OXIDATION AND REMOVAL OF INDUSTRIAL TEXTILE DYES BY A NOVEL PEROXIDASE EXTRACTED FROM POST-HARVEST LENTIL (*Lens culinaris* l.) STUBBLES

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Abstract

The degradation and removal of series of dyes used in textile industries for polyesters/wool (PES/WO) blends and present in their effluents, such as Green, Ash-Grey, Black, Navy Blue, Red and Yellow Domalan and Orange and Red Bemacid, by the catalytic action of extracts of a novel peroxidase from post-harvest lentil stubbles, in the presence of H_2O_2, was investigated. The extracts of this peroxidase were effective in degrading these industrial dyes, especially Green Domalan, Orange Bemacid, Grey and Black Domalan. A sensitivy study has been carried out for Green Domalan biodegration to know the effects of process parameters, such as aqueous phase pH, H_2O_2, enzyme and dye concentrations, contact time, centrifugation and temperature. Standard ecotoxicity studies performed with *Vibrio fischeri* showed that the dye solutions treated with peroxidase and H_2O_2 were less ecotoxic than the untreated.

Keywords: Industrial, textile, dyes, wastewaters, azoic, oxidation, peroxidase, hydrogen peroxide

1. Introduction

Most synthetic industrial dyes are complex aromatic compounds with an azo bond connected to various aromatic structures. Some, however, are polymeric structures containing metals.

It is estimated that there are over 10 000 commercially available dyes and pigments of industrial use, representing an annual consumption of around 7 x 10^5 tonnes worldwide (Akhtar *et al.* 2005). However, about 10-15% of the synthetic dyes produced are discharged into industrial effluents (Spadaro et al., 1992), causing environmental problems. Then, dye contamination of water bodies is a great problem in many countries.

Removal of dyes can be carried out by means of oxidative enzymes (Mohorcic *et al.* 2006). Peroxidases are versatile enzymes that catalyze the oxidation of a large number of aromatic structures through a reaction with hydrogen peroxide, being applied in the chemical, environmental, pharmaceutical and biotechnological industries (Zamorano *et al.* 2007).

The objectives of the present study were to extract potent peroxidases from agricultural biowastes, particularly from post-harvest fresh leaves from lentils, and to use their reaction mechanism to the oxidation/removal of a series of dyes commonly found in the contaminated effluents of textile industries. This study is aimed at documenting the capabilities of such peroxidase extracts and H_2O_2 coupled oxidation of those textile dyes and particularly of Green Domalan BL. The effect of parameters such as pH, H_2O_2, enzyme and dye concentrations, contact time, centrifugation, temperature on the decoulorization and detoxification of the dye was investigated.

2. Methods

Dye assay

Quantitative estimation of the dyes in aqueous phase was carried out by colorimetry, scanning the absorbance spectrum at wavelengths between 350 nm and 700 nm by means of a Beckman DU-7 UV/VIS spectrophotometer. For the dye Green Domalan solution (76 mg L^{-1}), the absorbance maximum was obtained at λ_{max} = 600 nm. At this wavelength, the corresponding Beer-Lambert correlation between absorbance and dye concentration gives a molar absorptivity of 8,6 $mM^{-1}cm^{-1}$ which was used for estimation of dye concentration. After the enzymatic treatment, the dye samples were centrifuged and the supernatants were assayed for the residual dye concentrations.

Assay of enzymatic dye removal

Experiments were carried out at a constant temperature (21°C) by varying the process parameters such as pH, dye concentration, peroxidase concentration, H_2O_2 concentration, centrifugation and incubation times. Initially, kinetics were carried out at 76 mg L^{-1} dye concentration by keeping aqueous phase pH at 4.0, enzyme concentration at 5.65 U L^{-1} and H_2O_2 at 10 mM. The reaction mixtures in vials were kept for agitation on shaker at 100 rpm for the requisite contact time and aliquots of the solution were analyzed for residual dye concentration in aqueous phase after centrifugation (10 000*g*, 2min, 20°C). Subsequent series if experiments were performed by varying the aqueous phase pH (from 2 to 9), dye concentration (from 20-270 mg L^{-1}), peroxidase concentration (0-6.78 U L^{-1}), H_2O_2 dose (from 0-11 mM), centrifugation time (2-10 min) and contact time (0-24 h) to know the optimum conditions for dye removal.

3. Results and discussion

Electronic spectra of dyes before and after their enzymatic treatment

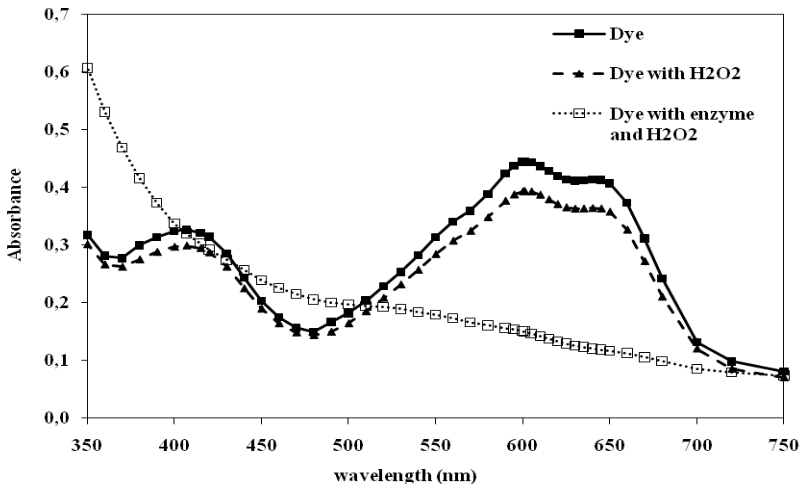

Figure 1. Electronic Spectrum of Green Domalan BL (76 mg L^{-1}) alone (■), after 24 h incubated with 10 mM H_2O_2 (▲) and with enzyme (5.65 U L^{-1}) plus 10 mM H_2O_2 ([]).

Kinetics of the dye removal

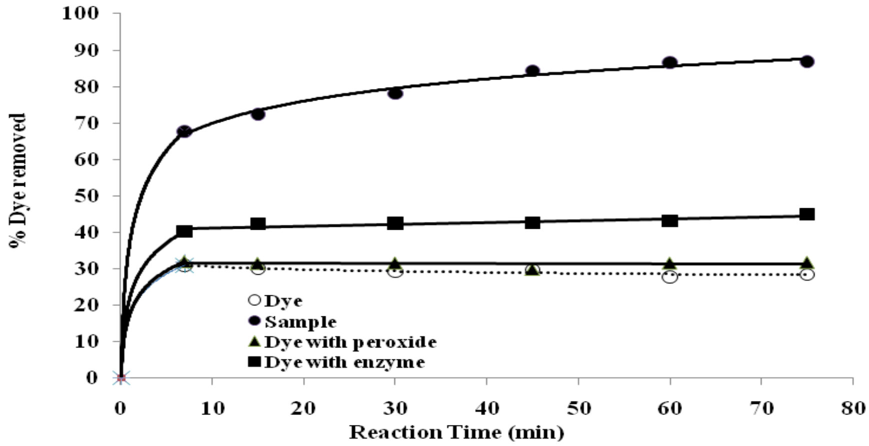

Figure 2. Kinetics of Green Domalan (76 mg L^{-1}) removal in 0.02 M acetate buffer (pH 4.0) at 25 °C: three reference samples (dye with buffer (O), dye with 5.65 U l^{-1} enzyme (■) and dye with 10 mM H_2O_2 (▲)) and the sample with dye, enzyme and H_2O_2. All the samples were centrifugate for a period of 2 min.

Activity versus pH profile

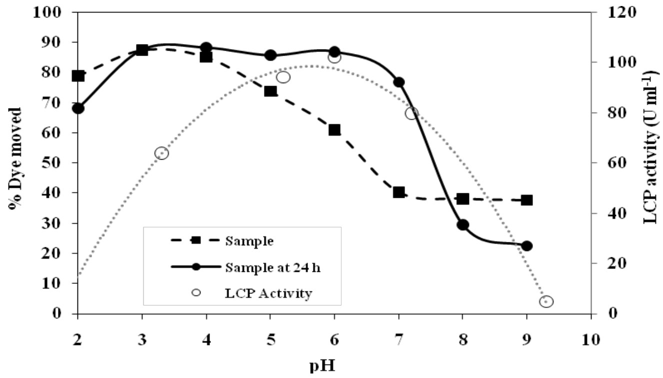

Figure 3. Effect of pH on the enzymatic removal of Green Domalan at 1 and 24 h of enzymatica reaction. Conditions: 20 mM universal buffer (pH 2–9), dye (76 mg L^{-1}), peroxidase (5.65 U L^{-1}), H_2O_2 (10 mM), reaction temperature (25 °C). About 87% of dye was removed at a pH range from 3 to 6. This wide dye removal activity (pH) profile indicates that a polymer is formed as a reaction product and causes the inmobilization of the peroxidase, limiting the free dye and H^+ diffusional approach to the enzyme.

Table.- Sensitivity analysis of Green Domalan oxidation by lentil peroxidase (+ H_2O_2)

Variable	Experimental conditions I	Experimental conditions II	Range studied	Optimum value
Time of centrifugation at 10000 rpm	20 mL 21 °C pH 4,0 50 min reaction	5,65 U/mL enz. 76 mg/L dye 10 mM H_2O_2	2 – 10 min	2 min
Reaction time	20 mL 21 °C pH 4,0 50 min centrifug.	5,65 U/mL enz. 76 mg/L dye 10 mM H_2O_2	0 – 24 h	1 h
[H_2O_2]	1,5 mL 21 °C pH 4,0 2 min centrifug. 1 h reaction	5,65 U/mL enz. 76 mg/L dye 10 mM H_2O_2	0 – 11 mM	0, 1 mM
[Peroxidase]	5,0 mL 21 °C pH 4,0 2 min centrifug. 1 h reaction	76 mg/L dye 0,3 mM H_2O_2	0 – 6,78 U/mL	2,26 U/mL

[Dye]	5,0 mL 21 °C pH 4,0 2 min centrifug. 1 h reaction	2,26 mg/L enz. 0,3 mM H_2O_2	20 – 270 mg/L	240 mg/L

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