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Xylanase Production in Solid State Fermentation by *Aspergillus niger* Mutant using Statistical Experimental Designs

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Abstract

The production of xylanase from *Aspergillus niger* mutant in SSF was optimized by using statistical experimental designs. An inoculum size of 5×10^5 spores/g, initial moisture content of 65 %, cultivation time of 5 days and 10 times concentration of basal medium containing 50 times concentration of CSL were optimum for xylanase production. Under the optimized conditions, the activity and productivity of xylanase obtained after 5 days of fermentation were 5,071 IU/gram of rice straw and 14,790 IU/l·h, respectively.

Introduction

A variety of microorganism, including bacteria, yeast and filamentous fungi, has been reported to produce xylanase[1]. In recent years, interest has markedly increased in xylanases mainly due to their usage in the pulp and paper industry. Solid state fermentation (SSF) offers advantages over submerged fermentation (SmF)[2]. The cost of enzyme is one of the main factors determining the economics of a process. Reducing the costs of enzyme production by optimizing the fermentation medium and process is the basic research for industrial application. Recently, the statistical designs for optimization have been successfully employed in enzyme production[3]. These statistical methods proved to be powerful and useful tools. The aim of this work is to evaluate the xylanase production of *Aspergillus niger* KK2 mutant grown on rice straw as a substrate in SSF. The effect and optimization of variables on the production of enzyme was studied by combining different statistical experimental designs.

Materials and methods

A fungal strain *Aspergillus niger* KK2 mutant was used. The basal medium contained in grams per 100 g: $CoSO_4 \cdot 7H_2O$, 0.01; $CuSO_4 \cdot 5H_2O$, 0.05; KH_2PO_4, 0.5; corn steep liquor (CSL), 1.0; industrial yeast extract (IYE), 0.05. The pH of basal medium was adjusted to 7.0. The concentration of basal medium was poured into each 250 ml Erlenmeyer flask containing 5 g rice straw according to experimental designs. The media was then autoclaved for 30 min at 121 ℃. After cooling, the flasks were inoculated with the inoculum level and were incubated at 28 ℃.

Results and discussions

Four variables were chosen (see Table 1) and the effect of each variable was investigated. The experimental design and the results of the 2^{4-1} fractional factorial design are shown in Table 2. The xylanase activity varied markedly with the conditions tested, in a range of 168−1177 IU/g. When ANOVA analysis reflects the significance of model with a confidence to greater than 99 % ($P < 0.01$), the F−value and P−value were 42.01 and 0.0097, respectively (Table 3). Consequently, analysis of ANOVA showed that X_2 and X_4 proved to be the two most important variables. To determine the optimal conditions of X_2 and X_4, design of experiment and results are presented in Table 4. As shown in Table 5, the tested model is statistically significant at the 1% level of significance. The coefficient of determination (R^2) of the model was calculated to be 0.97. The response equation obtained is as follows:

$$Y = 4900.3 + 1460.9\, x_2 + 513.1\, x_4 - 1598.0\, x_2^2 - 598.2\, x_4^2 + 510.0\, x_2 x_4$$

According to these results, optimal cultivation time and concentration of basal medium for xylanase production were calculated to be 5.1 days and 60.0 times, respectively. The maximum value of enzyme activity predicted from model was 5,484 IU/g. With 50 times concentration of CSL containing 10 times concentration of basal medium, the maximum activity (5,034 IU/g) was obtained among all the concentrations of CSL used (Fig. 2). Consequently, an inoculum size of 5×10^5 spores/gram, initial moisture content of 65 %, cultivation time of 5 days and 10 times concentration of basal medium containing 50 times concentration of CSL were optimum for xylanase production in SSF. The time course profile of xylanase activity under the optimized condition is shown in Fig. 3. The highest xylanase activity of 5,071 IU/g was obtained after 5 days of fermentation. The productivity of xylanase by *A. niger* KK2 mutant in SSF was 14,790 IU/l·h. In conclusion, *A. niger* KK2 mutant is a potential microorganism for the production of cellulase and hemicellulase by using SSF.

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Table 1. Range of variables at different levels for the fractional factorial design.

Independent variables X_i	−1	0	+1
X_1 Initial moisture content (%)	50	65	80
X_2 Cultivation time (day)	5	7	9
X_3 Inoculum size (spores/gram)	10^4	5×10^5	10^6
X_4 Concentration of basal medium (magnitude)	×1	×5	×9

Table 2. Experimental design and results of 2^{4-1} fractional factorial design.

Runs	Code levels				Activity
	x_1	x_2	x_3	x_4	(IU/g)
1	−	−	−	−	557
2	−	−	+	+	1177
3	−	+	−	+	213
4	+	−	−	+	1049
5	−	+	+	−	168
6	+	−	+	−	487
7	+	+	−	−	294
8	+	+	+	+	736
9	0	0	0	0	457
10	0	0	0	0	411
11	0	0	0	0	487
12	0	0	0	0	377

Table 3. Results of the regression analysis of the of 2^{4-1} fractional factorial design.

Factors	Mean square	F−value	P−value
x_1	25425	10.72	0.049
x_2	431985	182.22	0.0028
x_3	25878	10.92	0.0478
x_4	348195	146.88	0.0033
x_1*x_2	89676	37.83	0.0117
x_1*x_3	60378	25.47	0.0181
x_1*x_4	14365	6.06	0.0918
x_2*x_3	14365	6.06	0.0918
x_2*x_4	60378	25.47	0.0181
x_3*x_4	89676	37.83	0.0117
Model	9959	42.01	0.0097

Table 4. Experimental design and results of the 2^2 full factorial central composite design.

Runs	x_2 (day)	x_4 (magnitude)	Activity (IU/g)
1	2 (−)	×35 (−)	1193
2	6 (+)	×35 (−)	3390
3	2 (−)	×65 (+)	671
4	6 (+)	×65 (+)	4908
5	1.2 (−1.414)	×50 (0)	12
6	6.8 (+1.414)	×50 (0)	3726
7	4 (0)	×28.79 (−1.414)	2769
8	4 (0)	×71.21 (+1.414)	4967
9	4 (0)	×50 (0)	4930
10	4 (0)	×50 (0)	4930
11	4 (0)	×50 (0)	4833
12	4 (0)	×50 (0)	4908

Table 5. Analysis of variance (ANOVA) for the full regression.

Source	Sum of squares	Degrees of freedom	Mean square	F-value	P-value
Model	37071482	5	7414296	46.69	0.0001
Error	952881	6	952881		
Total	38024362	11			

$R^2=0.97$

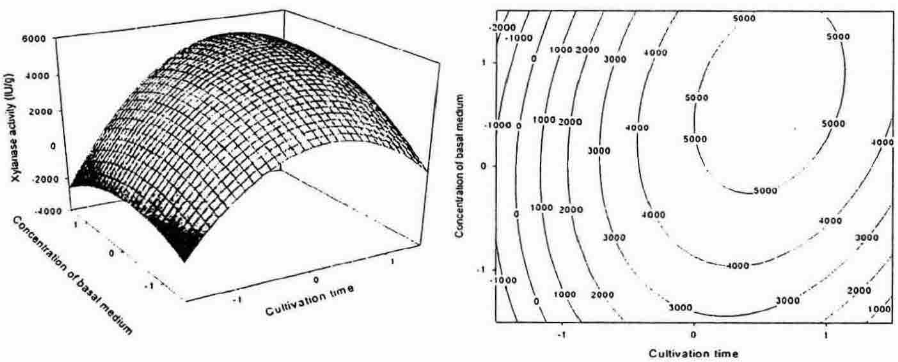

Fig. 1. 3-D response surface plot of the central composite design experiment and contour plot of the calculated response surface.

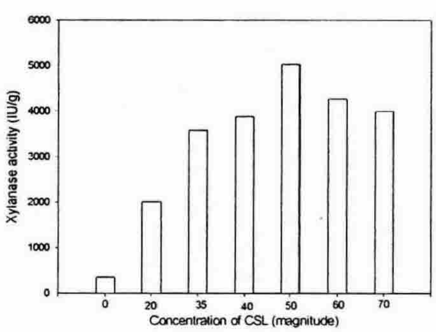

Fig. 2. Effect of concentration of CSL on xylanase production under the optimized conditions in SSF. Concentration of basal medium except CSL was fixed at 10 times.

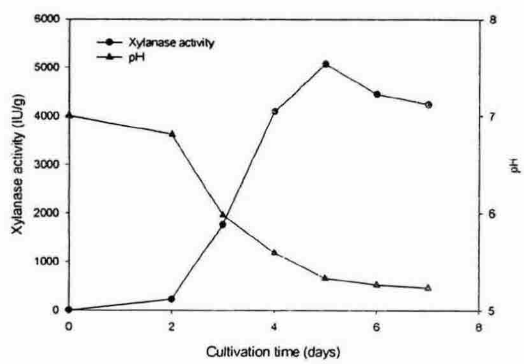

Fig. 3. Time course profile of xylanase production by *A. niger* KK2 mutant in SSF under the optimized condition.