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ORIGINAL PAPER

Response surface optimization of fermentation conditions for producing xylanase by *Aspergillus niger* SL-05

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Abstract Fermentation conditions were statistically optimized for producing extracellular xylanase by *Aspergillus niger* SL-05 using apple pomace and cotton seed meal. The primary study shows that culture medium with a 1:1 ratio of apple pomace and cotton seed meal (carbon and nitrogen sources) yielded maximal xylanase activity. Three significant factors influencing xylanase production were identified as urea, KH_2PO_4, and initial moisture content using Plackett–Burman design study. The effects of these three factors were further investigated using a design of rotation–regression–orthogonal combination. The optimized conditions by response surface analysis were 2.5% Urea, 0.09% KH_2PO_4, and 62% initial moisture content. The analysis of variance indicated that the established model was significant ($P < 0.05$), "while" or "and" the lack of fit was not significant. Under the optimized conditions, the model predicted 4,998 IU/g dry content, whereas validation experiments produced an enzymatic activity of xylanase at 5,662 IU/g dry content after 60 h fermentation. This study innovatively developed a fermentation medium and process to utilize inexpensive agro-industrial wastes to produce a high yield of xylanase.

Keywords Apple pomace · Cotton seed meal · *Aspergillus niger* SL-05 · Medium optimization · Response surface methodology · Solid-state fermentation · Xylanase

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Introduction

Xylanases play a key role in xylan hydrolysing to xylo-oligosaccharides. Microbial xylanases mainly include xylanase or endoxylanase (1,4-β-D-xylan xylanohydrolase, E.C. 3.2.1.8) and β-xylosidase (1,4-β-xylan xylohydrolase, E.C. 3.2.1.37) [1]. Endoxylanase (EC 3.2.1.8) primarily cleaves β-1, 4-linked xylan back bone and β-xylosidase (EC 3.2.1.37) hydrolyses xylo-oligomers. From a commercial viewpoint, xylanases are an important group of carbohydrolases and have a worldwide market of around $200 million each year [2]. Xylanases have been widely applied in food, animal feed, bioconversion, textile, and in paper and pulp industries [3]. However, high cost and low yields of xylanase have been the main problems for its industrial production [4]. Therefore, there is a great need to develop a new fermentation medium with inexpensive substrates that provides a high xylanase yield.

China has the largest production of apple and cotton in the world. Millions of tons of apple pomace and cotton seed meal are also produced each year. In 2006, the yields of apple pomace and cotton seed meal were about 1 and 6 million tons, respectively. These relatively cheap agro-industrial residues, containing abundant nutrients (hemicellulose and cellulose), have a great potential to be utilized as alternative fermentation substrates. Therefore, in this research, apple pomace and cotton seed meal were selected and used as basic carbon and nitrogen sources for production of xylanase.

Among existing technologies in the fermentation industry, solid-state fermentation (SSF) shows many advantages over fermentation with submerged culture, such as lower cost and much higher reactor volume [5]. The application of SSF process has a considerable economical

potential in the food, feed, pharmaceutical, and agricultural industries. There are a great number of literatures reported to use the SSF process for producing enzymes with industrial importance, such as protease, cellulase, polygalacturonase, xylanase, pectinase, amylase, and glucoamylase [6–8]. However, it has not been reported using the SSF for production of xylanase using apple pomace and cotton seed meal.

It is well known that 30–40% of the production cost of industrial enzymes is taken up by the cost of growth medium [9]. Carbon and nitrogen sources together with fermentation time have been reported to play significant roles in the determination of the final morphology of the culture [10]. Therefore, it is significant to optimize these conditions for low-cost enzyme production using powerful statistical techniques. Response surface methodology has been used as a successful statistical tool for optimization of medium compositions in a fermentation process for enzyme production [11–13]. However, there are few literatures reported to use the second-order regression for rotation-orthogonal composite design for process optimization [14].

Therefore, the aim of this study was to optimize the nutrient medium with apple pomace and cotton seed meal for producing xylanase using the SSF with a strain of *Aspergillus niger* SL-05, by applying different statistical experimental designs and data analysis methods.

Materials and methods

Microorganism and preparation of spore suspension

A strain of *A. niger* SL-05 was provided by our laboratory. It was cultivated in wheat bran medium and incubated at 30°C for 5 days. The 5-day-old culture in wheat bran medium was transferred into a 250 ml flask, mixed with 100 ml sterile deionized water, and stirred for 20 min. Then the mixture was separated with double sterile gauze and the filtrated solution was diluted to 1×10^7 spore/ml with sterile deionized water. Direct microscopic counts were taken using a blood counting chamber.

Basic fermentation medium materials

Apple pomace was obtained from a local apple juice concentrate company in Shandong Province, China. It was dried in an oven at 60°C and ground in a hammer mill. The ground material was passed through 30- and 50-mesh sieves. The fraction which passed through the 30-mesh sieve but retained by the 50-mesh sieve was collected and used as basic fermentation media. The cottonseed meal was obtained from a local company at the Tai'an district of the Shandong Province of China. The meal was made after cotton seed oil extraction using a compression method and was pretreated in the same way as the apple pomace. The moisture content, total protein content and crude fiber of apple pomace were 9, 6 and 15%, respectively; and those of cotton seed meal powder were 8, 41 and 12%, respectively.

Soy bean powder and wheat bran obtained from local market was pretreated as same as for the apple pomace.

Solid-state fermentation

Solid-state fermentation was carried out in a 500 ml Erlenmeyer flask that contained 15 g powdered apple pomace and 15 g cotton seed powder per bottle. The flask was sealed with a six-layer sterile gauze. The initial moisture content was adjusted to 55% (w/w). The pH of the medium was under natural, and it was not changed after autoclaving at 121°C for 30 min. Such a prepared medium was used as the basic solid substrate.

The culture medium was inoculated with 2 ml spore suspension (1×10^7 spore/ml). After mixing well, the inoculated medium was incubated at 30°C for 60 h. Each flask was gently tapped intermittently for mixing well during incubation period.

Single factor experiments

The purpose of this study was to screen carbon and nitrogen sources for optimal xylanase production. Apple pomace was used to be a basic carbon source in nutrient medium composition. Different nitrogen sources were also primarily selected, including cotton seed powder, soy bean meal, and wheat bran.

Supplementation of carbon and nitrogen sources in SSF is always necessary. Which extra-addition carbon and nitrogen sources is the best and how much of them should be supplemented depend on the availability of carbon and nitrogen from the substrates and the requirement of the organism [15]. In this study, extra-addition carbon (Sodium carboxymethylcellulose, Konjac, Oat xylan, Glucose, Starch) and nitrogen sources [$(NH_4)_2SO_4$, Urea] have been studied in the basic substrate. Oat xylan was purchased from Sigma Chemical Co. (X-0627), and other chemicals used in this investigation were analytical reagent grade and purchased from local chemical suppliers in China.

Plackett–Burman (PB) design

Initial screening of the most significant fermentation parameters affecting xylanase production by *A. niger* SL-05 was performed by PB design as reported by Plackett [16]. In this study, the same technique was applied to

determine the most significant factor affecting the xylanase production.

A total of seven (n) variables including urea, ammonium sulphate, initial moisture content, KH_2PO_4 and two inorganic ions ($MgSO_4$, $CaCl_2$), and one dummy or unassigned variables were studied in eight experiments. In addition, three center points were added for the variables that could be assigned numerical values. The experimental design with the name, symbol code, and actual level of the variables is shown in Tables 1 and 2. The analysis of variance (ANOVA) for the data and the model coefficients were computed with Microsoft Excel 2003 software.

Response surface methodology

Urea, KH_2PO_4, and initial moisture content, primarily selected by PB design experiments, were taken into consideration for optimizing xylanase production. The experimental design with the name, symbol code, and actual level of the variables is shown in Tables 3 and 4.

The SAS statistical package (version 8.2, SAS Institute Inc., Cary, NC, USA) was employed for regression analysis of data to obtain optimal working parameters and to generate response surface graphs.

Enzyme extraction

After fermentation (incubation), the cultured medium was dried at 50°C for 24 h in a constant temperature oven. One gram dry content was added to 100 ml of fresh distilled water and stirred for 30 min. Mixture was separated using Xinhua No.1 filter paper (Xinhua Paper Co., Hangzhou, China), and the filtrated solution was used for analyzing xylanase activity.

Table 1 Experimental variables at different levels used for the production of xylanase by *Aspergillus niger* SL-05 using Plackett-Burman design

Variables	Units (w/w)	Symbol code	Actual factor level at coded factor levels		
			Lower (−1)	Center (0)	Higher (1)
$(NH_4)_2SO_4$	%	*X1*	1.0	1.25	1.5
Urea	%	*X2*	1.0	1.25	1.5
KH_2PO_4	%	*X3*	0.05	0.063	0.075
$CaCl_2$	%	*X4*	0.2	0.25	0.3
$MgCl_2$	%	*X5*	0.050	0.075	0.100
Initial moisture content	%	*X6*	44	50	55

The radio of apple pomace to cotton seed powder was 1:1 (w/w)

Table 2 Eleven-trial Plackett-Burman design matrix for seven variables with the observed xylanase activity, including three replications at the center point

Runs	X_1	X_2	X_3	X_4	X_5	X_6	X_7	Xylanase activity (IU/g)
1	1	1	1	1	1	1	1	4,005
2	1	1	1	−1	−1	−1	−1	3,013
3	1	−1	−1	1	1	−1	−1	2,426
4	1	−1	−1	−1	−1	1	1	3,866
5	−1	1	−1	1	−1	1	−1	4,142
6	−1	1	−1	−1	1	−1	1	2,934
7	−1	−1	1	1	−1	−1	1	2,102
8	−1	−1	1	−1	1	1	−1	3,387
9	0	0	0	0	0	0	0	3,296
10	0	0	0	0	0	0	0	3,351
11	0	0	0	0	0	0	0	3,514

Enzyme activity assay

Xylanase activity was determined according to the widely recognized method by Bailey et al. [17]. Prior to analysis, 0.5 ml of diluted enzyme solution was mixed with 1 ml 1% (w/v) oat xylan (Sigma) in 0.05 M citrate buffer (pH 5.0) and incubated at 40°C for 10 min. The release of reducing sugars was determined by the 3,5-dinitrosalicylic acid method [18] using xylose (Fluka, 95730) solution as a standard reference. The OD value was measured at 540 nm using a UV-Vis spectrophotometer (Ultrospec 4300 pro, Pharmcia, USA).

Three samples were taken and each sample was analyzed in triplicate. The nine values were calculated to the mean to represent the observed values of xylanase activity. One unit (IU) of enzyme activity was defined as the amount of enzyme required to release 1 μmol reducing sugars per min. The results of these analyses are expressed as units per gram of present dry matter (IU/g).

Table 3 Values of independent variables at different levels of the design of rotatio–regression–orthogonal combination

Variables	Units	Symbol code	Actual factor levels at coded factor levels				
			−1.682	−1	0	1	1.682
Urea	%	X_1	0.50	1.11	2.00	2.89	3.50
KH_2PO_4	%	X_2	0.01	0.04	0.09	0.14	0.17
Initial moisture content	%	X_3	50	55	60	64	67

The radio of apple pomace to cotton seed powder was 1:1 (w/w). The glucose was fixed at the 2%

Table 4 Experimental design and results of the second-order regression for rotation–orthogonal composite design

Runs	X_1 (Urea)	X_2 (KH_2PO_4)	X_3 (initial moisture content)	Xylanase activity (IU/g)	Standard deviation
1	1	1	1	4,685	255
2	1	1	−1	4,817	247
3	1	−1	1	4,875	211
4	1	−1	−1	4,243	255
5	−1	1	1	4,274	183
6	−1	1	−1	4,240	157
7	−1	−1	1	4,159	146
8	−1	−1	−1	4,110	250
9	−1.682	0	0	3,787	54
10	1.682	0	0	4,385	261
11	0	−1.682	0	4,012	94
12	0	1.682	0	3,957	68
13	0	0	−1.682	3,934	150
14	0	0	1.682	4,553	47
15	0	0	0	5,029	157
16	0	0	0	5,147	72
17	0	0	0	5,199	33
18	0	0	0	5,246	138
19	0	0	0	4,716	20
20	0	0	0	5,079	105
21	0	0	0	4,619	111
22	0	0	0	4,411	20
23	0	0	0	4,573	20

Relationship between cell growth and xylanase production

Crude protein, pure protein, total sugar, and reducing sugar were determined as a measure of cell growth. Crude protein and pure protein content were estimated by the Kjeldahl Method using a Kjeldahl test unit (KDY-9820, Ketuo, Beijing). Reducing sugar was determined by the dinitrosalicylic acid method [18], using glucose as a standard reference. Total sugar was analyzed according to the method of Geatera [19].

Results and discussion

Effects of organic nitrogen sources on xylanase activity

Our primary study results indicated that the xylanase activity was strongly influenced by the nitrogen sources (Fig. 1). Bean powder (100%) seemed to be the best carbon source and cotton seed powder (99%) was the second, while wheat bran (19%) was the least. Wang et al. [20] also reported a higher activity of hemicellulase with the mixture of cotton seed powder and soy bean meal compared with wheat bran when two mixed strains of *Aspergillus niger* were used. The total protein contents of cotton seed powder, soy bean meal and wheat bran in this research were 41, 43 and 14 g/100 g dry weight, respectively. Therefore, higher protein content in cotton seed powder and soy bean meal might have contributed to higher xylanase production compared to wheat bran. However, because soy bean meal is more expensive than cotton seed powder, cotton seed powder was selected to be the basic nitrogen source in our following studies.

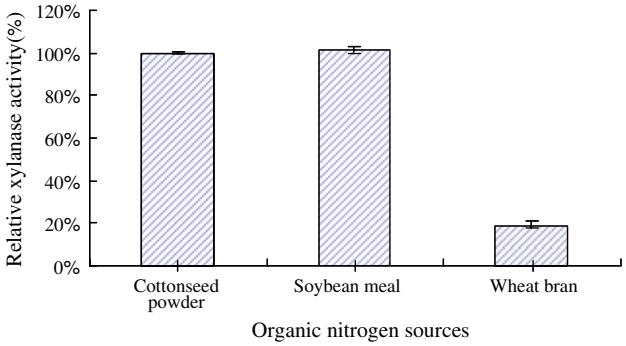

Fig. 1 Effect of organic nitrogen sources on yield of xylanase by *Aspergillus niger* SL-05. The radio of carbon to nitrogen sources is 1:1 (w/w) and initial moisture content (w/w) is 55%. The xylanase activity of cotton power was regarded as 100%

Effects of carbon–nitrogen ratio on xylanase activity

The carbon–nitrogen ratio is one of the most important indexes affecting solid-state fermentation. Therefore, a wide range of the ratios from 1:9 to 9:1 between apple pomace and cotton seed powder were studied for achieving an optimal xylanase activity. The results suggested that the peak yield of xylanase appeared at the ratio of 5:5, whereas the ratio at 9:1 yielded the lowest xylanase activity (Fig. 2). As the total protein content in apple pomace was not high enough (6%) for xylanase production, a substantial increase in xylanase activity was observed when cotton seed powder content increased until the ratio was up to 5:5. This result agreed with the report of Silva et al. [21], in which maximum pectinase was produced using bagasse and wheat bran (1:1) as carbon and nitrogen sources by the filamentous fungus *Penicillium viridicatum* RFC3.

Results of variance analysis showed that the ratio of apple pomace to cotton seed powder between 1:9 and 9:1 had extremely significant influence on xylanase activity ($P < 0.01$), but it was not significant when the ratio was between 6:4 and 4:6 ($P > 0.05$).

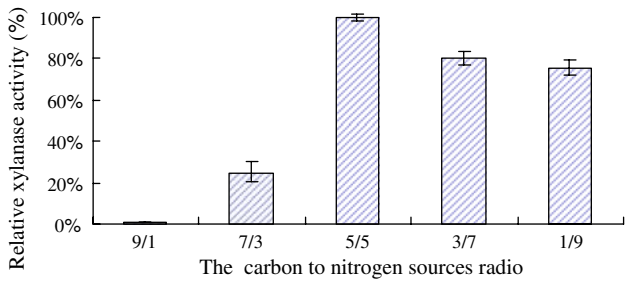

Fig. 2 Effect of the apple pomace to cottonseed powder ratio on yield of xylanase by *A. niger* SL-05. Initial moisture content (w/w) was 55%. The highest xylanase activity was regarded as 100%

Effects of extra-addition carbon and nitrogen sources on xylanase activity

It was observed that extra-addition carbon sources except for starch improved xylanase activity after 60 h of incubation (Table 5). The high xylanase activity was observed in the presence of hemicellulose and cellulose substrates. Xylanase could be specifically induced by hemicellulose [22]. The addition of glucose also increased some xylanase activity comparing to the control within the same incubation time, suggesting that the second carbon source (glucose) facilitated microbial growth and enzyme production. This finding would partially support why extra-addition glucose shortened the incubation time [23]. Although xylan and sodium carboxymethylcellulose showed high improvement in xylanase production, they are much more expensive than glucose. Therefore, in this research, we chose inexpensive glucose as the extra-addition carbon and energy source.

In the presence of extra-addition nitrogen sources, xylanase production was also positively affected. An increase of xylanase activity was observed when the concentrations of $(NH_4)_2SO_4$ and Urea increased. The interactions between these two factors and with other nutrient sources were further defined in the following studies.

Table 5 Effect of extra-addition carbon sources and nitrogen sources on xylanase activity

		Levels (w/w)	Relative xylanase activity[b]
Extra carbon sources	Control		100%
	Sodium carboxymethylcellulose	2%	125%
	Konjac[a]	2%	102%
	Xylan	1%	112%
	Glucose	2%	108%
	Starch	2%	86%
Extra nitrogen sources	Control		100%
	$(NH_4)_2SO_4$	1%	108%
		2%	110%
	Urea	1%	100%
		2%	117%

[a] Konjac(Amorphophallus konjac), scientific name: Glucomannan, a food species of Yunnan and Sichuan

[b] The radio of apple pomace to cotton seed powder was 1:1 (w/w) and initial moisture content (w/w) was 55%. The xylanase activity of control was set as 100%

Selection of influential media components for process modeling

Production of cellulase or hemicellulase is known to be sensitive to nitrogen source and nitrogen levels in the medium [24]. According to single factor experiments described earlier, the production of xylanase was positively influenced by higher levels of $(NH_4)_2SO_4$ and organic nitrogen sources (Urea). Based on other similar researcher's findings, the initial moisture content, KH_2PO_4 and two inorganic ions ($MgSO_4$, $CaCl_2$) were selected and evaluated for optimization of the medium formulation. The experimental PB design and results were given in Tables 1 and 2. The xylanase activity varied considerably within the tested conditions in the range of 2,102–4,142 IU/g. This suggested that these variables strongly affected xylanase production.

The adequacy of the model was tested, and the parameters with statistically significant effects were identified using the Fisher's test for ANOVA. The ANOVA for the selected factorial model showed that the model was significant at the 5% level of significance with a model *P*-value of 0.02. The coefficient of determination (R^2) of the model was calculated to be at 0.99, indicating that 99% of the variability in the response could be explained by the model.

Factors having a confidence level greater than 95% ($P < 0.05$) were considered to have a significant effect on the response and were selected for further studies. KH_2PO_4, one of the three screened significant variables, exerted a negative effect, whereas the other variables, initial moisture content and urea, showed positive effects on xylanase production (Table 6). Initial moisture content with *P*-value of 0.0079 was found to be the most significant factor, followed by urea and KH_2PO_4.

The model was reconfirmed by the three center points via Student's *t*-test.

$$|t| = 2.4069 < t_{0.05}(3) = 3.18$$

The non-significant *t*-value also suggested that there was no significant difference between the obtained data at center points and the intercept.

Table 6 Coefficient estimates of the regression model

Parameter	Coefficients	*t*-value	$P > \|t\|$
Intercept	3234.38	430.12	0.0015
$(NH_4)_2SO_4$	93.13	12.41	0.0520
Urea	93.13	38.44	0.0168
KH_2PO_4	−107.63	−14.33	0.0450
$CaCl_2$	−65.63	−8.71	0.0736
$MgCl_2$	−46.38	−6.17	0.1037
Initial moisture content	615.63	81.87	0.0079

Optimization of medium composition with response surface methodology

Based on the previous PB screening test, response surface methodology was applied to determine the optimal conditions of the three significant factors including initial moisture content, urea, and KH_2PO_4. A three-factor and five-level second-order regression for rotation–orthogonal composite design consisting of 23 experimental runs was employed including 9 replicates at the center point. The experimental design with observed xylanase activity is shown in Tables 3 and 4.

Regression analysis was performed to fit the response function with the experimental data. The model *P*-value was 0.03 and the *P*-value for lack of fit was 0.39, respectively. The tested model was statistically significant at the 5% level of significance, and the non-significant lack of fit also indicated that the model was a good fit.

The ANOVA analysis of the optimization study indicated that the X_1, X_1X_1, and X_2X_2 were significant at the 5% level (Table 7). These results indicated that the nitrogen source correlated a direct relationship with xylanase production. The interactions were non-significant, with the high *P*-value (>0.4). The regression equation coefficients were calculated, and the data were fitted to a second-order polynomial equation. The regression equation obtained was as follows:

$$Y_0 = 4883 + 208X_1 + 39X_2 + 119X_3 - 208X_1^2 - 244X_2^2 - 153X_3^3$$

where Y_0 was the yield of xylanase, and X_1, X_2 and X_3 were coded independent variables for urea, KH_2PO_4, and initial moisture content, respectively.

To determine the optimal levels of each variable, three-dimensional response surface plots were constructed by plotting the response on the *Z*-axis against two independent variables, maintaining initial moisture content at its optimal level (Fig. 3). As shown in Fig. 3, a linear increase in xylanase secretion was observed when the amount of urea or KH_2PO_4 increased. The optimum levels of each variable were determined to be as follows: 2.5% urea, 0.09% KH_2PO_4 0.09%, and 62% (w/w) initial moisture content.

Table 7 Test of significance for regression coefficients

Parameter	DF	Estimate	Standard Error	*t*-value	$P > \|t\|$
Intercept	1	4883	107.75	45.32	<0.001
X_1	1	208	87.53	2.38	0.0334
X_2	1	39	87.53	0.45	0.6610
X_3	1	119	87.53	1.36	0.1974
X_1X_1	1	−208	81.13	−2.57	0.0235
X_2X_1	1	17	114.36	0.15	0.8816
X_2X_2	1	−244	81.13	−3.01	0.0101
X_3X_1	1	52	114.36	0.46	0.6561
X_3X_2	1	−97	114.36	−0.85	0.4099
X_3X_3	1	−153	81.13	−1.88	0.0827

Validation of the model

To confirm these optimal conditions obtained earlier, a validation experiment was performed. Under these optimized conditions, the predicted response for xylanase production is 4,998 IU/g, and the observed experimental value was 5,662 IU/g, which was 664 IU/g higher than the predicted value. The increased amount of xylanase production in the validation experiment might be due to that the stain of *A. niger* SL-05 was more adapted to produce xylanase after long time cultivation.

In the previous work as summarized in Table 8, the yields of xylanase using SSF were mostly reported to be relatively low. Only two reports showed similar enzyme activities to our result. Park et al. [28] reported the highest

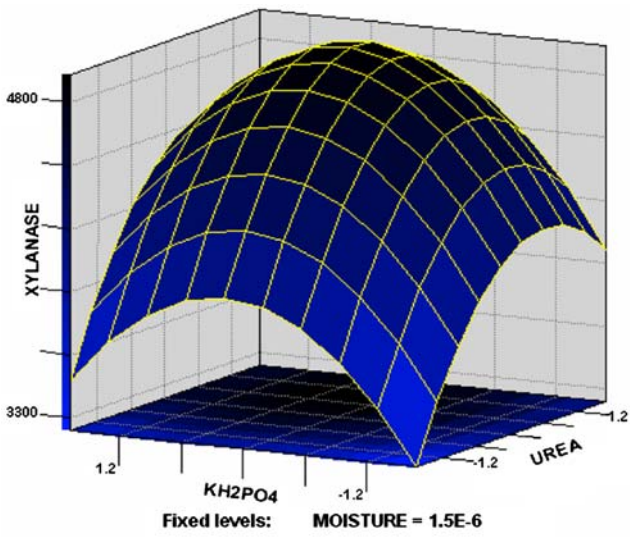

Fig. 3 Three-dimensional response surface plot of the second-order regression for rotation-orthogonal composite design

Table 8 Comparisons of xylanase production by other strains in SSF

Organism	Substrate	Cultivation condition	Xylanase (IU/g)	Reference
Aspergillus foetidus	Corncob	Static, 30°C, 4 days	3,065	[15]
Trichoderma longibrachiatum	Wheat bran and wheat straw:	Static, 25°C, 4 days	479	[25]
Thermoascus aurantiacus	Bagasse	Static, 45°C, 10 days	2,700	[26]
Scytalidium thermophilum	Rice straw and wheat bran	Static, 45°C, 7 days	196	[27]
Aspergillus niger	Rice straw	Static, 28°C, 5 days	5,071	[28]
A. niger	Lignocellulosic material	Static, 28°C, 3 days	6,320	[6]
A. niger SL-05	Apple pomace and cotton seed meal	Static, 30°C, 2.5 days	5,662	This work

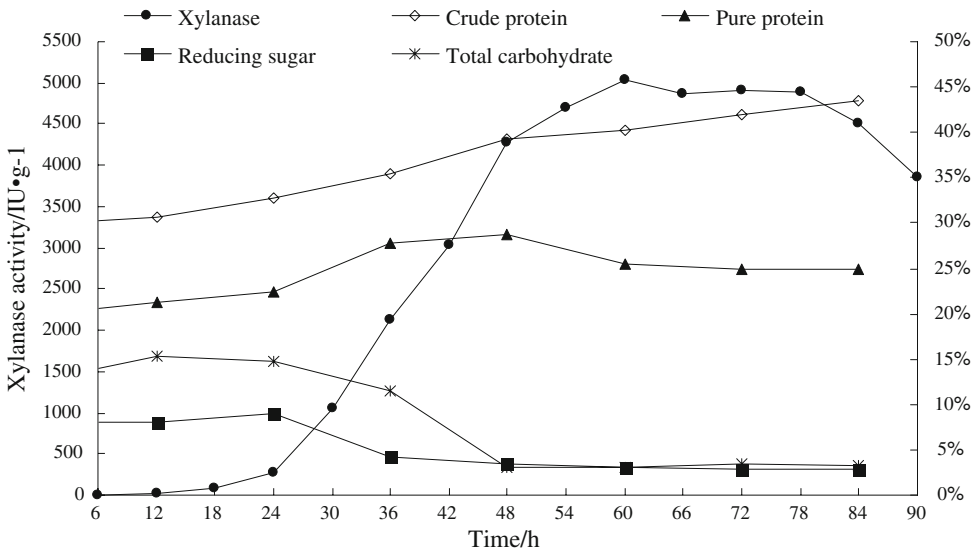

Fig. 4 Time course profile of xylanase production (IU g^{-1}), crude protein (%, w/w) and pure protein (%, w/w), total carbohydrate (%, w/w) and reducing sugar (%, w/w) by *A. niger* SL-05 in SSF. Experimental conditions: urea 2.5%, KH_2PO_4 0.09% and initial moisture content 62%, inoculum size 2 ml (1×10^7 spore/ml). The fermentation was carried out at the constant temperature of 30°C under optimized conditions

xylanase production (5071 IU/g), but used uneconomical 5 days fermentation time. Min Wu et al. [4] obtained the highest xylanase production (6,320 IU/g) grown on the material with high hemicellulose content, in which hemicellulose could efficiently induce xylanase [22]. In comparison, inexpensive apple pomace and cotton seed powder were used as basic substrates and the fermentation time was less than 60 h in our studies. The selected strain of *A. niger* SL-05 was also demonstrated to be appropriate for this fermentation process developed.

Relationship between cell growth and xylanase production

The relationship between cell growth and xylanase production using *A. niger* SL-05 under the above optimized conditions were studied. The time course profiles of xylanase production, crude protein and pure protein, total carbohydrate and reducing sugar were shown in Fig. 4. The xylanase production curve seems to be in a close correlation with a typical microbial growth curve that includes lag, exponential (log), stationery, and death phases. The major xylanase secretion was observed during 24–48 h of inoculation. Then, small increase in secretion was continuously observed for up to 60 h, after which slight decrease in the xylanase amount was seen. The highest xylanase activity of 5,662 IU/g was obtained at the 60 h of fermentation.

Mycelia were not observed in preceding 24 h in experimental process, which indicated that microorganisms were at the lag phase. Many mycelia appeared during 24–48 h and showed that the mold grown in exponential growth phase during this time. In this period, the amount of total carbohydrate and reducing sugar reduced dramatically, demonstrating the microorganisms metabolized quickly. The increased crude protein and pure protein during this period also indicated high cellular metabolism activities. As spores were produced after 48 h up to 60 h, the xylanase production tended to be slower. After that, the medium began to dry due to the heat generated during microbial growth and the organisms went to death phase. Therefore, the best fermentation time for xylanase

production under the optimized conditions would be between 48 and 60 h.

Conclusions

The response surface methodology using the second-order regression for rotation–orthogonal composite design was a successful tool for the optimization of medium compositions for xylanase biosynthesis by *A. niger* SL-05 in solid-state fermentation. The primary study showed that culture medium with a 1:1 ratio of apple pomace and cotton seed meal (carbon and nitrogen sources) with 2% glucose as additional carbon source yielded maximal xylanase activity. Three significant factors influencing xylanase production were identified as urea, KH_2PO_4, and initial moisture content using Plackett–Burman design study. The optimal medium compositions through the response surface modeling were: 2.5% urea as the second nitrogen source, 0.09% KH_2PO_4 and 62% (w/w) initial moisture content. Under the optimized conditions, the model predicted 4,998 IU/g dry content, while validation experiments produced an enzymatic activity of xylanase at 5,662 IU/g dry content after 60 h fermentation. The results from this study would be of significance for the agriculture and enzyme industries to develop innovative techniques to utilize these inexpensive agro-industrial wastes for enzyme production using SSF process. Future research is needed to scale-up such a developed fermentation process and to investigate more potential for utilizing these agro-industrial wastes for producing valuable bioproducts using fermentation technology.

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