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Optimization of laccase immobilization conditions and mathematical model

Zhang Jie[1]*, Zheng Shengliang[2], Zhang Ying[1], Ren Jing[1], Liu Yongqiang[1], Wang Binsong[2]*
[1]School of Life Science, Northeast Forestry University, Harbin 150040, (CHINA)
[2]Institute of Chemistry and Chemical Engineer, Heilongjiang University, Harbin, 150080, (CHINA)
E-mail: 875817856@qq.com; 609291321@qq.com

ABSTRACT

D380 resin utilized as a support for laccase immobilization which applied to pollution degradation. Plackett-Burman design showed cross-linking temperature, immobilization pH and dilution ratio were major factors among cross-linker concentration, cross-linking temperature, cross-linking time, immobilization pH, enzyme dilution ratio, ion concentration, immobilization time, immobilization temperature and the consumption of enzyme. For further practical application in pollution degradation area, the optimized model conditions are needed. The mathematical model of optimized conditions was obtained through the regression equation of the response surface methodology. In this work, the optimum technological conditions were investigated as cross-linker concentration of 10%, cross-linking time of 2 h, cross-linking temperature of 31°c pH =7.2, enzyme dilution ratio of 900, consumption of enzyme of 10 ml, PEB ion concentration of 0.2 mol/L, immobilization time 6 h and immobilization temperature of 30 °c, respectively. Compared average immobilized enzyme activity 15.53 U/g of the verification test with theoretical value 15.95 U/g, the results proved that the mathematical model was feasible.

KEYWORDS

Macroporous resin;
Laccase;
Immobilization;
Mathematical model.

INTRODUCTION

Laccase can catalyze a variety of phenolic and non-phenolic compound oxidation, the substrate widely used in the pulp waste water treatment, industrial dyes decolonzation and other areas of important applications[1,2]. However, the free enzyme vulnerable to environmental conditions and variability of inactivation, and easily lost, is difficult to recycle, in order to overcome the shortcomings of the free enzyme, people began to explore the combination of enzyme and carrier, prepared immobilized enzyme. Enzyme immobilization technology is a continuous and effective means to improve the enzyme stability[3-6].

The purpose of this paper is to use D380 macroporous weakly basic resin as the carrier, the value of a large industrial application of laccase immobilized cross-linking studies, including immobilization, immobilized condition optimization, a immobilized amount of enzyme protein, the catalytic activity as well as immobilized the nature and stability of enzymes such as research, and laccase immobilization conditions a mathematical model for the study of the conditions immobilized in order to broaden the immobilized laccase in in-

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dustry application[7-9].

MATERIALS AND METHODS

Test materials

Materials: laccase, D380 macroporous styrene alkaline ion exchange resin.

Reagents: glutaraldehyde solution (50%) (GA), disodium hydrogen phosphate, citric acid, ABTS, ethanol, Congo red, deionized water.

Test method

Application of Plackett-Burman (PB) experimental design method for screening an important factor[10,11], and in accordance with the relevant references and single-factor test results, designed Plackett-Burman (PB) test factors and levels. Based on previous studies and reported in the literature, the choice of the number of trials N = 12 of the experimental design. The cross-linking agent concentration (*X*1), crosslinking temperature (*X*2), cross-linking time (*X*3), pH of immobilization (*X*4), enzyme dilution multiple (*X*5), ion concentration (*X*6), time of immobilization (*X*7), temperature of immobilization (*X*8) enzyme dosage (*X*9),9 factors to study, the response was immobilized enzyme activity (*Y*)0According to Plackett-Burman (PB) experimental design method of screening out of three important factors, statistical analysis using SAS V8.2 software for Box-Behnken test design and response surface analysis carried out to determine the best level of three important factors[12]. Those tests of this study were done by 17 times, including analysis of 12 trials for analysis of test points, and set the repeat test five times, used to estimate the experimental error; enzyme activity in response to a immobilized value, according to Box-Behnken design of test results, the use of SAS software for quadratic regression analysis of the results.

Analysis program

Low temperature air drying, the system was dried immobilized laccase. This article is based on ABTS [13] as the substrate for selection of the light absorption method for determination of laccase activit[14,15].

Immobilized laccase adding 2 mL of pH 3.0 disodium hydrogen phosphate-citrate buffer solution and 1 mL distilled water, centrifuged and the supernatant is blank. Corresponding quality of laccase immobilized by adding 2 mL of pH 3.4 disodium hydrogen phosphate-citrate buffer solution and 1 mL 1 mmol / LABTS solution, reaction in 3 min then ice in 10 min to stop the reaction, at 4 °c,under 8000 rpm centrifuge for 5 min, the supernatant measured in absorbance at 420 nm. Immobilized laccase enzyme activity is calculated as follows:

$$P(unit/(g\cdot min)=\frac{\frac{AV}{\varepsilon t}\times 1000}{M_0}$$

Where M_0 is the quality of the immobilized laccase, A (A <0.5) is the absorbance value at time t, ε as the substrate extinction coefficient 36000 L/(mol • cm) at 420 nm, V is added to the pool than the color amount of solution, usually 3 mL, t is reaction time.

Immobilized enzyme and immobilized enzyme obtained after supernatant, the supernatant combined with coomassie blue staining, also known as Bradford determination of the supernatant protein content, in order to calculate the efficiency of enzyme immobilization.

RESULTS AND DISCUSSION

According to the Plackett-Burman design method for screening an important factor

Designed by the Plackett-Burmen obtained results, level of each factor should select the appropriate value, and analyze the cross-linking temperature, pH of immobilization and enzyme dilution effect of the three immobilized enzyme activity as the main factors, according to the size and value, their positive and negative effects of surface response test arrangements and level of the three factors, as shown in TABLE 1.

Response surface analysis and results

The quadratic regression equation $Y = 15.68600 + 0.49400 \times X1 - 1.23088 \times X2 + 0.42413 \times X3 - 0.27100 \times X1 \times X2 + 0.050500 \times X1 \times X3 + 1.60325 \times X2 \times X3 - 2.99638 \times (X1)^2 - 3.3286 \times (X2)^2 - 2.62513 \times (X3)^2$ ($X1$:cross-linking temperature (°c), $X2$: pH of immobilization, $X3$:enzyme dilution)

From the analysis of variance model can be seen in TABLE 2, $\alpha = 0.01$ level in the return of significant one-

TABLE 1 : Results of statistic analysis

Factor symbol	Factor	Estimated effect	Standard deviation	T value	Prob>|T| value	Sort
*X*1	Crosslinker concentration	-0.225	0.228	-0.163	0.428	7
*X*2	Crosslinking temperature	-0.513	0.228	-0.373	0.153	3
*X*3	Crosslinking time	-0.396	0.228	-0.288	0.224	5
*X*4	pH of immobilization	0.827	0.228	0.602	0.068	1
*X*5	Enzyme dilution	0.610	0.228	0.444	0.116	2
*X*6	Ion concentration	-0.222	0.228	-0.161	0.433	8
*X*7	Immobilized time	0.082	0.228	0.059	0.781	9
*X*8	Immobilized temperature	0.426	0.228	0.310	0.202	4
*X*9	To enzyme	-0.240	0.228	-0.175	0.402	6

time items, the second term on the response have a significant impact, but the interaction term was not significant, indicating that the interaction between the selected factors is not obvious, using the model can not consider the interaction between factors; loss of the proposed items reflect the experimental data and the model does not match the situation, $P = 0.164 > 0.1$, shows no loss to be significant, so that the correct model selection.

TABLE 2 : Regression analysis of variance

Source of variation	DF	SS	MS	F value	Pr>F
*X*2	1	1.95	1.95	3.74	0.0943
*X*4	1	12.12	12.12	23.23	0.0019
*X*5	1	1.44	1.44	2.76	0.1408
*X*2*X*2	1	0.29	0.29	0.56	0.4775
*X*2*X*4	1	0.010	0.010	0.020	0.8927
*X*2*X*5	1	10.28	10.28	19.70	0.0030
*X*4*X*4	1	37.80	37.80	72.44	0.0001
*X*4*X*5	1	46.65	46.65	89.39	0.0001
*X*5*X*5	1	29.02	29.02	55.60	0.0001
Model	9	152.74	16.97	27.27	0.0001
One-time items	3	5.21	1.74	0.15	0.9284
Quadratic	3	19.76	6.56	0.50	0.6899
Interaction terms	3	3.99	1.33	15.34	0.0117
Error term	7	4.34	0.62	--	--
Loss of the proposed items	3	3.31	15.34	0.0117	0.164
Pure error	4	0.35	0.087	--	--
All items	16	156.40	--	--	--

Analysis diagram drawn by the regression equation, the Institute of the corresponding shape of the surface fitting, response surface analysis chart and the corresponding three-dimensional contour map, respectively, shown in Figure.1, Figure.2, Figure.3, Figure.4, Figure.5 and Figure.6. From the figures, and software analysis shows that the existence of a stable point of the regression equation, while stable point of great value‰

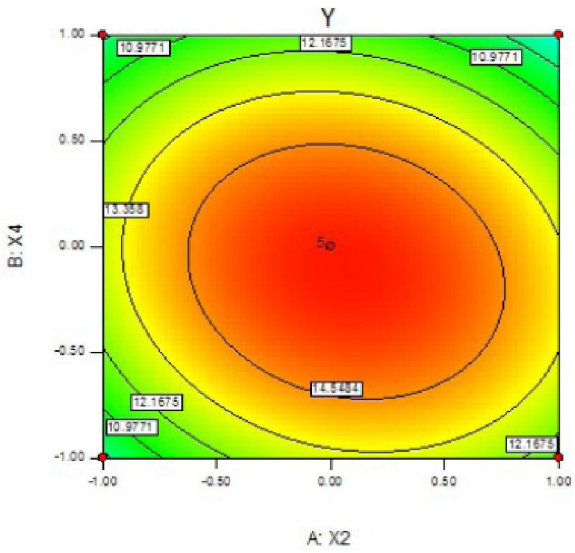

Figure 1 : *Y*=f(*X2*,*X4*) Contour map

From Figures.1 and Figures.2 can be seen: the picture shows the oval-shaped contour, indicating that *X*2 (crosslinking temperature (°c)) and *X*4 (pH of immobilization) a significant interaction between two factors; and from the density of lines can be obtained: *X*2 (crosslinking temperature (°c))has more effect than *X*4 (pH of immobilization) on the value response.

From Figure.3 and Figure.4 can be seen in the contour map: contour map of its near-round, indicating that the interaction between two factors was not significant; and from the density of lines can be drawn: *X*2 (cross-

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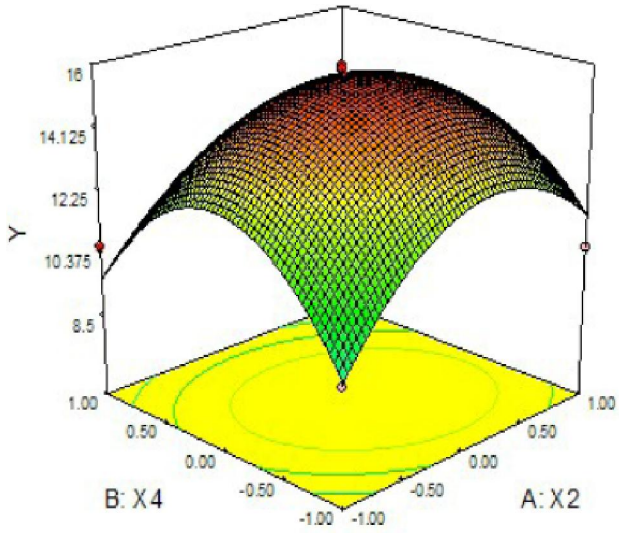

Figure 2 : *Y*=f(*X2*,*X4*)Three-dimensional response surface analysis chart

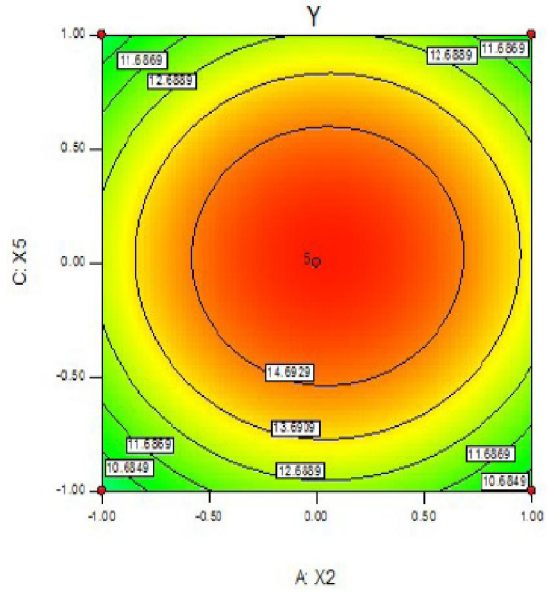

Figure 3 : *Y*=f(*X2*,*X5*)Contour map

linked temperature (°c)) has more effect than the *X*5 (enzyme dilution factor) on the response value.

Figures.5 and Figures.6 can be seen: the picture shows the oval-shaped contour, indicating that *X*4 (pH of immobilization) and *X*5 (enzyme dilution) a significant interaction between two factors; and from the density of lines can be drawn: *X*4 (crosslinking temperature (°c)) has more effect than the *X*5 (enzyme dilution factor) on the response value.

Experimental verification

According to the actual situation crosslinking temperature (°c) (*X*2), pH of immobilization (*X*4), the en-

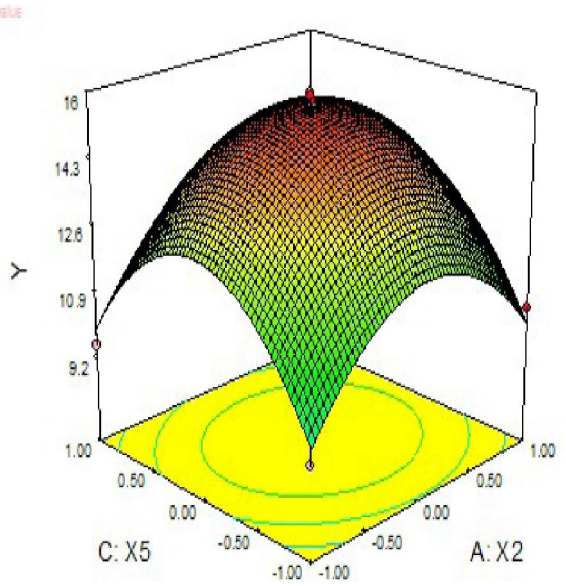

Figure 4 : *Y*=f(*X2*,*X5*)Three-dimensional response surface analysis chart

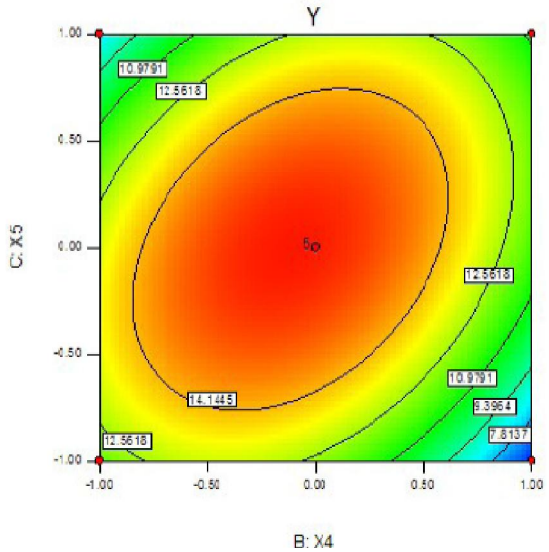

Figure 5 : *Y*=f(*X4*,*X5*)Contour map

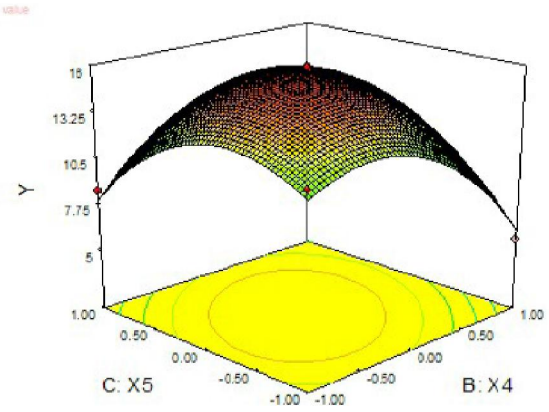

Figure 6 : *Y*=f(*X4*,*X5*)Three-dimensional response surface analysis chart

zyme dilution factor (*X*5) set at the level of cross-linking temperature of 31 °c, pH of immobilization is 7.2, the enzyme dilution factor of 900 times, respectively. In the above verification experiment under optimal conditions, a total of 5 batches of experiments have been ran, the average results for the immobilized enzyme activity 15.53 U/g, the difference between experimental value (15.53 U/g) and the model of the theoretical value (15.95 U/g) of theoretical value only 0.42 U/g, showing that the model can better shows the use of D380 resin immobilized laccase conditions.

CONCLUSIONS

(1)In a typical Plackett-Burman design of laccase immobilization,Cross-linker concentration (*X*1), crosslinking temperature (*X*2), cross-linking time (*X*3), pH of immobilization (*X*4), the enzyme dilution factor (*X*5), ion concentration (*X*6), a immobilized time (*X7*), immobilized temperature (*X*8) and to the enzyme (*X*9),those 9 factors associated with immobilized screened crosslinking temperature (°c) (*X*2), pH of immobilization (*X*4), the enzyme dilution factor (*X*5) as 3 main factors. (2) The use of response surface methodology to get optimized optimum conditions: the best cross-linker concentration of 10%, the best time of cross-linking is 2 hours, cross-linking optimum temperature of 31 °c, the best pH of immobilization is 7.2, the best enzyme dilution of 900, the amount of enzyme best to 10 ml, the best PEB ion concentration of 0.2 mol / L, the best immobilized time of 6 hours, the best immobilized temperature of 30 °c. (3) Validation test results under the optimized conditions as follows: average results for the immobilized enzyme activity of 15.53 U/g, the difference between experimental value (15.53 U/g) and the model of the theoretical value (15.95 U/g) only 0.42 U/g of the theoretical value, shows that the model can better shows the use of D380 resin immobilized laccase conditions.

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