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Decolorization of the phthalocyanine dye reactive blue 21 by turnip peroxidase and assessment of its oxidation products

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ABSTRACT

Peroxidases can be used in decolorization processes and the treatment of textiles effluents. This study evaluates the potential of the turnip peroxidase enzyme in the decolorization of the phthalocyanine textile dye Reactive Blue 21 (RB21). Some factors such as pH, the amount of H_2O_2 and the enzyme were evaluated in order to determine the optimum conditions for the enzyme performance. The reaction products formed during the decolorization of the RB21 dye were analyzed by high-performance liquid chromatography–mass spectrometry coupling (LC–ESI/MS). LC–ESI/MS analysis showed that the decolorization of the dye RB21 by turnip peroxidase is due to the breaking up of the chromatogenous system. The tests for toxicity towards lettuce seeds showed an increase of the toxicity after enzymatic treatment of the dye. This study verifies the viability of the use of the turnip peroxidase enzyme in the biodegradation of textile dyes.

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1. Introduction

The removal of dyes from textile wastewater prior to its discharge or reuse is a challenging task. The presence of color hinders the absorption of solar radiation, which can modify photosynthetic activity, causing changes in aquatic biota. Moreover, many of these dyes present acute or chronic toxicity on the ecosystems [1].

Unfortunately, the exact data on the quantity of dyes produced in the world or discharged in the environment are not available. It is assumed a production of 10,000 tons per year, while a loss of 1–2% in production and 1–10% loss in use are a fair estimate [2].

Phtalocyanine (PC) dyes are among the dyes which resist to bacterial degradation. These dyes constitute the main category of the reactive dyes which are one of the most important class of textile dyes [3].

Phthalocyanine reactive dyes are metallic complexes used to produce blue and green shades. Most of these dyes are copper phthalocyanines. They are potentially mutagenic and of special toxicity concern because of their metal Cu content [4].

The inherent properties of reactive phthalocyanine dyes, such as color fastness, stability, and resistance towards oxidative degradation, have made color removal from textile wastewaters a particularly difficult task. Reactive phthalocyanine dyes are highly water-soluble, resistant to biological degradation under aerobic conditions, and are not effectively removed by adsorption to the biomass in wastewater treatment plants, resulting in colored effluents [4].

Researchers have been focusing their attention to study enzymatic pretreatment as a potential and viable alternative to conventional methods, due to its highly selective nature [5–7]. Enzymes can act on specific recalcitrant pollutants to be removed by their precipitation or transformation into other innocuous products [8,9].

The removal of phthalocyanines dyes in aqueous solution by peroxidase has been widely reported, in last years, especially by white-rot fungi. Peroxidases can catalyze degradation/transformation of aromatic dyes either by precipitation or by opening the aromatic ring structure [10].

The decolorization of two reactive PC dyes, Reactive blue 15 (RB15) and Reactive blue 38 (RB38), by *Bjerkandera adusta*, *Trametes versicolor* and *Phanerochaete chrysosporium* was shown by Heinfling et al. [11]. Meanwhile, the PC dye RB21 (Reactive Blue 21) was oxidized by Horsehadish peroxidase. The decolorization of the dye RB21 in this case was approximately 59% [12]. The degradation of dye RB21 was also evaluated by Marchis et al. [13], using soybean

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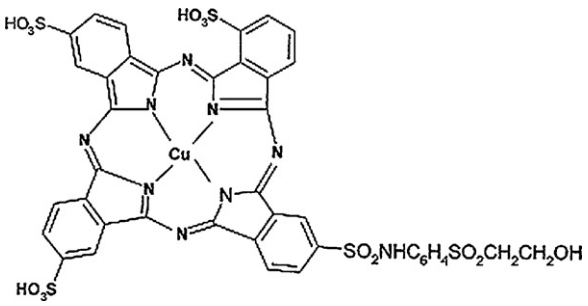

Fig. 1. The molecular-structure of reactive blue 21 dye.

peroxidase as biocatalyst. The decolorization obtained was 95–96% after 4 h of reaction at pH 3.0.

Despite these previous investigations have shown that PC dyes can be decolorized by white-rot fungi or by plant peroxidases, the degradative pathway and potential metabolites, mostly, remain unknown. The knowledge of the metabolites formed during the decolorization of textile dyes by plant peroxidases is a way for the understanding of the break up mechanism of complex structures chemically stable, by the enzymes [11].

Many treatments can be efficient in the decolorization, but it is essential to know if there is formation of toxic products during the process. A valuable technique to evaluate the toxicity of the reaction products is the use of bioindicators [14].

In general it becomes very important for a bioremediation technology to assess the toxicity of the pollutants and metabolites formed after their degradation in order to test out the feasibility of the technique [15].

There are few studies to assess the toxicity of dyes and the products formed during their enzymatic degradation. Da Silva and coworkers [16] observed the reduction of *Artemia salina* mortality after decolorization reactions of Drimarene Blue X-3LR (DMBLR), Drimarene Rubinol X-3LR (DMR), and Drimarene Blue CL-R (RBBR) by horsehadish peroxidase.

The metabolites formed after degradation of remazol red by *Pseudomonas aeruginosa* BCH were more toxic than the parental molecule [15].

In this work study, the use of the turnip peroxidase in the decolorization of the PC dye RB21 is studied. In this context, the effect of parameters such H_2O_2, dye and enzyme concentrations, as well as contact time has been investigated to optimize the system conditions. The toxicity of the dye both before and after the enzymatic dye was evaluated by utilizing lettuce seeds (*Lactuca sativa*) as a bioindicator.

Moreover, we here report the identification of the major metabolites of the treatment of the PC dye RB21 with turnip peroxidase by liquid chromatography–mass spectrometry (HPLC–MS).

2. Material and methods

2.1. Dye

The textile dye RB21 was kindly provided by DyStar (Brasil) and were used for degradation experiments without any further purification. The molecular-structure of the dye is shown in Fig. 1 [17].

2.2. Obtention of the enzymatic extract

The enzyme was extracted from turnip roots purchased from local market. The roots (with peel) were washed in water and cut into small uniform pieces. Turnip roots (300 g) were homogenized in a blender with 100 mL of 0.05 mol L^{-1} pH 6.5 phosphate buffer for 30 s. The homogenate was filtered in organza cloth and centrifuged at 10,000 × *g* for 15 min, at 4 °C [18]. The obtained solution was subjected to precipitation by adding cold acetone until reaching 65% (v/v). After a rest from 12 to 14 h, at −18 °C, the homogenate was centrifuged at 11,000 × *g* for 15 min, at 4 °C. The supernatant was collected and acetone was subsequently recovered by distillation in a rotary evaporator, at controlled temperature of 56 °C. The obtained precipitate after the removal of the acetone by a treatment in gridge during 72 h was redissolved in 15 mL sodium phosphate buffer, pH 6.5 and then used in the dye removal studies.

2.3. Determination of enzyme activity

The activity was determined according to Khan and Robinson [19], using as reaction medium: 1.5 mL of guaiacol (Vetec; 97%, v/v) 1% (v/v); 0.4 mL of H_2O_2 (Vetec, PA) 0.3% (v/v); 0.1 mL of enzyme and 1.2 mL of 0.05 mol L^{-1} phosphate buffer pH 6.5. The reaction was monitorized during 5 min at 30 °C using a Spectrovision spectrophotometer coupled to a thermostatic bath.

One unit of peroxidase activity represents the oxidation of μmol of guaiacol during 1 min in the assay conditions and it was calculated using data relative to the linear portion of the curve.

2.4. Dye removal studies

Experiments were conducted to assess the turnip peroxidase catalyzed removal of phthalocyanine dye in aqueous phase. The experiments were carried out at a constant temperature (30 °C) by varying the process parameters such as dye, H_2O_2 and enzyme concentrations [20]. Initially the enzymatic reactions were conducted in sodium phosphate buffer, 0.05 mol L^{-1}, pH 7.0 (1.2 mL), containing: (1) H_2O_2 100 μmol L^{-1} (0.4 mL), (2) the dye Remazol Turquoise G 133%, at concentration of 50 mg L^{-1} (1.5 mL) and 0.1 mL of enzymatic solution for estimated the optimum contact time.

The reaction mixture was incubated in a spectrophotometer coupled to a thermostatic bath. The monitorization of the substrate consumption was carried out at 624 nm which corresponds to the maximum absorption of Remazol Turquoise G 133%. The calculation to determine the color removal percentage of the dyes was made according to the equation:

$$\frac{\text{absorbancy}_{\text{initial}} - \text{absorbancy}_{\text{final}}}{\text{absorbancy}_{\text{initial}}} \times 100$$

Subsequent series of experiments were performed by varying the concentrations of dye concentration (from 10 to 50 mg L^{-1}), H_2O_2 dose (from 50 to 500 μmol L^{-1}) and enzyme concentration (from 1.62 to 26.16 U mL^{-1}) to understand the optimum conditions for dye removal. Reactions were performed also using multiple or single additions of H_2O_2.

2.5. HPLC–MS

The HPLC–MS analyses were performed using a liquid chromatographic system (Thermo Surveyor with gradient pump, auto sampler and diode array detector – DAD) coupled to a mass spectrometer Thermo LXQ Linear Ion Trap with electrospray ionization (ESI+) and a diode array detector.

The samples were filtered through ultrafiltration membranes (Millipore) with molecular weight cut 50 kDa, before injection into the chromatograph.

A volume of 20 μL of sample was injected and the chromatographic separation was performed on a Hypersil GOLD column (100 mm × 4.6 mm). Methanol/water (acidified 1%, v/v) was used as mobile phase at a flow rate of 0.4 mL min^{-1}.

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