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Full Length Research Paper

Statistical optimization of xylanase production by *Aspergillus niger* AN-13 under submerged fermentation using response surface methodology

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Response surface methodology (RSM) was performed to evaluate the effects of cultivation time, pH and substrate concentration on production of xylanase by *Aspergillus niger* AN-13. Agricultural residue wheat bran was used as main substrate under submerged fermentation. Xylanase production was optimized by Box-Behnken design (BBD). Statistical analysis of results showed that, the linear and quadric terms of these three variables had significant effects, and evident interactions existing between pH and substrate concentration were found to contribute to the response at a significant level. Furthermore, Box-Behnken design (BBD) used for the analysis of treatment combinations gave a second-order polynomial regression model, which was in good agreement with experimental results, with R^2=0.9959 (P<0.05). By response surface methodology and canonical analysis, the optimal fermentation parameters for enhanced xylanase production were obtained. Under these conditions, namely cultivation time of 53.3 h, pH of 7.92 and wheat bran concentration of 54.2 g·L^{-1}, the model predicted a xylanase activity of 125.14 U·mL^{-1}. Verification of the optimization showed that xylanase production of 127.12 U·mL^{-1} was observed under the optimal condition, which had a marked increase compared with a xylanase activity of 4.80 U·mL^{-1} in experiments according to Box-Behnken design.

Key words: *Aspergillus niger* AN-13, xylanase, statistical optimization, response surface methodology, Box-Behnken design.

INTRODUCTION

Xylanase (endo-1,4-β-xylanase) and β-xylosidase (β-D-xyloside xylohydrolase) are the main constituents of the xylanolytic enzyme system, converting xylan (the main hemicellulosic polysaccharide) into a more readily fermentable form (Ghosh et al., 1993). Xylanases are useful in several industrial applications. They are extensively used in pre-treatment of forage crops and other lignocellulosic biomass, added to swine and poultry cereal-based diets to improve nutrient utilization, flour modification for bakery products, and saccharification of agricultural, industrial and municipal wastes (Sá-Pereira et al., 2002). Moreover, it is reported that xylanases have been widely used for clarifying fruit juices and wine (Hang and Woodams 1997), food processing in combination with cellulases (Biely, 1985), and improving the nutritional properties of agricultural silage and grain feed (Kuhad et al., 1993).

Xylanases are produced by numerous microorganisms among which the fungi are the most potent producers (Pham et al., 1997). *Aspergillus niger* has been used for the production of enzymes such as pectinases (Castilhoa et al., 2000; Debing et al., 2006) and invertase (Montiel-González et al., 2004) by solid state fermentation, feruloyl esterases (Benoit et al., 2006), cellobiase (Xueliang and Liming, 2004), cellulases and hemicellulases (Kang et al., 2004), xylanase (Qi-peng et al., 2005) and so on. There are advantages of application of *A. niger* as suitable strain: not only the performance of the organism is invariable but also it can produce enzymes more steadily.

Large amounts of agro-industrial residues are generated every year from diverse economic activities. These

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Table 1. Coded values of variables used in Box-Behnken experimental design.

Independent variables	Level		
	-1	0	1
Time (X_1) (h)	24	48	72
pH (X_2)	7	8	9
Wheat bran (X_3) (g·L^{-1})	10	50	90

residues represent one of the most energy-rich resources available on the planet and when not properly discharged or used, add to the environmental pollution (Francis et al., 2003). On the other hand, the cost of an enzyme is one of the main factors determining the economic of a process. Reducing the costs of enzyme production by optimization the fermentation medium and cultivation condition is the goal of basic research for industrial application. Most of the reports concerning xylanase are dealt with the purification and characterization of these enzymes, with very few studies regarding optimizing their production (Bocchini et al., 2002). But in order to develop good fermentation, some parameters should be optimized according to the limits of the process, such as pH, substrate concentration, cultivation time, and so on.

The classical method of 'one-variable-at-a-time' bioprocess design may be effective in some situations, but fails to consider the combined effects of all involved factors (Silva and Roberto, 2001). Factorial design optimization and response surface methodology fulfill this requirement. RSM is a collection of mathematical and statistical techniques widely used to determine the effects of several variables and to optimize different biotechnological process (Rao et al., 2000).

The collective role diversified fermentation parameters play in the yield of xylanase by *A. niger* AN-13 have not been reported yet. The objective of the present work was to apply statistical methods to optimize culture conditions for higher production of xylanase under submerged fermentation. In this paper, the optimum parameters including cultivation time, pH and wheat bran concentration in the medium were obtained by response surface methodology.

MATERIALS AND METHODS

Microorganism and cultivation conditions

The *A. niger* AN-13 strain was isolated from soil and was maintained at 4ºC on potato dextrose agar (PDA). Spores suspensions were made from six-day-old cultures that had been grown on PDA slopes at 30ºC. Sterile distilled water was aseptically added to each slope and a suspension of the spores made by lightly brushing the mycelium with a sterile wire loop. The suspension, was diluted with sterile distilled water to give a final spore count of 10^7 spores·mL^{-1}.

The medium used for xylanase production was composed of (g·L^{-1}): NH_4Cl 9; KH_2PO_4 1; $NaNO_3$ 1; $MgSO_4$•$7H_2O$ 1; $CaCl_2$•$2H_2O$ 0.3; and yeast extract 1. The medium pH, also with varying concentrations of wheat bran and cultivation time, was adjusted according to the experimental design. The microorganism was cultured in 75 mL of medium in 250 mL Erlenmeyer flasks on a rotary shaker (150 rev·min^{-1}). Shake flasks were then maintained at 35ºC. At the end of fermentation, the mycelium was separated from the enzyme-containing broth by centrifugation at 10000×*g* for 15 min to obtain the crude enzyme preparation.

Box-Behnken design

A Box-Behnken (Box at al., 1960) factorial design with three factors and three levels, including three replicates at the centre point, was used in order to generate 15 treatment combinations, with cultivation time, pH and substrate concentration as variables. According to the Box-Behnken design, the total number of experimental combinations is 2^k+2k+n_0, where k is the number of independent variables and n_0 is the number of repetitions of the experiments at the centre point. In this design, both k and n_0 are equal to three. For statistical calculation, the experimental variable X_i has been coded as x_i according to the following transformation equation:

$$x_i = \frac{X_i - X_0}{\delta X} \quad (1)$$

Where x_i is the dimensionless coded value of the variable X_i, X_0 the value of X_i at the centre point and δX the step change.

Table 1 and Table 2 show the actual levels corresponding to the coded settings, and the experimental design, respectively. This design is represented by a second-order polynomial regression model as follows:

$$Y = \beta_0 + \sum_{i=1}^{3} \beta_i X_i + \sum_{i=1}^{3} \beta_{ii} X_i^2 + \sum \sum_{\substack{i=1 \\ i<j}}^{3} \beta_{ij} X_i X_j \quad (2)$$

Where Y is the predicted response variable; β_0, β_i, β_{ii} and β_{ij} are constant regression coefficients of the model, X_i, X_j and (i =1, 3; j = 1, 3, i ≠ j) represent the independent variables in the form of coded values. The accuracy and general ability of the above polynomial model could be evaluated by the coefficient of determination R^2.

Data analysis

Data from Box-Behnken design for the optimization of xylanase production were subjected to a second-order multiple regression analysis using the least squares regression methodology to obtain the parameter estimates of the mathematical model. The regression analysis and analysis of variance (ANOVA) were carried out using the RSREG procedure (Zeng and Biebl, 2002; Yin et al., 2006) to fit second order polynomial equations for all response variables. Response surface was made by the fitted quadratic polynomial equation obtained from RSREG analysis, holding independent variables with two parameters at a constant value, and changing the other two variables.

Analytical method

The xylanase activity was determined by measuring the release of reducing sugars from oat spelt xylan (1%, w/v) using the dinitrosalicylic acid method (Miller 1959; Yin et al., 2006, 2007). Reaction

Table 2. Box-Behnken design matrix with experimental and predicted values of xylanase production by *Aspergillus niger* AN-13.

Trial number	Variables			Xylanase (U·mL^{-1})	
	X_1 (Time)	X_2 (pH)	X_3 (Wheat bran)	Experimental	Predicted
1	-1	-1	0	17.94	16.95
2	-1	1	0	5.22	2.19
3	1	-1	0	52.31	56.25
4	1	1	0	41.42	41.49
5	0	-1	-1	26.50	23.59
6	0	-1	1	51.85	51.77
7	0	1	-1	20.50	20.55
8	0	1	1	22.40	25.29
9	-1	0	-1	4.80	6.42
10	1	0	-1	44.52	45.72
11	-1	0	1	20.50	22.88
12	1	0	1	67.39	62.18
13	0	0	0	119.50	122.22
14	0	0	0	126.95	122.22
15	0	0	0	120.22	122.22

mixture containing 1 mL of a solution of 1% oat spelt xylan in citrate buffer (50 mM, pH 5.0) and 1 mL of the diluted crude enzyme, was incubated for 30 min at 50ºC. One unite of xylanase was defined as the amount of enzyme required to released 1 µmol of xylose from xylan in 1 min under the assay condition.

RESULTS AND DISCUSSION

Regression model of response from Box-Behnken design and RSM strategies

In this study, wheat bran was used as main substrate under submerged fermentation. For one thing, the use of purified xylan enhanced the cost of enzyme production and was a major limitation to the economic feasible of bioconversion and utilization of lignocellulosic materials. For another, agricultural residue was not only inexpensive, but also it was abundant and easily available, supplying the microorganism better nutrition. In order to obtain optimum levels of xylanase by *A. niger* AN-13, optimization of cultivation conditions variables that had a significant impact on xylanase production was necessary. Bocchini et al. (2002) have reported that cultivation time, xylan concentration and their interactions have significant effects on xylanase production from *Bacillus circulans* D1. Heck et al. (2006) and Techapun et al. (2002) both have found that optimization of initial pH and temperature can improve xylanase production enormously.

Table 1 shows the maximum and minimum levels of variables chosen for trials in Box-Behnken design. For response surface methodology (RSM) based on the Box-Wilson, which was used to optimize cultivation conditions for xylanase production, 15 experimental runs with different combinations of three factors and three levels were carried out (Table 2). The variables used for the factorial analysis were cultivation time, pH and wheat bran, named X_1, X_2, X_3 in this design, respectively. The effects of the three independent variables on xylanase production and the experimental response along with the predicted response obtained from the regression equation for each run are shown in Table 2. It can be seen from Table 2, there was a considerable variation in the xylanase production depending on the three chosen variables. The maximum xylanase production (126.95 U·mL^{-1}) was achieved in run number 14, while the minimum xylanase production (4.80 U·mL^{-1}) was observed in run number 9. The former was much higher than the latter, which adequately indicated that choosing appropriate cultivation conditions could evidently enhance the yield of xylanase. In order to estimate the error, the centre point in the design was repeatedly carried out for three times.

By applying multiple regression analysis on the experimental data, the following second order polynomial equation was found to explain the xylanase production by only considering the significant terms and was shown as below:

$$Y = 122.22 + 19.65X_1 - 7.83X_2 + 8.23X_3 - 44.50X_1^2 - 48.50X_2^2 - 43.42X_3^2 - 5.86X_2X_3 \quad (3)$$

Where Y is the predicted response, X_1, X_2 and X_3 are coded values of cultivation time, pH and wheat bran, respectively.

The independent variables were fitted to the second-order model equation and examined for the goodness of fit. Several indicators were used to evaluate the adequacy of the fitted model and the results are shown in Table 3. The determination coefficient R^2 value, correlation coefficient R value, coefficients of variation (CV) and

Table 3. Analysis of variance (ANOVA) for the quadratic polynomial model of xylanase production[a].

Source	Degree of freedom	Sum of squares	Mean square	*F*-value	*P*>*F*
Linear	3	4065.74	-	68.12	0.0002
Quadratic	3	19907	-	333.55	<0.0001
Cross product	3	151.16	-	2.58	0.1708
Total model	9	24124	-	134.73	<0.0001
Total error	5	99.47	19.89	-	-

[a]Coefficient of variation (CV) =9.02; coefficient determination (R^2)=0.9959; correlation coefficient (R)=0.9979.

Table 4. Regression coefficients and their significances for xylanase production from the results of Box-Behnken experimental design.

Model term	Degree of freedom	Estimate	Standard Error	*t* value	P
Intercept	1	122.22	2.58	47.46	<0.0001
X_1	1	19.65	1.58	12.46	<0.0001
X_2	1	-7.38	1.58	-4.68	0.0054
X_3	1	8.23	1.58	5.22	0.0034
X_1^2	1	-44.50	2.32	-19.17	<0.0001
X_1X_2	1	0.46	2.23	0.21	0.8456
X_2^2	1	-48.50	2.32	-20.89	<0.0001
X_1X_3	1	1.79	2.23	0.80	0.4580
X_2X_3	1	-5.86	2.23	-2.63	0.0466
X_3^2	1	-43.42	2.32	-18.70	<0.0001

*Significant at 5% level (P<0.05).

model significance (*F*-value) were used to judge the adequacy of the model. R^2, or coefficient of determination, is the proportion of variation in the response attributed to the model rather than to random error (Henika, 1972). Joglekar and May (1987) have suggested for a good fit of a model, R^2 should be at least 80%. The determination coefficient (R^2) implies that the sample variation of 99.59% for xylanase production is attributed to the independent variables, and only about 0.4% of the total variation can not be explained by the model. The closer value of *R* (correlation coefficient) to 1, the better is the correlation between the experimental and predicted values. Here the value of *R* (0.9979) for Eq. (3) being close to 1 indicated a close agreement between the experimental results and the theoretical values predicted by the model equation. The coefficient of variation (CV) is the ratio of the standard error of estimate to the mean value of the observed response, expressed as a percentage. A model can be considered reasonably reproducible if the CV is not greater than 10% (Joglekar and May, 1987). Usually, the higher the value of CV, the lower is the reliability of experiment. Here, a lower value of CV (9.02) indicated a greater reliability of the experiments performed. The model significance (*F*-value) indicates the level of confidence that the selected model can not be due to experimental error (Henika, 1972). Linear and quadratic terms were significant at the 1% level. Therefore, the quadratic model was selected in this optimization study.

The Student *t*-distribution and the corresponding *P*-value, along with the parameter estimate, are given in Table 4. The *P*-values are used as a tool to check the significance of each of the coefficients which, in turn, are necessary to understand the pattern of the mutual interactions between the best variables. The parameter estimates and the corresponding *P*-values showed that among the independent variables, X_1 (cultivation time), X_2 (pH) and X_3 (wheat bran) had a significant effect on xylanase production. Positive coefficients for X_1 and X_3 indicated a linear effect to increase xylanase production, while negative coefficient of X_3 (wheat bran) revealed the opposite effect. It was included that X_1 (cultivation time) was the key factor influencing xylanase production, due to its largest t-value among the three variables. The quadric term of these four variables also had a significant effect. As could be seen, evident interactions existed in X_2 and X_3, but no interactions between the other variable pairs were found to contribute to the response at a significant level, also could be seen from the P values in Table 4. So, compared with the traditional 'one-variable-at-a-time' approach which is unable to detect the frequent interactions occurring between two or more factors although they often do occur, RSM has immeasurable effects and tremendous advantages.

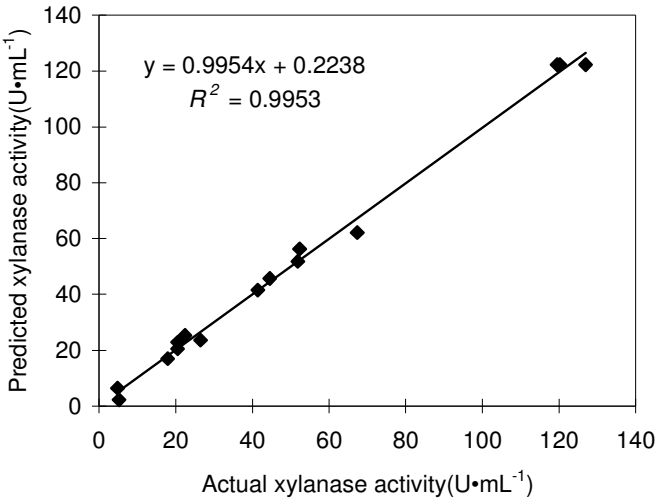

Figure 1. Experimental xylanase activities versus predicted xylanase activities under optimum fermentation conditions.

Comparison of observed and predicted xylanase activity

A regression model could be used to predict future observations on the response Y (xylanase activity) corresponding to particular values of the regressor variables. In predicting new observations and in estimating the mean response at a given point, one must be careful about extrapolating beyond the region containing the original observations. It was very possible that a model that fit well in the region of the original data would no longer fit well outside the region. Figure 1 shows observed xylanase activities (the response) versus those from the empirical model equation (3). The figure proved the predicted data of the response from the empirical model was in agreement with the observed ones in the range of the operating variables.

Localization of optimum condition

Three-dimensional response plots and their corresponding contour plots for the xylanase production by the above model are shown in Figures 2 - 4. The contour plots affirm that the objective function is unimodal in nature which shows an optimum in the boundaries. The boundary optimum point was evaluated using gradient method in the direction of steepest ascent. The graphical representation provides a method to visualize the relation between the response and experimental levels of each variable, and the type of interactions between test variable in order to deduce the optimum conditions.

Figure 2 depicts the three-dimensional plot and its respective contour plot showing the effects of cultivation time (X_1) and pH (X_2) on xylanase production, while wheat bran concentration (X_3) is fixed at its middle level. The interaction relationship between the two chosen variables and the response variable could be easily understood by examining the contour plots. The circinal nature of the contour plots indicated that no interactions between cultivation time and pH were found to contribute to the response at a significant level, due to the high *P*-value (0.8456>0.05) in Table 4. It could be seen from Figure 2, xylanase production increased gradually with the increasing cultivation time and pH. While cultivation time was at a low level, the effect of pH on the response was insignificant. When pH in medium was at a higher level, xylanase production steadily increased with increasing cultivation time, but decreased slowly beyond the range. It was evident that the activity of xylanase was higher than 120 U·mL^{-1} when cultivation time was in the range of -0.1 to 0.5 (coded value) and pH in the range of -0.4 to 0.2 (coded value).

Figure 3 shows the effects of cultivation time (X_1) and wheat bran ($X_{3)}$ on xylanase production, while the third variable is fixed at its middle level. Similarly, there were no evident interaction relationships existing between the two independent variables and the response variable. It was evident that at low wheat bran concentration, the effect of cultivation time on xylanase production was negligible. When the wheat bran concentration in medium was at a high level (0 - 0.2, coded value), xylanase production steadily increased with increasing cultivation time up to 0.2 - 0.4 (coded value). In this case, the yield of xylanase production could keep a higher level that was simply over 125 U·mL^{-1}. However, when the wheat bran concentration was enhanced further more, much higher than a level of 0 - 0.2 (coded value), xylanase production decreased slowly with the increasing cultivation time. This indicated that under optimal cultivation time and wheat bran concentration, excessive increase of extraction time would not increase the yield of xylanase any more. These facts are important in shortening fermentation periods during the potential industry application by keeping appropriate substrate concentration in medium, which can make the whole process more economical and feasible.

Figure 4 shows the effects occurring between X_2 (pH) and X_3 (wheat bran), while X_1 (cultivation time) is fixed at its middle level. The elliptical nature of the contour plots indicated that the interactions between pH and wheat bran were significant. Also could been proved from the P value (0.0466 <0.05) in Table 4. It was noticed that, xylanase production tended to increase with gradually increasing value in wheat bran concentration. Simultaneously, it was illuminated that *A. niger* AN-13 could make better use of wheat bran to produce more xylanase, which agrees well with the report by Beg et al. (2000) that wheat bran can effectively induce the higher xylanase production by *Streptomyces* sp. QG-11-3. Maybe these facts can be accounted for by the report that the enzyme involved in substrate degradation is generally inducible and is formed only when the corresponding

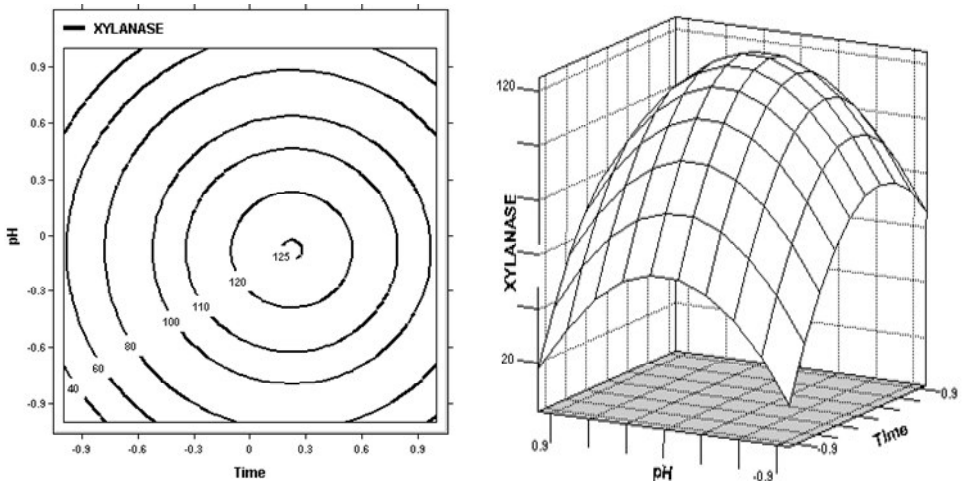

Figure 2. Response surface plot and contour plot of the combined effects of culture time (X_1) and pH (X_2) on the xylanase production by *Aspergillus niger* AN-13.

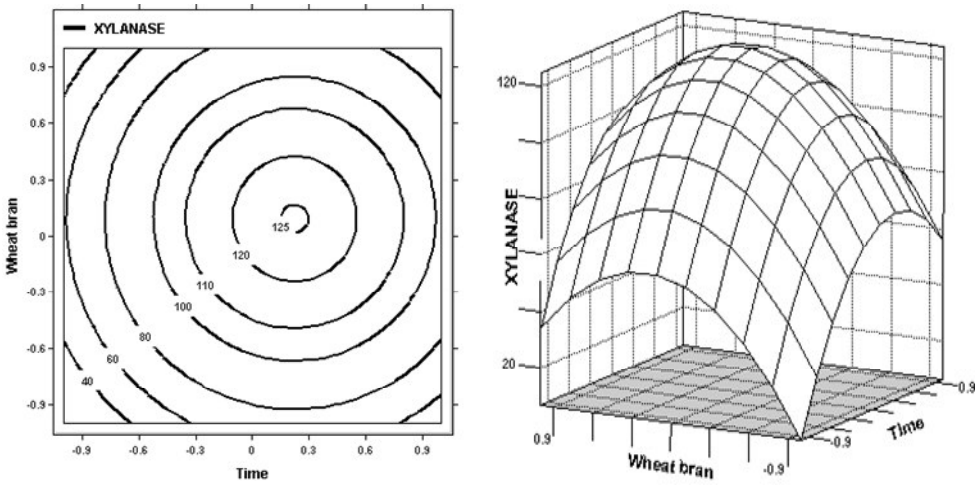

Figure 3. Response surface plot and contour plot of the combined effects of culture time (X_1) and wheat bran (X_3) on the xylanase production by *Aspergillus niger* AN-13.

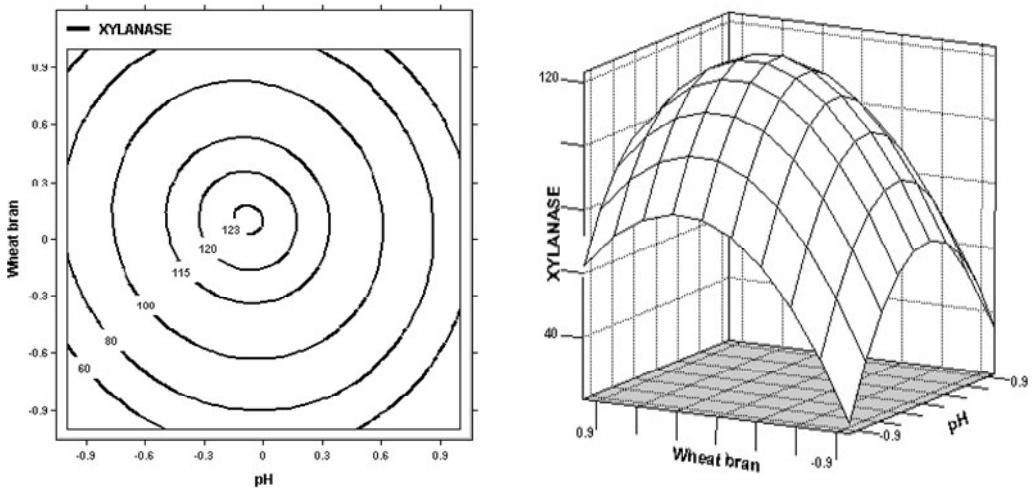

Figure 4. Response surface plot and contour plot of the combined effects of pH (X_2) and wheat bran (X_3) on the xylanase production by *Aspergillus niger* AN-13.

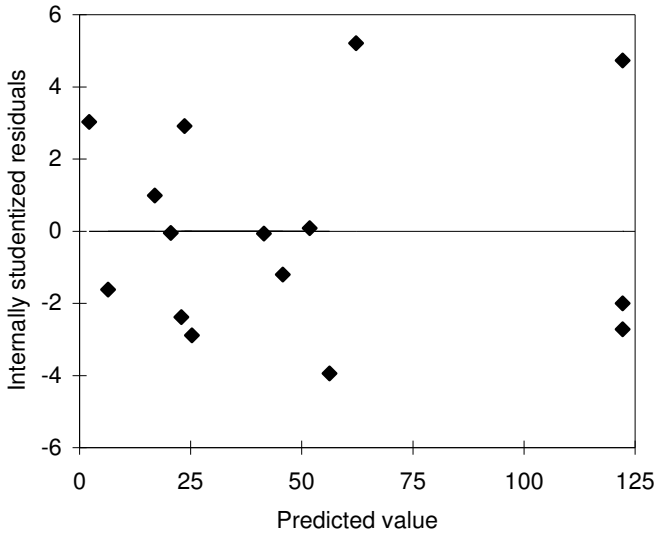

Figure 5. Plot of internally studentized residuals versus predicted values.

substrate is present in the nutrient solution by Schlegel et al. (1989). As could be seen, when pH and wheat bran concentration in medium were in the range of -0.2 to 0 (coded value) and 0 to 0.2 (coded value) respectively, the activity of xylanase could keep a high level, more than 123 U·mL^{-1}. Consequently, in order to obtain a good xylanase production the pH and wheat bran concentration should be kept a proper range.

Model adequacy checking

Usually, it is necessary to check the fitted model to ensure that it provides an adequate approximation to the real system. Unless the model shows an adequate fit, proceeding with the investigation and optimization of the fitted response surface likely gave poor or misleading results. The residuals from the least squares fit play an important role in judging model adequacy (Henika, 1972). Figure 5 presents a plot of residuals versus the predicted response. The general impression is that the residuals scatter randomly on the display, suggesting that the variance of the original observation was constant for all values of Y. As from Figure 5, it was satisfactory based on the judge of model adequacy. So it is concluded that the empirical model is adequate to describe the SOD activity by response surface.

Validation of the model

Validation of the experiment was repeated three times under optimal conditions in order to confirm the mathematical model, the maximal of which was 127.12 U·mL^{-1}. This value was found to have a marked increase compared with a lowest value of 4.80 U·mL^{-1} at run 9 in experiments according to Box-Behnken design.

Conclusion

Response surface methodology was proved to be a powerful tool for optimization of culture conditions and culture medium composition. Box-Behnken design was employed to evaluate the effects of cultivation time, pH and substrate concentration on production of xylanase by *A. niger* AN-13. Analysis of contour plots brought the following optimum parameters: cultivation time 53.3 h, pH 7.92 and wheat bran concentration 54.2 g·L^{-1}. Under these conditions, the predicted and verifiable xylanase activities were 125.14 and 127.12 U·mL^{-1}, respectively, the two of which agreed very well.

In this work, the focus was using statistical analysis methods to optimize xylanase production by *A. niger* AN-13 under submerged fermentation with agricultural residue wheat bran as main substrate. Based on the above work, further researches are concentrated on optimizing culture medium composition in order to obtain much higher xylanase production and now in process.

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REFERENCES

Beg QK, Bhushan B, Kapoor M, Hoondal GS (2000). Production and characterization of thermostable xylanase and pectinase from *Streptomyces* sp. QG-11-3. J. Ind. Microbiol. Biotechnol. 24: 396-402.

Benoit I, Navarro D, Marnet N, Rakotomanomana N, Lesage-Meessen L, Jean-Claude S, Asthera M, Asther M (2006). Feruloyl esterases as a tool for the release of phenolic compounds from agro-industrial by-products. Carbohydr. Res. 341: 1820-1827.

Biely P (1985). Microbial xylanolytic systems. Trends Biotechnol. 3: 286–290.

Bocchini DA, Alves-Prado HF, Baida LC, Roberto IC, Gomes E, Da-Silva R (2002). Optimization of xylanase production by *Bacillus circulans* D1 in submerged fermentation using response surface methodology. Process Biochem. 38: 727-731.

Box GEP, Benhnken DW (1960). Some new three level design for the study of quantitative variable. Technometrics 2: 455-475.

Castilhoa LR, Medronho RA, Alves TLM (2000). Production and extraction of pectinases obtained by solid state fermentation of agroindustrial residues with *Aspergillus niger*. Bioresour. Technol. 71: 45-50.

Debing J, Peijun L, Stagnitti F, Xianzhe X, Li L (2006). Pectinase production by solid fermentation from *Aspergillus niger* by a new prescription experiment. Ecotoxicol. Environ. Saf. 64: 244-250.

Francis F, Sabu A, Nampoothiri KM, Ramachandram S, Ghosh S, Szakacs G, Pandey A (2003). Use of response surface methodology for optimizing process parameters for the production of a-amylase by *Aspergillus oryzae*. Biochem. Eng. J. 15: 107-115.

Ghosh M, Amitabha D, Mishra AK, Nanda G (1993). *Aspergillus sydowii* MG 49 is a strong producer of thermostable xylanolytic enzymes.

Enzyme Microb. Technol. 15: 703-709.
Hang YD, Woodams EE (1997). Xylanolytic activity of commercial juice-processing enzyme preparations. Lett. Appl. Microbiol. 24: 389-392.
Heck JX, FlÕres SH, Hertz PF, Ayub MAZ (2006). Statistical optimization of thermo-tolerant xylanase activity from Amazon isolated Bacillus circulans on solid-state cultivation. Bioresour. Technol. 97: 1902-1906.
Henika RG (1972). Simple and effective system for use with response surface methodology. Cereal Sci. Today 17: 309-334.
Joglekar AM, May AT (1987). Product excellence through design of experiments. Cereal Foods World 32: 857-868.
Kang SW, Park YS, Lee JS, Hong SI, Kim SW (2004). Production of cellulases and hemicellulases by *Aspergillus niger* KK2 from lignocellulosic biomass. Bioresour. Technol. 91: 153-156.
Kuhad RC, Singh A (1993). Lignocellulose biotechnology: current and future prospects. Crit. Rev. Biotechnol. 13: 151-172.
Miller GL (1959). Use of dinitrosalicylic acid reagent for determination of reducing sugars. Anal. Chem. 31: 426-428.
Montiel-González AM, Viniegra-González G, José Fernández F, Loera O (2004). Effect of water activity on invertase production in solid state fermentation by improved diploid strains of *Aspergillus niger*. Process Biochem. 39: 2085-2090.
Pham PL, Taillandier P, Delmas M, Strehaiano P (1997). Optimization of a culture medium for xylanase production by *Bacillus sp.* using statistical experimental designs. World J. Microbiol. Biotechnol. 14: 185-190.
Qi-peng Y, Jian-dong W, Huai Z, Zhong-ming Q (2005). Effect of temperature shift on production of xylanase by *Aspergillus niger.* Process Biochem. 40: 3255-3257.
Rao KL, Kim CH, Rhee SK (2000). Statistical optimization of medium for the production of recombinant hirudin from Saccharomyces cerviasae using response surface methodology. Process Biochem. 35: 639-647.
Sá-Pereira P, Mesquita A, Duarte JC, Barros MRA, Costa-Ferreira M (2002). Rapid production of thermostable cellulase-free xylanase by a strain of Bacillus subtilis and its properties. Enzym. Microb. Technol. 30: 924-933.
Schlegel HG (1989). The Metabolism of Microorganisms. In: Präve P, Faust U, Sitting W, Sukatsch DA (eds) Basic Biotechnology-A Student's Guide. VHC-Publishers, pp. 67-101.
Silva CJSM, Roberto IC (2001). Optimization of xylitol production by *Candida guilliermondi* FTI 20037 using response surface methodology. Process Biochem. 36: 1119-1124.
Techapun C, Charoenrat T, Watanabe M, Sasaki K, Poosaran N (2002). Optimization of thermostable and alkaline-tolerant cellulose-free xylanase production from agricultural waste by thermotolerant *Streptomyces* sp. Ab106 using the central composite experimental design. Biochem. Eng. J. 12: 99-105.
Xueliang S, Liming X (2004). Production and immobilization of cellobiase from *Aspergillus niger* ZU-07. Process Biochem. 39: 1363-1367.
Yin L, Jia L, Dejing M, Jian L, Guoxian G, Zhonggui M (2006). Effect of pH, cultivation time and substrate concentration on the endoxylanase production by *Aspergillus awamori* ZH-26 under submerged fermentation using central composite rotary design. Food Technol. Biotechnol. 44: 473-477.
Yin L, Zhiqiang L, Hui Z, Yingying X, Fengjie C (2007). Statistical optimization of xylanase production from new isolated *Penicillium oxalicum* ZH-30 in submerged fermentation. Biochem. Eng. J. 34: 82-86.
Zeng AP, Biebl H (2002). Bulk-chemicals from biotechnology: The case of 1, 3-propanediol production and the new trends. Adv. Biochem. Eng. Biotechnol. 74: 239-259.