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Full Length Research Paper

Effect of pretreatments on cellulose hydrolysis of industrial wastes by *Pleurotus sajorcaju*

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An effective method was developed to convert the cellulose of delignified and non-delignified waste-cellulose fibers of paper and woodwork industry by the cellulolytic fungi *Pleurotus sajorcaju*. These cellulosic materials are strongly resistant to direct enzymatic hydrolysis. For this reason, the cellulose fibers were effectively pretreated with physical and chemical pretreatments. It was observed that evolution of cellulases activity depends on the type of pretreatment, the substrate utilized and the culture age. However, the highest ignocellu activity was recorded in acid pretreatment of delignified and non-delignified waste-cellulose fibers of paper and woodwork industry.

Keywords: Cellulolytic fungi; Lignocellulose materials; Pretreatments; Cellulases; *Pleurotus sajorcaju.*

INTRODUCTION

Enormous amounts of agricultural, industrial and municipal ignocelluloses wastes are constantly piled up or used quite inefficiently, because of the very high cost of their utilization processes. This becomes a problem of primary importance for the ecology, chemical and biotechnological industries. Therefore, there is a considerable economic interest in the development of processes for effective pretreatment and utilization of cellulosic wastes as inexpensive carbohydrate sources (Gould, 1984; David et al., 1985; Viesturs et al., 1996). Cellulose, the major component of waste-cellulose fibers of paper and woodwork industry can be converted into glucose suitable for further microbial processing through its hydrolysis (Philippidis et al., 1993; Gregg and Saddler, 1996; Olson and Hahn-Hagerdal, 1996).

Numerous pretreatment methods have been developed in searching for ways to remove the lignin Barrier and enhance the accessibility of cellulose to hydrolytic degradation, but little is known about the pretreatment of already delignified waste-cellulose fibers (Rao and Setta, 1983; Gharpuray et al., 1983; Gould, 1984; Viesturs et al., 1996).

It is generally known that cellulose is resistant to enzymatic hydrolysis due to high crystalline structure and lignin contents which blocks cellulolytic enzymes resulting in a slow and incomplete hydrolysis. For this reasons pretreatments for cellulose materials have been suggested for complete cellulolytic efficiency (Scriban, 1985). The pretreatments are used in order to destroy the crystalline structure of cellulose as much as possible and also to expend the surface area and modify the polymerization level, which has been confirmed by earlier studies (Tofan and Segal, 1992).

Based on this rationale, we evaluated the effectiveness of several pretreatment methods in order to enhance the accessibility of waste-cellulose fibers to enzymatic hydrolysis, by *Pleurotus sajorcaju*. This paper discusses the results concerning the physical and chemical (acid and alkaline) pretreatments on cellulasic activity: endoglucanase – E.C.3.2.1.4.; exocellu-

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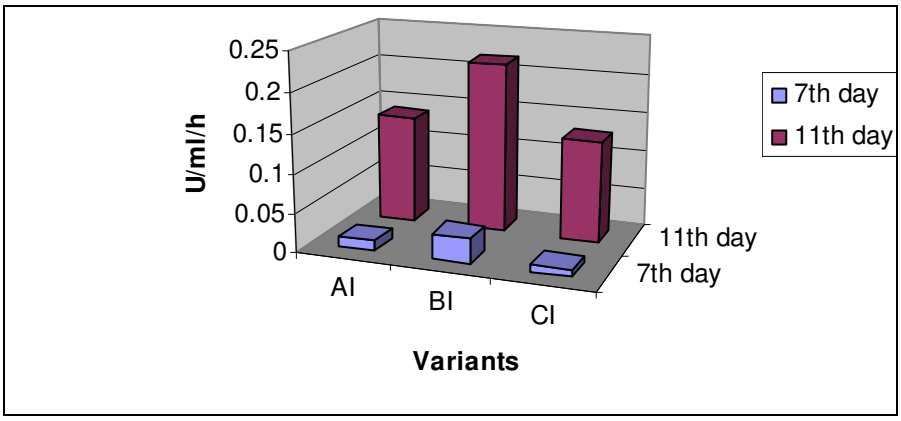

Figure 1. The influence of the pretreatments on endoglucanase activity in monoculture of *pleurotus sajorcaju* cultivated on non-delignifed waste-cellulose fibers of woodwork industry.

lobiohydrolase–E.C.3.2.1.91.;β–glucosidase E.C.3.2.1.21, in monoculture of *Pleurotus sajorcaju* on delignified and non-delignified waste-cellulose fibers of paper and woodwork industry, respectively.

MATERIALS AND METHODS

The culture of *Pleurotus sajorcaju* was obtained from Indian Agricultural Research Institute, New Delhi. It was cultivated in Czapek-Dox medium with following contents: $NaNO_3$ (3g), H_2PO_4 (1g), $FeSO_4.7H_2O$ (0.01g), KCl (0.01g), $MgSO_4.7H_2O$ (0.5g), Sucrose (40g) and Distilled Water (1000 ml). The carbon source (sucrose) was replaced with delignified and non-delignified waste-cellulose fibers of paper and woodwork industry. Before adding into the culture medium, residuals were pretreated physically (mechanically) and chemically (acidic and alkaline).

The mechanical pretreatment was performed by fine grinding of residual types. The acid pretreatment was performed with 3% HCl for 30 minutes at 120°C, then fast and abundantly washed with distilled water. Alkaline pretreatment was realized with distilled water with 2% NaOH for 30 minutes at 120°C followed by a distilled water wash. At the end, the following working variants were obtained: A1 – non-delignified waste-cellulose fibers (mechanical pretreatment), A2 – delignified waste-cellulose fibers (mechanical pretreatment), B1 – non-delignified waste-cellulose fibers (acid pretreatment), B2 – delignified waste-cellulose fibers (acid pretreatment), C1 – non-delignified waste-cellulose fibers (alkaline pretreatment), C2 – delignified waste-cellulose fibers (alkaline pretreatment).

The estimation of endoglucanase activity was made by Peterson method which provides testing of hydrolytic action of enzyme mixture on the filter paper whatman no. 1. The resulted sugars were measured using 3, 5-dinitrosalicilic acid (DNS) reagent.

For measuring the exocellobiohydrolase activity, we have followed Gould (1984) which consists of dosing freed sugars by enzyme from the carboxymeticellulose substrate with 3,5-dinitrosalicilic acid (DNS) reagent.

The dosage of the β-glucosidase activity is based on determination of the increasing reductive power of the medium with DNS reagent. The cellulosic activity was measured on 7^{th} and 11^{th} day from inoculation.

RESULTS AND DISCUSSION

The results concerning the pretreatments influences on cellulasic activity are presented in Figures 1-6. Data on influence of different pretreatments on endoglucanase activity in *pleurotus sajorcaju* cultivated on non-delignified waste-cellulose fibers from woodwork industry are presented in Figure 1 which shows that after 7^{th} day from inoculation, the enzyme activity had the highest value at B1- 0.0312 U/ml/h followed by A1 – 0.0134 U/ml/h and C1 – 0.008 U/ml/h. However, on 11^{th} day from inoculation, the enzyme activity increased in all medium variants, but the best value was observed again for B1 – 0.218 U/ml/h variant, followed by A1 – 0.1385 U/ml/h, and C1 – 0.1301U/ml/h.

The results concerning the pretreatment influence on endoglucanase activity in monoculture of *p. sajorcaju* cultivated on delignified waste-cellulose fibers from paper industry are shown in Figure 2. After 7^{th} day from inoculation, the maximum value of this enzyme was observed at B2- 0.0488 U/ml/h, followed by C2 – 0.038 U/ml/h, and A2 – 0.0194 U/ml/h. After 11^{th} day from inoculation, endoglucanase activity enhanced in all variants *i.e.* A2 – 0.1261 U/ml/h; B2 – 0.1511 U/ml/h; and C2 – 0.1309 U/ml/h.

Analyzing the dynamics of endoglucanase activity of monoculture on two types of the waste-cellulose fibers

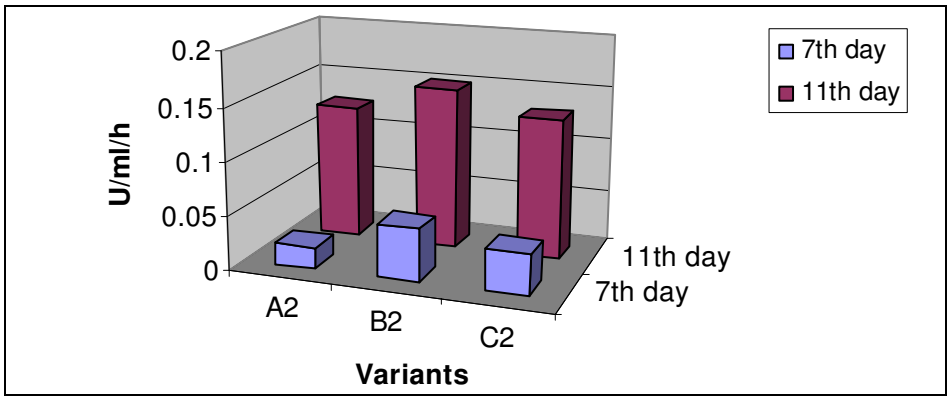

Figure 2. The influence of the pretreatments on endoglucanase activity in monoculture of *Pleurotus sajorcaju* cultivated on delignified waste-cellulose fibers of paper industry.

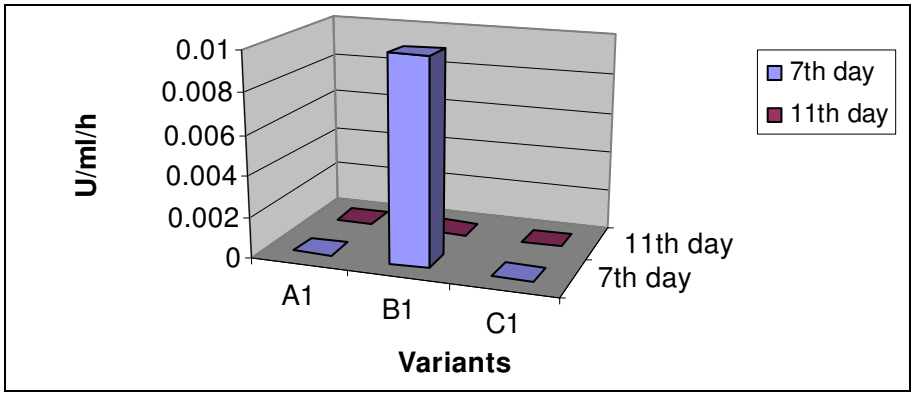

Figure 3. The influence of pretreatments on exocellulobiohydrolase activity in monoculture of *Pleurotus sajorcaju* cultivated on non-delignified waste-cellulose fibers of woodwork industry.

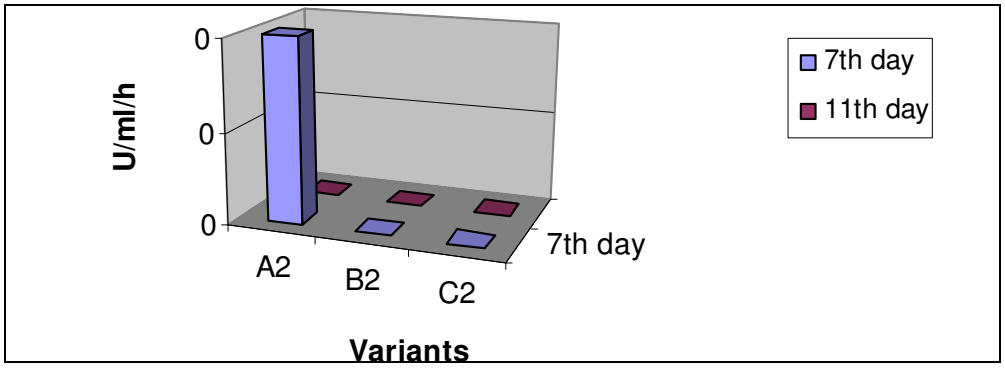

Figure 4. The influence of pretreatments on exocellulobiohydrolase activity in monoculture of *Pleurotus sajorcaju* cultivated on delignified waste-cellulose fibers of paper industry.

utilized, it was observed that in all variants this was much higher on 11th day compared to 7th day from inoculation as shown in the present study: A1 – from 0.0134 U/ml/h to 0.1385 U/ml/h; B1 – from 0.0312 U/ml/h to 0.218 U/ml/h; C1 – from 0.008 U/ml/h to 0.1301 U/ml/h; A2 – from 0.0194 U/ml/h to 0.1261 U/ml/h; B2 – from 0.0488 U/ml/h to 0.1511 U/ml/h; C2 – from 0.038 U/ml/h to 0.1309 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 B1 on 7th day from inoculation. In all other

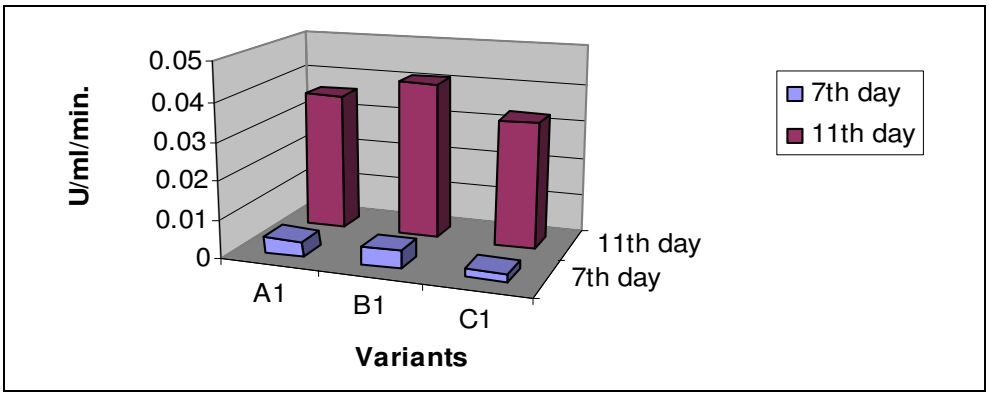

Figure 5. The influence of pretreatments on β-glucosidase activity in monoculture of *Pleurotus sajorcaju* cultivated on non-delignified waste-cellulose fibers of woodwork industry.

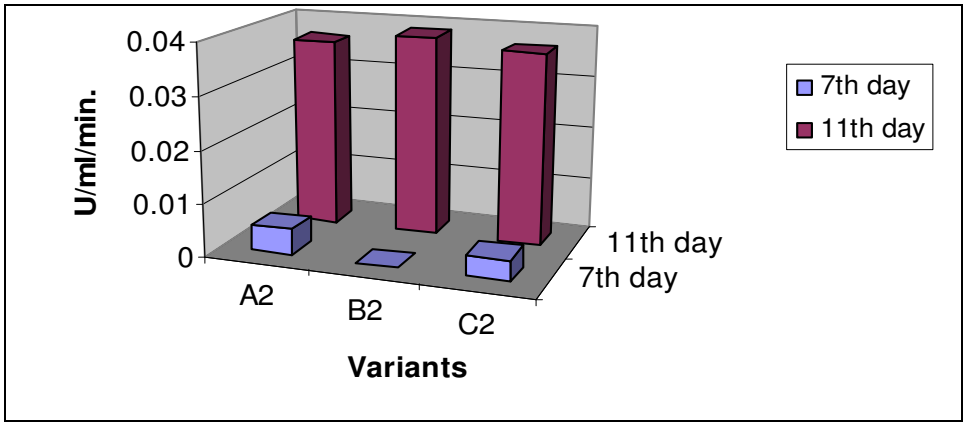

Figure 6. The influence of pretreatments on β-glucosidase activity in monoculture of *Pleurotus sajorcaju* cultivated on delignified waste-cellulose fibers of paper industry.

variants, the exocellobiohydrolase activity had zero value at both 7th and 11th day from inoculation.

The dynamics of the β-glucosidase activity in *psajorcaju* cultivated on non-delignified waste-cellulose fibers which was previously physically and chemically pretreated is presented in Figure 5. On 7th day from inoculation, the maximum enzyme activity was observed at B1- 0.0046 U/ml/h. On 11th day from inoculation, enzyme activity increased in all variants as follows; A1 – 0.036 U/ml/min, B1 – 0.0410 U/ml/min, C1 – 0.033 U/ml/min. The results concerning the pretreatment influence on β-glucosidase activity in *p.sajarcaju* cultivated on delignified waste-cellulose fibres of paper industry are shown in the Figure 6. These indicate that on 7th day from inoculation the enzyme activity in A2 – 0.0050 U/ml/min., while at C2 – 0.0040 U/ml/min and finally at B2-Zero. While on 11th day from inoculation the values were superior from 7th day follows: A2 – 0.0364 U/ml/min, B2 – 0.0385 U/ml/min, C2 – 0.0368 U/ml/min.

By analyzing β-glucosidase evolution in function of culture age it was observed that in all variants on 11th day from inoculation the activity of this enzyme was higher compared to that of 7th day *i.e.* in A1 activity of β-glucosidase increased from 0.0040 U/ml/min to 0.036 U/ml/min, B1 – from 0.0046 U/ml/min to 0.0410 U/ml/min, C1 – from 0.002 U/ml/min to 0.033 U/ml/min, A2 – from 0.0050 U/ml/min to 0.0364 U/ml/min. B2 – from 0 to 0.0385 U/ml/min and C2 – from 0.0040 U/ml/min to 0.0368 U/ml/min.

CONCLUSION

In all variants taken in the present study cellulasic activity was maximum under acid pretreatment utilization which may be an effective method for bioconversion of delignified and non-delignified waste-cellulose fibers of paper and woodwork industry.

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