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Triphenylmethane dye decoloration using hydrogen peroxide-resistant manganese peroxidase

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Abstract: [Objective] We produced a novel manganese-dependent peroxidase (MnP) by *Trametes* sp. SQ01 and studied its resistance of MnP to hydrogen peroxide, substrate specificity and ability of decolorizing triphenylmethane dyes. **[Methods]** MnP was purified through acetone precipitation and DEAE-celluose 52 anion-exchange chromatography. The resistance of MnP against H_2O_2 and dye decolorization were measured with a UV-visible spectrophotometer. **[Results]** The homogenous MnP has been obtained through two-step purification. The optimum pH and temperature for the purified MnP were 4.5 and 70 °C, respectively. The enzyme was highly stable in the pH range 3.0−8.0. The enzyme oxidizes various substrates in the presence of manganese, such as 2,6-dimethoxyphenol, guaiacol, 2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) and H_2O_2, simultaneously, the MnP also regards Mn^{2+} as its substrate. Among all the substrates tested, the optimum was H_2O_2 (K_m, 3.7 μmmol/L). Interestingly, the enzyme was resistant to H_2O_2 bleaching. The enzyme retains 70% integrity of its heme after incubation with 2.5 mmol/L H_2O_2 for 60 min. All of the tested dyes could be discolored by MnP, among which Crystal violet was observed with the highest decolorization rate at 65.8%. The effects of Mn^{2+} and H_2O_2 on dye decolorization by MnP were also studied, which revealed that both H_2O_2 and Mn^{2+} had less influence on the decolorization of Remazol Brilliant Blue R (RBBR) than Malachite Green. **[Conclusion]** The resistance of MnP to hydrogen peroxide and its ability of decolorizing triphenylmethane dyes demonstrated its potential application on dye decolorization.

Keywords: Manganese peroxidase (MnP), Hydrogen peroxide, Triphenymethane dye, Dye decolorization

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耐过氧化氢的锰过氧化物酶对三苯甲烷类染料的脱色

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摘 要: 【目的】从一株白腐菌 *Trametes* sp. SQ01 中获得一种新型的锰过氧化物酶, 探讨该酶的底物特异性和对过氧化氢的耐性, 以及其对三苯甲烷类染料的脱色能力。【方法】通过丙酮沉淀和 DEAE-cellulose 52 柱层析法纯化锰过氧化物酶。利用 UV-2010 紫外可见分光光度法研究锰过氧化物酶对过氧化氢的耐性, 同时, 用紫外可见分光光度计对三苯甲烷类染料脱色效果进行分析。【结果】通过两步纯化, 获得了均一性的锰过氧化物酶。该酶的最适 pH 和温度分别是 4.5 和 70 °C, 在 pH 3.0−8.0 时, 酶活相对稳定。该酶在二价锰离子存在下能够氧化 2,6-二甲氧基苯酚、愈创木酚、2,2′-连氮-双-(3-乙基苯并噻唑啉磺酸)和过氧化氢等化合物, 同时也能作用二价锰离子。在与这些底物反应中, 最适底物为过氧化氢(K_m 为 3.7 μmmol/L)。该酶具有抗过氧化氢漂白能力, 锰过氧化物酶与高浓度的过氧化氢(2.5 mmol/L)作用 60 min 后仍能保持 70%的活性。在所测试的染料中, 锰过氧化物酶对结晶紫的脱色率最高达到 65.8%。二价锰离子和过氧化氢对锰过氧化物酶脱色能力的影响进行研究, 与孔雀绿相比, 锰离子和过氧化氢对活性艳蓝脱色的影响很小。【结论】*Trametes* sp. SQ01 锰过氧化物酶对过氧化氢的耐受性, 以及对三苯甲烷类染料的高效脱色能力表明该酶在染料脱色降解方面有着广阔的应用前景。

关键词: 锰过氧化物酶, 过氧化氢, 三苯甲烷类染料, 染料脱色

Synthetic dyes are extensively used in textile dyeing, paper, printing, color photography, pharmaceutics, cosmetics, and other industries. The versatile azo and triphenylmethane dyes account for most of the textile dyestuffs produced, thereinto, triphenylmethane dyes are known to be highly toxic to mammalian cells, mutagenic and carcinogenic to humans[1]. Human contact with Malachite Green causes skin irritation and eye injury[2]. Crystal Violet is toxic to mammalian cells and is also a mutagen and mitotic poison[3].

In recent years, increasing interest has arisen regarding the ability of white rot fungi to produce extracellular ligninolytic enzymes, such as MnP from *Phanerochaete sordida* and white rot fungi L-25[4], lignin peroxidase (LiP) and laccase, which are responsible for the degradation of various xenobiotic compounds including dyes[5]. However, few reports have focused on the decolorization of triphenylmethane dyes by MnP.

MnP is considered as the key enzyme in the degradation of lignin and aromatic xenobiotics. MnP catalyzes the oxidation of Mn^{2+} into Mn^{3+} by utilizing H_2O_2 and forms complexes with an organic acid. The Mn^{3+} chelate complex is a highly reactive non-specific oxidant capable of oxidizing a variety

of environmental pollutants[6]. This powerful oxidant has been proposed to be important for the degradation of sterically bulky compounds that are unable to gain access to the active site of peroxidases. This mediated system of degradation is potentially valuable for applications, such as in the pulp and paper industry and in the degradation of synthetic polymers and environmental pollutants. However, MnP is very sensitive to inactivation by H_2O_2. In terms of industrial applications, the stability of MnP in the presence of high H_2O_2 concentrations is very important. MnP requires H_2O_2 for its activity, but, H_2O_2 also inactivates MnP quickly at high concentrations[7]. The MnP from *Lenzites betulinus* reportedly retains more than 60% of the initial activity after 5 min of exposure to 10 mmol/L hydrogen peroxide at 37 °C[8]. Some attempts to increase the resistance of MnP to H_2O_2 has also been reported, such as the engineering of the H_2O_2-binding pocket of a recombinant manganese peroxidase for H_2O_2 resistance[4] and improvement of H_2O_2 stability of manganese peroxidase by combinatorial mutagenesis and high-throughput screening using *in vitro* expression with protein disulfide isomerase[9].

In a previous study, we reported the decolorization of some dyes by a newly isolated *Trametes* sp. SQ01 and its laccase[10]. In the present study, we describe the purification and characterization of a new MnP, which retains certain amount of activity under high H_2O_2 concentration for an hour. In addition, the role of MnP in the decolorization of five triphenylmethane dyes was investigated *in vitro* in enzymatic experiments.

1 Materials and methods

1.1 Chemicals

The 2,2′-azio-di-(3-ethylbenzthiazolie sulfonic acid) (ABTS) was purchased from Sigma (St. Louis, Missouri). Five triphenylmethane dyes (Malachite Green, Crystal Violet, Cresol Red, Bromophenol Blue and CBB G-250) and RBBR were of analytical grade.

1.2 Culture medium

The *Trametes* sp. SQ01 was maintained on potato dextrose agar (PDA)[11]. The growth medium for the production of MnP and the decolorization of dyes was prepared with 10 g/L glucose as the carbon source. An inoculum of *Trametes* sp. SQ01 for liquid culture was prepared as follows: five agar plugs (5 mm in diameter) punched from the periphery of a 7 days of agar plate were cultivated in a 250 mL flask containing 50 mL culture solution. The flask was incubated for 7–8 days at 30 °C in a shaking incubator (170 r/min).

1.3 Enzyme activity assay

MnP activity was determined through the formation of Mn^{3+}-tartrate complex [ε_{238}= 6 500 mol/(L·cm)][4], during the oxidation of 0.1 mmol/L $MnSO_4$ in 20 mmol/L sodium tartrate (pH 4.5), using 0.1 mmol/L H_2O_2. One unit of enzymatic activity was defined as the amount of enzyme that transforms 1 μmol/L of substrate per minute.

1.4 Purification of MnP

For the preparation of extracellular MnP, *Trametes* sp. SQ01 was cultivated as described above. Unless otherwise stated, all procedures were performed at 4 °C. The supernatant liquid was obtained by centrifugation of the culture broth at 12 000×*g* for 20 min. Proteins were precipitated with pre-chilled acetone (−20 °C) from 25% to 66% saturation (*V*/*V*) and incubated at −20 °C for 6 h, followed by centrifugation at 12 000×*g* for 30 min. The proteins were dissolved in 20 mmol/L Tris-HCl buffer (pH 7.0) and dialyzed against the same buffer. After dialysis, the sample was centrifuged, and the resulting supernatant liquid was loaded onto a DEAE-cellulose 52 chromatography column (26 mm×150 mm; Amersham) previously equilibrated with 20 mmol/L Tris-HCl buffer (pH 7.0). The column was washed at a flow rate of 2 mL/min with 200 mL buffer and eluted with a linear gradient of 0 to 0.6 mol NaCl in the same buffer. The elution of absorbing material form the column was simultaneously monitored at 280 nm and 406 nm to detect total protein and heme, respectively. Fractions containing MnP activity were pooled, desalted, and stored at 4 °C until further use.

1.5 Characterization of MnP

The molecular mass of the enzyme was deter-

mined by sodium dodecyl sulfate polyacrylamide gel electrophoresis. After electrophoresis, the gels were stained and the protein bands were visualized with Coomassie Brilliant Blue R-250. For the determination of molecular weight, a low-molecular-weight protein calibration kit was used. The enzyme absorbance spectrum of MnP was determined by scanning the absorbance of the enzyme at wavelengths ranging from 350 nm to 700 nm using a UV-2010 spectrophotometer.

1.6 Effect of pH and temperature on the activity and stability of MnP

To determine the optimum temperature and pH of the purified MnP, enzyme activities of MnP were assayed at different temperatures ranging from 25 °C to 80 °C, and with different pH values (pH 3.0−8.0) under standard conditions, respectively. Thermal stability was investigated by incubating the enzyme at 20 °C, 30 °C, 40 °C, 50 °C, 60 °C, 70 °C and 80 °C for 30 min. Immediately afterwards, the enzyme was immersed in an ice bath and the residual activity was tested at room temperature. The pH stability was assayed by incubating the enzyme at different pH (pH 3.0−8.0) for 12 h at room temperature. The remaining activities were measured under standard conditions.

1.7 Effect of metal ions, surface active agent, and inhibitor on the activity of MnP

To determine the effect of the metal ions (Cu^{2+}, Zn^{2+}, Mn^{2+}, Mg^{2+}, Ca^{2+}, Co^{2+}), surface active agent, and inhibitor (SDS, Tween-80, EDTA, DTT), MnP solutions were incubated in the presence of 1 mmol/L and 5 mmol/L chemicals for 30 min in 0.1 mol/L tartrate buffer (pH 4.5) at 25 °C. MnP activity was measured as described above.

1.8 Resistance of MnP to H_2O_2

MnP (2 μmol/L) was incubated with 2.5 mmol/L H_2O_2 in 20 mmol/L sodium tartrate (pH 4.5) at room temperature for 1, 2, 4, 10, 20 and 60 min. The solution without H_2O_2 was designated as the control. The absorbance was recorded using a UV-visible spectrophotometer from 380 nm to 440 nm.

1.9 Kinetic analysis

Steady-state kinetic constants were obtained by measuring the initial rates of various substrates at 30 °C in 20 mmol/L sodium tartrate buffer, pH 4.5. The kinetic constants of MnP activities for H_2O_2 and Mn(II) were calculated through the formation of Mn(III) malonate complex at 238 nm. The rates of oxidation of phenolic [6-dimethoxyphenol (DMP) and guaiacol] and non-phenolic (ABTS), were measured at wavelengths of 469, 420 and 465 nm, respectively.

1.10 Treatment of dyes with *Trametes* sp. SQ01 MnP

Stirred dye solutions (20 g/L) buffered with 0.1 mol/L sodium tartrate, pH 5.0, were incubated with 20 μL of *Trametes* sp. SQ01 MnP (final concentration 0.5 U/mL) at room temperature. Dye decolorization was measured in a UV-visible spectrophotometer at different times, and the percentage of dye decolorization was calculated from these data.

The experiments were performed in triplicate with one controls (without enzyme added). All data represent the mean of three triplicate tubes, and error bars represent standard deviation.

1.11 Effect of the Mn^{2+} and H_2O_2 on the decolorization

Decolorization of malachite green and RBBR was performed by measuring A_{615} and A_{595}, respectively. The mixtures contained sodium tartrate buffer (pH 4.5, 100 mmol/L), $MnSO_4$ (1 mmol/L), H_2O_2 (0.1 mmol/L), Dye (100 mg/L RBBR, 20 mg/L malachite green) and 0.5 U/mL MnP in a total volume of 1 mL, or without $MnSO_4$ or H_2O_2. The mixtures were complemented with the sodium tartrate buffer when without the H_2O_2 or $MnSO_4$. The reaction mixtures were incubated for 7 hours at 30 °C. Control samples, without H_2O_2 and $MnSO_4$, were done in parallel identical conditions.

2 Results and discussion

2.1 Purification of MnP

After acetone fractionation and DEAE-cellulose 52 column chromatography, the enzyme was purified homogeneously, which appeared as a single protein band on the SDS-PAGE gel with a molecular weight of 44 kD (Fig. 1). This value falls within the range of molecular weight values for the MnP

family (38 kD to 50 kD)[12]. The molecular weight of purified MnP is almost identical to those of *Nematoloma frowardii* b19 (44 kD) and *Bjerkandera adusta* (44 kD) MnP[6,13].

2.2 Characterization of MnP

The absorption spectrum of the purified protein shows a maximum absorbance peak at 406 nm, typical for hemoglobin (Fig. 2). The figure shows a single, symmetrical peak at 406 nm similar to the MnP from *Schizophyllum* sp. F17, which was also detected by heme absorbance at 409 nm[14]. Its absorbance at 520 nm and 550 nm were also observed, which are similar to the MnP-LiP hybrid isozyme produced by *Bjerkandera* strain BOS55 oxidized by hydrogen peroxide[15]. With Mn^{2+} as a substrate, the MnP activity is relatively stable at the 3.0−8.0 pH range, which is a wide range for a MnP. The optimum pH for the enzyme was 4.5. The optimum temperature for substrate oxidation was 70 °C. The purified enzyme was stable at temperatures ranging from 25 °C to 50 °C for 30 min. At 4 °C, the enzyme was stable for at least 3 months. Compared with that from *Schizophyllum* sp. F17[14], the enzyme from *Trametes* sp. SQ01 showed greater stability at temperatures up to 60 °C.

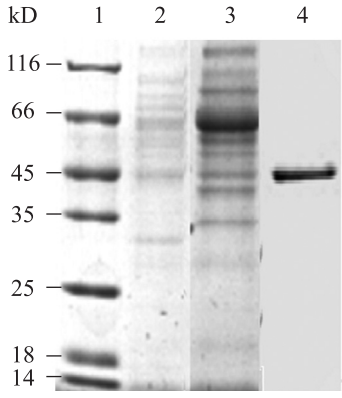

Fig. 1 SDS-PAGE analysis of MnP

图 1 锰过氧化物酶纯化的 SDS-PAGE 分析

Note: 1: Protein marker; 2: Crude enzyme; 3: Acetone precipitation; 4: Purified MnP.

注: 1: 蛋白分子量标准; 2: 粗酶液; 3: 丙酮沉淀; 4: 纯化后的锰过氧化物酶.

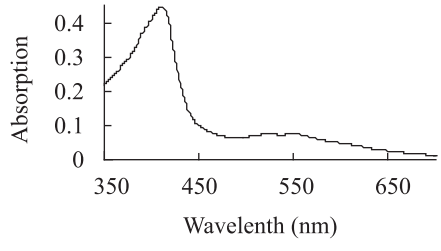

Fig. 2 The spectrum of purified MnP in the visible region

图 2 纯化后的锰过氧化物酶在可见光区域的光谱

Fakoussa and Hofrichter reported that the pH range for MnP was between 2.0 and 6.0, with an optimum range between 4.0 and 4.5[16], but the *Irpex lacteus* MnP was an exception, which has an optimum pH of up to 5.5[17]. At pH ranging from 4.0 to 7.6, the relative activity of the *I. lacteus* MnP was 39%–59% of the maximum activity (maximum activity=100%, pH=5.5), whereas at pH 3.0, it was only 3%[8]. However, the MnP from *Trametes* sp. SQ01 showed more than 50% activity in a wider rage (pH 4.0−8.0), which is a desirable feature for an enzyme for various industrial applications, especially under neutral pH.

2.3 Effect of metal ions, surface active agent and inhibitor on the activity of MnP

The effects of several chemicals on MnP activity were determined (Table 1). MnP was completely inhibited by EDTA, similarly, treatment of the enzyme with DTT also resulted in a complete loss of enzyme activity, which is similar to findings for *Phanerochaete chrysosporium* MnP[18]. Zn^{2+} and Mg^{2+} did not alter MnP activity. No activity loss was observed with MnP activity in the presence of Ca^{2+} and Co^{2+} at low concentrations (1.0 mmol/L), but was strongly inhibited at 5.0 mmol/L, with a relative activity of less than 40%. As a componet of MnP, Mn^{2+} did not affect MnP activity as well. MnPs are generally abundant in wood and soils, which are the natural habitats of white rot fungi. The physiologic chelator of Mn^{2+} in the fungal environment is thought to be oxalate, produced by the fungus at millimolar concentrations[19]. The proposed role of Mn^{2+} chelators in the MnP mechanism is the release Mn^{3+} from the enzyme. Additionally, chelators were suggested to stabilize the Mn^{3+} in solution. Partial inhibition was observed using 1 mmol/L SDS, in contrast, the enzyme activity was substantially increased by Tween-80 at the same

concentration, which was 2.5 times that of the control. However, when the concentration of both compounds was increased to 5 mmol/L, the enzyme activity was completely inhibitive. Ürek also reported that the highest MnP activity was obtained at low concentrations[20].

2.4 Substrate specificities of purified enzyme

MnP was able to oxidize different phenolic (DMP and guaiacol) and non-phenolic (ABTS) substrates in the presence of Mn^{2+}, and can also act on Mn^{2+} and H_2O_2 as substrates. Table 2 shows the kinetic constants of the MnP-catalyzed oxidation of the five substrates ABTS, 2,6-dimethoxyphenol, guaiacol, Mn^{2+} and H_2O_2. The MnP exhibited a similar K_m for both phenolic and non-phenolic substrates, which were much higher than Mn^{2+} and H_2O_2. The lowest K_m value (3.7 μmol/L) was attributed to H_2O_2. The highest K_m value was obtained with ABTS (200 μmol/L) followed by 2,6-dimethoxyphenol (167 μmol/L). For Mn^{2+}, the K_m value obtained from the *Trametes* sp. SQ01 MnP was at an intermediate level, higher than almost all *Bjerkandera* MnPs, but lower than the *Schizophyllum* sp. F17 MnP (Table 3). The K_m value of the MnP for H_2O_2 was slightly higher than that of the MnP from *Bjerkandera* sp. BOS1[21], and lower than those of most *Bjerkandera* spicece and *Schizophyllum* sp. F17[14−15, 22−23] (Table 3).

2.5 Resistance of MnP to H_2O_2

The mixture of 2.5 mmol/L H_2O_2 and MnP exhibited maximum absorption at 406 nm, which was the characteristic electronic absorption maxima for the heme group of compound III in the MnP. The absorption reduced greatly after the reaction for 1 min, and decreased from 0.37 to 0.29. After 2 min to 60 min, the absorbance decreased slightly, and achieved a value of 0.265 at final (Fig. 3). SQ01 MnP is thus demonstrated to be capable of enduring a certain amount of H_2O_2, which is unique among MnPs reported other species. The enzyme from

Table 1 Effects of some metal ions, surfactants, and inhibitors on the activity of MnP
表 1 金属离子、表面活性剂和抑制剂对 MnP 活性影响

Metal ions 金属离子	Relative activities 相对活性		Surfactants/Inhibitors 活性剂/抑制剂	Relative activities 相对活性	
	1 mmol/L	5 mmol/L		1 mmol/L	5 mmol/L
Control	100	100	SDS	82	2
Cu^{2+}	42	23	Tween-80	248	0
Zn^{2+}	100	100	EDTA	2	0
Mn^{2+}	110	97	DTT	8	0
Mg^{2+}	94	97			
Ca^{2+}	94	61			
Co^{2+}	97	32			

Table 2 Kinetic parameters for MnP from *Trametes* sp. SQ01
表 2 *Trametes* sp. SQ01 MnP 的动力学参数

Substrate 底物	Concentration 底物浓度 (mmol/L)	Wave length 最大波长 (nm)	K_m 米氏常数 (μmol/L)	K_{cat} 转化数 (s^{-1})	K_{cat}/K_m 转化效率 [L/(mol·s)]
ABTS	0.5	420	200.0	12.00	6.00×10^4
2,6-DMP	0.5	469	167.0	8.25	4.90×10^4
Guaiacol	0.5	387	200.0	2.25	1.13×10^4
Mn^{2+}	0.1	238	30.0	58.00	1.90×10^6
H_2O_2	0.1	240	3.7	10.00	2.70×10^6

Table 3 Comparison of K_m (Mn^{2+}, H_2O_2) of MnP from different fungi
表 3 不同真菌 MnP 的 K_m (Mn^{2+}, H_2O_2)的比较

Enzyme 酶	K_m 米氏常数(μmol/L Mn^{2+})	K_m 米氏常数(μmol/L H_2O_2)
Schizophyllum sp. F17 MnP	35.2	6.7
Bjerkandera sp. BOS55 MnP	51.0	31.0
Bjerkandera sp. BOS1 MnP	16.5	3.1
Bjerkandera sp. BOS2 MnP	25.4	4.7
Bjerkandera adusta DSM11310 MnP1	20.0	5.5
Bjerkandera adusta UAMH8258 MnP	17.0	4.5

Lentinula edodes was very stable in the presence of up to 0.25 mmol/L H_2O_2. However, at higher H_2O_2 concentrations, its MnP activity rapidly decreases[2]. In contrast, the characteristic electronic absorption maxima for the heme group of compound III in the MnP from *P. chrysosporium* disappeared during further incubation with H_2O_2 for up to 60 min, which indicates that the enzyme is irreversibly inactivated[24]. In terms of industrial applications, the MnP stability at high H_2O_2 concentrations is a very important property. Compared with MnPs from other white rot fungi, the *Trametes* sp. SQ01 MnP purified in this study appeared to be more resistant to high H_2O_2 concentrations.

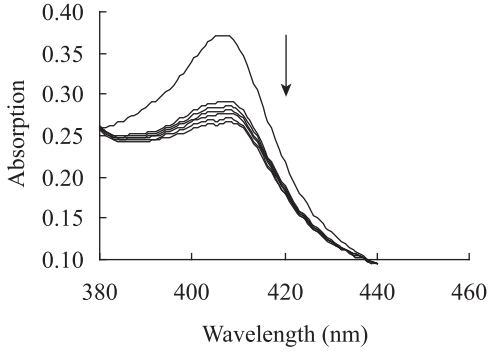

Fig. 3 Heme bleaching of MnP by excess H_2O_2
图 3 过量 H_2O_2 对 MnP 亚铁血红素的漂白

Note: Reaction mixtures contained 2 μmol/L MnP and 2.5 mmol/L H_2O_2 in 20 mmol/L sodium tartrate buffer, pH 4.5. The spectra were recorded after the addition of H_2O_2 at 1, 2, 4, 10, 20 and 60 min in the sequence shown by the arrow.

注: 在 20 mmol/L、pH 4.5 的酒石酸钠缓冲液中含有 2 μmol/L MnP 和 2.5 mmol/L H_2O_2. 在反应体系中添加 H_2O_2 后依次检测 1, 2, 4, 10, 20 和 60 min 后 MnP 的吸收谱.

2.6 Treatment of dyes with *Trametes* sp. SQ01 MnP

Decolorization of various triphenylmethane dyes was conducted using the *Trametes* sp. SQ01 MnP (Fig. 4). The *Trametes* sp. SQ01 MnP showed the highest decolorization rate against Crystal Violet at 71% (as compared with other triphenylmethane dyes: Cresol Red 57%, Malachite Green 43%, CBB G-250 32%, and Bromophenol Blue 31%). The five tested triphenylmethane dyes have similar decolorization rates, which could be due to their similar structure. Until now, most reports on the biodegradation of triphenylmethane dyes are about strain decolorization. For example, *Aeromonas hydrophila* was used to decolorize Crystal Violet and Malachite Green, achieving decolorization efficiencies of more than 90% by cultivation for

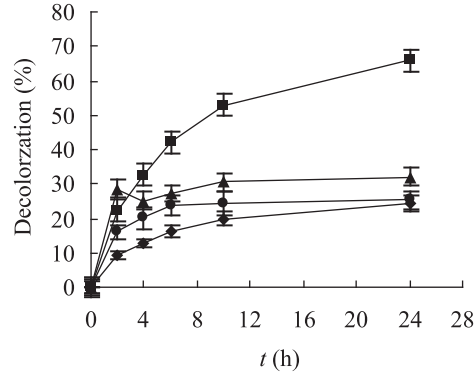

Fig. 4 Decolorization of dyes by MnP
图 4 MnP 对染料的脱色

Note: ◆: Crystal Violet; ▲: CBB G250; ●: Cresol Red; ■: Malachite Green.

注: ◆: 结晶紫; ▲: 考马斯亮蓝 G250; ●: 甲酚红; ■: 孔雀绿.

one day using an initial dye concentration of 50 mg/L[25]. Liu et al. investigated the decoloration of four triphenylmethane dyes by *Fome lignosus*, after 10 days, the drcolorizaton percent achieved 66%−99%[26].

We studied the effect of Mn^{2+} and H_2O_2 on MnP during decolorization using one-factor-at-a-time analysis in 7 hours (Fig. 5). Compared with the control, the single addition of H_2O_2 could not increase rate of decolorization of RBBR, while incubated with Mn^{2+} and MnP, the rate of decolorization of RBBR increased to 67%. When incubated with Mn^{2+}, H_2O_2 and MnP, the rate of decolorization of RBBR was 66.67%. It was suggested that Mn^{2+} had greater influence on the decolorization of RBBR than that of H_2O_2. On the contrary, for Malachite Green, the rate of decolorization was almost not changed with the addition of H_2O_2 or Mn^{2+} compared to control. However, the rate of decolorization of Malachite Green in the presence of H_2O_2 and Mn^{2+} was markedly higher than that of individual H_2O_2 or Mn^{2+}, in the case, the rate of decolorization increased from 5.87% to 37.65%. These results suggested that Mn^{2+} and H_2O_2 were an essential cofactors for decolorization of Malachite Green by MnP.

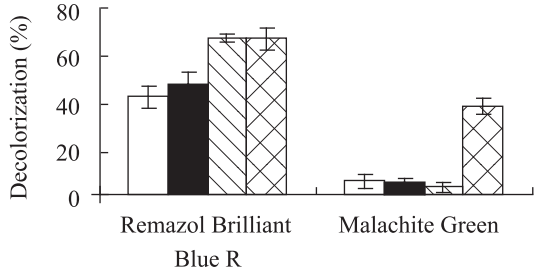

Fig. 5 One-factor-at-a-time analysis

图 5 单因子单次实验分析

Note: □: Decolorization with none of the H_2O_2 and Mn^{2+} (control); ■: Decolorization with no Mn^{2+}; ▨: Decolorization with no H_2O_2; ▩: Decolorization with both of the H_2O_2 and Mn^{2+}.

注: □: 反应体系中无 H_2O_2 和 Mn^{2+}的染料脱色(对照); ■: 反应体系中无 Mn^{2+}的染料脱色; ▨: 反应体系中无 H_2O_2 的染料脱色; ▩: 反应体系中含有 H_2O_2 和 Mn^{2+}的染料脱色.

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