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Enzymatic polymerization of catechol under high-pressure homogenization for the green coloration of textiles

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ABSTRACT

Laccase from *Myceliophthora thermophila* was used to catalyze the polymerization of catechol under high-pressure homogenization for the green coloration of textile substrates. The oxidation reactions were conducted using different forms of laccase, namely native laccase, PEGylated laccase and PEGylated laccase immobilized onto an epoxy resin. The three enzyme forms were deposited inside a polyester fabric bag during the experiments. The amount of polymer obtained was similar when using the three enzyme forms and its dispersion in water/DMSO mixture lead to powder particles of about 30–60 nm. The immobilized and PEGylated enzymes lead to poly(catechol) with 13 and 10 units, respectively, while the native form gave rise to shorter polymers (DP = 8). We have shown that the oxidation of catechol conducted under high-pressure homogenization can be an efficient methodology for the *in situ* coloration of textiles. The polymers produced by this methodology stained strongly the textile container, revealing this experimental set-up as a promising greener coloration/coating methodology involving milder conditions than the normally used in textile processes.

1. Introduction

Phenolic compounds, such as catechol, are released to the environment from a variety of industrial sources since they are often used as industrial reagents in the production of rubber, dyes, pesticides, colors, plastics, pharmaceuticals, and cosmetics (Tušek et al., 2017). When organic compounds like catechol are released into the environment, they can accumulate in the soil, groundwater and surface water, and therefore become an issue of great environmental concern. The presence of these potentially toxic chemicals may be able to transform into teratogenic or carcinogenic agents to life (Aghapour et al., 2013; Cheng et al., 2016, 2018; Cohen et al., 2009; Liu et al., 2017). The increased demand of the industry to develop environmentally friendly methodologies lead to the development of enzymatic processes for its pollutant removal from wastewater (Shinji et al., 1993; Tušek et al., 2017).

Laccase-catalyzed polymerization has received much attention from researchers in the last decades due to its ability to oxidize both phenolic and non-phenolic compounds as well as highly recalcitrant environmental pollutants, making it useful for applications on several biotechnological processes (Riva, 2006; Rodríguez Couto and Toca Herrera, 2006). Laccases, a family of multi-copper containing oxidoreductases, are probably one of the most promiscuous enzymes considering their excellent catalytic properties. They are capable of oxidizing a wide range of different substrates with or without mediators under mild conditions, and for these reasons several researchers have been applying them on the polymer synthesis (Jeon et al., 2012; Kunamneni et al., 2008; Witayakran and Ragauskas, 2009). As typical laccase substrates, phenols, namely catechol, and its derivatives, can generate various functional polymers based on diverse monomers, which may be applied in several fields like medicine, cosmetics, food and textiles. During synthesis, laccases are known to provide a unique alternative to organic synthesis compared with conventional chemical-catalyzed synthetic processes. The oxidation of catechol using laccase as catalyst has been studied. Saha et al. investigated a two-step treatment method for the removal of phenol, benzenediols, and an equimolar mixture of phenol and benzenediols from water and demonstrated

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that the proposed enzymatic method is a viable alternative means to remove phenol and benzenediols from industrial wastewaters (Saha et al., 2011). Tušek et al. tested two different methods to immobilize laccase from *Trametes versicolor* and compared them for the catechol polymerization using different reactors (Tušek et al., 2017). They found the successful catechol oxidation using immobilized laccase in different reactor systems, batch and continuous, micro and macro size.

Different reactors have been explored to proceed the enzyme-catalyzed reactions (Gupta and Roy, 2004; Henriksson et al., 2007; Shah and Gupta, 2008). Generally, water bath is the most common device used to promote mechanical agitation and improve the mass transport effects. However, the absence of external stimulus reveal low polymerization yields mainly due to inefficient agitation and thus low mass transport is achieved (Zerva et al., 2016). These results lead the researchers to investigate the use of different devices able to promote higher levels of conversion. They found that when ultrasound devices were applied, cavitation effects enhanced the transport of substrate molecules to the enzyme improving catalysis and accelerating the reactions (Delgado-Povedano and Luque de Castro, 2015; Lobo et al., 2013; Yachmenev et al., 2009; Yachmenev et al., 2002). Several works have been reporting enzyme catalysis assisted by high-pressure homogenization (HPH) (Gonçalves et al., 2014; Martins et al., 2015), and their results reveal HPH as a promising method to improve the enzyme application under cavitation effects. High-pressure homogenization, as well as ultrasound, are known to produce cavitation, which may accelerate reactions and increment the mass transport phenomena (Gall et al., 2016; Romanski et al., 2011). Until now and from our knowledge, the use of HPH on the polymer synthesis is still poorly explored.

In previous studies, we conducted the catechol polymerization with laccase from *Myceliophthora thermophila* using different apparatus, namely a water bath, an ultrasonic bath and a high-pressure homogenizer (Su et al., 2018). The results obtained showed higher conversion yields and polymerization degrees when both high-energy reactors were used, compared to the water bath reactor. Molecular dynamic simulations performed also demonstrated that under these conditions the enzyme presented a more open active site which we consider the main factor for the higher substrate accessibility to the enzyme therefore favoring the production of longer polymers. High-pressure homogenization was thus considered as a promising technique for catechol polymerization.

In this study, our goal was to evaluate the catalytic ability of different forms of laccase to polymerize catechol under high-energy/pressure environments. The catalysis was thus conducted using native laccase, PEGylated laccase and laccase immobilized onto an epoxy resin (Epoxy-PEGylated laccase) under high-pressure homogenizer (HPH) (Fig. 1). In this study, the non-soluble enzyme form was contained within a polyethylene terephthalate (PET) bag, as well as the other forms for control purposes. The objective was to constrain the immobilized enzyme movement along the machine and allow only the liquid solution to recirculate. The polymerization was followed during time by UV–Vis spectra analysis to monitor colour change. The produced polymers were characterized by weight measurement, ^{1}H NMR, and TGA. The particle size of the polymer powders was analyzed by dynamic light scattering analysis. We aim with this work to evaluate the potentialities of the high-pressure homogenization as energy source for the enzymatic-catalysis reactions with different laccase forms and explore the possibilities to obtain polymers with coloring performance via a green methodology. Generally, fabric dyeing processes imply the use of extreme conditions like high temperatures and/or highly acidic or alkaline pHs. Polyphenolic components from laccase reactions are produced at milder conditions and could be used for green coloration.

2. Materials and methods

2.1. Materials

2.1.1. Enzyme and reagent sources

Laccase from *Myceliophthora thermophila* (Novozymes A/S, batch number: OMN07012) was supplied by Novozymes, Denmark. Catechol, poly(ethylene glycol) methyl ether and sodium carbonate were purchased from Sigma Aldrich, Spain. Deuterated dimethyl sulfoxide was obtained by Cortecnet, France.

2.2. Methods

2.2.1. PEGylation of laccase

Laccase from *Myceliophthora thermophila* was PEGylated as previously reported (Su et al., 2017) using the procedure of Daly et al. (2005). Briefly, 14.0 mL of 12 mg/mL laccase were reacted with 20 kDa, poly(ethylene glycol) methyl ether at pH 5.0 phosphate solution 100 mM with 20 mM sodium cyanoborohydride. A control reaction without $_{m}$PEG was also conducted in every experiment. The reactions were stirred rapidly for 17 h at 4 °C. After 10 min of mixing, the reactants were completely dissolved, and an aliquot (namely time 0 h) was taken, as well as at each time point of reaction. These samples were ultrafiltrated, and washed several times with water, using a 30 kDa cellulose membrane mounted in an ultrafiltration apparatus, to separate the free PEG. Afterwards the final solution was freeze-dried.

2.2.2. Immobilization of PEGylated laccase onto epoxy resin supports

The immobilization of PEGylated laccase onto epoxy methacrylate resins (Purelite Lifetech ECR enzyme immobilization resins: 300-600 Å) was conducted as follows: 2 mg/mL PEGylated laccase in 0.5 M acetate buffer (pH 5.0) were mixed with epoxy methacrylate (50 mg/mL) and then stirred for 48 h at 4 °C. The powder was then washed several times with water by centrifugation and dried under vacuum.

2.2.3. Evaluation of enzyme activity and stability

The effect of high-pressure on the activity and stability of native laccase, PEGylated laccase and Epoxy-PEGylated laccase, was evaluated. For this, the three forms of laccase were incubated under the same conditions used for catechol polymerization: 100 U/mL enzyme were incubated in acetate buffer (pH = 5) at 40 °C for 3 h, using a high-pressure homogenizer. The enzymes were exposed for longer time in order to ensure the accurate stability during time. Aliquots of enzyme solution were taken at different periods of incubation and the activity of laccase was measured against ABTS according to the methodology described by Childs and Bardsley (1975).

2.2.4. Enzyme-assisted polymerization of catechol

Catechol polymerization was processed by incubating 50 mM of monomer in different solutions: a) 100 U/mL native laccase and b) 100 U/mL PEGylated laccase, c) 100 U/mL Epoxy-PEGylated laccase, in acetate buffer (pH = 5). The immobilized enzyme was confined in a polyethylene terephthalate (PET) bag and placed in the sample receptor of the high-pressure homogenizer. For control reasons, the other enzyme forms were also placed inside the PET bag. Afterwards the catechol solution was added and the homogenization proceed for 2 h (corresponding to 360 homogenization cycles). During the reaction, the top of the feed port was covered with a