H_2O$ – 0,5g, KCl - 0,01g, $FeSO_4 \times 7H_2O$ - 0,01g, sucrose– 40g, distilled water –1000 ml.

In this medium the carbon source (sucrose) was replaced with 4g residuals (wheat straw and maize stalk). Before adding into the culture medium, residuals were physically (mechanically) and chemically (alkaline and acid) pretreated.

The mechanical pretreatment was performed by fine grinding of the two residual types. The acid pretreatment was performed with HCl – 3% for 30 minutes at 1200C, then fast and abundantly washed with

distilled water. Alkaline pretreatment was realized with 2% NaOH for 30 minutes at 120°C, followed by a distilled water wash.

At the end, the following working variants were obtained: V1– maize stalk (mechanical pretreatment), V2– maize stalk (alkaline pretreatment), V3 – maize stalk (acide pretreatment), V4– wheat straw (mechanical pretreatment), V5– wheat straw (alkaline pretreatment), V6 – wheat straw (acid pretreatment).

The estimation of endo-β-1,4-D-glucanase activity was made by Peterson method which consist in testing of hydrolytic action of enzymic mixture on the filter paper Whatman no. 1. The resulted sugars were measured using 3,5-dinitrosalicilic acid (DNS) reagent.

For measure the exocellobiohydrolase activity we used Peterson and Porath method which consist in dosing freed sugars by enzyme from the carboxymetilcellulose substrate with 3,5-dinitrosalicilic acid (DNS) reagent.

The dossage of the β-glucosidase activity is based on determination of the increasing reductive power of the medium with DNS reagent. The cellulolytic activity was measured at 7 and 11 days from inoculation.

RESULTS AND DISCUSSION

The results concerning the pretreatments influences on cellulasic activity are presented in figures 1-6.

Thus, the data concerning the influence of different pretreatments on enduglucanase activity in mixed cultures of *Chaetomium globosum* and *Trichoderma viride* cultivated on maize stalk medium are presented in Figure 1 which shows that after 7 days from inoculation, the enzymic activity had the highest value at V1 – 0,0342 U/ml/h followed by V2 - 0,0134 U/ml/h and V3 - 0,009 U/ml/h. At 11 days from inoculation, the enzymic activity increased in all medium variants, but the best value was observed again for V1 - 0,202 U/ml/h, variant followed by V2 - 0,1385 U/ml/h and V3 - 0,1307 U/ml/h.

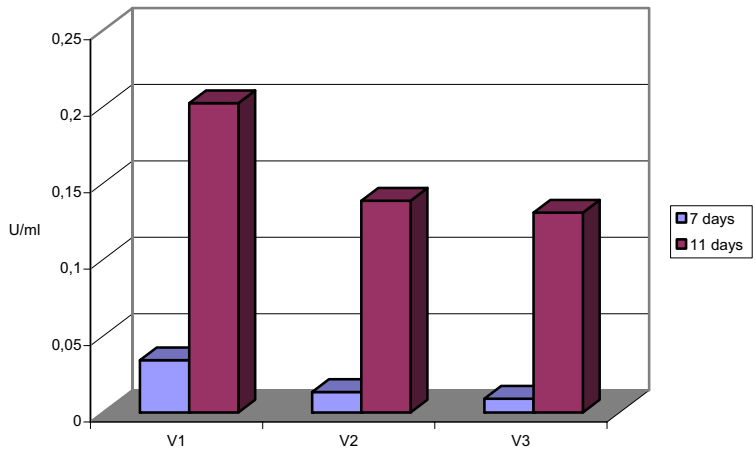

Fig. 1 The influence of the pretreatments on endoglucanase activity in mixed cultures of *Chaetomium globosum* and *Trichoderma viride* cultivated on maize stalk medium

The results concerning the pretreatment influence on endoglucanase activity in mixed culture of *Chaetomium globosum* and *Trichoderma viride* cultivated on media with wheat straw are shown in figure 2. After 7 days from inoculation, the maximum value of this enzyme was observed at V4 – 0,0488 U/ml/h, followed by V5 – 0,0194 U/ml/h and V6 – 0,031 U/ml/h. After 11 days from inoculation endoglucanase activity enhanced in all variants: V4 – 0,1511 U/ml/h, V5 – 0,1261 U/ml/h and V6 – 0,1379 U/ml/h.

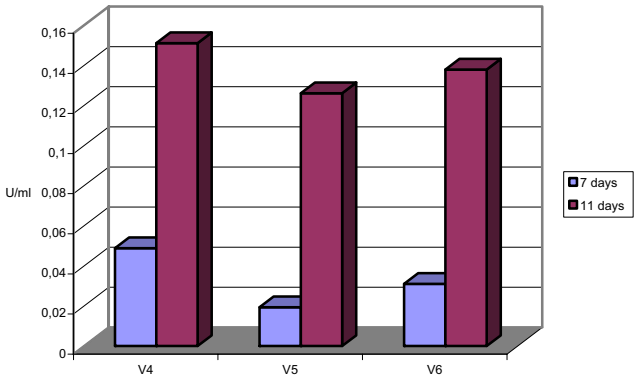

Fig. 2 The influence of the pretreatments endoglucanase activity in mixed cultures of fungi *Chaetomium globosum* and *Trichoderma viride* cultivated on wheat straw medium

By analyzing the dynamic of endoglucanase activity of mixed cultures on the two types of substrates utilized, it was observed that in all variants this was much bigger at 11 days compared to that at 7 days from inoculation as follows: V1 – from 0,0342 U/ml/h to 0,2023 U/ml/h; V2 – from 0,0134 U/ml/h to 0,1387 U/ml/h; V3 – from 0,009 U/ml/h to 0,137 U/ml/h; V4 – from 0,0488 U/ml/h to 0,1511 U/ml/h; V5 – from 0,0194 U/ml/h to 0,1261 U/ml/h; V6 – from 0,0130 U/ml/h to 0,1379 U/ml/h.

In figures 3 and 4 are presented the data concerning exocellobiohydrolase under physical and chemical pretreatments. The activity of this enzyme was observed only at V4 at 7 days from inoculation. In all other variants, the exocellobiohydrolase activity had zero value, at both 7 and 11 days from inoculation.

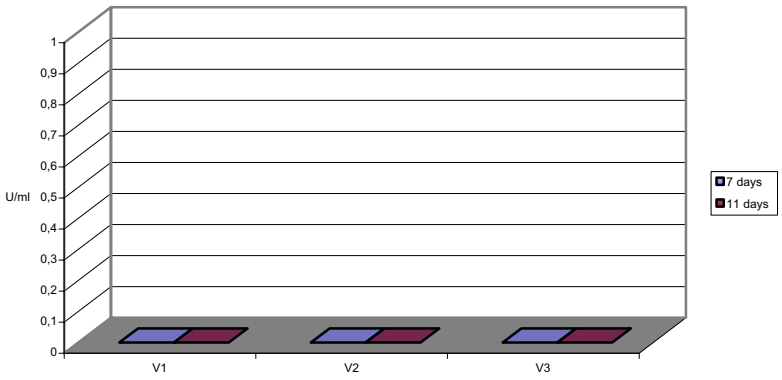

Fig. 3 The influence of the pretreatments on exocellobiohydrolase activity in mixed cultures of fungi *Chaetomium globosum* and *Trichoderma viride* cultivated on maize stalk medium

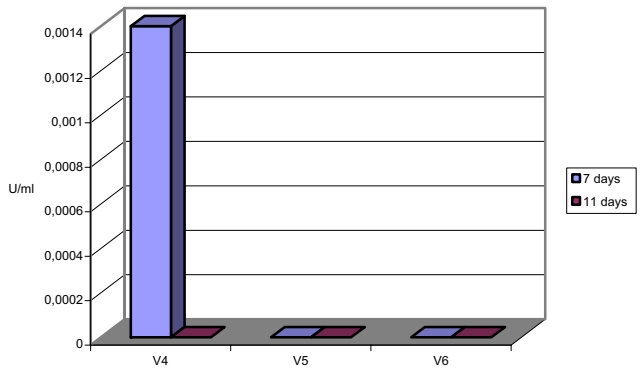

Fig. 4 The influence of the pretreatments on exocellobiohydrolase activity in mixed cultures of fungi *Chaetomium globosum* and *Trichoderma viride* cultivated on wheat straw medium

The dynamics of the β-glucosidase activity in mixed cultures of *Chaetomium globosum* and *Trichoderma viride* cultivated on maize stalk medium which were previously physically and chemically pretreated is presented in the figure 5. At 7 days from inoculation the maximum enzymic activity was observed at V1 – 0,0047 U/ml/min., followed very closely by V2 - 0,0042 U/ml/min. and V3 – 0,002 U/ml/min. At 11 days from inoculation enzymic activity increased in all variants as follows: V1 – 0,041 U/ml/min., V2 – 0,035 U/ml/min., V3 – 0,034 U/ml/min.

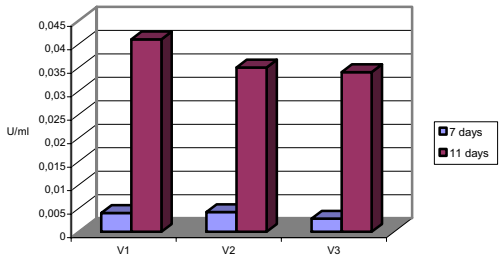

Fig. 5 The influence of the pretreatments on β-glucosidase activity in mixed cultures of fungi *Chaetomium globosum* and *Trichoderma viride* cultivated on maize stalk medium

The results concerning the pretreatments influence on β-glucosidase activity in mixed cultures of those two fungi cultivated on wheat straw medium are shown in the figure 6. At 7 days from inoculation at V5 it was recorded the maximal activity of this enzyme - 0,0051 U/ml/min., while at V6 - 0,0042 U/ml/min. and finally at V4 - zero. At 11 days from inoculation the values were superior those from 7 days as follows: V4 – 0,0395 U/ml/min., V5 – 0,0364 U/ml/min., V6 – 0,0361 U/ml/min.

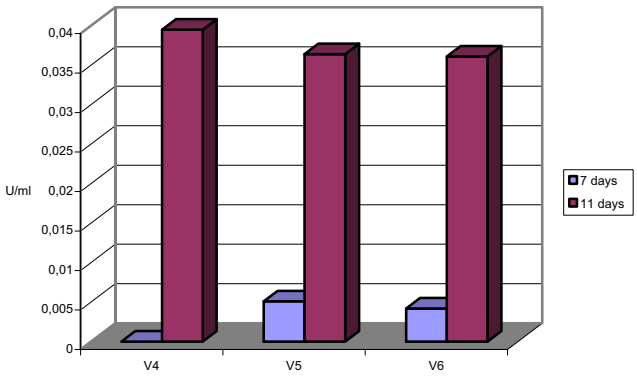

Fig. 6 The influence of the pretreatments on β-glucosidase activity in mixed cultures of fungi *Chaetomium globosum* and *Trichoderma viride* cultivated on wheat straw medium

By analyzing β-glucosidase evolution in function of culture age it was observed that at 11 days from inoculation the activity of this enzyme was bigger compared to that at 7 days: V1 – activity of β-glucosidase increased from 0,0047 U/ml/min. to 0,0419 U/ml/min., V2 – from 0,0042 U/ml/min. to 0,0358 U/ml/min., V3 –from 0,0028

U/ml/min. to 0,0341 U/ml/min., V4 – from 0 to 0,0395 U/ml/min., V5 – from 0,0051 U/ml/min. to 0,0364 U/ml/min., V6 – from 0,0042 U/ml/min. to 0,0361 U/ml/min.

CONCLUSIONS

In all variants taken in study cellulasic activity was maximal under physical pretreatment utilization.

The values of the cellulasic activity were nonuniform when chemical pretreatment was performed and in all variants also varied with culture age.

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