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Efficiency of enzymatic and non-enzymatic catalysts in the synthesis of insoluble polyphenol and conductive polyaniline in water

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Abstract

The present work analyzes the potential use of white-rot fungi (WRF) and hematin for phenol and aniline polymerization, as a low-cost alternative to horseradish peroxidase (HRPC). The objective is to evaluate the capability of these catalysts to produce tailor-made aniline as well as to eliminate phenols by precipitation from aqueous solution. 4-Aminoantypirine (4AAP) was used to test phenoxide formation by crude protein preparations of white-rot fungi at selected conditions. The crude extracts of *Pleurotus sajor-caju* (PSC) were selected because of the promising values obtained for the phenoxide formation rate. HRPC/H_2O_2 and *P. sajor-caju* derived enzymes/H_2O_2 (PSC/H_2O_2) systems produced soluble polyaniline in the presence of polystyrene sulphonated (PES), with high aniline conversions at 45 °C. For the case of insoluble polyphenol production, the PSC-derived enzymes, in absence of hydrogen peroxide, produced insoluble polyphenol with similar efficiencies as those found with HRPC or hematin in a one step phenol treatment (near 40% phenol conversion). For the aniline process, at least 75% aniline conversion was obtained when using PSC enzymes at room temperature. After long reaction times, the lignin-modifying enzymes derived from PSC only produced a conductive form of polyaniline (PANI) at lower temperatures than those required when employing HRPC. Fungal enzymes look promising for eliminating aniline/phenol from wastewaters since the obtained results demonstrated that they are able to polymerizate and precipitate them from aqueous solutions.

Keywords: Polyaniline; Polyphenol; White-rot fungi; Peroxidases

1. Introduction

Peroxidases from horseradish (HRPC) or soybean (*Glycine max*) (SBP) are typical oxidoreductases, which are able to catalyze different reactions using H_2O_2 as an H acceptor. They have been used in the oxidative polymerization of aromatic compounds to generate polyaromatics and as catalysts for phenol elimination as a clean alternative for detoxifying wastewater [1,2]. For example, the products from the HPRC/H_2O_2/phenol reaction are mainly polymers and phenol oligomers insoluble in water. Both polymers and insoluble phenol oligomers can be removed by sedimentation and filtration [3].

The enzymatic polymerization of anilines appears to be more efficient and promising than chemical or electrochemical technology [1,2,4]. HRPC, in the presence of H_2O_2, catalyzes the polymerization of aromatic amines to polymers with high molecular weight. When adding a polyelectrolite, such as sulphonated polystyrene (PES), aniline polymerizes and forms complexes with the polyanion, resulting in the conductive form of polyaniline [5]. PES, acting as a template, directs the para-link of the aniline radicals. The conductive, emeraldine form of polyaniline (PANI) has a para-link of the aniline moieties.

A methodology that involves "crude" enzymes to polymerize monomers is also an attractive and feasible proposal, especially because of the already known limitations in the use of HRPC. The so-called "white-rot fungi" (WRF) produces lignin-modifying extracellular enzymes (LME) that are able to degrade lignin: two glycosilated peroxidases (lignin peroxidase-LiP and Mn peroxidase-MnP), a phenoloxidase laccase (Lac) and an aryl alcohol oxidase (AAO). The latter produces hydrogen peroxide continuously. All these enzymes are able to synthesize aromatic polyalcohols and polyamines using substituted phenols

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Nomenclature

E	concentration of peroxidase in native form (mol/L)
E_1	Compound I concentration (mol/L)
E_2	Compound II concentration (mol/L)
E_3	Compound III concentration (mol/L)
k_1	reaction rate constant for native enzyme oxidation
k_2	reaction rate constant for phenol oxidation by intermediate active compound (E_1)
k_3	reaction rate constant for phenol oxidation by intermediate active compound (E_2)
k_{app}	reaction rate constant for E_2 oxidation to E_3
k_a	reaction rate for the decomposition of the intermediate compound E_3 to the native enzyme form
k_b	reaction rate for the decomposition of E_3 to E_1

and anilines as substrates [6]. Biomimetics are another alternative to the use of pure or crude enzymes.

This work presents the results for aniline and phenol polymerization using HRPC, the broth of the liquid culture from WRF obtained at the end of the tropophase (with emphasis on *Pleurotus sajor-caju* (PSC)), and hematin. Kinetic studies were also carried out using WRF and HRPC. Kobayashi et al. [7] and Tonami et al. [8] reported the quantitative production of soluble polyphenol by selecting of the reaction conditions appropriately. On the contrary, the aim of this work is to produce insoluble polyphenol (POFE) at high yields using peroxidase-like catalysts. These catalysts may be employed in decontamination of wastewaters, based on the ideas that were reported elsewhere [7–16] in terms of activity and polyphenol characterization. Besides, Fe-salen as biomimetic catalyst (*N*,*N*′ethylen-bis (salicylideneamine) has been developed by Kobayashi et al., as it was detailed in [7,8], achieving the efficient production of organic soluble functional phenolic polymers.

The goal of this manuscript is to present a comparative analysis of the capabilities of different enzymatic and non-enzymatic catalysts in specific reactions at the following selected conditions: aqueous soluble PANI synthesis with a template and insoluble POFE precipitation by phenol polymerization from aqueous solution. We demonstrate that low-cost crude enzymes from WRF (*P. sajor-caju*, mainly) can be used as catalysts for soluble PANI synthesis with a template and for insoluble POFE precipitation from wastewaters containing phenol. Firstly, a screening of the phenoxide radical generation rate was performed on different kinds of WRF. The best fungus (in terms of initial reaction rate of phenoxide formation) was selected to perform the PANI and POFE synthesis. This study also examines hematin (hydroxiferriprotoporfirine), both soluble and magnetite-supported, as a biomimetic catalyst and model for the active site of peroxidases. The effect of temperature was also addressed. Although synthesis of soluble polyaniline and insoluble polyphenol is not essentially new [3,17], the use of WRF is unusual and there are almost no reports on the use of magnetite-supported hematin.

2. Experimental

2.1. Materials and methods

Sulphonated polystyrene (PES, 99%) used for PANI synthesis was provided by Sigma and it was employed without further purification.

2.1.1. HRPC, hematin

Horseradish peroxidase was kindly provided by Amano Inc. and it was used without further purification. Hematin from Sigma Chemical Co. was employed as provided. Phenol (99%), aniline, 4-aminoantypirine (4AAP), and a pH 7 buffer were supplied by Merck. Other buffer solutions (pH 4 and 7) were provided by Anedra. Fe $(NO_3)_3{\cdot}9H_2O$ from Mallinckrodt was used.

2.1.1.1. Magnetite-supported hematin preparation. Magnetite was prepared by controlled oxidation in aqueous alkaline medium, using 36.8 g of $FeSO_4{\cdot}7H_2O$, 14 g of NaOH and 4.5 g of $NaNO_3$ in a total volume of 300 mL. The ferrous salt was added at a constant rate (ca. 2.8 mL/min), while it was vigorously stirred. A green-brownish gel was obtained, whose structure (or formula composition) was probably $Fe(OH)_2{\cdot}xH_2O$ $Fe_2O_3{\cdot}nH_2O$. This gel was kept at room temperature during 12 days to increase its oxidation. The final color was dark black. This product was carefully washed 10 times with twice-distilled water. Afterwards, it was centrifuged twice.

Magnetite and hematin were coupled using glutaraldehyde. One milliliter of 25% glutaraldehyde was put in contact with 160 mg of magnetite in 20 mL of phosphate buffer (pH 7) for 24 h. The recovered solid was reacted with 20-mg hematin in another buffer (pH 4). The latter was the optimum pH for the coupling reaction. After 2 h at 40 °C, the slurry was filtered through acetate filters. The product yield was ca. 86%, as tested with the 10-phenantroline method for Fe quantification. This yield implies that nearly 9.5% of the supported catalyst is hematin.

2.1.2. White-rot fungi

The following fungi were tested: *Lentinula edodes* (Shiitake) (Somycel 4055, Francia), *Ganoderma lucidum* (Reishi 34-D, Fungi Perfecti, Olympia WA USA), *P. sajor caju* (Fungi Perfecti Olympia WA USA), *Pleurotus florida* (ATCC 96997) and *Trametes versicolor* (Spanish Collection of Type Cultures, Universidad de Valencia CECT 20148). The different nutrient media for liquid cultivation of fungi were as follows: (1) *Lentinula edodes* (Shiitake): MYPA medium (20 g/L of malt extract, 10 g/L of yeast extract, 1 g/L of peptone) at pH 6.0, with 30 g/L of glucose and 25 g/L of milled sunflower hull; (2) *G. lucidum*: MYPA medium with 10 g/L of glucose and 65 g/L of sunflower seed; (3) *P. sajor caju* and *P. florida*: MYPA medium with 65 g/L of sunflower seed; and (4) *T. versicolor*: MYPA medium with 40 g/L of glucose and 65 g/L of sunflower seed. Two triangular sections of young mycelium from each strain, approximately 0.2 mg/mL, were inoculated in 200 mL of liquid culture medium in 1-L Erlenmeyer flasks to obtain an air-medium ratio of 5:1. The inoculated cultures were incubated in the dark at 25 ± 1 °C for