# EFFECT OF STATIC MAGNETIC FIELD ON SYNTHESIS OF ENDOGLUCANASE BY TRICHODERMA REESEI – M7

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## ABSTRACT

*Studies have been carried out with the micromycetic strain Trichoderma reesei M-7, producer cellulase enzymes. Spores and vegetative inoculums of the enzyme producer were treated with a static magnetic field with intensity of 5 - 70 mT. The influence of the magnetic field on the activity of the produced endoglucanase, the quantity of extracellular protein and biomass was studied at the conditions of batch cultivation.*

*Higher activity and differences in biosynthetic dynamics of endoglucanase was observed under the applied pretreatment of the spore inoculums.*

**Keywords:** endoglucanase, static magnetic field (SMF), *Trichoderma reesei*

## Introduction

Numerous published works confirm that static magnetic field (SMF) with low magnetic induction may induce effects on microbial and mammalian cells (9). Some of studies showed a negative effect (4), while most of them showed an enhancement in growth (5, 11, 13). Previous experiments indicated that the magnetic field tended to increase the bacterial activity (14).

Motta et al, (7) showed an increase of the *S. cerevisae* metabolism after magnetic stimulation. Muniz et al. (8) reported that the application of static magnetic field (220mT) enhanced biomass growth.

In this study spores and vegetative inoculums of strain Trichoderma reesei M7 were treated with static magnetic field with intensity of 5 -70mT. The influence of magnetic field on the activity of the produced endoglucanase, the quantity of extracellular protein and biomass was studied at the conditions of batch cultivation.

## Materials and methods

The investigated strain M7 of *Trichoderma reesei* was obtained by mutagenesis with nitroso- guanidine on the parent strain *Trichoderma s*p. 914. (1)

Experiment was conducted with 10 days-old culture of *Trichoderma reesei* strain M-7 grown on PDA (potato-dextrose agar) at 28°C.

A SMF was generated by permanent magnets pair. Installation with magnets was scaling by using teslameter.

Inoculums was obtained in 500 $cm^3$ flasks which contained 100 ml Mandels mineral salt medium (6) with added 2% glucose and 1% maize extract (starting pH after autoclaving 4.8-5.0) at 28°C with constant shaking (220 rpm) for 24 hours. Fermentation mixture (50 ml) was composed from Mandels mineral salt medium with added 1% microcrystaline cellulose Micricel® and 1% wheat bran (starting pH after autoclaving 5.8-5.9). Fermentation process was held at 28°C with constant shaking (220 rpm). Endo-1,4-β-glucanase (Cx) activity was measured at every 24 h. Cultivation medium was centrifuged at 5000 rpm for 15 min to remove mycelia, and the supernatant was used to test Cx activity.

Endo-1,4-β-glucanase activity was detected on sodium carboxy-methyl cellulose (Na-CMC) as substrate according to Wood and Bhat (12). Reaction mix containing 0.5 ml 1% solution of Na-CMC in 0.05 M Sodium-acetate buffer, pH 4.8 and 0.5ml enzyme solution were incubated at 50°C for 30 min.

Samogyi-Nelson assay (10) has been used to assess the level of reducing sugars. One unit activity was defined as the amount of enzyme that released 1µmol glucose per 1 min at experimental conditions (50°C, pH 4.8).

Soluble protein was determined by dye binding technique (3) using bovine serum albumin as a protein standard. All

measurements were made in triplicates and the standard error was calculated for the three separate samples.

Biomass was measured by determining the biomass dry weight (mg/ml).

## Results and Discussion

Effect of SMF on endoglucanase activity and total protein has been studied at 48h, 72h, 96h and 120h of batch (submerged) cultivation.

### Effect of SMF 10mT on 10 days' agar growth cultures on the enzyme production, total protein in batch fermentation and biomass growth

The results presented in **Fig. 1 a)** and **Fig. 1 b**) show that the endoglucanase activity in SMF treated culture was higher than that in the non-treated ones, from 48h to the end of the fermentation. The final mean value of the enzyme activity in magnetized samples was 14.5 % (at 120h) higher compared to the final non-magnetized culture. The total protein in the magnetized culture was 11.5 % greater, at the end of fermentation, than the non-treated ones.

After SMF treatment biomass dry weight of vegetative inoculums was 7.7% higher than in control.

### Effect of SMF (70mT, 30mT, 10mT and 5mT) on spores for 1 hour, studied on the enzyme production, total protein in batch fermentation and biomass growth

In this experiment four different conditions of magnetization were applied (70mT, 30mT, 10mT and 5mT). All of the applied magnetic field inductions resulted in higher endoglucanase activity and total protein except for 70mT, which results were similar to the control experiment.

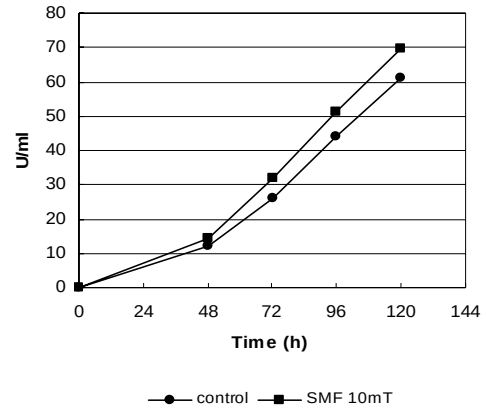
The highest enzyme activity and total protein values were observed when the spores were exposed on SMF with 10mT intensity (24.2 % and 18.0 % respectively, increasing compared to non-exposed control).

Biomass dry weight of vegetative inoculums after influence of SMF on spores was measured. The increasing of biomass at 30mT, 10mT and 5mT SMF intensity was 5.7%, 11.4%, 9% higher than in the control, respectively. SMF with 70mT intensity has not influence on biomass growth.

### Effect of SMF 10mT for 24hours on vegetative inoculums, measured on the produced enzyme, total protein in batch fermentation and biomass growth

The endoglucanase activity in SMF exposed vegetative inoculums (**Fig.3a**) was 27.4 % higher (at 120h) than that in the unexposed control. The total protein in the treated inoculums was 15.0 % greater, at the end of fermentation, than the non-treated ones (**Fig. 3b**). Biomass dry weight was 4.32% higher than control.

a)




b)

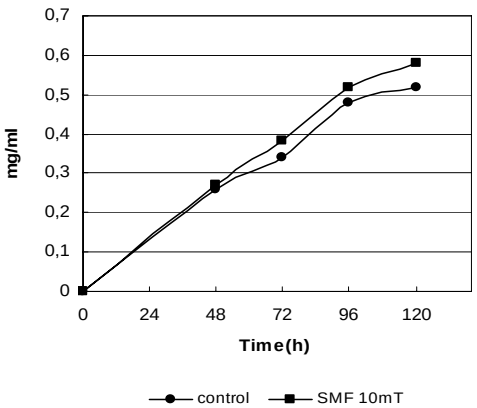
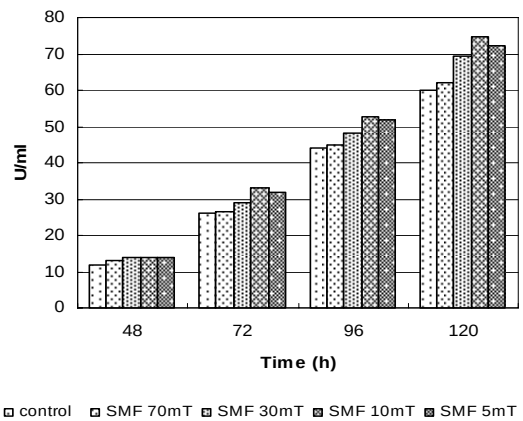



**Fig.1.** Effect of SMF 10mT on 10 days' agar growth cultures resulting on the endoglucanase production **(a)** and on total protein **(b),** measered in batch cultivation. Data are mean values ± S.D. from 0.02 to 0.05, n=3.

a)



b)

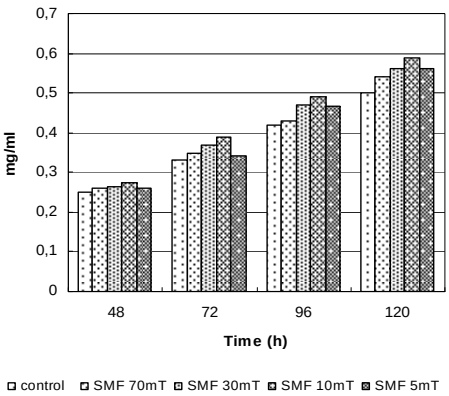


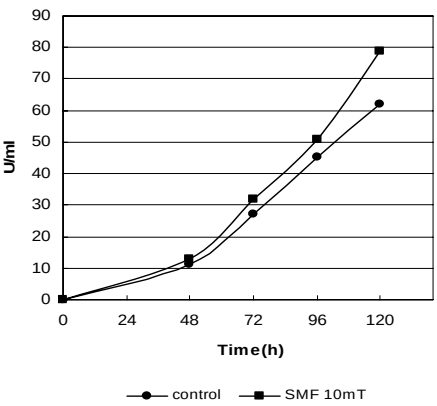

**Fig. 2 a)** Effect of SMF (70mT, 30mT, 10mT and 5mT) on spores for 1 hour, studied on the produced enzyme in batch fermentation.

Data are mean values ± S.D. from 0.04 to 0.09, n=3

**Fig. 2 b)** Effect of SMF (70mT, 30mT, 10mT and 5mT) on spores for 1 hour, studied on synthesis of total protein in batch fermentation.

Data are mean values ± S.D. from 0.04 to 0.09, n=3.

a)




b)

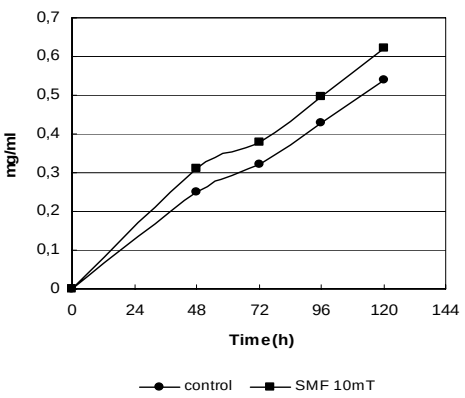



**Fig.3 a)**. Effect of SMF 10mT for 24hours on vegetative inoculums, measured on the produced enzyme in batch fermentation.

Data are mean values ± S.D. from 0.01 to 0.05, n=3.

**Fig.3 b)**. Effect of SMF 10mT for 24hours on vegetative inoculums, measured on the total protein in batch fermentation.

Data are mean values ± S.D. from 0.01 to 0.05, n=3.

The results show that the SMF (5mT-70mT) has a positive effect on endoglucanase activity and total protein synthesis in *Trichoderma reesei*-M7.

The highest values of enzyme activity were observed in treatment of vegetative inoculums for 24 h with SMF-10mT intensity (27.4 % increase compared to control).

The enhancement of enzyme synthesis and total protein production may be resulted from the increasing of biomass. Anyway, confirmation of these results needs other future experiments to be carried out.

In all experimens, presented in this study, a higher endoglucanase activity and total protein, and increased biomass are observed. That would be a good beginning for next researches with the main goal the practical application of magnetic field during the cultivation of *Trichoderma ressei*.

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