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The use of soybean peroxidase in the decolourization of Remazol Brilliant Blue R and toxicological evaluation of its degradation products

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ABSTRACT

This study evaluated the potential use of soybean peroxidase in the decolourization of reactive textile dye Remazol Brilliant Blue R (RBBR) and its synthetic effluent. The following parameters were studied: reaction time, dye concentration (10–60 mg L^{-1}), enzyme load (4.96–140 U mL^{-1}) and H_2O_2 concentration (20–1100 μmol L^{-1}). The maximum removal of RBBR (86%) was obtained after 13 min of reaction, using H_2O_2 100 μmol L^{-1}, enzyme 70.4 U mL^{-1} and RBBR 40 mg L^{-1}. The toxicity of the products formed after enzymatic treatment was assessed by using *Artemia salina* and lettuce seeds (*Lactuca sativa*). Although soybean peroxidase was very efficient in colour removal, the products obtained after enzymatic decolourization presented higher toxicity. The inhibition concentration (IC_{50}) obtained for lettuce seeds was 27.9%, and the lethal concentration (LC_{50}) for *A. salina* was 59.3%. The aforementioned results emphasize the importance of toxicological evaluation after enzymatic treatment. The potential application of peroxidases for colour removal and the increase in the products' toxicity reinforce the need of combined treatments.

1. Introduction

The removal of dyes from textile wastewater prior to its discharge or reuse is a challenging task. Chemical and photo stability are required characteristics for industrial dyes. As a consequence, these kinds of structure are recalcitrant in natural [1]. Unsuitable treatments of wastewater containing industrial dyes can damage natural systems, reducing water transparency and the incidence of solar radiation, which can modify photosynthetic activity and the dynamics of gas solubility [2]. Moreover, the degradation of some synthetic dyes may result in toxic and carcinogenic metabolites [3].

No single conventional technology can remove all classes of dyes [4,5]. In conventional biological treatment plants, the dyes are adsorbed into activated sludge and are poorly degraded. Additionally, this process also has the disadvantage of producing large amounts of solid waste [6]. Currently, the methods of textile wastewater treatment involve physico-chemical processes (coagulation/flocculation, adsorption, precipitation) and/or chemical processes (electrolysis, chemical reduction and advanced chemical oxidation). However, most of these processes are expensive, can generate large volumes of sludge and usually require the addition of environmentally hazardous chemical additives [5,7].

Remazol Brilliant Blue R (RBBR) dye has been used as a model substance in several studies on dye degradation and different kinds of physical, chemical and biological processes have been tested for its removal. Table 1 lists chemical and physical methods used for RBBR removal. Although these methodologies are effective, disadvantages of each process should be considered, such as: sludge generation (adsorption and fenton oxidation), short half life (ozonization) and high cost of electricity (electrochemical).

Enzymes from different plants and microorganisms like peroxidases, lacases, mono and dioxegenases have shown a great capacity to degrade a wide range of persistent organic pollutants, which includes textile dyes [16,17]. Enzyme-based dye decolourization methods are very attractive, due to the minimal impact on the ecosystem [18]. However, the use of biocatalysts demands high production cost [5]. Moreover, enzymes are biodegradable and are easily removed from contaminated streams and are also able to efficiently convert complex chemical structures under mild conditions.

The limitation for the use of plant peroxidase is the low yield and high production cost compared to microbial enzymes. Plant peroxidases, such as: horseradish [19–22], turnip [23,24], white radish

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Table 1
Chemical and physical methods studied for RBBR dye removal.

Methodology	Concentration range (mg L^{-1})	Temperature (°C)	pH	Time reaction (min)	Decolorization (%)	References
Adsorption on agro-industrial waste materials (wheat bran)	50	20	1.5	300	97.5	[8]
Adsorption on MgO nanoparticles	50–300	20	8	5	98	[9]
Fenton oxidation	100	20	3	60	>99	[10]
Ozonization	800	25	3–10	45	100	[11]
Hydrolysis	100	120	4	120	74.39	[12]
Electrochemical	400	25		80	100	[13]
Ultrasound assisted electrochemical process	50	–	8	120	90	[14]
Biosorption on *Candida sp.*	203	25	2	5760	69	[15]

[25] and soybean [26] have shown an excellent potential for dye decolourization. However, the high costs associated with biocatalysts production and application still hinder their large scale use under environmental purposes. The extraction of enzymes from agro industrial residues constitutes one alternative for reducing costs in biocatalysts production [27].

Soybean (*Glycine max L.*) is an agricultural product of great importance due to its versatile application in food and feed. It is also important to emphasize the economic relevance of soybean in the national and international markets. In addition, Brazil is among the largest producers of soybean in the world [28]. Soybean seed hulls have been identified as a rich source of peroxidases, and, as a soybean-processing industry by-product, they constitute one low-cost alternative [29].

Many treatments can be efficient in the decolourization, but it is essential to know if toxic products are formed during the process. The use of bioindicators is a valuable option to evaluate the toxicity of degradation products [30].

This work evaluated the use of a crude extract of soybean peroxidase from soybean seed hulls in order to catalyze colour removal from aqueous solution containing the reactive dye Remazol Brilliant Blue R (RBBR) and from simulated dyebath wastewater containing the same dye.

This dye is widely used in Brazilian textile industries. The following parameters were studied: reaction time, enzyme load and dye and H_2O_2 concentrations. The toxicity of the dye RBBR and its degradation products after enzymatic treatment were also evaluated through the use of *A. salina* and lettuce seeds (*L. sativa*) as bioindicators. Furthermore, a comparative study was performed among current methodologies and the present study. The most interesting point in this work lies on the promising use of a low cost enzyme obtained from an agro residue widely available in the Brazilian food industry.

2. Materials and methods

2.1. Dye

The textile dye Remazol Brilliant Blue R (RBBR), which presents an anthraquinone as chromophore, was kindly provided by DyStar (Porto – Portugal) and used as received without further purification. Dye solution used for degradation experiments was prepared with distilled water.

2.2. Soybean seed hulls extracts

The soybean hulls (25 g) were homogenized in a blender with 100 mL of 0.05 mol L^{-1} pH 6.5 phosphate buffer, containing NaCl 0.2 mol L^{-1} for 30 s. The homogenate was filtered in organza cloth and centrifuged at 10,000 × g for 15 min, at 4 °C [31]. The enzymatic extract obtained was subjected to a precipitation by adding cold acetone until reaching 65% (v/v). After 12–14 h at −18 °C, the sedimentation was concluded and the precipitate was separated by centrifugation at 11,000 × g for 15 min, at 4 °C. The precipitate containing the peroxidases was left at 4 °C for removal of waste acetone for approximately 72 h, and then, re-suspended in 25 mL pH 6.0 of citrate phosphate buffer 0.1 mol L^{-1}. The obtained suspension was stored at 4 °C and used in the decolourization assays.

The supernatant was collected, and the acetone was recovered by simple distillation in rotary evaporator with control temperature at 56° C. The recovered acetone can be reutilized in the enzyme obtention process, thus reducing the cost of the process, which yields the enzyme through an economically simple and viable process.

2.3. Enzymatic activity

The enzymatic activity was determined according to Khan and Robinson [32] by using the reaction medium of: 1.5 mL of guaiacol 1% (v/v) (Vetec, 97%, v/v), 0.4 mL of H_2O_2 0.3% (v/v) (Vetec, PA), 0.1 mL of enzyme (kept in ice bath) and 1.2 mL of 0.05 mol L^{-1} phosphate buffer pH 6.5. The reaction was carried out for 5 min at 30 °C in a Spectrovision spectrophotometer coupled to a thermostatic bath. One unit of peroxidase activity represents the oxidation of 1 μmol of guaiacol per minute in the assay conditions and it was calculated by using data relative to the linear portion of the curve [32].

2.4. Decolorization assay

The experiments were carried out at 30 °C [33] by varying the following parameters: reaction time, dye concentration (10–60 mg L^{-1}), enzyme activity (4.96–140 U mL^{-1}) and H_2O_2 concentration (20–1100 μmol L^{-1}). All reactions were performed at pH 6.0, according to Silva et al. [33] (sodium citrate buffer 0.1 mol L^{-1}, 1.2 mL) containing dye RBBR (1.5 mL), enzyme (0.1 mL) and H_2O_2 (0.4 mL), using multiple or single additions of H_2O_2. Controls were carried out in the absence of H_2O_2.

The reaction mixture was analyzed by using a spectrophotometer coupled to a thermostatic bath. The consumption of RBBR was monitored at 596 nm, which corresponds to the maximum absorption wavelength of this dye. The amount of oxidized dye was estimated according to the Eq. (1):

$$\text{oxidized dye (mg L}^{-1}) = \left[\frac{\text{dye concentration}_{\text{initial}} \times \text{removal percentage of dye}}{100}\right] \quad (1)$$