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环境生物技术

裂褶菌 F17 锰过氧化物酶酶活力影响因素的响应面优化

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摘　要：锰过氧化物酶是真菌分泌的一种糖基化的含有血红素辅基的胞外蛋白，在染料降解和脱色过程中起着重要作用。本实验利用本实验室保存的的白腐真菌裂褶菌 *Schizophyllum* sp. F17 产锰过氧化物酶（MnP），研究 MnP 的酶学性质，并对酶活条件进行优化。实验通过超滤浓缩、DEAE-纤维素、DE52 离子交换层析和 Sephadex G-75 凝胶过滤等步骤，分离纯化得到电泳纯的锰过氧化物酶。该酶蛋白含量为 23 μg/mL，分子量大小为 49.2 kDa，在 0.1 mmol/L H_2O_2 中半衰期为 5~6 min。Mn^{2+}、H_2O_2 以及酶的用量可以影响 MnP 酶促反应的效率，在单因子分析法的基础上，通过全因子中心组合设计响应面分析表明：H_2O_2 以及 H_2O_2 与酶用量之间的交互作用对酶促反应的作用是最显著的。在优化条件下，酶对偶氮染料金橙 G、刚果红显示出较强的脱色能力。

关键词：锰过氧化物酶，纯化，优化，相对酶活，全因子试验，偶氮染料

Optimization of process variables for the manganese peroxidase of the white-rot fungus *Schizophyllum* sp. F17 by full factorial central composite design

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Abstract: White-rot fungus manganese peroxidase (MnP) that has great potential in degrading azo dyes is one of the extracellular glycolsylated heme proteins. MnP from *Schizophyllum* sp. F17 was isolated and purified by Sephadex G-75 gel filtration chromatography followed by DEAE-cellulose anion exchange chromatography. The molecular weight of the puried enzyme was 49.2 kDa, while the half-life of the MnP in the presence of 0.1 mmol/L H_2O_2 was 5−6 min. The efficiency of MnP-catalyzed reactions were determined by three key factors: the concentrations of Mn^{2+}, H_2O_2, and the amount of MnP. Using single factor analysis, an optimized concentration of Mn^{2+}, H_2O_2 and enzyme were optimized to be 1.2 mmol/L, 0.1 mmol/L, and 0.4 mL, respectively. A response surface methodology (RSM) employing two-level-three-factor full factorial central composite design was used to optimize the catalytic conditions. The result showed that the concentration of H_2O_2 and the interaction between H_2O_2 and MnP mostly affect the MnP catalytic efficiency. Finally, we show that the azo dyes could be efficiently decolorized by the purified MnP under optimized conditions.

Keywords: manganese peroxidase, purification, optimization, relative MnP activity, full factorial central composite design, azo dyes

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Introduction

Azo dyes are extremely versatile colorants and are the largest group of synthetic known [1]. As a consequence, they are also the most common group of synthetic colorants released into the environment [2]. It is estimated that about 10% and 20% of 0.7 million tons of dyestuff being manufactured each year and used in dyeing processed may be found in wastewater [3]. The treatment of dye wastewater from textile and dyestuff industries is one of the most challenging among industrial wastewater [4]. Compared with physical and/or chemical methods, microbial decolorization has been claimed to be less expensive and less environmentally intrusive alternative [5].

White-rot fungi have been shown to possess a remarkable potential for degrading azo dyes because they produce oxidase and peroxidase, which are highly oxidative and substrate-nonspecific[6]. One of the most understood enzymes is manganese peroxidase (MnP, EC 1.11.1.13), first described in *Phanerochaete chrysosporium*[7]. MnP oxidizes a wide range of substance, rendering it an interesting enzyme for potential applications. MnP is an extracellular glycolsylated heme protein. MnP can catalyze the H_2O_2-dependent oxidation of Mn^{2+} to Mn^{3+}, which is stabilized by chelators such as organic acid. Chelated Mn^{3+} acts as a highly reactive, low molecular weight, diffusible redox-mediator[2]. Thus, MnP is able to oxidize and depolymerize its natural substrate, i.e., ligin and a range of diverse environmental pollutants such as nitroaromatic compounds and textile dyes [8]. Although they have enormous potential, their industrial application is hampered by their high price and low operational stability[9]. Heme peroxidases are swiftly inactivated in the presence of catalytic amounts of hydrogen peroxide, which acts as an electron acceptor during the catalytic cycle. Although the inactivation mechanism is not completely explained, several events such as heme destruction, intermolecular crosslinking and oxidation of low redox potential amino acid residues are known to lead to activity loss[10]. The inactivation is considered a suicide process, as the main inactivating species are the enzymatic intermediates involved in the catalytic cycle[11].

The MnP catalytic cycle process is a complicated system. The three main factors that decide the enzyme activity of MnP are concentrations of H_2O_2, Mn^{2+} and enzyme itself. It is important to analyze the factors as a whole and understand the combination interactions among these three factors. The classic method of determining optimum conditions by varying one parameter while keeping the other at specified constant level is a single-dimensional, laborious and time-consuming method, often dose not guarantee determination of optimal conditions [12]. In order to overcome these problems, optimization studies have been done using response surface methodology (RSM), a statistically designed experimental protocol in which several factors were simultaneously varied. This multivariate approach has its advantages in terms of reductions in the number of experiments, improved statistical interpretation possibilities and reduced time requirements from overall analysis. RSM has been found to be much successful and economical during optimization of various industrial processes [13]. Easy way to estimate response surface, factorial designs are the most useful schemes for the optimization of variables with a limited number of experiments. A variety of factorial designs are available to accomplish this task. The most successful and best is the central composite design (CCD), which is accomplished by adding two experimental points along each coordinate axis at opposite sides of the origin and at a distance equal to the semi-diagonal of the hyper cube of the factorial design and new extreme values (low and high) for each factor added in the design. The model is also used to predict the result by iso-response contour plot and three-dimensional surface plots [14]. Here two-level-three-factor full factorial central composite design model was used to acquire deeper understanding of the catalytic mechanism of MnP. However, MnPs from different white-rot fungi may have very different properties. For our studies on the properties of MnP, we chose the MnP from *Schizophyllum* sp. F17 which has been previously shown potential for dye decolorization [15].

1 Materials and methods

1.1 Microorganisms and culture conditions

Schizophyllum sp. F17 was isolated from a decayed wood chip pile in the vicinity of Hefei, China. The fungus was cultured on potato dextrose agar (PDA) slants for one week at 28°C. The grown mycelium mat was washed with sterile water. Mycelia obtained from several slants were blended with 100 mL of sterile water in three cycles of 15 s. Five hundred millilitre flasks containing a 100 mL liquid medium were

inoculated with a mycelial suspension and grown at 28°C on a rotary shaker at 130 r/min for 2 days. The liquid medium contained 20 g potato extrace, 2 g dextrose, 0.3 g KH_2PO_4, 0.15 g $MgSO_4$, 1 mg thiamine, and was autoclaved at 121°C for 20 min. Homogenized mycelia, which were obtained after crushing the mycelial pellets in a Waring blender, were used as the inoculums for 250 mL flasks containing 5 g SSF medium. SSF medium containing 90% rice hull and 10% soybean cake meal was humidified with 7.5 g water and autoclaved at 121°C for 20 min [16]. Incubation was carried out at 28°C for 4 days.

To the culture flasks, 100 mL of sodium acetate buffer (10 mmol/L, pH 5.9) was added. Contents were gently beaten and incubated on the rotary shaker at 130 r/min for 30 min. The crude enzyme obtained was then filtered and spun (10 000 r/min, 20 min, 4°C).

1.2 Enzyme activity assay of MnP

The level of MnP activity was determined by monitoring the formation of the Mn^{3+}-lactate complex (ε_{240}= 6500 $M^{-1}cm^{-1}$) at 240 nm at 25°C, during oxidation of 1 mmol/L $MnSO_4$ in 0.1 mol/L sodium lactate(pH 4.5) in the present of 0.1 mmol/L H_2O_2. One unit of MnP was defined as the amount of enzyme producing 1 μmol of the Mn^{3+}-lactate complex per minute[17]. The relative MnP activity was defined as the ratio of the enzyme activity and the corresponding maximum activity. The maximum MnP enzyme activity was 100%.

1.3 Enzyme purification and examination of protein concentration

The crude enzyme was concentrated 20-fold by ultrafiltration (20 kDa-cut-off polyethersultone membranes, Model 8400, Millipore Corporation, USA), then applied to a DEAE-cellulose anion exchange column (Whatman DE52, England) (2.6 cm× 30 cm) previously equilibrated with sodium acetate buffer (10 mmol/L, pH 5.9). The MnP was eluted with a liner gradient of 0–0.5 mol/L NaCl in the same buffer at a low rate of 100 mL/h. The fractions containing MnP activity were collected and concentrated 100-fold using Stirred Ultrafiltration Cell Model 8010 (20 kDa-cut-off polyethersultone membranes, Millipore Corporation, USA). The concentrated samples were loaded onto a Sephadex G-75 gel filtration column (Fluka, USA) (1.7 cm× 100 cm) equilibrated with sodium acetate buffer (10 mmol/L, pH 5.9). The column was run using sodium acetate buffer (10 mmol/L, pH 5.9) at a low rate of 6 mL/h. MnP fractions were pooled and concentrated with centrifugal ultrafiltration units, then analyzed by SDS-PAGE. The enzyme solution was kept at 4°C to be used for characterization experiments.

Protein concentration was estimated using Bradford with crystalline bovine serum albumin (BSA) as standard[18]. Protein concentrations in the fractions from the chromatography were determined from absorbance values at 280 nm.

1.4 H_2O_2 sensitivity and the kinetics of MnP with H_2O_2 and Mn^{2+}

The inactivation of the enzyme in the presence of H_2O_2 was studied as follows: MnP was incubated with 0.1 mmol/L or 1 mmol/L H_2O_2 respectively and the enzyme activities were monitored from 0 to 10 min every minute and afterward, every 5 minutes up to 25 min.

The kinetics of MnP with H_2O_2 and Mn^{2+} was studied as follows: The oxidation of Mn^{2+} was measured at wavelengths of 240 nm. Reaction with all substrates was quantitated in 0.1 mol/L sodium lactate buffer, pH 4.5. The K_m of Mn^{2+} and H_2O_2 were done in 0.1 mol sodium lactate buffer and 0.1 mL MnP using fixed concentrations of H_2O_2 (0.1 mmol/L) and varying Mn^{2+} concentrations (0.001–0.01 mmol/L) or fixed concentrations of Mn^{2+} (1 mmol/L) and varying H_2O_2 concentrations (0.004–0.04 mmol/L). The kinetic data were analyzed using double-reciprocal plots of rates versus substrate concentration.

1.5 The one-factor-at-a-time analysis

1.5.1 *Effect of concentrations of Mn^{2+} on the MnP activity*

MnP activity was measured in 4 mL 0.1 mol/L sodium lactate (pH 4.5) containing 0.1 mL MnP, 0.1 mmol/L H_2O_2 and a predetermined concentration of Mn^{2+} (0.2–2 mmol/L), The reaction was carried out for 5 min at 25°C. All data were the values for at least three samples.

1.5.2 *Effect of concentrations of H_2O_2 on the MnP activity*

To study the H_2O_2 dependence for the enzyme, MnP activity was measured in 4 mL 0.1 mol/L sodium lactate(pH 4.5) containing 0.1mL MnP, 1 mmol/L $MnSO_4$ and a predetermined concentration of hydrogen peroxide H_2O_2 (0.02–0.5 mmol/L), The reaction was carried out for 5 min at 25°C. All data were the values for at least three samples.

1.5.3 *Effect of amounts of MnP on the MnP activity*

MnP activity was measured in 4 mL 0.1 mol/L sodium lactate (pH 4.5) containing 0.1 mmol/L H_2O_2,

1 mmol/L Mn^{2+} and different amounts of MnP (0.05–0.6 mL), The reaction was carried out for 5 min at 25°C. All data were the values for at least three samples.

1.6 Design of experiments

A two-level-three-full factorial Central Composite Design (CCD) was designed to assess the influence of the main factors on the MnP activity, as well as their interactions between those factors. The three factors considered are (ⅰ) concentration of Mn^{2+}(low 0.8 mmol/L and high 1.6 mmol/L) (ⅱ) concentration of H_2O_2(low 0.05 mmol/L and high 0.15 mmol/L) (ⅲ) amounts of MnP(low 0.25 mL and high 0.55 mL).

1.7 Azo dye decolorization by manganese peroxidase

Dye decolorization was measured spectrophotometrically at the following wavelengths: Orange G, 474 nm; Congo red, 506 nm. The decolorization was carried out directly in the spectrophotometer cuvette. The reaction mixture contained sodium lactate buffer (pH 4.5, 100 mmol/L), dye (1 mmol/L) and optimized dosage of Mn^{2+}, H_2O_2 and purified MnP in a total volume of 3 mL. Data were noted every 5 min during the 60 min reaction. Control samples, without H_2O_2, were done in parallel identical conditions.

2 Results

2.1 Physicochemical properties of purified MnP

MnP was the main oxidoreductase produced in solid cultures of *Schizophyllum* sp. F17. Additionally, the fungus produced lower level of LiP and no laccase. The crude MnP was fractionated by anion-exchange chromatography at pH 5.9, then further purified by gel filtration chromatography (Sephadex G-75). The purified protein appeared to be homogeneous when analyzed by SDS-PAGE. The molecular mass of MnP was calculated to be 49.2 kDa under denaturing conditions (Fig.1). The protein concentration of this purified enzyme was 23 μg/mL estimated by Bradford method. The enzyme solution was kept at 4°C to be used for the next experiments.

The susceptibility of MnP to some concentrations of H_2O_2 was studied. The half-life of the MnP in the presence of 0.1 mmol/L or 1 mmol/L H_2O_2 were shown below (Fig.2). When the H_2O_2 concentration was 0.1 mmol/L, the MnP activity decreased by 50% in the first 5–6 min and continued to decrease but at a lower rate. When the H_2O_2 concentration was 1 mmol/L, the MnP activity decreased rapidly by 80% at the beginning of the catalytic process, and remained the same thereafter.

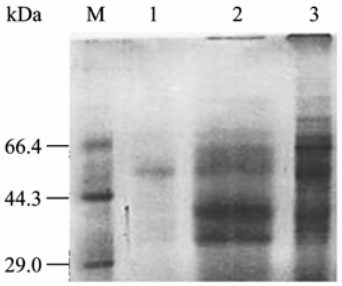

Fig. 1 SDS-PAGE of samples containing MnP from various purification steps, which were stained with Coomassie blue. M: protein standards molecular size markers; 1: purified MnP after gel filtration; 2: concentrated samples after DEAE-Cellulose DE52 column chromatography; 3: crude extract.

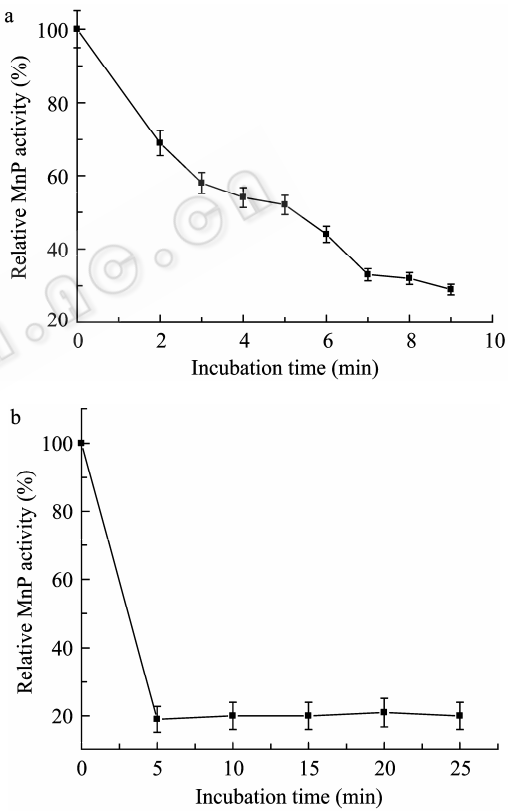

Fig. 2 Stability of MnP at two different concentrations of H_2O_2. (a) 0.1 mmol/L H_2O_2. (b) 1 mmol/L H_2O_2.

The kinetics of MnP with H_2O_2 and Mn^{2+} were also studied. The K_m for H_2O_2 and Mn^{2+} were 5.2 μmol/L and 13.1 μmmol/L respectively. The Km of H_2O_2 was a little higher than that of the MnPs from most *Bjerkandera* species when the K_m of Mn^{2+} was much lower than that of *Bjerkandera* Species [19-22].

2.2 The one-factor-at-a-time analysis

In this section, the single-factor effects of H_2O_2, Mn^{2+} and MnP dosage on MnP enzyme activity were

investigated. The effect of Mn^{2+} on the MnP activity is shown in Fig.3a. As the Mn^{2+} concentration reached 1.2 mmol/L, the MnP had the maximum activity then slowly decreased over the concentration of 1.2 mmol/L. MnP showed its strong activity (more than 80% of the maximum) at a board concentration of H_2O_2 ranging from 0.1 to 0.2 mmol/L. The maximum was observed when the H_2O_2 was 0.1 mmol/L, but decreased sharply at the concentration over 0.25 mmol/L (Fig.3b). The optimum MnP dosage for the enzyme activity was 0.4 mL. The activity increased with the increase in amount of purified enzyme used up to 0.4 mL, however amounts above 0.4 mL decreased the MnP enzyme activity (Fig.3c).

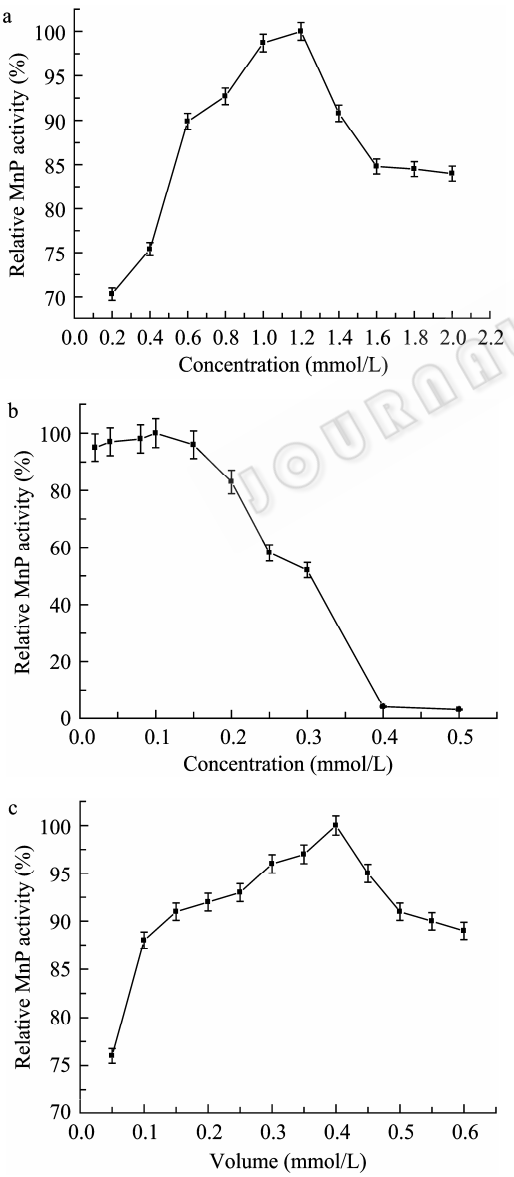

Fig. 3 The effect of single factor on MnP activity. (a) Mn^{2+}. (b) H_2O_2. (c) MnP.

2.3 Response surface factorial design for the optimization of the MnP enzyme activity

In this study, the two-level-three-full factorial Central Composite Design (CCD) experiment was chosen to investigate the factors predominantly affecting the MnP enzyme activity.

Table 1 shows the experiments performed according to the experimental plan and the response thus obtained for each combination of the variables; also in this table is the predicted value from the model.

Table 2 shows the response surface regression results, which give the coefficients for all the terms in the model. The R^2 value was 96.4%, which means 96.4% results of the total variations could be explained by this model. Except square terms, all the P values were very small (<0.05) suggesting that these three factors and there interactions may be important in this model.

Analysis of variance (ANOVA) utilized for statistical testing is shown in Table 3. The p-values (0.000) for Liner and Interaction terms meant these three factors and their interactions had serious effect on the activity of the MnP, and the model was applicable.

Table 1 Two-level-three-full factorial Central Composite Design matrix and experiments results of dependent variables (data processed by MINITAB software)

Run	Mn^{2+}	H_2O_2	Enzyme	Relative MnP activity (%)	Predicted value (%)
1	0.8	0.05	0.25	38.8	41.4
2	1.2	0.1	0.15	39.4	41.499
3	1.2	0.1	0.4	54.7	51.93
4	1.6	0.05	0.25	41.1	40.089
5	0.8	0.15	0.25	50.2	44.341
6	0.53	0.1	0.4	36.9	40.839
7	1.6	0.05	0.55	35.6	40.494
8	1.2	0.02	0.4	23.9	27.173
9	1.87	0.1	0.4	65.3	63.021
10	0.8	0.05	0.55	30.8	26.855
11	1.2	0.1	0.4	52.8	51.93
12	1.2	0.1	0.65	59.5	62.361
13	1.2	0.1	0.4	53.6	51.93
14	1.2	0.1	0.4	54.8	51.93
15	1.2	0.02	0.4	68.1	76.687
16	1.6	0.15	0.25	54.1	57.08
17	1.6	0.15	0.55	100	96.435
18	1.2	0.1	0.4	54.1	51.93
19	1.2	0.1	0.4	56.2	51.93
20	0.8	0.15	0.55	68.7	68.746

Central composite design: factor, 3; base runs, 20; base blocks,1; replications, 1; total runs, 20; total blocks, 1. Two-level factorial: full factorial: cube points, 8; center points in cube, 6; axial points, 6; center points in axial, 0. alpha: 1.68179. Response Surface Regression: relative MnP activity 100% versus Mn^{2+}(mmol/L), H_2O_2(mmol/L), enzyme(mL).

Based on the statistical analysis above, the mathematical expression of relationship to the MnP activity with variables Mn^{2+}, H_2O_2 and enzyme is as follows:

Relative MnP activity (%)=73.3358−21.0042×Mn^{2+}−264.820×H_2O_2−130.669×enzyme−2.07844×$(Mn^{2+})^2$−854.269×$(H_2O_2)^2$−40.7023×$(enzyme)^2$+175.625×Mn^{2+}×H_2O_2+62.2917×Mn^{2+}×enzyme+1298.33×H_2O_2×enzyme (1)

As the square terms were insignificant (5%), they were deleted from the equation, hence a new regression model as follows:

Relative MnP activity (%)=89.0738−25.9924×Mn^{2+}−435.674×H_2O_2−163.235×enzyme+175.625×Mn^{2+}×H_2O_2+62.2917×Mn^{2+}×enzyme+1298.33×H_2O_2×enzyme (2)

The predicted values of relative MnP activity obtained using Eq.(2) were close to the experimental values(see Table 1) proving that the model was applicable.

2.3.1 *Residual plots for the relative MnP activity*

The normal probability plot (Fig.4) showed that the distribution of the residual value, which was defined as the difference between the observed and the predicted, could form a straight line, and these residual value were normaly distributed on both sides of the line. It showed that experimental point was reasonably aligned with the predicted value.

Table 2 Estimated regression coefficient for relative MnP activity

Term	Coef	SE Coef	T	*P*
Constant	54.2408	1.780	30.480	0.000
Mn^{2+}	6.5947	1.181	5.586	0.000
H_2O_2	14.7205	1.181	12.468	0.000
Enzyme	6.2023	1.181	5.523	0.000
Mn^{2+}×Mn^{2+}	−0.3326	1.149	−0.289	0.778
H_2O_2×H_2O_2	−2.1357	1.149	−1.858	0.093
Enzyme×enzyme	−0.9159	1.149	−0.797	0.444
Mn^{2+}×H_2O_2	3.5125	1.543	2.277	0.046
Mn^{2+}×enzyme	3.7375	1.543	2.423	0.036
H_2O_2×enzyme	9.7375	1.543	6.312	0.000

R-Sq = 96.4%; *R*-Sq(adj) = 93.2%

Table 3 Analysis of variance for relative MnP activity

Source	DF	Seq SS	Adj SS	Adj MS	F	*P*
Regression	9	5120.62	5120.62	568.96	29.89	0.000
Liner	3	4078.63	4078.63	1359.54	71.41	0.000
Square	3	72.99	72.99	24.33	1.28	0.334
Interaction	3	969.00	969.00	323.00	16.97	0.000
Residual error	10	190.38	190.38	19.04		
Pure error	5	6.77	6.77	1.35		
Total	19	5311.00	5311.00			

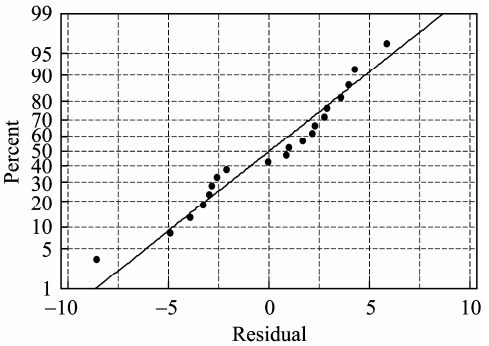

Fig. 4 The normal probability plot.

Fig.5 shows that the residuals distribute randomly about zero, indicating that the regression terms were incorrelated with one another. This plot rules out the impact of order which may influence the results.

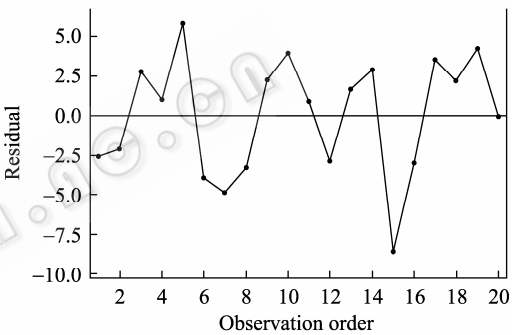

Fig. 5 Residual versus the order of the data.

2.3.2 *Pareto chart for the effect of different factors on the MnP activity*

Mn^{2+}, H_2O_2 and enzyme itself, as well as there interactions among them are main factors that decide the enzyme activity of MnP, In this work, we tried to find out the most important factor for the enzyme activity by Pareto chart (Fig.6). The Pareto Chart is a vertical bar chart, the graph shows the relative frequency of numerical way from left to right in descending order. Plato can be effectively applied to analysis of the primary concerns. Thus we found that the concentration of H_2O_2 was the most important

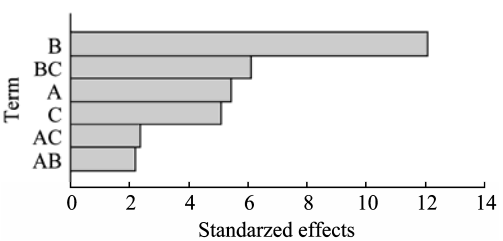

Fig. 6 Pareto chart of the standardized effects. A: concentration of Mn^{2+}(mmol/L); B: concentration of H_2O_2(mmol/L); C: amounts of enzyme(mL).

factor for the MnP, as well as the interaction between H_2O_2 and enzyme. The liner terms owned the stronger ability to influence the MnP activity then the interaction terms. According to the Pareto chart, the effect of different factors from serious to minimal was:

H_2O_2> (H_2O_2×enzyme) >Mn^{2+}> enzyme > (Mn^{2+}× enzyme) > (Mn^{2+}×H_2O_2)

2.3.3 *The three-dimensional response surface plots and Interaction Plot for the mutual effect between two variable factors*

The three-dimensional response surface plots are the graphical representations of the regression equation. The main goal of response surface is to track efficiently for the optimum values of the variables such that the response is maximized [23]. The surface plots (Fig.7a-c) were in 3D graphs in which the relative MnP activity was represented by varying two of the three main factors which have effect on the enzyme activity.

It was shown that in different concentrations of H_2O_2, the MnP had larger activity when the concentration of Mn^{2+} was higher (Fig.7a). According to the sharply slope, H_2O_2 had more significant effect on the MnP activity compared with Mn^{2+}.

From Fig.7b it was shown that in different amounts of MnP, the MnP activity became larger when the model had more Mn^{2+}. However, when the concentration of Mn^{2+} was low, the change of enzyme activity was significant if the amounts of enzyme increased.

Fig.7c showed the combined effect of varying concentrations of H_2O_2 and amounts of MnP on the MnP activity. From this response surface plot it was shown that relative MnP activity could reach its maximum if the concentration of H_2O_2 should be near 0.15 mmol/L and amounts of enzyme should be 0.55 mL.

The interaction plot shows the change of the one factor if the other one is changed between the two relative variables. It is very important to study the plot because it can enlarge or eliminate the effect of the main effect. Two variables' interactions are the lines of different slope. (Fig.8) The graph shown in Fig.6 confirmed the model of three-dimensional response surface plots which reflected the relationship between the variables.

2.4 Studies on the decolorization of azo dyes by MnP

Azo dyes such as Orange G, Congo red were treated with optimized dosage of Mn^{2+}(1.6 mmol/L), H_2O_2(0.15 mmol/L) and purified MnP(0.42 mL). The

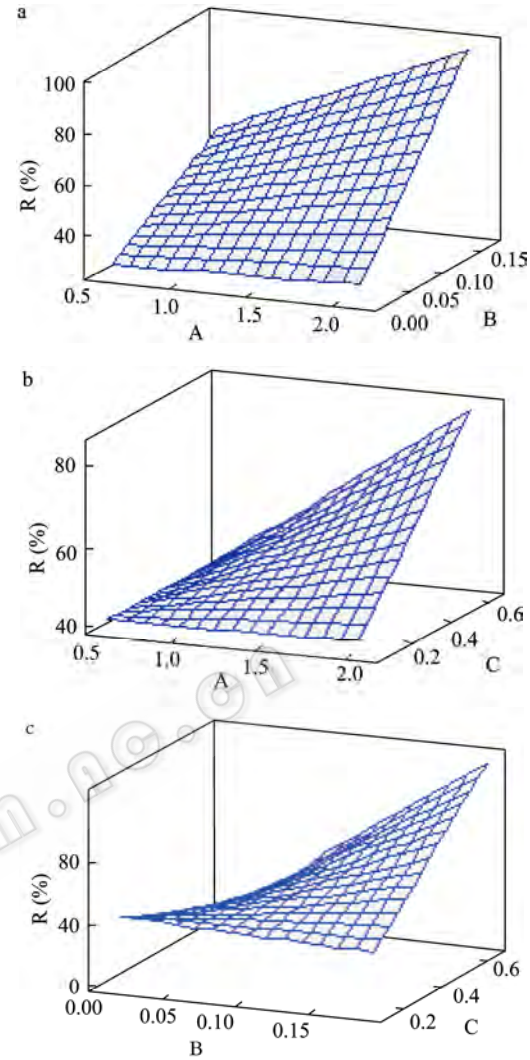

Fig. 7 (a) 3D surface plot of the combined effect of concentration of Mn^{2+} and concentration of H_2O_2 on relative MnP activity. (b) 3D surface plot of the combined effect of amounts of enzyme and concentration of Mn^{2+} on relative MnP activity. (c) 3D surface plot of the combined effect of amounts of enzyme and concentration of H_2O_2 on relative MnP activity. R: relative MnP activity; A: concentration of Mn^{2+} (mmol/L); B: concentration of H_2O_2 (mmol/L); C: amounts of enzyme (mL).

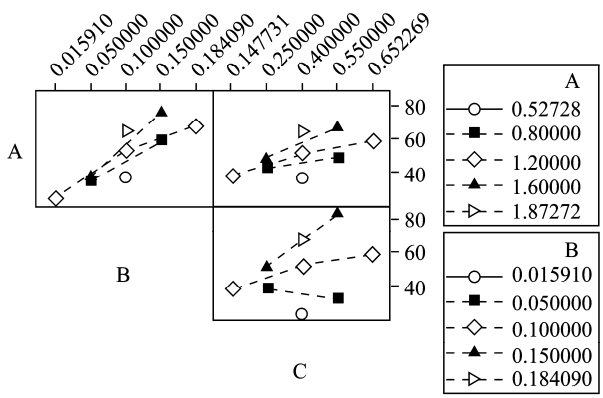

Fig. 8 Interaction Plot for MnP activity. A: Mn^{2+} (mmol/L); B: H_2O_2 (mmol/L); C: MnP (mL).

results of decolorization are shown in Fig.9. The decolorization for Orange G reached nearly 35% after 60 min of treatment as well as for the Congo red. The purified MnP was able to play a role in the decolorization of the azo dyes.

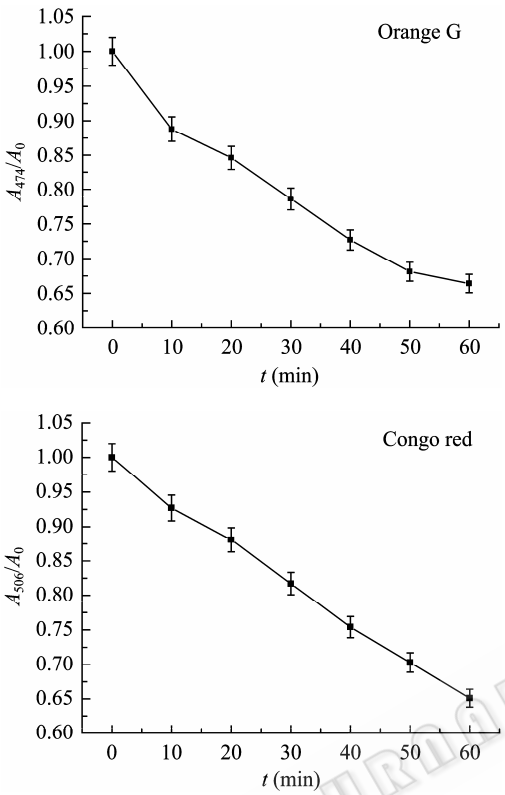

Fig. 9 Decolorization of azo dyes by MnP.

3 Conclusions

Statistically designed experimentation has been applied in optimization of medium for MnP production as well as in the enzymatic decolorization with MnP. But to our knowledge little attention has been paid to the interactions among those factors which had serious effect on the MnP activity.

In this study, the statistically process analyzed the key factors and their interactions in the biochemical reaction, which was useful to investigate the enzymatic mechanism of MnP. H_2O_2 was proved to be the most important factor in the action, so did its interactions with the amounts of the enzyme. But the MnP is easily inactivated by H_2O_2 present in the action. Meanwhile the half-life time of MnP presented in H_2O_2 is too short to effectively apply to the manufacture. However, this powerful oxidant[24] is potentially valuable for some applications, such as in the pulp and paper industries and the degradation of environmental pollutants[25-27]. Therefore, investigation of the effect of H_2O_2 on the MnP is desirable for practical use. Here a model was set up by central composite design which can predict the value of MnP activity and show the effect of Mn^{2+}, H_2O_2, amounts of enzyme on the reaction, so do their inactions.

According to the result of decolorization experiment, the purified MnP has a good potential for the application in the optimized system.

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