



# Polymerisation of Kraft lignin from black liquors by laccase from *Myceliophthora thermophila*: Effect of operational conditions and black liquor origin



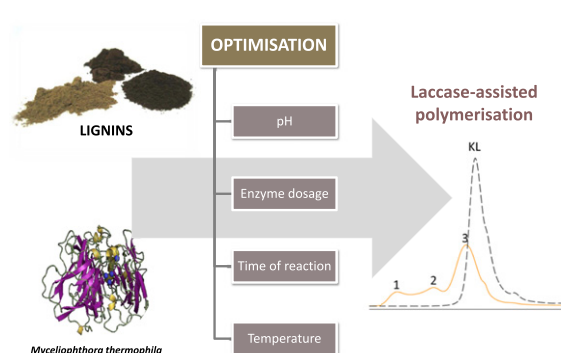
S. Gouveia, C. Fernández-Costas, M.A. Sanromán, D. Moldes\*

Department of Chemical Engineering, University of Vigo, Lagoas Marcosende s/n, E-36310 Vigo, Spain

## HIGHLIGHTS

- ▶ Reaction conditions play a crucial role in KL polymerisation by laccase.
- ▶ Several polymers are produced in laccase-assisted polymerisation of KL.
- ▶ Hardwood lignin may be extensively polymerised in contrast to softwood one.

## GRAPHICAL ABSTRACT



## ARTICLE INFO

### Article history:

Received 1 August 2012  
 Received in revised form 21 December 2012  
 Accepted 25 December 2012  
 Available online 3 January 2013

### Keywords:

Laccase  
 Kraft lignin (KL)  
 KL source  
 Lignin fractionation  
 Polymerisation

## ABSTRACT

The capacity of laccase from *Myceliophthora thermophila* to promote the oxidative polymerisation of Kraft lignin (KL) was evaluated in several conditions of pH, temperature, enzyme dosage and treatment time. Moreover, different black liquors from the Kraft cooking of *Eucalyptus globulus* and mixture of *Pinus pinaster*/*E. globulus* were evaluated in order to determine the effect of the KL source on the polymerisation reaction. Furthermore, one of these black liquors was fractionated by sequential organic solvent fractionation and the polymerisation of the corresponding fractions was tested.

Polymerisation products were analysed by size exclusion chromatography and Fourier transform infrared spectroscopy. The results provide evidence of notable lignin modifications after incubation with laccase. Structural oxidation and a notably molecular weight increase were attained, reaching a polymer of 69-fold its initial molecular weight depending on the raw lignin. Moreover, optimum values of reaction conditions were obtained: pH 7.3, 70 °C, 2 U mL<sup>-1</sup> and 2 h.

© 2012 Elsevier Ltd. All rights reserved.

## 1. Introduction

Enzymatic processes, besides being environmentally friendly, can be very effective in the transformation of specific substrates due to their high selectivity. Because of these characteristics the enzymatic processes have arisen as a feasible alternative to several traditional chemical processes. In the forest based industry, a wide

range of enzymatic applications have being researched: pulp biobleaching (Moldes and Vidal, 2011), mill effluent treatment (Monje et al., 2010), wood pre-treatment in composite boards such as medium-density fibreboard (Felby et al., 2002; Widsten and Kandelbauer, 2008a) or paper and wood properties improvement (Chandra et al., 2004; Kudanga et al., 2008) are some examples of potential applications of the enzymatic technology. Particularly, one of the most common enzymes to be applied in relation to the forest industry is laccase (Widsten and Kandelbauer, 2008b). Laccases (EC 1.10.3.2) are phenoloxidases capable of oxidising a

\* Corresponding author. Tel.: +34 986 818723; fax: +34 986 812380.  
 E-mail addresses: [diego@uvigo.es](mailto:diego@uvigo.es), [diegomoldes@gmail.com](mailto:diegomoldes@gmail.com) (D. Moldes).

wide range of substrates such as phenolic compounds, including those characteristic of the lignin structure.

Lignin is a complex, three-dimensional biomolecule that represents from 15% to 35% of the wood (Onnerud et al., 2002; USDA, 2007) and makes up about 20% of the total mass of the biosphere (Kleinert and Barth, 2008). Industrial lignins are by-products of the pulp and paper industry, where several processes are carried out to separate lignin from lignocellulosic materials to obtain an enriched cellulose pulp. The Kraft cooking is the dominant pulping process in the pulp and paper industry. The nearly 130 million tons of pulp produced worldwide every year represent 90% of the chemical pulp and two-thirds of the world's virgin pulp production (Tran and Vakkilainen, 2008). The delignification process involves the digestion of wood in a strong alkaline solution. After cooking, the undesired lignin is concentrated (black liquor) and incinerated for steam and energy production. Although burning lignin reduces the use of fossil fuels, lignin could be isolated from the black liquor and used as raw material for a variety of new products (Gargulak and Lebo, 2000) obtained by processes involving enzymatic or chemical modifications (Sena-Martins et al., 2008).

Regarding its composition, lignin mainly consist of three major phenolic components, namely *para*-coumaryl alcohol (H), coniferyl alcohol (G) and sinapyl alcohol (S) (Ralph et al., 2004). The relative amount of each of these monomeric lignin precursors and the total lignin content, mostly depend on wood species. Lignin from softwood is essentially made up of G structures while hardwood has a higher content of S monomers. In nature, polymerisation of lignin precursors is initiated by an electro-abstracting enzyme, such as laccase, and followed by coupling reactions that may involve different types of carbon-carbon or ether linkages (Widsten and Kandelbauer, 2008a). Contrary to other natural polymers like cellulose or proteins, lignin does not have a regular or ordered structure. One of the major problems that industrial lignins must overcome for their application in certain fields is their heterogeneity (Crestini et al., 2010). Molecular weight distribution has a great influence on polymer properties like mechanical ones, solubility or flow behaviour. Some authors (Gouveia et al., 2012; Ropponen et al., 2011) stated that lignin fractionation by solvent extraction allows to attain several lignin fractions separated according to their molar mass. Therefore, this methodology could be employed to obtain polymers with homogeneous properties.

In the literature, laccase oxidation capacity was studied over lignin model compounds (Areskog et al., 2010a; Onnerud et al., 2002; Rittstiege et al., 2002, 2003) and to a lesser extent in technical lignins (Areskog et al., 2010b; Moya et al., 2011). Recently, the influence of lignin fractionation on the polymerisation capacity of laccase has been studied by van de Pas et al. (2011) and Gouveia et al. (2012). However, more studies are necessary to determine the reaction conditions that permit to increase the polymerisation degree obtained in these previous studies.

In the present work, laccase capability to oxidise and polymerise lignin was studied under different operational conditions of pH, temperature, enzyme dosage and treatment time in order to establish the optimal polymerisation conditions. Moreover, to determine the influence of the type of lignin used in the enzymatic polymerisation, three different unfractionated lignins (from the black liquor of several Kraft pulp mills) and a solvent fractionated lignin were used. Fourier transform infrared spectroscopy (FTIR) and size exclusion chromatography (SEC) were used to study lignin polymerisation. As far as we know, there are not published works where the oxidation capacity of laccase was optimised over KL. This fact points out the relevance and novelty of the results obtained in the present study.

## 2. Methods

### 2.1. Lignin

Black liquors from the Kraft cooking of *Eucalyptus globulus* were supplied by Ence (Pontevedra, Spain) and Portucel-Soporcel (Cacia, Portugal). Europac (Viana do Castelo, Portugal) supplied a black liquor from cooking of the mixture of *Pinus pinaster/E. globulus* (75/25). The corresponding Kraft lignins (KL) named EN-KL, P-KL and EU-KL for Ence, Portucel-Soporcel and Europac, respectively, were obtained by acidic precipitation: the black liquor was diluted with distilled water and then pH was lowered to pH 2.5 with sulphuric acid 1 M under stirring at 50 °C. After filtrating, the precipitated lignin was washed twice with acidified water (pH 2.5) and oven dried at 60 °C overnight. The dried samples were then milled with mortar and pestle and stored.

#### 2.1.1. Organic solvent fractionation

The solvent fractionation was performed to EN-KL following the method described by Gosselink et al. (2010) and detailed in a previous report (Gouveia et al., 2012). All solvent fractions were obtained from the same EN-KL sample.

### 2.2. Enzyme

A commercial laccase from *Myceliophthora thermophila* (NS51003) was kindly supplied by Novozymes (Bagsvaerd, Denmark). Before polymerisation experiments, the enzyme was cleaned in a PD-10 Desalting column, Sephadex G-25 Medium (General Electric) following the protocol recommended by the supplier.

### 2.3. Determination of laccase activity

Laccase activity was determined spectrophotometrically at 436 nm ( $\epsilon = 29300 \text{ M}^{-1} \text{ cm}^{-1}$ ), as described Nugroho Prasetyo et al. (2009), at pH 7.3 and 20 °C with 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) 0.5 mM, as substrate. One activity unit was defined as the amount of enzyme that oxidised 1  $\mu\text{mol}$  of ABTS per min. The activities were expressed in  $\text{U mL}^{-1}$ .

### 2.4. Laccase-lignin reactions

Lignin was solubilised in phosphate buffer (100 mM) to attain a concentration of  $1.5 \text{ g L}^{-1}$ . The laccase was added to 90 mL of the latter solution and the reaction was carried out under stirring. Several conditions were tested in this lignin polymerisation reaction; pH: 6–8, temperature: 60–80 °C, time of reaction: 2–6 h, laccase activity: 0.5–4  $\text{U mL}^{-1}$ .

Control experiments with heat-denatured laccase were carried out to guarantee that the products obtained are result of the enzymatic effect. Reactions were concluded by lowering the pH to 2.0. The reaction products were filtered and washed twice with acidified water (pH 2.5) and oven dried at 60 °C overnight.

All experiments were carried out at least in duplicate. Experimental error from  $M_w$  determinations was always lower than 5%.

### 2.5. Determination of phenolic content

The phenolic content was analysed following the method described by Areskog et al. (2010a). A phenol-free sample was employed as control and vanillin was used for calibration purposes. All experiments were carried out in duplicate. Experimental error was always lower than 5%.

Download English Version:

<https://daneshyari.com/en/article/7084071>

Download Persian Version:

<https://daneshyari.com/article/7084071>

[Daneshyari.com](https://daneshyari.com)