

## Mini Review

# Laccases in the textile industry

Susana Rodríguez Couto\* and José Luis Toca-Herrera\*

Department of Chemical Engineering, Rovira i Virgili University. Av. Països Catalans 26, 43007 Tarragona, Spain.

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The search for efficient and green oxidation technologies has increased the interest in the use of enzymes to replace the conventional non-biological methods. Among the different existing oxidant enzymes, laccases (benzenediol: oxygen oxidoreductases; EC 1.10.3.2) have been subject of intensive research in the last decades due to their low substrate specificity. The use of laccases in the textile industry is growing very fast, since besides to decolourise textile effluents, laccases are used to bleach textiles, modify the surface of fabrics and synthetise dyes. Therefore, laccase-based processes might replace the traditionally high chemical, energy and water-consuming textile operations. The present paper offers an overview of the laccase applications in the textile industry up to date.

**Keywords:** bleaching, decolouration, denim, laccase, textile industry, wastewater

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## 1.0 INTRODUCTION

The search for efficient and green oxidation technologies has increased the interest in the use of enzymes to replace the conventional non-biological methods. Among the different existing oxidant enzymes, laccases (benzenediol: oxygen oxidoreductases; EC 1.10.3.2) have been subject of intensive research in the last decades because they have the following properties: low substrate specificity, do not need the addition or synthesis of a low molecular weight cofactor, as their cosubstrate – oxygen – is usually present in their environment, most laccases

are extracellular enzymes, making the purification procedures very easy, they generally exhibit a considerable level of stability in the extracellular environment, the inducible expression of laccases in most fungal species also contributes to their easy applicability in biotechnological processes.

Yoshida (1883) first described laccase in 1883 when he extracted it from the exudates of the Japanese lacquer tree *Rhus vernicifera*, from which the name laccase was derived and was characterised as a metal-containing oxidase by Bertrand (1895). This makes it one of the oldest enzymes ever described. Laccases have also been detected in insects (Sidjanski et al., 1997) and bacteria (Alexandre and Zhulin, 2000) but they are especially abundant in white-rot fungi, which are the only micro-org-

\*Corresponding authors E-mails: [susana.rodriguez@urv.cat](mailto:susana.rodriguez@urv.cat); [jose Luis.toca@urv.cat](mailto:jose Luis.toca@urv.cat). Phone: +34 977 55 9617; Fax: +34 977 55 9667

**Table 1.** Status of copper in fungal laccases (extracted from Claus 2003). Three types of copper have been distinguished.

Cu type	Cu atoms/protein	EPR signal	Features	Coordination	Functions
1	1	+ Paramagnetic	"Blue Cu <sup>2+</sup> ", absorbance at 610 nm (oxidation), redox potential +785 mV	2 His, 1 Cys, 1 Leu	Substrate oxidation (one-electron step)
2	1	+ Paramagnetic	"Non-blue Cu <sup>2+</sup> " (affinity to azide, fluoride, cyanide)	Trinuclear center	Reoxidation of type 1 Cu <sup>2+</sup> Stabilisation of an H <sub>2</sub> O <sub>2</sub> intermediate
3	2	- Diamagnetic	Spin-coupled Cu <sup>2+</sup> -Cu <sup>2+</sup> pair Absorbance at 330 nm (oxidation)	8 His	O <sub>2</sub> reduction by enzyme oxidation (four electron transfer)

organisms able to degrade the whole wood components.

Laccases catalyse one electron oxidation of a wide range of inorganic and organic substances, coupled with electron reduction of oxygen to water (Xu 1996). The molecular mass of the monomer ranges from about 50 to 100 kDa (Sjoblad and Bollag 1981; Dean and Eriksson 1994; Thurston 1994). An important feature of fungal laccases is a covalently linked carbohydrate moiety (10–45%), which may contribute to the high stability of the enzyme (Durán et al., 2002). For the catalytic activity, a minimum of four copper atoms per active protein unit is needed. The copper atoms differ in their light absorbance and electron-paramagnetic behaviour (Table 1).

Laccases have relatively lower redox potential (450–800 mV) compared to those of ligninolytic peroxidases (>1 V), so it was initially thought that laccases would only be able to oxidise phenolic substrates (Kersten et al. 1990). However, the range of substrates oxidised by laccases can be increased through a mediator-involved reaction mechanism. Mediators are low molecular weight compounds that are easily oxidised by laccases producing, in some cases, very unstable and reactive cationic radicals, which can oxidise more complex substrates before returning to their original state. The electrons taken by laccases are finally transferred back to oxygen to form water (McGuirl and Dooley, 1999; Wong and Yu, 1999).

The laccase mediator system (LMS) has yet to be applied on large scale due to the cost of mediators and the lack of studies that guarantee the absence of toxic effects of these compounds or their derivatives. The use of naturally-occurring laccase mediators would present environmental and economic advantages. Recently, Camarero et al. (2005) reported that several lignin-derived phenols (such as syringaldehyde and acetosyringone) represented ecofriendly alternatives to synthetic mediators for the degradation of different types of dyes and other recalcitrant compounds by laccase in terms of both efficiency and velocity of oxidation. Figure 1 shows

the structure of different synthetic and natural mediators of laccases.

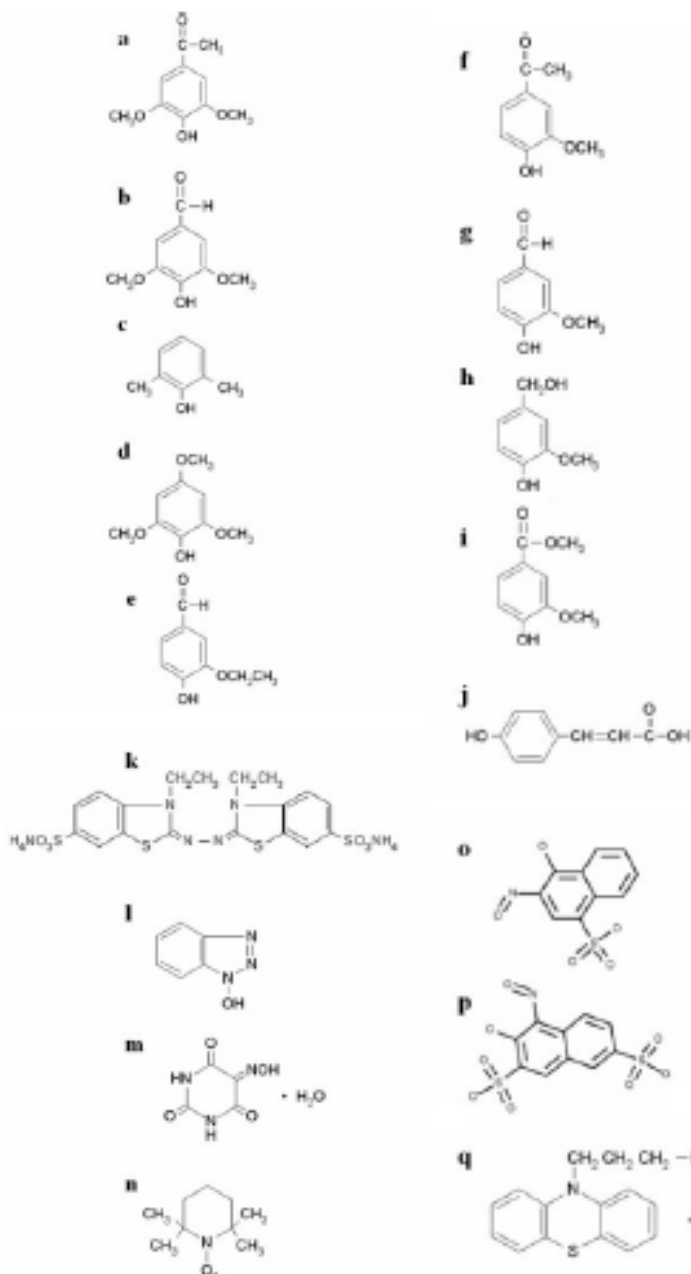
The use of laccases in the textile industry is growing very fast, since besides to decolourise textile effluents, laccases are used to bleach textiles (Vinod 2001), synthesise dyes (Setti et al. 1999) and modify the surface of fabrics (Zille 2005). The first commercial use of laccases in the textile industry was in the denim-washing process, where LMS was used to reduce backstaining, enhance abrasion levels and bleach indigo.

## 2.0 Potential applications of laccases in the textile industry

### 2.1 Wastewater treatment

The pollution problems due to the textile industry effluents have increased in the last years. The dyeing processes have, in general, a low yield and the percentage of the lost dye in the effluents can reach up to 50% (Pierce, 1994; Pearce et al., 2003). From the available literature it can be estimated that approximately 75% of the dyes, discharged by Western European textile processing industries, belong to the following classes: reactive (~36%), acid (~25%) and direct (~15%) (Øllgaard et al., 1998). In these classes, the azo dyes are the most important chemical class of synthetic dyes. Azo dyes are characterised by the presence of at least one azo bond (-N=N-) bearing aromatic rings and have high photolytic stability and resistance towards major oxidising agents Reife et al. (1993).

Textile dye effluents are complex, containing a wide variety of dyes, natural impurities extracted from the fibers and other products such as dispersants, levelling agents, acids, alkalis, salts and some times heavy metals (Laing, 1991). In general, the effluent is highly coloured with high biological oxygen demand (BOD) and chemical oxygen demand (COD), it has a high conductivity and is alkaline in nature. The degradation products of textile dyes are often carcinogenic (Das et al., 1995; Banat et



**Figure 1.** Chemical structures of natural (a to j) and synthetic mediators (k to r). (a) Acetosyringone; (b) syringaldehyde; (c) 2,6-dimethylphenol; (d) 2,4,6-trimethoxyphenol; (e) ethyl vanillin; (f) acetovanillone; (g) vanillin; (h) vanillyl alcohol; (i) methyl vanillate; (j) *p*-coumaric acid; (k) ABTS (2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid)); (l) HBT (1-hydroxybenzotriazole); (m) VIO (violuric acid); (n) TEMPO (2,2,6,6-tetramethylpiperidin-1-yloxy); (o) HNNS (2-nitroso-1-naphthol-4-sulfonic acid); (p) NNDS (1-nitroso-2-naphthol-3,6-disulfonic acid); (q) PZ (promazine) (extracted from Camarero et al. 2005).

al., 1996). Furthermore, the absorption of light due to textile dyes creates problems to photosynthetic aquatic plants and algae.

Most currently existing processes to treat dye wastewater are ineffective and not economical (Cooper, 1995; Stephen, 1995). Therefore, the development of

processes based on laccases seems an attractive solution due to their potential in degrading dyes of diverse chemical structure (Abadulla et al., 2000; Blázquez et al., 2004; Hou et al., 2004; Salony and Bisaria, 2006), including synthetic dyes currently employed in the industry (Rodríguez Couto et al., 2004; Rodríguez Couto

et al., 2005).

## 2.2 Denim finishing

In the textile finishing industry, enzymatic degradation of indigo could have a potential both in stone-wash process and for the treatment of dyeing effluents. Several steps are involved in the manufacture of denim garments between dyeing and the final stone-washing where excessive amounts of indigo are removed from the fabrics and discharged with the wastewater. The traditional technology of producing a stone-washed look in denim fabric involves the wash of the fabrics in the presence of pumice to generate the desired erosion of the fabrics. Subsequently, the fabrics are partially bleached by a treatment with sodium hypochlorite, followed by neutralisation and a rinsing step all causing substantial environmental pollution (Pedersen and Schneider 1998).

In 1996, Novozyme (Novo Nordisk, Denmark) launched a new industrial application of laccase enzyme in denim finishing: DeniLite™, the first industrial laccase and the first bleaching enzyme acting with the help of a mediator molecule. In 1999, USA launched DeniLitell™ based on a new type of laccase with higher activity than that of DeniLite™. Also, in 2001, the company Zytex (Zytex Pvt. Ltd., Mumbai, India) developed a formulation based on LMS capable of degrading indigo in a very specific way. The trade name of the product is Zylite.

Campos et al. (2001) reported the degradation of indigo both in effluents and on fabrics using purified laccases from *Trametes hirsuta* and *Sclerotium rolfsii* in combination with redox-mediators and reported that bleaching of fabrics by the laccases correlated with the release of indigo degradation products. More recently, Pazarloglu et al. (2005) showed that a phenol-induced laccase from *Trametes versicolor* was an effective agent for stone-washing effects of denim fabric without using a mediator. Moreover, they found that *T. versicolor* laccase without a mediator was more effective than commercial laccase (obtained from recombinant *Aspergillus niger*, Novo Nordisk, Denmark) with a mediator.

## 2.3 Cotton bleaching

The purpose of cotton bleaching is to decolourise natural pigments and to confer a pure white appearance to the fibres. Mainly flavonoids are responsible for the colour of cotton (Hedin et al., 1992; Ardon et al., 1996). The most common industrial bleaching agent is hydrogen peroxide, which is usually applied at alkaline pH and temperatures close to boiling. However, radical reactions of bleaching agents with the fibre can lead to a decrease in the degree of polymerisation and, thus, to severe damage. Furthermore, a huge amount of water is needed to remove hydrogen peroxide from fabrics, which can cause problems in dyeing. Therefore, replacement of hydrogen peroxide by an enzymatic bleaching system would not only lead to better product quality due to less fibre damage but

also to substantial savings on washing water needed for the removal of hydrogen peroxide.

Tzanov et al. (2003a) reported for the first time the enhancement of the bleaching effect achieved on cotton fabrics using laccases in low concentrations. In addition, the short time of the enzymatic pre-treatment sufficient to enhance fabric whiteness makes this bio-process suitable for continuous operations. Also, Pererira et al. (2005) showed that a laccase from a newly isolated strain of *T. hirsuta* was responsible for whiteness improvement of cotton most likely due to oxidation of flavonoids. More recently, Basto et al. (2006) proposed a combined ultrasound-laccase treatment for cotton bleaching. They found that the supply of low ultrasound energy (7 W) enhanced the bleaching efficiency of laccase on cotton fabrics.

## 2.4 Rove scouring

Flax processing into yarn essentially still follows traditional methodologies. As an alternative to the chemical scouring of rove, Ossola and Galante, (2004) studied the effects of several enzymes under slightly alkaline pH conditions. They found that the treatment with laccase plus mediator performed better than the chemical one. Also, Sharma et al. (2005) showed that laccase enzyme could be used for roving treatment to improve yarn regularity. The advantage of the use of laccase in rove scouring is that the process is performed under mild reaction conditions resulting, thus, in an ecologically-friendly process.

## 2.5 Wool dyeing

Shin et al. (2001) showed that laccase was able to colour wool fabric that was previously padded with hydroquinone. More recently, Tzanov et al. (2003b) and Zille (2005) also proved the ability of laccases for wool dyeing. They used a dye bath prepared with a dye precursor (2,5-diaminobenzenesulfonic acid), dye modifiers (catechol and resorcinol) and laccase, without any dyeing auxiliaries. Additionally, the enzymatic reaction was carried out at pH and temperature values safe to the wool material. Furthermore, they showed that by pro-longing the contact time between wool, enzyme, precursor and modifier deeper colours were obtained in contrast to the conventional process in which deeper colours are attained by increasing the amount of dye. This makes wool dyeing with laccase an economically attractive alternative to the conventional process, which uses high amounts of water, auxiliaries, acid and energy. This laccase application is a promising technology especially for the coating of natural and synthetic materials.

## 2.6 Anti-shrink treatment for wool

A process conventionally used for wool shrink-proofing is chlorination. This process degrades the exo-cuticle of the

wool, forming cysteic acid residues and protein losses. This process has been replaced by proteinases treatment due to their high specificity and much lower environmental impact. However, proteinase treatment leads to protein degradation, resulting in deterioration of fiber strength and limited shrink resistance (Breier 2000; Breier 2002). A patent application about the use of laccase from *T. versicolor* plus a mediator to increase the shrink resistance of wool was published (Yoon 1998). Also, Lantto et al. (2004) found that wool fibers can be activated with laccase if a suitable mediator is present. Therefore, the use of laccase for anti-shrink treatment of wool seems very attractive.

## 2.7 Dye synthesis

Setti et al. (1999) described the ability and efficacy of laccases from *Pyricularia oryzae* to form red azo dyes by the oxidative coupling of 3-methyl-2-benzothiazolinone hydrazone (MBTH) and phenols. More recently, Mustafa et al. (2005) showed that the oxidation of ferulic acid by laccase in a biphasic hydro-organic medium led to the production of stable yellow coloured products, which were only soluble in the organic phase being, thus, easily recovered. The synthesis of colourants by laccase in this medium is currently extended to other phenolic and polyphenolic compounds.

## 3.0 Future outlook

Laccases are promising enzymes to replace the conventional chemical processes of the textile industry. However, one of the problems to commercialise the use of laccase is the lack of sufficient enzyme stocks. Thus, efforts have to be made in order to achieve cheap overproduction of laccase in heterologous hosts and also their modification by chemical means or protein engineering to obtain more robust and active enzymes. Another additional problem is the cost and toxicity of redox mediators. Further investigations should consider different and less polluting mediators such as the natural mediators produced by laccase in a bio-environment during lignin degradation.

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