J Ind Microbiol Biotechnol (2008) 35:1123–1129
DOI 10.1007/s10295-008-0391-0

ORIGINAL PAPER

Decolourization of recalcitrant dyes with a laccase from *Streptomyces coelicolor* under alkaline conditions

Etienne Dubé · François Shareck · Yves Hurtubise · Marc Beauregard · Claude Daneault

Received: 1 November 2007 / Accepted: 18 June 2008 / Published online: 17 July 2008

Abstract Colored wastewater from textile industries is a consequence of dye manufacturing processes. Two percent of dyes that are produced are discharged directly in aqueous effluent and more than 10% are subsequently lost during the textile coloration process. It is not surprising that these compounds have become a major environmental concern. In that context, we have evaluated the potential use of *Streptomyces coelicolor* laccase for decolourization of various dyes with and without a mediator. Results showed that in all cases the combination of laccase and the mediator acetosyringone was able to rapidly decolourize, to various degrees, all the dyes tested. In 10 min, decolourization was achieved at 94% for acid blue 74, 91% for direct sky blue 6b and 65% for reactive black 5. Furthermore, decolourization was achieved at 21% for reactive blue 19 and at 39% for the direct dye Congo red in 60 min. These results demonstrate the potential use of this laccase in combination with acetosyringone, a natural mediator, for dye decolourization.

Keywords Laccase · Mediator · Textile · Dye · Decolourization · Wastewater

E. Dubé · Y. Hurtubise · M. Beauregard · C. Daneault (✉)
Département de chimie-biologie, Université du Québec à Trois-Rivières, 3351 Des Forges, C.P. 500, Trois-Rivières, Quebec G9A 5H7, Canada
e-mail: claude.daneault@uqtr.ca

F. Shareck
INRS - Institut Armand-Frappier, Université du Québec, 531 boul. des Prairies, Ville de Laval, Quebec H7V 1B7, Canada

Introduction

The first laccase studied was from *Rhus vernicifera* in 1883, a Japanese lacquer tree, from which the designation laccase was derived [1]. Laccases are defined in the Enzyme Commission nomenclature as oxidoreductases acting on diphenols and related substances using molecular oxygen as acceptor (EC 1.10.3.2). They are multicopper proteins found mostly in plants and fungi but also in some bacteria [2–4]. The protein structure acts as an intricate ligand for the catalytically active coppers, providing them with a coordination sphere where changes between the reduction states are thermodynamically possible. They contain at least one type-1 (T1) copper, which is associated to the oxidation site, and typically harbor at least three additional coppers: one type-2 (T2) and two type-3 (T3) coppers arranged in a trinuclear cluster. This cluster is associated with the site where reduction of molecular oxygen occurs [5–8].

Laccases are of particular interest with regard to various commercial applications because of their ability to oxidize a wide range of relevant substrates. Thus, research is being carried out in various fields of interest: textile, pulp and paper, food and cosmetics industries, as well as in bioremediation, biosensor, biofuel and organic synthesis applications [3, 9–13]. Few commercialized laccase products are already available: Denilite I and II from Novozymes (Denmark) and Zylite from Zytex (India). These products are used in the textile industry for denim bleaching. Usually, mediators are used with laccase in order to optimize the process. Once oxidized by the enzyme, these molecules act as electron scavenger and attack the dye, which eventually results in bleaching in the end [14, 15].

Dyes are used in numerous industries. While textile mills predominantly use them, dyes can also be found in the

food, pharmaceutical, paper printing and cosmetics industries. These compounds retain their color as well as their structural integrity under exposure to sunlight and they exhibit a high resistance to microbial degradation [16, 17]. Because of these properties many of them find their way into the environment via wastewater treatment facilities. It is not surprising that these compounds have become a major environmental concern.

Recently, we have cloned a laccase gene from *Streptomyces coelicolor* and expressed the protein with the goal of investigating its potential uses for industrial applications [18]. Using an efficient bacterial expression system, large quantities of this laccase (SLAC) could be produced with a high purity yield and no extensive purification steps were required. The enzyme produced possesses a broad pH working range and has a high thermostability. This enzyme also showed a good potential for rapid decolourization of Indigo carmine, with syringaldehyde as redox mediator, at pH 9.0 [18]. Since most wastewaters from textile industries are characterized by a neutral to alkaline pH (around 7–11) [19, 20], the potential use of SLAC could be advantageous compared to typical fungal laccases which are generally acidic by nature and are not active in this pH range. The decolourization properties of this bacterial laccase have never been extensively studied before. Therefore, the main objective of this work was to determine if this enzyme was able to oxidize various dyes, with and without a mediator, to help in the reduction of their environmental impact.

Materials and methods

Substrates and chemicals

Unless specified otherwise, all substrates and chemicals were of analytical grade or better and purchased from Sigma-Aldrich.

Organisms and vectors

Streptomyces lividans IAF10-164 (msiK−), a xylanase- and cellulase-negative mutant, served as host strain for the recombinant plasmid [21]. Plasmid pIAFD95A was used for gene expression.

Cloning of the laccase gene

The coding sequence for the laccase of *S. coelicolor* A3(2) was available from Genbank (accession number CAB45586). The structural gene was amplified from total DNA of *S. coelicolor* A3(2) by PCR using the following primers: 5-GAAACGC*ATG*CACAGGCGAG-GCTTTAACCG-3 (F) and 5-CCTGGAGCTC*AGT*GCTCGTGTTC-GTGTGCG-3 (R). The forward primer (F) introduced a *Sph*I site (underlined) at the start codon (italic) and the reverse primer (R) introduced a *Sac*I (underlined) after the stop codon (italic). The amplification product was digested with *Sph*I–*Sac*I and cloned into plasmid pIAFD95A. Protoplasting, transformation of the mutant IAF10-164 and spore preparations were performed as described by Kieser et al. [22]. Screening of *Streptomyces* transformants was carried out using the colony-PCR technique [23]. DNA extracts were sequenced and analyzed with an ABI Prism 3100 to confirm the sequence of the cloned gene.

Culture conditions

The strain was maintained on a 7-day-old fresh Bennett agar containing a modified yeast-extract malt-extract medium, in which 0.4% maltose was substituted for glucose. Spore suspensions prepared from such slants were used as inocula for vegetative cultures in 100 mL trypticase soy broth (TSB) with glass beads. Incubation was carried out at 34 °C on a rotary shaker at 240 rpm for a period of 24 h. Enzyme production was carried out in 1 L Erlenmeyer flasks containing 80 ml of M_{14} medium, as previously described by Kluepfel et al. [24], using 1% (v/v) of D-xylose as main carbon source. The flask cultures were incubated at 34 °C on a rotary shaker at 240 rpm for 72 h.

Protein purification

The fermentation broth was recovered by centrifugation at 11,000×*g* for 30 min at 4 °C. Prior to protein purification, the laccase supernatant was incubated overnight with 25 µm of $CuSO_4$ to restore the enzymatic activity. The supernatant was then filtered on a 0.2 µm membrane to retain any floating particles and concentrated fivefold by ultrafiltration with an Amicon system (Millipore) using a 10 kDa cut-off membrane.

Enzymatic activity and protein determination

Laccase activity was determined at 25 °C using 2,2′-azino-bis(3-ethylbenzthiazoline-6-sulfonate) (ABTS) or 2,6-dimethoxyphenol (DMP) as the substrate. The oxidation of the substrate was detected by measuring the absorbance at 420 nm for ABTS ($\varepsilon = 36{,}000\ M^{-1}\ cm^{-1}$) [25] and at 470 nm for DMP ($\varepsilon = 27{,}500\ M^{-1}\ cm^{-1}$) [26]. The reaction mixture (300 µl) contained 5 µl of appropriately diluted enzyme sample, 150 µl of MES–glycine buffer (0.1 M, pH 4.0), 20 µl of 60 mM ABTS or DMP and 125 µl of H_2Odd. Activity was calculated in international unit (IU) which is the amount of laccase that oxidizes 1 µmol of substrate per min. The protein content was determined at 595 nm using the Pierce Coomassie Plus protein assay reagent [27] with

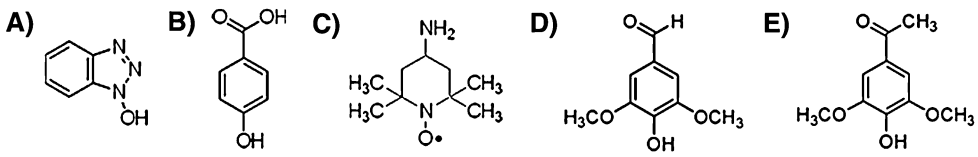

Fig. 1 Mediators used in this study. **a** 1-hydroxybenzotriazole, **b** 4-hydroxybenzoic acid, **c** 4-acetamido-TEMPO, **d** syringaldehyde and **e** acetosyringone

bovine serum albumin as a standard. The absorbance was measured with a Multiskan Ascent spectrophotometer (Thermo electron, Inc., USA).

Screening of mediators

Screening of mediators was done via the decolourization of acid blue 74 by SLAC with the various mediators (Fig. 1). The mediators selected were 1-hydroxybenzotriazole (HBT), 4-hydroxybenzoic acid (HBA), 4-acetamido-TEMPO (TEMPO), syringaldehyde and acetosyringone (60 mM solution prepared with ethanol). Those compounds are typical laccase mediators used for dye decolourization [14, 15, 28]. The reaction mixture (300 µl) contained 50 mM MES–glycine buffer (pH 9.0), dye (final concentration 25 µM), concentrated enzyme (100 mU) and mediator solution (final concentration 1 mM). Reactions were initiated by enzyme addition and incubated at 45 °C for 60 min. Control samples were run in parallel with a deactivated enzyme (boiled for 20 min) under identical conditions. Dye decolourization was determined by measuring the absorbance at 595 nm. The results are expressed in percentage of decolourization.

Effect of pH on decolourization

The effect of pH on decolourization was evaluated via the same method used for the mediators screening except that the tests were run for 10 min. The pH range tested was from 3.0 to 11.0 in 50 mM MES–glycine buffer. Only syringaldehyde and acetosyringone were evaluated.

Mediators concentration

For mediator concentration evaluation, we used the same method as in the mediators screening except that the tests were run for 10 min. The concentration range tested was from 0 to 2 mM. Only syringaldehyde and acetosyringone were evaluated.

Dyes decolourization by laccase

For this study five dyes with different chemical structures were selected (Fig. 2). The decolourization of reactive blue 19 (RB19), direct sky blue 6b (DSB6b), acid blue 74 (AB74), reactive black 5 (RB5) and congo red (CR) by laccase were tested with and without a mediator. The mediators selected were syringaldehyde and acetosyringone. The reaction mixture (300 µl) contained 50 mM MES–glycine buffer (pH 9.0), dye (final concentration 10, 15 or 25 µM depending on the dye), purified enzyme (100 mU) and mediator solution (final concentration 1 mM). Reactions were initiated by enzyme addition and incubated at 45 °C for 60 min. Control samples were run in parallel with a deactivated enzyme (boiled for 20 min) under identical conditions. Dye decolourization was determined by measuring the absorbance at 595 nm for RB19, DSB6b, AB74, RB5 and 492 nm for CR. The results are expressed in percentage of decolourization.

Data analysis

Enzymatic activity, protein determination, mediator screening and decolourization assays were performed in triplicate. All standard deviations were below 10% of the mean values presented.

Results

Mediator screening and dye decolourization conditions

Among the five phenolic compounds screened as laccase mediators, two allowed a high decolourization of AB74 (indigo carmine) with the use of SLAC (Fig. 3). While HBT, HBA and TEMPO did not give a better decolourization than laccase alone, syringaldehyde and acetosyringone produced decolourization of 89 to 95%, respectively, in 20 min. Since they yielded the highest decolourization as rate means per min, they were selected for additional studies with different types of dyes.

The optimum pH for phenolic compound oxidation by a laccase may vary depending on the substrate used. For instance, the optimal pH for ABTS and DMP oxidation by SLAC was pH 4.0 and 9.0, respectively (Fig. 4). With syringaldehyde or acetosyringone the best decolourization results in 10 min, for AB74, were obtained between pH 7.0–9.0 (Fig. 5). Thus, subsequent decolourization assays were performed at pH 9.0.

For economical purposes, the concentration of various mediators was determined for the assays. The lowest mediator concentration used, for syringaldehyde or acetosyringone, to reach the highest decolourization of AB74 in 10 min, was achieved at 1.0 mM (Fig. 6). Thus, subsequent

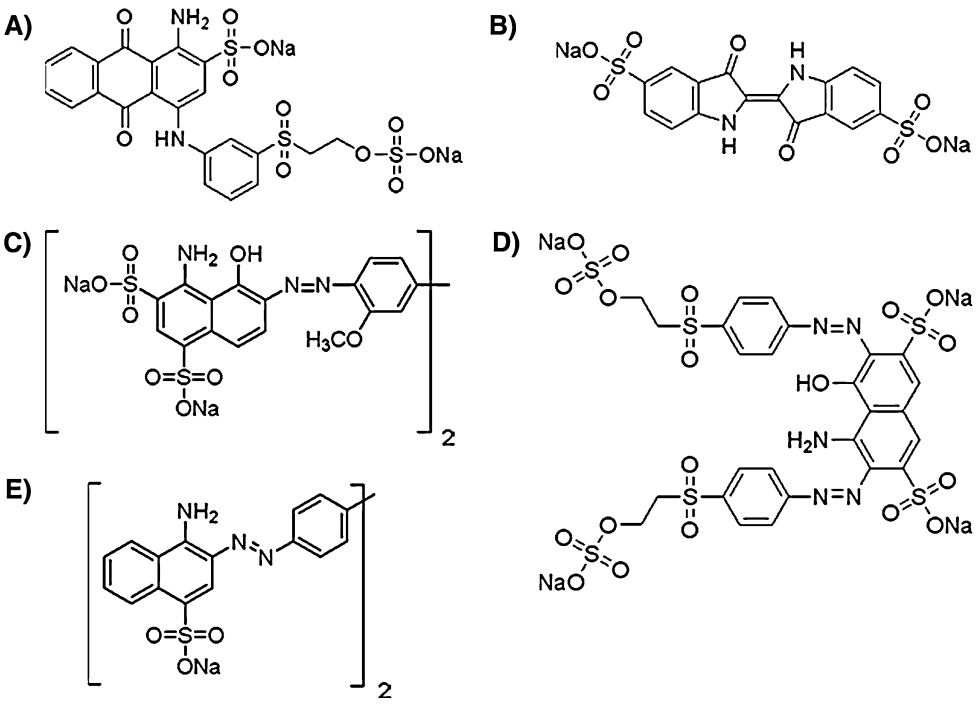

Fig. 2 Dyes used in this study. **a** Reactive blue 19, **b** acid blue 74, **c** direct sky blue 6b, **d** reactive black 5 and **e** Congo red

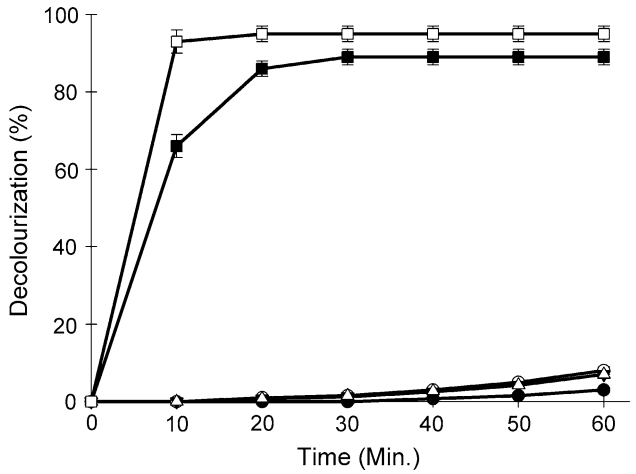

Fig. 3 Mediators screening via the decolourization of AB74 with SLAC. SLAC alone (*open circle*), SLAC with TEMPO (*closed circle*), SLAC with HBT (*closed triangle*), SLAC with HBA (*open triangle*), SLAC with syringaldehyde (*closed square*) and SLAC with acetosyringone (*open square*) after 60 min at 45 °C, pH 9.0. No decolourization was achieved when mediators were used without SLAC (data not shown). All standard deviations were below 10% of the mean values presented

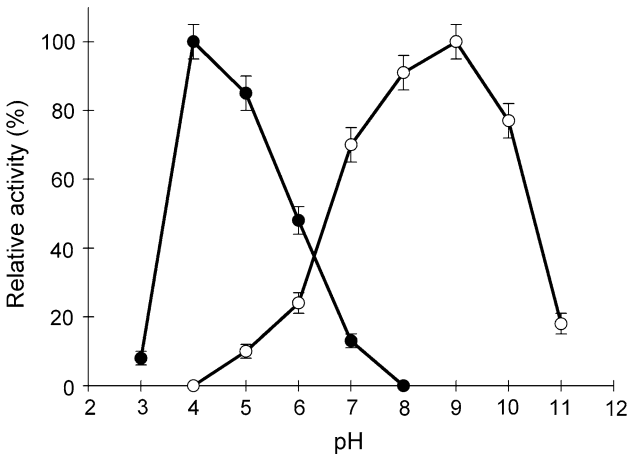

Fig. 4 Effect of pH on the activity of SLAC. Activity was measured in the presence of 6 mM ABTS (*closed circle*) or DMP (*open circle*). Relative activity was normalized to the maximal activity achieved when using either substrate at the optimal pH. All standard deviations were below 10% of the mean values presented

decolourization assays were performed with a mediator concentration of 1.0 mM.

Decolourization of different types of dyes

The enzyme SLAC alone or in combination with a mediator was used to evaluate the oxidation of the various dyes. Syringaldehyde or acetosyringone were used as laccase mediator. Table 1 show that SLAC alone was able to decolourize all the dyes tested but the process is not efficient and needs a longer time treatment. Almost no decolourization was observed after 1 h. After 5 h, a decolourization of 11–40% was achieved for the different dyes tested. The addition of syringaldehyde or acetosyringone provided better results than the laccase treatment alone. The combination SLAC-syringaldehyde efficiently decolourized the diazo dye DSB6b and the indigoid dye AB74 by 81% or more after 1 h. However, this combination was inefficient for the decolourization of the anthraquinoid dye RB19 and the azo dye CR. No decolourization was achieved. Our results also

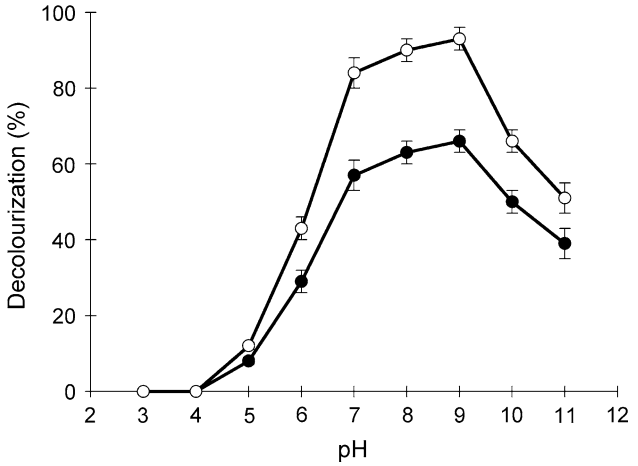

Fig. 5 Effect of pH on mediator oxidation of AB74. SLAC with syringaldehyde (*closed circle*) and SLAC with acetosyringone (*open circle*) in MES–glycine buffer pH 3.0–11.0 after 10 min at 45 °C. All standard deviations were below 10% of the mean values presented

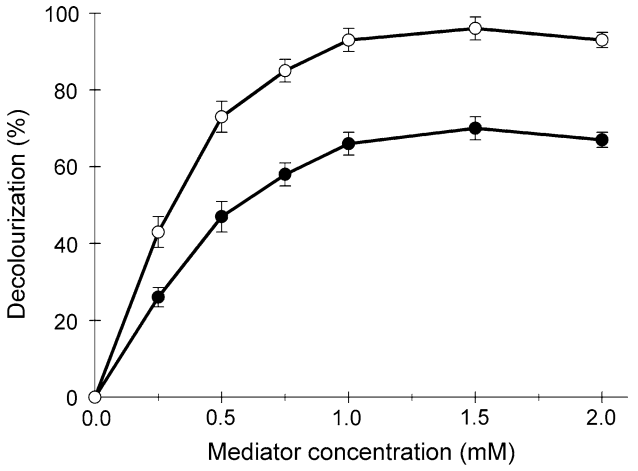

Fig. 6 Effect of mediator concentration on the oxidation of AB74. SLAC with syringaldehyde (*closed circle*) and SLAC with acetosyringone (*open circle*) in MES–glycine buffer pH 9.0 after 10 min at 45 °C. All standard deviations were below 10% of the mean values presented

demonstrated that the combination SLAC-acetosyringone gave better results than the combination SLAC-syringaldehyde. In 1 h, this combination could decolourize all the dyes evaluated in this study. RB19 and CR were the most resistant to oxidation by the treatment. They were decolourized by 21 and 39%, respectively, in 1 h. This treatment has a better efficiency toward DSB6b, AB74 and the diazo dye RB5. DSB6b and AB74 were decolourized by 91% or more and RB5 by 65% in only 10 min.

Discussion

Colored wastewater is a consequence of batch processes both in the dye manufacturing industries and in the dye-consuming industries. Two percent of dyes that are produced are discharged directly in aqueous effluent, and 10% are subsequently lost during the textile coloration process [29]. Since there are thousands of commercially available dyes with over 7×10^5 tons of dye-stuff produced annually [30], it is not surprising that these compounds have become a major environmental concern. The ability of laccase enzymes to degrade different varieties of dyes such as azo, anthraquinoid, triarylmethane and indigoid implies that it offers potential application in textile dye bleaching processes [14, 28]. A study from Claus et al. has demonstrated that a laccase from *Trametes versicolor* was able to decolourize 3% of RB5, 82% of AB74 and 63% of RB19 in 16 h [28]. In comparison, under the conditions used our results showed that SLAC alone was able to decolourize 11% of RB5, 40% of AB74 and 23% of RB19 in only 5 h at pH 9.0.

Until recently, the development of a successful enzymatic solution for the textile industry has been hindered by poor kinetics between the enzyme and various dyes. The use of small molecules that are able to act as electron transfer mediators between the enzyme and the dye has opened up new possibilities in this field of research [14, 28]. A previous study suggested that syringaldehyde and acetosyringone were two of the best redox mediators to use for dye decolourization [14]. In this study, we tested the decolourization of Indigo carmine with SLAC and different synthetic and natural mediators (HBT, HBA, TEMPO, syringaldehyde and acetosyringone). Our results did not show higher decolourization rate with HBT, HBA and TEMPO than laccase alone. These poor results could be attributed to bad kinetics between the enzyme and these mediators since SLAC is described as a low redox potential laccase [31]. Nevertheless, our results also demonstrated that syringaldehyde

Table 1 Decolourization of five dyes by SLAC without or with mediators, for different time treatment, at pH 9.0, 45 °C

	Decolourization of dye (%)				
	RB19	DSB6b	AB74	RB5	CR
SLAC					
10 min	0	0	0	0	0
60 min	0	4	8	0	0
5 h	23	20	40	11	13
SLAC + SYR					
10 min	0	64	66	2	0
60 min	0	81	89	17	0
SLAC + ACE					
10 min	1	91	94	65	3
60 min	21	92	95	89	39

SYR Syringaldehyde, *ACE* acetosyringone, concentration of 1 mM
All standard deviations were below 10% of the mean values presented

and acetosyringone were the best redox mediators to use for dye decolourization. Since these compounds are involved in the degradation of lignin by white-rot fungi in the environment; they are therefore naturally occurring laccase mediators [32]. The use of natural mediators will present environmental and economic advantages for future industrial applications.

The main reason for dye loss is the incomplete exhaustion of dyes on to the fiber. This loss varies from one dye to the other and depends on the classification of the dye [33]. Up to 20% of the initial dye mass used in the dyeing process is lost in the effluent for acid dyes, up to 30% for direct dyes and up to 50% for reactive dyes. Reactive dyes make up approximately 30% of the total dye market [29]. This impact is considerable for the environment and needs to be addressed.

While laccase alone has a limited potential for dye decolourization, the LMS (laccase-mediator system) can improve significantly the decolourization properties of laccase toward various dye class [14, 15, 28]. In a previous study, the addition of HBT as mediator increased the laccase decolourization level of RB5 from 3 to 70% [28]. Under the conditions used, our results showed that in all cases decolourization with the combination SLAC-acetosyringone was better than with laccase alone. This combination rapidly decolourized all the dyes tested to various degrees. In 10 min, decolourization was achieved at 94% for AB74, 91% for DSB6b and 65% for RB5. In comparison, using fungal laccases from *Pycnoporus cinnabarinus* and *Trametes villosa*, with acetosyringone as mediator, other researchers achieved a decolourisation level of 100% for AB74 and of 84% for RB5 in less than 10 min at pH 5.0 [14]. As stated before, most wastewaters from textile industries are characterized by a neutral to alkaline pH (around 7–11) [19, 20]. For this application, SLAC offers an important advantage compared to typical acidic fungal laccases which are not active in this pH range.

Conclusion

This study confirms that the particular laccase investigated here, SLAC, can use natural mediators and decolourize dyes under alkaline conditions. Because of practical applications in the textile industry for denim bleaching and wastewaters treatment at a neutral to alkaline pH, the potential use of this alkaline laccase opens up new possibilities for the development of green technology alternatives to existing chemical treatment. Future work is under way that will examine decolourization potential of this laccase toward others dyes and also investigates whether or not the resulting products from the dye oxidation are toxic in any way.

Acknowledgments This work was supported by the Canada research chair on value-added papers from the Centre Intégré en Pâtes et Papiers (Trois-Rivières, Canada), AgroTerra Biotech (Trois-Rivières, Canada), the Institut National de la Recherche Scientifique–Institut Armand-Frappier (Laval, Canada) and the Natural Sciences and Engineering Research Council of Canada.

References

1. Yoshida H (1883) Chemistry of lacquer (urushi). J Chem Soc Trans 43:472–486
2. Alexandre G, Zhulin IB (2000) Laccases are widespread in bacteria. Trends Biotechnol 18:41–42
3. Claus H (2004) Laccases: structure, reactions, distribution. Micron 35:93–96
4. Sharma P, Goel R, Capalash N (2007) Bacterial laccases. World J Microbiol Biotechnol 23:823–832
5. Bertrand T, Jolivalt C, Briozzo P, Caminade E, Joly N, Madzak C, Mougin C (2002) Crystal structure of a four-copper laccase complexed with an arylamine: insights into substrate recognition and correlation with kinetics. Biochemistry 41:7325–7333
6. Enguita FJ, Martins LO, Henriques AO, Carrondo MA (2003) Crystal structure of a bacterial endospore coat component. A laccase with enhanced thermostability properties. J Biol Chem 278:19416–19425
7. Hakulinen N, Kiiskinen LL, Kruus K, Saloheimo M, Paananen A, Koivula A, Rouvinen J (2002) Crystal structure of a laccase from Melanocarpus albomyces with an intact trinuclear copper site. Nat Struct Biol 9:601–605
8. Piontek K, Antorini M, Choinowski T (2002) Crystal structure of a laccase from the fungus *Trametes versicolor* at 1.90-A resolution containing a full complement of coppers. J Biol Chem 277:37663–37669
9. Burton SG (2003) Laccases and phenol oxidases in organic synthesis—a review. Curr Org Chem 7:1317–1331
10. Kenealy WR, Jeffries TW (2003) Enzyme processes for pulp and paper: a review of recent developments. In: Goodell B, Nicholas DD, Schultz TP (eds) Wood deterioration and preservation: advances in our changing world. ACS symposium series, vol 845. American Chemical Society, Washington, pp 210–239
11. Mayer AM, Staples RC (2002) Laccase: new functions for an old enzyme. Phytochemistry 60:551–565
12. Minussi RC, Pastore GM, Duran N (2007) Laccase induction in fungi and laccase/N-OH mediator systems applied in paper mill effluent. Bioresour Technol 98:158–164
13. Rodriguez Couto S, Toca Herrera JL (2006) Industrial and biotechnological applications of laccases: a review. Biotechnol Adv 24:500–513
14. Camarero S, Ibarra D, Martinez MJ, Martinez AT (2005) Lignin-derived compounds as efficient laccase mediators for decolorization of different types of recalcitrant dyes. Appl Environ Microbiol 71:1775–1784
15. Moldes D, Sanroman MA (2006) Amelioration of the ability to decolorize dyes by laccase: relationship between redox mediators and laccase isoenzymes in Trametes versicolor. World J Microbiol Biotechnol 22:1197–1204
16. Hao OJ, Kim H, Chiang PC (1999) Decolorization of wastewater. Crit Rev Environ Sci Technol 30:449–505
17. Kandelbauer A, Guebitz G (2005) Bioremediation for the decolorization of textile dyes—a review. In: Environmental Chemistry, Springer, Berlin, pp 269–288
18. Dubé E, Shareck F, Hurtubise Y, Daneault C, Beauregard M (2008) Homologous cloning, expression and characterization of a laccase from *Streptomyces coelicolor* and enzymatic decolourization of an indigo dye. Appl Microbiol Biotechnol (in press)

19. Manu B, Chaudhari S (2002) Anaerobic decolorisation of simulated textile wastewater containing azo dyes. Bioresour Technol 82:225–231
20. Jahmeerbacus MI, Kistamah N, Ramgulam RB (2004) Fuzzy control of dyebath pH in exhaust dyeing. Coloration Technol 120:51–55
21. Hurtubise Y, Shareck F, Kluepfel D, Morosoli R (1995) A cellulase/xylanase-negative mutant of *Streptomyces lividans* 1326 defective in cellobiose and xylobiose uptake is mutated in a gene encoding a protein homologous to ATP-binding proteins. Mol Microbiol 17:367–377
22. Kieser T, Bibb MJ, Buttner MJ, Chater KF, Hopwood DA (2000) Practical *Streptomyces* genetics. The John Innes Foundation, Norwich
23. Ward AC (1992) Rapid analysis of yeast transformants using colony-PCR. BioTechniques 13:350
24. Kluepfel D, Vats-Mehta S, Aumont F, Shareck F, Morosoli R (1990) Purification and characterization of a new xylanase (xylanase B) produced by *Streptomyces lividans* 66. Biochem J 267:45–50
25. Childs RE, Bardsley WG (1975) The steady-state kinetics of peroxidase with 2, 2′-azino-di-(3-ethyl-benzthiazoline-6-sulphonic acid) as chromogen. Biochem J 145:93–103
26. Jaouani A, Guillen F, Penninckx MJ, Martinez AT, Martinez MJ (2005) Role of *Pycnoporus coccineus* laccase in the degradation of aromatic compounds in olive oil mill wastewater. Enzyme Microb Technol 36:478–486
27. Bradford MM (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Analyt Biochem 72:248–254
28. Claus H, Faber G, Konïg H (2002) Redox-mediated decolorization of synthetic dyes by fungal laccases. Appl Microbiol Biotechnol 59:672–678
29. Pearce CI, Lloyd JR, Guthrie JT (2003) The removal of colour from textile wastewater using whole bacterial cells: a review. Dyes Pigments 58:179–196
30. Robinson T, McMullan G, Marchant R, Nigam P (2001) Remediation of dyes in textile effluent: a critical review on current treatment technologies with a proposed alternative. Bioresour Technol 77:247–255
31. Machczynski MC, Vijgenboom E, Samyn B, Canters GW (2004) Characterization of SLAC: A small laccase from *Streptomyces coelicolor* with unprecedented activity. Protein Sci 13:2388–2397
32. Camarero S, Ibarra D, Martinez AT, Romero J, Gutierrez A, del Rio JC (2007) Paper pulp delignification using laccase and natural mediators. Enzyme Microb Technol 40:1264–1271
33. Blackburn RS (2004) Natural polysaccharides and their interactions with dye molecules: applications in effluent treatment. Environ Sci Technol 38:4905–4909