

Effect of Magnetic Fields on Superoxide Dismutase Activity Using Response Surface Methodology

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Abstract: The effect of three variables (temperature, magnetic intensity, and exposure time) on the activity of superoxide dismutase (EC 1.15.1.1) isolated from *Saccharomyces cerevisiae* was studied by Response Surface Methodology (RSM). This multivariate methodology offers an empirical approach to the study of enzyme assays and allows to detect the interaction between different variables of the system. Analysis of variance (ANOVA) showed that a second order polynomial model (SOP) generated response surface and contour plots, and the model may predict the experimental data. Lack-of-fit tests did not result in a significant F-value. Determination coefficients (R^2) were greater than 94%. The optimum treating parameters were obtained as: temperature, 15.5 °C; magnetic intensity, 0.95T; time of magnetic field exposure, 64.5 minutes.

Key words: superoxide dismutase; *Saccharomyces cerevisiae*; response surface methodology

响应曲面法研究磁场对超氧化物歧化酶活性的影响

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摘要: 通过响应曲面法(response surface methodology, RSM)研究了温度、磁场强度及曝磁时间这三个磁场处理的不同因素, 对于由酿酒酵母中分离出的超氧化物歧化酶(EC 1.15.1.1)活性效果的影响。这种多变量统计方法可用于

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源, GC-MS 鉴定其挥发性物质成分主要为脂肪烃; 蛋黄中的极性脂(磷脂)参与了鸡蛋香味的形成, GC-MS 鉴定极性脂加热后产生了大量醛类; 非极性脂(甘油三酯)对蛋黄的风味贡献不大。

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进行酶分析,并可研究体系内不同变量的交互作用,建立了可用于预测实验数据的二次多项式模型。失拟项不显著,决定系数(R^2)大于94%。根据模型优化出可以取得最大酶活性的工艺参数为:温度15.5,磁场强度0.95T,曝磁时间64.5min。

关键词:超氧化物歧化酶;酿酒酵母;响应曲面法

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Superoxide dismutase (SOD) is an enzyme that can catalyze the dismutation of superoxide anion radical (O_2^-) to hydrogen peroxide (H_2O_2) and O_2 ^[1]. It plays an important role in longevity and degenerative disease and has great potential being used in medicine, nutrient, cosmetic, etc. Thus, its industrial production is in expectation. In comparison with animal blood, the main current sources of SOD production, microbial fermentation may be more economic approach to yield the enzyme^[2] and taking spent beer yeast as raw material is promising^[3]. It was proved by our previous work that treating *Saccharomyces cerevisiae* with static magnetic fields can effectively increase the SOD activity in it, and single-factor experiments verified that temperature, magnetic intensity and time of magnetic fields exposure are significant influencing factors (data not shown). The purpose of this study is to discuss the interaction of these three variables in the system.

Response surface methodology (RSM) consists of a group of mathematical and statistical procedures and can be widely used to detect the interaction between different variable of a system. RSM was already used to optimize different bioconversion processes^[4], such as the optimization of medium composition^[5,6], the parameters of food preservation^[7] and fermentation^[8]. Many applications deal with the study of the effects of two or more factors.

1 Materials and Methods

1.1 Yeast strain and growth conditions

The yeast strain *Saccharomyces cerevisiae* was provided by Microbial Culture Collection Center of Guangdong Institute of Microbiology. The strain was grown in liquid Malt Extract Medium. The medium used were sterilized at 115 for 20min. The culture was grown aerobically in 500ml conical flasks with shaking (130r/min) for 18h at 29 ± 1 .

1.2 Analytical methods

The cell-free crude SOD extract were obtained from *Saccharomyces cerevisiae* using the method of Tan^[9].

Total SOD activities were determined by the method of pyrogallol autoxidation with the optical density (OD) value at 325nm. The rate of pyrogallol's autoxidation was manipu-

lated to be 0.070 OD/min, and 50% depression of autoxidation rate of pyrogallol by SOD was known as 1 U. Results were expressed as U/mg protein.

Protein content was determined by the method of Bradford^[10].

1.3 Experimental design of RSM

Box and Behnken^[11] presented some new three-level designs for the study of quantitative variables and these designs are capable of being regressed using the RSM. The different parameters such as temperature, magnetic intensity, and time of magnetic field exposure were chosen as key variables and designated as X_1 , X_2 and X_3 , respectively. The low, middle and high levels of each variable were designated as -1, 0 and +1, respectively, and are given in Table 1. The variables were coded according to the Eq. (1):

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

Table 1 Code and level of factors chosen for the trials

Factor	Symbol s		Level ⁽¹⁾		
	Coded	Uncoded	-1	0	1
Temperature()	x_1	X_1	5	15	25
Magnetic intensity(Tesla)	x_2	X_2	0.5	1.0	1.5
Time of MF exposure(minute)	x_3	X_3	30	60	90

Note: ⁽¹⁾ $x_1 = (X_1 - 15)/10$; $x_2 = (X_2 - 1.0)/0.5$; $x_3 = (X_3 - 60)/30$.

where x_i is the dimensionless coded value of the variable X_i , X_0 is the value of X_i at the centre point and X is the step change. Table 2 shows the actual design of the experiments. The behaviour of the system was explained by the following second-degree polynomial equation.

$$Y = B_0 + \sum_{i=1}^n B_i X_i + \sum_{i=j=1}^n B_{ij} X_i X_j \quad (2)$$

where Y =predicted response, it can be observed that in the present study, three variables are involved and hence n takes the value 3. Thus, by substituting the value 3 for n , Eq. (2) becomes:

$$Y = B_0 + B_1 X_1 + B_2 X_2 + B_3 X_3 + B_{12} X_1 X_2 + B_{13} X_1 X_3 + B_{23} X_2 X_3 + B_{11} X_1^2 + B_{22} X_2^2 + B_{33} X_3^2 \quad (3)$$

where X_1 , X_2 and X_3 are put variables (viz., temperature, magnetic intensity, time of magnetic exposure); B_0 is a

Table 2 Box-Bohnken experimental design and results

Trial No.	X_1	X_2	X_3	Response	
				Observed	Predicted
1	-1	0	-1	150.225	155.017
2	1	0	-1	181.306	172.630
3	-1	0	1	150.225	158.902
4	1	0	1	158.514	153.722
5	-1	-1	0	162.658	154.369
6	1	-1	0	151.261	156.442
7	-1	1	0	146.081	140.901
8	1	1	0	142.973	151.261
9	0	-1	-1	175.608	179.105
10	0	-1	1	163.176	162.787
11	0	1	-1	160.586	160.974
12	0	1	1	165.766	162.269
13	0	0	0	203.090	206.491
14	0	0	0	203.919	206.491
15	0	0	0	211.351	206.491
16	0	0	0	211.748	206.491
17	0	0	0	202.347	206.491

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} , B_{33} are quadratic coefficients. Y_i is predicted response.

The Design Expert (Version 7.0.1, State-ease Inc., Minneapolis, MN, USA, 2005) was used for regression analysis of the data obtained and to estimate the coefficients of the regression equation. Isoresponse contour plots were also obtained by using Design Expert.

2 Results and Discussions

2.1 Regression models of response

The mean value ($n=9$) of the response (superoxide dismutase activity) obtained under the different experimental conditions are summarized in Table 2. The experimental data (Table 2) were analyzed using statistical methods appropriate to the experimental design used. Multiple regression analysis of the experimental data gave the following second order polynomial equation:

$$Y = 206.49 + 3.11x_1 - 4.66x_2 - 3.76x_3 - 30.98x_1^2 - 24.77x_2^2 - 15.44x_3^2 + 2.07x_1x_2 - 5.70x_1x_3 + 4.40x_2x_3 \quad (4)$$

Each of the observed values, Y_0 , is compared with the predicted value, Y_i , which is calculated from the model, as depicted in Fig. 1. We can see that Y_0 accords with Y_i .

2.2 Analysis of variance

Statistical testing of the model was done by the Fisher's F-test for analysis of variance (ANOVA) and the results are shown in Table 3. The analysis of variance of the quadratic regression model demonstrates that the model is highly significant, as is evident from the Fisher's F-test (F_{model} , mean

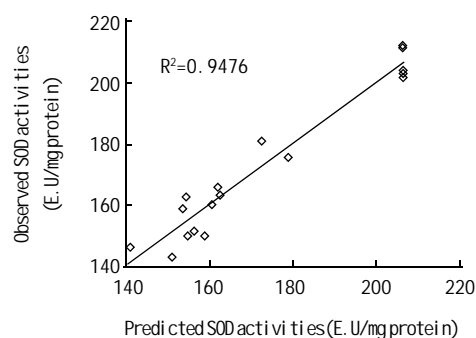


Fig. 1 Comparison between predicted and observed SOD activity

Table 3 Analysis of variance (ANOVA) for regression equation

Source	Sum of squares	df	Mean square	F value	Prob > F
Model	9027.74	9	1003.08	14.07	0.0011
Residual	498.92	7	71.27		
Lack of fit	412.31	3	137.44	6.35	0.0531
Pure error	86.61	4	21.65		
Total	9526.66	16			

$R=0.9734$ $R^2=0.9476$ Adj $R^2=0.8803$

square regression/mean square residual = 14.07) with a low probability value [$(p_{\text{model}} > F) = 0.0011$]. The lack-of-fit test, which measures the fitness of the model obtained, did not result in a significant F-value, indicating that the model is sufficiently accurate for predicting the SOD activity. The value of R (0.9734) for Eq. (4) being close to 1 indicates a high degree of correlation between the observed and predicted values. The goodness of fit of the model was checked by determination coefficient (R^2). In this case, the value of the determination coefficient R^2 is 0.9476 and adjusted R^2 is 0.8803. The adjusted R^2 corrects the R^2 value for the sample size and for the number of terms in the model. The high value of adjusted R^2 indicated that the model well fit the observed data.

The significance of each coefficient was determined by F-value and p-values which are listed in Table 4. The smaller the magnitude of the p-value the more significant is the corresponding coefficient. It can be seen from this table that all the quadratic terms were significant, the p-values being very small ($p < 0.05$). However, linear parameters and interactions did not produce a significant effect in this case. Thus, quadratic effects of independent variables were the primary determining terms that may cause significant effects in the response.

2.3 Optimization of the conditions

The graphical representation of the regression Eq. (4), called the response surfaces and the contour plots were obtained using the Design Expert and are presented in Fig. 2 ~ Fig. 5. Fig. 2 ~ Fig. 3 shows the effect of temperature and

Table 4 Test of significance for regression coefficient

Model term	Coefficient estimate	df	Standard error	95% CI Low	95% CI High	Prob > F
Intercept	206.49	1	3.78	197.56	215.42	
X_1 (Temperature)	3.11	1	2.98	-3.95	10.17	0.3324
X_2 (Magnetic intensity)	-4.66	1	2.98	-11.72	2.40	0.1623
X_3 (Time of MF exposure)	-3.76	1	2.98	-10.81	3.30	0.2487
X_1^2	-30.98	1	4.11	-7.91	12.05	0.0001
X_2^2	-24.77	1	4.11	-15.68	4.28	0.0005
X_3^2	-15.44	1	4.11	-5.58	14.38	0.0071
X_1X_2	2.07	1	4.22	-40.71	-21.25	0.6385
X_1X_3	-5.70	1	4.22	-34.49	-15.04	0.2191
X_2X_3	4.40	1	4.22	-25.17	-5.71	0.3316

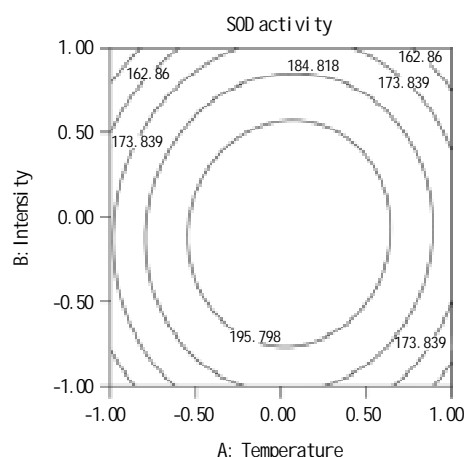


Fig.2 Contour plot of SOD activity as a function of temperature and magnetic intensity

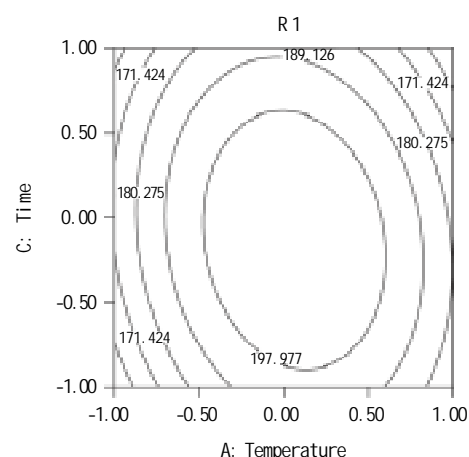
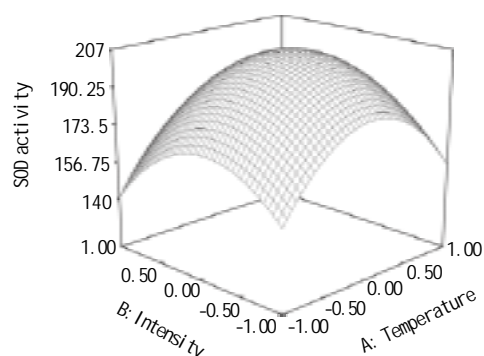
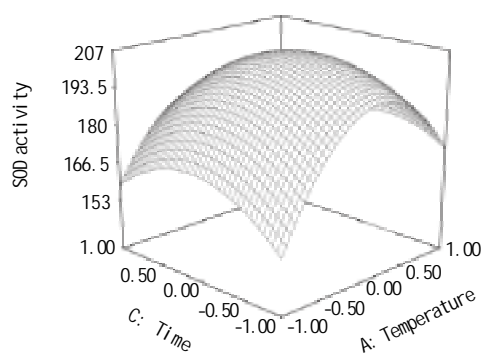


Fig.4 Contour plot of SOD activity as a function of temperature and time of magnetic field exposure

Fig.3 Three-dimensional response surface plot of the effect of treating temperature and magnetic intensity on SOD activity of *Saccharomyces cerevisiae*Fig.5 Three-dimensional response surface plot of the effect of treating temperature and time of magnetic field exposure on SOD activity of *Saccharomyces cerevisiae*

magnetic intensity on SOD activity. By solving the inverse matrix of the derivation of Eq. (4), fixing that optimal magnetic intensity is 0.95T and the optimal temperature is 15.6 °C. SOD activity increased with increasing magnetic intensity under 15.6 °C.

Fig. 4 ~ Fig. 5 shows the effect of temperature and time of magnetic field exposure on SOD activity. By solving the inverse matrix of the derivation of Eq. (4), fixing that optimal time of magnetic field exposure is 64.5 minutes and the opti-

mal temperature is 15.6 °C. SOD activity increased with increasing exposure time under 15.6 °C.

3 Conclusion

Statistical analysis using response surface methodology appears to be a valuable tool for studying the optimization of combined effect of temperature, magnetic intensity and time of magnetic field exposure on the SOD activity of *S. cerevisiae*. The RSM, a multi factorial statistical approach that

considers interaction of independent variables, can be used to predict and optimize the SOD activity of yeast treated with magnetic fields. The optimum magnetic fields treating parameters for SOD activity were obtained as: temperature, 15.5 °C; magnetic intensity, 0.95T; time of magnetic field exposure, 64.5 minutes. Thus, RSM provided a basis for the model to search for a non-linear nature of the response in a short term experiment.

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