Research Article

Optimizing enzymatic dyeing of wool and leather

Alexandre António Antunes Barros[1] · Ana Patrícia da Rocha Cardoso[2] · Ana Cristina Rodrigues[3] · Carla Joana dos Santos Marinho da Silva[2] · Andrea Zille[4]

Abstract
This work reports on the environmental friendly enzymatic dyeing of wool and leather performed at low temperature and mild pH conditions without any dyeing auxiliaries. The substrates have been dyed with "in situ" generated pigment by means of laccase-catalyzed oxidative coupling of dye modifier 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) and dye precursor 1,3-benzenediol in a batchwise process. The process reaction variables (laccase, precursor and modifier concentrations, temperature and dyeing time) were optimized by response surface methodology using an appropriate experimental design. The temperature, precursor concentration, interaction between precursor and modifier and time are the most important factors in the dyeing process. The best-optimized wool dyeing conditions (2 h reaction time, 50 µl laccase (0.1 U), 500 mM precursor, 10 mM modifier at 40 °C) were then successfully applied onto leather material. The enzymatic-dyeing optimized process can be successfully performed on wool and leather at low temperature and mild pH obtaining different hues and depths of shades by varying the modifier concentration and time. The colouring enzymatic system has a good reusability (which has a huge advantage in terms of cost reduction) and washing durability and is comparable in terms of fastness properties to the traditional dyeing process for both wool and leather.

Keywords Enzymatic dyeing · Laccase · Modifiers · Full factorial design · Wool · Leather

1 Introduction

The potential of enzymes as catalysts has been greatly explored and applied over the past years to obtain new and more sustainable textiles processes [1, 2]. The use of enzymes, especially oxidoreduxtases, to synthesize colourants "in situ" is an efficient way for textiles dyeing at mild pH and temperature conditions without the use of harsh chemicals, but few studies have been conducted in this field [3–8]. Several patents report on enzymatic colouration; however, the knowledge about the process parameters is quite limited [9–12].

Laccase (EC 1.10.3.2) belongs to the class of multicopper oxidoreductases enzymes that are able to oxidize colourless aromatic compounds such as phenols, aminophenols and diamines to aryloxy radicals capable of undergoing further non-enzymatic oxidation resulting in coloured dimeric, oligomeric or polymeric compounds [13–15]. The synthesis of colourants by laccase has been widely studied with different kinds of substrates such as phenols and polyphenolic compounds [16–20]. Altering the type of phenols, amine and mediator, it will be possible to obtain a broad range of enzymatically generated new colours with different properties and affinity toward a wide spectra of substrates. There are some publications focusing on the enzymatic colouration of wool, cotton and human hair by laccase via several reaction substitutes [21, 22]. However, only one publication that uses

✉ Andrea Zille, azille@2c2t.uminho.pt | [1]3B´s Research Group-Biomaterials, Biodegradables and Biomimetics, Headquarters of the European Institute of Excellence on Tissue Engineering and Regenerative Medicine, University of Minho, AvePark Barco, 4805-017 Guimarães, Portugal. [2]CeNTI – Centre for Nanotechnology and Smart Materials, 4760-034 Vila Nova de Famalicão, Portugal. [3]IPVC - Viana do Castelo Polytechnic Institute, 4901-909 Viana do Castelo, Portugal. [4]2C2T - Centro de Ciência e Tecnologia Têxtil, Universidade do Minho, Campus de Azurém, 4800-058 Guimarães, Portugal.

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phenolic breakdown products of laccase-oxidized Kraft lignin for dyeing leather is available [23]. In Suparno work, the enzymatic dyeing of leather was achieved, but no control on the type and intensity of the colour as well as on the reactant concentrations was performed. The traditional leather dyeing is a complex and toxic process. It is very difficult to obtain colours with consistent shade and level due to the unique nature of the raw material [24, 25].

The present study intends to develop an environmentally friendly alternative process for colouring wool and leather avoiding the use of commercial dyes and other chemicals used in the traditional dyeing process and all the problems associated with this type of recalcitrant compounds. To maximize the attained results and minimize the number of assays performed, the technique of response surface methodology (RSM) was used for wool dyeing. The obtained best results were then applied for leather colouration. The RSM is a statistical technique of planning experiences, which can be used in laboratory-scale research or studies in an industrial environment, allowing the modelling and optimization of processes avoiding the variation in one factor at a time, maintaining the others fixed [26]. Factors such as the concentrations of precursor resorcinol, modifier ABTS and laccase enzyme, as well as temperature and reaction time, were studied to the process of enzymatic colouring in a 2^5 full factorial design. Moreover, reusability of the colouring bath, washing resistance and colour fastness were evaluated in both wool and leather materials.

2 Materials and methods

2.1 Materials

The textile material used in this study was a 100% wool fabric, subjected to a bleaching pre-treatment in a ratio bath of 1:10 with 10 mL/L of hydrogen peroxide, 2 mL/L of ECE detergent for 30 min at 40 °C and pH of 8. The wet white leather was used as received. Wet white leather tanning is a method using zirconium(IV) and aluminium(III) salts to avoid the use of chromium tanning which is harmful for humans and environment. *Trametes versicolor* laccase pure enzyme in solid state was purchased from Fluka (23.1 IU/mg). One mg of enzyme was dilute in 1 mL acetate buffer (0.1 M) at pH 5 for a final activity of 23.1 U/mL. ABTS (2,2′-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid), resorcinol (1,3-benzenediol) and acetic acid were provided by Sigma-Aldrich and used without further purification.

2.2 Enzymatic dyeing process

The colouring process was performed in wool and leather under continuous agitation in the presence of laccase, dye modifier (ABTS) and dye precursor (resorcinol) in an aqueous solution of acetic acid 0.1 M at the pH of maximum enzyme activity (pH 5). The wool and leather were dyed at a liquor ratio 1:50 (2 g of substrate in 100 mL of acetic acid buffer at pH 5). The factorial design (RSM) experiments were performed in the following variable ranges: 30–50 °C, 10–90 µL *Trametes versicolor* laccase, 2–18 mM dye modifier (ABTS), 200–800 mM dye precursor (resorcinol) and 0.5–3.5 h. After dyeing, the substrates were thoroughly washed by boiling in non-ionic 0.2% ECE detergent, until no more dye was released in the washing bath.

2.3 *K*/*S* Measurements

Colour strength was evaluated in terms of *K*/*S* values, and these were calculated using Kubelka–Munk's equation: $K/S = (1-R)^2/2{*}R$, where *K* is the absorption coefficient of the substrate, *S* is the scattering coefficient of the substrate and R is the reflectance of the dyed fabric at λ_{max}. For each sample, three reflectance measurements were taken and the arithmetic mean of these determinations was used for the *K*/i. The reflectance values were measured with a Datacolor reflectance spectrophotometer at standard illuminant D_{65} (LAV/Spec. Incl., *d*/8, D_{65}/10°).

2.4 Optical microscope and photograps

The distribution of the dye into the wool fibres was studied with a fluorescence optical microscopy (Leica DM 2500) in the biocoloured wool samples using the fluorescent rhodamine B cationic dye and in an optical microscope (Leica. The images were acquired at a magnification of 20×. The samples were prepared with the aid of a microtome (Leica RM 2255), resorting to the use of a resin (Technovit 7100) to fix the fibre. Transversal cuts of the leather samples were captured using a reflection optical microscope Olympus BH (Japan) coupled to a JVC TK1280E (Japan) camera and a Micron Measurement video recorder associated with the Leica Quantimet 500 (Germany) software using a magnification of 20×. Images were optimized in terms of contrast and brightness using the function "auto level" of the Graphic Converter 9.7.5 program of the Lemke Software GmbH, Germany. The adjustment applied to every pixel in the image was done without obscuring, eliminating or misrepresenting any information given by the original images. Optical photos of the dyed leather were taken

with a Nikon CoolPix4300 digital camera. The pictures were acquired in a light chamber under a D65 light source.

2.5 Washing test, colour fastness and reusability

The biocoloured wool was submitted to several washing cycles, according to ISO 6330:2000X, before evaluating *K/S* values. The washings were performed in equipment Mathis Labomat BFA (Werner Mathis AG) using 4 g/L of standard detergent without optical brightener and a bath ratio of 1:20 and 20 rpm agitation. It was added a metallic sphere for each 100 mL bath to simulate mechanical abrasion. The washings were performed at 40° C for 30 min (gradient of 3.5 °C/min). The colour fastness was performed according to ISO 11640/ISO 11641 for biocoloured leather and ISO 105-B02 for biocoloured wool and their quality in colour change, and staining was assessed using the grey change scale. On this scale, 5 indicates that no colour was lost, and 1 indicates that most colour was lost. The reusability of leather and wool dyeing bath was evaluated, by mean of the *K/S* values, performing five consecutive cycles of dyeing in the same colouring bath.

2.6 Experimental design

The influence of the dyeing process variables (A = precursor´s concentration [mM]; B = modifier´s concentration [mM]; C = laccase´s amount [µL]; D = temperature [°C]; E = dyeing time [h]) on the colour of the wool fabrics was studied using a 2^5 full factorial design with two blocks and two centre points per block rise to 36 sets of experiments (Table 1). The range and the levels of the independent variables (Table 1) correspond to the ranges of variation in these parameters in the enzymatic recipe for dyeing stated earlier (30–50 °C, 10–90 µL laccase, 2–18 mM ABTS, 200–800 mM resorcinol and 0.5–3.5 h). The dependent variable, in the context of design of experiments, is called the response, and the independent variables are called factors. Experiments are run at different factor values, called levels. Each run of an experiment involves a combination of the levels of the investigated factors. The response analyzed was the colour strength (*K/S*), measuring the colour intensity on the substrate by reflectance spectroscopy. To simplify the model, the statistically insignificant terms were eliminated. We used the program Design Expert (version 8.0.3) for regression and graphical analysis of experimental data, as well as for obtaining the optimal set of variables, based on the criterion of desirability. The Student's *t* test and Fisher test were used to determine statistical significance of regression coefficients and equations of the model. The proportion of variance explained by the model was given by the coefficient of multiple determinations R^2.

Table 1 Levels of the factors studied in the planning factor 2^5

Variables	Level		
	− 1	0	1
A: Resorcinol (mM)	200	500	800
B: ABTS (mM)	2	10	18
C: Enzyme (µl)	10	50	90
D: Temperature (°C)	30	40	50
E: Time (H)	0.5	2	3.5

3 Results and discussion

3.1 Experimental design of biocolouration

The effects of variables were simultaneously studied using a full factorial planning 2^5. Table 2 records the values of the dependent variable of colour strength (*K/S*) obtained for each of the biocoloured wool samples under the conditions indicated for each levels reported in Table 1.

As previously demonstrated, the values of *K/S* are directly proportional to the amount of dye that was adsorbed and fixed on the substrate [22]. The values of *K/S* measured after each set of experiments in the RSM ranging from 1.42 to 27.10. The highest value of *K/S* was obtained at the highest level (Level 1) for all five variables, while the lowest value was not obtained at the corresponding minimum level (Level − 1) for all variables but using high amount of precursor and enzyme.

The significance of the considered factors was determined based on the statistical parameters *t* of student and the value of *p*. It was assumed that a confidence level above 99.99% is considered to be significant for the model. Screened parameters were represented on Pareto chart of standardized effects (Fig. 1). In the Pareto chart, significant and non-significant factors were placed above and below the black horizontal line (*t*-value limit), respectively, in descending order of significance (Rank). The vertical line shown in the chart indicted the statistical significance ($p > 0.0001$), at 99.99% confidence level. According to the results of Student's *t* test, obtained in Design Expert software for each parameter, the model is statistically significant for the following factors: temperature (D), precursor concentration (A), the interaction between the concentrations of precursor and modifier (AB), reaction time (E), third-order interaction between the concentration of modifier, precursor and reaction temperature (ABD), the interaction between temperature and precursor concentration (AD) and modifier concentration (B). Factors A, D, E and AB are in the range $p < 0.0001$ (High significance) while the significance of factors B, AD and

Table 2 Experimental design results according to 25 factorial designs

Std #	Block	Variables					Response
		A	B	C	D	E	*K/S*
1	Block 1	−1	−1	−1	−1	−1	1.67
2	Block 1	1	−1	−1	−1	−1	3.07
3	Block 1	−1	1	−1	−1	−1	3.00
4	Block 1	1	1	−1	−1	−1	4.73
5	Block 1	−1	−1	1	−1	−1	1.70
6	Block 1	1	−1	1	−1	−1	1.42
7	Block 1	−1	1	1	−1	−1	1.58
8	Block 1	1	1	1	−1	−1	6.77
9	Block 2	−1	−1	−1	1	−1	6.68
10	Block 2	1	−1	−1	1	−1	5.94
11	Block 2	−1	1	−1	1	−1	5.52
12	Block 2	1	1	−1	1	−1	25.89
13	Block 2	−1	−1	1	1	−1	9.51
14	Block 2	1	−1	1	1	−1	12.95
15	Block 2	−1	1	1	1	−1	4.61
16	Block 2	1	1	1	1	−1	19.67
17	Block 1	−1	−1	−1	−1	1	9.38
18	Block 1	1	−1	−1	−1	1	8.10
19	Block 1	−1	1	−1	−1	1	3.07
20	Block 1	1	1	−1	−1	1	15.25
21	Block 1	−1	−1	1	−1	1	6.56
22	Block 1	1	−1	1	−1	1	7.52
23	Block 1	−1	1	1	−1	1	3.52
24	Block 1	1	1	1	−1	1	13.14
25	Block 2	−1	−1	−1	1	1	14.74
26	Block 2	1	−1	−1	1	1	13.64
27	Block 2	−1	1	−1	1	1	8.44
28	Block 2	1	1	−1	1	1	24.78
29	Block 2	−1	−1	1	1	1	17.46
30	Block 2	1	−1	1	1	1	15.79
31	Block 2	−1	1	1	1	1	7.29
32	Block 2	1	1	1	1	1	27.10
33	Block 1	0	0	0	0	0	18.78
34	Block 1	0	0	0	0	0	18.93
35	Block 1	0	0	0	0	0	17.06
36	Block 1	0	0	0	0	0	19.06

A = resorcinol concentration, B = ABTS concentration, C = laccase amount, D = temperature, E = dyeing time

ABD are inserted between 0.001 and 0.01 of *p* value below the Bonferroni correction limit. The Bonferroni correction is used to reduce the chances of obtaining false-positive results when multiple pairwise tests are performed on a single set of data [27].

The determination of statistical significance of our model was assessed using analysis of variance (ANOVA) based on Fischer's *F* test. The model provides a confidence level of 99.99%, presenting the response of *K*/*S* a coefficient of determination $R^2 = 0.79$, explaining 79% of the variability in response, the remaining 21% explained by systematic errors or impurities. The model obtained after the execution of full factorial plan defined by "design expert" showed a significant curvature. This indicates the nonlinearity of the model and the need to expand the area under study to allow a rigorous analysis of the experimental results. For the analysis of this planning, we used the response surface methodology (RSM) with face-centreed, where α is equal to one. The study of this response surface corresponded in practice to carry out a number of

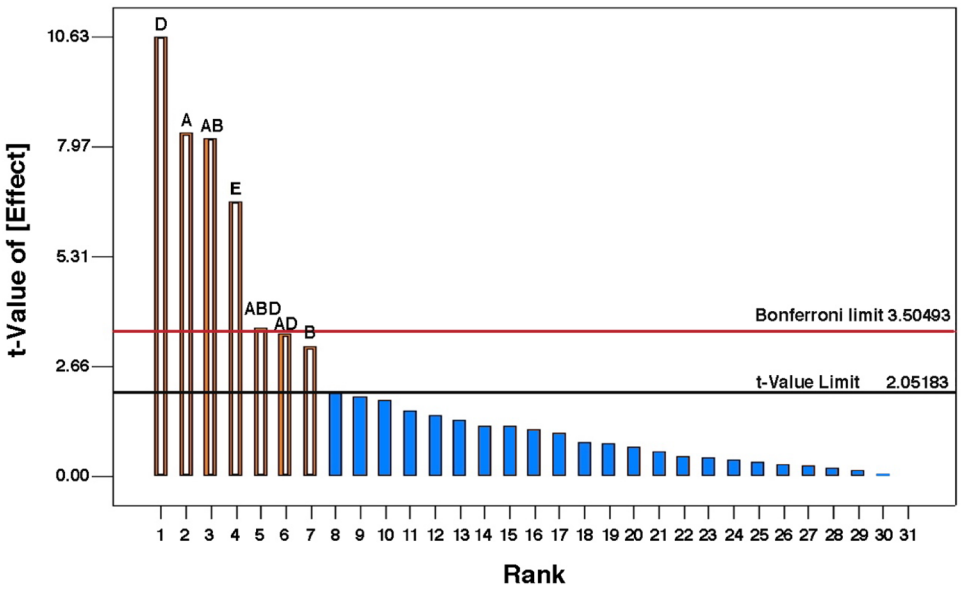

Fig. 1 Pareto chart of standardized effects ranking. (D) Temperature, (A) precursor concentration, (AB) interaction between the concentrations of precursor and modifier, (E) reaction time, (ABD) third-order interaction between the concentration of modifier, precursor and reaction temperature, (AD) interaction between temperature and precursor concentration and (B) modifier concentration. Unlabelled bars are the insignificant parameters. Bonferroni limit (red line) indicates absolute significance, and *t*-value limit (black line) indicates line of significant effect

Table 3 Experimental design results according to the response surface methodology with face-centreed

Std #	Block	Variables					Response
		A	B	C	D	E	*K/S*
37	Block 1	200	10	50	40	2	19.5
38	Block 1	800	10	50	40	2	22.27
39	Block 1	500	2	50	40	2	14.69
40	Block 1	500	18	50	40	2	20.56
41	Block 1	500	10	10	40	2	21.23
42	Block 1	500	10	90	40	2	20.56
43	Block 1	500	10	50	30	2	12.14
44	Block 1	500	10	50	50	2	25.75
45	Block 1	500	10	50	40	0.5	10.41
46	Block 1	500	10	50	40	3.5	23.76
47	Block 1	500	10	50	40	2	21.23
48	Block 1	500	10	50	40	2	20.11

A = precursor concentration, B = modifier concentration, C = laccase amount, D = temperature, E = dyeing time

additional tests. Table 3 shows the executed additional experimental tests as defined by the optimization's tool and the respective values of *K/S* obtained for each of the biocoloured samples.

According to the results, the most significant factors for *K/S* response, after RSM, are the precursor concentration (A), modifier concentration (B), the interaction between both (AB), temperature (D), the interaction between the precursor concentration and temperature (AD), the reaction time (E) and the quadratic terms of variable concentration of modifier (B2) and the reaction time (E2). Only the factors (A), (D), (E) and (AB) showed a confidence level better than 99.99% ($p < 0.0001$). The other variables were found to be in the range $0.001 < p < 0.01$. Then, a quadratic mathematical model for the *K/S* response was obtained and showed in the equation below:

$$K/S = -9.10807 - 0.020284 * \text{Resorcinol} + 1.03343 * \text{ABTS} + 0.20230 * \text{Temperature} + 11.45586 * \textit{Tempo} + 1.29648E^{-003} * \text{Re}\textit{sorcinol} * \textit{ABTS} + 4.37396E^{-004} * \text{Re}\textit{sorcinol} * \textit{Temperature} - 0.075977 * \text{ABTS}^2 - 2.40112 * \text{Time}^2$$

Statistical significance of the quadratic equation of the model (Table 4) was evaluated by analysis of variance (ANOVA) based on Fischer's *F* test. A significant model with a confidence level of 99.99% ($p < 0.0001$) to the *K/S* response was obtained.

Table 4 Analysis of variance (ANOVA) the quadratic model representative for the response *K/S*

K/S					
Font	SQ	GL	MQ	*F* value	*p* value
Model	2543.693241	8	317.9616551	43.65609948	< 0.0001
Lack of fit	274.2597593	34	8.066463509	4.119746429	0.0594
Pure error	9.79	5	1.958		
Total	2827.74	47			
$R^2 = 0.90$	C.V. = 21.01%				

SQ sum of squares; *GL* degrees of freedom; *MQ* mean square, *F value* ratio of the mean regression sum of squares divided by the mean error sum of squares, *P value* probability of getting a result at least as extreme as the one that was actually observed, given that the null hypothesis is true

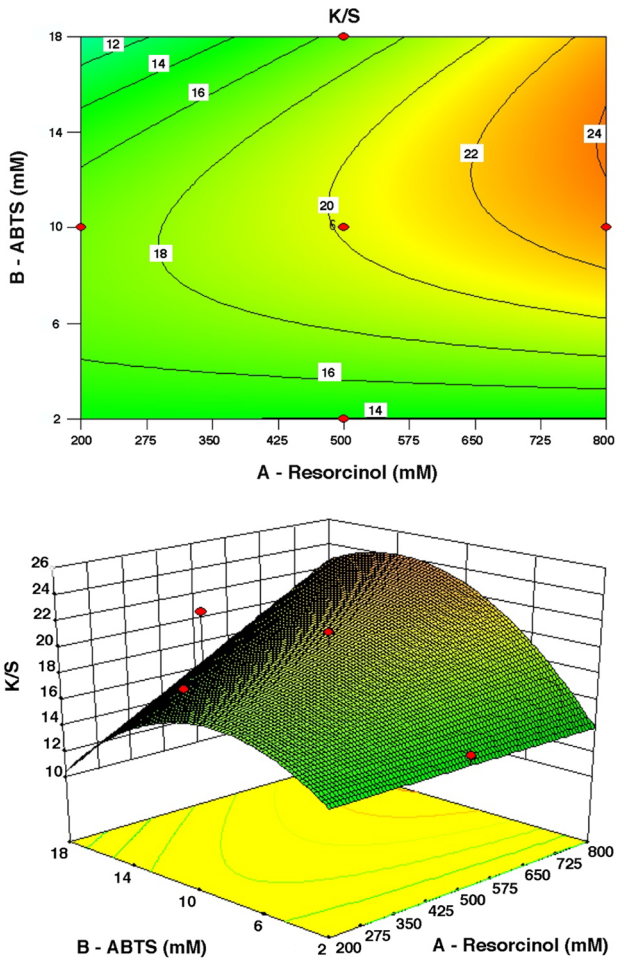

Fig. 2 3D response surface plots and contour plots showing the interactions between two parameters (concentrations in mM of ABTS and resorcinol) and their effect on *K/S* response

The response surface for the values of *K/S* (Fig. 2) shows that it is possible to improve the value of *K/S* increasing the concentration of precursor and modifier.

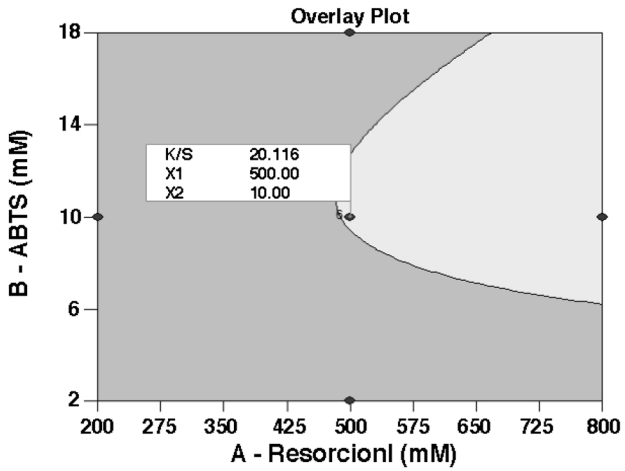

Fig. 3 Optimum region defined by overlaid plots of the response *K/S* evaluated for precursor resorcinol concentration (mM) as function of modifier ABTS concentration (mM). The other factors were kept constant in the middle level (level 0)

3.2 Validation of the model

Based on the obtained model, a graphic optimization was performed using the statistical program Design-expert®. The optimal conditions were defined to maximize colour strength (*K/S*) for a value higher than 20. A graph of modifier concentration versus precursor concentration, being the other factors in the middle level (Level 0), was generated (Fig. 3). The light grey region in the graph satisfies the imposed conditions showing an optimization point at 2 h of reaction time at 40 °C with 50 µl of enzyme dose, 500 mM precursor concentration and 10 mM of modifier concentration. At these conditions, the program predicts a *K/S* response of 20.12, with a standard deviation of 2.70, within a confidence level of 95%. The experimental confirmation showed a *K/S* response of 18.76 well within the ranges set by the program validating the model.

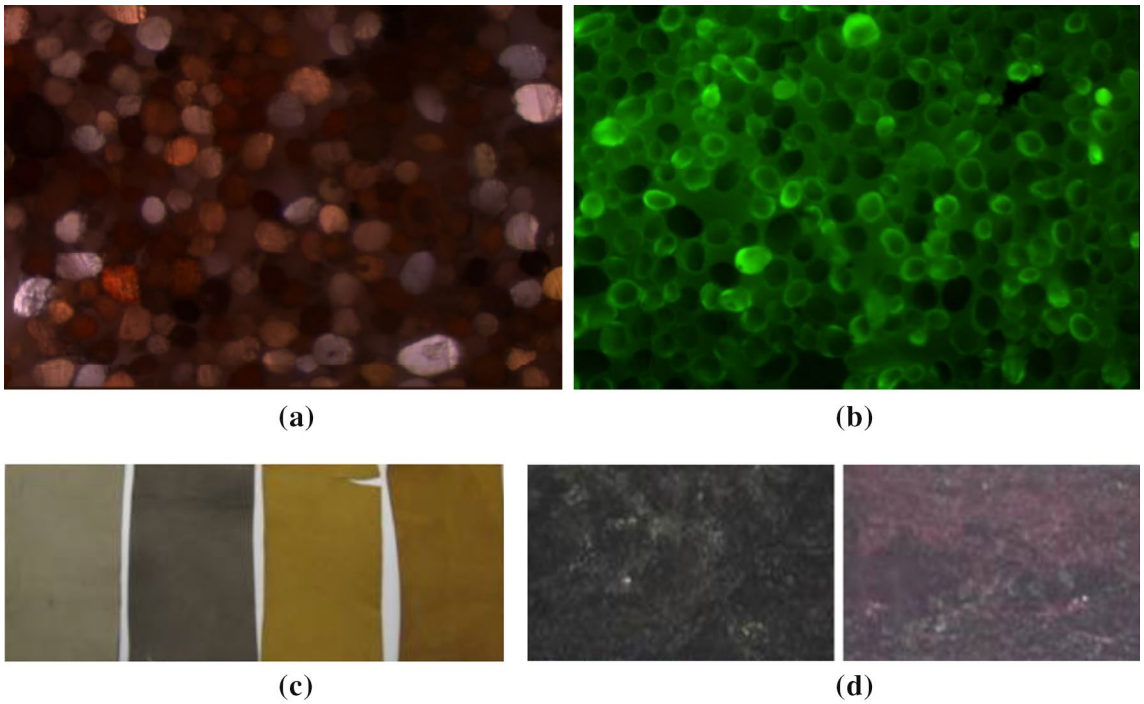

Fig. 4 Fluorescence microscopic images of the cross section of the wool fibre with 23 µm of diameter (original magnification: ×20), **a** biocoloured fibres, **b** fibres coloured with fluorescent rhodamine B cationic dye, **c** photographs of enzymatically coloured leather using different phenol precursors (the second from the left is the resorcinol/ABTS sample developed in this work) and optical microscopic images of the transversal cuts of enzymatically coloured leather (left d) and conventionally dyed leather (right d)

3.3 Optical microscope

In Fig. 4 clearly observs the deep penetration of the enzymatically "in situ" developed dye into the wool fibres (Fig. 4a). Despite the deep penetration of the dye onto the fibres, the enzymatic dyeing is not uniform at microscopic scale showing different shades in colour depending on the reactants access, contact and penetration in the fabric structure. Comparatively to the enzymatic dyeing, the traditional dyeing process, simulated in this work using a fluorescent dye, is not able to include the dye into the interior of the keratin fibre (Fig. 4b). The second sample from the left in Fig. 4c represents the enzymatically dyed leather using resorcinol and ABTS and the same conditions for wool. Figure 4c also shows other enzymatically dyed leather samples using different phenol precursors demonstrating the wide range of colour palette that can be obtained in leather. It is possible to observe the deep penetration of the "in situ" generated dye into the leather compared with a conventionally dyed leather observing the transversal cuts (Fig. 4d). Small molecules of the dye, modifier and precursor, can penetrate the cuticle of wool or the collagen matrix of leather and continue to interact non-enzymatically forming part of the dye in the fibre or itself. The small size of the molecules also delivers high colour levelness, an important dyeing quality parameter. Moreover, the presence of sulphur groups in the ABTS molecule provides both solubility of the dye and substantivity towards the proteinaceous materials [28].

3.4 Reusability and washing fastness

The traditional dyeing process produces highly polluting effluents with a high consumption of water [29]. Thus, the reusability of the colouring bath is an important parameter in order to reduce the environmental impact and the costs of the dyeing process. After the first reusability cycle, a decrease of 10% of the initial *K/S* was observed in the wool fibres. The enzyme continues to catalyze the reactions between resorcinol and ABTS during the reutilization baths alternating depolymerization, polymerization and radical reactions forming new dye. In this dynamic environment, it is very difficult to provide a reliable measure of the exhaustion in the bath. Only the final colour on the fabric or leather can be used as indicator of the reducing performance of the enzyme. After five dyeing process using the same bath, the value of *K/S* showed a loss of 40% of the initial value (Fig. 5a). This decrease is associated with the loss of enzyme activity and availability of phenolic compounds. However, depending on the quality and intensity of the desired colour, this reusability can be

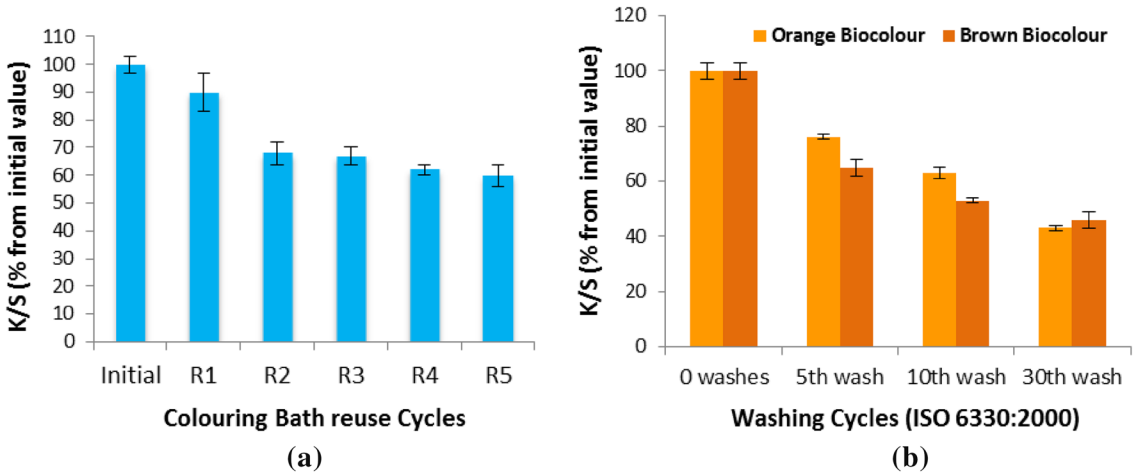

Fig. 5 **a** Colour variation in the biocoloured wool (% of the initial attained *K*/*S*), after reusing the colouring bath by five consecutive cycles. **b** Colour variation in the biocoloured wool (% of the initial attained *K*/*S*), after several washing cycles, according to the standard ISO 6330:2000

an attractive method due to the good level and uniformity that can be achieved.

Colour durability to washing and light exposure is one of the major concerns of textile industry. The colour variation in the enzymatically dyed wool was evaluated after five washing cycles showing a decrease of 20% of the initial *K*/*S* value. However, after thirty washing cycles wool fabric was able to retain about 50% of the initial colour (Fig. 5b).

3.5 Colour fastness

Table 5 shows the colour fastness properties of the enzymatically dyed wool and leather samples. The used standard methods determine the transfer of dye and the behaviour of the surface of leather and wool on rubbing with an undyed felt. The felt may become coloured through transfer of any kind of coloured matter. The change in colour of the felt and of the leather and wool is assessed with a standard grey scale. On this scale, 5 indicates that to no colour was lost, and 1 indicates that most colour was lost. The high values of the grey scale obtained in the tested samples demonstrate that enzymatic dyeing has good fastness properties comparable to the traditional dyeing process [30, 31].

The results suggest that laccase-oxidized ABTS-resorcinol dye has a relatively strong affinity to wool and leather. The laccase-mediated oxidation of ABTS and resorcinol results in highly reactive radicals, which can undergo either self- or cross-propagation with the respective monomers in a very complex way. When ABTS was added to the laccase-polymerized resorcinol, a clear shift in colour due to coupling and polymerization reactions could be observed. This is in contrast to the reported depolymerizing effects of laccase-ABTS system on Kraft lignin and laccase-HOBT system on the synthetic guaiacyl lignin [32, 33]. Laccase-oxidized resorcinol developed a clear bright orange colour; however, after ABTS addition a different colour shape is observed and reported here for the first time. The result seems to indicate that with both resorcinol and laccase, ABTS not only reacts as a reversible electron carrier mediator but underwent coupling reactions with the substrate, leading to a copolymer [34]. Moreover, resorcinol hydroxyl groups develop a weak acidity because of the electron delocalization promoted by the ABTS-coupled structure after the dissociation of the hydrogen ions. This dissociation provides to the newly formed dye a high affinity towards the substrates under acidic conditions due to: (1) the ionic attractions between dye negative charges

Table 5 Colour fastness properties of enzymatically dyed wool and leather

Standard	Results (grey scale 1–5)
Leather—colour fastness to cycles of to-and-fro rubbing (ISO 11640)	Dry (100 cycles): 5
	Wet (50 cycles): 4
Leather—colour fastness to perspiration (ISO 11641)	4
Textiles—colour fastness to artificial light: Xenon arc fading lamp test (ISO 105-B02)	4

Grey scale: from 1 poor to 5 very good

and positively charged amine groups of wool and collagen proteins and (2) the van der Waals forces that induced dipoles and $\pi - \pi$ interactions among the conjugated systems [35]. Resorcinol is a meta-substituted diphenol, but different colours and shades could be obtained using other diphenols that differ for the position of the second OH group in their structure, phenols or amines [22].

4 Conclusion

The model attained for the colour intensity was significant, showing a confidence level higher than 99.99% ($p < 0.0001$). The most significant factors to colour strength (K/S) response were temperature, precursor concentration, interaction between precursor and modifier and time. The optimization point was with 2 h reaction time, 50 µl enzyme amount, 500 mM precursor concentration, 10 mM modifier concentration and 40 °C. Microscopic observations of the enzymatic-dyed wool and leather demonstrated the penetration of the dye into the materials and the absence of damages to the cuticle layers of wool. The enzymatic-dye bath solution can be reused several times, which has a huge advantage in terms of cost reduction. The laccase-assisted dyeing process displays good reusability, washing durability and fastness properties comparable to the traditional dyeing process for both wool and leather.

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Compliance with ethical standards

Conflict of interest On behalf of all authors, the corresponding author states that there is no conflict of interest.

References

1. Madhu A, Chakraborty JN (2017) Developments in application of enzymes for textile processing. J Clean Prod 145:114–133
2. Chatha SAS, Asgher M, Iqbal HMN (2017) Enzyme-based solutions for textile processing and dye contaminant biodegradation-a review. Environ Sci Pollut Res 24:14005–14018
3. Varadarajan G, Venkatachalam P (2016) Sustainable textile dyeing processes. Environ Chem Lett 14:113–122
4. Pezzella C, Giacobbe S, Giacobelli VG et al (2016) Green routes towards industrial textile dyeing: a laccase based approach. J Mol Catal B-Enzym 134:274–279
5. Mirjalili M, Nazarpoor K, Karimi L (2011) Eco-friendly dyeing of wool using natural dye from weld as co-partner with synthetic dye. J Clean Prod 19:1045–1051
6. Munteanu FD, Basto C, Gubitz GM, Cavaco-Paulo A (2007) Staining of wool using the reaction products of ABTS oxidation by Laccase: synergetic effects of ultrasound and cyclic voltammetry. Ultrason Sonochem 14:363–367
7. Kanth SV, Venba R, Jayakumar GC, Chandrababu NK (2009) Kinetics of leather dyeing pretreated with enzymes: role of acid protease. Bioresource Technol 100:2430–2435
8. Vankar PS, Shanker R, Verma A (2007) Enzymatic natural dyeing of cotton and silk fabrics without metal mordants. J Clean Prod 15:1441–1450
9. Aaslyng D. Laccases with improved dyeing properties. Google Patents 1999
10. Barfoed M, Kirk O, Salmon S. Enzymatic method for textile dyeing. Google Patents 2001
11. Hadzhiyska H, Calafell M, Gibert JM, Dagà JM, Tzanov T (2006) Laccase-assisted dyeing of cotton. Biotech Lett 28:755–759
12. Barfoed M, Kirk O, Salmon S. Enzymatic method for textile dyeing. Google Patents 2004
13. Mate DM, Alcalde M (2017) Laccase: a multi-purpose biocatalyst at the forefront of biotechnology. Microb Biotechnol 10:1457–1467
14. Nasir M, Hashim R, Sulaiman O, Nordin NA, Lamaming J, Asim M (2015) Laccase, an emerging tool to fabricate green composites: a review. BioResources 10:6262–6284
15. Su J, Fu J, Wang Q, Silva C, Cavaco-Paulo A (2018) Laccase: a green catalyst for the biosynthesis of poly-phenols. Crit Rev Biotechnol 38:294–307
16. Polak J, Jarosz-Wilkolazka A, Szuster-Ciesielska A et al (2016) Toxicity and dyeing properties of dyes obtained through laccase-mediated synthesis. J Clean Prod 112:4265–4272
17. Kumar D, Kumar A, Sondhi S, Sharma P, Gupta N (2018) An alkaline bacterial laccase for polymerization of natural precursors for hair dye synthesis. 3 Biotech 8:182
18. Giacobelli VG, Pezzella C, Sannia G et al (2018) Laccase-based synthesis of SIC-RED: a new dyeing product for protein gel staining. Biocatal Agric Biotechnol 15:270–276
19. Sousa AC, Baptista SR, Martins LO, Robalo MP (2019) Synthesis of azobenzene dyes mediated by CotA laccase. Chem Asian J 14:187–193
20. Polak J, Jarosz-Wilkolazka A (2012) Fungal laccases as green catalysts for dye synthesis. Process Biochem 47:1295–1307
21. Kim SY, Zille A, Murkovic M, Guebitz G, Cavaco-Paulo A (2007) Enzymatic polymerization on the surface of functionalized cellulose fibers. Enzyme Microb Technol 40:1782–1787
22. Tzanov T, Silva CJ, Zille A, Oliveira J, Cavaco-Paulo A (2003) Effect of some process parameters in enzymatic dyeing of wool. Appl Biochem Biotechnol 111:1–13
23. Suparno O, Covington AD, Evans CS (2005) Kraft lignin degradation products for tanning and dyeing of leather. J Chem Technol Biotechnol 80:44–49
24. Rosu L, Varganici CD, Crudu AM, Rosu D, Bele A (2018) Ecofriendly wet–white leather vs. conventional tanned wet–blue leather. A photochemical approach. J Clean Prod 177:708–720
25. Kanth SV, Venba R, Madhan B, Chandrababu NK, Sadulla S (2008) Studies on the influence of bacterial collagenase in leather dyeing. Dyes Pigments 76:338–347
26. Silva CJSM, Roberto IC (2001) Optimization of xylitol production by Candida guilliermondii FTI 20037 using response surface methodology. Process Biochem 36:1119–1124
27. Abu ML, Nooh HM, Oslan SN, Salleh AB (2017) Optimization of physical conditions for the production of thermostable T1 lipase in Pichia guilliermondii strain SO using response surface methodology. BMC Biotechnol 17:78
28. Shin H, Guebitz G, Cavaco-Paulo A (2001) "In situ" enzymatically prepared polymers for wool coloration. Macromol Mater Eng 286:691–694

29. Sundrarajan M, Vishnu G, Joseph K (2007) Ozonation of light-shaded exhausted reactive dye bath for reuse. Dyes Pigments 75:273–278
30. Guesmi A, Ben Hamadi N, Ladhari N, Sakli F (2012) Dyeing properties and colour fastness of wool dyed with indicaxanthin natural dye. Ind Crop Prod 37:493–499
31. Velmurugan P, Kamala-Kannan S, Balachandar V, Lakshmanaperumalsamy P, Chae JC, Oh BT (2010) Natural pigment extraction from five filamentous fungi for industrial applications and dyeing of leather. Carbohydr Polym 79:262–268
32. Bourbonnais R, Paice M, Reid I, Lanthier P, Yaguchi M (1995) Lignin oxidation by laccase isozymes from *Trametes versicolor* and role of the mediator 2, 2′-azinobis (3-ethylbenzthiazoline-6-sulfonate) in kraft lignin depolymerization. Appl Environ Microbiol 61:1876–1880
33. Srebotnik E, Hammel KE (2000) Degradation of nonphenolic lignin by the laccase/1-hydroxybenzotriazole system. J Biotechnol 81:179–188
34. Rittstieg K, Suurnakki A, Suortti T, Kruus K, Guebitz GM, Buchert J (2003) Polymerization of guaiacol and a phenolic beta-O-4-substructure by Trametes hirsuta laccase in the presence of ABTS. Biotechnol Prog 19:1505–1509
35. Han SY, Yang Y (2005) Antimicrobial activity of wool fabric treated with curcumin. Dyes Pigments 64:157–161