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Toxicity of enzymatically decolored textile dyes solution by horseradish peroxidase

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ABSTRACT

The oxidative systems including enzymatic systems have been widely studied as an alternative for textile effluents treatment. However, studies have shown that some oxidative processes can produce degradation products with higher toxicity than the untreated dye. In this work, enzymatic dye decolorization was evaluated by horseradish peroxidase enzyme (HRP) and the toxicity of discoloration products was evaluate against *Daphnia magna*, *Euglena gracilis* algae, and *Vibrio fischeri*. Dye decolorization kinetics data were evaluated and the pseudo-second-order model showed the best-fitting to the experimental data. In addition, it was observed an increased acute and chronic toxicity associated with the decolorization efficiency. The Reactive Blue 19 and Reactive Black dye showed the highest toxicity against *D. Magna* (16 toxicity factor) and *V. Fischeri* (32 toxicity factor) after enzymatic decolorization. For the chronic toxicity against *D. Magna*, Reactive Red was the only dye with no fertility inhibition. In relation to toxicity tests with *E. gracilis* algae, it was not observed photosynthetic inhibition for all dyes. This study verified the viability of the enzyme horseradish peroxidase in the textile dyes decolorization and the importance to evaluate the decolorization products.

1. Introduction

Since the first synthetic dye was reported, the use of dyes in chemical, food, textile, paper, and pharmaceutical areas has increased, resulting in an increased volume and complex wastewater discharge [1,2]. During textile processing, dyeing inefficiencies and poorly wastewater treatment resulted in a large amount of dyestuff being directly lost in wastewater, making the water treatment difficult, because the color tends to persist even after the conventional removal processes [3–5]. In this sense, the concerning about toxic agents detections and determinations also increased in the last years, mainly due to the aquatic ecosystem.

According to the Baban et al. [6], among 10–15% of total dye used in the dyeing process are eliminated as effluents due to the non-fixation on the fiber, and these dyes, when released into the environment present a high impact mainly due to the difficult molecule degradation, since the chemical constituents of the dye are mainly phenolic compounds. The molecules are often toxic and hard to degrade in the conventional wastewater treatment systems, thus studies in this area showing new technologies that allow the degradation of these compounds are required [4,7,8].

With regard to the effluents treatment, oxidative processes have been seen as a promising technology, promoting dye decolorization and the degradation of recalcitrant organic substances when compared to the traditional processes [9]. Recently, the enzymatic approach has attracted interest in the textile dyes decolorization [5,10]. The peroxidase enzyme is known for the capacity to remove phenolic compounds and aromatic amines from aqueous solutions and to decolorize textile effluents [3,11]. O'Brien and O'Fagain [12] emphasize that the enzymatic approach has many advantages over conventional procedures (such as membrane separation, aerobic and anaerobic degradation using microorganisms, and activated carbon adsorption), however, it may require high cost, formation of toxic byproducts, and limited applicability at a determined concentration range [5].

Traditionally, the effluents monitoring has been carried out through physicochemical parameters, however, these parameters are global indicators, and it is not possible to determine the chemical composition of all components. According to Einicker-Lamas et al., [13], the metabolites formed during the dyes decolorization can be monitored using sensitive organisms to toxic substances as bioindicator [14]. Based on

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the chemical tests limitations, such as a substances range in effluents, low analytical detection capacity, and interferences by chemical phenomena of antagonism and synergism, the establishment of correlations between the toxicity of untreated and treated effluent by biological indicator is a very useful tool that allows toxic substances determination [15].

In order to overcome these limitations and to complement physicochemical analyzes, tests using living organisms as indicator have been developed and applied to determine toxic effects [14]. Therefore, biological, ecotoxicological, chemical, physical and physicochemical evidence must be used at integrated level, to obtain information to subsidize decisions and actions to protect the environment [16,17].

Among the main groups of organisms to represent the most diverse ecosystems and trophic levels used in effluent toxicity tests, microalgae, microcrustaceans, echinoids, polychaetes, oligochaetes, fish, and bacteria are highlighted [14,18]. Different species have a different sensibility, according to their metabolism, eating habits, behavior, development phase and other aspects. For a more adequate evaluation, the use of a set of assays using organisms representing different levels in the ecosystem is necessary.

Studies regarding dye decolorization by HRP enzymes reported in the literature, usually not mention the toxic effect of decolorization products. Thus, the mean goal of this work is to presents a study of the factors that influence the dye decolorization by Horseradish peroxidase enzyme, with the toxicological evaluation of decolorization products.

2. Material and methods

2.1. Material

Reactive Black 5 (azo, λ = 597 nm), Reactive Blue 19 (anthraquinone, λ = 593 nm), Reactive Red 239 (azo, λ = 541 nm), and Reactive Blue 21 (phthalocyanine, λ = 621 nm), were donated by Karsten and Döhler SA. The Horseradish peroxidase enzyme was donated by Toyobo Brazil (2012). For the aquatic toxicity tests, the *Daphnia magna* (1820, Cladocera, Crustacea) and *Euglena gracilis*, cultivated from the algae culture from University of Göttingen collection, Germany (strain Z), and Bioluminescent bacterium *Vibrio fischeri* lyophilized (Bioluz, Umwelt Ltda.) were used. *E. gracilis* culture was grown in mineral medium [19] obtained from Sigma-Aldrich (Sigma- Aldrich, Brazil).

2.2. Analytical procedure

The enzymatic dye decolorization by HRP was performed in batch mode (125 mL erlenmeyer) under optimized conditions (Table 1) at 30 °C and 50 mg/L of dye concentration according to the previous experiments (data not shown). Dye decolorization was spectrophotometrically evaluated (Shimadzu, Model 1601PC) in triplicate (n = 3) in a range from 400 to 800 nm at room temperature. After complete decolorization, 20 mL of supernatant was centrifuged at 10,000 rpm for 20 min in order to remove the enzymes, then after centrifugation process, the samples were analyzed and/or used in the toxicity assays. It is important to be mention that even at low concentration, according to reported by Meinertz et al. [20], Radtke et al. [21], and Junges et al. [22] the studied concentration range of H_2O_2 in this work, is not toxic against *D. magna, Euglena gracilis,* and *V. fischeri* bacteria, respectively.

Table 1
Reaction conditions used for dye decolorization and maximum decolorization obtained under studied conditions.

	Reactive Blue 19	Reactive Blue 21	Reactive Black	Reactive Red
Enzyme concentration (U/mL)	10.5	5.2	31.5	21.0
H_2O_2 concentration (mM)	1×10^{-4}	2.5×10^{-5}	5×10^{-5}	5×10^{-5}
pH	6	4	4	4
Dye decolorization	96%	90%	87%	17%

2.3. Organisms and culture conditions

Euglena gracilis strain was cultured in mineral and organic medium at 20 °C with a photoperiod of 12 h (20 Wm^2). *Daphnia magna* was cultured in distilled water (pH 7.0–8.0, 175–225 mg/L $CaCO_3$). The freshwater was kept under aeration for total solubilization of the salts, saturation of the dissolved oxygen and pH stabilization. The organisms were kept in glass flasks (4 L) with up to 25 adults/L, with a photoperiod of 16 h at 18 ± 4 °C. For the culture maintenance, organisms with up 60 days were used. Green alga *Scenedesmus subspicatus* (Chlorophyta) was used to feed the organism, providing approximately 106 cells/mL per adult organism daily. *Vibrio fischeri* bacteria was hydrated at the time of the test with deionized water and the salinity was adjusted. For the toxicity tests, the samples were adjusted to pH 6.0–7.0 with HCl or NaOH.

2.4. Acute toxicity test against Daphnia magna

The acute toxicity tests were carried out with young organisms (6–24 h of life), not fed during the test period. Each sample was tested under 6 dilutions (ratio 2). Each dilution was prepared in duplicate with 25 ml of test solution placed in a beaker with 10 organisms and incubated for 48 h at room temperature. After 48 h, the number of immobile organisms was counted. The organisms were considered immobile if not showed any mobility during 20 s of observation. Monthly, *D. magna* organisms were evaluated by sensitivity test against potassium dichromate ($K_2Cr_2O_7$) as standard, in order to guarantee the validity of the performed tests. The results were expressed in terms of dilution factor (DF), equivalent to the lowest dilution in which the immobility is higher than 10% and in terms of mean effective concentration (EC_{50}). Dilution water was prepared according to the ISO 6341 methodology [23]. A negative control was carried out in parallel using dilution water.

2.5. Chronic toxicity test against Daphnia magna

The chronic toxicity test was carried out for 21 days. Briefly, young organisms (6–24 h of life), fed 3 times a week with *Scenedesmus subspicatus* (Chlorophyceae) during the test, were split into 9 groups (Control, Reactive Black, Reactive Blue 19, Reactive Red, and Reactive Blue 21 untreated and after enzymatic decolorization) with 10 neonates each. The chronic toxicity was evaluated in terms of longevity and fecundity at 1, 5 and 10 mg/L over 21 days. The longevity was calculated by survival *D. magna* number and the fecundity was evaluated by the total number of neonates per female, and then, the average number of neonates per female was calculated.

For the average number of neonates, in case of adult death, this mother was not considered and the neonates were excluded. In case of a male adult presence in the organisms, only the longevity data was used, not including this individual in the fecundity calculations. A negative control was carried out in parallel using dilution water. Dilution water, used as control and diluent, was prepared according to the ISO 6341 protocol [23]. The assays were performed at 20 °C ± 2 with 16 h of photoperiod in 10 replicates (n = 10) with the test solution (25 ml) weekly replaced.

2.6. Acute toxicity test against Vibrio fischeri bacteria

The acute toxicity test evaluated the natural luminescence of the *V. fischeri* bacteria after 30 min of exposition to untreated and treated