Optimization for the DXAMase production from *Lipomyces starkeyi* using statistically designed experiments

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Abstract

The optimal condition for the production of DXAMase, containing the both characteristics of dextranase and amylase, was studied based on different levels of pH, temperature, and aeration rate. Response surface methodology was applied to find the optimatic condition showing the relationship between the fermentation response(dextranase and amylase activity of DXAMase) and the fermentation variables(pH, temperature, and agitation rate). In case of dextranase activity, the condition of pH 4.06, 28.08℃, and 235.14 rpm showed the highest activity, 2.26 U/ml, and for amylase activity, the condition of pH 4.01, 27.96℃, and 212.01 rpm showed the highest activity, 3.52 U/ml. For the production of DXAMase, dextranase and amylase, the optimum condition was pH 4.06, 28.08 ℃, and 234.80 rpm.

Introduction

In fermentation biotechnology, improvement in the productivity of the microbial metabolite is carried out by improving the strain genetically and by manipulating the nutritional and physical parameters of the fermentation.[1] The optimal design of fermentation condition is an important aspect to be considered in the development of fermentation processes. One of the worthwhile techniques to identify the explanatory variable in the system is experimental design technique, so called response surface methodology (RSM). It can provide statistical models with a relatively small number of experiments.[3] From these models, the relative influence of various factors studied can be determined and their optimal conditions were calculated for a given target such as maximal activity or maximal metabolite production. Temperature, pH, and agitation rate effect on both bacterial growth and metabolism. Modeling the DXAMase production during

fermentation of *L. starkeyi* in terms of temperature, pH, and agitation rate is useful in improving the fermentation process such as planning and using the production facility. In the present study, we tried to obtain the best-suited level of temperature, pH, and agitation rate for the maximal production of DXAMase prepared from *L. starkeyi* using starch by using Box-Behnken Design of Experiments.

Material and Method

Organisms and growth condition

L. starkeyi KCCM 10181 was grown for about 36 h in 8 L vessel containing 6 L LM medium with 1% soluble starch. LM medium consists of 0.3%(w/v) yeast extract, 0.3%(w/v) KH_2PO_4, and 0.5% glycerol. The pH of the medium was controlled with 30% NaOH and 30% HCl.

Enzyme assay

Cell-free medium after centrifugation was used for determination of a combined amylase and dextranase activity (DXAMase). The formation of reducing sugar from dextran or starch was determined with dinitrosalicylic acid (DNS) reagent prepared by dissolving 10g 3,5-dinitrosalicylic acid and 10g NaOH in 1 L water containing 18.2 % Rochelle salt (potassium sodium tartarate), 0.2 % phenol and 0.05 % sodium pyrosulfite. Assays on dextran or starch solution were carried out at 37°C by mixing 50 $\mu\ell$ enzyme with 450 $\mu\ell$ of 2% dextran or starch dissolved by boiling in 20 mM citrate phosphate buffer, pH 5.5. The reaction was stopped after 10 or 12 min by mixing with 50 $\mu\ell$ reaction solution and 150 $\mu\ell$ DNS reagent. The samples were then boiled for 10 min and cooled. The absorbance was read at 595 nm and values for reducing sugar were expressed as maltose or isomaltose equivalents. One unit of DXAMase activity corresponds to the formation of 1 μmol maltose and isomaltose equivalent per min.

Metholology and design of experiments

The variables were pH, temperature and agitation rate and the responses observed were both activities of DXAMase. We coded the levels in standardized units so that the values taken by each of the three variables X_1, X_2, and X_3 were -1, 0 and +1, respectively. The significant independent variables can be approximated by the quadratic model equation.

$$Y = b_0 + b_1X_1 + b_2X_2 + b_3X_3 + b_{11}X_1^2 + b_{22}X_2^2 + b_{33}X_3^2 + b_{12}X_1X_2 + b_{13}X_1X_3 + b_{23}X_2X_3, \quad (1)$$

Where, **Y** = predicted response, X_1, X_2, and X_3 are input variables, b_0 is a constant; b_1, b_2, and b_3 are linear coefficients; b_{12}, b_{13} and b_{23} are cross product coefficients; b_{11}, b_{22}, and b_{33} are quadratic coefficients.[5-8] By solving *Eq.* (1), it was found that a total of 17 experiments was necessary in order to estimate the 10 coefficients for optimization of the growth condition.

Results and discussion

Second order polynomial regression model containing 3 linear, 3 quadratic and 3 interaction terms plus 1 block term was employed using the Design Expert(Stat-Ease, Minneapolis, MN ver. 6. 0. 6, 2002). All terms regardless of their significance are included in the following equation:

For dextranase activity

$$Y_1 = 2.21 + 0.13X_1 - 0.048X_2 + 0.22X_3 - 0.77X_1^2 - 0.68X_2^2 - 0.35X_3^2 + 0.075X_1X_2 + 0.17X_1X_3 + 0.22X_2X_3$$

For amylase activity

$$Y_2 = 3.49 + 0.075X_1 - 0.012X_2 + 0.38X_3 - 1.38X_1^2 - 1.04X_2^2 - 0.80X_3^2 + 0.18X_1X_2 + 0.11X_1X_3 - 0.024X_2X_3$$

Reference

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Table 1. Experimental and theoretically predicted values for DXMAse activity

Trial no.	Dextranase activity(U)		Amylase activity(U)	
	Experimental value	Predicted value	Experimental value	Predicted value
1	0.60	0.75	0.81	1.19
2	0.73	0.87	0.81	0.98
3	0.64	0.50	0.98	0.80
4	1.07	0.92	1.70	1.32
5	1.03	0.91	1.41	0.97
6	0.94	0.84	1.13	0.91
7	0.90	1.00	1.29	1.51
8	1.51	1.62	1.44	1.87
9	1.27	1.23	1.22	1.27
10	0.45	0.70	0.69	1.29
11	1.48	1.23	2.68	2.07
12	1.53	1.57	2.05	2.00
13	2.20	2.21	3.50	3.49
14	2.21	2.21	3.52	3.49
15	2.18	2.21	3.45	3.49
16	2.28	2.21	3.50	3.49
17	2.20	2.21	3.50	3.49

Table 2. Regression analysis for DXAMase and quadratic response surface model fitting(ANOVA)

Source	S.S	DF	M.S	*F* value	Prob>F
For dextranase activity					
Model	6.38	9	0.71	18.95	0.0004
Residual	0.26	7	0.037		
Lack of fit	0.26	3	0.085	57.66	0.001
pure error	5.920E-003	4	1.480E-003		
Correlation Total	6.64	16		R-Squared	0.9606
				Adj R-Squared	0.9099
For amylase activity					
Model	18.28	9	2.03	9.07	0.0041
Residual	1.57	7	0.22		
Lack of fit	1.57	3	0.52	767.46	0.0001
pure error	2.720E-003	4	6.800E-004		
Correlation Total	19.85	16		R-Squared	0.9210
				Adj R-Squared	0.8194

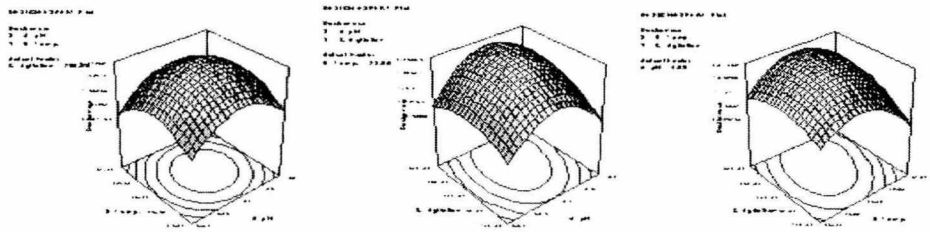

Fig 1. 3-D graphics of response surface for dextranase activity of DXAMase.