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A straightforward bioprocess for a cleaner paper decolorization

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Abbreviations

MtL: *Myceliophthora thermophila* laccase

TvL: *Trametes villosa* laccase

MeS: methyl syringate

SA: syringaldehyde

AS: acetosyringone

K_L: control treatment with laccase

P: hydrogen peroxide stage

Z: ozone stage

F: formamidine sulfinic acid stage

DRI: dye removal index

k/s: absorption and scattering coefficients

C*: chroma

Abstract

A new biotechnological sequence for decolorizing red and black colored paper was developed to reduce the environmental impact of the chemicals used in paper recycling processes. Commercially available low-redox potential laccase from *Myceliophthora thermophila*, which operates optimally under alkaline conditions, was used in combination with natural mediators to make the process even greener. Based on the optical properties of the resulting decolorized paper, red and black dyes were efficiently removed by all laccase–mediator systems. The best results were provided by the laccase–methyl syringate combination, followed by the laccase–acetosyringone system and the laccase–syringaldehyde system. The decolorization rate for red paper achieved with the laccase–methyl syringate treatment exceeded that obtained with ozone. Red was removed by about 98% by combining two enzymatic stages and hydrogen peroxide stage, and black by 65%, without altering the physical properties of the colored paper in either case. A sequence combining oxidative and reductive (formamidine sulfinic acid) chemical treatments led to comparable optical and physical properties for the two types of paper. The effects of *Myceliophthora thermophila* laccase and methyl syringate were similar to those of high-redox potential laccase from *Trametes villosa* combined with either methyl syringate or the synthetic mediator violuric acid.

Keywords: Recycled paper, laccase, natural mediators, ozone, color removal

1. Introduction

Paper, a material obtained from a natural resource (lignocellulosic biomass), is recyclable, biodegradable and widely used globally. Recycling paper has economic and environmental advantages since it reduces waste and helps conserve natural resources, (Lopez et al., 2003). Mixed office waste and colored paper often constitute an underused waste paper resource owing to their difficult decolorization by removal of the dyes they contain. Dyes are added to papers to obtain specific optical properties. The most widely used synthetic dyes for this purpose are azo dyes, which possess N=N groups and are allegedly toxic (Gholami-Borujeni et al., 2011), mutagenic and carcinogenic (Gregory, 1986). Conventional paper recycling processes use vast amounts of chemicals to remove dyes and are uneconomical and environmentally hazardous. A variety of chemicals including hydrogen peroxide (Ibarra et al., 2012), oxygen, ozone, and reductive bleachers such as sodium dithionite and formamidine sulfinic acid (Vidal et al., 2000) have been used to bleach recycled paper.

Using enzymes in biotechnological processes could reduce the impacts of conventional recycling methods on global warming and the environment (Jegannathan and Nielsen, 2013). Singh et al., (2012) obtained lower COD and BOD values by introducing enzymes in a deinking process. Nathan et al., (2018) found enzymes to produce nontoxic effluents during the deinking process. Various types of enzymes, which can act directly on paper fibers or ink, have been used for deinking. These enzymes include cellulases, xylanases, pectinases (Shing et al., 2012), and amylases, lipases, esterases and laccases (Leduc and Daneault, 2011). Enzymatic deinking with cellulases (Ibarra et al., 2012) and hemicellulases (Xu et al., 2011) alone or in combination has been thoroughly characterized. The adverse effects of these enzymes on strength-related properties has boosted a search for less aggressive alternatives. Laccases in combination with synthetic (Valls et al., 2010a) or natural mediators (Valls et al., 2013) have been extensively used in recent years to biodelignify wood pulp.

Combinations of laccases with synthetic (Mirzadeh et al., 2014) and natural mediators (Grassi et al., 2011) have proved effective in removing dyes. They are less toxic to bacteria (Forootanfar et al., 2016) and *Saccharomyces cerevisiae* yeasts (Pereira et al., 2009). Direct application of laccase–mediator systems to paper remains largely unexplored. Mohandass et al. (2008) used laccase to decolorize blue colored pulp. Later, Xu et al. (2011) and Virk et al. (2013) observed a synergistic deinking action of hemicellulases and laccase–mediator systems applied to old newsprint. According to Virk et al. (2013), no mediator was needed. Laccase–mediator systems have proved effective in removing flexographic inks (Fillat et al., 2015). Ibarra et al. (2012) found laccase–mediator systems not to deink newspaper or magazine fibers. In these previous studies, the enzyme was applied in a single step or combined with a flotation or bleaching stage with hydrogen peroxide. The authors failed to specify where the enzymatic stage fell in the treatment sequence. They did not compare the results with those other chemical oxidants such as ozone. In relation to black dies, no study of the direct removal of black color from paper has seemingly been reported to date. As regard mediators, synthetic chemicals such as HBT (1-hydroxybenzotriazole) or violuric acid have proved the most effective for pulp delignification (Valls et al., 2010a). Their industrial use can pose environmental problems owing to their potential toxicity. Natural mediators are environmentally friendly and can be obtained as byproducts of the pulp industry or from industrial effluents. Natural mediators have been found to provide high decolorization rates for various dyes (Camarero et al., 2005); their use can make enzyme based treatments more sustainable.

The main purpose of this work was to develop an environmentally friendly alternative sequence for decolorizing commercially red and black colored paper. Commercially available laccase from *Myceliophthora thermophila*, which operates optimally under alkaline conditions (Ibarra et al., 2006), was used in combination with natural mediators. Chemical stages using hydrogen peroxide, ozone or formamidine sulfinic acid were studied to identify the most efficient sequence for completely decolorizing paper. The best natural mediator was compared with a synthetic mediator, and *Myceliophthora thermophila* with a high-redox potential laccase. As a novelty, the optical properties of decolorized paper were thoroughly examined to better understand the behavior of each bleaching agent. The impact on the final physical properties of the paper was assessed.

2. Materials and Methods

2.1. Raw Material

Red and black colored papers from Motif® and Liderpapel were used. A *Eucalytus globulus* ECF (elemental chlorine free) bleached pulp supplied by ENCE S.A. (Spain) was used as reference. These papers and pulp were disintegrated at 30,000 revolutions.

2.2. Enzymatic treatments (L)

A low-redox potential laccase from the ascomycete *Myceliophthora thermophila* (MtL, NOVOZYMES®, Bagsvaerd, Denmark) was used in combination with syringaldehyde (SA), acetosyringone (AS) (Sigma–Aldrich Quimica S.A., Madrid, Spain) and methyl syringate (MeS) (NOVOZYMES®). A high-redox potential laccase from the basidiomycete *Trametes villosa* (TvL, NOVOZYMES®, Bagsvaerd, Denmark) was tested with violuric acid (VA, Sigma–Aldrich Quimica S.A., Madrid, Spain), and MeS. A control treatment with laccase and without mediator was performed and designated as K_L.

Treatments were performed in a Datacolor Easydye reactor at 5 % consistency, 20 U g^{-1} odp (oven-dried pulp) laccase and at 1.5 % or 3 % (w/w) of mediator concentration, during 4 h at 50 ºC, in 50 mM sodium phosphate buffer (at pH 7 for MtL) or in 50 mM sodium tartrate buffer (at pH 4 for TvL). The resulting pulp was washed with decalcified water three times and once with distilled water (Valls et al., 2014).

2.3. Hydrogen peroxide stage (P)

Treatments were performed in a Datacolor Easydye reactor with 1.5 % odp of NaOH, 3 % odp H_2O_2, 1 % odp DTPA and 0.2 % odp $MgSO_4$, at 90 ºC, 5 % consistency for 120 min. The pulp was extensively washed after P (Valls et al., 2014).

2.4. Ozone treatments (Z)

Ozone treatments were performed at a pH of 2.5, low consistency (0.5 %), and at 0.8 % odp of ozone dose. The pulp was extensively washed after Z (Roncero and Vidal, 2007).

2.5. Formamidine sulfinic acid treatment (F)

The reductive F stage was performed on polyethylene bags at 60 ºC with 1% odp of formamidine sulfinic acid and 0.5 % odp of NaOH, at 5 % consistency for 120 min. After this stage, the pulp was extensively washed (Vidal et al., 2000).

2.6. Optical properties of papers

Handsheets of 75 ± 2 g m^{-2} grammage and with an area of 0.03142 m^2 were prepared on Rapid-Köhten equipment according to ISO 5331. The optical properties of paper sheets obtained were analysed using a reflectance measuring Technidyne Color Touch apparatus at standard illuminant D_{65} (LAV/Spec. Excl., d/8, D_{65}/10º). Two paper sheets per sample were obtained and six measures were performed in each paper sheet. The reflectance spectra of paper sheets were obtained from scattering (s) and absorption (k) coefficients using the Kulbelka–Munk theory (ISO 9416). The intrinsic reflectance factor (R∞) was measured. Sample color was described in terms of the CIE $L^*a^*b^*$ color coordinates, namely: lightness (L^*), red–green (a^*) and yellow–blue (b^*) sensations. Chroma (C^*), which is the perpendicular distance of a point from the lightness axis [$C^*= (a^{*2} + b^{*2})^{1/2}$] and represents the amount of color of a sample, was used to characterize the process (Hunt, 1998; Jordan, 1996).

Color removal was evaluated by the dye removal index (DRI), that indicates the reduction of the distance from an ideal bleach point expressed in percentage, representing the quantity of color removed by the treatment: DRI= $(\Delta R^2/R_1^2)*100$; $\Delta R^2=(R_1^2 - R_2^2)$; $R_1^2= (100 - L_1^*)^2+(a_1*)^2+(b_1*)^2$. ($L^*_1$, a^*_1, b^*_1): color coordinates of initial papers; (L^*_2, a^*_2, b^*_2): color coordinates after each decolorizing stage. Initial papers were used as reference, positive values represents color removal and negative ones represents coloration. The Color difference (ΔE^*), distance between two color locations of CIE $L*a*b*$ space was measured: $\Delta E^*= (\Delta L^{*2}+\Delta a^{*2}+\Delta b^{*2})^{1/2}$. Initial papers were used as reference (Jordan, 1996). Whiteness (ISO 11475) and yellowness (ISO 17223) were determined.

2.7. Laccase residual activity

The residual enzymatic activity was measured following the oxidation of ABTS based on the absorptivity increment at 436 nm ($\varepsilon_{436} = 29300\ M^{-1}\cdot cm^{-1}$) at 25 ºC (Valls et al., 2012). The reaction mixture contained 5 mM ABTS, 100 mM sodium acetate buffer at pH 5, and the effluent solution. A Shimadzu 1603 UV–Vis spectrophotometer was used. Enzymatic activity was defined as the amount of enzyme needed to convert 1 µmol of the substrate ABTS min^{-1}.

2.8. Mechanical properties of papers

Handsheets were tested mechanically in accordance with the following standards: bulk (ISO 12625-3), tensile index and breaking length (ISO 1924), burst index (ISO 2758) and tear index (ISO 1974).

3. Results and discussion

3.1. Selecting the best mediator for decolorizing red paper

Red color removal was studied by applying MtL alone or in combination with three different natural mediators in an LP sequence (Fig. 1a).

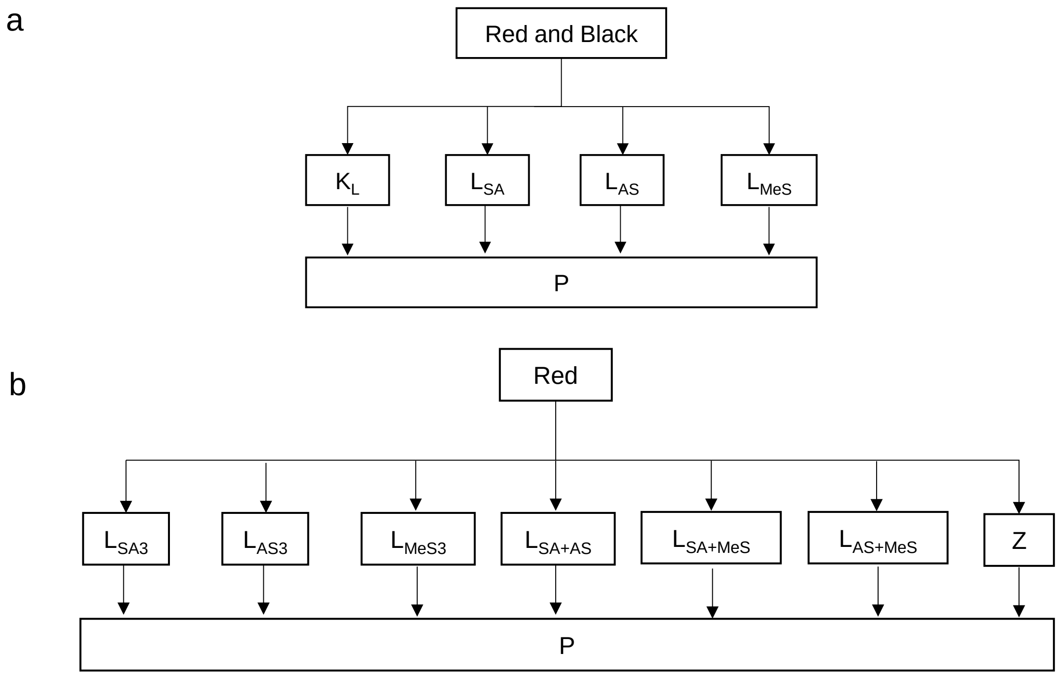

Fig. 1 a) Treatments performed on red and black papers with laccase alone (K_L) and with laccase and natural mediators (L_{SA}, L_{AS} and L_{MeS}) applied at a dose of 1.5 % followed by hydrogen peroxide stage (P); b) Laccase-mediator treatments at a dose of 3 % (L_{SA3}, L_{AS3} and L_{MeS3}), mediator combinations at a dose of 1.5 % each (L_{SA+AS}, L_{SA+MeS}, L_{AS+MeS}) and ozone (Z) sequence performed on red paper.

Fig. 2a shows the *L*a*b** color coordinates for treated paper after the L stage. Although the control treatment with laccase alone (K_L) slightly decreased *a** and *b** (two measures of color desaturation), the greatest reduction in color saturation was obtained in combination with a mediator (particularly MeS). The presence of a mediator, but particularly MeS, substantially increased lightness *(L*)* (Fig. 2a). Introducing a P stage in the sequence further decreased *a** and *b** and increased *L** (Fig. 2c). The same trend in relation to the L stage was observed as regards color reduction, being MeS the best mediator. The effects were visually apparent from the decolorized paper sheets (see supplementary Fig. S1). Decolorization was homogeneous along all the surface of the paper sheet.

Figs. 2b and 2d, show the k/s curves after the L and P stage. Red paper exhibited an absorbance peak at 500 nm typical of red. Changes in area under a k/s curve reflect changes in the amount of chromophores present. The control treatment (K_L) reduced the area only slightly. A considerable drop in area was observed with the addition of natural mediators. They were required for efficient decolorization. The results differed among mediators; SA had little effect, whereas AS and MeS, had strong effects on paper color. Although the need for a mediator to remove synthetic dyes is widely assumed, Pereira et al. (2009) found azo dyes to be efficiently removed with a bacterial laccase and no mediator. Virk et al. (2013) found that no mediator was needed to deink old newsprint.

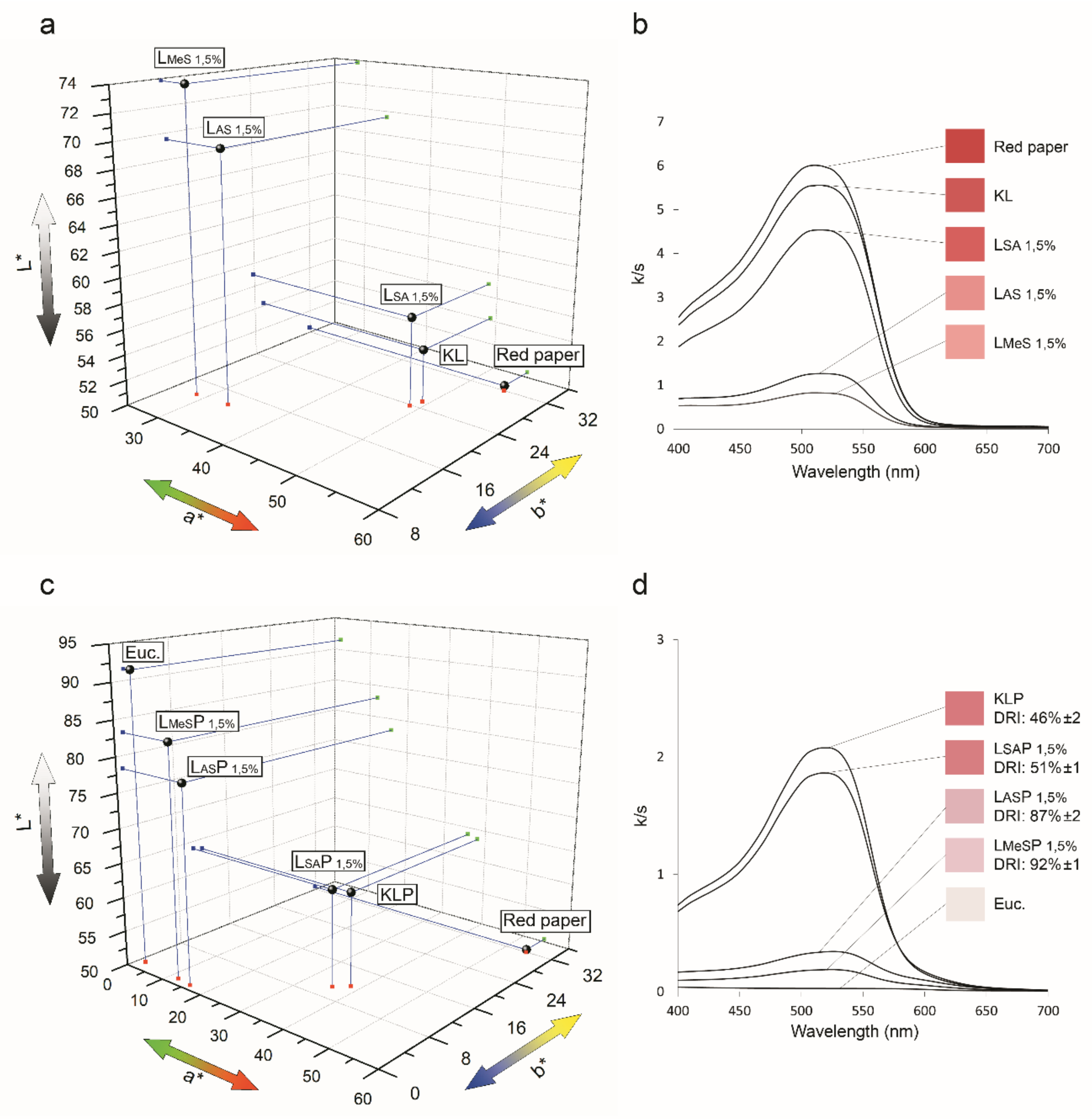

Fig. 2 *L*a*b** color coordinates and light absorption (k/s) of red paper treated with the natural mediators at a concentration of 1.5 % after L (a and b) and P (c and d) stages. The dye removal index (DRI) of the papers after P stage is shown in d. The confidence interval of *L**, *a** and *b** color coordinates was lower than 1 in all the cases.

The increased decolorization rate observed with MeS may have resulted from its increased redox potential (690 mV; Aracri et al., 2013) relative to SA and AS (589 and 575 mV; González Arzola et al., 2009). All three mediators (SA, AS and MeS) have two methoxy groups in ortho-phenol position. According to Andreu and Vidal (2011) and Barneto et al. (2012), the intermediate radical cation needed is more easily formed with electron-releasing substituents (e.g., methoxy groups) in ortho than in para-phenol positions. The substituents present in para-phenol position may have contributed to the differences. The mediators contained a different group in para, namely: aldehyde in SA, ketone in AS and methyl in MeS. These natural compounds were previously used to remove lignin from kenaf (Andreu and Vidal, 2011), flax (Fillat et al., 2010), eucalyptus (Valls et al., 2014) and softwood (Quintana et al., 2013) pulp. Their effects were found to be considerably smaller than with synthetic mediators. Valls et al. (2014) observed similar effects with SA and AS, and found MeS to be scarcely effective in removing lignin —which contradicts its high decolorization capacity. The mediators performed similarly in the L and P stages (see Figs. 2b and d). The absorbance intensity was lower by effect of color removal during the hydrogen peroxide stage (P) (Fig. 2d). The P stage converted colored organic molecules into colorless molecules because hydrogen peroxide is a nondegrading chemical, so its action is limited to destroying carbonyl or azo groups (Lachenal and Chirat, 1999).

The natural mediators studied had the ability to remove red from paper. They were applied at a higher dose (3 %) and in mutual combinations (1.5 %) to check for a potential synergistic effect (Fig. 1b). The total mediator dose used in each treatment was 3 %. The result of increasing the mediator dose differed with the particular mediator (Fig. 3). Using both mediators in combination detracted from their individual effects, probably as a result of competition between the two for the enzyme active sites. The best treatment was that using a 1.5 % dose of MeS alone.

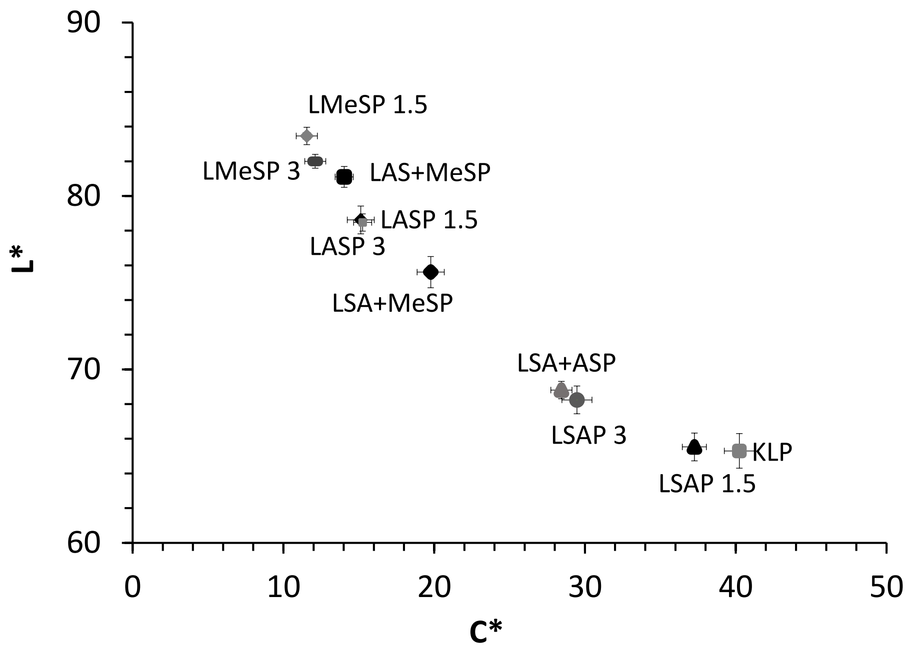

Fig. 3 *L** vs. *C** of red paper treated with laccase and natural mediators combined (at 1.5 %) or alone (at 3 %) after P stage

Enzymes can be inactivated during the enzymatic treatment. Only 20 % of the initial amount of enzyme remained active in the absence of a mediator. The enzyme retained most of its activity when incubated with the least efficient mediator (SA) at a dose of 1.5 or 3 %. Laccase was completely inactivated (1 % residual activity) by the end of the enzymatic treatments with AS and MeS, and with the two mediators in combination. Some authors have shown the inactivation of laccase during treatment conditions in sisal (Aracri et al., 2009), flax (Fillat et al., 2010) and eucalyptus pulp (Valls et al., 2014). These authors demonstrated that inactivation of the enzyme was more marked with synthetic mediators than with natural mediators. Fillat et al. (2012) found paper additives to affect laccase stability.

3.2. Selecting the best mediator for decolorizing black paper

The laccase–natural mediator systems were tested on a different color: black. Black paper absorbed similarly across the spectral region examined (Fig. 4b) but exhibited a small peak at ca. 650 nm. Forootanfar et al. (2016) previously observed an absorbance peak at 602 nm for the dye Direct Black 166. The removal of black dyes with laccases has been the subject of some study. Daâssi et al. (2012), Murugesan et al. (2007) and Sayahi et al. (2016) succeeded in removing the diazo dye Reactive Black 5 with laccase and HBT (1-hydroxybenzotriazole). Camarero et al. (2005) obtained better results with natural mediators such as SA and AS. Forootanfar et al. (2016) examined the removal of the triazo dye Direct Black 166 with laccase and HBT. They found the resistance of azo dyes to laccase-catalyzed oxidation to be enhanced by an increased number of azo groups. No study of the direct removal of black color from paper has seemingly been reported to date. Black color as assessed from *L*a*b** coordinates and k/s absorbance curves was removed from paper during the enzymatic stage with the three natural mediators used here (Fig. 4). Although the coordinates decreased in the same sequence as in red paper (L_{MeS} > L_{AS} » L_{SA}), *a** and *b** were both increased by the enzymatic treatments (Fig. 4a). L_{SA} increased *a** more markedly than the other combinations, whereas L_{AS} and L_{MeS} led to greater *b** values than the other treatments. Substantial color removal was observed during the hydrogen peroxide stage that resulted in a huge increase in Lightness (Fig. 4c) and a decrease in k/s (Fig. 4d). This result testifies to the effectiveness of the oxidative treatment in removing black colored papers.

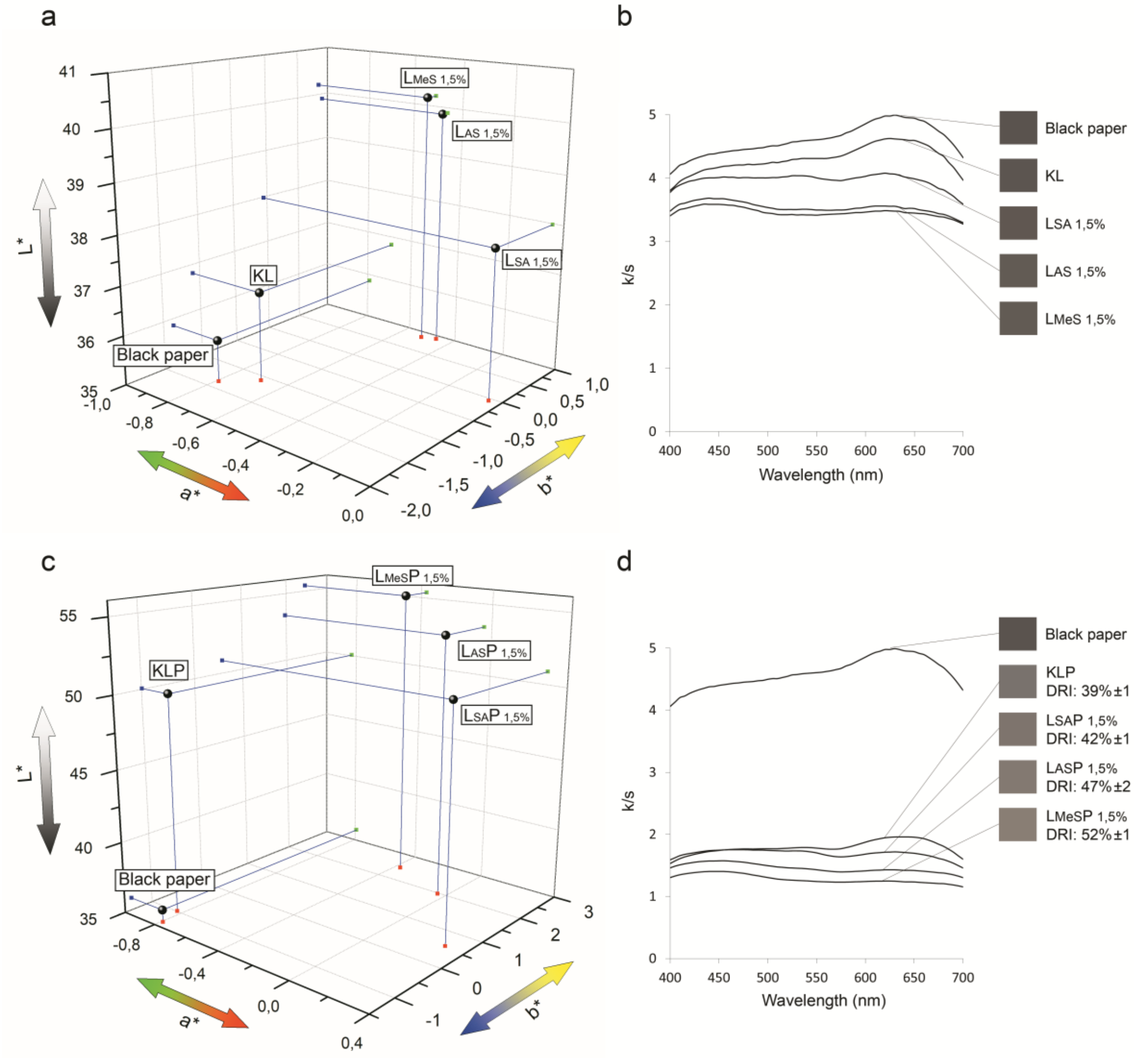

Fig. 4 *L*a*b** color coordinates and light absorption (k/s) of black paper treated with the natural mediators at a concentration of 1.5 % after L (a and b) and P (c and d) stages. The dye removal index (DRI) of the papers after P stage is shown in d. The confidence interval of *L**, *a** and *b** coordinates was lower than 1 in all the cases.

Dye removal index (DRI) was useful to compare decolorization after the P stage between red and black paper. The enzymatic sequence was more efficient in removing red than black (Figs. 2d and 4d). Forootanfar et al. (2016) found red to be more efficiently removed than black by a laccase–mediator system. The $L_{MeS}P$ sequence removed almost all red (more than 90 %).

It is important that the enzymatic and chemical treatments performed remove colorant without affecting cellulose and producing deterioration of the physical properties of papers. Neither bulk, tensile, burst indexes nor breaking length were affected by the enzymatic or hydrogen peroxide stages (supplementary Table S1).

3.3. Decolorization of red paper. Ozone versus laccase–mediator systems

Once a biotechnological treatment was shown to be effective in avoiding contamination from a dye in recycled paper, its oxidative capacity was compared with that of ozone, a powerful oxidant (Fig. 1b). Unlike hydrogen peroxide, ozone can destroy phenolic groups, carbon–carbon double bonds and conjugated aromatic structures (Roncero et al., 2003). Upon ozonation, dyes lose their color by effect of oxidative cleavage of their chromophores (Sevimli and Sarikaya, 2002). The rate of this process depends on the particular dye (Gomes et al., 2012).

Ozone was less effective than the biotechnological treatments despite its oxidative power (Fig. 5). DRI after the LP and ZP sequences was 92.2 % and 73.2 %, and the differences were apparent to the naked eye (supplementary Fig. S1).

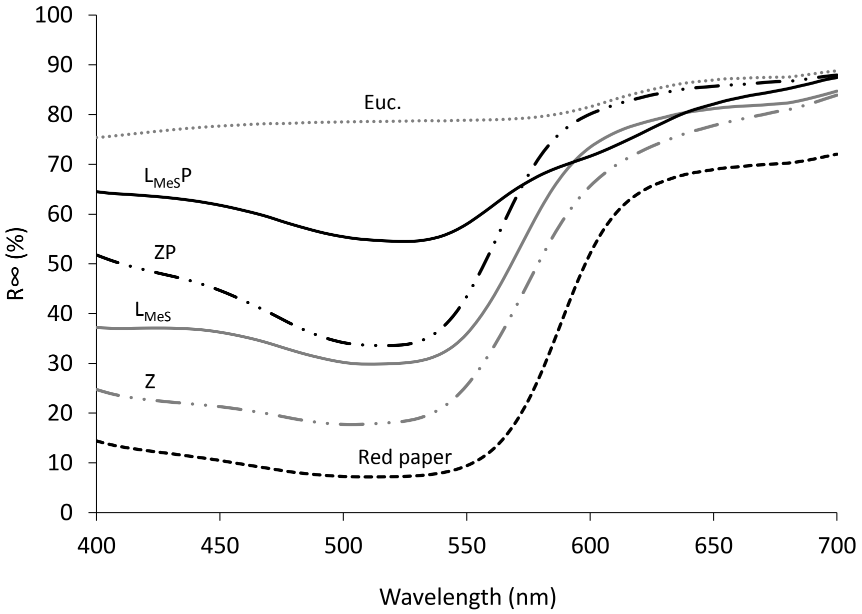

Fig. 5 Reflectance curves of red paper treated with the biotechnological ($L_{MeS}P$) and ozone (ZP) sequences

One of the drawbacks of applying ozone to pulp fiber is that the reagent is not completely selective for the dye and lignin. Ozone usually alters cellulose (Roncero and Vidal, 2007) and detracts from the physical properties of the resulting paper. Ozone did not deteriorate the physical properties in this case (supplementary Table S1).

3.4. Complete decolorizing sequences for black and red paper

Further treatment was required with the $L_{MeS}P$ sequence to obtain the same reflectance results as with the reference eucalyptus pulp (Fig. 5). Extensive sequences combining enzymatic, hydrogen peroxide and ozone treatments were investigated to maximize dye removal (Fig. 6a). Using ozone in the first stage resulted in the lowest DRI for red paper. Supplementary Fig. S2 shows the evolution of color removal from paper sheets during the best biotechnological sequence. The most effective sequences were those combining the two enzymatic stages, which were used to decolorize black paper (Fig. 6b). DRI obtained in this case was lower than with red paper.

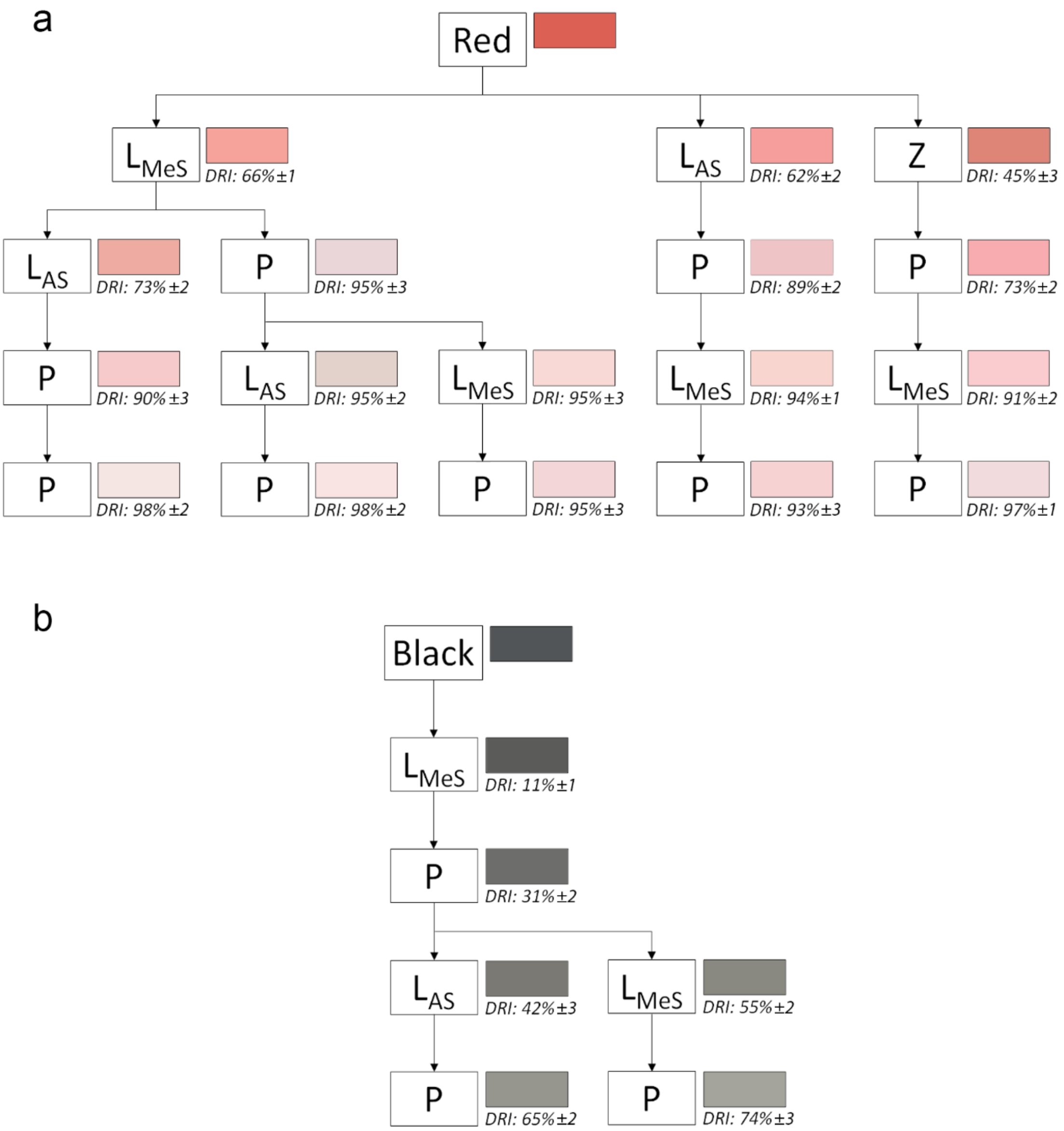

Fig. 6 Complete decolorizing sequences performed on red (a) and black (b) papers, combining biotechnological and chemical treatments. The dye removal index (DRI) after each stage stage is shown.

Fig. 7a shows the color coordinates for decolorized paper samples. Lightness in red paper was higher than that for the reference eucalyptus pulp. *a** and *b** were higher and suggestive of reddish hues and more saturated color in all cases. The low lightness values in black paper resulted in very dark hues. Chroma for the red paper sheets was gradually decreased by the different treatments (Fig. 7b). It was very low for all black samples —even lower than that for eucalyptus paper in the initial black paper—. The decolorizing sequence increased *C** relative to the initial black paper by increasing *b** (i.e., by increasing yellowness). *L** was increased from 35.6 to 62–67; these values are still far from that for eucalyptus paper (91.7).

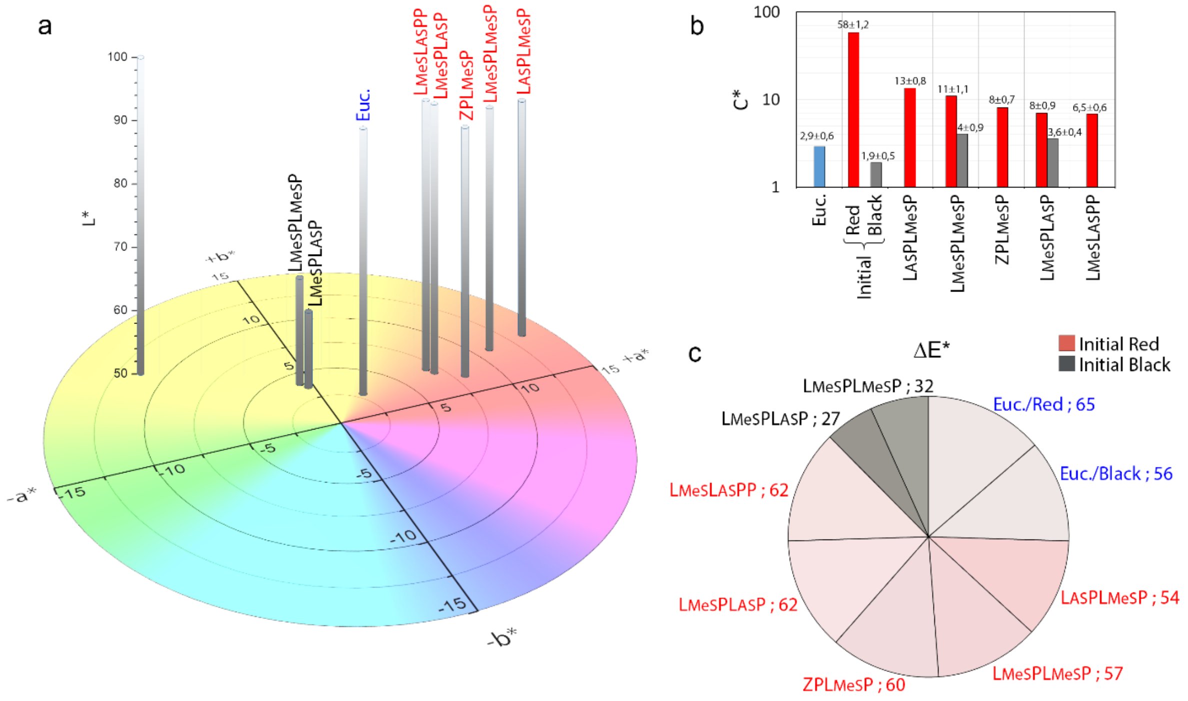

Fig. 7 *L*a*b** color coordinates of the complete decolorizing sequences performed on red and black papers. The length and grey value of the bars indicate the lightness of the colors (a). Chroma value of papers in the different treatments for red and black papers (b). ΔE* values of the treated papers with respect to the initial paper, and representation of the color obtained (c).

Red paper can be thoroughly biodeinked by combining an enzymatic stage and a P stage. Whether the P stage is inserted between the enzymatic stages or applied at the end of the sequence seemingly has no effect on the final optical properties of the paper. The physical properties of red papers were only slightly deteriorated during the complete sequence. The worst effect was produced when the two P stages were performed at the end (supplementary Table S1). The physical properties of black papers were not deteriorated during the complete sequence; they were slightly increased (supplementary Table S2).

Black paper required further treatment for the dye to be completely removed. An identical conclusion can be drawn from the ΔE^* values of Fig. 7c. ΔE^* is the color difference from the red or black initial paper. A ΔE^* value higher than 3 is detectable by the human eye, whereas a lower one is not. ΔE^* values were calculated with respect to the initial pulp in each case (*i.e.*, initial red or black). The ΔE^* value for eucalyptus was calculated with respect to the initial red or black paper. ΔE^* for red paper treated with the $L_{MeS}L_{AS}PP$ and $L_{MeS}PL_{AS}P$ sequences was only 3 points smaller than that for eucalyptus. The appearance, as indicated by ΔE^*, of paper decolorized with these sequences was similar to that of eucalyptus paper. Decolorized black paper was still far from white.

3.5. Complete decolorizing sequence with high-redox potential laccase

The above-described treatments were all performed with a low-redox potential laccase that was unable to oxidize synthetic mediators owing to their high redox potential. High-redox potential laccase from *Trametes villosa* (TvL) has been successfully used for pulp delignification in flax (Fillat and Roncero, 2010) and eucalyptus (Valls et al., 2010b, 2010c) pulps. In these works, it has proved more efficient than MtL. Synthetic mediators such as violuric acid, HBT or NHA are seemingly the most effective for this purpose by virtue of their containing N–OH groups (Valls et al., 2010a). These mediators have some problems associated with their potential toxicity.

In this work, TvL was used in combination with the synthetic mediator violuric acid (VA) and compared with the natural mediator MeS. Combinations of oxidative and reductive treatments were studied. F was used as reductive treatment (Imamoglu et al., 2013) in an LPFZ sequence. In red paper, whiteness initially had negative values that were strongly increased by the chemical stages (Table 1). Yellowness started with high positive values that decreased during the treatments. In black paper whiteness started with positive values and was only slightly increased by the treatments, the final value being higher for red paper than for black paper. Yellowness started with negative values and evolved differently depending on the particular chemical agent (oxidative or reductive). At the end of the sequence, yellowness was lower for black paper than it was for red paper. No significant differences were observed between synthetic and natural mediators. MtL was less effective in decolorizing both papers.

Table 1. Whiteness and Yellowness of red and black papers during LPFZ sequence, with two laccases (MtL and TvL) and two mediators (VA and MeS)

		Whiteness	Yellowness
Red	Initial	-136.6±0.3	133.5±0.6
TvL + VA	L	-114.1±0.4	128.0±0.9
	P	-76.8±0.3	104.4±1.0
	F	31.9±0.2	23.4±0.8
	Z	57.2±0.1	7.2±0.5
TvL + MeS	L	-115.8±0.9	129.9±0.4
	P	-69.4±0.1	99.1±0.9
	F	28.1±0.3	27.7±1.0
	Z	56.5±0.2	8.4±0.9
Black	Initial	23.4±0.2	-7.1±0.8
MtL + MeS	L	25.6±0.3	-3.2±0.8
	P	26.4±0.1	-1.0±0.7

	F	28.6±0.3	-3.6±0.4
	Z	29.4±0.1	2.9±0.2
TvL + MeS	L	28.6±0.2	-4.5±0.6
	P	27.8±0.1	-2.2±0.1
	F	31.5±0.2	-4.3±0.7
	Z	37.6±0.1	1.2±0.2
TvL + VA	L	22.2±0.3	-3.5±0.9
	P	28.5±0.1	-1.5±0.8
	F	31.7±0.1	-3.9±0.7
	Z	38.8±0.1	0.8±0.1

Other optical properties were examined (results not shown). Red and black dyes were removed by the reductive (F) and oxidative (Z) chemical stages. F was more effective than Z with red paper. The reflectance curve after F exhibited an increased peak at ca. 450 nm (results not shown) which was previously observed by Vidal et al. (2000). The final Z stage had a greater effect on black paper than on red paper. Black dyes were successfully removed with ozone by Colindres et al. (2010) and Zheng et al. (2016). Ozone allowed black to be removed from colored paper.

The LPFZ sequence afforded nearly complete color removal from red paper (DRI = 97 %) with TvL in combination with synthetic or natural mediators. TvL was less effective with black paper (DRI = 78 %). An identical sequence with the low-redox potential laccase (MtL) had a less marked decolorization effect on black paper (DRI = 73 %). Natural and safer mediators can be efficiently applied for decolorization. Supplementary Fig. S3 shows actual images of black paper sheets subjected to the LPFZ sequence. The physical properties of the paper were not significantly affected by the enzymatic or chemical treatments (Table 2). The values at the end of each decolorizing sequence were similar. The only substantial difference was that in tear index, which was greater in red paper than in black paper.

Table 2. Physical properties of red and black papers during LPFZ sequence, with two laccases (MtL and TvL) and two mediators (VA and MeS)

		Bulk (cm^3 g^{-1})	Tensile index (Nm g^{-1})	Burst index (KPa·m^2 g^{-1})	Breaking length (m)	Tear Index (mN·m^2 g^{-1})
Red	Initial	1.60±0.10	33.9±2.7	1.91±0.02	3,459	7.2±0.1
TvL + VA	L	2.69±0.10	30.9±3.0	1.64±0.17	3,154	9.0±0.3
	P	1.97±0.12	27.7±2.7	1.55±0.13	2,823	8.6±0.1
	F	1.94±0.09	27.8±3.2	1.57±0.04	2,830	9.0±0.1
	Z	1.85±0.08	27.2±2.8	1.55±0.10	2,780	8.8±0.2
TvL + MeS	L	2.63±0.12	28.9±3.0	1.64±0.02	2,941	8.6±0.1
	P	2.40±0.07	27.5±3.4	1.39±0.07	2,800	7.9±0.3
	F	1.89±0.09	28.8±3.6	1.53±0.09	2,935	8.5±0.2
	Z	1.83±0.07	32.6±2.4	1.82±0.23	3,325	8.9±0.4
Black	Initial	1.72±0.10	33.8±0.1	1.66±0.10	3,449	5.2±0.3
MtL + MeS	L	1.85±0.10	30.9±2.4	1.34±0.09	3,149	5.7±0.3
	P	1.81±0.12	31.7±2.8	1.52±0.14	3,228	5.8±0.2
	F	1.83±0.09	31.3±1.9	1.40±0.08	3,188	6.0±0.2
	Z	1.87±0.06	28.9±3.1	1.26±0.05	2,949	5.8±0.1
TvL + MeS	L	1.84±0.08	32.0±3.2	1.42±0.04	3,260	5.7±0.5
	P	1.76±0.07	36.0±3.5	1.49±0.47	3,667	6.4±0.4
	F	1.83±0.09	34.0±2.7	1.56±0.11	3,467	5.8±0.4
	Z	1.87±0.10	31.7±2.8	1.37±0.14	3,228	6.3±0.4
TvL + VA	L	1.83±0.10	34.5±2.3	1.65±0.11	3,519	5.8±0.5

P	1.79±0.08	33.5±1.8	1.55±0.13	3,411	6.0±0.5
F	1.81±0.10	35.7±3.7	1.66±0.08	3,634	5.9±0.4
Z	1.83±0.09	33.2±2.4	1.63±0.09	3,385	6.1±0.7

No significant differences in optical (Fig. 6) or physical properties (supplementary Table S1) between the LPFZ sequence and that using two enzymatic stages (LPLP) were observed. Our results testify to the effectiveness of biotechnological sequences as more sustainable alternatives to chemical decolorizing agents.

4. Conclusions

An environmentally friendly process for removing dyes during paper recycling was developed. In this process an enzymatic stage was included in order to reduce the hazardous chemical products typically used for paper decolorization. In the bioprocess developed, color was removed by 98 % in red paper and 73–74 % in black paper. Their physical properties were not deteriorated. The advantage was that most part of color removal was produced by the biotechnological treatment. This treatment included a laccase enzyme combined with natural phenolic compounds. The most efficient natural phenolic compound (methyl syringate) was proved to be more efficient than ozone, a powerful oxidant. Methyl syringate afforded the same decolorization rate as the more toxic, well-known synthetic compound violuric acid. This natural compound was also compatible with both, low- and high-redox potential laccases. The results found show that chemical agents needed to decolorize paper can be reduced by introducing an enzymatic stage. The bioprocess developed will contribute to a cleaner decolorization during paper recycling.

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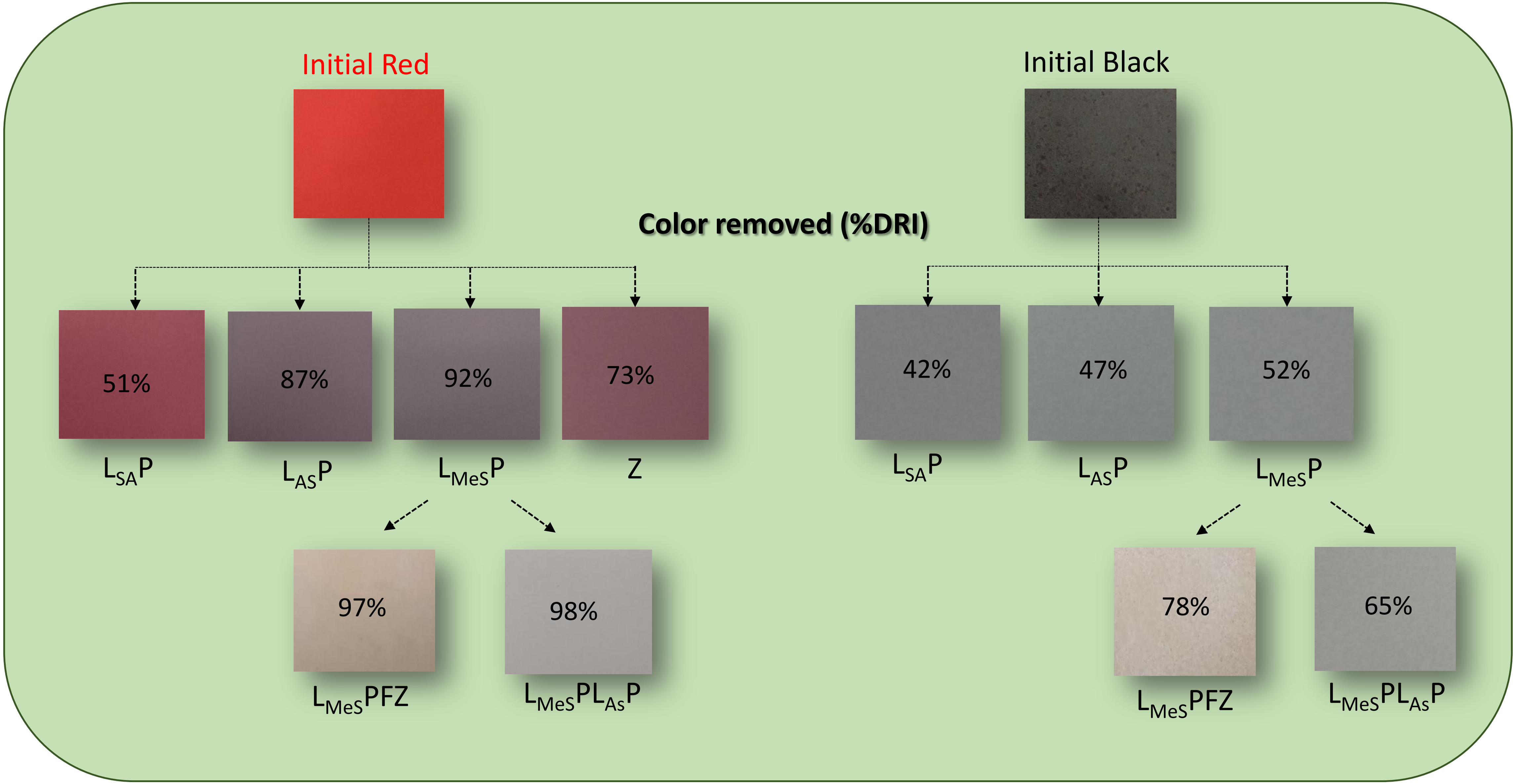
Graphical Abstract



Highlights (for review)

- The laccase-natural mediator system was able to remove red and black dyes from paper
- Methyl syringate was the best mediator to decolorize papers
- Higher dye removal was produced by biotechnological stages than by chemical stages
- Both, low and high-redox potential laccases can be used to produce decolorization
- Several optical parameters were measured to assess paper decolorization