

INFLUENCE OF PH ON β -XYLANASE ACTIVITY IN THE FILAMENTOUS FUNGI *TRICHODERMA REESEI*, *TRICHODERMA VIRIDE* AND *PHANEROCHAETE CHRYSOSPORIUM*

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Abstract

Xylanases are industrially important enzymes. They are produced by a wide range of fungi, particularly filamentous fungi, such as *Trichoderma reesei*, *Trichoderma viride* and *Phanerochaete chrysosporium*. Xylanases are enzymes that catalyze the hydrolysis of 1, 4- β -D-xylosidic bonds in xylan, resulting xylose, a primary carbon source in cellular metabolism.

Cellulose and xylan are two polysaccharides that induce effectively the synthesis of xylanolytic enzymes produced by the fungi mentioned above. Xylan is found in high quantities in the cell wall of annual plants. One of the main parameters influencing the activity of xylanases is the hydrogen ion concentration, pH of these enzymes fluctuating from one species to another.

To assess the impact pH has on xylanase activity, especially on β -xylanase, filamentous fungi *Trichoderma reesei*, *Trichoderma viride* and *Phanerochaete chrysosporium* were grown at different levels of pH, on a modified Mandels medium, where the main carbon source consists of byproducts from local agricultural practices (wheat straws and corn stalks). A dynamic profile of the activity was mapped, during a ten day period. The results indicated that β -xylanase activity is influenced by both the hydrogen ions concentration and the nature of the carbon source.

Key words: *Trichoderma reesei*, *Trichoderma viride*, *Phanerochaete chrysosporium*, β -xylanase

Xylan is one of the the most abundant polysaccharide in nature. It is found in high amounts in hardwood from angiosperms, the softwood from gymnosperms and the cell wall of annual plants (Singh et al., 2003).

Due to structural complexity, xylan degradation requires the combined action of several hydrolytic enzymes (Biely, 1985). These enzymes, also known as xylanases, act in a synergistic manner, depolymerizing xylan molecules into xylose units that are used by microorganisms as a primary source of carbon.

Two groups of xylanases act together to cleave the xylan backbone: endo- β -xylanases, enzymes that hydrolyze xylan and xylo-oligosaccharides and β -D-xylosides, involved in the hydrolysis of xylo-oligosaccharides to D-xylose.

Xylanases are produced mainly by bacteria and fungi (Gilbert, Hazlewood, 1999; Sunna, Antranikian, 1997). However, some freshwater mollusks are known for their ability to synthesize these enzymes (Yamura et al., 1997). Xylanases are required for many applications ranging from prebleaching of kraft pulp to minimize the

application of strong chemicals in the subsequent treatment stages of kraft pulp; improve feed digestibility (Kuhad, Singh, 1993); increase the clarification process of fruit juices (Biely, 1985).

Lately, the potential applications of xylanases in the bioconversion of lignocellulosic material and agro-wastes to fermentative products was taken under consideration (Subramaniyan, Prema, 2002). The use of regenerable low cost substrates for the production of industrial enzymes can reduce production costs. Wheat straws and corn stalks are some of the most abundant agricultural wastes.

Filamentous fungi are powerful producers of xylanases. Their use has several advantages, namely: are non-pathogenic, are able to produce high levels of extra cellular enzymes and can be grown easily.

Trichoderma reesei, *Trichoderma viride* and *Phanerochaete chrysosporium* are three of the most intensively used filamentous fungi in the production of xylanases.

The metabolic activity and enzyme productivity of these fungi is influenced by environmental conditions such as pH, cultivation

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period and temperature, concentration and the nature of the substrate used (Haltrich et al., 1996; Fadel, 2000; Lenartovics et al., 2003).

Therefore, this study aims to investigate the manner, in which, β -xylanase activity is influenced by the initial pH of the culture medium and the nature of the carbon source.

MATERIALS AND METHODS

Microorganisms. *Trichoderma reesei* and *Phanerochaete chrysosporium* were acquired by the Institute of Biological Research, Iași from the Institute Scientifique de la Santé Publique, Belgium. *Trichoderma viride* was isolated in our laboratory. The stock cultures were maintained on potato dextrose agar (PDA) slants.

Culture medium. To determine β -xylanase activity, the fungi were grown on a liquid medium, distributed in 250 ml Erlenmeyer flasks, each of these containing 100 ml of a modified Mandels medium: (Ferreira et al., 2009), with the following composition: 2.0 g/L KH_2PO_4 ; 1.4 g/L $(\text{NH}_4)_2\text{SO}_4$; 0.0027 g/L $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$; 0.0016 g/L $\text{MnSO}_4 \cdot \text{H}_2\text{O}$; 0.0014 g/L $\text{ZnSO}_4 \cdot \text{H}_2\text{O}$; 0.0037 g/L $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$; 0.6 g/L $\text{MgSO}_4 \cdot 7 \text{H}_2\text{O}$; 0.4 g/L $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$; 0.75 g/L peptone; 2.0 ml/L Tween 80; 0.3 g/L urea; the carbon source-glucose was replaced with by 30 g/L wheat straws and corn stalks. Prior to inoculation, the fungi were grown on a similar solid medium. Solid cultures were maintained at 28°C for 7 days. Liquid medium was inoculated with 8 mm in diameter discs from the solid medium and incubated at 28°C for 10 days.

During this period, 2 ml of culture liquid were withdrawn every two days, and used as enzyme solution. The experiments were carried out in triplicate and mean values were calculated.

Enzyme assay. Endo-1, 4- β -xylanase (EC 3.2.1.8) activity was assayed according to Bailey et al. (1992) using 1% beechwood xylan as substrate for enzyme reaction.

The reaction mixture contained 1 ml of 1% beechwood xylan (Sigma), dissolved in 0.05 M citrate buffer and 0, 2 ml of enzyme solution. Blanks were also made. The reaction mixture was incubated at 50°C for 10 min. Both samples and blanks were incubated at 50°C for 10 minutes. Then, the reaction was stopped by adding dinitrosalicylic acid (DNS).

The total amount of reducing sugars released from xylan was estimated according to Miller (1959). A standard curve of 1% D-xylose was used as reference. One unit of xylanase activity (IU) was defined as the amount of enzyme that liberated 1 μmol reducing sugar (xylose) from the substrate solution per minute.

RESULTS AND DISCUSSIONS

Carbon source is one of the nutritional parameters essential for the biosynthesis of xylanases. Xylan in its pure form efficiently induces the synthesis of these enzymes. However, xylan is an expensive substrate, therefore, the use of cheap and abundant lignocellulosic materials as carbon source is an effective alternative. Two carbon sources were compared in this study, wheat straw and corn straws in a final concentration of 30 g/L.

The fungi *Trichoderma reesei* and *Phanerochaete chrysosporium* showed a β -xylanase activity higher when grown on medium with wheat straw, recording values of 25.679 IU/ml and 21.976 IU/ml. In contrast, *Trichoderma viride* showed higher activity when the carbon source used was corn (94.007 IU/ml).

Another parameter that significantly influences xylanase biosynthesis is the initial pH value of culture medium. The results indicate a variation in activity both by the initial concentration of H^+ ions and the carbon source used.

In fig. 1 and 2 is plotted β -xylanase activity in the fungus *Trichoderma reesei* grown on medium with wheat and corn. The highest value was recorded at pH 5 (25.679 IU/ml) for wheat and at pH for corn (21.669 IU/ml).

Results indicate a variation in enzyme activity correlated with incubation period of the culture. The highest values of β -xylanase activity in the fungus *Trichoderma reesei* were obtained at 10 days after inoculation, for all three pH studied, regardless of the carbon source used.

Enzyme activity was low during the first 3 days in *Trichoderma reesei*, 6 days following inoculation an increase in activity was recorded, followed by a slight decline, eventually reaching a maximum value.

The lowest results were reported at pH 6 in both the wheat and corn medium (0.338 IU/ml; 1.94 IU/ml).

H^+ ion concentration is an important parameter in the synthesis of enzymes by *Trichoderma reesei* xylanases (Denison, 2000). Some studies show that *Trichoderma reesei* RUT C-30 has a high enzymatic activity at pH equal to 7, but the best mycelial growth at a lower pH, equal to 4 (Xiong, 2004).

Cultivation of the fungus *Trichoderma reesei* on a medium in which the nitrogen source is represented by ammonium salt induces a decrease in pH, while the use of urea causes an increase in pH (Xiong, 2004).

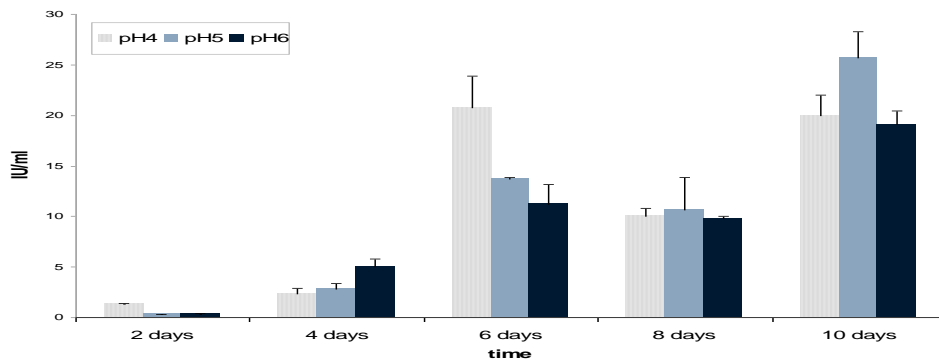


Figure 1 β -xylanase activity - *Trichoderma reesei* (carbon source-wheat straws)

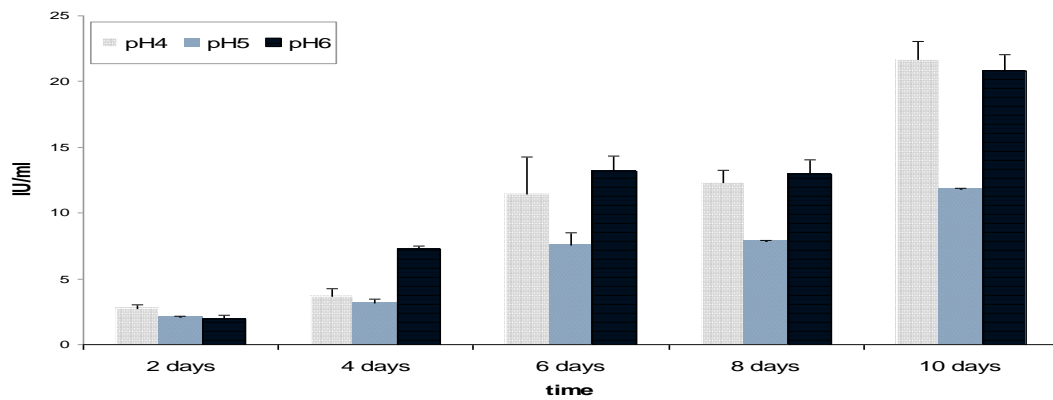


Figure 2 β -xylanase activity - *Trichoderma reesei* (carbon source-corn stalks)

The pH of the culture medium was recorded at the end of the assay.

The results indicate a change of the initial pH of the culture for all three fungi (tab. 1).

Table 1

Final pH of the cultivation medium

Initial pH	<i>Trichoderma reesei</i>		<i>Phanerochaete chrysosporium</i>		<i>Trichoderma viride</i>	
	wheat	corn	wheat	corn	wheat	corn
pH4	6.68	5.65	5.44	5.48	6.57	5.79
pH5	7.13	6.68	5.81	5.24	6.89	6.63
pH6	7.23	7.23	5.68	5.24	6.85	6.85

Fig. 3, and 4, depicts β -xylanase activity of the fungus *Phanerochaete chrysosporium*. The enzyme reached high values when *P. chrysosporium* was grown on wheat medium, reaching a maximum of 21.976 IU/ml.

β -xylanase activity was higher at pH value of 6 in both the corn and wheat medium. Cultivation medium, in which the initial

concentration of hydrogen ions was 4, exhibited a low enzyme activity of 0.548 IU / ml (wheat), and 0.183 IU / ml (corn).

Phanerochaete chrysosporium recorded an increase in β -xylanase activity on the sixth day of cultivation, a decline in the next period, followed by an increase and a maximum at 10 days.

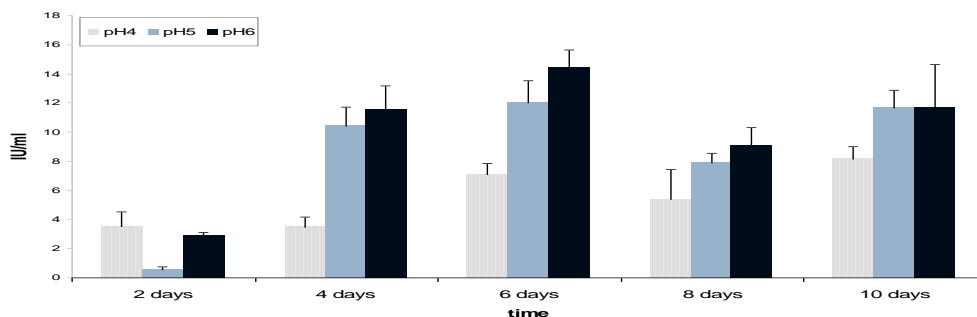


Figure 3 β -xylanase activity - *Phanerochaete chrysosporium* (carbon source-wheat straws)

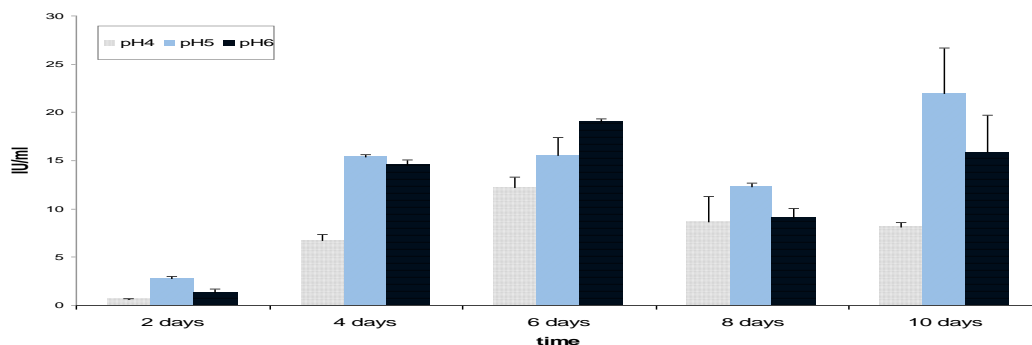


Figure 4 β -xylanase activity - *Phanerochaete chrysosporium* (carbon source-corn stalks)

The β -xylanase activity of *Trichoderma viride* is depicted in fig. 5 and 6. The highest activity was recorded on the corn medium (94.007 IU/ml).

Enzymatic activity was influenced by the initial concentration in H^+ ions: on corn stalks medium the optimum pH was 6, while on wheat straws was 4.

The evolution of β -xylanase during the 10 days cultivation period was as follows: on wheat medium, at pH 4 the activity was low in the first days, but by the sixth day it reached a peak, followed by a slight fall and a new peak in the tenth day. At pH 5, β -xylanase recorded an increase in activity at 6 days, followed by a decrease.

At pH 6 the activity was at its highest in the last day of cultivation. On corn medium the activity increased in the first six days, slightly decreased by day 8 and increased again reaching its highest at ten days.

CONCLUSIONS

The xylanase activity of *Trichoderma reesei* is the result of the combined action of four xylanases (I, II, III and IV), enzymes that have different optimal pH, for xylanases I and II the pH ranges between 4 and 6.5 (Tenkanen et al., 1992; Xu et al., 1998) and for xylanase IV between 3,5-4 (Clarkson și colab., 2001).

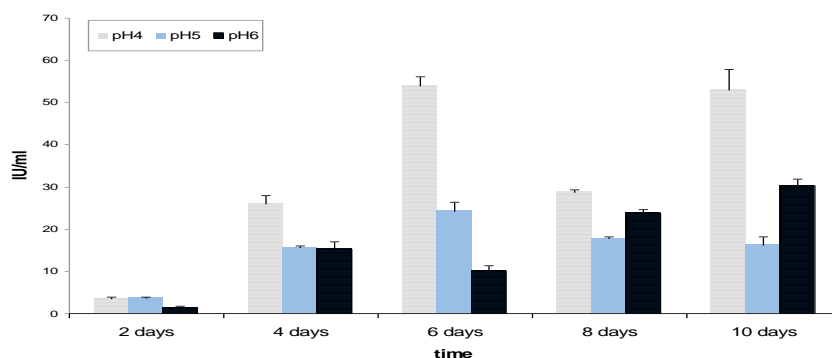


Figure 5 β -xylanase activity - *Trichoderma viride* (carbon source-wheat straws)

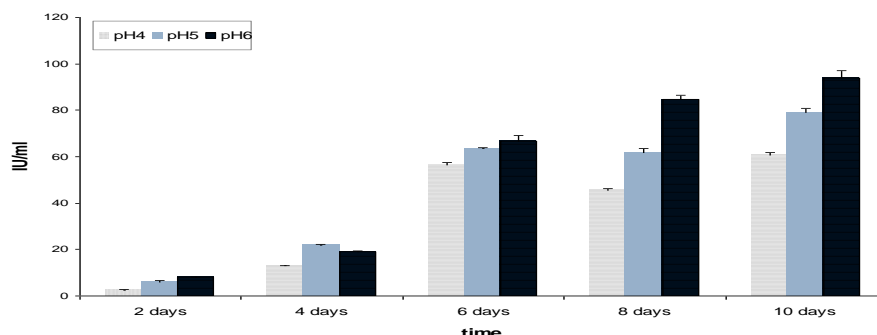


Figure 6 β -xylanase activity - *Trichoderma viride* (carbon source-corn stalks)

Phanerochaete chrysosporium produces three xylanases (I, II and III), with high activities around a pH of 4-5.5 (Dobozi et al., 1992).

The results recorded in this study indicate the profound influence of initial culture medium pH on β -xylanase activity, the studied fungi displayed various activities, each organisms having different optimal pH values.

Another factor influencing xylanase activity of these three fungi is the carbon source. β -xylanase activity was higher when *Trichoderma reesei* and *Phanerochaete chrysosporium* were grown on medium with wheat straws, while the enzyme activity in *Trichoderma viride* is stimulated by medium with corn.

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