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Improvement of xylanase production by *Aspergillus niger* XY-1 using response surface methodology for optimizing the medium composition*

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Abstract: Objective: To study the optimal medium composition for xylanase production by *Aspergillus niger* XY-1 in solid-state fermentation (SSF). Methods: Statistical methodology including the Plackett-Burman design (PBD) and the central composite design (CCD) was employed to investigate the individual crucial component of the medium that significantly affected the enzyme yield. Results: Firstly, $NaNO_3$, yeast extract, urea, Na_2CO_3, $MgSO_4$, peptone and $(NH_4)_2SO_4$ were screened as the significant factors positively affecting the xylanase production by PBD. Secondly, by valuating the nitrogen sources effect, urea was proved to be the most effective and economic nitrogen source for xylanase production and used for further optimization. Finally, the CCD and response surface methodology (RSM) were applied to determine the optimal concentration of each significant variable, which included urea, Na_2CO_3 and $MgSO_4$. Subsequently a second-order polynomial was determined by multiple regression analysis. The optimum values of the critical components for maximum xylanase production were obtained as follows: x_1 (urea)=0.163 (41.63 g/L), x_2 (Na_2CO_3)=−1.68 (2.64 g/L), x_3 ($MgSO_4$)=1.338 (10.68 g/L) and the predicted xylanase value was 14374.6 U/g dry substrate. Using the optimized condition, xylanase production by *Aspergillus niger* XY-1 after 48 h fermentation reached 14637 U/g dry substrate with wheat bran in the shake flask. Conclusion: By using PBD and CCD, we obtained the optimal composition for xylanase production by *Aspergillus niger* XY-1 in SSF, and the results of no additional expensive medium and shortened fermentation time for higher xylanase production show the potential for industrial utilization.

Key words: Xylanase, Optimization of medium composition, Plackett-Burman design (PBD), Central composite design (CCD), Response surface methodology (RSM), *Aspergillus niger* XY-1

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INTRODUCTION

Xylan, the major hemicellulose polysaccharide, is widely distributed in agricultural by-products including corncob, rice husk, wheat straw and wheat bran. In China billions of tons of those by-products have been produced every year and some of them have been used in animal feed as feed ingredients for economic reasons. However, due to the high content of arabinoxylans, which might reduce animal digestibility of dietary protein and the absorption of amino acids, the utilization of those by-products in feed is limited and impractical (Slominski *et al*., 2004; Barrera *et al*., 2004). Hence the removal of arabinoxylans from agricultural by-products becomes highly desirable.

To degrade arabinoxylans completely, a number of enzymes are required including xylanases, β-xylosidase, α-arabinofuranosidase, acetyl and feruloyl esterases and α-D-glucuronosidases (Courtin *et al.*, 2006). Among them xylanase (EC 3.2.1.8), which can catalyze the hydrolysis of the xylopyranosyl linkages of β-1,4-xylan and randomly cut the arabinoxylan backbone to produce a wide range of arabinoxylan fragments, plays the most important role. Supplementation of xylanase into the feed has thus been reported as an efficient method to increase the apparent faecal digestibility coefficients of crude protein, crude fat, crude fibre and organic matter, and

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improved energy absorption (Lindberg *et al*., 2007; Tapingkae *et al*., 2008; Sterk *et al*., 2007; Nortey *et al*., 2007). So far commercial xylanase products have already appeared on the market and most of them are produced by *Bacillus* (Heck *et al*., 2006; Gessesse and Mamo, 1999), *Aspergillus* (Wu *et al*., 2005; Senthilkumar *et al*., 2005; Botella *et al*., 2007), *Penicillium oxalicum* (Li *et al*., 2007a; 2007b), *Paecilomyces themophila* (Yang *et al*., 2006) and *Thermomyces lanuginosus* (Sonia *et al*., 2005).

Production of xylanase by solid-state fermentation (SSF) using various lignocellulosic substrates has been reported previously (Ghanem *et al*., 2000; Li *et al*., 2007c) and was found to have several advantages, such as higher productivity as well as lower operational and capital costs. Because the cost of the enzyme is the major factor for broad application, approaches that either decrease the medium cost or increase production efficiency should be investigated. Since SSF can be performed on a variety of lignocellulosic materials, such as wheat straw, wheat bran and corncob, the cost of xylanase production can be reduced greatly. On the another hand, high yield xylanase production strains have been screened by Yang *et al*.(2006), but the fermentation time was 4~12 d and that was not adequate in meeting industrial needs (Narang *et al*., 2001; Park *et al*., 2002; Bakri *et al*., 2003; Wu *et al*., 2005; Yang *et al*., 2006). Recently an *Aspergillus niger* strain XY-1 was isolated for high-level xylanase production in our lab and its fermentation time was shortened to 2 d. It provided great expectations for use as an industrial strain.

The aim of this work was to apply statistical methods to optimize the fermentation medium compositions for the improvement of xylanase production by *A. niger* XY-1. A Placket-Burman design (PBD) (Plackett and Burman, 1946) was firstly used to identify the medium components which had significant effects on xylanase production. A central composite design (CCD) (Box and Wilson, 1951) was then employed to optimize the factors that had a significant influence on xylanase production.

MATERIALS AND METHODS

Microorganism and chemicals

The organism used in the current study was *A. niger* XY-1, identified by the China General Microbiological Culture Collection Center. The strain was grown on potato dextrose agar (PDA) slants at 28 °C for 5 d and then stored at 4 °C. Inoculum was prepared by suspending the spores from a PDA slant by adding sterile distilled water to give a final spore count of 1×10^6 spores/ml. Unless indicated otherwise, all chemicals and reagents were purchased from Huadong Medicine Co., Ltd. Equipment Chemical Reagent Branch (Hangzhou, China).

SSF for xylanase production

Wheat bran was purchased from a local market and used as the solid substrate in the experiments. The fermentation medium was made by adding 10 g of wheat bran to a 300-ml Erlenmeyer flask, then mixed with 10 ml of additive nutrient solution (changed according to experimental needs) to give the final moisture ratio of 1:1 (w/v). After sterilization by autoclaving at 121 °C for 30 min, the medium in the flask was cooled down and inoculated with 1 ml spores suspension, then incubated at 28 °C for 48 h. To assess the effects of medium ingredients on the production of xylanase by using PBD and CCD, the composition of the nutrient solution varied according to the experimental designs.

PBD for valuating the factors effect

There are a range of factors that need to be tested for their importance in xylanase production. PBD is a useful tool to screen 'n' variables ('$n-3$' actual factors and '3' invented variables for estimating errors) in just '$n+1$' experiments (Plackett and Burman, 1946), which will reduce the enormous total number of experiments in comparison with full factorial designs which require 2^N (N denotes the number of factors) experiments. The factors investigated in the current study included eight easily metabolized sugars (glucose, xylose, maltose, galactose, lactose, sucrose, mannose and raffinose), seven nitrogen sources (yeast extract, peptone, beef extract, $(NH_4)_2SO_4$, $NaNO_2$, $NaNO_3$ and urea) and five salt additives ($CaCl_2$, $FeSO_4$, Na_2CO_3, $MgSO_4$ and KH_2PO_4). Each variable was tested at two levels. The high level was 5 g/L (+) and the low level was 0 g/L (−). The impact of each variable on xylanase activity was estimated based on comparison of the difference in the mean between the high level (+) and the low level (−).

CCD for ingredient optimization

Based on the results from PBD experiments, three factors that significantly affected the xylanase production were identified and optimized further using the response surface methodology (RSM). The CCD with three factors and five levels including six replicates at the centre point was used to fit the second order response surface. Table 1 shows the factors and values. This methodology allows the modeling of a second-order equation that describes the process. Xylanase production was analyzed by multiple regressions through the least squares method to fit the following equation:

$$Y = a_0 + \sum a_i x_i + \sum a_{ii} x_i^2 + \sum a_{ij} x_i x_j,$$

where Y represents response variable, a_0 is the interception coefficient, a_i is the coefficient of the linear effect, a_{ii} is the coefficient of quadratic effect and a_{ij} is the coefficient of interaction effect. x_i and x_j denote the coded levels of variable X_i and X_j in experiments. The variable X_i was coded as x_i according to the following transformation equation:

$$x_i=(X_i-X_0)/\Delta X_i,$$

where x_i is the dimensionless coded value of the variable X_i, X_0 is the value of X_i at the center point, and ΔX_i is the step change.

Table 1 Coded values of variables used in CCD

Coded value x_i	Independent variables X_i (g/L)		
	Urea (i=1)	Na_2CO_3 (i=2)	$MgSO_4$ (i=3)
−1.68	23.18	2.64	4.64
−1	30.00	4.00	6.00
0	40.00	6.00	8.00
1	50.00	8.00	10.00
1.68	56.82	9.36	11.36

Crude enzyme extraction

After 48 h incubation the sample was transferred into the drier at 40 °C until its moisture content of the medium was below 10%, then pulverized through a 0.25-mm sieve. A 1 g sample was soaked with 160 ml distilled water and kept in 40 °C water bath for 30 min. The enzyme extract was filtered by a Whatman #1 analytical filter and the suitable dilution was stored at 4 °C for further analysis.

Xylanase assay

The xylanase activity was determined using Oat spelts xylan (Sigma Co., USA) as substrate. The reducing sugars produced were quantified by the dinitrosalicylic acid method using D-xylose as standard. In brief, the reaction containing 0.5 ml of appropriate enzyme solution and 0.5 ml of 1% (w/v) oat spelts xylan (pH 5.0 acetate buffer) was incubated in a water bath at 40 °C for 15 min, then determinated by adding 1.5 ml of dinitrosalicylic acid solution. After incubation in a boiling bath for 5 min, the liberated reducing sugars were measured at 540 nm wavelength with spectrophotometer (VIS-7220). One unit (U) of xylanase activity was defined as the amount of enzyme that released 1 μmol of xylose per minute under the assay conditions. Xylanase production of fermentation was expressed as U/g dry substrate.

Statistical analysis

Quantification of enzyme activity was carried out in duplicate experiments and the mean values were given. The significance of each variable in the PBD experiment was determined by applying the Student's t-test using MINITAB statistical software (6sigma, Mb13). For the CCD experiment the working parameters were calculated and generated response surface graphs using MINITAB statistical software. The accuracy and general ability of the above polynomial model could be evaluated by the determination coefficient (R^2).

RESULTS AND DISCUSSION

PBD and response values

PBD for 20 factors made a total of 24 experimental treatments. The PBD factors and response values are listed in Table 2. From the listed data, treatment 11 with A−B−C+D−E+F−G−H+J+K−L−M+N+O−P+Q−R+S+T+U+ gave a better value than the others.

Table 3 shows test factors and the rank of significance. Among them the reliability of $NaNO_3$, yeast extract, urea, Na_2CO_3, $MgSO_4$, $CaCl_2$, peptone, xylose, $(NH_4)_2SO_4$, sucrose and raffinose reached 90%, suggesting a significant effect on xylanase production. When the concentrations of $CaCl_2$, xylose,

sucrose and raffinose were increased, xylanase production declined. In contrast, positive effects on xylanase production were observed when the levels of $NaNO_3$, yeast extract, urea, Na_2CO_3, $MgSO_4$, peptone and $(NH_4)_2SO_4$ were increased. The factors resulting in a negative effect on xylanase production were no longer taken into account, while the positive factors were included in the next CCD optimization.

Table 2 The PBD and the experimental results

Trial	Coded value x_i																			Y (U/g dry substrate)
	A	B	C	D	E	F	G	H	J	K	L	M	N	O	P	Q	R	S	T	U	
1	1	−1	1	−1	−1	1	1	−1	−1	1	1	−1	1	−1	1	1	1	1	1	−1	6048
2	−1	−1	−1	−1	1	−1	1	−1	−1	1	1	−1	−1	1	1	−1	1	−1	1	1	6628
3	−1	1	−1	1	−1	−1	1	1	−1	−1	1	1	−1	1	−1	1	1	1	1	1	7344
4	−1	−1	−1	−1	−1	−1	−1	−1	−1	−1	−1	−1	−1	−1	−1	−1	−1	−1	−1	−1	5466
5	1	−1	1	−1	1	1	1	1	1	−1	−1	−1	−1	1	−1	1	−1	−1	1	1	6932
6	1	−1	1	1	1	1	1	−1	−1	−1	−1	1	−1	1	−1	−1	1	1	−1	−1	5934
7	−1	−1	1	1	−1	−1	1	1	−1	1	−1	1	1	1	1	1	−1	−1	−1	−1	5722
8	−1	−1	1	1	−1	1	−1	1	1	1	1	1	−1	−1	−1	−1	1	−1	1	−1	7164
9	1	−1	−1	1	1	−1	−1	1	1	−1	1	−1	1	1	1	1	1	−1	−1	−1	6242
10	1	−1	−1	−1	−1	1	−1	1	−1	−1	1	1	−1	−1	1	1	−1	1	−1	1	6068
11	−1	−1	1	−1	1	−1	−1	1	1	−1	−1	1	1	−1	1	−1	1	1	1	1	7626
12	−1	1	1	−1	−1	1	1	−1	1	−1	1	1	1	1	1	−1	−1	−1	−1	1	6118
13	1	1	−1	1	−1	1	1	1	1	1	−1	−1	−1	−1	1	−1	1	−1	−1	1	6162
14	1	1	−1	−1	1	1	−1	1	−1	1	1	1	1	1	−1	−1	−1	−1	1	−1	6454
15	1	1	1	1	1	−1	−1	−1	−1	1	−1	1	−1	−1	1	1	−1	−1	1	1	5798
16	1	−1	−1	1	1	−1	1	−1	1	1	1	1	1	−1	−1	−1	−1	1	−1	1	6014
17	1	1	1	−1	−1	−1	−1	1	−1	1	−1	−1	1	1	−1	−1	1	1	−1	1	6682
18	−1	1	−1	1	1	1	1	1	−1	−1	−1	−1	1	−1	1	−1	−1	1	1	−1	5950
19	−1	1	−1	−1	1	1	−1	−1	1	1	−1	1	−1	1	1	1	1	1	−1	−1	5630
20	1	1	−1	−1	−1	−1	1	−1	1	−1	−1	1	1	−1	−1	1	1	−1	1	−1	6270
21	−1	1	1	−1	1	−1	1	1	1	1	1	−1	−1	−1	−1	1	−1	1	−1	−1	5342
22	1	1	1	1	−1	−1	−1	−1	1	−1	1	−1	−1	1	1	−1	−1	1	1	−1	6350
23	−1	1	1	1	1	1	−1	−1	−1	−1	1	−1	1	−1	−1	1	1	−1	−1	1	5408
24	−1	−1	−1	1	−1	1	−1	−1	1	1	−1	−1	1	1	−1	1	−1	1	1	1	6600

A: Glucose; B: Xylose; C: Maltose; D: Lactose; E: Sucrose; F: Mannose; G: Galactose; H: Yeast extract; J: Peptone; K: Raffinose; L: Beef extract; M: $(NH_4)_2SO_4$; N: KH_2PO_4; O: $MgSO_4$; P: $FeSO_4$; Q: $CaCl_2$; R: Na_2CO_3; S: $NaNO_2$; T: $NaNO_3$; U: Urea

Table 3 The factors included in the PBD and the importance ranking

Variable	Coefficient	t-test	$P>\|t\|$	Ranking	Variable	Coefficient	t-test	$P>\|t\|$	Ranking
Interception	6248	272.46	0		L	17.00	0.75	0.508	17
A	−1.84	−0.08	0.943	20	M	97.16	4.24	0.024	9
B	−122.34	−5.34	0.013	7	N	13.16	888.90	0.570	18
C	12.34	0.53	0.631	19	O	138.34	6.03	0.009	5
D	−24.00	−1.05	0.372	16	P	−52.84	−2.30	0.105	12
E	−84.84	−3.71	0.034	10	Q	−131.00	−5.71	0.011	6
F	−42.34	−1.85	0.161	15	R	180.16	7.84	0.004	4
G	−42.66	−1.87	0.158	14	S	51.00	2.23	0.112	13
H	226.00	9.85	0.002	2	T	349.00	15.21	0.001	1
J	122.84	5.36	0.013	7	U	200.34	8.73	0.003	3
K	−61.00	−2.66	0.076	11					

A: Glucose; B: Xylose; C: Maltose; D: Lactose; E: Sucrose; F: Mannose; G: Galactose; H: Yeast extract; J: Peptone; K: Raffinose; L: Beef extract; M: $(NH_4)_2SO_4$; N: KH_2PO_4; O: $MgSO_4$; P: $FeSO_4$; Q: $CaCl_2$; R: Na_2CO_3; S: $NaNO_2$; T: $NaNO_3$; U: Urea

It was postulated that the positive effects of $NaNO_3$, yeast extract, urea, peptone and $(NH_4)_2SO_4$ on xylanase production could be attributed to the requirement for nitrogen sources during fermentation, whereas beef extract and $NaNO_2$ had little effect. The optimal nitrogen source for xylanase production was diversified depending on the microorganisms tested. It was reported that *Aspergillus fischeri* Fxn 1, *Thermomyces lanuginosus* D2W3, *Aspergillus terreus* and *Aspergillus* sp. Zh-26 can use $(NH_4)_2SO_4$ and $NaNO_2$ as the best optimal nitrogen sources (Ghanem *et al*., 2000; Senthilkumar *et al*., 2005; Sonia *et al*., 2005; Li *et al*., 2007c), while *Paecilomyces themophila* J18 used yeast extract as the best nitrogen source (Yang *et al*., 2006). Based on our knowledge it was the first report showing that $NaNO_3$, yeast extract and urea have the most significant effect on xylanase production. For inorganic nitrogen sources like $NaNO_2$, it was reported that this could reduce sporulation by decreasing the conidiation level (Sanchez and Pilosof, 2000). Senthilkumar *et al*.(2005) found that $NaNO_2$ has a significant effect on xylanase production by *Aspergillus fischeri* Fxn 1, and that the reduced level of protease activity was directly responsible for the retention of xylanase activity in the optimized medium. In current study $NaNO_2$ had no significant effect on xylanase production. A fermentation medium lacking an exogenous supply of nitrogen yielded relatively high enzyme activity, indicating that the substrate already supplied the organism with an adequate amount of nitrogen.

Metabolizable sugars such as glucose, xylose, sucrose, lactose, maltose, raffinose, galactose and mannose demonstrated different influences on xylanase production for different microorganisms. Glucose, maltose and xylose acted as the induction materials for a few strains but showed repression for other strains (Gessesse and Mamo, 1999). In contrast, galactose acted as the repression material for most strains (Sun *et al*., 2007). Wheat bran systems were resistant to catabolic repression even at high concentrations of glucose (Gessesse and Mamo, 1999; de Souza *et al*., 2001). In the current experiment, only maltose had a positive but not statistically significant effect on xylanase production by *A. niger* XY-1. Other sugars such as xylose, sucrose and raffinose showed a significantly negative effect. A slight repression effect was observed with galactose, mannose and lactose, while, due to its wheat bran buffering capacity, glucose showed little effect on xylanase production.

Ca^{2+} was found to be the essential element for xylanase productivity and stability by *Aspergillus terreus* and *Chainia* sp. (Bandivadekar and Deshpande, 1994; Ghanem *et al*., 2000). However, in the current study a negative effect was detected on xylanase production. All these results indicated the need for a nutrients experiment for xylanase production using different strains.

Optimal nitrogen source for *A. niger* XY-1

From PBD, $NaNO_3$, yeast extract and urea were recognized as the suitable nitrogen sources for *A. niger* XY-1 xylanase production. To compare those three nitrogen sources, different concentrations of $NaNO_3$, yeast extract and urea were tested for xylanase production. The fermentation medium was composed of 10 g of wheat bran and different concentrations of the nitrogen sources. As indicated in Table 4, the maximal xylanase enzyme activity reached 9543, 9432 and 9531 U/g dry substrate when 40 g/L of $NaNO_3$, 300 g/L of yeast extract and 30 g/L of urea were added respectively. It was interesting to note that the maximal enzyme activity achieved by the three nitrogen sources was similar, while the concentration of yeast extract in the medium was much higher than those of $NaNO_3$ and urea. For future cost concerns, urea was chosen as the best nitrogen

Table 4 Effect of nitrogen concentration on xylanase production

Nitrogen source	Concentration (g/L)	Xylanase activity (U/g dry substrate)
$NaNO_3$	10	6512±110
	20	6934±92
	30	7746±118
	40	9543±59
	50	7464±68
Yeast extract	50	5502±95
	100	7109±62
	200	7642±130
	300	9432±200
	400	4579±64
Urea	10	6620±122
	20	7987±31
	30	9531±189
	40	7970±128
	50	4776±105

source. Li *et al*.(2007d) reported that *Alternaria mali* ND-16 used NH_4Cl and urea as nitrogen sources for xylanase production, but NH_4Cl acted as the primary nitrogen source and urea acted as the secondary nitrogen source.

Outcome of CCD experiment

CCD is a very useful tool for determining the optimal level of medium constituents and their interaction. Based on the results from PBD and nitrogen optimal experiments, urea, Na_2CO_3 and $MgSO_4$ were selected for the further evaluation of their effects on xylanase production by CCD. Table 1 shows the maximum and minimum levels of variables chosen in the CCD. For RSM analysis based on the CCD, 20 experiments were carried out and their response values with different combinations of three factors are demonstrated in Table 5.

Table 5 Experimental design and results of the CCD

Run No.	Coded variables			Response (U/g dry substrate)	
	x_1 (urea)	x_2 (Na_2CO_3)	x_3 ($MgSO_4$)	Actual	Predicted
1	−1	1	1	13110	12842.1
2	0	0	0	14158	13958.2
3	0	0	−1.68	13110	13328.6
4	−1	−1	1	13022	12759.0
5	−1	−1	−1	12948	12274.7
6	0	0	0	13922	13958.2
7	1	1	−1	10900	10753.4
8	−1	1	−1	12936	13198.8
9	0	0	0	13684	13958.2
10	0	0	0	14084	13958.2
11	0	−1.68	0	13048	13892.4
12	0	0	0	13860	13958.2
13	1	−1	−1	12774	12632.4
14	1	−1	1	14158	13485.7
15	0	0	0	14144	13958.2
16	−1.68	0	0	10988	11351.0
17	1.68	0	0	9690	9907.4
18	0	1.68	0	12648	12383.9
19	0	0	1.68	13384	13745.8
20	1	1	1	10502	10765.8

From Table 5 it can be seen that the centre points were set up at runs of 2, 6, 9, 10, 12, 15 and the maximum xylanase production was achieved at the centre points. The minimum xylanase production (9690 U/g dry substrate) was detected in run No. 17.

From multiple regression analysis, we found that the second-order polynomial equation can explain xylanase production regardless of the significance of coefficients:

$$Y = 13958 - 430x_1 - 449x_2 + 124x_3 - 1179x_1^2 - 291x_2^2 - 149x_3^2 - 701x_1x_2 + 92x_1x_3 - 210x_2x_3,$$

where Y is the response value. In current experiment, Y value is the level of xylanase production (U/g dry substrate). x_1, x_2 and x_3 represent the coded levels of urea, Na_2CO_3 and $MgSO_4$, respectively.

The statistical significance of the regression model was checked by F-test, and the analysis of variance for the response surface quadratic model is shown in Table 6. The model was highly significant, as manifested by the F-value and the probability value [$(P_{\text{total model}}>F)$=0.000]. The linear terms, quadratic terms and cross terms were all statistically significant based on the F-value. The data from the analysis of variance showed that the second-order model was well fitted to the experimental data. The goodness of fit was manifested by the determination coefficient (R^2). In this case the R^2 value of 92.2% indicated that the response model can explain 92.2% of the total variations. In general, a regression model having an R^2 value higher than 0.9 is considered to have a very high correlation (Haaland, 1989). The value of the adjusted determination coefficient ($R^2_{\text{Adj}} = 85.3\%$) was also high enough to indicate the significance of the model.

Table 6 Analysis of variance for the response of xylanase production

Source	*df*	$\sum x^2$ ($\times 10^6$)	F-value	$P>F$
Linear product	3	5.47	7.18	0.007
Quadratic product	3	20.43	26.78	0.000
Cross product	3	4.35	5.70	0.015
Total model	9	30.26	13.22	0.000
Total error	10	2.54		

The Student's t-distribution and the corresponding P-value, along with the parameter, are given in Table 7. The P-values are used as a tool to check the significance of each coefficient, which will help to explain the pattern of mutual interactions between the best variables. The parameter coefficient and the

corresponding *P*-value suggested that, among the independent variables, x_1 (urea) and x_2 (Na_2CO_3) have a significant effect on xylanase production.

The 3D response surfaces plots were employed to determine the interaction of the basal medium components and the optimum levels that have the most significant effect on xylanase production. Fig.1 illustrated the relationship between the response and the experimental data. Fig.1a describes the effects of Na_2CO_3 and $MgSO_4$ on xylanase production, when urea was fixed at its middle level (40 g/L). From Fig.1a it can be seen that the yield of xylanase production decreased gradually while the Na_2CO_3 concentration increased at a high level of $MgSO_4$. With the increase in the concentration of $MgSO_4$, the xylanase production steadily increased at a low concentration of Na_2CO_3. This observation was consistent with the results demonstrated in Table 7, which suggest a negative interaction of Na_2CO_3 and $MgSO_4$. Fig.1b demonstrates the effects of urea and Na_2CO_3 on xylanase production when the $MgSO_4$ concentration was fixed at its middle level (8 g/L). The xylanase production was predominantly affected by the urea concentration and the interaction between urea and Na_2CO_3 was significantly negative for xylanase production. Similarly Fig.1c shows the effects of urea and $MgSO_4$ on the xylanase production when the concentration of Na_2CO_3 was set at the middle level (6 g/L).

Table 7 Results of the regression analysis of the CCD

Source	Coefficient	*SE*	*t*-test	*P*>|*t*|
Interception	13958	205.7	67.868	0.000
x_1	−430	136.5	−3.147	0.010
x_2	−449	136.5	−3.288	0.008
x_3	124	136.5	0.910	0.384
x_1^2	−1179	133.1	−8.864	0.000
x_2^2	−291	133.1	−2.183	0.054
x_3^2	−149	133.1	−1.121	0.288
x_1x_2	−701	178.3	−3.930	0.003
x_1x_3	92	178.3	0.517	0.616
x_2x_3	−210	178.3	−1.179	0.266

In general it is necessary to compare the fitted model with the real system and confirm that the fitted model provides an adequate approximation. Unless the model shows an adequate fit, proceeding further with a fitted response surface may end in misleading results. The residuals from the least squares fit play an important role in judging the model adequacy. Fig.2 presents a plot of residuals versus the predicted response. The plot shows the residuals scattered randomly on the display, suggesting that the variance of the original observation was constant for all values of xylanase enzyme activity (*Y*). Because the plot of xylanase production was satisfactory, the conclusion can be drawn that the empirical model was adequate to describe the xylanase activity by response surface.

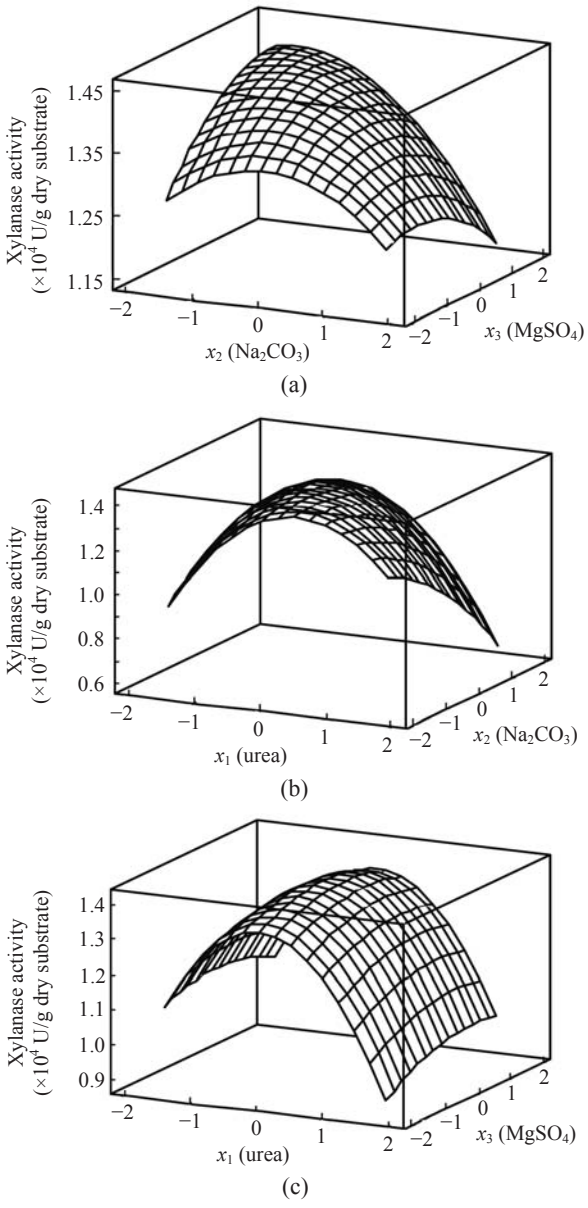

Fig.1 Response surface for xylanase production by *A. niger*. The interaction between Na_2CO_3 and $MgSO_4$ (a), urea and Na_2CO_3 (b) and urea and $MgSO_4$ (c)

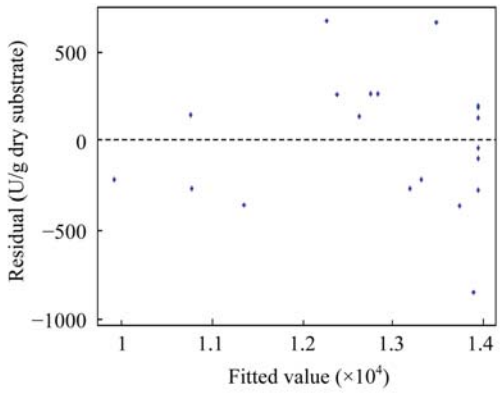

Fig.2 Plot of residuals versus fitted value of xylanase production

The prediction from response optimizer analysis gives a maximum level of xylanase as 14374.6 U/g dry substrate in the medium containing x_1 (urea)= 0.163 (41.63 g/L), x_2 (Na_2CO_3)=−1.68 (2.64 g/L), x_3 ($MgSO_4$)=1.338 (10.68 g/L). Verification of the predicted values was conducted by using optimal conditions in fermentation. The practical corresponding response was 14637 U/g dry substrate, which corroborated the validity and the effectiveness of the current model.

Time course curve

To evaluate the effect of the optimized medium on xylanase production, the time course of enzyme activities was conducted for 72 h. Maximum production was observed at 48 h (Fig.3). Further incubation beyond this time point did not show any improvement in any aspect of enzyme production.

In comparison with other xylanase production strains (Table 8), *A. niger* XY-1 showed a shortened fermentation time and a relatively high enzyme yield.

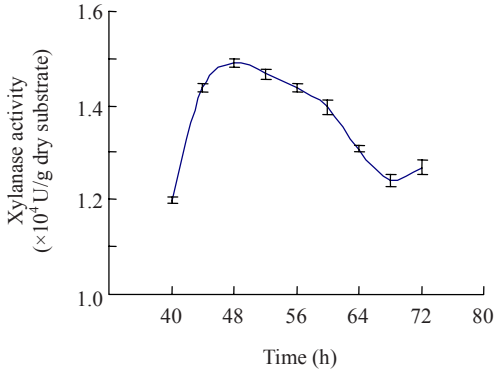

Fig.3 Time course of xylanase production by *A. niger* XY-1

Table 8 Comparison of xylanase production by different strains in SSF

Strains	Xylanase activity (U/g dry substrate)	Fermentation time (h)	References
Aspergillus XY-1	14637	48	This work
Aspergillus fischeri Fxn1	1024	72	Senthilkumar *et al*., 2005
Thermomyces lanuginosus D_2W_3	48000	144	Sonia *et al*., 2005
Bacillus sp.	720	72	Gessesse and Mamo, 1999
Aspergillus niger N218	6320	64	Wu *et al*., 2005

CONCLUSION

Xylanase production by *A. niger* XY-1 was optimized using response analysis, which was found to be an efficient tool. From PBD and nitrogen screening experiments, urea, Na_2CO_3 and $MgSO_4$ were shown to be critical components for xylanase production by *A. niger* XY-1. The CCD experiment estimated the optimum values of the critical components for maximum xylanase production. Under the following conditions: x_1 (urea)=0.163 (41.63 g/L), x_2 (Na_2CO_3)= −1.68 (2.64 g/L), x_3 ($MgSO_4$)=1.338 (10.68 g/L) and with a substrate of wheat bran, the predicted xylanase activity was 14374.6 U/g dry substrate. In the current experiment, xylanase production reached 14637 U/g dry substrate after 48 h of fermentation in the shake flask experiment, which accorded with the predicted value. In summary, the facts that no additional expensive medium was required and that there was a shortened fermentation time for higher xylanase production by *A. niger* XY-1 show the potential for industrial utilization.

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