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Experimental design to enhance xylanase production from *Paenibacillus curdlanolyticus* B6 utilizing corn cob as carbon source

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Abstract

Possibility of xylanase production from *Paenibacillus curdlanolyticus* B6 utilizing corn cob as carbon source was studied. The results indicated that xylanase activity was found to be 2.66 U/mg. Consequently, fractional factorial design was employed to study the effects of initial pH, cultivation temperature, carbon source concentration and nitrogen source concentration on xylanase production. The results suggested that cultivation temperature and corn cob concentration were significantly affected to the yield of xylanase ($p < 0.05$). The parameters for enhanced xylanase production up to 450% (14.63 U/mg) were found to be initial pH of 7.0, cultivation temperature of 40 °C, corn cob and $NaNO_3$ concentrations of 1.0 % (w/v). Therefore, it is possible to use corn cob as carbon source for the production of xylanase from *P. curdlanolyticus* B6.

Keywords: xylanase production, corn cob, experimental design, *Paenibacillus curdlanolyticus*

1. Introduction

As the main waste and residue in corn production, corn cob is produced in large amounts all over the world. It can be utilized as animal feed, fertilizer in soil, or burned as a fuel. Corn cob contains 40–45% cellulose, 30–35% hemicellulose and 10–20% lignin (1). Xylan, the major constituent of hemicellulose, consists of a β-1,4-linked D-xylose backbone branched with other pentoses, hexoses and uronic acids. Xylanases and complementary debranching enzymes, produced by a variety of micro-organisms, including bacteria, yeasts and filamentous fungi, bring about the hydrolysis of hemicellulose (2-4). From a commercial point of view, xylanases have attracted substantial research interest because of their potential industrial applications, such as hydrolysis of lingo-cellulose to fermentable sugars for ethanol production, bread making and clarification of beer and juices (5-7). Xylanases are also crucial in improving the nutritional quality

of animal feed (8). Alkaliphilic xylanases can be used as bleaching agents in the paper and pulp bleaching industry (9). Among xylanase-producing microorganisms, only few reports on xylanase production from *Paenibacillus* sp. have been investigated (10-13). A facultatively anaerobic bacterium *P. curdlanolyticus* B6, isolated from an anaerobic digester fed with pineapple waste, primarily produced xylanase, when it was grown on xylan as a sole carbon source under aerobic condition (14). Since this bacterium could grow on mineral media supplemented with a carbon source, commercial production of xylanase was relatively economical. The aim of this study was to evaluate the xylanase production of *P. curdlanolyticus* B6 grown on mineral media supplemented with corn cob as a sole carbon source. The effects of variables (initial pH, cultivation temperature, concentrations of carbon and nitrogen sources) on the production of enzyme were examined by fractional factorial design.

2. Materials and Methods

2.1 Bacterial strain and culture medium

P. curdlanolyticus B6 was the facultatively anaerobic bacterium, isolated from an anaerobic digester fed with pineapple wastes and was identified by 16S rRNA gene analysis (14). The bacterium was grown on Berg's mineral salt medium at pH 7.0 (15) containing 0.2% $NaNO_3$, 0.05% K_2HPO_4, 0.02% $MgSO_4 \bullet 7H_2O$, 0.002% $MnSO_4 \bullet H_2O$, 0.002% $FeSO_4 \bullet 7H_2O$, 0.002% $CaCl_2 \bullet 2H_2O$, and supplemented with 0.5% commercial oat spelt xylan (Sigma-Aldrich Chemical Inc.). The culture was incubated in a rotary incubator at 200 rpm and 37 °C.

2.2 Production of xylanase in submerged culture utilizing corn cob as carbon source

P. curdlanolyticus B6 was grown on Berg's mineral salt medium containing 1% glucose up to the late exponential phase, and was washed 4 times with fresh sterilized mineral salt medium without any carbon sources, then resuspended in the same fresh medium. Aliquots of this suspension were rapidly mixed with media containing 1% corn cob (Yongsuwat Agritrade Ltd., Thailand), consisted of 32.23% hemicellulose. The culture supernatant was separated from cells by centrifugation (10,000 rpm, 10 min, 4 °C) at a time intervals up to 84 h for assays of xylanase activity, protein content and reducing sugar concentration.

2.3 Experimental design

The influence of the variables (initial pH, cultivation temperature, concentrations of corn cob and $NaNO_3$) on the xylanase production was studied employing a 2^{4-1} fractional factorial design. The levels of the examined variables were shown in Table 1.

2.4 Analytical methods

Xylanase activity was measured by determining the amount of reducing sugar released from oat spelt xylan (Sigma-Aldrich Chemical Inc., USA). The reaction mixture consisted of 0.5 ml of 1% oat spelt xylan in 100 mM Tris-HCl buffer (pH 7.0) and 0.1 ml enzyme (16). After incubation for 15 min at 50 °C, reducing sugar was determined by using the Somogyi–Nelson method with

xylose as standard (17). One unit of the xylanase activity was defined as the amount of enzyme that liberated 1 μmol of reducing sugar in 1 min under the assay conditions. Furthermore, reducing sugar content in the culture supernatant was determined by using the Somogyi–Nelson method with glucose as standard (17), whereas, protein concentration was measured according to Bradford method, using bovine serum albumin as standard (18).

3. Results and discussion

3.1 Production of xylanase from *P. curdlanolyticus* B6 utilizing corn cob as carbon source

At various time intervals during incubation of *P. curdlanolyticus* B6 in the medium containing corn cob as carbon source (Figure 1), protein concentration was sharply increased after 24 h of incubation and reached to 109.08 μg/ml at 84 h of incubation. Furthermore, xylanase activity was steadily enhanced to 0.29 U/ml (2.66 U/mg) at 84 h of incubation. However, reducing sugar content in the culture medium was gradually reduced from the beginning until 24 h of incubation and then nearly stable. The results indicated that the bacterium was initially grown on the reducing sugar from corn cob and steadily produced xylanase to hydrolyze xylan in corn cob to constantly release reducing sugar the culture medium. The results were analogous to previous study using corn hull

Table 1. Range of variables at different levels for the 2^{4-1} fractional factorial design.

Independent variables, X_i	Levels		
	-1	0	+1
X_1; Corn cob concentration (%)	0.50	0.75	1.00
X_2; $NaNO_3$ concentration (%)	0.2	0.6	1.0
X_3; Initial pH	6.0	6.5	7.0
X_4; Cultivation temperature (°C)	30	35	40

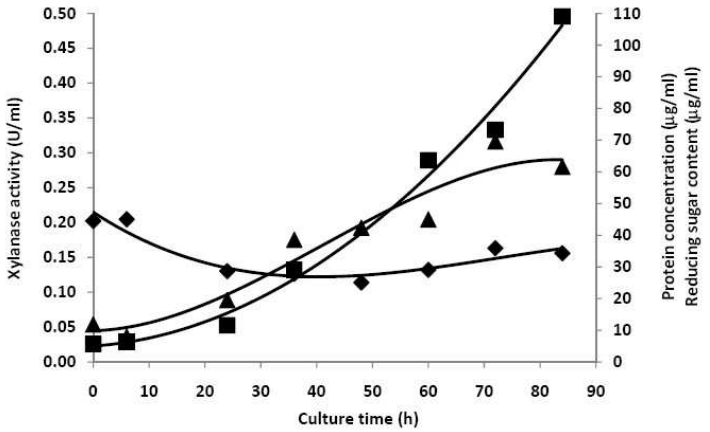

Figure 1. Production of xylanase (▲), protein concentration (■) and reducing sugar content (♦) by *P. curdlanolyticus* B6 during cultivated on mineral medium supplemented with corn cob as a sole carbon source.

as a carbon source, xylanase activity from *P. curdlanolyticus* B6 was found to be 2.45 U/mg (19).

3.2 Influence of culture conditions on the xylanase production from *P. curdlanolyticus* B6

To study the effects of initial pH, cultivation temperature, concentrations of carbon source and nitrogen source on xylanase production, experiments were carried out using statistical experimental design to verify the conditions in which the enzyme was highly produced. The ranges of the examined parameters were displayed in Table 1. The experimental treatment and specific activity of enzyme (U/mg) were showed in Table 2. As shown in Table 3, the results were clearly exhibited that the yield of enzyme was significantly affected by cultivation temperature and corn cob concentration (p<0.05). In addition, interaction of corn cob concentration and initial pH was also significantly influenced (p<0.05). The parameters for enhanced xylanase production up to 450% were found to be initial pH of 7.0, cultivation temperature of 40 °C, corn cob and $NaNO_3$ concentration of 1.0% (w/v). The improved

Table 2. Experimental treatments of the 2^{4-1} fractional factorial design and the values of xylanase production by *P. curdlanolyticus* B6.

Treatment	Coded levels				Response
	X_1	X_2	X_3	X_4	Xylanase activity (U/mg)
1	-1	-1	-1	-1	2.44
2	+1	-1	-1	+1	14.39
3	-1	+1	-1	+1	5.37
4	+1	+1	-1	-1	7.07
5	-1	-1	+1	+1	14.15
6	+1	-1	+1	-1	5.85
7	-1	+1	+1	-1	6.59
8	+1	+1	+1	+1	14.63
9	0	0	0	0	7.80
10	0	0	0	0	5.85
11	0	0	0	0	10.24
12	0	0	0	0	8.05

Table 3. Analysis of variance for the response of xylanase production.

Source	Degree of freedom	Mean of squares	F – value	p – value
Model	7	23.24	7.20	0.0141
X_1	1	22.41	6.94	0.0388
X_2	1	1.26	0.39	0.5557
X_3	1	17.85	5.53	0.0569
X_4	1	88.38	27.39	0.0020
X_1X_2	1	4.64	1.44	0.2759
X_1X_3	1	24.19	7.49	0.0338
X_1X_4	1	3.93	1.22	0.3119

xylanase activity up to 450% was found to be 14.63 U/mg. As shown in Table 2, the model was statistically significant (p<0.05). Thus, the mathematical model representing the specific activity of enzyme in the range studied can be expressed by equation 1.

$$Y = 1.67 X_1 + 3.32 X_4 - 1.74 X_1X_3 + 8.81 \ldots.[1]$$

Where Y was the value predicted for the specific activity of enzyme (U/mg), X_1, X_4 and X_3 the coded value for was the coded value for corn cob concentration, cultivation temperature and initial pH, respectively.

The observed actual xylanase activity against predicted enzyme activity from the equation 1 are shown in figure 2 which suggested that the two values were in agreement. Figure 3 displayed a 3D plot and its corresponding contour plots showing the effects of X_1 (corn cob content) and X_3 (initial pH) on xylanase production by *P. curdlanolyticus* B6, while others were fixed at their middle level. As shown in Figure 3,

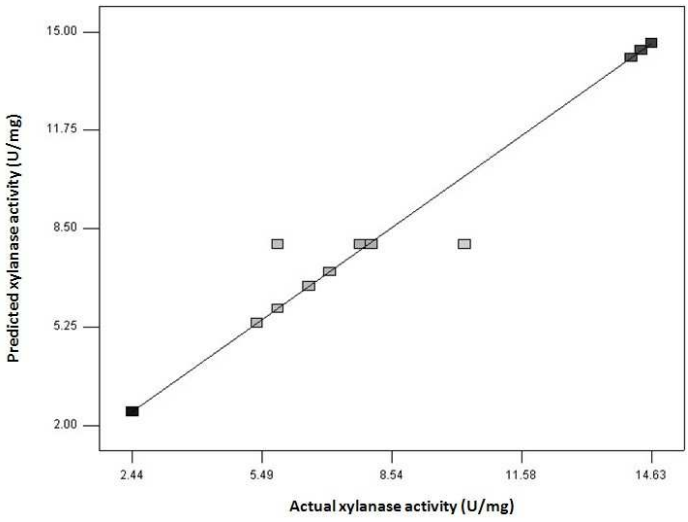

Figure 2. Predicted and observed xylanase activity of experimental treatments in the 2^{4-1} fractional factorial design.

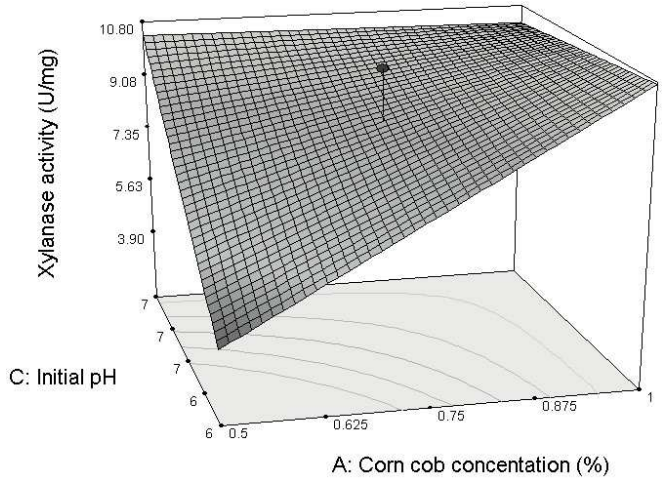

Figure 3. Effect of initial pH and corn cob concentration on the xylanase production by *P. curdlanolyticus* B6 with constant $NaNO_3$ concentration (0.6%) and cultivation temperature (35 °C).

higher level of corn cob favored the xylanase production. As indicated in previous report, this bacterium was able to produce multiple xylanases when it was grown on mineral medium supplemented with corn hull as carbon source (18). Higher amount of added corn cob in the medium could be resulting in more xylooligo-saccharides to greatly induce the bacterium to regularly produce a large amount of xylanase. Therefore, the bacterium *P. curdlanolyticus* B6, grown on an economical medium containing mineral salts and agricultural waste, proved to be a promising microorganism for the production of commercial xylanase.

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