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Selective laccase-catalyzed dimerization of phenolic compounds derived from lignin: Towards original symmetrical bio-based (bis) aromatic monomers

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ABSTRACT

A laccase-catalyzed process was developed to prepare, selectively, in high yield, dimers of lignin-based phenolic compounds without any purification. The influence of experimental parameters such as laccase loading, nature of solvent and the presence of oxygen on the conversion of vanillin was investigated. After the dimerization, the product obtained as a precipitate is filtered off and the solution containing the enzyme can be re-used several times, which improves the process economics. A phenolic-substrate screening reveals that such process enables to dimerize regioselectively, six *ortho*-methoxy-*para*-substituted phenols (vanillin, 4-hydroxy-3-methoxybenzonitrile, acetovanillon, methyl vanillate, 2-methoxy-4-methylphenol, and eugenol) with yields ranging from 87% to 96% and one *ortho*-disubstituted phenol (2,6-dimethoxyphenol) with 80% yield.

1. Introduction

Nowadays, the partial replacement of fossil feedstocks by renewable resources attracts a thriving interest due to the petrol depletion and the growth of environmental concerns [1,2]. In this purpose, new molecules and monomers issued from available biomass have to be developed [3–6]. Lignin is the main source of aromatic bio-based substrates. Despite extensive researches on efficient ways of recovering aromatic products from lignin [7], nowadays, the only commercial process is the production of vanillin and vanillic acid by hydrothermal pretreatment under alkaline conditions of lignosulfonates, by-products of the sulfite paper industry [8–12].

Vanillin can be derived into divanillin, also called dehydrodivanillin, which is used mainly as flavoring [13] and antioxidant agent in food, cosmestic and pharmaceutical industry but can also be employed in microlithography [14] and in polymer synthesis [15,16]. Over the years, divanillin has been synthesized by different methods. It is commonly produced by oxidative phenol-coupling using iron(III) chloride ($FeCl_3$) or iron(II) sulfate ($FeSO_4$) [17–22]. These non-sustainable processes require a high amount of iron catalyst, long reaction times and are not fully selective, thus generating a mixture of products and a difficult work up. In order to avoid the use of inorganic salts and toxic agents (sodium persulfate), enzymatic pathways were developed. In 1972, the formation of divanillin was observed for the first time after oxidation of vanillin in aqueous solution with peroxidase in the presence of hydrogen peroxide [23]. In 2004, Dordick and coworkers studied the structural diversity of peroxidase-catalyzed oxidation products of o-methoxyphenols, leading to oligomers in the case of vanillin [24]. Further improvements of the conditions were needed to reach a rather good selectivity in dimer formation [25].

Laccase is another very well-known class of oxidative enzyme studied since 1883 [26]. The latter were identified in several plants, insects, bacteria and fungus, where they have different biological functions [27–29]. Contrary to peroxidases, laccases employ dioxygen as oxidant. Currently, a lot of studies report the use of laccases as biocatalysts for the oxidation of functional moieties or the oxidative coupling of phenolic substrates [30–37]. Laccases generate radical intermediates on phenolic compounds, which can undergo self-coupling reactions generally resulting in the forma-

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tion of a mixture of products from dimers to higher oligomers. The selectivity of the coupling and the size of the oligomers depend on a broad range of parameters such as laccase source, pH, temperature, substitution of the phenolic compound, solvents, etc. The use of laccases is limited due to their lack of selectivity. Recently, Beifuss and coworkers described a method on the coupling of vanillidene derivatives catalyzed by laccase from *Trametes versicolor* which provided the best result, in terms of yield/selectivity, of dimer synthesis by laccase catalysis [38]. Some specific substrates selectively led to one dimer in yield of over 80% but the authors did not investigate further the coupling reaction.

This study extends previous works in laccase-catalyzed dimer formation of *ortho*-methoxy-*para*-substituted phenols by improving dimerization process, product yields and extending the range of molecules studied for this reaction. Indeed, different reaction parameters such as reaction time, laccase loading and type of solvent were investigated on the example of vanillin. A refill procedure was also developed in order to recycle the catalyst solution. Afterwards, the same reaction conditions were applied on several phenolic substrates and the structures of the resulting products investigated.

2. Results and discussion

2.1. Coupling process development and optimization on the example of vanillin

Vanillin dimerization, catalyzed by laccase from *Trametes versicolor*, was performed at room temperature in a solution saturated in oxygen (Scheme 1). Prior to the addition of the acetate buffer (90 vol%, pH 5), vanillin was dissolved into acetone (10 vol%). Hence, the reactant stays in solution while the resulting product precipitated. After addition of the laccase, the colorless solution turned yellow, which either indicate the formation of radicals or quinone structures. After few minutes, a brown solid precipitated. The first reactions were performed on 1.5 g scale of vanillin, employing 100 U of laccase, in 200 mL solvent, for 24 h. The precipitate was filtered off, washed with water and analyzed by mass spectrometry, NMR and HPLC (Figs. S1–S4, SI). These analyses revealed the selective formation of a symmetric dimer, divanillin **1** (Scheme 1). Particularly, the NMR spectroscopy analyses were in agreement with the study of Eswaran et al. [14].

The selectivity and yield of coupling reactions catalyzed by laccase depend on the reaction conditions [39]. In this work, the influence of various parameters (enzyme loading, solvent, pH and saturation in oxygen) was investigated. The enzyme loading can be decreased down to 20 U without affecting the yield of divanillin, which after 24 h, remained over 80% (Fig. 1). Below this value, the yield decreased drastically to 50%. Thus, the quantity of laccase for the following reactions was set at 20 U for 1.5 g of substrate.

Vanillin conversion under different reaction conditions was followed by ^{1}H NMR spectroscopy (Fig. 2).

After 8 h, 85% conversion of vanillin into divanillin was achieved for an acetone/acetate buffer ratio of 10/90 under O_2 bubbling (a). In the following samples, the concentration of vanillin in the solution was too low to be detected (Fig. S5, SI). Instead of bubbling O_2, the reaction was carried out in a beaker with a large surface in contact with air, under vigorous stirring. The vanillin conversion (b) was similar to the conversion obtained in a solution saturated in oxygen (a). However, if the quantity of O_2 in the solution is limited by bubbling N_2 into the solution, after 25 h, the vanillin conversion (d) only reached 25%. The saturation of the solution with O_2 is thus a key parameter to reach high yield in divanillin.

When the acetate buffer is substituted by water, the conversion profile (c) follows the reference curve (a) until 5 h of reaction.

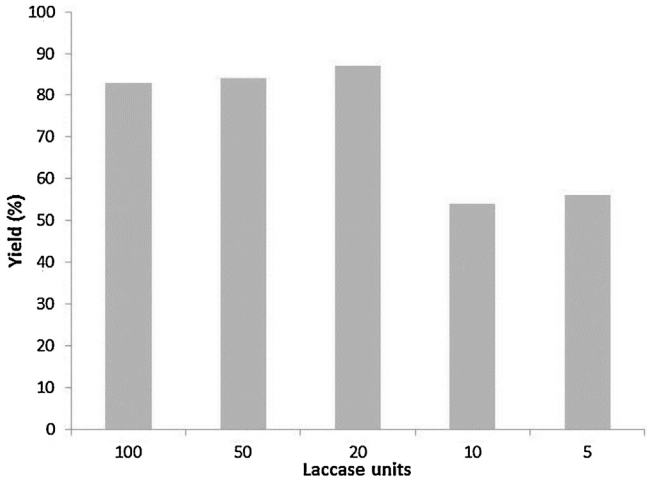

Fig. 1. Divanillin yields depending on laccase quantity for 1.5 g of vanillin, in 200 mL of solvent, after 24 h.

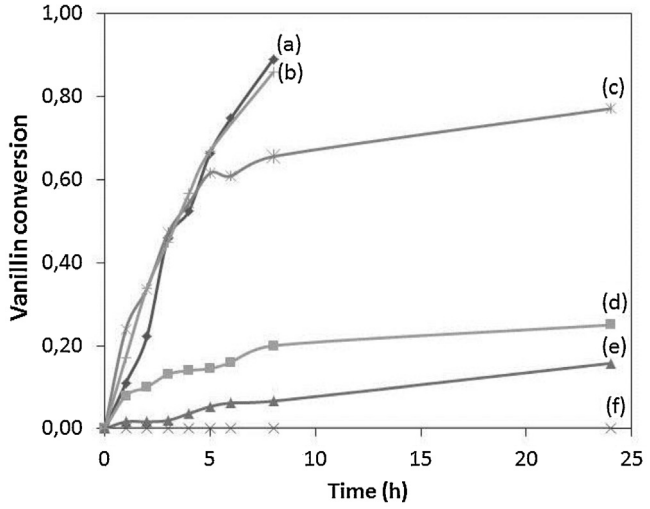

Fig. 2. Vanillin conversion versus time using data extracted from ^{1}H NMR spectra (aldehyde signal): different conditions: (a) 10% acetone–90% acetate buffer/O_2, (b) 10% acetone–90% acetate buffer/air, (c) 10% acetone–90% water/O_2, (d) 40% acetone–60% acetate buffer/O_2, (e) 10% acetone–90% acetate buffer/N_2, (f) 70% acetone–30% acetate buffer/O_2.

Beyond this time, the reaction speed decreased and, only 75% conversion was achieved after 24 h. An increase of the pH from 5–7 was observed after the reaction was stopped, that can explain the low conversion in the last hours. Indeed, the optimal pH zone for laccase ranges from 4–6; off this range, the laccase activity decreases.

The amount and nature of solvent also influence the laccase activity. Increasing the acetone/buffer ratio to 40% dramatically decreased the reaction speed (e). When the reaction was carried out with 70% of acetone, no conversion of the starting compound was observed (f). It is thus crucial to use the minimum amount of solvent required to dissolve the starting material in order to achieve high yields. Acetone can be substituted by other organic solvents provided the latter do not inhibit the laccase. However, depending on the solvent and quantity, the reaction yield can be affected [40,41]. For instance, 10% of DMSO was tested as alternative co-solvent and led to a yield around 90% after 8 h.

In this process, the recovery of divanillin as a precipitate presents three advantages: (i) as vanillin is soluble into the solution, the purity of the obtained dimer is very high (95% by NMR) and no purification is needed (ii) the precipitation shifts the reac-