Optimisation of Solid-state Fermentation of *Aspergillus niger* JL-15 for Xylanase Production and Xylooligosaccharides Preparation

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Abstract

Dai X.-J., Liu M.-Q., Jin H.-X., Jing M.-Y. (2011): **Optimisation of solid-state fermentation of** *Aspergillus niger* JL-15 **for xylanase production and xylooligosaccharides preparation**. Czech J. Food Sci., **29**: 557–567.

The production of xylanase (XylA) by *Aspergillus niger* JL-15 in solid-state fermentation (SSF) on orange peel was optimised by the response surface methodology (RSM). The results revealed that four factors had significant effects on the XylA production ($P < 0.05$), that is the concentrations of the added glycerin and ammonium sulfate, the moisture content, and fermentation time. Exploying orange peel as the solid substrate, maximum xylanase activity (917.7 U/g dry fermentation product) was obtained at 4.2% glycerin, 3.1% $(NH_4)_2SO_4$, 61% moisture content, and 73.4-h fermentation, this activity being close to the predicted one and 3.2 times higher than that of the basic medium (218.5 U/g). Optimum temperature and pH for XylA activity were 55°C and pH 5.0, respectively. SDS-PAGE analysis showed that the relative molecular mass of XylA was about 30.0 kDa. XylA exhibited K_m and V_{max} values of 9.24 mg/ml and 54.05 μmol/min/ml, respectively. XylA liberated mainly xylotriose from birchwood xylan and wheat bran, respectively. XylA was an endo-acting xylanase with transglycosylation activity, with the ability to hydrolyse, xylobiose, xylotriose, xylotetraose, xylopentaose, and xylohexaose.

Keywords: xylanase; response surface methodology (RSM); solid-state fermentation (SSF); xylooligosaccharides; HPLC

Biomass from the plant material is the most abundant and widely spread renewable raw material for the production of high-value bioproducts. Xylan, a major component of the plant cell wall, consists of a backbone of β-(1,4)-linked D-xylosyl residues with substitutions by arabinosyl, acetyl, and glucuronosyl residues (Thomson 1993; Cazemier *et al*. 1999; Collins *et al*. 2002). The hydrolysis of the xylan backbone involves several enzymes, and the most important one is endo-β-(1,4)-xylanase (EC 3.2.1.8). Based on the sequence similarities and hydrophobic cluster analysis, endoxylanases have been grouped into families 10 and 11 of glycosyl hydrolases (Henrissat & Bairoch 1993). Xylanases have attracted considerable research interest in recent years mainly due to their potential application in food, animal feed, and paper and pulp industries. For example, there are many researches that describe

Supported by the Zhejiang Provincial Natural Science Foundation of China, Grant No. Y3090503, the Scientific Research Found of Hangzhou Science and Technology Bureau, Project N. 20110232B81, and the Scientific Research Fund of Zhejiang Provincial Education Department, Project No. Y200805864.

the production of xyloses by enzymatic hydrolysis of xylan from xylan-rich materials (Vazquez *et al.* 2000; Yang *et al.* 2005; Moure *et al.* 2006; Yuan *et al.* 2006; Akpinar *et al.* 2007).

Fungi, due to their reproductive and growth characteristics, are well adapted to a large variety of substrates, being excellent decomposers of the vegetal material. The filamentous fungi belonging to the genus *Aspergillus* have been used throughout the world for the production of hemicellulase and other enzymes (van den Broeck *et al.* 1992; Krengel & Dijkstra 1996). In recent years, there has been an increasing trend towards efficient utilisation and value-addition of agriculture-industrial residues. The orange is the third widely cultivated fruit crop in China and its total production was about 17.9 million tonnes in 2007, with the peel amount being over 5 million tonnes in the citrus industries, however, most of it was discarded as garbage, which led to a great waste of resources and to environmental pollution.

The solid-state fermentation (SSF) has gained a renewed interest in recent years for the production of many enzymes due to lower operation costs and energy requirements, and simpler plant and equipment projects as compared to submerged fermentation (SmF) (Pandey 2003). The optimal design of the culture medium is a very important aspect in the development of SSF processes. Traditional approach for the optimisation of the medium components production takes into account one factor at a time, which is time consuming and laborious and often does not yield reliable results because the interactions of the different medium components are neglected. Response surface methodology (RSM) is a very useful tool for the optimal selection of nutrients, which can provide statistical models aid in understanding the interactions between the process parameters at different levels, and calculating the best level of each factor for the given target (Reddy *et al.* 2003; Elibol 2004; Chen *et al.* 2077; Faramarz *et al.* 2007).

In this study, *Aspergillus niger* JL-15, a xylanase producer, was cultured optimally by RSM on orange peel powder in SSF. The hydrolytic products released from xylan and xylooligosaccharides by xylanase (XylA) were determined and specifically quantified.

MATERIAL AND METHODS

Material. Birchwood xylan was obtained from Sigma Chemical Co. (St. Louis, USA). The standard xylooligosaccharides (XO2–XO6) came from Megazyme (Bray, Ireland). Xylose (XO) was obtained from Merck (Darmstadt, Germany). Wheat bran-insoluble xylan was provided by Dr. Chen (Southern Yangtze University, Wuxi, China). All other chemicals used were of reagent grade and obtained from standard sources.

Microorganism and solid-state fermentation. *Aspergillus niger* JL-15 was stored as spores on sterile soil in a test tube at –20°C in the Central Laboratory of Food Science Department, China Jiliang University. For xylanase production, *Aspergillus niger* JL-15 was cultivated using solid-state fermentation. The basic medium consisted of orange peel powder (9.8 g), $MgSO_4$ (0.05 g), K_2HPO_4 (0.15 g), and H_2O (18.6 g). The basic medium was sterilised at 121°C for 20 minutes. The strain was cultivated in a 500 ml flask containing 50 g of the medium at 30°C.

Optimisation of xylanase productions via response surface methodology (RSM). Optimisation of the solid-state fermentation for XylA production focused on the concentrations of the added glycerin (carbon source) and ammonium sulfate (nitrogen source) employing the orange peel powder of the basic medium, moisture content, fermentation time, and their interactions between one another. Based on the central composite design (CCD, 4-variable and 5-level), total 31 experiments with eight star points, 16 two-level points, and seven replicas of the central point were employed to fit the polynomial model:

$$Y = \beta_0 + \beta_1X_1 + \beta_2X_2 + \beta_3X_3 + \beta_4X_4 + \beta_{11}X_1^2 + \beta_{12}X_1X_2 + \beta_{13}X_1X_3 + \beta_{14}X_1X_4 + \beta_{22}X_2^2 + \beta_{23}X_2X_3 + \beta_{24}X_2X_4 + \beta_{33}X_3^2 + \beta_{34}X_3X_4 + \beta_{44}X_4^2$$

where:

Y – dependent or response variable

β – regression coefficient

X – coded levels of the independent variables

The corresponding coefficients of both variables and interaction variables were estimated by SAS 9.0 (SAS Institute Inc., Beijing, China) while their response surface graphs were drawn by Matlab 6.5 (Math Works, Portola Valley, USA). Statistical analysis of the significance of the coefficient estimations was performed via Student's *t*-test. The optimum values of the selected variables were obtained by solving the regression equation and also by analysing the response surface plots.

Purification and SDS-PAGE analysis of XylA. The fermentation products were suspended in

McIlvaine's buffer (pH 5.0) with a constant shaking rate and then they were filtered with spinning paper. To precipitate xylanase, the supernatant was treated with ammonium sulphate to 65% saturation. The precipitate was resuspended in 25 ml of McIlvaine's buffer (pH 5.0), loaded onto a Sephadex G-25 column (Pharmacia, 40.0 cm × 2.4 cm), and eluted with McIlvaine's buffer (pH 5.0). The fractions containing xylanase activity were pooled and concentrated. The enzyme solution was applied to SephacrylTM S-100 HR column (Pharmacia, Stockholm, Sweden; 70.0 cm × 1.6 cm) and eluted with the same buffer at a flow rate of 24 ml/hour. The active fractions were pooled for xylanase assays.

The purified fractions were subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). In the Laemmli system (Laemmli 1976), the stacking and separating gels consisted of 5% and 15% polyacrylamide, respectively. Proteins were visualised with Coomassie brilliant blue R-250 staining.

Enzyme assay. The xylanase activity was assayed using 1% birchwood xylan (w/v) as the substrate. The liberation of reducing sugars was estimated by the dinitrosalicylic acid (DNS) method (Miller *et al*. 1959). Protein concentration was measured by the Bradford method, with bovine serum albumin (BSA) having been used as the standard (Bradford 1976). The kinetic parameters for xylanase activity were calculated from the initial velocities using the concentration range from 1 mg/ml to 10 mg/ml birchwood xylan.

One unit of xylanase activity was defined as the amount of the enzyme that catalysed the formation of 1.0 μmol of reducing sugar as xylose equivalent from xylan in l min under its optimal conditions (at 55°C, pH 5.0). For each assay in this study, triplicate measurements were conducted to obtain the mean values of activity.

Effect of temperature on the activity and thermal stability of XylA. The effect of temperature on xylanase activity was measured at pH 5.0 (McIlvaine's buffer) from 30°C to 80°C. Thermal stability of xylanase was determined by assaying the residual activity after incubation from 30°C to 80°C at pH 5.0 for 4 min, respectively.

Effect of pH on the activity and stability of XylA. The effect of pH on xylanase activity was measured over the pH ranges of 3.0 to 7.0 (McIlvaine's buffer) and 8.0 to 9.0 (Glycine-NaOH buffer) at 55°C. To determine the pH stability, xylanase was incubated in various pH buffers at 25°C for 1 h, and the residual activities were measured at 55°C and pH 5.0.

Changes in viscosity and reducing sugars concentration during hydrolysis. The relationship between reducing sugars and viscosity of xylan solution in the presence of the xylanase was determined as follows: 200 ml of the reaction mixture containing 4.0% birchwood xylan and purified enzyme (25.0 U) was incubated at 30°C. The viscosity and the amount of reducing sugars released were estimated at different time intervals.

Xylooligosaccharides released by XylA from xylans. 1.0% (w/v) birchwood xylan and wheat bran insoluble xylan solutions in McIlvaine's buffer (pH 5.0) were hydrolysed by purified xylanase at 40°C for 24 h with constant shaking (100 rpm). In the two reaction mixtures, the substrates (10 mg) were excessive and the amounts of xylanase (1.5 U) were the same. The hydrolysis products and standard xylooligosaccharides were analysed by HPLC with Sugar-Pak TM1 column (6.5 mm diameter and 300 mm length; Waters, Milford, USA), pure water as the mobile phase (0.5 ml/min), and injection volumes of 20 μl. The column was maintained at 85°C. The sugar peaks were screened using Waters 2410 refractive index detector. The hydrolysates of xylan and standard xylooligosaccharides were quantified based on their own standard curves.

The mode of action of XylA on xylooligosaccharides. The mode of xylanase action was determined using different xylooligosaccharides (XO2–XO6) as substrates. The standard xylooligosaccharides solutions (water, pH 7.0) were incubated with purified xylanase at 40°C. The samples taken at different time intervals were determined and quantified by HPLC. The injection volume was 20 μl.

RESULTS AND DISCUSSION

Optimization of XylA production by RSM

The statistical optimisation method for the fermentation process could overcome the limitation of the classic empirical methods and was proved to be a powerful tool for the optimistion of the production of xylanase by *A. niger* JL15. The response results shown in Table 1 were analysed using SAS 9.0 software. The *t*-test and *P* values were used to identify the effect of each factor on xylanase yield (Table 2). The concentrations of glycerin and

Table 1. The variables in central composite design (CCD) and the xylanase production

Run	X_1 (%)	X_2 (%)	X_3 (%)	X_4 (h)	Yield (U/g dry product)
1	0 (4.0)	0 (3.0)	0 (60)	0 (72)	918.9
2	1 (5.0)	1 (4.0)	1 (67)	–1 (60)	875.9
3	–1 (3.0)	–1 (2.0)	–1 (53)	1 (84)	840.5
4	0	0	0	0	911.7
5	0	–2 (1.0)	0	0	811.1
6	–1	–1	1	1	831.4
7	2 (6.0)	0	0	0	867.4
8	0	0	0	0	920.9
9	0	0	0	0	919.9
10	1	–1	1	–1	853.7
11	–2 (2.0)	0	0	0	841.3
12	1	–1	–1	1	855.8
13	0	0	0	–2 (48)	770.1
14	1	1	1	1	870.1
15	0	0	0	2 (96)	860.6
16	–1	1	–1	–1	864.4
17	1	1	–1	1	888.2
18	–1	–1	–1	–1	796.4
19	0	2 (5.0)	0	0	832.9
20	1	–1	1	1	861.1
21	1	–1	–1	–1	799.1
22	–1	1	1	1	816.2
23	1	1	–1	–1	831.7
24	0	0	0	0	920.2
25	–1	1	–1	1	847.7
26	–1	–1	1	–1	827.9
27	0	0	2 (74)	0	879.1
28	0	0	–2 (46)	0	827.9
29	–1	1	1	–1	861.4
30	0	0	0	0	917.7
31	0	0	0	0	915.5

Coded levels (+2, +1, 0, –1, –2) and actual values (in parentheses) of the independent variables in CCD; X_1 and X_2 – concentration of glycerin and ammonium sulphate added to basic medium, respectively; X_3 – moisture content; X_4 – fermentation time

ammonium sulphate added to the basic medium, the moisture content, and fermentation time had significant effects on the XylA production ($P < 0.05$). The Fisher F-test with a very low probability value ($P < 0.0001$) showed a high statistical significance of the regression model. The goodness of fit of the model was checked by the determination coefficient ($R^2 = 0.9351$), which indicated that the following second order polynomial model could explain 93.51% of the total variation. The model can be shown as follows:

$$Y = 917.8285 + 8.4125X_1 + 9.7208X_2 + 7.3458X_3 + 11.7292X_4 + 0.1688X_1X_2 + 6.1313X_1X_3 + 8.0687X_1X_4 - 5.6688X_2X_3 - 7.6813X_2X_4 - 11.2938X_3X_4 - 14.4040X_1^2 - 22.4915X_2^2 - 14.6165X_3^2 - 24.1540X_4^2$$

where:

Y – xylanase yield

X_i – coded independent variables (X_1 and X_2 – concentrations of the added glycerin and ammonium sulfate, respectively; X_3 – moisture content; X_4 – fermentation time)

Table 2. Analysis of variance and coefficient estimates of central composite design

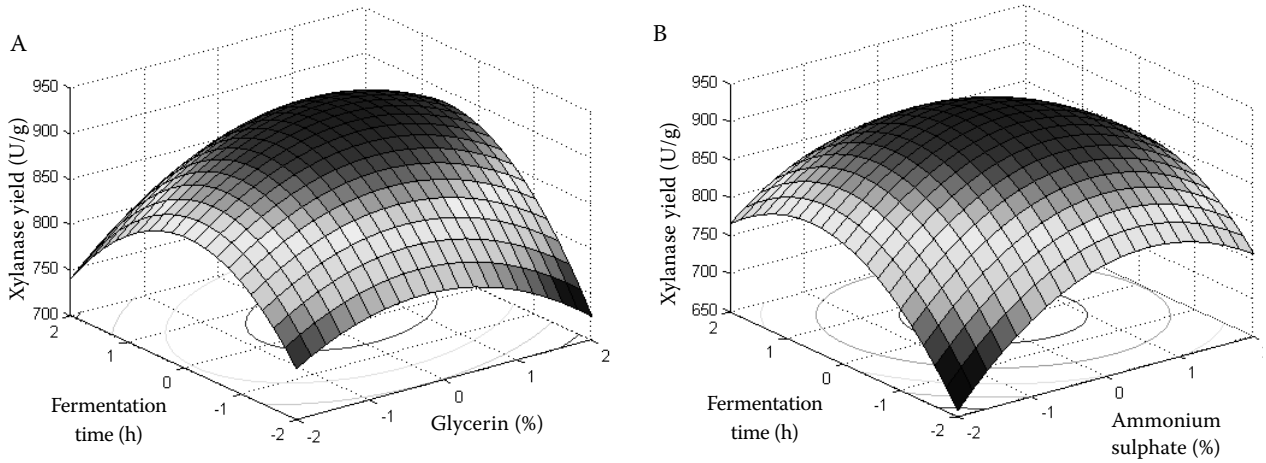
Source of variation	Degree of freedom	Sum of squares	Mean square	*F*-value	*P*-value
Regression	14	47 264.0	3376.0	16.46	< 0.0001**
Linear	4	8 563.2	2140.8	10.44	0.0002**
Quadratic	4	36 679.0	9169.8	40.90	< 0.0001**
Interaction	6	5 142.6	857.1	4.18	0.0102*
Lack of fit	10	3 218.5	321.9	30.41	0.0002**
Pure error	6	63.5	10.6		
Total	16	3 282.0	205.1		
Factors	Degree of freedom	Coefficient estimate	Standard error	*t*-value	*P*-value
Intercept	1	917.8285	5.41	169.55	< 0.0001**
X_1	1	8.4125	2.92	2.88	0.0109*
X_2	1	9.7208	2.92	3.33	0.0041**
X_3	1	7.3458	2.92	2.51	0.0200*
X_4	1	11.7292	2.92	4.01	0.0009**
$X_1 \times X_2$	1	0.1688	3.58	0.05	0.9630
$X_1 \times X_3$	1	6.1313	3.58	1.71	0.1061
$X_1 \times X_4$	1	8.0687	3.58	2.25	0.0386*
$X_2 \times X_3$	1	–5.6688	3.58	–1.58	0.1329
$X_2 \times X_4$	1	–7.6813	3.58	–2.15	0.0461*
$X_3 \times X_4$	1	–11.2938	3.58	–3.15	0.0061**
X_1^2	1	–14.4040	2.67	–5.38	< 0.0001**
X_2^2	1	–22.4915	2.67	–8.40	< 0.0001**
X_3^2	1	–14.6165	2.67	–5.46	< 0.0001**
X_4^2	1	–24.1540	2.67	–9.02	< 0.0001**

*$P < 0.05$; **$P < 0.01$; X_1 and X_2 – concentration of glycerin and ammonium sulphate added to basic medium, respectively; X_3 – moisture content; X_4 – fermentation time

The application of RSM yielded a regression equation showing positive linear and negative quadratic effects and expressing the relationship between the XylA production and the independent variables.

Three-dimensional response surface and contour plots (Figure 1) were obtained. The relations between the factors and response can be understood by examining three-dimensional response surface and contour plots as a function of two factors at a time and holding all other factors at fixed levels. It was evident from the plots that the addition of higher concentrations of glycerin and ammonium sulphate to orange peel, middle levels of the moisture content, and a longer fermentation time are responsible for the enhancement of xylanase production. Based on the analysis of the regression equation and plots, optimums of the four variables were found to be as follows: glycerin 4.2%, ammonium sulphate 3.1%, moisture content 61%, and fermentation time 73.4 h, with the production of 922.4 U/g (dry fermentation products). The predicted yield was verified by performing an experiment with the optimised variables in the basic medium, and the recovery of xylanase was 917.7 U/g which was close to the predicted one, and was 3.2 times higher than that of the basic medium (218.5 U/g).

RSM method is used for some similar topics. Xu *et al.* (2008) reported that the optimum amounts of the critical components for maximum xylanase production by *A. niger* XY-1 strain in solid-state fermentation were: urea (41.63 g/l), Na_2CO_3 (2.64 g/l), and $MgSO_4$ (10.68 g/l), and the predicted xylanase value was 14374.6 U/g dry substrate, which was higher than that with *A. niger* JL-15 strain. The effects of the cultivation time, pH, and substrate



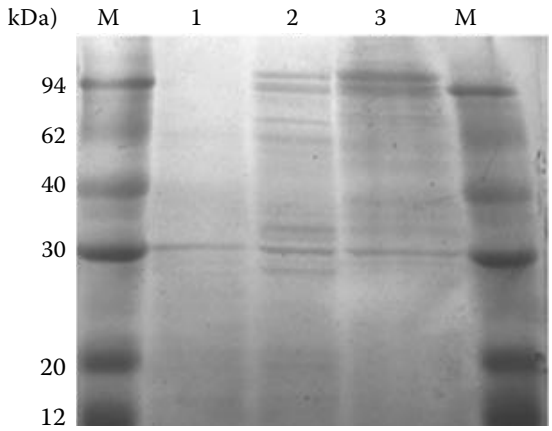
A – effect of glycerin (X1) and fermentation time (X4) on the production of xylanase with the other two variables at 0 level; B – effect of ammonium sulfate (X2) and fermentation time (X4) on the production of xylanase with the other two variables at 0 level

Figure 1. Response surface/contour plot showing effect of independent variables on XylA production.

concentration on the production of xylanase by *A. niger* AN-3 were studied by Cao *et al.* (2008). The optimal fermentation parameters for an enhanced xylanase production were: cultivation time 53.3 h, pH 7.92, and wheat bran concentration 54.2 g/l. Under these conditions, the xylanase production was 127.12 U/ml (Cao *et al.* 2008).

Purification and kinetic parameters of XylA

Partial purification by ammonium sulfate precipitation and gel filtration resulted in a 7.1-fold increase in the specific activity of xylanase (from 243.2 U/mg to 1969.7 U/mg) with 22.7% recovery. SDS-PAGE analysis showed that the relative molecular mass of XylA was about 30.0 kDa (Figure 2). Kinetic parameters that reflect the effect of the substrate concentration on the reaction velocity were depicted. The rate dependence of the enzyme reaction on the birchwood xylan concentration followed the Michaelis-Menten kinetics. The values of K_m and V_{max} were 9.24 mg/ml and 54.05 μmol/min/ml, respectively, being similar to those of xylanase from *Streptomyces cyaneus* SN32 (Ninawe *et al.* 2008). These values are consistent with the reported range of the kinetic values for microbial xylanases (Beg *et al.* 2001).

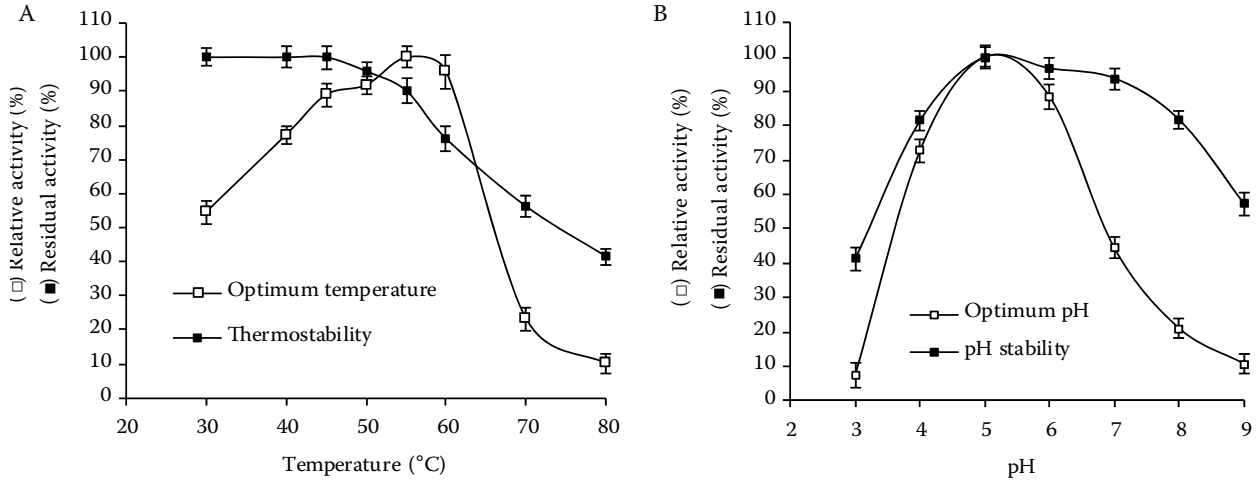


Lanes M – standard protein marker; Lane 1 – purified xylanase after chromatography on Sephacryl S-100 HR; Lanes 2 and 3 – crude enzyme

Figure 2. SDS-PAGE analysis of XylA

Effect of temperature on the activity and stability of XylA

The xylanase activity increased with the rise of temperature, reaching the maximum at 55°C; and then decreased with further rise of temperature. To determine the thermal tolerance, the enzyme was incubated at various temperatures for 4 min, and then the residual activities were determined at 55°C, pH 5.0. When treated at 70°C, pH 5.0 for 4 min, the residual activity of xylanase was 56.2%. The xylanase was stable below 60°C (Figure 3A).



These assays were performed using 1% birchwood xylan as substrate; the highest xylanase activity was taken as 100% in assay of optima (□); the xylanase activity under optimal conditions (55°C, pH 5.0) was taken as 100% in assays of stability (■)

Figure 3. The optima (□) and stability (■) of XylA

Effect of pH on the activity and stability of XylA

The xylanase showed a high activity in the pH range of 4.0–7.0, with the optimal pH at 5.0 (Figure 3B). It was very stable from pH 4.0 to 8.0. Over 80% of the xylanase activity was retained after the treatment of the enzyme by preincubation over a pH range of 4.0–8.0 at 25°C for 1 hour.

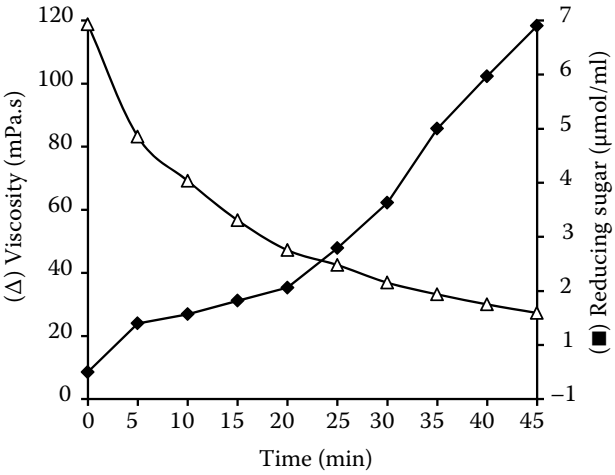
Decrease in viscosity and the release of reducing sugars from xylan

The relationship between the change in viscosity and release of reducing sugars during hydrolysis of xylan is showed in Figure 4. The enzyme from *A. niger* rapidly decreased the viscosity of xylan solution by 30% and 77% after 5 min and 45 min, respectively, but released a small amount of reducing sugars, which was similar to the xylanase from *Sporotrichum thermophile* (Katapodis *et al*. 2003). The enzyme mediates a decrease in the viscosity of xylan associated with a release of only small amounts of reducing sugar.

Xylooligosaccharides released from xylans by XylA

Xylan represents about 40% of dry matter of wheat bran, which is an important by-product of the cereal industry (Beaugrand *et al*. 2004). The hydrolysis products released by xylanase from birchwood xylan and wheat bran insoluble xylan were xylose to xylohexaose (XO–XO6) with xylotriose (XO3) as the major product (Figure 5). After 24-h reaction, about 32.4% and 22.5% of the hydrolysis products from birchwood xylan and wheat bran insoluble xylan were xylotriose in concentrations of 2.268 mg/ml and 0.551 mg/ml, respectively (Table 3).

Xylooligosaccharides can be produced from xylan-rich materials by chemical methods, di-



The viscosity of reaction mixture was measured at 5 min interval by DV-II prime viscometer (Brookfield, spindle: sp-2, speed: 100 rpm); 1.0 ml reaction mixture withdrawn and the reducing sugars released were quantified using DNS method

Figure 4. The changes in viscosity and reducing sugars from xylan solution

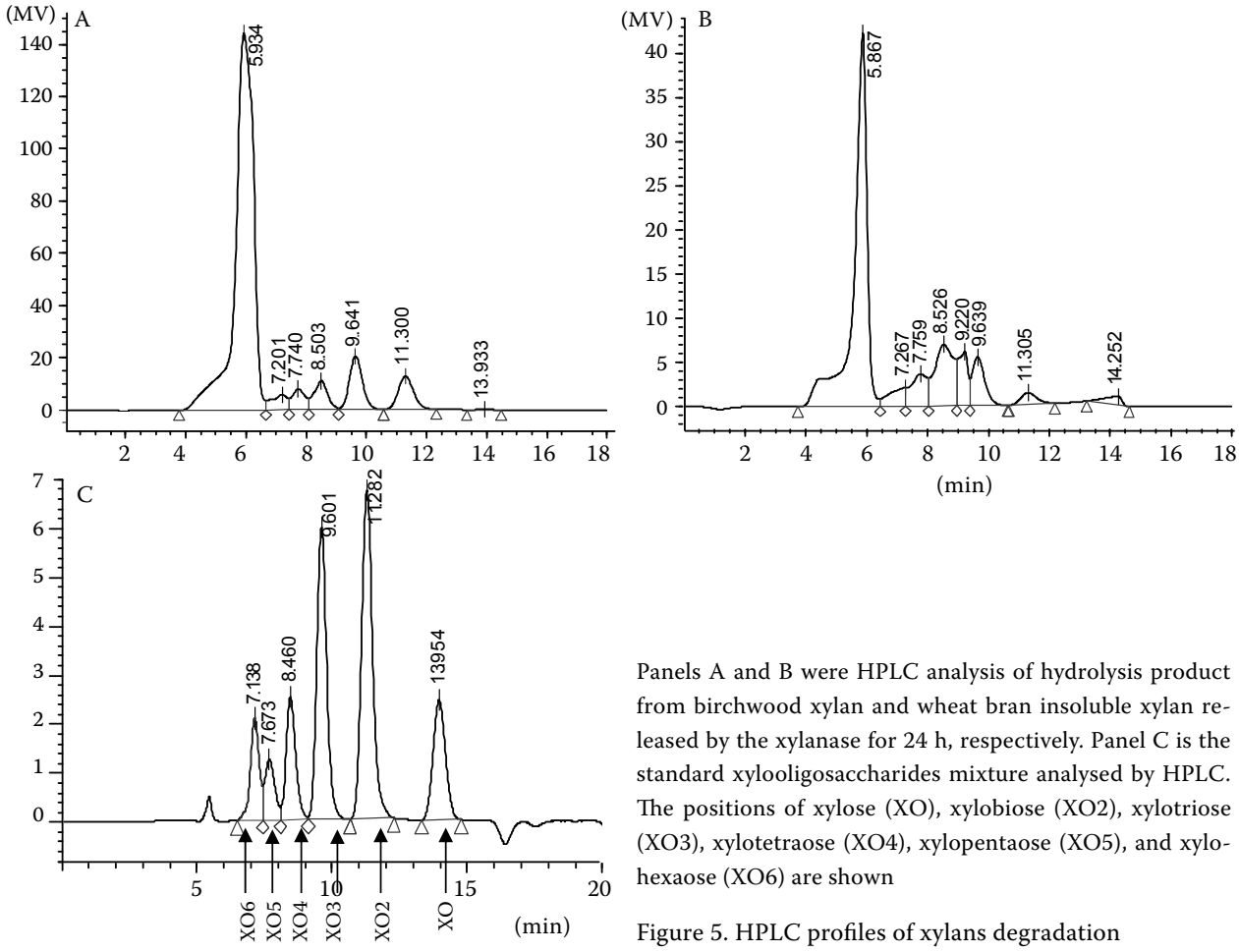
Table 3. Concentration of hydrolysis products from xylans by XylA

Xylans	Xylose	Hydrolysis products concentration (mg/ml)				Xylohexaose
		xylobiose	xylotriose	xylotetraose	xylopentaose	
Birchwood xylan	0.011	1.599	2.268	1.265	0.934	0.814
Wheat bran insoluble xylan	0.137	0.358	0.551	0.493	0.535	0.376

rect enzymatic hydrolysis of a susceptible substrate (Katapodis & Christakopoulos 2005), or a combination of chemical and enzymatic treatments (Yuan *et al.* 2004). Out of these methods, enzymatic hydrolysis is more desirable because it does not produce undesirable byproducts or high amounts of monosaccharides and does not require special equipment. Xylooligosaccharides used as a functional food in many countries can be selectively used by the beneficial gastrointestinal microflora, *Bifidobacterium* spp. (Petschow & Talbott 1990; Suwa *et al.* 1999; Moure *et al.* 2006; Zeng *et al.* 2007). The reported beneficial effects of *Bifidobacterium* spp. on human health include: suppressing the activity of entero putrefactive bacteria, preventing the proliferation of pathogenic intestinal bacteria, and facilitating the digestion and absorption of nutrients (Loo *et al.* 1999; Yuan *et al.* 2005).

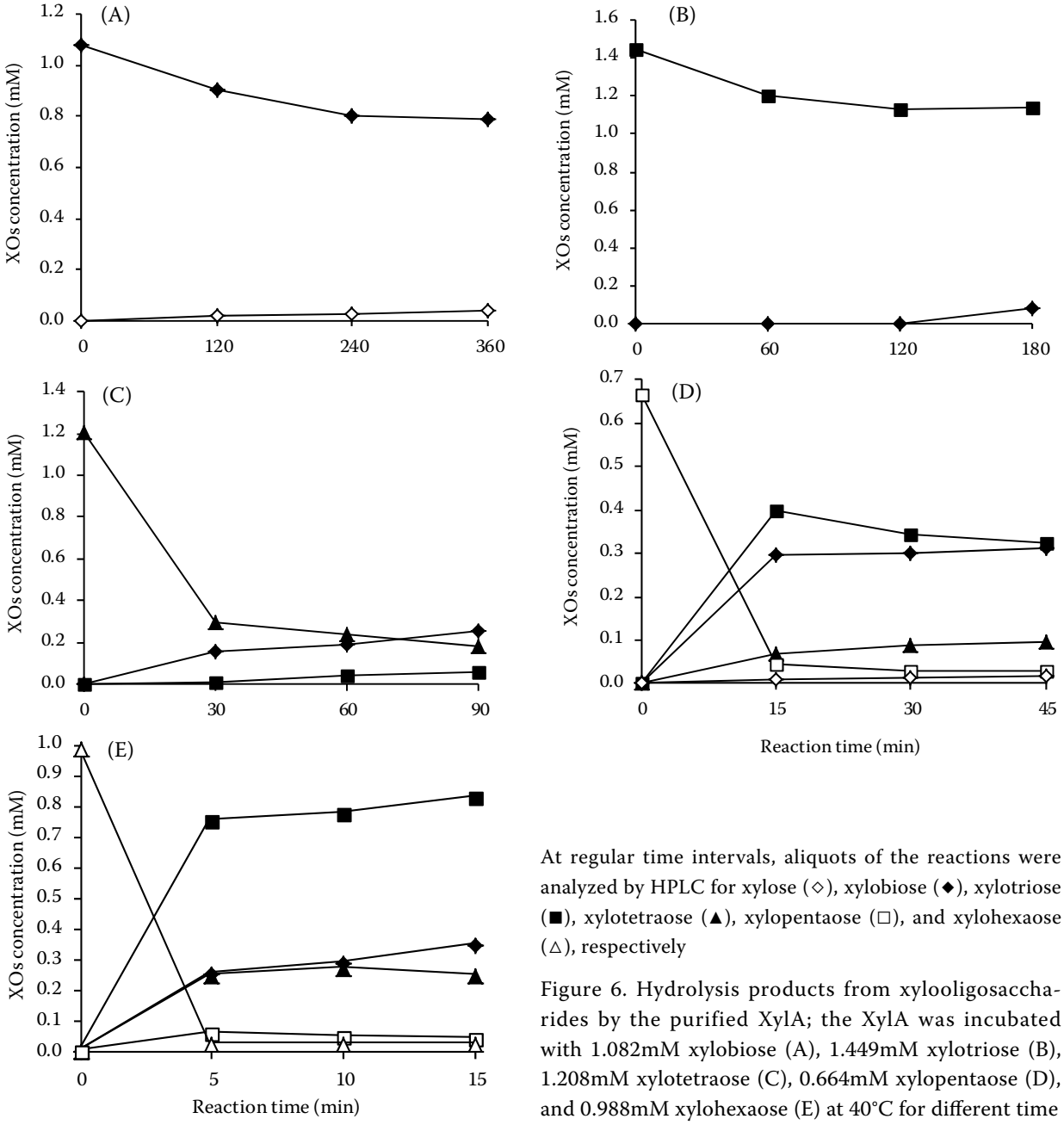
The mode of action of XylA on xylooligosaccharides

The mode of action of Xy1A was determined using different standard xylooligosaccharides (XO2–XO6) as substrates. XO2–XO6 could be further hydrolysed by XylA. The XylA showed a very low activity on xylobiose and xylotriose (Figures 6A and B). Xylobiose (XO2) and xylotriose (XO3) were the main products released separately from xylotetraose (XO4) and



Panels A and B were HPLC analysis of hydrolysis product from birchwood xylan and wheat bran insoluble xylan released by the xylanase for 24 h, respectively. Panel C is the standard xylooligosaccharides mixture analysed by HPLC. The positions of xylose (XO), xylobiose (XO2), xylotriose (XO3), xylotetraose (XO4), xylopentaose (XO5), and xylohexaose (XO6) are shown

Figure 5. HPLC profiles of xylans degradation



At regular time intervals, aliquots of the reactions were analyzed by HPLC for xylose (◇), xylobiose (◆), xylotriose (■), xylotetraose (▲), xylopentaose (□), and xylohexaose (△), respectively

Figure 6. Hydrolysis products from xylooligosaccharides by the purified XylA; the XylA was incubated with 1.082mM xylobiose (A), 1.449mM xylotriose (B), 1.208mM xylotetraose (C), 0.664mM xylopentaose (D), and 0.988mM xylohexaose (E) at 40°C for different time

xylopentaose (XO5) (Figures 6C and D). Xylohexaose (XO6) was rapidly hydrolysed by xylanase and generated a xylooligosaccharides mixture (XO2–XO5). XO3 was the major product released from XO6 (Figure 6E). XO5 was among the hydrolysis products of XO6, but no XO was detected, suggesting that the formation of XO5 was the result of transglycosylation reaction. The overall reaction might be:

XO6 + E = XO6(E); XO6(E) = XO3 + XO3(E);
XO6(E) = XO4(E) + XO2; XO3(E) + XO2 = XO5 + E

where: E – free xylanase; XO6(E) – xylanase xylohexaose complex; XO2(E) – xylanase xylobiose complex

The presence of trace amounts of XO generated from xylooligosaccharides by XylA revealed that XylA preferentially cleaved the internal glycosidic bonds of xylooligosaccharides, thus being an endo-acting xylanase. Endo-mode enzyme shows low susceptibility of the substrates of DP = 2, such as xylobiose, chitobiose, and maltose (Biely *et al*. 1981). Jiang *et al*. (2004) reported that a transglycosylation reaction might be involved in the degradation of xylobiose. No XOs with the DP > 2 was detected in the hydrolysis product mixture of XO2, suggesting that the xylanase might hydrolyse XO2 directly, not by transglycosylation reaction. These results

were similar to those for xylanases from *Aspergillus sojae*, *Thermomonospora fusca*, and *Bacillus licheniformis* (Kimura & Tajima 1998; Sun *et al.* 2007, 2009; Lu & Lu 2008).

CONCLUSION

Response surface methodology proved to be a powerful tool for optimisation of the culture conditions and medium composition. The study of contour plots showed that the optimum parameters of solid-state fermentation for xylanase production by *A. niger* JL-15, employing orange peel as the solid substrate, were 4.2% glycerin, 3.1% $(NH_4)_2SO_4$, 61% moisture content, and 73.4 h fermentation time. Under these conditions, the predicted and verifiable xylanase yields were 917.7 U/g and 917.7 U/g dry fermentation products, respectively.

XylA was found to be a mesophilic enzyme with the optima of 55°C and pH 5.0, respectively. It is an endo-acting xylanase with transglycosylation activity. The oligomer present in minimal amount in XylA hydrolysate was XO2. Xylan was hydrolysed by XylA to a mixture of xylooligosaccharides with XO3 as the major product. XO2 and XO3 are the main functional components of xylooligosaccharides. The results indicated a potentiality of XylA for the production of xylooligosaccharides, which were recognised as functional food additives.

Acknowledgments. The authors thank Dr. Shang-Wei Chen for his kind assistance in HPLC analysis.

Abbreviations

RSM – response surface methodology; XylA – *Aspergillus niger* xylanase A; SSF – solid-state fermentation; CCD – central composite design; HPLC– high performance liquid chromatography; XO – xylose; XO2 – xylobiose; XO3 – xylotriose; XO4 – xylotetraose; XO5 – xylopentaose; XO6 – xylohexaose; DP – degree of polymerisation; XOs – xylooligosaccharides

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Received for publication March 29, 2010
Accepted after corrections November 1, 2010

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