



New Laccase-Mediated System Utilized for Bio-Discoloration of Indigo-Dyed Denim Fabrics

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Abstract

In this study, indigo-dyed denim fabric was decolorized via separate and simultaneous applying of laccase, sodium hydrosulfite, and cellulase. In this regard, the surface reflectance and color coordinates of the discolored fabrics were evaluated and scanning electron microscopy (SEM) images of the cellulase treated fabric were prepared to analyze their surfaces. Finally, the characterization of the treated samples was investigated, including moisture content, crease recovery angle, air permeability, and abrasion resistance. The color experiments showed that simultaneous applying of laccase, sodium hydrosulfite, and cellulase had a 55.79% improvement in the samples' lightness (L^*). Furthermore, the color coordinate test of specimens revealed that the hue of the treated samples was changed to blue and green, and the purity of color (C^*) was modified. The increment in the moisture content and air permeability of the treated specimens indicated that the comfort of the jeans clothing had been enhanced. As a result, sodium hydrosulfite demonstrates a high-efficiency denim discoloration in the laccase-mediated system.

Keywords Cellulase · Laccase · Enzyme · Sodium hydrosulfite · Discoloration · Indigo dye

Introduction

Nowadays, jeans are an important part of the fashion industry due to their adaptability to social and cultural changes. The demand for distressed jeans has created a great change in finishing these clothes [1]. Jeans have many fans from all age groups and have a steady growth rate in the global market [2]. The global consumption of denim in 2014 was about 4.500 million meters per year and its demand increases by 5% annually [3]. This type of garment has various applications like pants, uniforms, and casual clothing [4]. Jean fabrics are called Indigo-dyed denim with a strong, thick, and rough surface and its common weave structure is 2/1 twill. Denim fabrics are fabricated from thick cotton yarns; However, many different varieties are available in the market nowadays [5, 6]. One of the main features of this fabric is that the warp yarns are blue while the weft yarns are not [7, 8]. The warp yarn is traditionally dyed with indigo dye, although other vat dyes or sulfur dyes

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are used as well. The vat dyes (including indigo dyes) become soluble via alkaline reduction, penetrate into the fabrics, and then become insoluble through oxidation resulting in the physical trapping of dyes into the fabric. In indigo dyeing process, ring dyeing occurs which means the outer surface of the fibers is intentionally indigo dyed, while the inner core remains white [9, 10].

Discoloration is performed as an important finishing treatment to achieve an aged appearance on denim by abrading or surface treating, in which the dye is removed from the outer surface of the fabric [11–13]. Stone washing is one of the first mechanical finishing treatment used for denim discoloration by applying pumice stones. This method produces the desired aged appearance, but at the same time reduces the resilience and strength of the fabric remarkably and damages the equipment and facilities [14, 15].

Oxidizing agents including sodium hypochlorite, potassium permanganate, nano materials with oxidative activity such as silver nanoparticles, and ozone are commonly used for denim discoloration [14]. Advanced oxidation process such as the combination of oxidizing agents and irradiation has been used in previous research [14]. However, oxidizing agents can cause undesirable adverse chemical effects, i.e., yellowness, tensile strength reduction, and fabric degradation [15, 16].

Discoloration has also been studied using plasma or laser irradiation [6, 14, 17–19]. While they provide good discoloration result and could provide special patterned discoloration, they need expensive technological equipment to perform plasma and laser irradiation for denim discoloration.

Enzymes are environmentally friendly biocatalysts that have been used for discoloration of denim fabrics. On the other hand, enzyme treatment applies only on the surface of the fabrics which cannot cause an extensive degradation of denim fabrics, unlike oxidative materials or mechanical stone washing. Enzyme treatment process also does not require special equipment, which makes them easy to use compared to plasma and laser irradiation techniques [20–25]. Cellulase and laccase are two typical enzymes used for this purpose [10, 26–30]. Cellulase enzymes degrade the surface piles and fibers on the outer layer of the fabric resulting in the discoloration. This process simultaneously improves the hand and flexibility of denim fabric [23, 31–36].

Laccases (benzenediol; oxygen oxidoreductases, EC1.10.3.2) are polyphenol oxidizers with multi-coppers that catalyze the oxidation of a wide range of organic and inorganic substrates by an oxygen as an electron acceptor [37–39]. Therefore, discoloration by laccase enzyme occurs by degradation of indigo dye molecules through oxidation-reduction mechanism (Fig. 1) [20].

It can be said that cellulase produces an aged appearance by abrasion, while laccase produces a lighter shade by degrading the indigo dye molecules and also reduces the back staining caused by the cellulase treatments [20, 40, 41].

However, laccase enzyme could not metabolize nonphenolic parts of the aromatic compound, because of its low redox potentiality. Furthermore, laccase cannot degrade dyes without any other chemicals in high efficiency. Researchers have developed small organic compounds called mediators that have high redox potential and can be oxidized and activated by laccase. To date, several mediators including 2,2'-azine-bis (3-ethylbenzothiazoline-6-sulfonic acid (ABTS)) and 2,2,6,6-tetramethyl-1-piperidinyloxyl (TMPO) have been introduced to degrade organic and nonorganic nonphenolic compounds and dye by laccase. The mediators have been commonly expensive materials which are not easily accessible in some cases [16, 42–44].

In the present study, the sodium hydrosulfite was considered as a possible and easily accessible laccase-mediated system to achieve high-efficiency denim discoloration. The

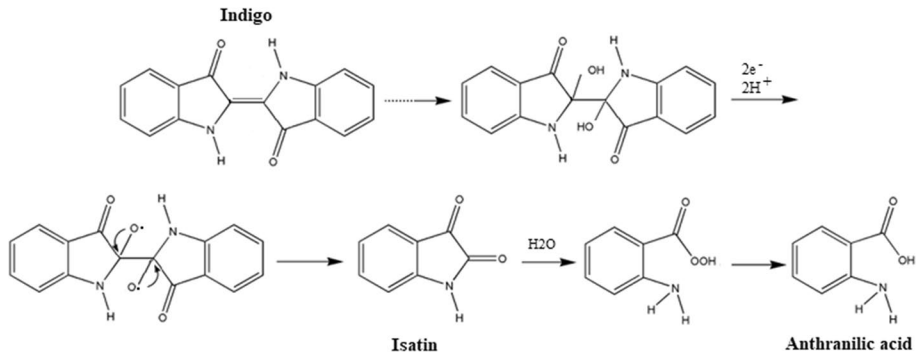


Fig. 1 Degradation of Indigo dye molecule by the usage of laccase enzyme

usage of this compound as a mediator was not reported so far. Also, the discoloration result, color, and fabric characterization were investigated in combination with the cellulase enzyme.

Experimental

Materials

Jean fabrics were woven as a 2×1 twill (surface density=358 g/cm²). Laccase enzyme (Setenzim Eco-L 30), neutral cellulase enzyme (Dsn 205-G), and acrylic thickener (Setaprint RST) were purchased from Setas Company. Sodium hydrosulfite was obtained from AZ Chemicals Inc. Disodium hydrogen phosphate (Na₂HPO₄) and citric acid (C₆H₈O₇) (99%) were bought from Sigma-Aldrich. Also, nonionic detergent was supplied by Saveh Resin Co.

Methods

Discoloration of Denim Fabrics

The laccase buffer solution (pH=5) was prepared according to the previous research [45]. For preparing 20 mL buffer solution, 10.30 mL Na₂HPO₄ (0.2M) was added to 9.70 mL C₆H₈O₇. Also, 12.63 mL Na₂HPO₄ solution (0.2M) was added to 7.37 mL C₆H₈O₇ to prepare the mixture of laccase and cellulase buffer solution (pH=6) [45].

In this research, jeans' fabric was decolorized by washing method (S1). Also, the effect of laccase as well as laccase and cellulase were investigated on the discoloration of jean fabrics via using sodium hydrosulfite as a mediator. For this purpose, jeans' fabrics were placed in baths and then put in a chamber for 1 h. The bath temperatures during and after treatment were 65°C and 50°C for the laccase and the mixture of laccase and cellulase, respectively. Afterward, jean fabrics were placed in boiling deionized water to deactivate enzymes and washed with non-ionic detergent. The compositions of washing solution are given in Table 1 (Sh, sodium hydrosulfite; L, laccase; L Sh, laccase and

Table 1 Washing solutions compositions for samples

Sample code	Sodium hydro-sulfite (g/L)	Laccase (g/L)	Neutral Cellulase (g/L)
Sodium hydrosulfite (Sh)	75	-	-
Laccase (L)	-	20	-
Laccase and sodium hydrosulfite (L Sh)	75	20	-
Laccase and cellulase (L C)	-	10	10
Laccase, cellulase, and sodium hydrosulfite (L C Sh)	75	10	10

sodium hydrosulfite; L C, laccase and cellulase; L C Sh, laccase and cellulase and sodium hydrosulfite).

Characterization

Color measurements were performed utilizing a reflective spectrophotometer (Texflash Data Color, Switzerland). Equation 1 was used to quantify the color difference (ΔE_{ab}^*) in the CIELAB color space of untreated and treated fabric through.

$$\Delta E_{ab}^* = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2} \quad (1)$$

The surface morphology of the specimens was evaluated through using scanning electron microscopy (SEM, EM3200 KYKY, China) and the images were presented with magnification of 2500 \times . For this purpose, the fabrics were first coated with a thin layer of gold using gold sputter coater (SBC-12, KYKY, China).

The moisture content of samples was calculated from Equation 2 according to ASTM D2654-1976.

$$\text{Moisture Content (\%)} = (M_2 - M_1) / M_1 \times 100 \quad (2)$$

while M_2 is the fabrics' weight after presence at the conditioning room and M_1 is the fabrics' weight after complete drying.

Air permeability was performed according to ASTM D737-96 using air permeability tester (SDL-Mo 21S, Shirley, England).

Crease recovery angle apparatus (model of 150, James H. Heal & Co. Ltd, England) was used to evaluate the fabrics' wrinkle resistance according to AATCC 66-2003.

Nu-Martindale abrasion and pilling tester (model of 404, James H. Heal & Co. Ltd, England) was utilized to measure abrasion fastness according to ASTM D 4158-01.

Result and Discussion

Enzyme processing is one of the promising approaches to replace conventional textile treatments. Laccases are oxidoreductase that are able to decolorize industrial dyes. However, some dyes' molecules are too large to enter the enzyme active site. Therefore, laccase mediators have been extended to enhance the process. On the other hand, cellulase

is another sustainable option to denim treatment by acting on the surface of the fabric. In this research, denim samples were treated using laccase, laccase plus mediator, and laccase plus mediator plus cellulase, and the results were investigated.

Appearance and Color Changes of the Treated Samples

The reflective curve indicates the lightness or darkness percentage of the sample surface. As was shown in the reflectance curves (Fig. 2), a remarkable increment was seen in the lightness of samples treated with the mixture of cellulase and laccase and sodium hydrosulfite which was remarkable compared to the lightness of samples treated with laccase [16], immobilized cellulase [36], the mixture of cellulase and laccase [40], and nanomaterials [46] such as nano silver [47] as well as nano clay [22]. Also, samples treated with enzymes and sodium hydrosulfite have provided desired shade due to their ability to discolor the surface of the denim fabric.

To gain a better understanding of the color changes in the treated samples, color coordinates in CIELAB color space were obtained (Table 2). The results presented in Fig. 3 and Table 2 show the improvement in the lightness of the specimens ranging from 0.40 to 27.42. The simultaneous using of laccase and sodium hydrosulfite resulted in a significant synergistic effect increase in the lightness, while the addition of cellulase has slightly enhanced the synergistic effect of the treated specimens. Researchers reported that the significant enhancement in the lightness of samples had been seen via utilization of KMnO_4 [48], advanced oxidation procedures [49], and ozone treatment [50] with the highest intensity resulting in extensive degradation of cellulosic fibers due to their high oxidative function. Also, in the previous studies, the increase in lightness was observed after four times treating [33]; while in the present study, the lightness enhancement was observed after only one treatment which is one of the most remarkable achievements of this research.

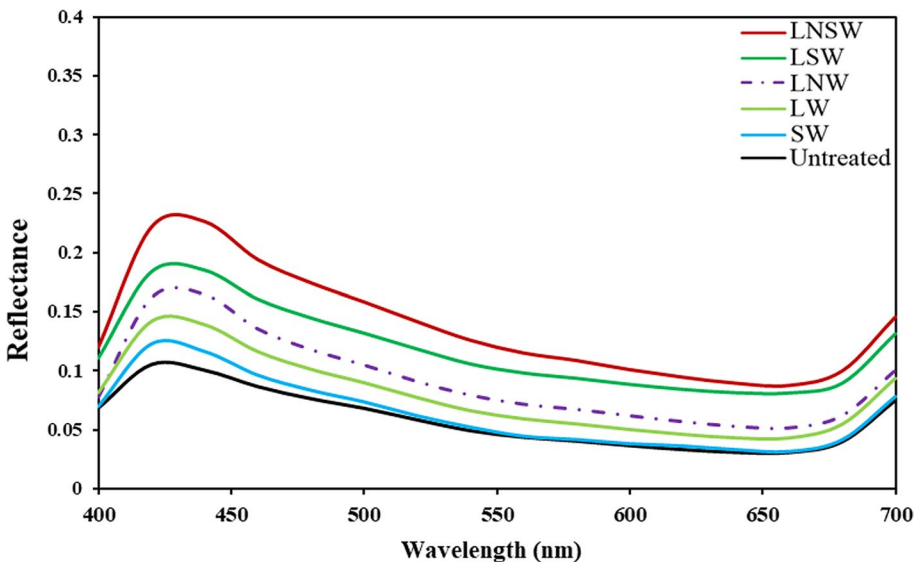


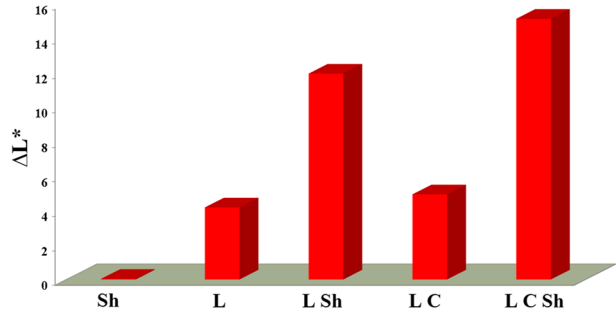
Fig. 2 Surface reflectance curves of the specimens

Table 2 Color indices of the untreated and the treated specimens

	L*	(±)σ	a*	(±)σ	b*	(±)σ	C*	(±)σ	h°	(±)σ	ΔL*	(±)σ	Δa*	(±)σ	Δb*	(±)σ	ΔE	(±)σ
Untreated	27.1	0.51	1.36	0.06	-12.2	0.31	12.28	0.31	-83.64	0.15	0.0	0.51	0.0	0.06	0	0.31	0	0.60
Sh	27.14	0.44	0.48	0.03	-15.72	0.44	15.73	0.44	-88.25	0.07	0.04	0.44	-0.88	0.03	-3.52	0.44	3.6286	0.43
L	31.28	0.33	0.66	0.04	-12.55	0.28	12.57	0.29	-86.99	0.10	4.18	0.33	-0.7	0.04	-0.35	0.28	4.2526	0.34
L Sh	39.04	0.17	0.32	0.03	-18.28	0.35	18.28	0.35	-89	0.09	11.94	0.17	-1.04	0.03	-6.08	0.35	13.4392	0.25
L C	32.04	0.16	-0.7	0.16	-18.38	0.45	17.46	0.45	87.819	0.52	4.94	0.15	-2.06	0.16	-6.18	0.45	8.1755	0.38
L C Sh	42.22	0.43	-0.5	0.09	-18.34	0.49	18.35	0.49	88.45	0.28	15.12	0.43	-1.86	0.09	-6.14	0.49	16.4248	0.50

The addition of the cellulase enzyme for jeans discoloration had no effect in the present study. The indigo dye molecules were released by degradation of the fibers' surface via cellulase enzyme [40]. On the other hand, the laccase enzyme destroyed the indigo dye molecules [51]. It can be assumed that sodium hydrosulfite has a synergistic effect in the

Fig. 3 The lightness improvement of the specimens



presence of the two enzymes (S2).

Fig. 4 shows the improvement of the specimens' lightness. In addition, the discoloration process changed the samples' hue (Table 2) which is shown in the a*b* diagram (Fig. 5). As shown, the transition from redness to greenness was important and the greenness of the treated specimens has been increased. The blueness also improved in all treated specimens. The improvement in blueness appears to have been noticeable in the treated specimens. Finally, color differences (ΔE*_{ab}) in CIELAB color-space were determined to investigate the color changes of the treated specimens compared to the untreated specimens (Fig. 6). The color differences were evident in all specimens (ΔE*_{ab}>3). Moreover, the greatest color differences were associated with the improved lightness and blueness of the treated specimens.

Surface Morphology Evaluation

According to the literature, laccase as an oxidative enzyme affects the dye molecule to treat wastewater from dyeing process while cellulase treatment changes the surface characteristic of the cotton fabric by hydrolysis of cellulose at the end or in the middle of the cellulose chain and yields a shorter polymer chain. Therefore, morphology of cellulase treated and untreated samples was investigated by SEM (Fig. 7). A comparison between Fig. 7a with b and c showed the formation of anchor fibers due to the surface destruction of the fibers [40].

On the other hand, the existence of sodium hydrosulfite reduces the destructive effect of the cellulase enzyme due to its reduction effect [52]. As enzymes are highly

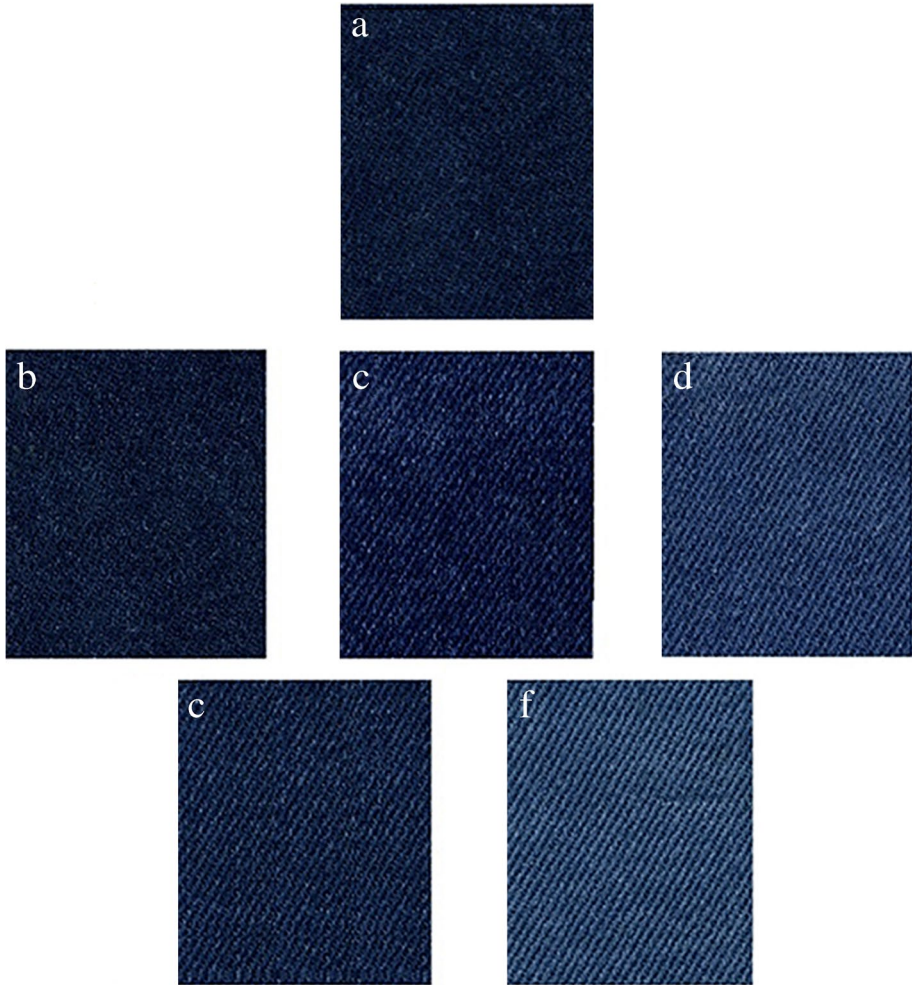


Fig. 4 Optical images of (a) untreated specimen, (b) discolored by sodium hydrosulfite, (c) discolored by laccase, (d) discolored by the combination of laccase and sodium hydrosulfite, (e) discolored by the combination of laccase and cellulase, and (f) discolored by the combination of laccase, cellulase, and sodium hydrosulfite

sensitive to pH, it seems that the reducing effect of sodium hydrosulfite changes the pH of the solution and leads to decreasing the cellulase function. Therefore, the degradation of the specimens treated with two enzymes along with sodium hydrosulfite was lower than the specimens treated with laccase and cellulase enzymes (comparing Fig. 7b and c).

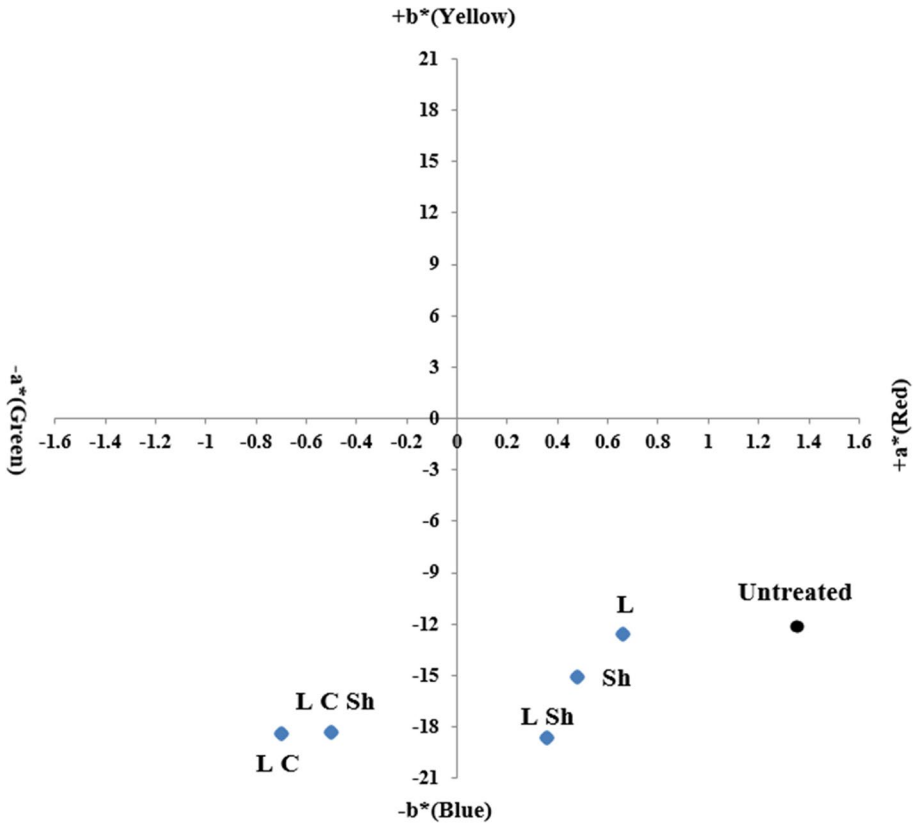
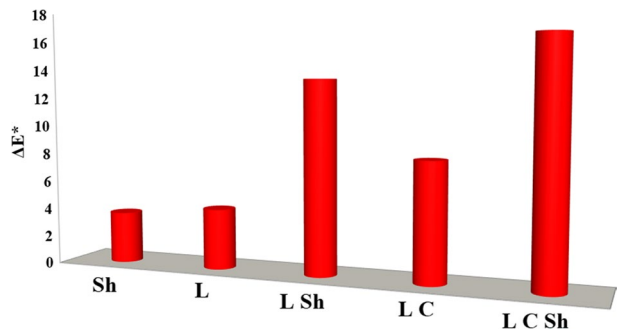


Fig. 5 Color changes of the specimens (redness-greenness and yellowness-blueness)

Fig. 6 Color differences of the treated specimens



Moisture Content Evaluation

As illustrated in Table 3, a significant increase was seen in the moisture content of the samples treated with cellulase individually and the mixture of laccase and cellulase with sodium hydrosulfite due to the formation of hydroxyl groups at both ends of the polymeric chain as a result of the enzymatic hydrolysis. Same results have been reported in previous

Fig. 7 SEM micrographs of (a) untreated specimen, (b) specimen treated using the combination of laccase and cellulase, and (c) specimen treated by the combination of laccase, cellulase, and mediator

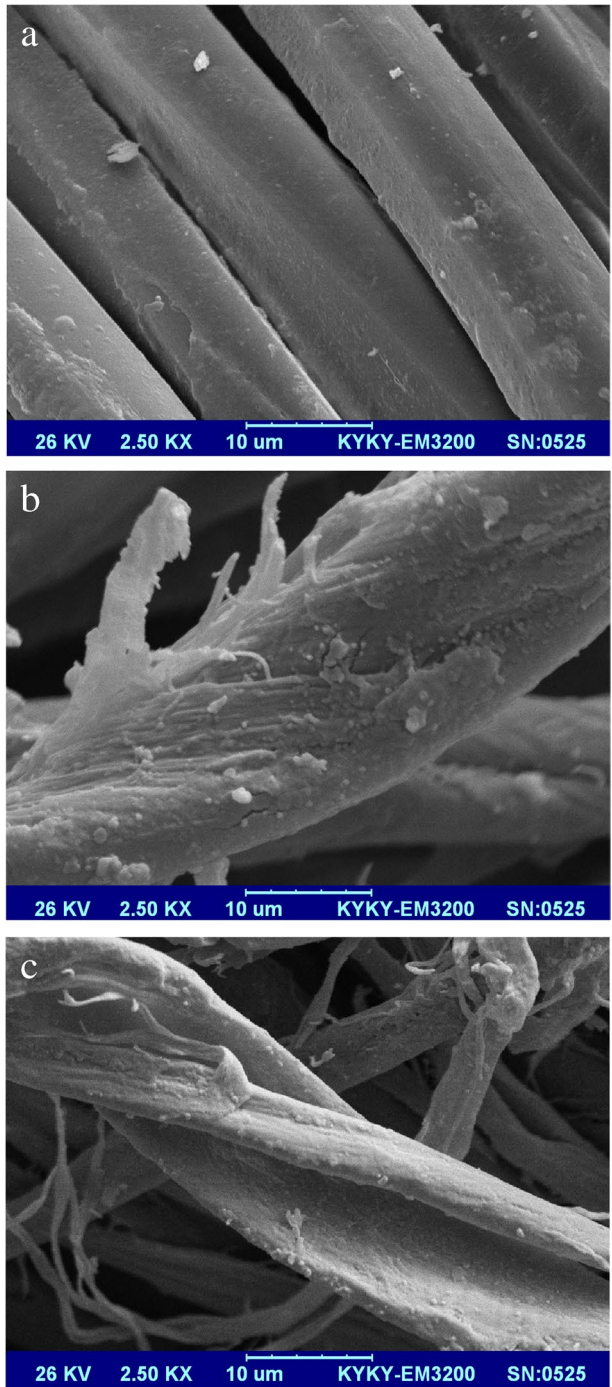


Table 3 Moisture content, crease recovery, and air permeability of untreated and treated specimens

Specimen code	Moisture content (%)	Crease recovery angle (°)	Air permeability (ml/s cm ²)
Untreated	4.14	62	6.39
Sh	4.08	78	6.62
L	4.11	61	6.68
L Sh	4.26	74	6.72
L C	4.54	53	7.62
L C Sh	4.43	55	7.43

studies [34, 35]. Compared to the previous research, performing the advanced oxidation process (AOP) did not result in any significant changes in the water absorption capacity and water retention properties of the denim fabric; hence, an immense destructive process happens [49]. Due to the reducing effect of sodium hydrosulfite, it can partially prevent the oxidation of cellulose [52]. It can be concluded that the combination of sodium hydrosulfite and cellulase enzyme reduces the destructive effect of the enzyme as shown in Fig. 7b and c.

Crease Recovery Angle Evaluation

According to the results presented in Table 3, sodium hydrosulfite increased the wrinkle resistance of the fabric. Specimens treated with cellulase enzyme demonstrated a significant decrease in the crease recovery angle due to the surface degradation of the cellulosic fibers. According to other literature, using other materials such as oxidizing agents leads to decreasing the wrinkling and stiffness of cotton fabrics indicating further destruction of the fiber structure [1, 49].

Also, the higher wrinkle resistance of the specimens treated with two enzymes and sodium hydrosulfite compared to the specimens treated with two enzymes confirmed that sodium hydrosulfite reduces the destructive effect of the cellulase enzyme due to its reduction function. In the previous research, the numerical values of the crease recovery angle increased for cellulase treated, two enzymes treated, laccase treated, and untreated specimens, respectively [53]. The reason for the increase in wrinkle resistance of the specimens treated with the two enzymes compared to the specimens treated with cellulase is that the enzyme has a large molecule and its function is limited to the fabric surface. Therefore, the existence of both enzymes in the same bath led to decreasing available surface for cellulase hydrolysis action.

Air Permeability Evaluation

The air permeability of the specimens treated with laccase and cellulase as well as the mixture of enzymes with sodium hydrosulfite are presented in Table 3. As cellulase can remove the pills of cotton fabrics and changes their mechanical properties, a higher amount of the air permeability was observed in the washed specimens [54]. In the previous study [1], it was shown that the air permeability of denim fabric decreased with time after treating with calcium hypochlorite due to the excessive damage, which leads to the formation of anchor fibers on the fabrics' surface [49]. In the present study, the less decrease

Table 4 Abrasion fastness of the untreated and the treated specimens

Specimen code	Before	After	Weight loss (%)
Untreated	0.4088	0.4054	0.8317
Sh	0.4103	0.4074	0.7068
L	0.4064	0.4035	0.7136
L Sh	0.4117	0.4087	0.7287
L C	0.3984	0.3947	0.9290
L C Sh	0.408	0.4045	0.8580

of the air permeability of the enzyme treated fabrics indicated the less degradation effect of the enzyme (Fig. 7). However, the lower air permeability in the specimens treated with two enzymes might be due to the fewer degradation on the fabric surface (Fig. 7b and c). Therefore, the air permeability and moisture content improve the comfort of the fabric after the discoloration process.

Abrasion Fastness Evaluation

Table 4 shows the results of the abrasion fastness test of the specimens. It can be said that the washing method produced anchor fibers and fuzzy fibers and pills due to the surface degradation caused by the cellulase enzyme [32, 40]. It was reported that the surface area of the fabric was increased by cellulase enzyme through mechanical treatments [54]. The specimens treated with two enzymes and sodium hydrosulfite showed lower abrasion fastness due to the destructive effect of the cellulase enzyme on the cellulose fibers (Fig. 7). Also, the abrasion fastness is higher for samples treated with two enzymes and sodium hydrosulfite compared to the specimens treated with the two enzymes. The reason for the mentioned mechanism is decreasing the destructive effect of the cellulase enzyme with sodium hydrosulfite due to its reduction effect [52].

Conclusion

In the present study, the denim discoloration was performed via separate and simultaneous applying of laccase, sodium hydrosulfite, and cellulase. The investigation of color index indicated that the lightness was improved for the samples treated with laccase and sodium hydrosulfite. Also, the significant enhancement was observed in the blueness and greenness of the treated samples. Furthermore, the increment in moisture content and air permeability represents the improvement in the comfort of the treated samples. Our results demonstrate the potential usage of sodium hydrosulfite as a mediator in laccase-mediated system to achieve high-efficiency denim discoloration.

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1007/s12010-022-04066-5>.

Author Contribution M.S. and F.A. conceptualized the research topic and prepared the research. M.S. did the main part of the experiments in IUT, and was supervised by F.A. All authors analyzed the data and interpreted the results. Also, all authors prepared and corrected the draft.

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Declarations

Ethics Approval Not applicable.

Consent to Participate Not applicable.

Consent for Publication Informed consent was obtained from all individual participants included in the study.

Conflict of Interest The authors declare no competing interests.

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