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Short communication

Decolorization of textile dyes and effluents using potato (*Solanum tuberosum*) phenoloxidase

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ABSTRACT

Potatoes are desirable source for polyphenol oxidase (PPO, EC 1.14.18.1) purification because this enzyme can be purified from the food industry waste such are potato peels from potato chips industry. This paper presents data concerning decolorization of 7 different, so far untested textile dyes and 3 real samples (industry effluents) by a partially purified PPO. Under optimized conditions 93–99.9% removal of dyes was achieved after 1 h using 424–1700 U ml^{-1} of PPO, depending on dye. Optimum pH for decolorization process of all dyes was found to be 3.0. Potato PPO was capable of removing reactive dyes and textile dye effluents without requiring any mediator. Decolorization was accomplished *via* insoluble polymers formations that were separated by filtration.

1. Introduction

Industrial dyeing of textile consumes large amounts of water and energy, while 5–40% of the dyestuffs used are released in the effluent (Silva et al., 2010). Released colors may seriously jeopardize both photosynthetic activities of surface water ecosystems since they interfere with the absorption of solar radiation and ecosystem integrity due to their toxicity. Since some of those dyes are recalcitrant to direct microbial degradation there is an ongoing search for improvement of existing technologies and introduction of clean and more efficient technologies that will enable degradation of these compounds (Verma et al., 2003). Promising alternative technology is the use of oxidative enzymes as crude or partially purified to reduce overall treatment costs. It is known that polyphenol oxidases (PPO) and peroxidases can quickly and non-selectively oxidize a broad spectrum of structurally diverse organic molecules. PPOs (EC 1.14.18.1) from potatoes are the enzymes with highest potential with respect to its availability and cost (Khan et al., 2006). It has been shown previously that PPO activity was the highest in the tuber exterior, including the skin and cortex tissue 1–2 mm beneath the skin (Thygesen et al., 1995). Potatoes are desirable source for PPO purification because this enzyme can be purified from the food industry waste such as potato peels from potato chips industry. The enzymatic treatment efficiency was found to be independent of the enzyme purity and therefore, it was possible to utilize a crude or partially purified preparation that is protected from inactivation due to the significant quantity of protein present instead of a purified one (Cooper and Nicell, 1996). However, use of partially purified enzymes is preferable over crude extracts due to many reasons such as removal of undesired phenolics from plant source during purification processes and avoidance of excess of proteins in crude extracts that may adversely increase biochemical oxygen demand (BOD) in the treated wastewater.

The present study was performed to extend our previous work on application of cheap enzyme source such as potato PPO (Lončar and Vujčić, 2011). We now describe the usability of PPO partially purified by ion-exchange chromatography for removal of recalcitrant textile dyes. Seven dyes widely used in textile industries for cotton dyeing have been selected for the study as well as three different dye effluents collected directly from local textile-dying industry. Optimum conditions for efficient removal of dyes by precipitation from real samples and model systems were investigated.

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2. Material and methods

2.1. Materials

Potato (*Solanum tuberosum*) tubers were obtained from the local market. All reagents used in this study were of analytical grade. They were purchased from Merck (Darmstadt, Germany) and Sigma–Aldrich (St. Louis, MO, USA). The textile effluents were collected at the outlet of the neutralization tank from a textile dyeing industry, located in Sombor, Serbia.

2.2. Polyphenol oxidase activity assay

PPO was purified as described previously (Lončar and Vujčić, 2011). Briefly, potatoes were cooled to 3 °C over night and then homogenized in commercial juicer. The homogenate was centrifuged and obtained supernatant was desalted against 10 mM Na-phosphate buffer pH 7.3 using Sephadex G25 Coarse column. Equilibrated and deaerated ion-exchanger QAE Sephadex A-50 was added to extract and mixed with magnetic stirrer 30 min in oxygen-free atmosphere. Unbound proteins were washed with 3 volumes of starting buffer and enzyme was eluted with 0.75M NaCl in starting buffer. Obtained enzyme preparation was stored at −20 °C until use.

PPO activity was determined using L-DOPA as a substrate at 25 °C by measuring the initial rate of dopachrome formation at 475 nm (Kwon and Kim, 1996).

2.3. Effect of pH on the decolorization of reactive textile dyes

Concentrations and characteristic wavelength maximums (λ_{max}) for four azo and three aminochlorotriazine dyes used in this study are given in Table 1. These concentrations were chosen in order to have starting dye solution with absorbance of 1.0 at specific λ_{max}. Each dye was incubated with PPO (424 U ml^{-1}) in the appropriate 50 mmol l^{-1} buffers (sodium acetate, pH 3.0 and pH 5.0; sodium phosphate, pH 7.0; glycine–sodium hydroxide, pH 9.0) at 25 °C for 1 h, after which the solutions were liberated from produced polymers by filtration through Whatman No. 1 or by centrifugation at 600 × g and tested for remaining dye content by measuring absorbance at λ_{max}. Starting absorbance at characteristic λ_{max} for each dye (control) was designated as 100%. The extent of decolorization rate (Rd) was defined by following formula: Rd (%) = $[(A_0 - A_1)/A0] \times 100$, where A_0 was the absorbance of the untreated dye and A_1 was the absorbance after treatment.

Table 1
Effect of initial solution pH on decolorization of tested dyes with PPO (424 U mL^{-1}).

	λ_{max}	C (mg L^{-1})	% of remaining dye[a]			
			pH 3	pH 5	pH 7	pH 9
Aminochlorotriazine dyes						
Procion yellow (PY)	420	65	7 ± 0.46	50 ± 1.06	95 ± 1.15	100
Procion red brown (PRB)	460	50	26 ± 0.86	67 ± 2.60	97 ± 1.2	100
Procion dark blue (PDB)	600	50	47 ± 1.60	93 ± 1.65	100	100
Azo dyes						
Reactive blue 52 (RB52)	615	50	5 ± 0.17	71 ± 2.15	91 ± 1.24	92 ± 1.7
Reactive green 15 (RG15)	625	100	9 ± 0.31	59 ± 1.69	93 ± 1.0	93 ± 1.2
Reactive yellow 125 (RY125)	390	65	11 ± 0.29	54 ± 1.87	97 ± 0.9	100
Congo red (CR)	522	65	0.5 ± 0.04	5 ± 0.03	83 ± 1.1	90 ± 0.8

[a] Statistical significance is confirmed by Student's *t*-test.

2.4. Effect of PPO concentration and time of incubation on the decolorization of dyes

To test effect of PPO concentration decolorization rates of all dyes were analyzed with increasing concentrations of PPO in range 212–1700 U ml^{-1} in 50 mmol l^{-1} sodium acetate buffer, pH 3.0 at 25 °C for 1 h. Effects of incubation time was monitored by incubation of dye solutions with PPO (283 U ml^{-1}) in the 50 mmol l^{-1} sodium acetate buffer, pH 3.0 at 25 °C for different period of time. Dye decolorization was monitored at specific λ_{max} as described above.

2.5. Vis and FTIR spectrometry analysis of textile effluents treated with PPO

The textile effluents were kept at an ambient temperature, original pH being 7.1. pH adjustments to pH 3.0 were done by addition of acetic acid. Acidification had no visible influence on effluents. Decolorization treatments were done by addition of 424 U ml^{-1} of PPO to each effluent. Vis spectrums of effluents were recorded using Cintra 40 Spectrometer in 380–800 nm range before and after treatment with PPO. FTIR spectra of Reactive blue 52 (RB52) and its polymer product were obtained in a range of 400–4000 nm by using an attenuated total reflectance (ATR) technique on FTIR spectrophotometer (Nicolet 6700FT-IR, Thermo Scientific) as described by Aktas et al. (2000, 2003). Polymer product was collected by centrifugation after treatment with PPO and dried until constant weight using Eppendorf Vacuum Concentrator Model 5301.

2.6. Statistics

All experimental results reported in the next sections were based on averaging results of repeated experimental runs (triplicates), with the SD ranging from 2 to 6% of the reported average. Statistical significance is confirmed by Student's *t*-test.

3. Results and discussion

Potato peels from chips industry are free source of enzymes for biotechnology due to potatoes availability during whole year (Vujčić et al., 2010; Lončar et al., 2011) Endogenous polyphenols must be removed during purification procedure since phenoxy

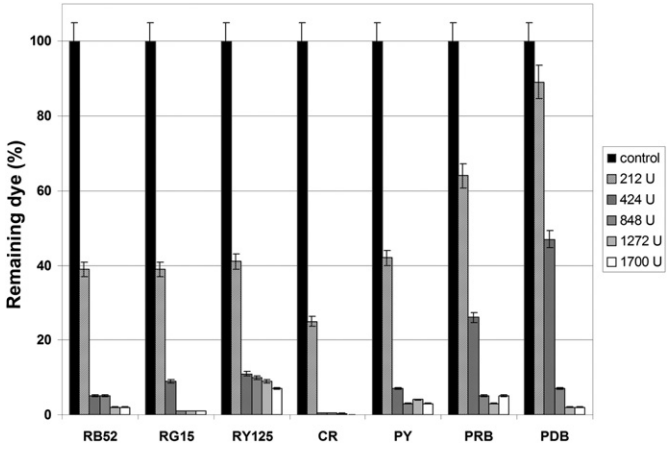

Fig. 1. Influence of PPO concentration on decolorization of reactive dyes.