

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

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## Introduction

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

It is estimated that there are over 10,000 commercially available dyes and pigments for industrial use, representing an annual consumption of around  $7 \times 10^5$  tons worldwide. However, about 10-15% of the synthetic dyes produced are discharged into industrial effluents, causing environmental problems. Accordingly, in many countries the contamination of water bodies by dyes is an important problem.

The colour of textile effluents is due to the dyes. Synthetic dyes vary in chemical composition, but they share a common feature: they must be highly stable against light and washing and they must be resistant to chemical and microbial attack. Among the chemical classes of dyes, azo dyes, the largest class of these compounds (84% of all dyes used), are considered to be recalcitrant, non-biodegradable by aerobic bacteria, and persistent. The anaerobic process has a major disadvantage in that the reduction of azo dyes generates colourless but potentially mutagenic and carcinogenic aromatic amines. Consequently, the treatment of dye-polluted effluents is considered to be one of the most challenging tasks among the environmental community.

Physicochemical methods, such as adsorption by activated carbon, ultrafiltration, coagulation, irradiation, oxidation (with chlorine, hydrogen peroxide, and ozone), reduction, precipitation, electrochemical treatment and ion-pair extraction, are effective in the removal of dyes. However, none of these methods has been found to be very suitable because they are expensive and produce toxic pollutants, and commonly used methods of chemical precipitation produce large quantities of sludge, which creates problems of disposal. Consequently, owing to its highly selective nature researchers have been focusing their attention on the study of enzymatic pretreatment as a potential and viable alternative to conventional methods. Further, in enzymatic treatment, inhibition by toxic substances is minimal and the process can operate over a broad concentration range of aromatics, with low retention times. Enzymes can act on specific recalcitrant pollutants to remove them by precipitation or transformation to other (innocuous) products and can also change the characteristics of a given waste to render it more amenable to treatment. Compared to chemical catalysts, the catalytic action of enzymes is extremely efficient and selective owing to higher reaction rates, milder reaction conditions, and greater stereospecificity. Enzymes can catalyze reactions at relatively low temperatures and across the whole aqueous pH range. Although much attention has been paid to the use of biocatalysts in several fields, their involvement has only been considered very recently for the resolution of environmental problems.

Dyes can be removed by means of oxidative enzymes. Peroxidases are versatile enzymes that catalyze the oxidation of a large number of aromatic structures through a reaction with hydrogen peroxide, and they are applied in the chemical, environmental, pharmaceutical and biotechnological industries. Peroxidases can act on specific recalcitrant pollutants either by free radical oxidative polymerization and consequent settling or by transformation into other products. Horseradish peroxidase (HRP) in the presence of  $H_2O_2$  is known to be effective in the removal of a broad spectrum of aromatic compounds (phenols, biphenols, anilines) and in the degradation and precipitation of industrial dyes.

The objectives of the present study were to extract potent peroxidases from agricultural biowastes, in particular from post-harvest fresh lentil leaves, and to use their reaction mechanism for the oxidation/removal of a series of dyes commonly found in the contaminated effluents of textile industries. The study is aimed at checking the capabilities of such peroxidase extracts coupled to  $H_2O_2$  in the oxidation of those textile dyes, in particular of Green Domalan BL. The effects of parameters such as pH,  $H_2O_2$ , enzyme and dye concentrations, contact and centrifugation times, the temperature of decolorization, and the detoxification of the dye were investigated.

## Materials and Methods

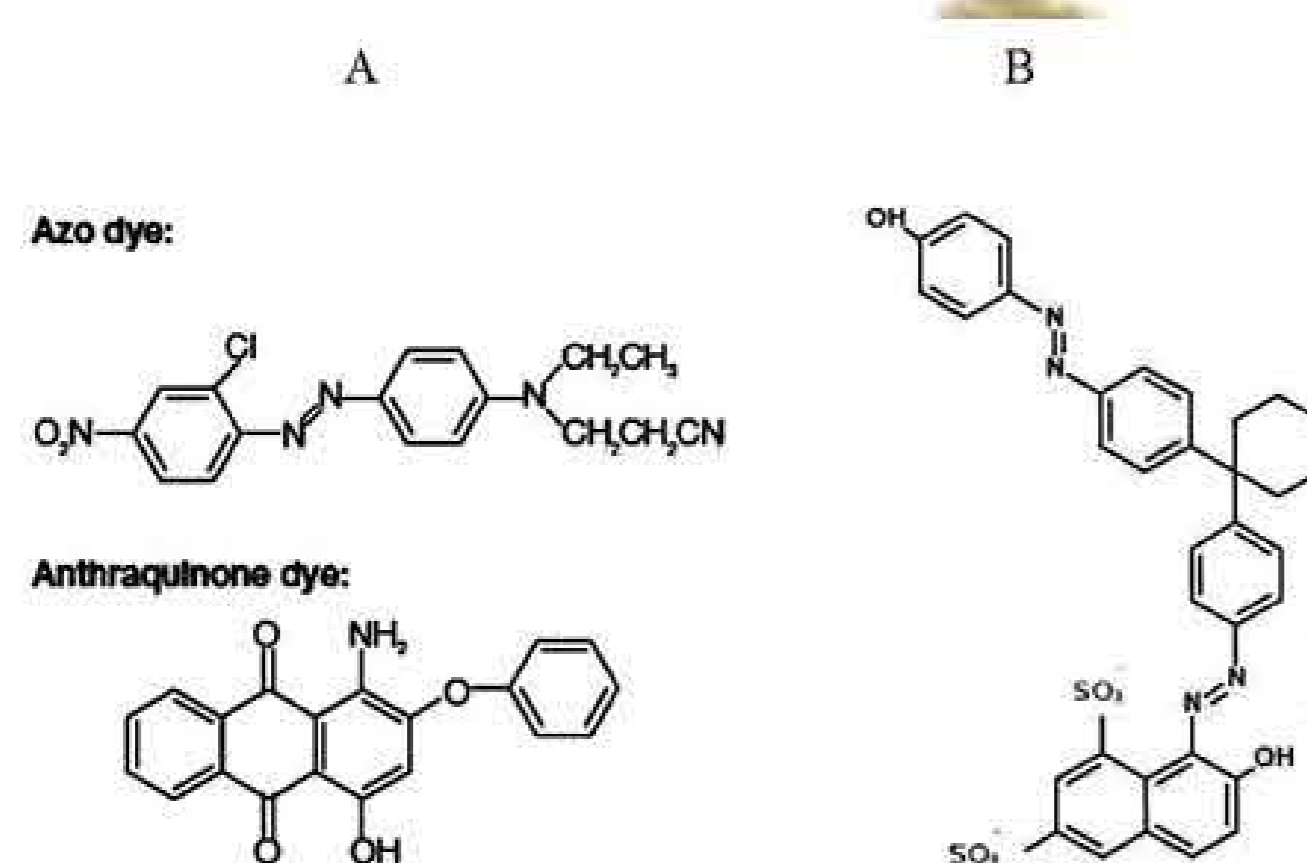
### Dye assay

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 ( $76 \text{ mg L}^{-1}$ ), the absorbance maximum was obtained at  $\lambda_{\text{max}} = 600 \text{ nm}$ . At this wavelength, the corresponding Beer-Lambert correlation between absorbance and dye concentration gives a molar absorptivity of  $8.6 \text{ mM}^{-1}\text{cm}^{-1}$  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 ( $21^\circ\text{C}$ ) 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  $76 \text{ mg L}^{-1}$  dye concentration by keeping aqueous phase pH at 4.0, enzyme concentration at  $5.65 \text{ U mL}^{-1}$  and  $H_2O_2$  at  $10 \text{ mM}$ . The reaction mixtures in vials were kept for agitation on shaker at  $100 \text{ rpm}$  for the requisite contact time and aliquots of the solution were analyzed for residual dye concentration in aqueous phase after centrifugation ( $10,000 \text{ g}$ ,  $2 \text{ min}$ ,  $20^\circ\text{C}$ ). Subsequent series of experiments were performed by varying the aqueous phase pH (from 2 to 9), dye concentration (from  $20$ – $270 \text{ mg L}^{-1}$ ), peroxidase concentration ( $0$ – $6.78 \text{ U mL}^{-1}$ ),  $H_2O_2$  dose (from  $0$ – $11 \text{ mM}$ ), centrifugation time ( $2$ – $10 \text{ min}$ ) and contact time ( $0$ – $24 \text{ h}$ ) to know the optimum conditions for dye removal.

## Results and discussion



Scheme 1. Azoic and anthraquinone structures of Domalan dyes (A) and the structure of Orange Bemacid dye (B).

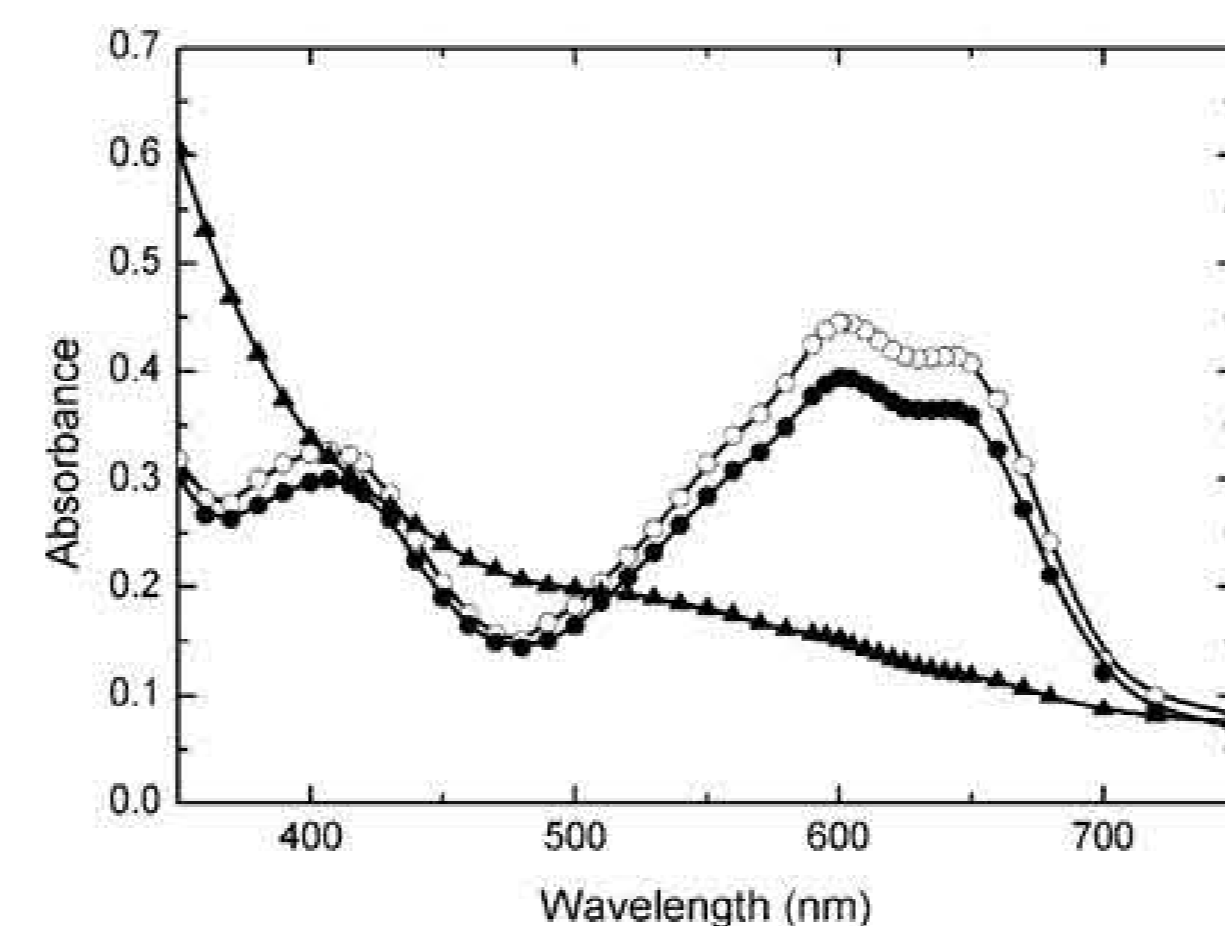


Fig. 1. Electronic spectra of Green Domalan BL ( $76 \text{ mg L}^{-1}$ ) in  $20 \text{ mM}$  acetic acid/acetate, pH 4.0, before ( $\circ$ ) and after being incubated for 24 h with  $H_2O_2$  ( $10 \text{ mM}$ ) ( $\bullet$ ) and with peroxidase ( $5.65 \text{ U mL}^{-1}$ ) and  $H_2O_2$  ( $10 \text{ mM}$ ) ( $\blacktriangle$ ). The decolorization yield in 24 h for the peroxidase process was 79.7%.

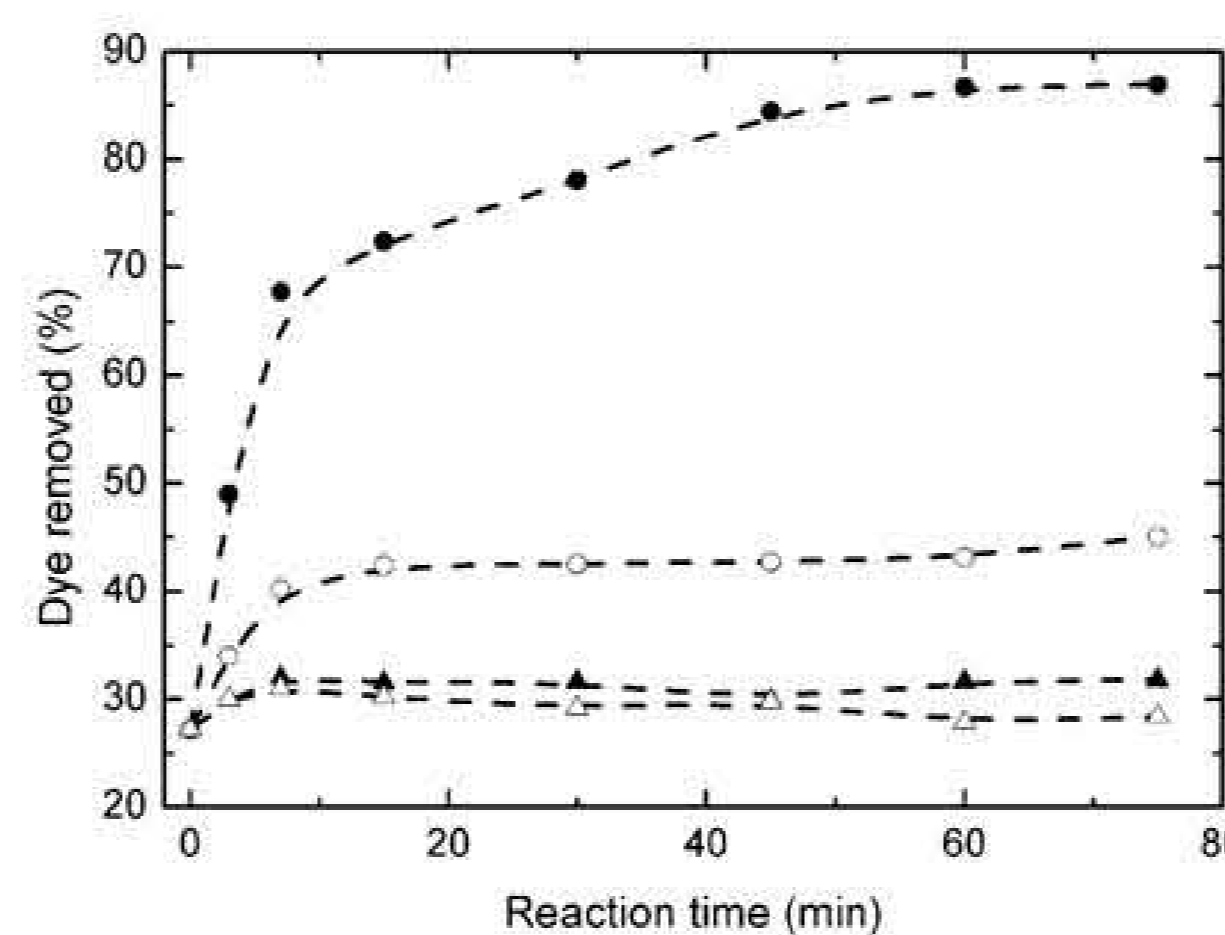


Fig. 2. Time-course for Green Domalan removal mediated by LSP. Dye solution ( $\Delta$ ), dye incubated with LSP ( $\circ$ ), dye incubated with  $H_2O_2$  ( $\blacktriangle$ ), and dye incubated with LSP plus  $H_2O_2$  ( $\bullet$ ). For experimental conditions, see text.

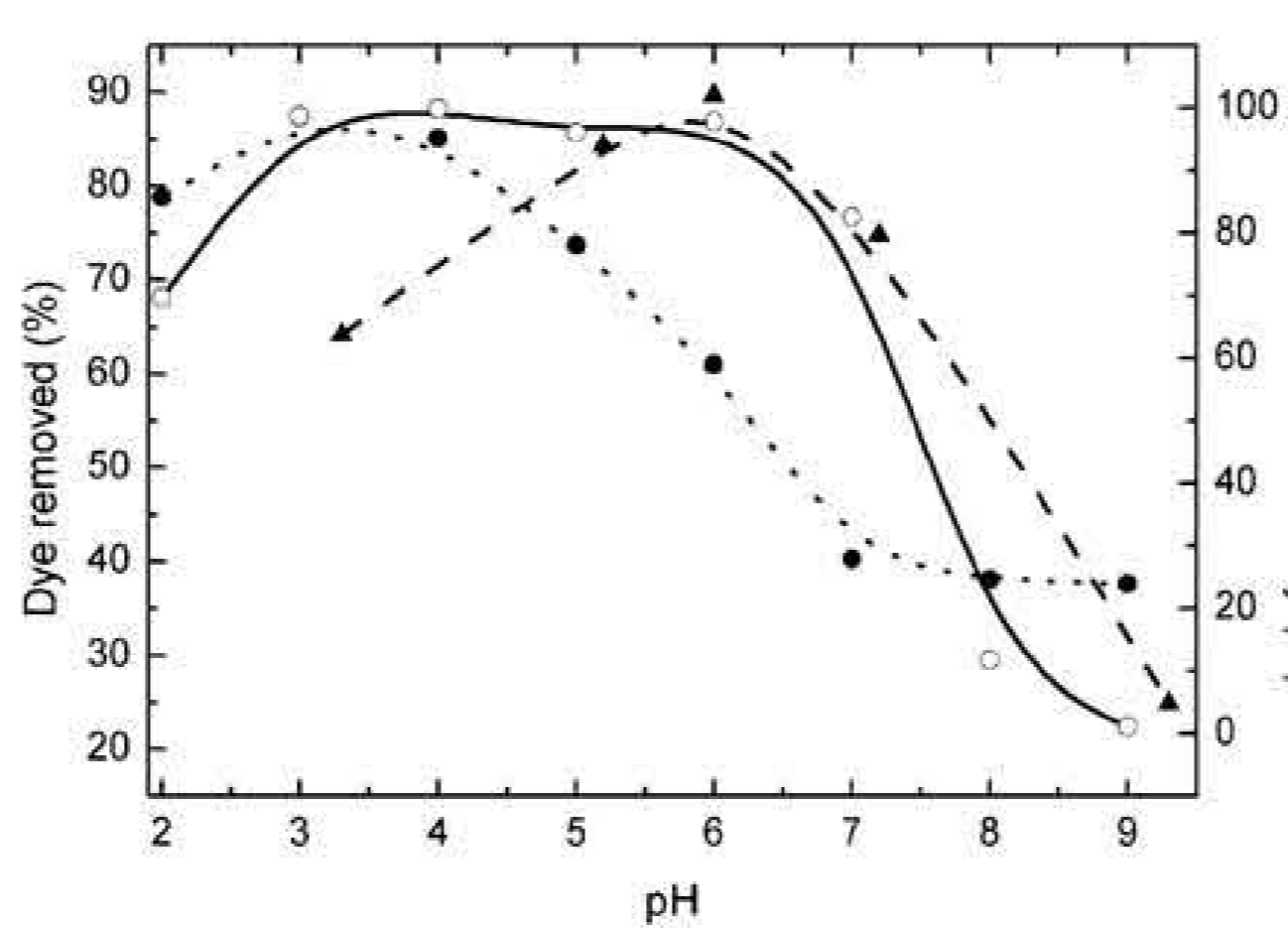


Fig. 3. Effect of pH on LSP catalyzed Green Domalan removal from aqueous solutions. Dye removal activity versus pH at 1 h ( $\bullet$ ) and 24 h ( $\circ$ ) reaction. LSP activity towards guayacol versus pH ( $\blacktriangle$ ). For experimental conditions, see text.

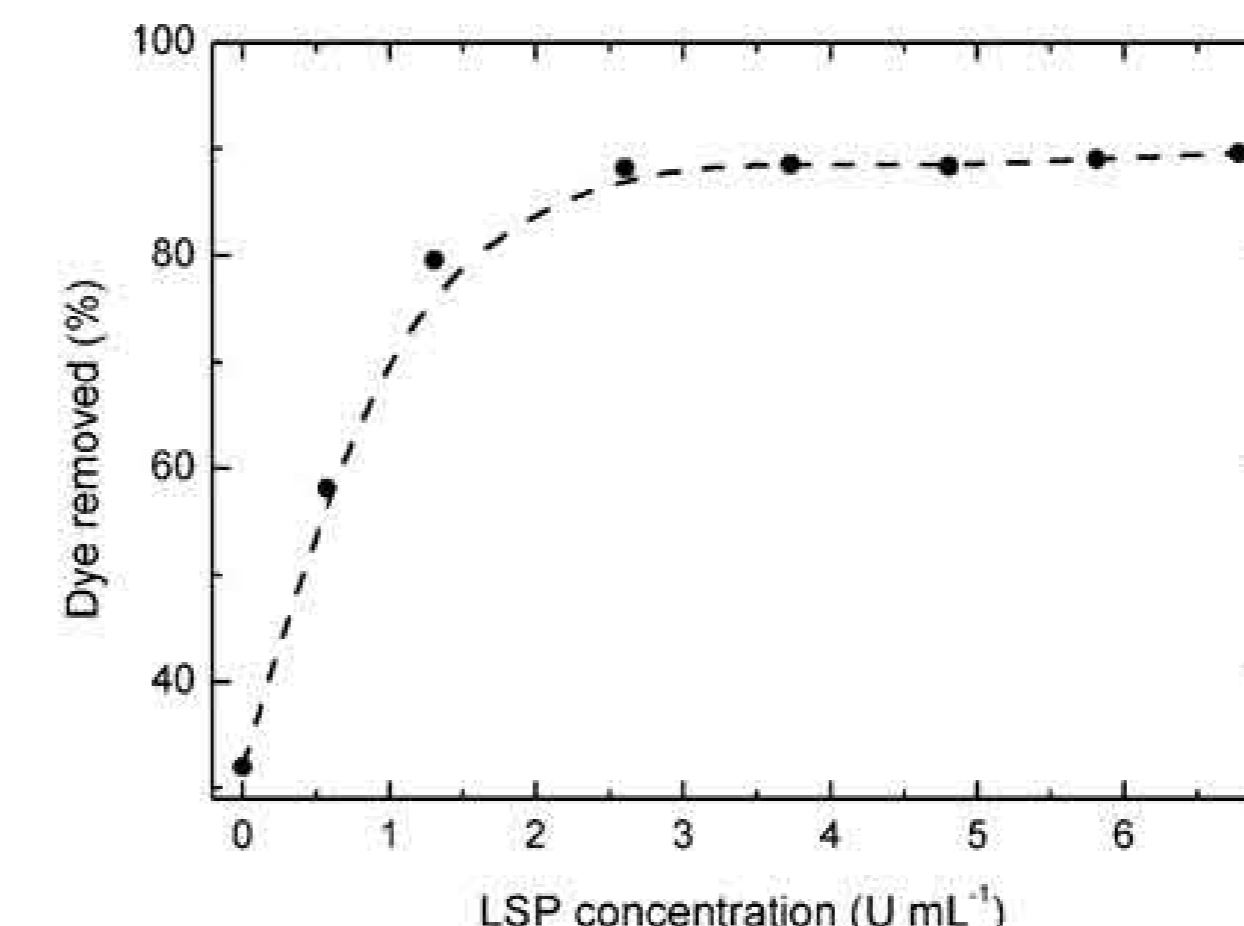


Fig. 4. Effect of LSP concentration on Green Domalan removal activity. See text for experimental conditions.

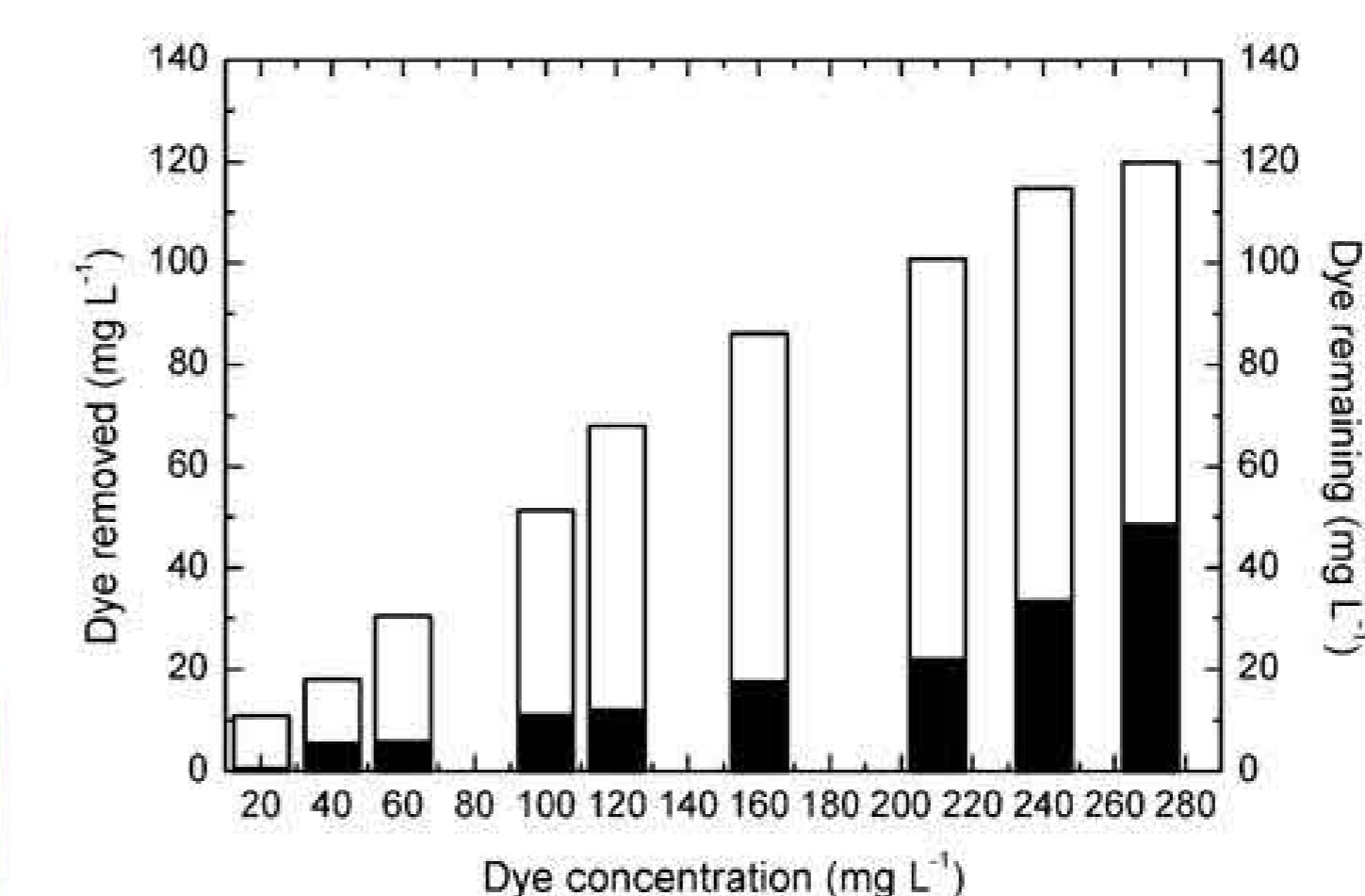


Fig. 5. Effect of dye concentration on LSP-catalyzed Green Domalan removal from aqueous solution. Dye removed ( $\square$ ) and remaining ( $\blacksquare$ ). For experimental conditions, see text.

Table 1

Sensitivity study for the determination of the optimum conditions for the performance of lentil stubble peroxidase (LSP) with regard to Green Domalan BL removal from aqueous solutions.

Parameters	Range evaluated	Optimized parameters
pH	2.0 – 9.0	3.0 – 5.0 <sup>a</sup>
Reaction time (h)	0 – 24	1 <sup>b</sup>
Centrifugation time (min)	2–10	2 <sup>c</sup>
Peroxidase activity ( $\text{U mL}^{-1}$ )	0 – 6.78	1.13 <sup>d</sup>
$[H_2O_2]$ (mM)	0 – 11	0.3 <sup>e</sup>
$[Dye]$ ( $\text{mg L}^{-1}$ )	20 – 270	240 <sup>f</sup>
Temperature ( $^\circ\text{C}$ )	18 – 38	25 <sup>g</sup>

Other conditions: a)  $20 \text{ mM}$  universal buffer,  $76 \text{ mg L}^{-1}$  dye,  $5.65 \text{ U mL}^{-1}$  LSP,  $10 \text{ mM}$   $H_2O_2$ , 24 h reaction time,  $25^\circ\text{C}$ . b)  $20 \text{ mM}$  acetic acid/acetate, pH 4.0,  $76 \text{ mg L}^{-1}$  dye,  $5.65 \text{ U mL}^{-1}$  LSP,  $10 \text{ mM}$   $H_2O_2$ , 50 min centrifugation,  $25^\circ\text{C}$ . c)  $20 \text{ mM}$  acetic acid/acetate, pH 4.0,  $76 \text{ mg L}^{-1}$  dye,  $5.65 \text{ U mL}^{-1}$  LSP,  $10 \text{ mM}$   $H_2O_2$ , 1 h reaction,  $25^\circ\text{C}$ . d)  $20 \text{ mM}$  acetic acid/acetate, pH 4.0,  $76 \text{ mg L}^{-1}$  dye,  $0.3 \text{ mM}$   $H_2O_2$ , 2 min centrifugation, 1 h reaction,  $25^\circ\text{C}$ . e)  $20 \text{ mM}$  acetic acid/acetate, pH 4.0,  $76 \text{ mg L}^{-1}$  dye,  $5.65 \text{ U mL}^{-1}$  LSP, 2 min centrifugation, 1 h reaction,  $25^\circ\text{C}$ . f)  $20 \text{ mM}$  acetic acid/acetate, pH 4.0,  $1.13 \text{ U mL}^{-1}$  LSP,  $0.3 \text{ mM}$  of  $H_2O_2$ ,  $240 \text{ mg L}^{-1}$  dye, 1 h reaction. g)  $20 \text{ mM}$  acetic acid/acetate, pH 4.0,  $1.13 \text{ U mL}^{-1}$  LSP,  $0.3 \text{ mM}$  of  $H_2O_2$ ,  $240 \text{ mg L}^{-1}$  dye, 1 h reaction.

Table 2

Substrate specificity of LSP for the decolorization/degradation of a series of dyes.

Dye	$\lambda_{\text{max}}$ (nm)	Time (h)	$[Dye]$ ( $\text{mg L}^{-1}$ )	$Abs_0$	$Abs_{\text{fin. time}}$	Decolorization yield (%)
Green Domalan BL	600	24	700	0.4137	0.1211	79.7
Orange Bemacid CMGL	491	72	40	0.6125	0.2140	65.1
Ash-Grey Domalan R	556	24	800	0.6042	0.3571	40.9
Black Domalan HEBD	570	24	700	0.5244	0.3325	36.6
Navy Blue Domalan HEND	590	24	100	1.2952	0.9872	23.8
Red Bemacid F-GS	518	72	110	1.1547	0.8862	23.3
Red Domalan 2BL	541	24	700	0.8541	0.6921	19.0
Red Domalan RL	490	24	150	1.4712	1.3197	10.3
Yellow Domalan 3RL	446	72	150	1.0087	0.9675	4.1

Other experimental conditions as in Fig. 1

## Conclusions

The experimental results obtained in the present work reveal the effectiveness of the peroxidase-catalyzed enzymatic reaction for the treatment of textile dyes in the aqueous phase. However, the performance of the LSP-catalyzed dye-removing reaction was found to be dependent on the centrifugation and reaction times, the dye, the  $H_2O_2$  and enzyme concentrations, and pH. Likewise, the absence of an effect of temperature on the dye equilibrium concentration was observed. The decrease in the ecotoxicity value of Green Domalan BL solutions due to the action of the LSP extract shows that the remaining products are less toxic than the starting products.

## Acknowledgements

Funding from the Consejería de Educación (projects SA129A07 and SA052A10-2) and Consejería de Agricultura y Ganadería (project SA06000), Junta de Castilla y León, is acknowledged.

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