Production and characterization of endoglucanase from *Chaetomium globosum* and its effect on lignocellulose degradation

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ABSTRACT

This work deals with optimization the production conditions of endoglucanase produced by *Chaetomium globosum* and its role in some lignocellulosic materials (corn stover) degradation. Optimization studies revealed that Reese and Mandel's basal medium was the best for maximum endoglucanase secretion by *C. globosum*. Maximum endoglucanase production was found at 2.5% cellulose and 0.3% urea. *C. globosum* gave maximum endoglucanase production on Reese and Mandel's basal medium at pH 6 and 8% (v/v) inoculum volume after 8 days of cultivation. Yield of endoglucanase and protein were 60.2 and 35.6%, respectively after concentration with ultrafiltration. Specific activity of endoglucanase was 2.17 U/mg. Kinetic studies of endoglucanase revealed 2.27 mg/mL as K_m and 0.1194 µmol/min/mL as V_{max}. Optimum temperature for endoglucanase activity was 65 °C, while the optimum pH was 5. Endoglucanase retained about 60 % of its activity when incubated at 65 °C for 2 h. While, the enzyme lost about 58% from its activity at 65 °C for 12h. Moreover, endoglucanase showed high stability for pH 5 when incubated for 12h. An isoelectric point (pI) around 4.5 was detected for endoglucanase. The molecular weight was calculated to be about 40 KDa. Application of endoglucanase on corn stover was assessed compared with cellulase produced from *T. reesei* and laminex enzyme. The three enzymes gave higher concentrations of reducing sugars when corn stover was treated with peroxidase (MsP1) than untreated one. Laminex enzyme showed the highest catalytic activity to hydrolyze polymeric carbohydrates of corn stover. Maximum level of lignin degradation can be reached after 24 hours with using 3 units of MsP1.

Keywords: Endoglucanase, *Chaetomium globosum*, production conditions, kinetic studies and corn stover.

INTRODUCTION

Cellulase, a family of enzymes that breaks down cellulose into glucose molecules, catalyzes the cellulose hydrolysis, ultimately yielding cellobiose and glucose as available carbon and microbial energy sources. Although all cellulolytic enzymes share the same chemical specificity for β-1,4-glycosidic bonds, they show difference in their specificities towards macroscopic properties of substrate (Dorland's Illustrated Medical Dictionary, 2003).

Endoglucanase (Endo-β-1,4-glucanase, EC 3.2.1.4) plays an important role in the cellulose hydrolysis by cleaving cellulose chains randomly and thus encouraging strong degradation (Cao and Huimin, 2002). Generally, the indiscriminate action of endoglucanase progressively increases the accessibility of cellulose chain ends, in this manner increasing the specific surface area of the substrate for exocellulase activity. Endoglucanase attacks the β-1,4 glycosidic bonds within the amorphous regions of cellulose chains. The products of this attack are oligosaccharides of various lengths and subsequently new chain reducing ends (Lynd *et al*., 2002).

Fungi are the main cellulase producing microorganisms, although a few bacteria and actinomycetes have also been reported to yield cellulase activity. Microorganisms of the genera *Trichoderma*, *Aspergillus* and *Chaetomium* are thought to be cellulase producers, and crude enzymes produced by these microorganisms are commercially available for agricultural use (Szengyel *et al*. 2000). The use of microorganisms or their enzymes for the conversion of cellulose into simple carbohydrates is receiving increased attention. This is the result of growing concern over the accumulation of wastes, and our awareness of the vast quantities of residues, rich in cellulose, which result from agricultural operations and the manufacture of wood products. Lignocellulose is converted to glucose and biofuels by a multistep process that includes pretreatment, enzymatic hydrolysis, and fermentation (Wyman *et al.,* 2005).

The aim of this work is to study the production and characterization of endoglucanase produced by *Chaetomium globosum*. In addition, the effect of endoglucanase and some other lignocellulose degrading enzymes on corn stover degradation was investigated.

MATERIALS AND METHODS

Microorganisms

The fungus *Chaetomium globosum* was obtained from Agric. Microbiol. Dept., Soil, Water and Environ. Res. Institute, Agric. Res. Centre, Giza, Egypt. The original culture was maintained on potato dextrose agar (PDA) slant supplied with 0.5 % cellulose. Stock cultures were kept at 5°C and monthly transferred.

Lignocellulose degrading enzymes and lignocellulosic materials

Endoglucanase produced from this work and three lignocellulose degrading enzymes were applied for the degradation of lignocellulosic materials as corn stover. Lignocellulose degrading enzymes (Table 1) and corn stover were obtained from Faculty of Biochemical and Chemical Engineering, Technical Biochemistry, Dortmund University, Germany.

Table 1. Lignocellulose degrading enzymes used for corn stover degradation

Enzymes	The source
1. Cellulase	Produced from *T. reesei* ATCC 26921, EC 3.2.1.4 (Sigma-Aldrich, C8546)
2. Laminex	Mixture of cellulase and xylanase (DSM, Batch:4900673869)
3. MsP1	Recombinant peroxidase enzyme produced from *Marasmius scorodonius*

Media

Five media were used for screening the highest production of endoglucanase by *C. globosum*, the used media were: Cellulase production medium (Camassola and Dillon, 2007) [(g L^{-1}) Cellulose 10.0; Proteose peptone 1.0; KH$_2$PO$_4$ 0.2; (NH$_4$)$_2$SO$_4$ 0.13; MgSO$_4$ .7H$_2$O 0.03; CaCl$_2$ 0.03; FeSO$_4$.7H$_2$O 0.005; MnSO$_4$.7H$_2$O 0.016; ZnSO$_4$.7H$_2$O 0.0014 and CoCl$_2$.2H$_2$O 0.002] Basal Mineral Salt medium, (Chen and Wayman, 1991) [(g L^{-1}) Cellulose 10.0; Proteose peptone 1.0; KH$_2$PO$_4$ 2.0; (NH$_4$)$_2$SO$_4$ 2.0; MgSO$_4$.7H$_2$O 0.3 and CaCl$_2$ 0.3] Cellulose broth, (Bagga *et al.*, 1990) [(g L^{-1}) Cellulose 5.0; Casein hydrolysate 0.5; Yeast extract 0.5; NaNO$_3$1.0; KH$_2$PO$_4$0.9; Na$_2$HPO$_4$.2H$_2$O 1.2; MgSO$_4$.7H$_2$O 0.5 and KCl 0.5] Czapek-Dox liquid medium containing 1% CMC, (Coral *et al.*, 2002) [(g L^{-1}) Carboxymethyl cellulose 10.0; NaNO$_3$ 3.0; K$_2$HPO$_4$ 1.0; MgSO$_4$ 0.5; KCl 0.5 and FeSO$_4$ 0.01] Reese and Mandel's basal medium, (Reese and Mandel, 1963) [(g L^{-1}) Cellulose powder 10.0; Proteose peptone 0.25; (NH$_4$)$_2$SO$_4$1.4; Urea 0.3; KH$_2$PO$_4$ 2.0; CaCl$_2$. 2H$_2$O 0.3; MgSO$_4$.7H$_2$O 0.3; 1 mL L^{-1} Tween 80 and 1 mL L^{-1} Trace metal solution [(mg L^{-1}) FeSO$_4$.7H$_2$O 5.0; MnSO$_4$.7H$_2$O 5.6; ZnSO$_4$.7H$_2$O 3.34; CoCl$_2$.2 H$_2$O 2.0]. Each medium was adjusted to pH 6.

Fermentation and optimization studies

About 95 milliliter of the production medium were dispended into 250 mL Erlenmeyer flasks, sterilized and inoculated with 5 mL of a 3-day-old fungal inoculum. The inoculated flasks were incubated with shaking at 150 rpm for 6 days at 25˚C. The cultures were centrifuged at 4000 rpm for 30 min at 4˚C. The supernatant was used for measurement of endoglucanase activity.

Optimization of enzyme production by *C. globosum* was carried out in the presence of some factors such as production media, cellulose concentration, nitrogen source and its concentration, initial pH, time course and the inoculum volume.

Enzyme assay

Endoglucanase activity was determined according to the method of Bailey (1981) as follow, 100 µL of culture filtrate was added to 300 µL of 1.0% hydroxyethylcellulose (HEC) in 0.05 M sodium-acetate buffer (pH 3.5). The reaction mixture was incubated at 50°C for 25 min. Finally, the released reducing sugars were determined and calculated using standard curve of

glucose. One international unit of enzyme activity is defined as the amount of enzyme that liberates one micromole glucose/min under the standard assay conditions.

Determination of reducing sugars

Reducing sugars was carried out using dinitrosalicylic acid method (Miller *et al*., 1960).

Concentration of endoglucanase

Culture of *C. globosum* was centrifuged at 4000 rpm for 30 min at 4°C and the clear supernatant was used as source of crude enzyme. Endoglucanase was concentrated by ultrafiltration technique (Jumbosep[TM] Centrifugal Devices, exclusion limit 10 kDa) at 3000 rpm and 4 °C. Following the ultrafiltration, protein concentration and endoglucanase activity in the retentate and filtrate were measured.

Kinetic constants

Kinetic parameters such as K_m and V_{max} of endoglucanase was determined under steady state conditions by Lineweaver-Burk double reciprocal plot of enzyme activity against substrate concentration. Activity assay was carried out at pH 3.5 and 30°C. Graphs were drawn using the enzyme kinetic module.

Effect of temperature on endoglucanase activity and thermal stability

The crude enzyme preparation was assayed for endoglucanase activity at temperature range of 30-75 °C for 25 min. Thermal stability of endoglucanase was detected by incubating the enzyme at its optimum temperature for different periods intervals, starting from 2 to 12 hours. Samples were removed periodically every 2 hours and assayed for residual activity under standard assay conditions.

Effect of pH on endoglucanase activity and pH stability

Enzyme activity was measured after incubating the reaction mixtures for 25 min at different pH values, ranging from pH 3 to 6 using 50 mM sodium acetate buffer. pH stability was investigated by incubation at the optimum pH value for different time intervals, starting from 2 to 12 hours.

Isoelectric focusing (IEF)

Isoelectric point was detected according to the methods of Kluepfel, (1988). Polyacrylamide gel (IEF-gel, Serva) with immobilized pH gradient ranging from 3 - 10 was used. 5 µL of marker (Servalyt®, precote® 3-10) and 6 µL as sample were individually applied to a flatbed electrophoresis apparatus. In parallel, 8 µL samples were used for the activity staining.

Protein bands were visualized by staining for 1 h. with 0.2 % Coomassie Brilliant Blue CBB-R250 dye in methanol-acetic acid-water solution (4:1:5, by volume). The same solution without dye was used for the destaining process. For the activity staining, the gel was placed on 1% agarose plate containing 0.1 % hydroxyethylcellulose and incubated at 40 °C for 4-5 h. The agarose plate was then stained with 0.1 % congo red solution for 30 min and destained in 1 M NaCl for 15 min. Clear zone (unstained) on the agarose plate indicates the presence of endoglucanase activity.

SDS –PAGE analysis

Active IEF band was cut, ground in 60 µL ultrapure water and mixed with 10 µL loading buffer. 50 µL as sample was applied to polyacrylamide gel electrophoresis. 5µL protein marker for SDS-PAGE (Roti®-Mark STANDARD Roth) was used. Protein bands were visualized by staining for 1 h. with 0.2 % Coomassie Brilliant Blue CBB-R250 dye in methanol-acetic acid-water solution (4:1:5, by volume). The same solution without dye was used for the destaining process. A plot of log molecular weight versus relative mobility (Rf) of standard proteins was used to estimate molecular weight of endoglucanase enzyme, Laemmli, (1970).

## Application of lignocellulose degrading enzymes for corn stover degradation

This experiment was performed in two steps, the first is pretreatment of lignocellulose materials by peroxidase (MsP1), and the second is to find out the effect of endoglucanase and some lignocellulose degrading enzymes on corn stover degradation. Pretreatment of corn stover with peroxidase (MsP1) was applied using 50 mM sodium acetate buffer. Concerning the blank sample, sodium acetate buffer was used instead of peroxidase. All treatments were incubated at 30 ºC for 24 h. under shaking. For the activity of peroxidase enzyme, 20 mM hydrogen peroxide was periodically added after an every complete consumption of hydrogen peroxide. After that, all treatments were centrifuged and the residual corn stover was washed three times with the same used buffer. In addition, at the last washing time, one unit of catalase enzyme was added to remove the remaining hydrogen peroxide.To investigate the effect of lignocellulose degrading enzymes on treated and untreated corn stover, 5 units of endoglucanase, cellulase produced from *T. reesei* and laminex were individually added. All treatments were incubated at 30 ºC for 24 h. under shaking.

Samples were taken after 0, 6, 12 and 24 h. to determine the catalytic activity of the enzymes on corn stover degradation by measuring the released reducing sugars, glucose and xylose, as abovementioned.

RESULTS AND DISCUSSION

Effect of different media on endoglucanase production

The influence of the production media on endoglucanase production by *C. globosum* was investigated. Five different media were tested for their ability to support growth and enzyme production (Fig. 1). Data indicated that Reese and Mandel's basal medium was the best medium for endoglucanase production. This may be attributed to the presence of Tween 80 within the constituent of the medium, which supports the release of the enzyme into the culture medium.
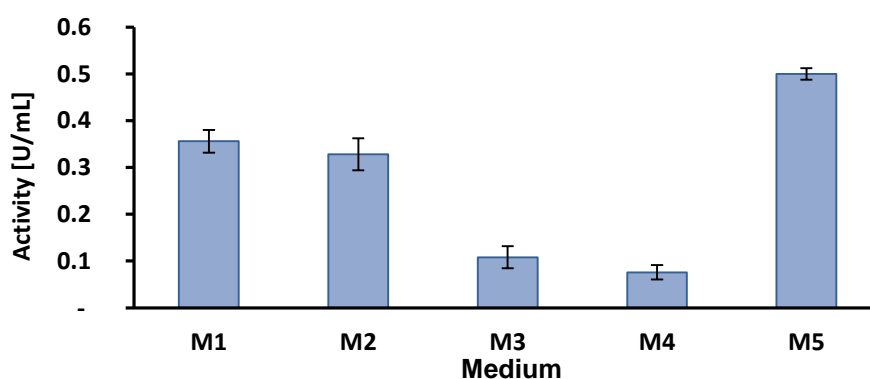
**Fig.1. Effect of different media on endoglucanase production.**

M1: Cellulase production medium (CPM).   **M2**: Basal mineral salt medium.
M3: Cellulose broth.   **M4**: Czapek-Dox liquid medium containing 1% CMC
M5: Reese and Mandel's basal medium.

The obtained results are in accordance with those reported by Vyas (2004) who demonstrated that the addition of Tween 80 as surfactant led to higher cellulase activities. Moreover, Tween 80 facilitates the release of cellulases into the medium by causing an increased permeability of cell membranes and/or by promoting the release of cell-bound enzymes.

Reese and Mandel's basal medium was followed by cellulase production medium (CPM). On the other hand, Czapek-Dox liquid medium containing 1% CMC gave the lowest activity. Consequently, Reese and Mandel's basal medium was used for the succeeding experiments.

Effect of cellulose concentration

Different concentrations of cellulose (0.5 - 2.5%) were added to Reese and Mandel's basal medium to study their effect on endoglucanase production (Fig. 2). Data show gradual increase of endoglucanase activity reaching a maximum activity of 0.94 U/mL at 2.5% cellulose.
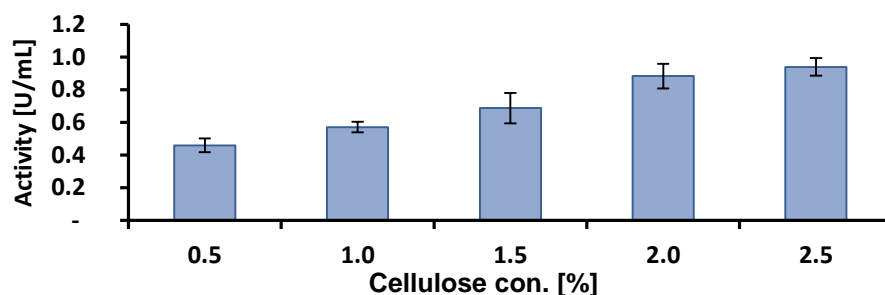
Fig.2. Effect of cellulose concentration on endoglucanase production.

Similar result was obtained by Vyas (2004) who stated that maximum CMCase activity of *Fusarium* sp. was obtained with 2.5% cellulose powder. Moreover, Rao *et al.* (2003) recorded maximum CMCase production of the extremophilic actinomycete *Thermomonospora* sp. when 4% cellulose powder was used as the carbon source. In addition, Narasimha *et al*. (2006) stated that the production of cellulase from *A. niger* in response to cellulose concentration.

The production of hydrolytic enzymes is directly related to the available substrate (Nybroe *et al.* 1992). Endoglucanase is an inducible enzyme system. Therefore, an increase in the concentration of a particular substrate may stimulate the microorganisms' specific enzyme production.

Effect of the nitrogen source

The effect of various nitrogen sources on endoglucanase production by *C. globosum* using Reese and Mandel's basal medium was illustrated in Fig. 4. Among the various organic and inorganic nitrogen sources, urea was found to be optimal for endoglucanase production by *C. globosum*. On the other hand, sodium nitrate yielded the lowest cellulolytic activity.
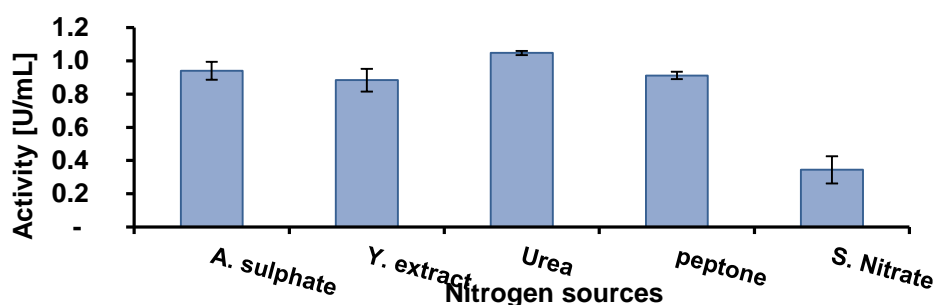
Fig.4. Effect of different nitrogen sources on endoglucanase production.

Previous experiments on the effect of various nitrogen sources on cellulase production demonstrated a substantial increase in the enzyme activity when the media were supplemented with nitrogen sources like urea (Vyas, 2004).

Effect of different concentrations of urea

Reese and Mandel's basal medium was supplemented with 0.1 – 0.4% urea, and endoglucanase activity was determined (Fig. 5).
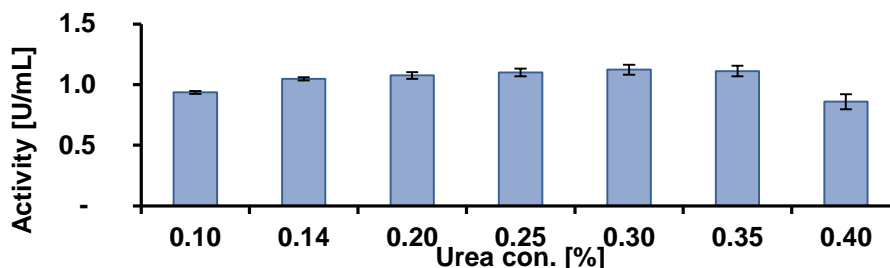

Fig.5. Effect of urea concentration on endoglucanase production

Data show that urea at concentration of 0.3% resulted in maximum enzyme activity by *C. globosum*. The activity increased with increased urea concentration up to 0.35%.

Jin and Toda, (1989) reported that an increase of urea concentration from 2 to 6 g/L improved endocellulase production of the bacterium *Clostridium thermocopriae*. In contrast, the growth of T*richoderma reesei* on production medium without nitrogen source increased cellulase production (Turker and Mavituna, 1987).

Effect of initial pH-value

The initial pH of Reese and Mandel's basal medium was adjusted to pH 4.0 to 8.0. After fermentation process, endoglucanase activity was determined (Fig. 6).
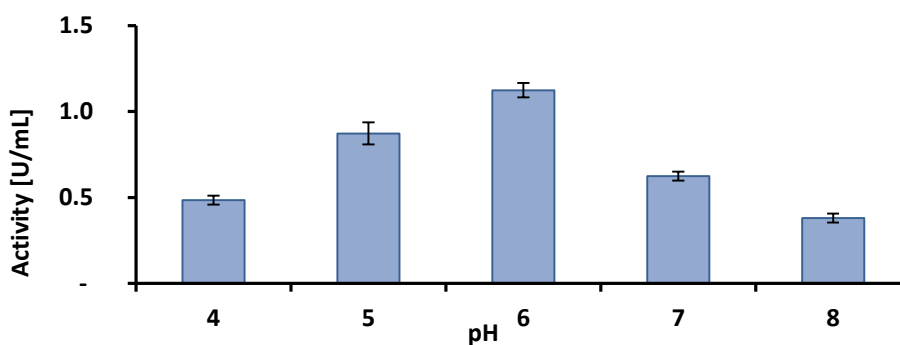

Fig.6. Effect of initial pH on endoglucanase production

Generally, endoglucanase formation strongly depended on the pH value. pH 6 was found to be optimum for enzyme production by *C. globosum*. Similarly, El-Baz (2003) found pH 5.0 to be most suitable for cellulase formation with *Trichoderma viride* and *Aspergillus niger*. In contrast, Bollok and Reczey (2000) investigated that pH 5.0 was unfavorable for cellulase enzyme production by *Trichoderma reesei* Rut C-30. Enzymes have a characteristic optimum pH at which their formation is maximal. Above or below this pH the enzyme secretion declines (El-Sawah, 2002).

Effect of the cultivation period

The time course of endoglucanase was studied to determine the point of time with maximum activity (Fig. 7). Endoglucanase activity gradually increased with increasing the culture period. Maximum enzyme activity was obtained after 8 days of cultivation.
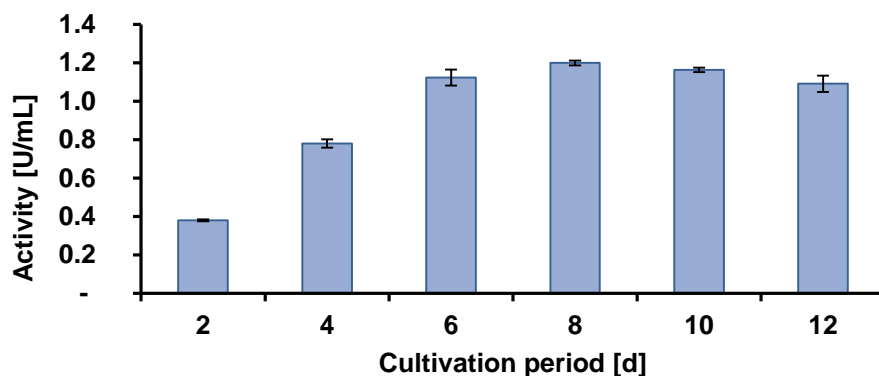
**Fig.7. Effect of cultivation period on endoglucanase production.**

Similarly, Vyas (2004) obtained maximum cellulase production by *Fusarium* sp. after 8 days of cultivation. Prolongation of the cultivation period above 8 days resulted in gradual decrease in the enzyme activity which can be explained as the end products inhibition as mentioned by Gan et al., (2003) and Zaldivar et al., (2001).

Effect of the inoculum volume

The inoculum volume is one of the most important factors that affect the enzyme yield. Inoculation was performed with inoculum volumes from 2.0 - 12.0%. The cultures were grown under the previously optimized conditions.

It is seen from the obtained results, which graphically illustrated in Fig. 8 that the enzyme production gradually increased with increasing the inoculum volume. Endoglucanase activity of *C. globosum* reached the maximum at 8% (v/v) inoculum volume. Moreover, increasing of the inoculum volume more than 8% led to slight decrease of the enzyme activity.

Similarly, Hao *et al*. (2006) used an inoculum volume of 10% for the production of cellulase by *Trichoderma reesei* WX-112.
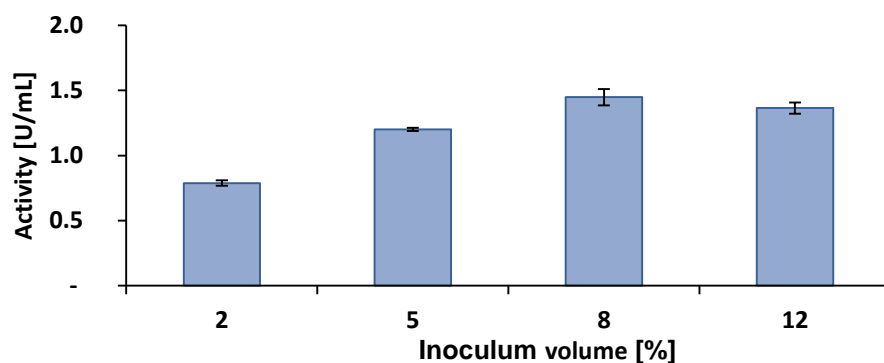
Fig.8. Effect of inoculum volume on endoglucanase production

## Concentration of endoglucanase produced by *C. globosum*

The culture supernatant of *C. globosum* was concentrated by ultrafiltration (Table 2). Endoglucanase and protein yields after concentration were 60.2 and 35.6%, respectively. The obtained specific activity was 2.17 U/mg. The protein after ultrafiltration was 6.76 mg/mL which was sufficient for analysis by means of isoelectric focused electrophoresis.

Table 2. Concentration of endoglucanase produced by *C. globosum* by ultrafiltration

|	Volume [mL]	Activity [U/mL]	Protein concentration [mg/mL]	Specific activity [U/mg]	Total activity [U]	Activity yield [%]	Total protein [mg]	Protein yield [%]
Initial supernatant	1,000	1.22	0.95	1.28	1,220	100	950	100
Retentate	50	14.69	6.76	2.17	734.5	60.2	338	35.6

Kinetic constants

Two kinetic parameters i.e. Michaelis-Menten constant (K_m) and maximum velocity (V_{max}) was worked out using different concentrations of the substrate (HEC). The obtained results are graphically illustrated in Fig. 10. Endoglucanase was found to have K_m value of 2.27 mg/mL. Maximum velocity was 0.1194 µmol/min/mL as determined from Lineweaver-Burk plot.
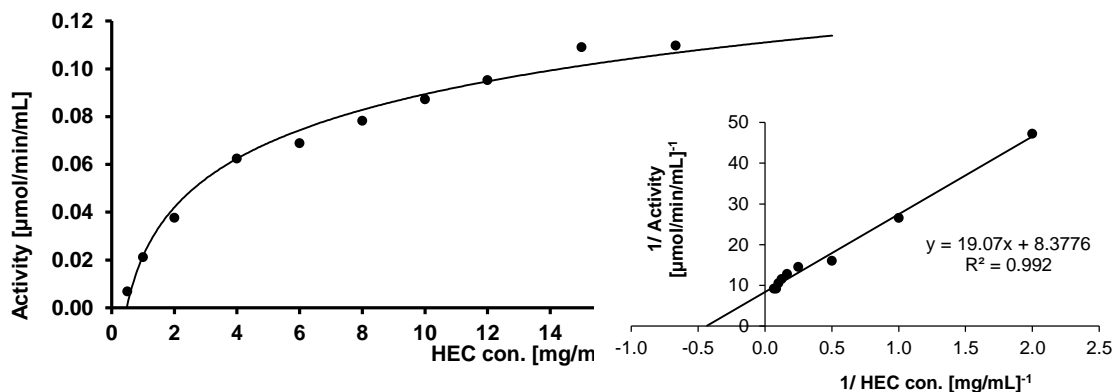
Fig.10.Michaelis-Menten curve and double reciprocal (Lineweaver-Burk) plot with HEC as substrate for endoglucanase activity
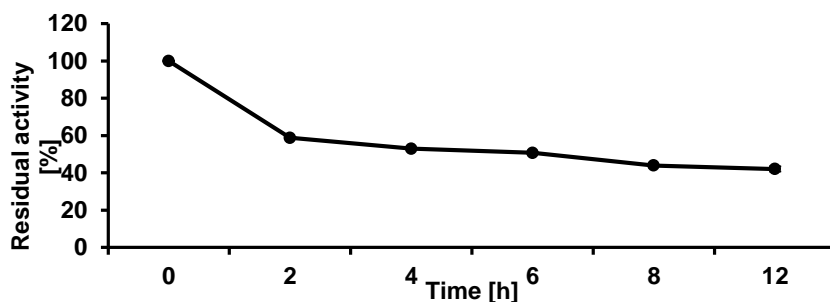
Effect of temperature on endoglucanase activity and thermal stability

Temperature optimum and thermostability of endoglucanase were determined under standard conditions (Figs.10a, 10b). The enzyme preparations were active in a broad temperature range of 30 °C to 75°C. The optimum temperature for endoglucanase activity was 65 °C. These results are in good agreement with those reported by Rao *et al.* (2003) and Wang *et al.* (2008). Moreover, Raza and Ur-Rehman (2009) mentioned that the use of thermostable enzymes to carry out hydrolysis at high temperature is advantageous because they speed up the reaction rate and prevent microbial contamination.
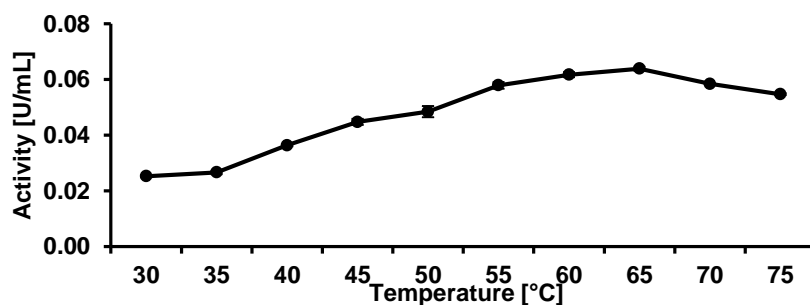
**Fig.10a. Effect of the temperature on endoglucanase activity**

Endoglucanase enzyme retained about 60 % of its activity when incubated at 65 °C for 2 h. Moreover, the enzyme lost about 58% from its activity at 65 °C for 12h. The loss of enzyme activity can be attributed to protein denaturation. The aforementioned results are in accordance with those obtained by Reis *et al.* (2003), Whiteley *et al.* (2003) and Wang *et al.* (2008) who stated that the loss of enzyme activity at elevated temperatures is a consequence of thermal denaturation of protein.

Fig.10b. Thermal stability of endoglucanase

## Effect of pH on endoglucanase activity and pH stability

To determine the pH optima and the pH stability of endoglucanase, the activity was checked at different pH values (3.0 – 6.0). The obtained results are illustrated in Figs.11a and 11b.
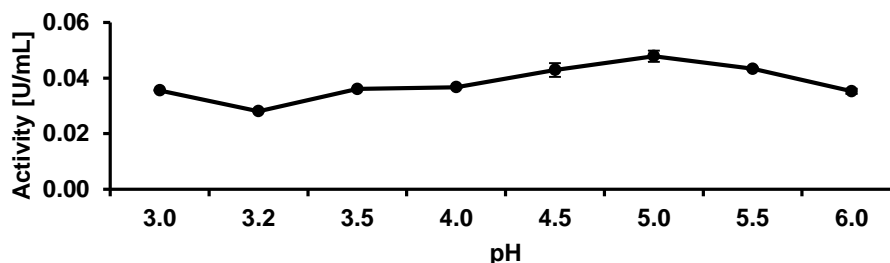
Fig.11a. Effect of pH on endoglucanase activity

Endoglucanase of *C. globosum* exhibited high activity in the pH range from 3.0 - 6.0 with an optimum at pH 5.0. These results are in good agreement with those of Coral *et al.* (2002). They studied the pH dependence of an endocellulase from a wild type strain of *Aspergillus niger* and reported a broad activity range of 3.0 - 9.0 with a maximum activity at pH 4.5. In addition, endoglucanase showed high stability for pH 5 when incubated for 12 h.

The ionization state of amino-acid residues of an enzyme depends on the pH value. Thus, enzyme activity is consequently pH dependent. Enzymes are often active over a narrow pH range with a specific pH optimum at which their catalytic activity is maximal (Wilson, 2000).
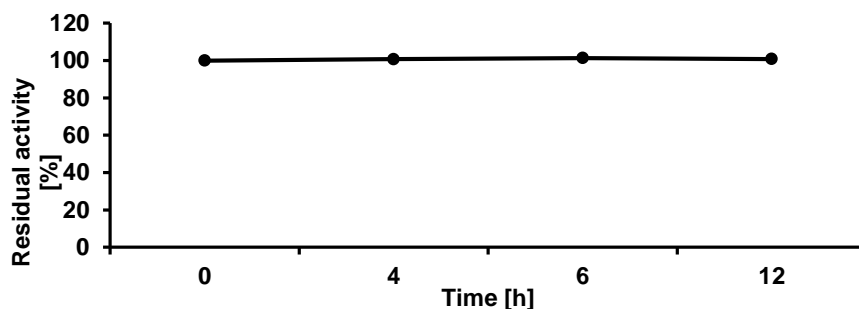

Fig.11b. pH stability of endoglucanase

Isoelectric focusing (IEF)

From the activity stained agarose gel, an isoelectric point (pI) around 4.5 was detected for endoglucanase of *C. globosum* (Fig. 12).

SDS –PAGE analysis

A plot of log molecular weight versus relative mobility (Rf) of standard proteins was used to estimate molecular weight of endoglucanase enzyme. Single band was detected on the SDS-PAGE gel (Fig. 13). The molecular weight was calculated to be about 40 KDa.
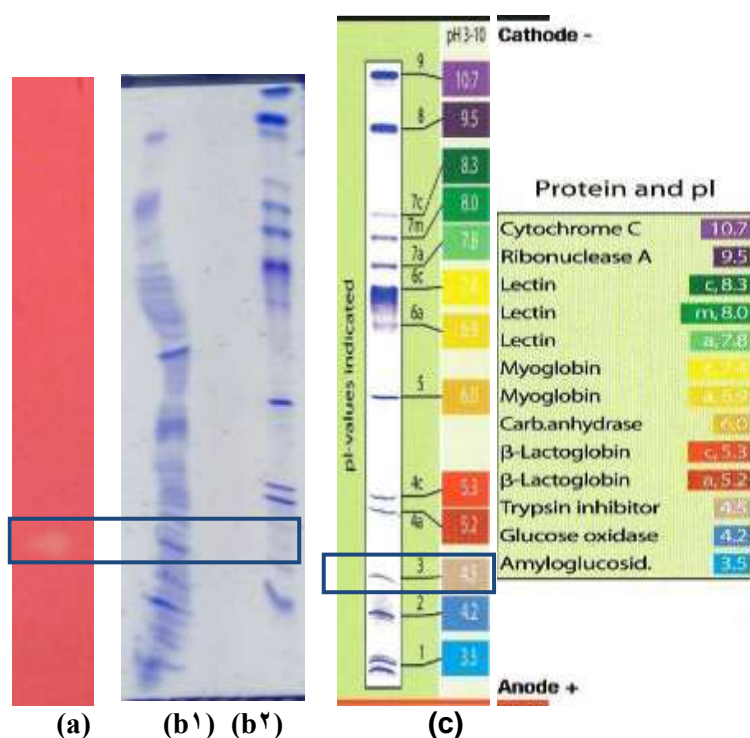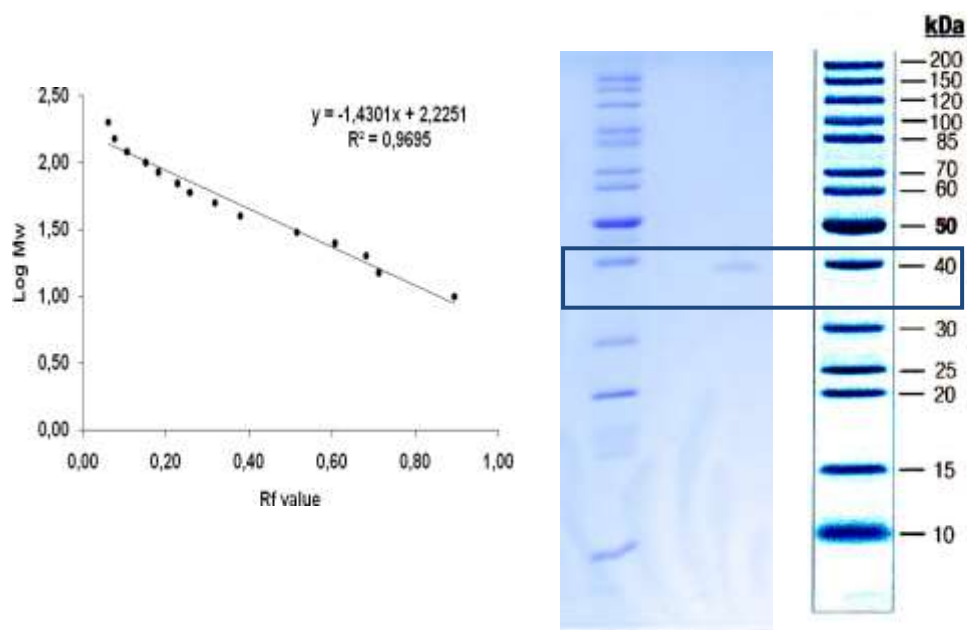

Fig. 12. Analysis of endoglucanase (*C. globosum*) by IEF electrophoresis, (a): activity staining with congo red, (b1, b2): Coomassie staining of sample (6µL) and protein marker (5µL), respectively. (c): protein marker.

Application of lignocellulose degrading enzymes for corn stover degradation

Pretreatment of lignin with enzymes, such as peroxidases, is very important to improve the accessibility of lignocelluloses yielding lignocellulosic materials that are much more susceptible to cellulases and hemicellulases attack.

MsP1 represents a peroxidase originally derived from the basidiomycete *Marasmius scorodonius*. 1.5 units were used for the degradation of corn stover lignin, followed by treatment with cellulases and hemicellulases (Fig.14).

Fig. 13. SDS-PAGE analysis of endoglucanase secreted by *C. globosum*

The amounts of released reducing sugars increased with increasing the incubation period up to 24 hours. Higher concentrations of reducing sugars were released after pretreatment with peroxidase, indicating a direct attack of MsP1 on the lignin fraction of corn stover.

Lignin is the most recalcitrant biomaterial to degradation because of its highly ordered crystalline structure. Enzymes are preferred for lignin degradation since they operate under mild reaction conditions, do not produce undesirable side-products and are environmentally compatible (Howard *et al.,* 2003).

Two families of lignolytic enzymes are widely considered to play a key role in the enzymatic degradation: phenol oxidase (laccase) and peroxidases (lignin peroxidase (LiP) and manganese peroxidase (MnP) (Krause *et al.,* 2003; Malherbe and Cloete, 2003). Moreover, Martinez *et al.,* (2005) stated that lignin peroxidase and manganese peroxidase were described as true ligninases because of their high redox potential. LiP degrades non-phenolic lignin units (up to 90% of the polymer), whereas MnP generates Mn^{3+}, which acts as a diffusible oxidizer on phenolic or non-phenolic lignin units via lipid peroxidation reactions.

Laminex enzyme showed the highest catalytic activity to hydrolyze polymeric carbohydrates of corn stover. These results may be explained by the fact that Laminex exhibits cellulase and hemicellulase activity.

These results are similar to those reported by Berlin *et al.,* (2007). They found that ability of a commercial *T. reesei* cellulase preparation to hydrolyze cellulose of corn stover was

significantly improved by supplementation with three types of crude commercial enzyme preparations enriched in xylanase, pectinase, and β-glucosidase. Moreover, high glucose yields from corn stover are obtained if xylanases are used to supplement cellulases during hydrolysis. Xylanases hydrolyse residual hemicellulose, thereby improving the access of enzymes to cellulose (Ohgren *et al.*, 2007).
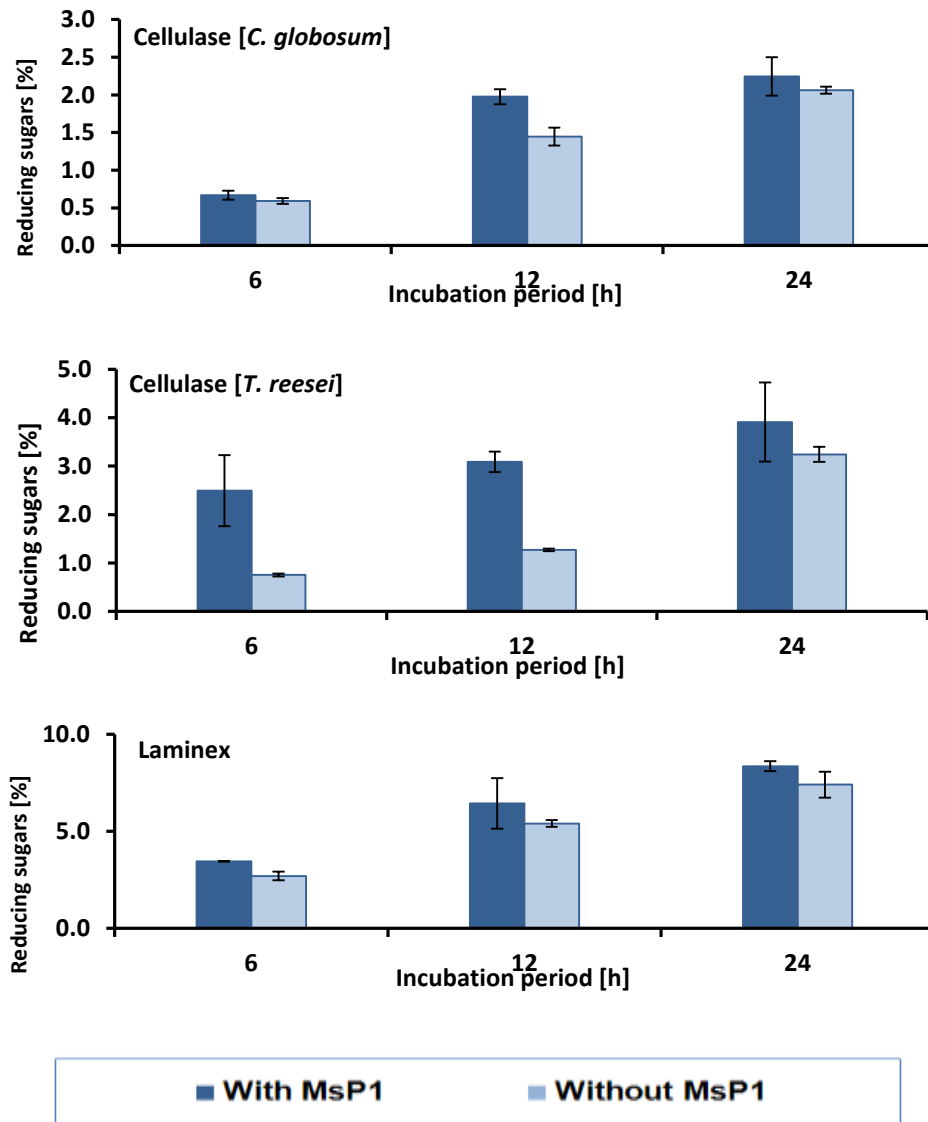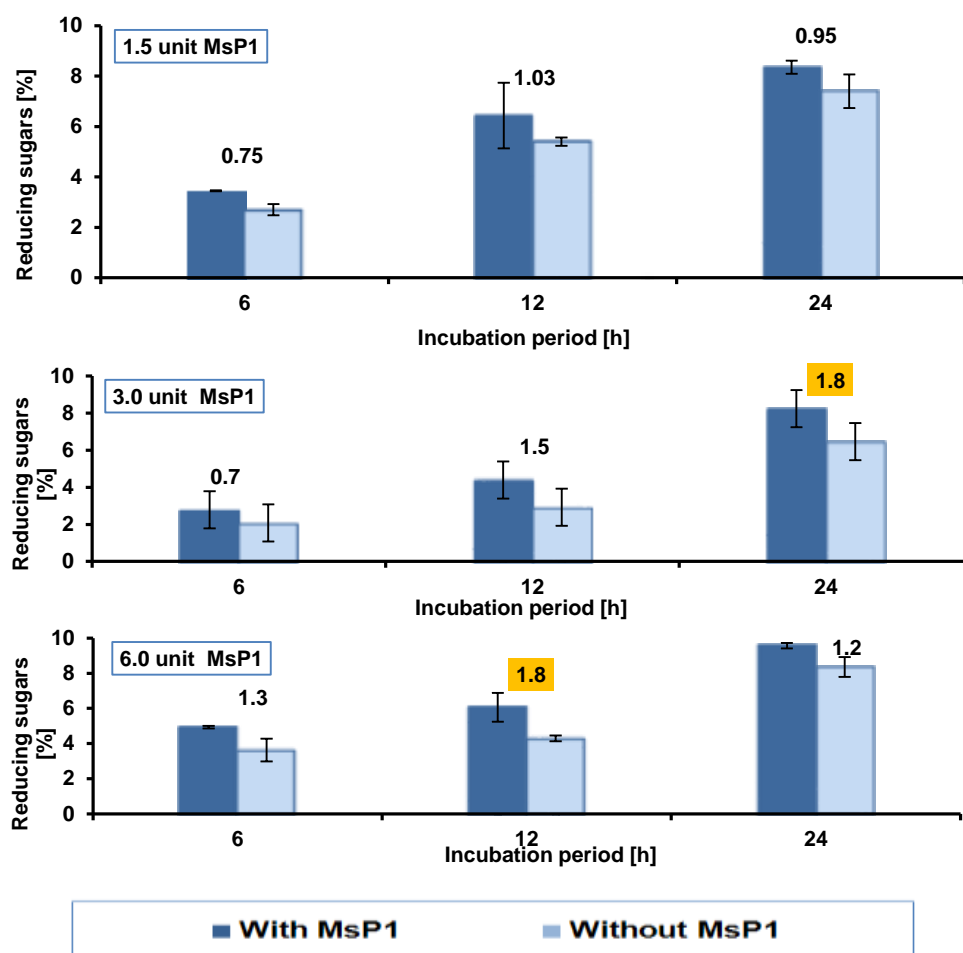
Fig.14. Application of MsP1 enzyme in corn stover degradation

Application of different amounts of MsP1 in corn stover degradation

Different amounts of MsP1 were examined for the degradation of corn stover lignin. 1.5, 3.0 and 6.0 units of MsP1 were applied using 50 mM Na-acetate buffer at pH 3.5. For the activity of MsP1, hydrogen peroxide was periodically added after its complete consumption. Then, laminex enzyme was applied to hydrolyze cellulose and hemicellulose of corn stover (Fig. 15).

The lowest lignin degradation was obtained when 1.5 unit of MsP1 were used. While, the high degradation rates were found after 12 and 24 hours incubation when 6.0 and 3.0 units of MsP1 were used, respectively. These results indicate that the maximum level of lignin degradation can be reached after 24 hours with using the half unit's level of MsP1.

Fig. 16. Application of MsP1 at different levels in corn stover degradation

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