ELSEVIER

Bioresource Technology 73 (2000) 113–121

BIORESOURCE TECHNOLOGY

Production of *Aspergillus terreus* xylanase in solid-state cultures: application of the Plackett–Burman experimental design to evaluate nutritional requirements

Nevine B. Ghanem *, Hoda H. Yusef, Heba K. Mahrouse

Botany Department, Faculty of Science, Alexandria University, Alexandria, Egypt

Received 12 July 1999; received in revised form 3 November 1999; accepted 5 November 1999

Abstract

Xylanase was produced by *Aspergillus terreus* cultivated on finely ground wheat straw in solid-state fermentation. The optimal medium composition was developed by applying the Plackett–Burman experimental design. Best enzymic activity was obtained in a medium containing 10 g wheat straw/flask moistened with a concentrated nutrient salt solution to 75% initial water content and incubated for 4 days at 30°C. *A. terreus* xylanase was fractionated by ammonium sulfate precipitation and purified by chromatography on DEAE Bio-Gel A followed by gel-filtration on Sephadex G-75. The enzyme was characterized by apparent V_{max} and K_m values of 333.3 U/mg protein and 16.7 mg xylan/ml, respectively, obtained for xylanase with oat spelt xylan as substrate. The optimal pH and temperature for maximum activity were 7 and 50°C, respectively. The enzyme showed high specificity towards oat spelt xylan and minute activities were observed with carboxymethyl cellulose and cellobiose. About 48.02% of the activity remained after the enzyme had been incubated at 60°C for 30 min. Metal ions such as Hg^{2+}, Cu^{2+}, Co^{2+}, Fe^{3+}, Pb^{2+} strongly inhibited xylanase, whereas, Ca^{2+} activated the enzyme. © 2000 Elsevier Science Ltd. All rights reserved.

Keywords: *Aspergillus terreus*; Xylanase; Solid-state fermentation; Wheat straw

1. Introduction

Endo-1,4-B-D-xylanases (EC 3.2.1.8) are produced by a wide variety of microorganisms, among which the fungi are the most potent producers (Purkarthofer et al., 1993; Ishihara et al., 1997). Xylanases have major applications in the pulp and paper industry (Vükari et al., 1991). A treatment with xylanases facilitates the chemical extraction of lignin from pulp. This leads to a significant saving of chemicals required for bleaching and for the reduction or the emission of toxic compounds into the environment (Haltrich and Steiner, 1994).

Production of xylanase enzyme by solid-state fermentation (SSF) using various lignocellulosic substrates has been reported and was found to have several advantages (Shamala and Sreekantiah, 1986; Purkarthofer et al., 1993).

For a broad application, the cost of enzymes is one of the main factors determining the economics of a process (Biely, 1985). Reducing the costs of enzyme production by optimizing the fermentation medium is the basic research for industrial application. The use of different statistical designs for medium optimization has been successfully employed especially for xylanase production (Gomes et al., 1993; Haltrich et al., 1993). These statistical methods, as compared to the common 'one-factor-at-a-time' method, proved to be powerful and useful tools.

The objective of this study was to evaluate the xylanase production of *Aspergillus terreus* grown on wheat straw as a substrate in solid-state culture. The effect of medium components on the production of the enzyme was also studied using Plackett–Burman experimental design. Purification and characterization of the produced enzyme were reported.

2. Methods

2.1. Microorganism

The fungal strain used in this study was isolated from a local wheat field soil and identified by Microcheck, Inc., USA as *A. terreus*. Stock cultures were grown at

* Corresponding author.

0960-8524/00/$ - see front matter © 2000 Elsevier Science Ltd. All rights reserved.
PII: S0960-8524(99)00155-8

Table 1
The eight-trial Plackett–Burman experimental design matrix for the evaluation of relative importance of selected nutrients for xylanase activity produced by *A. terreus*

Trial	Factor(s) under study	Variable						
		W[a]	N[b]	K[c]	Fe[d]	Mg[e]	Ca[f]	P[g]
1	W N K Mg Ca P	+	+	+	−	+	−	−
2	W N Fe P	+	+	−	+	−	−	+
3	W K Ca P	+	−	+	−	−	+	+
4	N Mg Ca P	−	+	−	−	+	+	+
5	W Fe Mg Ca	+	−	−	+	+	+	−
6	K Fe Mg P	−	−	+	+	+	−	+
7	N K Fe Ca	−	+	+	+	−	+	−
8	–	−	−	−	−	−	−	−

[a] Waste at a high concentration of 20 g/l and a low concentration of 10 g/l.
[b] $(NH_4)_2SO_4$ at a high concentration of 0.47 g/l and a low concentration of 0.33 g/l.
[c] KH_2PO_4 at a high concentration of 2.79 g/l and a low concentration of 1.41 g/l.
[d] $FeSO_4 \cdot 7H_2O$ at a high concentration of 0.15 g/l and a low concentration of 0 g/l.
[e] $MgSO_4 \cdot 7H_2O$ at a high concentration of 0.39 g/l and a low concentration of 0 g/l.
[f] $CaCl_2 \cdot 2H_2O$ at a high concentration of 0.39 g/l and a low concentration of 0 g/l.
[g] pH at a high level of 7 and a low level of 5.5.

30°C for 3–4 days, maintained on glucose-peptone and stored at 4°C with subsequent transfers every 6–7 weeks.

2.2. Substrate

Lignocellulosic substrate (wheat straw) was used as the carbon source in fermentation medium. It was ground in a Wiley mill to 2 and 5 mm size. Steam treatment was carried as described by Abdullah et al. (1985). Delignification of wheat straw was done with alkali (Araujo and D'souza, 1980) or sodium hypochlorite (Lynch et al., 1981).

2.3. Fermentation medium

The medium used for solid-state culture was that reported by Purkarthofer et al. (1993) with slight modification. Wheat straw (10 g) was placed in a 250 ml Erlenmeyer flask and a concentrated nutrient salt solution containing $(NH_4)_2SO_4$, 0.4; KH_2PO_4, 2.1; $FeSO_4 \cdot 5H_2O$, 0.11; $MgSO_4 \cdot 7H_2O$, 0.3; and $CaCl_2 \cdot 2H_2O$, 0.3 g/l was used to moisten the solid substrate with initial water content of 50% unless otherwise stated, pH was adjusted to 6.0.

2.4. Inoculum preparation and solid-state cultivation (SSC)

Inocula were prepared as spore suspensions by scraping a 7 days old agar slant with 10 ml sterile distilled water. Each flask received a standard inoculum (2 ml) containing a number of spores equivalent to 1.2×10^4. The cultures were incubated statically at 30°C in a H_2O-saturated atmosphere. Every 24 h the flasks were shaken by hand to prevent solidification of the medium.

2.5. Preparation of the crude enzyme and estimation of protein

The crude enzyme was obtained after cultivation by soaking the mouldy substrate with 1 mM sodium phosphate buffer pH 6 and extracting at room temperature (22–28°C) for 1 h. The contents were then filtered through a nylon cloth and centrifuged for 15 min at 12,000 rpm in a cooling centrifuge to remove cells and residual wheat straw (Shamala and Sreekantiah, 1986). The clarified extract represented the crude enzyme. The protein content of the crude enzyme extract was determined according to the method of Lowry et al. (1951).

2.6. Estimation of xylanase activity

Xylanase (1,4,β-D-Xylanase) activity was assayed according to Kluepfel et al. (1986) by incubating the diluted enzyme extract with an aqueous solution of 1% (w/v) oat spelt xylan dissolved in 50 mM sodium phosphate buffer pH 7 at 50°C for 15 min. The released reducing sugars (as D-xylose) were assayed by adding 1 ml of DNS (3,5 dinitrosalicylic acid) reagent, boiling for 5 min, cooling and measuring the absorbance at 540 nm (Miller, 1959).

Two assays for cellulase activity were used. Filter paper cellulase activity (FPase), as an indication of overall cellulolytic activity, was determined using the filter paper assay according to IUPAC (1987) with Whatman No. 1 filter paper as a substrate in 0.05 M citrate buffer, pH 5.0. Carboxymethylcellulase (CMCase) activity was assayed with a 1% solution of carboxymethylcellulase in 0.05 M citrate buffer, pH 5.0, as a substrate. One unit of xylanase activity represents 1 μm of xylose, or glucose, equivalents released per minute under the given conditions.

2.7. Evaluation of nutritional effects

The Plackett–Burman experimental design (Haaland, 1989) was used to evaluate the relative importance of various nutrients for xylanase production in SSF. Seven variables representing six nutritional components and initial pH of the medium were used. The rows in Table 1 represent the eight different trials and each column represents a different variable. For each nutrient variable, a high (+) and a low (−) concentration were tested. The main effect of each variable upon xylanase activity was estimated as the difference between both averages of measurements made of the high level (+) and at the low (−). The significance of each variable was determined by applying the student's *t*-test (Plackett and Burman, 1946).

2.8. Enzyme fractionation and purification

The crude enzyme extract of 7 days old culture of *A. terreus* grown under optimal fermentation condition was precipitated using ammonium sulfate (65% saturation), which was slowly added to the enzyme extract (3:1, v/v). The mixture was stirred and kept at 4°C overnight, then centrifuged at 10,000 rpm for 15 min in a cooling centrifuge. The supernatant was discarded and the precipitated protein was dissolved in 10 ml of 20 mM Tris/HCl buffer (pH 7.4) and dialyzed overnight against the same buffer. Enzyme purification was conducted by the following two successive chromatographic steps.

2.8.1. Ion-exchange chromatography on DEAE-Bio-Gel A

Portions of the concentrated dialyzed ammonium sulfate fraction were carefully applied to a DEAE-Bio-Gel A ion exchange chromatography column ($2.5 \times 60\ cm^2$) equilibrated with 20 mM Tris/HCl pH 7.4. The proteins were eluted with the same buffer with a linear gradient of NaCl (0–0.5 M). Fractions (2.5 ml) were then collected at a flow rate of 0.5 ml/min, and the most active fractions showing xylanase activity were pooled, concentrated and dialyzed against 20mM Tris/HCl buffer (pH 7.4) at 4°C.

2.8.2. Gel filtration

The concentrated DEAE-Bio-Gel A pooled sample was loaded onto a pre-equilibrated column ($2.5 \times 60\ cm^2$) of Sephadex G-75. The protein samples were eluted with 20 mM Tris/HCl buffer, pH 7.4 at a flow of 1 ml/min at 4°C. Those fractions that showed xylanase activity were combined and lyophilized (Li et al., 1993).

2.9. Enzyme characterization

The effect of substrate concentration on xylanase activity was determined using oat spelt xylan (Sigma) concentrations varying from 2 to 10 mg/reaction mixture, and the Lineweaver Burk plot was used (Lineweaver and Burk, 1934). Different substrates (1% w/v) were incubated for 1 min at pH 7 and 50°C with the purified enzyme to detect the substrate specificity.

pH and temperature optima on xylanase activity were carried out over the range of 4–10°C and 30–80°C, respectively. For pH adjustment a 50 mM, acetate buffer (pH 4–6), phosphate buffer (pH 7–8) and glycine sodium hydroxide buffer pH (9–10) were used.

Temperature stability was determined by incubation of the enzyme at the desired temperature for different time intervals (15, 30 and 60 min) in 50 mM sodium phosphate buffer, pH 7. Residual enzyme activity was determined after incubation with the substrate for 15 min.

Influence of some ions and enzyme inhibitors on the enzymatic activity was studied by incubating the enzyme with each of the tested substances (0.01 M) at room temperature for 10min and then the residual activity was measured by adding the substrate, and carrying out the enzyme assay under the optimum conditions. The activity of untreated enzyme was taken as 100%.

3. Results and discussion

3.1. Induction of xylanase by different agroindustrial byproducts

It has been reported that the ratio of cellulose to xylan of the growth substrate is important for production of xylanase (Haltrich and Steiner, 1994). Therefore, lignocellulosic materials from various sources with different cellulose contents were compared for their potentiality to induce xylanolytic enzymes when used as substrates for SSF. Wheat straw (containing 50.7% cellulose) showed the highest inducing effect (Fig. 1), yielding the highest activity titre (16.16 U/ml). Moreover, this substrate showed minimal filter paper and CMase activities. This property is of tremendous importance for pulp bleaching, since treatment of pulp with xylanase preparations containing cellulose has resulted in a reduction of the polymerization degree of the cellulose fibers and a drop in product quality (Senior et al., 1988).

Wheat straw was selected as substrate for further experimentation, not only because of its commercial potential as an agroindustrial byproduct and its suitability for xylanase formation but also because it has received much attention as a substrate for SSF (Yadav, 1988). Compared to wheat straw, the other substrates supported different but lower levels of xylanase activity due to variations in the accessibility to the substrate.

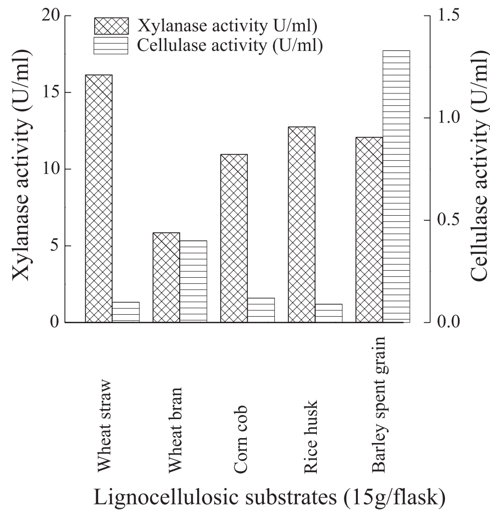

Fig. 1. Effect of various lignocellulosic substrates on enzyme formation by *A. terreus*.

3.2. Pretreatment of substrate

Utilization of pretreated substrate, i.e. autoclaved, steamed or delignified wheat straw, resulted in a drastic (70%) reduction in xylanase activity (data not shown). Thus, the highest xylanase titre was recorded with the finely ground (2 mm) wheat straw which was subsequently used for further experiments.

3.3. Cultivation time

The time course of protein and xylanase production by *A. terreus* in basal medium containing 10 g/flask wheat straw under SSF is displayed in Fig. 2. Maximum xylanase activity (22.03 U/ml) was reached after 4 days; thereafter the enzyme activity declined. Similar observations were reported for the production of pectinases and cellulase by fungi on SSF (Desgranges and Durand, 1990).

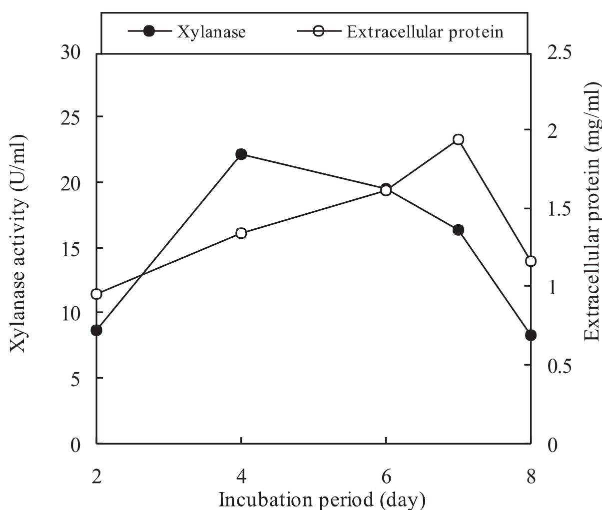

Fig. 2. Protein content and xylanase activity in culture filtrate of *A. terreus* grown on 15 g/flask wheat straw in SSF.

3.4. Effect of nitrogen source

The nitrogen source (ammonium sulfate) in the basal medium was replaced by various inorganic and organic nitrogen compounds to test their effect on xylanase formation by *A. terreus*. The concentration of nitrogen was fixed at 84.86 mg N/l.

Fermentation medium lacking an exogenous supply of nitrogen source yielded an enzyme of considerable activity (10.04 U/mg protein), indicating that the substrate supplied the organism with adequate amount of nitrogen. Ammonium sulfate which proved to be the best nitrogen source among the tested compounds enhanced xylanase formation and a 1.7-fold increase in enzyme activity was produced compared to the control (Fig. 3). Our results are in good agreement with others (Hoq and Deckwer, 1995; Haapala et al., 1995) that showed the suitability of this compound as a nitrogen source for xylanase production by fungi.

3.5. Effect of moisture content

Low moisture content is known to decrease the metabolic and enzymatic activity probably due to reduced solubility of nutrients from the solid substrate, low substrate swelling and higher water tension (Ramesh and Lonsane, 1990). Therefore, to study the effect of moisture level, the substrate was moistened with different volumes of nutrient solution. It was taken into

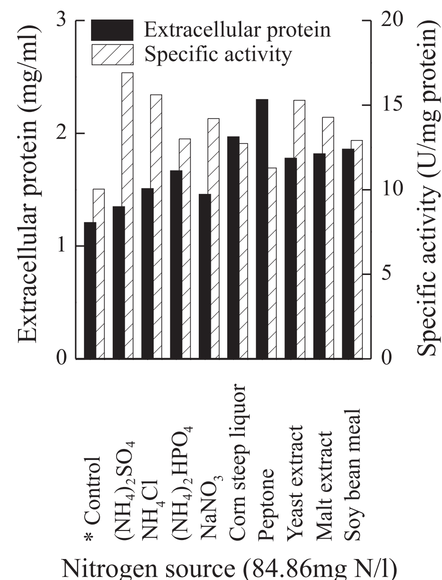

Fig. 3. Effect of various compounds serving as nitrogen source in the fermentation medium on xylanase activity during cultivation of *A. terreus* in SSF.

consideration that the concentration of medium ingredients was not changed. Data in Table 2 reveal that enzyme production in the medium with 40–75% initial moisture was extremely high. Maximal activity (23.98 U/ml) was attained in the medium with 75% initial moisture content (wheat straw: nutrient solution ratio 1:3, v/v). This could be attributed to faster growth of the organism at higher moisture content and the subsequent early initiation of the enzyme production.

3.6. Inoculum size

Inoculum density is an important consideration for SSF process since overcrowding of spores can inhibit germination and development. Under our experimental condition, maximal xylanase activity was achieved upon using an inoculum of 1.2×10^4 spores/flask (Fig. 4). Similar observations were obtained by Shamala and Sreekantiah (1986).

3.7. Plackett–Burman experimental design

Statistical methods for medium optimization have proved to be a powerful and useful tool for biotechnology. Therefore, we attempted to improve the composition of the medium by simultaneous comparisons between two levels of several factors. The factors tested included different concentrations of the medium components, wheat straw, $(NH_4)_2SO_4$, KH_2PO_4, $CaCl_2 \cdot 2H_2O$, $FeSO_4 \cdot 5H_2O$ and $MgSO_4 \cdot 7H_2O$; and the initial pH value prior to sterilization.

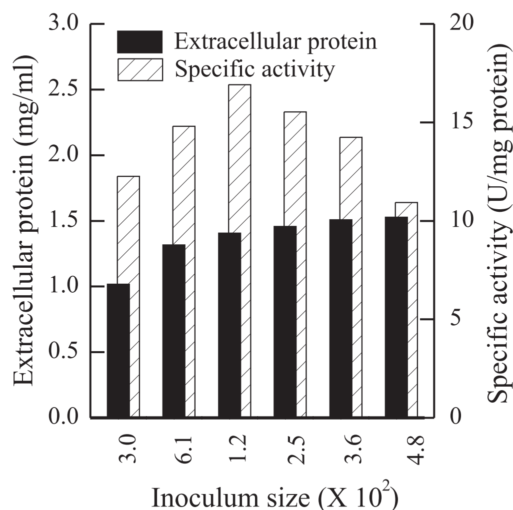

Fig. 4. Effect of different inoculum size on xylanase yield using *A. terreus* in SSF.

From the data displayed in Table 3, it was found that the presence of low concentrations (10 g/flask, 0.33 g/l and 1.41 g/l) of wheat straw, $(NH_4)_2SO_4$ and KH_2PO_4, respectively enhanced xylanase production. The same ranges of 2 concentrations were reported to be optimal for other fungal strains (Haltrich et al., 1993). $CaCl_2 \cdot 2H_2O$ (0.39 g/l) supported high enzyme titre, a result that coincides with that previously found by Bandivadeker and Deshpanda (1994), indicating that Ca is essential for xylanase productivity and stability.

The experimental design employed indicated that the enzyme production by *A. terreus* is not much affected by

Table 2
Effect of moisture level on xylanase formation by *A. terreus* on SSF

Moisture content (%)	Extracellular protein (mg/ml)	Xylanase activity (U/ml)	Specific activity (U/mg protein)
25	1.29	14.96	11.59
40	1.31	21.43	16.36
50	1.35	22.20	16.44
60	1.37	22.58	16.48
75	1.41	23.98	17.01
85	1.45	18.32	12.63

Table 3
Degree of positive or negative effects of various nutrients on xylanase activity produced by *A. terreus* according to the Plackett–Burman experimental design

Factor under study	Specific activity (U/mg protein) Main effect	Significance level (*P*-value)
Wheat straw	−3.44[a]	$P < 0.05$[a]
$(NH_4)_2SO_4$	5.92	ns[b]
KH_2PO_4	1.15	ns[b]
$FeSO_4 \cdot 7H_2O$	−1.35	ns[b]
$MgSO_4 \cdot H_2O$	−0.64	ns[b]
$CaCl_2 \cdot 2H_2O$	11.05[c]	$P < 0.05$[c]
pH	−5.38	ns[b]

[a] Indicates a significant negative effect.
[b] Non-significant at $P < 0.01$.
[c] Indicates a significant positive effect.

pH variation in the range 5–7. Comparative studies showed an optimal pH for *Humicola lanuginora* of 5–6 (Grajek, 1987a). Also Dubeau et al. (1987) reported a pH near neutrality for *Chaetomium cellulolyticum*.

From this point, the medium was modified to be of the following composition; 10 g wheat straw moistened with mineral solution composed of (g/l): $(NH_4)_2SO_4$, 0.33; KH_2PO_4, 1.41 and $CaCl_2 \cdot 2H_2O$, 0.39, pH was adjusted to 5.5 and the initial water content was 75%. *A. terreus* xylanase produced in this simplified low cost medium possessed high activity (64.14 U/ml) representing a 2.7-fold increase compared to that obtained previously before applying the Plackett–Burman experimental design.

3.8. Purification of A. terreus xylanase

The enzyme was purified from the crude culture extract of *A. terreus* (Table 4). All operations were carried out at 4°C unless otherwise indicated. The enzyme was partially purified from the culture extract by salting out using 65% ammonium sulphate with 34.5% recovery of the xylanase activity (Li et al., 1993). The precipitate was suspended in a small volume of 20 mM tris/HCl buffer pH 7.0 and was applied to an anion-exchange column (DEAE Bio-Gel A) equilibrated in the same buffer. Elution was performed with starting buffer to remove unabsorbed protein and thereafter by a peak-controlled gradient (0.1–0.5 M NaCl) in 20 mM/HCl buffer.

The xylanase activity was enriched after DEAE-Bio-Gel A and the specific activity yielded 103.12 U/mg protein (Fig. 5a) with a purification factor of 2.5 and the total protein was reduced from 35.5 to 20.6 mg. The affinity of the enzyme to the DEAE Bio-Gel A was found to be low since it did not bind to the matrix or was eluted from the column with a low concentration of NaCl (Olama et al., 1998).

The highest active xylanase fractions obtained from DEAE-Bio Gel A anion exchange column were loaded on the Sephadex G-75 column. Elution of the protein sample was performed using 20 mM tris/HCl buffer pH 7.0. Xylanase activity was detected only in the eluted protein component forming a sharp, distinctive xylanase peak (Fig. 5b). The specific activity determined (292.88 Umg/protein), showed about 7.0-fold purification of the crude enzyme. These values are similar to those obtained for xylanase from other microbial sources (Düsterhöft et al., 1997).

It can be seen that the overall yield of enzyme is low with most of the losses occurring during the different steps of purification (Magnuson and Gawford, 1997).

The purity of the xylanase obtained from gel filtration was examined by Disc-PAGE gel electrophoresis showing a single distinctive band indicating the high purity of the isolated enzyme preparation (data not shown). Similar purification steps were used by other workers (Kubata et al., 1994; Düsterhöft et al., 1997).

3.9. Kinetics of purified xylanase

The effect of substrate (oat spelt xylan) concentration on the activity was examined using different concentrations ranging from 2–10 mg/reaction mixture. The initial velocity of xylanase reaction was measured as a function of substrate concentration and plotted as a double reciprocal in accordance with the Lineweaver–Burk analysis in Fig. 6. The plot gives a K_m of 16.7 mg xylan/ml and a V_{max} of 333.3 U mg/protein, indicating high affinity of the enzyme for the substrate. The K_m values of xylanases were shown to range between 0.5 and 19.6 mg/ml (Bansod et al., 1994). The K_m reported in this study is higher than that reported for *Trichoderma longibrachiatum* (10.14 mg/ml) (Chen et al., 1997) and that of *Fusarium oxysporium* was 4.76 mg/ml (Ruiz et al., 1997). These differences could be, to a certain extent, attributed to different xylanases or temperatures used in the xylanase assay and to the methods used in determination of sugar concentration.

Table 4
Summary of the purification of xylanase enzyme from *A. terreus* in SSF

Purification step	Collected volume (ml)	Activity (U/ml)	Protein (mg/ml)	Total activity (U)	Total protein (mg)	Specific activity (U/mg protein)	Purification factor (fold)	Yield (%)
Crude enzyme	100	64.14	1.54	6414.00	154.00	41.65	1.00	100.0
Ammonium sulfate fraction (65%)	25	88.63	1.42	2215.75	35.50	62.42	1.50	34.5
Anion exchange chromatography on DEAE-Bio-Gel A.	20	106.72	1.03	2134.40	20.60	103.12	2.50	33.3
Gel filtration chromatography on Sephadex G-75	15	133.80	0.73	2007.00	10.95	183.29	4.42	31.3

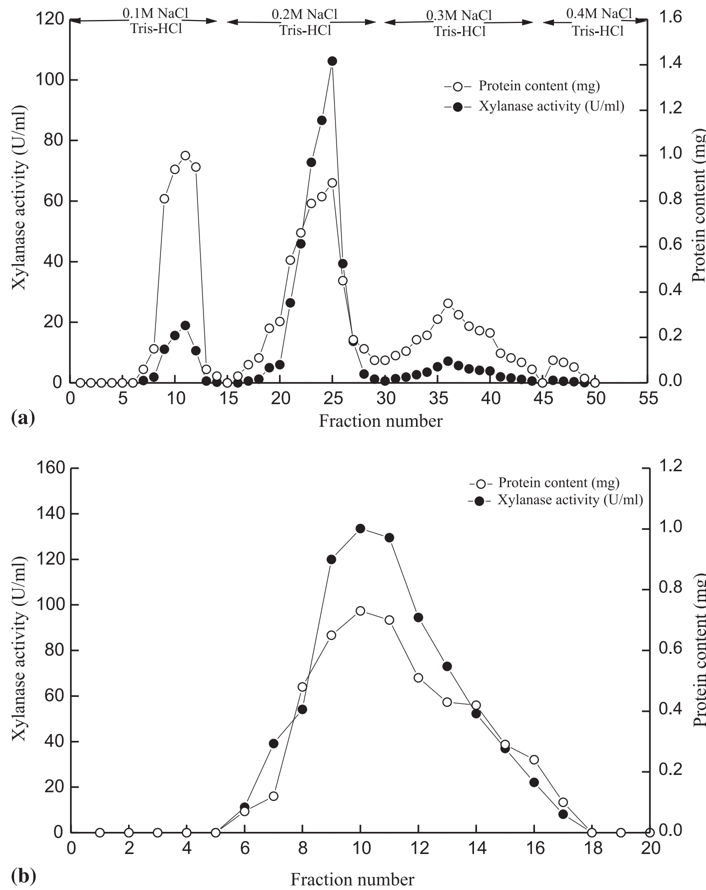

Fig. 5. (a) DEAE Bio Gel A. Chromatography of the partially purified xylanase produced by *A. terreus* in SSF using different NaCl gradients. (b) Gel filtration on Sephadex G-75 of the DEAE Bio-Gel A filtrate xylanase produced by *A. terreus* in SSF.

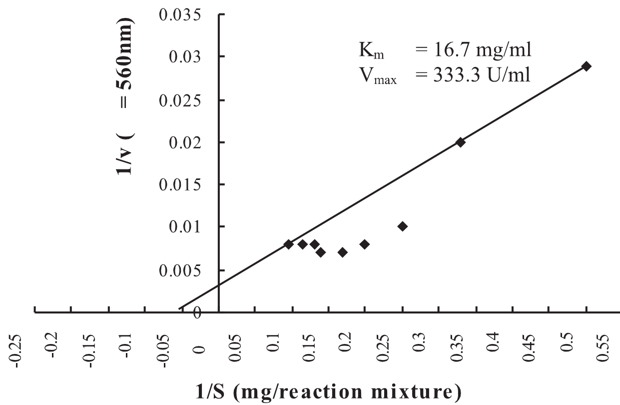

Fig. 6. Lineweaver–Burk plot of *A. terreus* xylanase using oat spelt as substrate.

3.10. Substrate specificity

The purified xylanase was specific and capable of hydrolyzing only xylan. It showed little activity on carboxymethyl cellulose and cellobiose (Table 5). This is similar to *T. longibrachiatum* xylanase (Chen et al., 1997), and *Sclerotium rolfssi* xylanase (Deshpande and Sadana, 1989).

The enzyme has optimal activity at 50°C (Fig. 7a) and about 60% of its activity was retained at 70°C. This fact is important for its utilization in paper manufacture. This temperature was comparable to the xylanase formed by *Humicola insolens* (Düsterhöft et al., 1997), and *Aureobasidium pullulans* Y 2311.1 (Li et al., 1993). The enzyme of *A. terreus* was active over a pH range of 4–10 with an optimum pH at 7, but retained 85.19% of its activity at pH 8 (Fig. 7b).

Thermal stability of xylanase was determined by incubating the enzyme extract at different temperatures (30–70°C). The enzyme was fairly stable at temperatures up to 60°C and retained 48.02% of its activity at this temperature after 30 min. (Fig. 7c). These results are in partial agreement with those reported for other microbial xylanases (Gomez and Fevre, 1993; Li et al., 1993; Magnuson and Gawford, 1997).

Different metallic ions exerted variable effects on xylanase activity. The enzyme was strongly inhibited by Hg^{2+}, Co^{2+}, Cu^{2+}, Fe^{3+} and Pb^{2+}. EDTA also showed an inhibitory effect on enzyme activity. Intermediate level of inhibition was observed upon addition of Zn^{2+}, Ba^{2+}, Na^{+}, Mn^{2+} and K^{2+} (Fig. 8). The inhibitory effects of these ions may be due to the formation of a complex

Table 5
Activity of the purified xylanase of *A. terreus* as affected by different substrates

Substrate (1% w/v)	Related enzyme	Relative activity (%)
Xylan (oat spelt)	1,4-β xylanase	100
Cellobiose	1,4-β glucosidase	2.14
Carboxymethyl cellulose (CMC)	1,4-β glucanase	3.49
Mannan (locust bean gum)	1,4-β mannanase	0.00

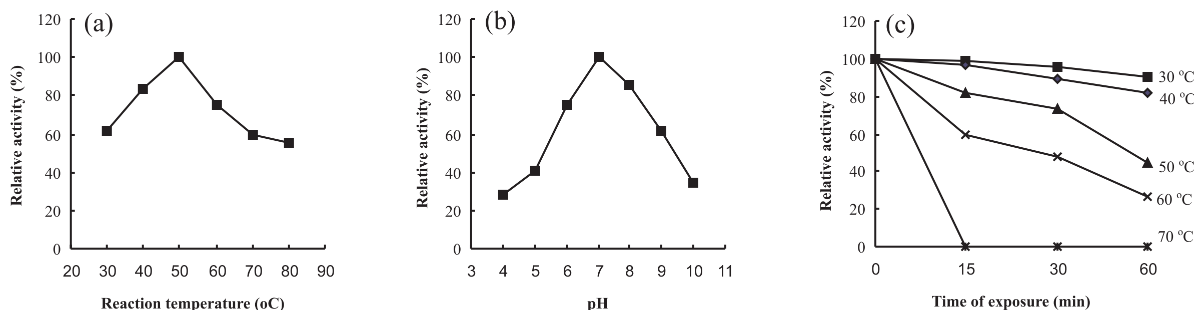

Fig. 7. Relative activity of purified xylanase of *A. terreus* (a) Effect of reaction temperature, (b) influence of pH and (c) thermal stability of the enzyme.

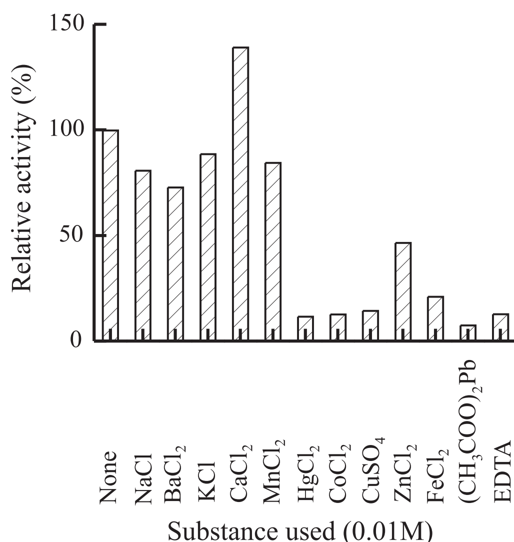

Fig. 8. Effect of some activators and inhibitors on the activity of the purified xylanase produced by *A. terreus* in SSF.

with the acidic amino acid residue at the active site (Magnuson and Gawford, 1997). On the other hand, Ca^{2+} ions slightly stimulated xylanase activity (1.4-fold increase), indicating its possible role as a metal ion cofactor in the enzyme–substrate reaction and having a stabilizing effect on various enzymes (Ghosh et al., 1993). These results are similar to those of endo 1–4 β-xylanases from *F. oxysporum* f.sp.melons (Alconada and Martinez, 1994), where enzyme activity was generally inhibited by addition of metallic ions. The fact that *A. terreus* xylanase could be produced on various cheap lignocellulosic wastes (wheat straw) with low levels of cellulase activity is significant from a practical point of view. This enzyme was reported to have growing importance for the paper–pulp, textile and food industries (Hrmová et al., 1989).

Acknowledgement

The authors wish to express their sincere gratitude and deep appreciation to Prof. Dr. Soraya Sabry for her assistance and valuable suggestions during the experimental work and the writing of the manuscript.

References

Abdullah, A.L., Tengerdy, R.P., Murphy, V.G., 1985. Optimization of solid substrate fermentation of wheat straw. Biotechnol. Bioeng. 27, 20–27.

Alconada, T.M., Martinez, M.J., 1994. Purification and characterization of an extracellular endo-1,4-β xylanase from *Fusarium oxysporum* F. sp. melons. FEMS Microbiol. Lett. 118, 305–310.

Araujo, A., D'souza, J., 1980. Production of biomass from enzymatic hydrolysate of agricultural waste. J. Ferment. Technol. 58, 399–401.

Bandivadeker, K.R., Deshpanda, V.V., 1994. Enhanced stability of cellulase free xylanase from *Chainia* sp. (NCL. 82.5.1). Biotechnol. Lett. 16, 179–182.

Bansod, S.M., DuttaChoudhary, M., Deshpanda, V.V., 1994. Enhanced stability of cellulose-free xylanase from *Chainia* sp. (NCL. 82.5.1). Biotechnol. Lett. 16, 179–182.

Biely, P., 1985. Microbial xylanolytic enzymes. Trends Biotechnol. 11, 286–290.

Chen, C., Chen, J.L., Lin, T.Y., 1997. Purification and characterization of a xylanase from *Trichoderma longibrachiatum* for xylooligosaccharide production. Enz. Microb. Technol. 21, 91–96.

Desgranges, C., Durand, A., 1990. Effect of pCO_2 on growth, conidiation and enzyme production in solid-state culture of

Aspergillus niger and *Trichoderma viride* TS. Enz. Microb. Technol. 12, 546–551.

Deshpande, M.V., Sadana, J.C., 1989. Purification and characterization of a cellulase-free endo-1,4-β-D-xylanase of *Sclerotium rolfsii* UV-8 mutant. Enz. Microb. Technol. 11, 678–684.

Dubeau, H., Chahal, D.S., Ishaque, M., 1987. Xylanase of *Chaetomium cellulolyticum*: its nature of production and hydrolytic potential. Biotechnol. Lett. 9, 275–280.

Düsterhöft, E.M., Linsen, V.A.J.M., Voragen, A.G.J., Beldman, G., 1997. Purification characterization and properties of two xylanases from *Humicola insolens*. Enz. Microbiol. Technol. 20, 437–445.

Ghosh, M., Das, A., Mishra, A.K., Nanda, G., 1993. *Aspergillus sydowii* MG 49 is a strong producer of thermostable xylanolytic enzyme. Enz. Microb. Technol. 15, 703–709.

Gomez, B., Fevre, M., 1993. Purification and characterization of two 1,4-β-xylan endohydrolases from the rumen fungus Neocallimastix frontalis. Appl. Environ. Microbiol. 59, 3654–3660.

Gomes, J., Purkarthofer, H., Hayn, M., Kapplmüller, J., Sinner, M., Steiner, W., 1993. Production of a high level of cellulase-free xylanase by the thermophilic fungus *Thermomyces lanuginosus* in laboratory and pilot scales using lignocellulosic materials. Appl. Microbiol. Biotechnol. 39, 700–707.

Grajek, W., 1987a. Production of D-xylanases by thermophilic fungi using different methods of culture. Biotechnol. Lett. 9, 353–356.

Haaland, P.D. (Ed.), 1989. Experimental Design in Biotechnology. Dekker, New York.

Haltrich, D., Preiss, M., Steiner, W., 1993. Optimization of a culture medium for increased xylanase production by a wild strain of *Schizophyllum* commune. Enz. Microb. Technol. 15, 854–860.

Haltrich, D., Steiner, W., 1994. Formation of xylanase by *Schizophyllum* commune: Effect of medium components. Enz. Microb. Technol. 16, 229–235.

Haapala, R., Parkkinen, E., Suominen, P., Linko, S., 1995. Production of extracellular enzymes by immobilized *Trichoderma reesei* in shaked flask cultures. Appl. Microbiol. Biotechnol. 43, 815–821.

Hoq, M.M., Deckwer, W.D., 1995. Cellulase free xylanase by thermophilic fungi: a comparison of xylanase production by two *Thermomyces lanuginosus* strains. Appl. Microbiol. Biotechnol. 43, 604–609.

Hrmová, M., Biely, P., Vrsanšká, M., 1989. Cellulose and xylan degrading enzymes of *Aspergillus terreus* and *Aspergillus niger*. Enz. Microb. Technol. 11, 610–615.

Ishihara, M., Nojiri, M., Hayashi, N., Nishimura, T., Shimizu, K., 1997. Screening of fungal β-xylanases for production of acidic xylooligosaccharides using in situ reduced 4-O-methylglucuronoxylan as substrate. Enz. Microb. Technol. 21, 170–175.

IUPAC, 1987. Measurement of cellulase activities. Pure Appl. Chem. 59, 257–268.

Kluepfel, D., Shareck, F., Mondou, F., Morosoli, R., 1986. Characterization of cellulase and xylanase activities of *Streptomyces lividans*. Appl. Microbiol. Biotechnol. 24, 230–234.

Kubata, B.K., Suzuki, T., Horitsu, H., Hawai, K., Takamizawa, K., 1994. Purification and characterization of *Aeromonas caviae* ME-1 xylanase V which produces exclusively xylobiose from xylan. Appl. Environ. Microbiol. 60, 531–535.

Li, X.L., Zhang, Z.Q., Dean, J.F.D., Eriksson, K.E.L., Ljungdahl, L.G., 1993. Purification and characterization of a new xylanase (APX-II) from the fungus *Aureobasidium pullulans* Y-2311-1. Appl. Environ. Microbiol. 59, 3212–3218.

Lineweaver, H., Burk, D., 1934. The determination of enzyme dissociation constants. J. Am. Chem. Soc. 56, 658–666.

Lowry, O.H., Rosebrough, N.J., Farr, A.L., Randall, R.J., 1951. Protein measurement with the Folin reagent. J. Biol. Chem. 193, 265–275.

Lynch, J.M., Slater, J.H., Bennett, J.A., Harpers, S.H.T., 1981. Cellulase activities of some aerobic microorganisms from soil. J. Gen. Microbiol. 127, 231–236.

Magnuson, T.S., Gawford, D.L., 1997. Purification and characterization of an alkaline xylanase from *Streptomyces viridosporus* T7A. Enz. Microb. Technol. 21, 160–164.

Miller, G.L., 1959. Use of the dinitrosalicylic acid reagent for determination of reducing sugar. Anal. Chem. 31, 426–428.

Olama, Z.A., Sabry, S.A., Mabrouk, M.M., 1998. Purification and characterization of cellulase free xylanase from *Streptomyces coriofaciens*. Adv. Food Sci. (CMTL) 20, 69–78.

Plackett, R.L., Burman, J.P., 1946. The design of optimum multifactorial experiments. Biometrica. 33, 305–325.

Purkarthofer, H., Sinner, M., Steiner, W., 1993. Cellulase-free xylanases from *Thermomyces lanuginosus*: Optimization of production in submerged and solid-state culture. Enz. Microb. Technol. 15, 677–682.

Ramesh, M.V., Lonsane, B.K., 1990. Characteristics and novel features of thermostable alpha-amylase produced by *Bacillus licheniformis* M27 under solid-state fermentation. Starch/Stärke 6, 233–238.

Ruiz, M.C., Pietro, A.D., Roncero, I.G., 1997. Purification and characterization of an acidic endo β-1-4 xylanase from the tomato vascular pathogen *Fusaruin oxysporum* and *lycopersici*. FEMS Microbiol. Lett. 148, 75–82.

Senior, D.J., Mayers, P.R., Miller, D., Sutcliffe, R., Tan, L., Saddler, J.N., 1988. Selective solubilization of xylan in pulp using a purified xylanase for *Trichoderma harzianum*. Biotechnol. Lett. 10, 907–912.

Shamala, T.R., Sreekantiah, K.R., 1986. Production of cellulases and D-xylanase by some selected fungal isolates. Enz. Microb. Technol. 8, 178–182.

Vükari, L., Kantelinen, A., Rätlö, M., Sundquist, J., 1991. Enzymes in pulp and paper processing. In: Leatham, G.F., Himmel, M.E. (Eds.), Enzymes in Biomass Conversion. ACS Symposium Series 460. American Chemical Society, Washington DC, pp. 12–21.

Yadav, J.S., 1988. SSF of wheat straw with alkaliphilic *Coprinus* sp. Biotechnol. Bioeng. 31, 414–417.