Jurnal Teknologi

Full paper

Biodegradation of Dye Using Free Laccase and Sol-gel Laccase

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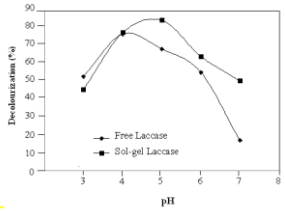
Article history

Received :18 May 2012
Received in revised form :18 July 2012
Accepted :13 August 2012

Graphical abstract



Abstract

Malachite green oxalate dyes are of synthetic origin and their environmental existence is not well understood. They are resistant to direct aerobic bacterial degradation and form potentially carcinogenic aromatic amines. This study shows that applying the oxidative processes of enzymatic treatment with free laccase and sol-gel laccase could lead to dye degradation. The degradation of dye malachite green oxalate using free laccase and sol-gel laccase, respectively were 37% and 13% at 1 hour reaction. The optimum pH for the free laccase and sol-gel laccase respectively were at pH 5 and pH 6. The optimal temperature for degradation of dye by sol-gel laccase was at 40°C. These results showed that free laccase and sol-gel laccase have good performance in the degradation of malachite green oxalate dyes.

Keywords: Free laccase; entrapment; sol-gel laccase; biodegradation; malachite green oxalate

Abstrak

Pewarna oxalate hijau Malachite adalah sintetik asli dan kewujudan semulajadinya tidak begitu diketahui. Ia bersifat kalis degradasi bakteria aerobik dan membentuk amina aromatik yang berpotensi karsinogen. Kajian ini menunjukkan bahawa penggunaan proses oksidatif untuk rawatan enzim dengan lakase bebas dan lakase sol-gel boleh membawa kepada degradasi pewarna. Degradasi pewarna malachite hijau oxalate yang menggunakan lakase bebas dan lakase sol-gel, masing-masing adalah sebanyak 37% dan 13% pada 1 jam reaksi. pH optimum untuk lakase bebas and lakase sol gel, masing-masing adalah pada pH 5 dan pH 6. Suhu optimum bagi degradasi pewarna oleh lakase sol-gel adalah pada 40°C.

Kata kunci: Lakase bebas; pemerangkapan; lakase sol-gel; penguraian biologi; malachite hijau oxalate

1.0 INTRODUCTION

Wastewater from textile industries is a serious environmental problem all over the world. Many dyes are difficult to decolorize due to their complex structure and synthetic origin. A variety of physical, chemical and biological methods are presently available for the treatment of textile wastewater [1-3].

There is a growing recognition that enzymes can be used in many remediation processes to target specific pollutants for treatment [4]. Different studies show that the extracellular ligninolytic enzymes of white-rot fungi can degrade a wide variety of recalcitrant compounds, such as dyes [5, 6]. Laccase (p-diphenol oxidase, EC 1.10.3.2) catalyzes the oxidation of phenolic compounds and aromatic amines and accepts a broad range of substrates [7] .Various studies on dye degradation by using laccase have been published [8]. Cristovao et al. [9] reported that a high decolourization percentage of practically all dyes in the first two cycles and an effective decolourization of the dye mixture had been obtained, showing the suitability of the immobilized commercial laccase using Green coconut fiber as a precursor for continuous colour removal from textile industrial effluents. Arıca et al. [10] showed that a laccase immobilized onto plain and spacer-arm attached poly(GMA/EGDMA) beads was operated in a batch system, and a textile dye Reactive Red 120 was successfully decolourized in the enzyme reactor.

It has been reported that the catalytic activity and stability of laccase could be enhanced by immobilization in silica sol-gel [11-14]. Therefore, it is of considerable value if malachite green oxalate dye decolourization could be catalyzed by using free laccase and sol-gel laccase. In present research, the important parameters that affect the efficiency of the biodegradation process, such as the reaction time or time course, pH, temperature and enzyme loading were investigated.

2.0 MATERIALS AND METHODS

2.1 Materials

The reagent water type 1 was obtained using Nanopure deonizer, purchased from Purite Ltd. (England). All gels were prepared using tetraethyl orthosilicate (TEOS) (99% purity) purchased from Fluka (Switzerland). Other chemicals were of analytical grade reagents from various suppliers and used without further purification. 2,6-dimethoxylphenol, potassium dihydrogen phosphate (KH_2PO_4), dipotassium hydrogen phosphate (K_2HPO_4), and Malachite green oxalate were purchased from Sigma (USA). Laccase from *Trametes* sp. was purchased from Daiwa Kasei Co. Ltd. (Japan). The purity of the laccase powder was 30% (w/w) of pure protein and 70% (w/w) of dextrin, having molecular weight of 62 kDa, and the *p*I was 3.

2.2 Preparation of Sol-gel Laccase

The encapsulation of oxidoreductases laccase was performed according to [15] with slight modification. Briefly, 2.5 ml of TEOS, 0.4 ml of reagent water and 0.1 ml of 0.04 M HCl (corresponding to 10 mmol, 28 mmol, and 0.004 mmol, respectively) were mixed by stirring. The mixture was sonnicated under ice cooling for 30 min and left at ambient temperature until it acquired an elastic consistency. The sonication of the sol during the initial hydrolysis and condensation reactions using NEY 57x ultrasonic bath, 27 ^{0}C eliminated the need for often used co-solvents such as alcohols, which can promote protein denaturation. A 2 ml aliquot of phosphate buffer (0.4 M, pH 7) to facilitate gelation was then added into the sol, immediately followed by the addition of 1 ml of laccase solution (5mg/ml); both additions needed vigorous stirring. As soon as gelation began (1-4 min), the stirring was stopped to avoid the formation of bubbles in the gel. The gels were then lyophilized at -33^{o}C and 6.1 mbar for 24 hours using Martin Christ Freeze Dryer ALPHA 1-2/LD Plus (Germany). The lyophilized gels were crushed with mortar in order to get homogenous samples and stored at 0^{o}C to maintain enzymatic activity.

2.3 Determination of Dye Decolourization

The degradation of dye is presented as the percentage of absorbance reduction at the maximum absorbance wavelength. Thus, the dye decolorization by using free laccase and sol-gel laccase was determined by monitoring the decrease in the absorbance peak at the maximum absorbance wavelength of 650 (nm). The percent decolorization was calculated as $(A_i-A_f)/A_i x100$; where A_i is the initial absorbance at a given wavelength or the total area under the initial spectrum and A_f is the final absorbance of the dye or the total area under the final spectrum. Figure 1 shows the spectral scans (200-1100 nm) of the Malachite green oxalate dye absorbance.

2.4 Main Degradation Experiments

To study the effect reaction of degradation of dye malachite green oxalate, 30 mg of free laccase and sol-gel laccase, respectively were added into the conical flask having 100 ml of 50 ppm dye. The flask was then shaken at 200 rpm using the temperature controlled shaker. 5 ml of the mixture was taken at selected time intervals. The extent of dye decolorization was calculated based on the decrease in concentration of dye against its initial.

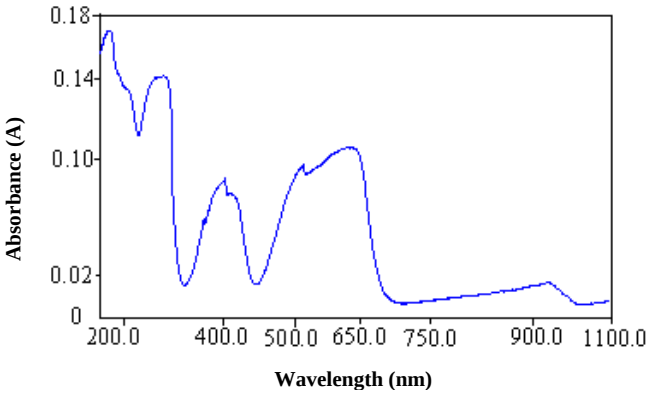


Figure 1 Spectral scans (200–1100 nm) of the Malachite green oxalate dye absorbance

2.5 Effect of pH against Dye Decolorization

In order to investigate the effect of pH on the decolorization of dye using free laccase and sol-gel laccase, 15 mg of free laccase and sol-gel laccase respectively were added into the conical flask having 50 ml of 50 ppm, (pH 3–8).

2.6 Effect of Temperature against Dye Decolorization

The effects of temperature on the decolorization of dye by free laccase and sol-gel laccase were investigated by adding 15 mg of free laccase and sol-gel laccase respectively into the conical flask having 50 ml of 50 ppm, pH6.

2.7 Effect of Enzyme Loading against Dye Decolorization

In order to study the effect of laccase loadings on the dye decolorization, 10-30 mg of free laccase and sol-gel laccase respectively were added into the reaction mixture having 50 ml of 50 ppm, pH6. The flask was then shaken at 200 rpm using the temperature controlled shaker for 1 hr. 5 ml of the sample was taken from each reaction media.

3.0 RESULTS AND DISCUSSION

3.1 Degradation of Dye by Free Laccase and Sol-Gel Laccase

The same amount of free laccase and sol-gel laccase (30 mg) having pure laccase content of 9 and 0.225 mg respectively were used to catalyze the biodegradation of dye. Figure 2 shows the degradation rate of dye malachite green oxalate or time course using free laccase and sol-gel laccase. The decolouration was carried out directly in the spectrophotometer cuvette. It showed that the degradation of dye malachite green oxalate using free laccase and sol-gel laccase at 3rd hour were 45% and 31% respectively. Decolorization using sol-gel laccase was improved since the pure laccase content in the sol-gel laccase was low. Rodr´ıguez [16] demonstrated that the nature and position of the dye substituents strongly affected the discoloration efficiency. Unfortunately, we were unable to discover the reason for higher discoloration of malachite green oxalate dyes by using laccase since their structures have not been disclosed. It is beyond the scope of the present work to provide mechanistic interpretations

of the observed results as this would require further analysis and identification of the reaction products.

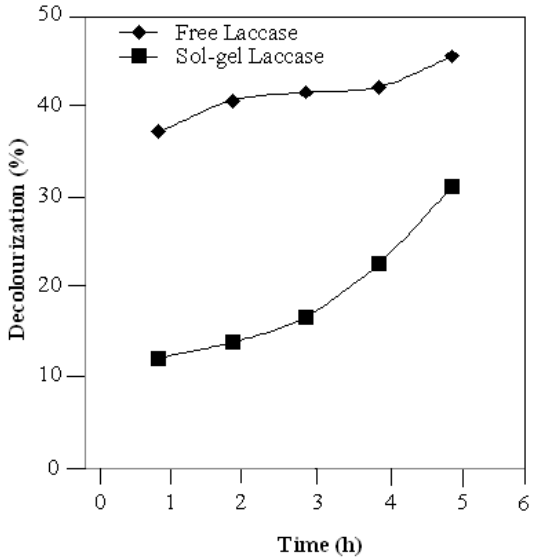


Figure 2 Degradation rate of dye malachite green oxalate using free laccase and sol-gel laccase

3.2 Effect of pH on Dye Degradation by Laccase

The effect of the pH of reaction media against decolorization catalyzed by using free laccase and sol-gel laccase is shown in Figure 3. It was observed that the optimum pH of free laccase and sol-gel laccase decolorization respectively were at pH 5 and 6. The decolorization rate decreased as the pH increased. It had been reported that the optimal pH of sol-gel laccase and free laccase respectively were at pH 5 and pH 6 [11]. The present result indicated that the optimal pH for sol-gel laccase shifted slightly toward a more acidic region compared to the free laccase. When the laccase was encapsulated in a charged matrix (support) as a result of a change in the microenvironment of the enzyme, the apparent bulk pH optimum of the sol-gel laccase shifted if compared to free laccase. The charged matrix would repel or attract substrate, product, cofactor, and H^+ depending on the type and quantity of surface charge [17, 18]. Such shifts have previously been detected for various immobilized enzyme using various supports [19-21].

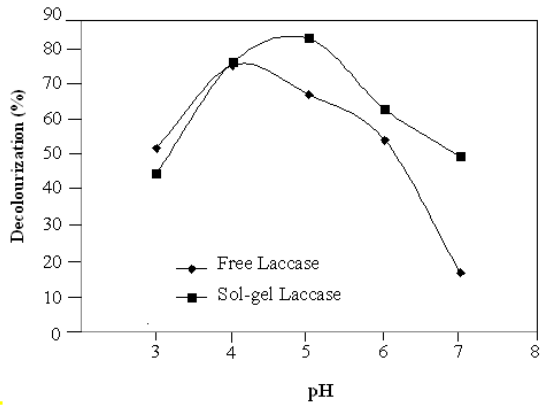


Figure 3 Effect of pH on decolourization of malachite green oxalate by using free laccase and sol-gel laccase

3.3 Effect of Temperature on Dye Degradation by Laccase

The effect of temperatures against dye decolorization is shown in Figure 4. It was observed that for the sol-gel laccase, the percentage of the dye decolorization increased with the temperature up to 40 ^{0}C, which then decreased with the increasing temperature. This decrease might be due to the laccase denaturation or unfolding. It can be explained by referring to the properties of enzyme itself. Shuler and Kargi [17] reported that most enzymes unfold (denature) at elevated temperatures; in fact, some unfold even at very low temperatures. Enzyme can almost always be unfolded by raising the temperature sufficiently. It also indicates that the laccase can be activated or inactivated at certain temperature ranges.

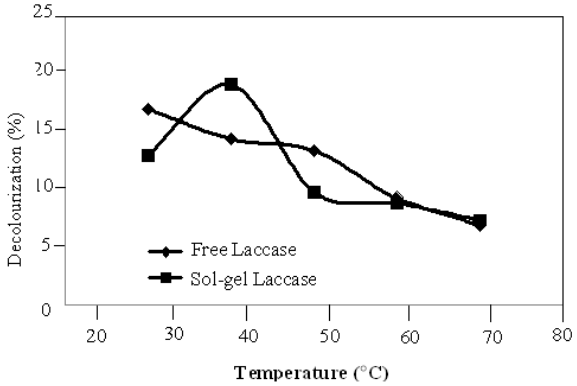


Figure 4 The effect of temperature on the decolourization of malachite green oxalate by using free laccase and sol-gel laccase

3.4 Effect of Laccase Loading on Dye Degradation.

The effect of free laccase and sol-gel laccase loading against dye decolorization was studied, and was depicted in Figure 5. It was observed that the dye decolorization increased with the increasing enzyme loading up to an optimum ($15x10^{-3}$g) and then dropped with the increase of enzyme loading. The decolorization of dye decreased at higher enzyme loading because of the agglomeration and denaturation of enzyme. Besides, Najera et al. [22] reported the effects of pH, temperature, $CaCl_2$ and enzyme concentrations on the rennet-clotting properties of milk. It showed that the coagulum firmness increased progressively as the concentration of enzyme increased.

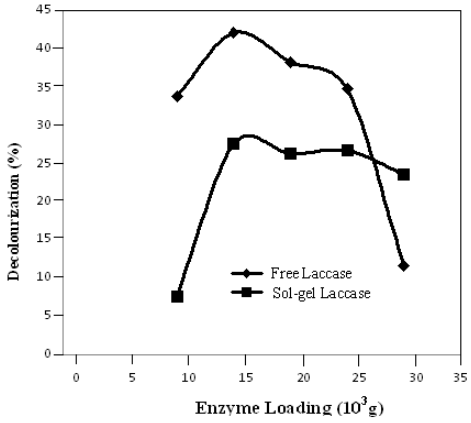


Figure 5 The effect of laccase loading on the decolourization of malachite green oxalate by using free laccase and sol-gel laccase at pH 6

4.0 CONCLUSION

The complex dye mixture is highly recalcitrant because they are composed of a great variety of dyes and, in addition, they contain different impurities, which make their degradation problematic. From the present results, it can be concluded that the degradation of dye malachite green oxalate using free laccase and sol-gel laccase at the 3rd hour were 45% and 31% respectively. The optimum pH for free laccase and sol-gel laccase against their degradation capacity respectively was at pH 5 and pH 6. The optimum temperature for both free laccase and sol-gel laccase was 40°C and, their optimum loadings were 30 mg against their degradation. The results obtained were very promising since the free laccase and sol-gel laccase were able to degrade Malachite green oxalate.

Acknowledgement

The financial support from the Ministry of Higher Education (MOHE), Malaysia under the Fundamental Research Grant Scheme (FRGS Project No. 78213) is gratefully acknowledged.

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