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Application of response surface methodology to optimize decolourization of dyes by the laccase-mediator system

Dalel Daâssi [a], Fakher Frikha [a], Hela Zouari-Mechichi [a], Lassaad Belbahri [b], Steve Woodward [c], Tahar Mechichi [a,*]

[a] Laboratory of Enzyme Engineering and Microbiology, University of Sfax, Ecole Nationale d'Ingénieurs de Sfax, Route de Soukra Km 4.5, BP «1173», 3038 Sfax, Tunisia
[b] Laboratory of Soil Biology, University of Neuchatel, Rue Emile Argand 11, CH-2009 Neuchatel, Switzerland
[c] University of Aberdeen, Institute of Biological and Environmental Sciences, Department of Plant and Soil Science, Cruickshank Building, St. Machar Drive, Aberdeen AB24 3UU, Scotland, UK

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ABSTRACT

Response surface methodology (RSM) was applied to optimize the decolourization of 3 dyes belonging to 3 dye families such as reactive black 5 (diazoic), indigo carmine (indigoid) and aniline blue (anthraquinonic). Crude laccase from *Trametes trogii* and the laccase-mediator 1-hydroxybenzotriazole (HBT) were used in this study. Box–Behnken design using RSM with six variables namely pH, temperature, enzyme concentration, HBT concentration, dye concentration and incubation time was used in this study to optimize significant correlation between the effects of these variables on the decolourization of reactive black 5 (RB5), indigo carmine (IC) and aniline blue (AB).

The optimum of pH, temperature, laccase, HBT, RB5 and reaction time were 4.5, 0.5 U ml^{-1}, 0.5 mM, 100 mg ml^{-1} and 150 min respectively, for a maximum decolourization of RB5 (about 92.92% ± 7.21). Whereas, the optimum decolourization conditions of both IC (99.76% ± 7.75) and AB (98.44% ± 10) were: pH 4.5, temperature of 45 °C, enzyme concentration of 0.1 U ml^{-1} and 0.5 U ml^{-1}, HBT concentration of 0.9 mM and 0.5 mM, dye concentration of 60 mg l^{-1} and reaction time of 150 and 90 min, respectively.

The experimental values were in good agreement with the predicted ones and the models were highly significant, the correlation coefficient (R^2) being 0.864, 0.663 and 0.776 for RB5, IC and AB, respectively. In addition, when the kinetic parameters for the three dyes decolourization were calculated according to Hannes–Wolf plot, the following values were obtained: Km of 268.4, 47.94 and 44.64 mg l^{-1} then V_{max} of 35.58, 10.43 and 9.23 mg l^{-1} min for the RB5, IC and AB decolourizations by laccase, respectively.

1. Introduction

Synthetic dyes are increasingly used in the textile, paper, cosmetics, leather dyeing, color photography, pharmaceutical and food industries. These compounds are classified as azo, anthraquinone, heterocyclic, triphenylmethane or phthalocyanine dyes (Husain, 2006). Most of them are toxic, mutagenic and carcinogenic and can cause serious environmental pollution (Al-Degs et al., 2000; Leechart et al., 2009).

Dyes are one of the major sources of wastewater pollution generated by the textile printing and dyeing industries. In these industries, dyes are first adsorbed on cellulose and then reacted with the fibers. However, reactive dyes pose particular problems. These dyes exhibit low levels of fixation with the fibers and approximately 10–20% of total dye used in the dyeing process remains in the spent dye bath, along with accessory chemicals, resulting in serious environmental problems, such as interference by the color with penetration of sunlight into waters, retardation of photosynthesis in aquatic plants, inhibition of growth of aquatic biota and inhibition of gas solubility in water bodies (Jain and Sikarwar, 2008). Hence, removal of these dyes from the effluents is necessary.

Many authors have reported physico-chemical treatments for the removal of color from industrial wastewaters (Arslan et al., 2000; Mittal, 2006; Gupta et al., 2006). Even though these procedures were efficient, the operational costs are relatively high and leads to other disadvantages like sludge formation, biomass accumulation, etc. (Mishra and Tripathy, 1993).

As a viable alternative, biological processes have received increasing interest owing to their cost effectiveness, ability to

* Corresponding author. Tel.: +216 74 274 088; fax: +216 74 275 595.
E-mail address: tahar.mechichi@enis.rnu.tn (T. Mechichi).

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produce less sludge, and environmental benignity (Banat et al., 1996). Therefore, to develop a practical bioprocess for treating dye-containing wastewater is of great significance.

In recent years, biological decolourization techniques have been considered as alternative, environmentally-friendly methods for dye detoxification and color removal. Enzyme methods applied in dye degradation have low energy costs, are easy to control and have low impacts on ecosystems. Recent studies have shown that fungi or enzymes from fungi are able to decolorize and detoxify industrial dyes (Cristóvão et al., 2008). Decolourization of dye wastewater by the action of the enzyme laccase has been the subject of many studies (Mechichi et al., 2006; Khlifi et al., 2010; Neifar et al., 2011).

Response surface methodology (RSM) is a collection of mathematical and statistical techniques useful for analyzing the effects of several independent variables (Montgomery et al., 2000). RSM has been utilized extensively in biotechnology for the optimization of substrate composition (Tavares et al., 2005; Aghaie-Khouzania et al., 2012), fermentations (Tavares et al., 2006) and food processing (Demarigny et al., 2005).

The application of experimental design and RSM in the textile effluent treatment process can result in improved decolourization, reduced process variability, time and overall costs. In addition, the factors that influence the degradation processes can be identified and optimized, and possible synergistic or antagonistic interactions that may exist between factors can be evaluated (Box and Behnken, 1978).

Several studies have been published recently on the application of Response surface methodology (RSM) in color removal optimization by enzymatic catalysis (Neifar et al., 2011). RSM was applied for the decolourization of several textile dyes such as azo dye reactive black 5 (RB5) (Murugesan et al., 2007), reactive red 239 (RR239), reactive yellow 15 (RY15) and reactive blue 114 (RB114) dyes (Tavares et al., 2009).

The most common and efficient design used in response surface modeling is the Box–Behnken design. Compared to the central composite and Doehlert designs, Box–Behnken presents some advantages such as requiring few experimental points for its application (three levels per factor) and high efficiency (Costa Ferreira et al., 2007).

In the present work, Box–Behnken design and response surface methodology (RSM) has been used to optimize the decolourization of three textile dyes, reactive black 5 (RB5), indigo carmine (IC) and aniline blue (AB) by crude laccase from culture of the white-rot fungus *Trametes* sp. strain CLBE 55.

2. Materials and methods

2.1. Chemicals

2,6-dimethoxyphenol (DMP), 1-hydroxybenzotriazole (HBT) and the dyes reactive black 5 (RB5) indigo carmine (IC) and aniline blue (AB) were obtained from Sigma Aldrich.

2.2. Fungal strain, media and culture conditions

The strain CLBE 55, identified as *Trametes trogii*, was isolated from a forest in the north west of Tunisia. The isolate is maintained in the culture collection of our laboratory on 2% malt extract agar incubated at 30 °C and stored at 4 °C.

For laccase production by *T. trogii*, 3.0 ml of homogenized mycelium were used for inoculation of 1000-ml Erlenmeyer flask containing 300 ml of culture medium. This basal medium (M7) was supplemented with $CuSO_4.5H_2O$ (300 μM) as inducer of laccase. The M7 composition is as described by Zouari-Mechichi et al. (2006).

2.3. Determination of laccase activity and properties

Laccase activity was measured by monitoring the increase in absorbance at 469 nm ($\varepsilon_{469\ nm} = 27{,}500\ M^{-1}\ cm^{-1}$) of a reaction mixture containing 10 mM 2,6-dimethoxyphenol in 100 mM acetate buffer, pH 5.0. Enzymatic reactions were carried out at room temperature (22–25 °C). One unit of enzyme activity was defined as the amount of enzyme oxidizing 1 μmol of substrate per minute.

Partial characterization of the laccase in the crude preparation, showed an optimal pH at approximately 4.5. Activity was stable in the crude extract at room temperature, pH 7.0 for 24 h; more than 50% activity was retained at pH 5.0. The laccase in the crude extract was also stable for 24 h at 50 °C; over 40% activity was lost at 60 °C.

2.4. Box–Behnken designs and response surface analysis

RSM using Box–Behnken was employed to optimize the six selected factors (X_1: pH, X_2: Temperature (°C), X_3: enzyme concentration (U ml^{-1}), X_4: HBT concentration (mM), X_5: dye concentration (mg l^{-1}) and X_6: incubation time (min)) for enhancing the decolourization yield of the three selected dyes. The six independent factors were investigated at three different levels (−1, 0, +1) (Table 1) and the experimental design used for study is shown in Table 2.

The decolourization yield of the three selected dyes was fitted using a second-order polynomial equation and a multiple regression of the data was carried out for obtaining an empirical model related to the factors. The general form of the second-order polynomial is:

$$Y = \beta_0 + \sum \beta_i X_i + \sum \beta_{ii} X_i^2 + \sum \beta_{ij} X_i X_j \quad (1)$$

Where Y is the predicted response, X_i and X_j are independent factors, β_0 is the intercept, β_i is the linear coefficient, β_{ii} is the quadratic coefficient and β_{ij} is the interaction coefficient.

Design expert, version 7.0 (STAT-EASE Inc., Minneapolis, USA) was used for the experimental designs and statistical analysis of the experimental data. The analysis of variance (ANOVA) was used to estimate the statistical parameters.

2.5. Decolourization of dyes by laccase-HBT system

All experiments in Box–Behnken design were performed using 50 ml disposable flasks in 5 ml final reaction volumes. The reaction mixture, containing 100 mM tartrate buffer, different concentrations of HBT, dye and laccase from culture filtrate, was incubated in the dark. These reactions were prepared as shown in Table 1 according to the experimental design (Table 2): different concentrations of enzyme (0.1, 0.5 and 0.9 U ml^{-1}), HBT (0.1, 0.5 and 0.9 mM) and dye (20, 60, 100 mg l^{-1}), increasing temperatures (30, 45 and 60 °C), pH (3.0, 4.5 and 6.0) and incubation times (30, 90 and 150 min) were used as variables.

For each textile dye a reaction mixture without enzyme was prepared under the same conditions to detect possible color changes not due to enzyme activity. Controls contained heat killed enzymes whereas blanks used all components of the reaction

Table 1
Levels of the factors tested in the Box–Behnken Design.

Factors	Units	Symbol	Levels		
pH	X_1		1	0	−1
Temperature	X_2	(°C)	6	4.5	3
Enzyme concentration	X_3	(U ml^{-1})	60	45	30
HBT concentration	X_4	(mM)	0.9	0.5	0.1
Dye concentration	X_5	(mg l^{-1})	0.9	0.5	0.1
Incubation time	X_6	(min)	100	60	20