THE INFLUENCE OF THE STATIC MAGNETIC FIELD (SMF) ON SOME BIOCHEMICAL PARAMETERS IN CELLULOLYTIC FUNGI *CHAETOMIUM GLOBOSUM* AND *TRICHODERMA VIRIDE* CULTIVATED ON MEDIA SUPPLEMENTED WITH PANIFICATION INDUSTRIAL WASTES

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This paper presents the influence of the static magnetic field (SMF) on cellulases: (endo-1,4-β-glucanase – E.C. 3.2.1.4., cellobiohydrolase – E.C. 3.2.1.91. and β-glucosidase – E.C. 3.2.1.21.), peroxidase – E.C. 1.11.1.7. and catalase – E.C. 1.11.1.6. activity in cellulolytic fungi *Chaetomium globosum* and *Trichoderma viride* cultivated on media with waste from industry of panification. The activity of these enzymes was influenced both by the cellulolytic species studied and by the time of exposure to the static magnetic field (SMF).

Key words: cellulolytic fungi, *Chaetomium globosum*, *Trichoderma viride*, static magnetic field, cellulases, catalase, peroxidase.

INTRODUCTION

The cellulose constitutes the major form of stocking glucose obtained through photosynthesis and at the same time the major component of solar energy conversion to the biomass. The cellulose is also a major constituent of all the vegetal materials and that is why it is the most abundant organic material in nature, which is renowned every year. Because of its highly ordered structure, the cellulose is very hard to be degraded and that is why it is unusable and stocked in nature as waste.

Lately, because of the energetic crisis and of the environment pollution, the notion of waste was reevaluated and it was accentuated the reuse of the cellulolytic wastes, some in particular, for the purpose of insuring the abundant and cheap resources in different biotechnological processes.

The chemical hydrolysis of cellulose into glucose was achieved for the first time with an efficiency of 96.5 % utilizing fumans chlorhydric acid. This was considered as a success because it was the first practical demonstration of the possibility of quantitative transforming of cellulose into glucose, although the chemical inertia of this polysaccharide was known in the saccharification process.

The chemical hydrolysis of cellulose did not become the basis of a technological process because of the high price of a hydrolysis agent and inclusively of the impossibility of its recovery, the decrease of global efficiency of the process through transformations of the final product because of the hydrolysis agent, the difficulty of rapid separation of glucose of the hydrolysis agent and then of the products appeared at the neutralisation, etc.

All these aspects could have led to giving up cellulose as a source of glucose, but seeing the fact that cellulose is the most abundant natural organic component in nature the idea of hydrolysis was an objective on which no one could give up. In those conditions it was imposed a new strategy, namely enzymatic hydrolysis of cellulose with the help of cellulolytic microorganisms.

The capacity to degrade the natural cellulose implies the synthesis of the entire cellulolytic system. The cellulases system consists of three types of enzymes: endo-1,4-β-glucanase (E.C. 3.2.1.4.), cellobiohydrolase (E.C. 3.2.1.91) and β-glucosidase (E.C. 3.2.1.21) (31, 36). No enzyme can determine by itself the extensive degradation of cellulose. So, it was demonstrated that all the micro organisms which can degrade the crystalline cellulose produce systems of cellulases more or less complex which are made of enzymes with specifications and way of action different which act in a cooperate way. They produce some cellulolytic enzymes, but they lack one of the essential enzymes.

As part of the complex biological, biochemical and biophysical studies accomplished in our laboratories on the cellulolytic fungi (*Chaetomium globosum, Trichoderma viride* and *Alternaria alternata*), the main efforts of the latest have been directed towards the influence of chemical and physical agents on some enzymes within this microorganism cell. So, we carried out experimental research concerning the modifications determined at the level of the biosynthesis of the cellulases complex by the aminoacids (11), centimetric waves (17), ferrofluids (15, 16), mineral nitrogen (10), trace elements (12), water-soluble vitamins (13), pH and temperature (14) and cultivated on media with different waste (18, 19). Such investigation clearly showed that the enzymatic activity is individualized function of the enzyme type, culture age and external factors. These data confirmed the conclusions of other authors concerning the factors influencing cellulases activity in several fungi (2, 3, 4, 6, 7, 9, 22, 23, 25, 26, 28, 29, 30, 33).

In this paper we carried out the influence of the static magnetic field (SMF) on cellulolytic fungi *Chaetomium globosum* and *Trichoderma viride*. The importance of studying the effects of the magnetic field upon the biological organisms was concretized by the appearance of a scientific branch – the biomagnetism. The magnetic fields are of two types: static (SMF) and oscillating (OMF). In case of static magnetic fields, the intensity is constant in time, and in case of oscillating magnetic fields, the intensity is varied, the constant amplitudes alternating with sinusoidal amplitudes. N. Yoshimura (35) classified the effects of magnetic fields on microbial growth and reproduction as inhibitory, stimulatory and non-observable. In the speciality literature, there are some papers regarding the influence of the magnetic field on the metabolism of the cellulolytic microorganisms. At international level, the researches regarding the influence of the magnetic field on the microorganisms have been conducted since 1937, when G.C. Kimball showed that the wine yeasts cells were not affected by exposure for

5, 10, 20, 40, 80 minutes to the magnetic field (8). V.F. Gerencser *et al.* (5) showed that the growth rate of the *Staphylococcus aureus* species increases further to the exposure to a magnetic field, for 3–6 hours, but the exposure for 7 hours does not have any effect; the growth rate of the *Serratia marcescens* species remains the same further to a 6 hours exposure to the magnetic field, but it increases following an exposure longer than 6 hours. F.E. Van Nostran *et al.* (34) studied the effects of high magnetic fields on the multiplication of *Saccharomyces cerevisiae*, and R.L. Moore (21) showed that the influence of the static magnetic field has stimulating effects on the growth of *Escherichia coli*, remarking that the growth stimulation is directly proportional to the increase of the magnetic field frequency. K. Tsuchiya *et al.* (32) studied the effect of the magnetic field on the growth and development of *Escherichia coli*; also, Romana Ruzic (27) performed exhaustive researches regarding the influence of the magnetic field on *Pisolithus tinctorius*.

MATERIAL AND METHODS

The researches were performed with cellulolytic species *Chaetomium globosum* and *Trichoderma viride*, selected within the Department of Microbiology of the Biological Reasearch Institute of Iaşi. For the research regarding the influence of the static magnetic field upon the activity cellulases, peroxidase and catalase, there was used the Czapek modified liquid culture medium ($NaNO_3$ – 3 g, KH_2PO_4 – 0.1 g, $MgSO_4 \cdot 7H_2O$ – 0.5 g, KCl – 0.5g, $FeSO_4 \cdot 7H_2O$ – 0.01g, 40 g wheat bran, 1 000 ml distilled water) which was seeded with 0.8 cm diameter disks from a 7 days culture of *Chaetomium globosum* and *Trichoderma viride*. The flasks containing the culture media with cellulolytic species were exposed for 7 days and 14 days, respectively, to the action of the static magnetic field having a magnetic induction with 80 mT, the following variants resulting: V_1 – *Chaetomium globosum* – control, V_2 – *Chaetomium globosum* – exposed to static magnetic field, V_3 – *Trichoderma viride* – control, V_4 – *Trichoderma viride* – exposed to static magnetic field. The activity of the cellulolytic complex was determined from the culture liquid and the peroxidase and catalase from the fungi mycelium and from the culture liquid. The endo-β-1,4-D-glucanase activity was performed by the Peterson method, the cellobiohydrolase activity by the Peterson and Porath method and the determination of the β-glucosidase activity was based on the determination of the increase of the medium reducing power, with DNS reactive agent, further to the hydrolytic action of the enzyme on the cellobiose (24). The determination of the catalase activity was performed by the Artenie and Tănase method (1) and the peroxidase activity by the K.M. Möller method (20).

RESULTS AND DISCUSSION

Cellulases activity

The results regarding the influence of the static magnetic field on the endo-β-1,4-D-glucanase in the *Chaetomium globosum* are presented in figure 1, resulting that, at 7 days from the seeding the value of this enzyme was 0.0363 U/ml at control and 0.0093 U/ml at the variant exposed to the action of the magnetic field. In 14 days from seeding, the activity of this enzyme was of 0.1834 U/ml in the control and 0.1030U/ml in the variant exposed to the magnetic field. From these data results that in both time intervals, the static magnetic field had an inhibitory effect upon the activity of this enzyme. However, dynamically analyzing the activity of the endo-β-1,4-D-glucanase in time it was established that while in the control the activity of this enzyme increased 7 times at 14 days comparatively to 7 days, in the variant exposed to the static magnetic field, the activity of this enzyme increased 11 times compared to the values from 7 days from seeding. In the same figure, there are shown the data regarding the influence of the static magnetic field on the endo-β-1,4-D-glucanase activity in *Trichoderma viride*, wherefrom resulting that at 7 days from seeding, this factor had a stimulating effect, the value of this enzyme being of 0.0526 U/ml in the variant exposed to the magnetic field compared with 0.0426 U/ml previously found in the control. In 14 days from seeding the activity of this enzyme had zero values in both variants.

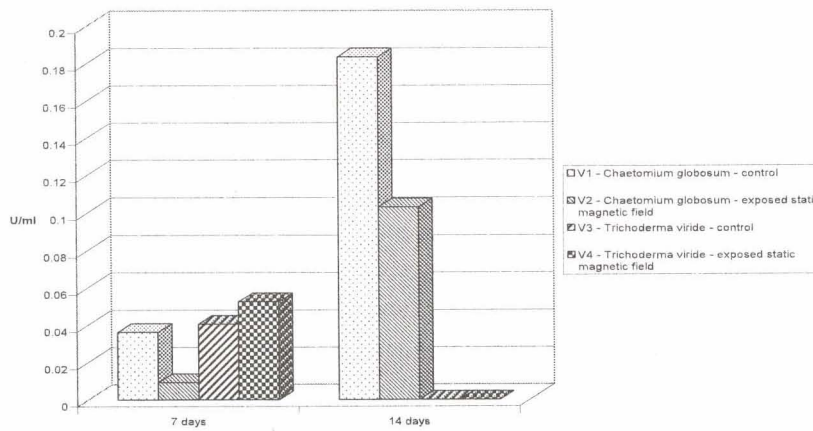

Fig. 1. The influence of static magnetic field on endo-β-1,4-D-glucanase activity in *Chaetomium globosum* and *Trichoderma viride* (U/ml).

The cellobiohydrolase activity in *Chaetomium globosum* is presented in Fig. 2, wherefrom results that at 7 days from seeding, the value of this enzyme was zero both in the control and in the variant exposed to the static magnetic field. In 14 days from seeding the value of this enzyme activity was 0.0303 U/ml in the

control and 0.0242 U/ml in the variant exposed to the static magnetic field, finding a weak inhibitory effect in the variant exposed to the action of the magnetic field. Figure 2 shows the cellobiohydrolase activity in *Trichoderma viride* wherefrom results that in 7 days from seeding the following values of this enzymes were determined: in control – 0.2286 U/ml, in the variant exposed to static magnetic field – 0.3076 U/ml, which represents an increase of 34.46% under the influence of the static magnetic field. In 14 days from seeding, the value of this enzyme was 0.1360 U/ml in the control and 0.3051 U/ml in the variant exposed to static magnetic field, thus an increase of 124% comparatively to the control. Analyzing the evolution of this enzyme dynamically shows a value decrease, in 14 days comparatively to 7 days from seeding, from 0.2280 U/ml to 0.130 U/ml in the control (a decrease of 40.41%), and from 0.3076 U/ml to 0.3051 U/ml in the variant exposed to the magnetic field (a decrease of only 0.81%).

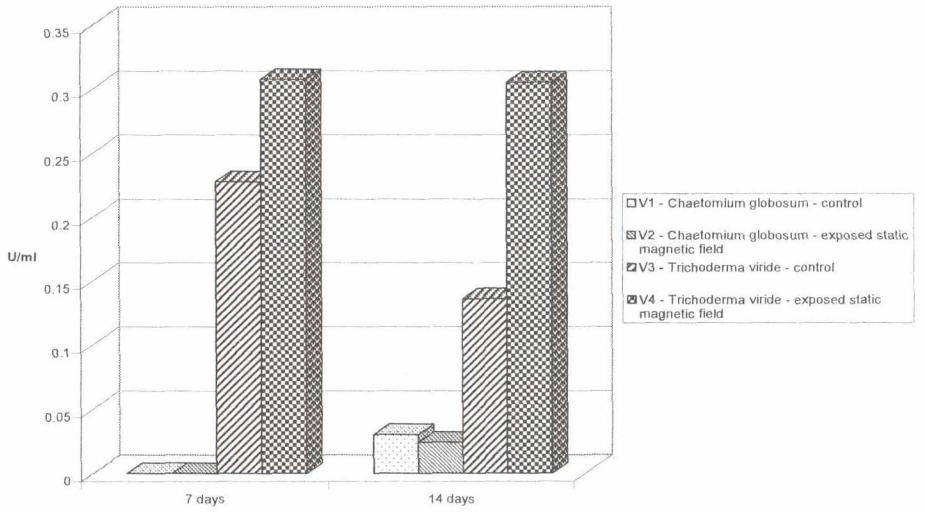

Fig. 2. The influence of static magnetic field on cellobiohydrolase activity in *Chaetomium globosum* and *Trichoderma viride* (U/ml).

Figure 3 shows the data regarding the influence of the static magnetic field on the β-glucosidase in *Chaetomium globosum*, wherefrom it shows that at 7 days from seeding the values of this enzyme were: 0.0072 U/ml in the control and 0.0147 U/ml in the variant exposed to the static magnetic field, thus an increase by 204%. In 14 days from seeding, the activity of this enzyme was of 0.0208 U/ml in the control and of 0.0053 U/ml in the variant exposed to the magnetic field, which represents a decrease of 74.52%. Analyzing the dynamics of this enzyme, an increase in control in 14 days from seeding comparatively with 7 days was found (from 0.0072 U/ml to 0.0208 U/ml), representing an increase of 288%; in the

exposed variant to magnetic field, the value of this enzyme decreased from 0.0147 U/ml in 7 days from seeding to 0.0053 U/ml in 14 days from seeding, a decrease of 64%.

The results regarding the influence of the static magnetic field on the β-glucosidase in *Trichoderma viride* are shown in figure 3, too, finding that in 7 days from seeding, the values of this enzyme were the following: the control – 0.0188 U/ml, the variant exposed to static magnetic field – 0.0477 U/ml, representing an increase by 254 % of the activity of this enzyme. In 14 days from seeding, the activity of this enzyme was in control – 0.0382 U/ml and – 0.0132 U/ml in the variant exposed to magnetic field, resulting that, in this time interval, the stimulating effect of the magnetic field was no longer maintained. Analyzing the dynamics of this enzyme, an increase at 14 days from seeding was found compared to 7 days from seeding, from 0.0188 U/ml to 0.0382 U/ml in the control variant and a decrease from 0.0477 U/ml to 0.0132 U/ml in the variant exposed to the action of the static magnetic field.

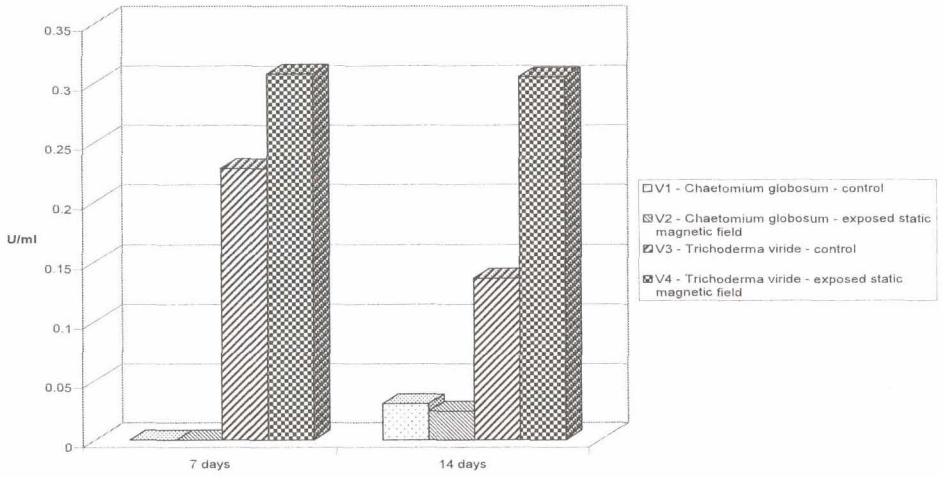

Fig. 3. The influence of static magnetic field on β-glucosidase activity in *Chaetomium globosum* and *Trichoderma viride* (U/ml).

Peroxidase activity

The results concerning the influence of the magnetic field on the peroxidase activity in *Chaetomium globosum* mycelium are shown in figure 4, wherefrom it results in 7 days from seeding that the values of this enzyme were the following: 17.61 UP/g × 10^{-3} in control and 8.56 UP/g × 10^{-3} in that exposed to magnetic field. This decrease of the peroxidase activity under the influence of the static magnetic field confirms the fact that this factor does not have stressing effect upon the biochemical processes within the cell, the increase of the activity of this enzyme being known under the conditions of involvement of certain disturbing

factors. In 14 days from seeding, the value of this enzyme was of 18.81 UP/g × 10^{-3} in the control and of 7.91 UP/g × 10^{-3} in the variant exposed to the magnetic field. Analyzing the dynamics of the evolution of this enzyme depending on the culture age it is found that in control, the peroxidase activity decreased from 8.56 UP/g × × 10^{-3} in 7 days from seeding to 7.91 UP/g × 10^{-3} in 14 days from seeding. In the same figure, the data concerning the influence of the static magnetic field upon the peroxidase activity in *Trichoderma viride* mycelium, wherefrom it results that in 7 days from seeding the values of this enzyme were the following: 7.57 UP/g × 10^{-3} in the control and 2.26 UP/g × 10^{-3} in the variant exposed to the magnetic field; in 14 days from seeding, the value of this enzyme was zero, these data confirming that in this species the exposure to the magnetic field was not a stressing factor upon the fungi metabolism.

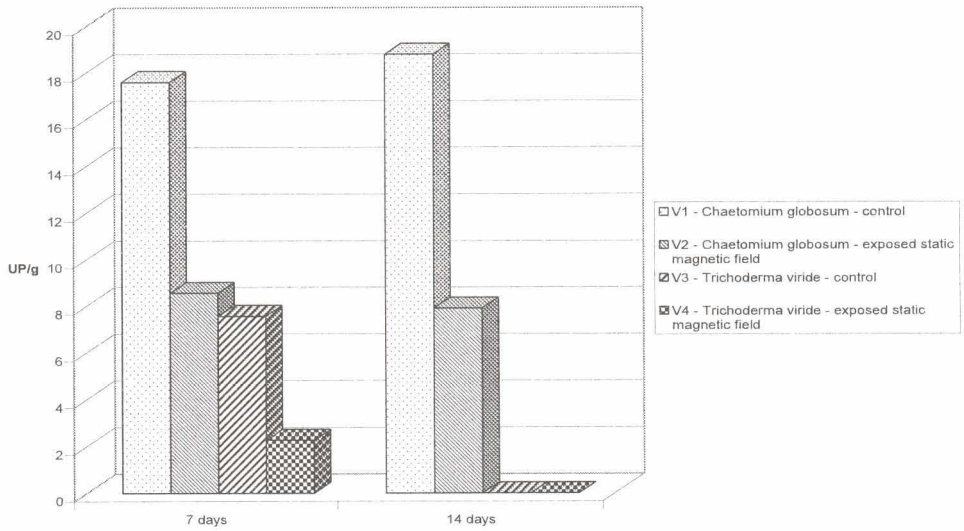

Fig. 4. The influence of the static magnetic field on the peroxidase activity in *Chaetomium globosum* and *Trichoderma viride* – mycelium (UP/g × 10^{-3}).

Figure 5 shows the results regarding the influence of the static magnetic field upon the peroxidase activity in the culture liquid at *Chaetomium globosum*, wherefrom it results that in 7 days from seeding the value of this enzyme was zero in the control and of 2.09 UP/ml × 10^{-3} in the variant exposed to the static magnetic field. In 14 days from seeding, the activity of this enzyme was of 0.12 UP/ml × 10^{-3} in control and of 1.22 UP/ml × 10^{-3} in the variant exposed to the static magnetic field. The dynamics of the evolution of this enzyme highlights that, in control, the peroxidase activity increased from zero in 7 days from seeding to 0.12 UP/g × 10^{-3} in 14 days from seeding. In the variant exposed to the static magnetic field the

peroxidase activity decreased from 2.09 UP/g × 10^{-3} in 7 days from seeding to 1.22 UP/g × 10^{-3} in 14 days from seeding.

The results regarding the influence of the static magnetic field on the peroxidase activity in the culture liquid in the *Trichoderma viride* are presented in figure 5, too, wherefrom it results that in 7 days from seeding the value of this enzyme was of 0.42 UP/ml × 10^{-3} in the control and of 0.80 UP/ml × 10^{-3} in the variant exposed to the magnetic field. In 14 days from seeding the activity of this enzyme was zero in both variants. Figure 5 shows the results regarding the influence of the static magnetic field upon the peroxidase activity in the culture liquid at *Chaetomium globosum*, wherefrom it results that in 7 days from seeding the value of this enzyme was zero in the control and of 2.09 UP/ml × 10^{-3} in the variant exposed to the static magnetic field. In 14 days from seeding, the activity of this enzyme was of 0.12 UP/ml × 10^{-3} in the control and of 1.22 UP/ml × 10^{-3} in the variant exposed to the static magnetic field. The dynamics of the evolution of this enzyme highlights, that in the control, the peroxidase activity increased from zero in 7 days from seeding to 0.12 UP/g × 10^{-3} in 14 days from seeding. In the variant exposed to the static magnetic field the peroxidase activity decreased from 2.09 UP/g × 10^{-3} in 7 days from seeding to 1.22 UP/g × 10^{-3} in 14 days from seeding. The results regarding the influence of the static magnetic field on the peroxidase activity in the culture liquid in the *Trichoderma viride* are presented in Figure 5, too, wherefrom it results that in 7 days from seeding the value of this enzyme was of 0.42 UP/ml × 10^{-3} in the control and of 0.80 UP/ml × 10^{-3} in the variant exposed to the magnetic field. In 14 days from seeding the activity of this enzyme was zero in both variants.

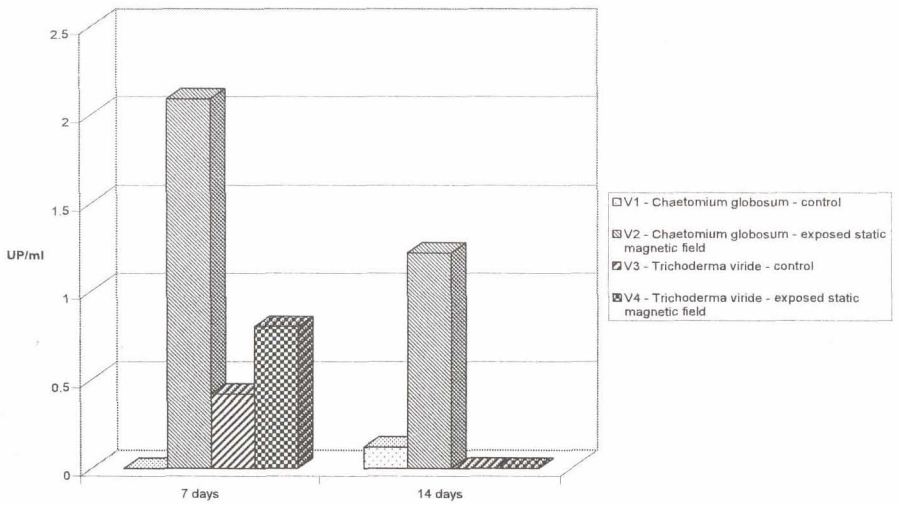

Fig. 5. The influence of the static magnetic field on peroxidase activity in *Chaetomium globosum* and *Trichoderma viride* – culture liquid (UP/ml × 10^{-3}).

Catalase activity

The data regarding the influence of the static magnetic field upon the catalase activity in the *Chaetomium globosum* mycelium are presented in figure 6, finding that in 7 days from seeding, the value of this enzyme was of 41.71 UC/g in the control, decreasing in the variant exposed to the magnetic field to 39.18 UC/g. In 14 days from seeding, this enzyme had the maximum value in the control, too – 44.19 UC/g, while the variant exposed to the action of the magnetic field the value of this enzyme decreased to 36.41 UC/g. Following the dynamics of this enzyme depending on the culture age, the catalase activity of the control was found to have decreased from 41.71 UC/g in 7 days from seeding to 44.19 UC/g in 14 days from seeding, while in the variant exposed to the magnetic field, the catalase activity decreased from 39.18 UC/g in 7 days from seeding to 36.41 UC/g in 14 days from seeding.

In the same figure the data regarding the influence of the static magnetic field on the catalase activity in the *Trichoderma viride* mycelium are presented, finding that in 7 days from seeding, the value of this enzyme was of 41.00 UC/g in the control, decreasing from the variant exposed to the magnetic field to 25.00 UC/g. In 14 days from seeding, the magnetic field had the same stimulating effect, the activity of this enzyme increasing from 36.00 UC/ml in the control to 64.00 UC/g in the variant exposed in the static magnetic field.

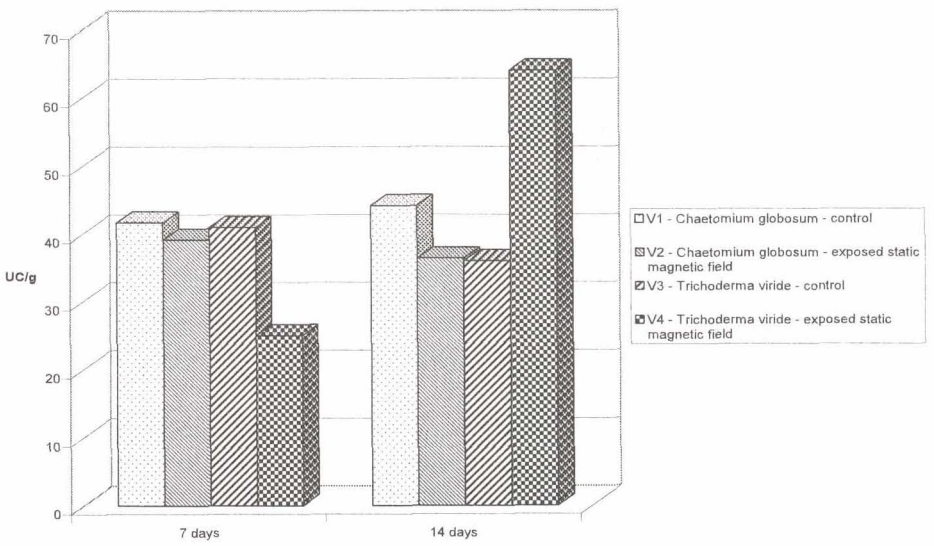

Fig. 6. The influence of static magnetic field on catalase activity in *Chaetomium globosum* and *Trichoderma viride* – mycelium (UC/g).

The results regarding the influence of the magnetic field upon the catalase activity in the culture liquid in *Chaetomium globosum* are presented in Fig. 7,

whereby it is found that in 7 days from seeding, these were: in the control – 28.00 UC/ml and in the variant exposed to the static magnetic field – 12.00 UC/ml. In 14 days from seeding, the activity of this enzyme had a value – 42.00 UC/ml in the control, while in the variant exposed to the magnetic field, the value decreased to 27.00 UC/ml. The evolution of this enzyme activity shows the increase in the control – 28.00 UC/ml in 7 days from seeding to 42.00 UC/ml in 14 days from seeding, and in the variant exposed to the static magnetic field the increase from 12.00 UC/ml in 7 days from seeding to 27.00 UC/ml in 14 days from seeding.

The values of the catalase activity in the culture liquid in *Trichoderma viride* are presented in the same figure, wherefrom it is found that in 7 days from seeding these were: in the control – 8.00 UC/ml and in the variant exposed to magnetic field – 26.00 UC/ml. In 14 days from seeding the activity of this enzyme decreased both in the control (3.00 UC/ml) and in the variant exposed to the magnetic field (0).

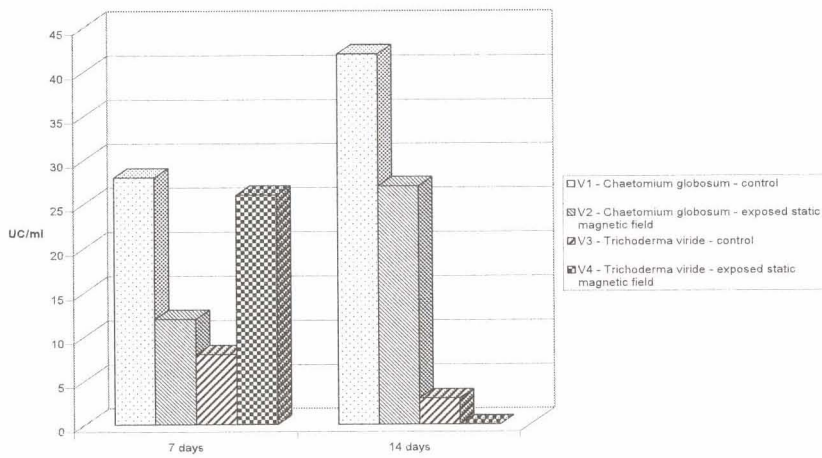

Fig. 7. The influence of the static magnetic field on the catalase activity in *Chaetomium globosum* and *Trichoderma viride* – culture liquid (UC/ml).

CONCLUSIONS

1. The activity of the cellulolytic system was influenced both by the cellulolytic species studied and by the culture age: the endoglucanase activity in 7 days from seeding was inhibited in the *Chaetomium globosum* and stimulated in the *Trichoderma viride*; in 14 days from seeding the activity of this enzyme was inhibited by exposure to the action of the static magnetic field in both species; the cellobiohydrolase activity was stimulated in the *Trichoderma viride* both in 7 days and in 14 days from seeding; the activity of β-glucosidase was stimulated by exposure to magnetic field in 7 days from seeding in both species, while in 14 days from seeding it was inhibited in both species studied.

2. The peroxidase activity in the mycelium had lower values in both species exposed to static magnetic field, in 7 days from seeding; in 14 days from seeding, the peroxidase activity had lower values in *Chaetomium globosum* in the variant exposed to the magnetic field, while in *Trichoderma viride* it had zero value both in the control and in the variant exposed to the static magnetic field. In the culture liquid, the peroxidase activity was stimulated by exposure to a static magnetic field in both species, in 7 days from seeding; in 14 days from seeding the enzyme activity was stimulated in *Chaetomium globosum* in the variant exposed to the magnetic field and had zero value in *Trichoderma viride* in both variants.

3. The catalase activity in the mycelium in 7 days from seeding was inhibited by exposure to the influence of the static magnetic field in both species, while in 14 days from seeding the activity of this enzyme was stimulated only at *Trichoderma viride*, the variant exposed to the static magnetic field, in other variants studied the catalase activity was inhibited by the action of the static magnetic field.

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