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 by washing and printing with separate and simultaneous applications of laccase enzyme, sodium hydrosulfite, and cellulase enzyme. In this regard, the surface reflectance and color coordinates of the discolored fabrics were analyzed, and SEM photographs of the treated fabrics were prepared to analyze their surfaces. Finally, the effects of the discoloration process and materials on various parameters of the treated samples were investigated, including moisture content, creaserecovery angle, air permeability, and abrasion resistance. The color experiments showed that the discoloration mechanism with the combined use of laccase enzyme, sodium hydrosulfite, and cellulase enzyme had a significant effect on the improvement of the lightness (L^*) of the samples, as the lightness of the treated samples was improved by 101.18 percent and 55.79 percent in both printing and washing, respectively. Furthermore, examination of specimen color coordinates revealed that the hue of the treated samples was changed to blue and green, and the purity of color (C^*) was improved. The increased moisture content and air permeability of the treated specimens suggested that the comfort of the jeans clothing provided by these treatment methods had improved. As a result, it should be noted that the mediating action of sodium hydrosulfite was significantly influential for discoloration of denim with the laccase enzyme.

Introduction

Nowadays clothing and 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 the field of finishing these clothes [1]. Jeans are one of the garments that have many fans from all age groups, and their use has a steady increase in the global market [2]. In today's time, jeans pants are still loved by the Americans, so according to Cotton Incorporated Lifestyle Monitor (2004), in 2003, fans of this clothing own an average of 8 pairs of pants and 16 pairs of jeans dresses [3]. It should be noted that this type of textile has various uses and can be used to make denim pants, uniforms, and casual clothing [4].

Jeans textiles are called as denim fabrics and often have a strong, thick, and rough surface with the weft thread running under two or more warp yarns [5, 6]. Many denim fabrics are made from thick or high-count cotton yarns. However, many different varieties are available in the market nowadays. The traditional and common texture consists of two upward facing rows and one downward facing row or three upward facing rows and one downwardfacing row [5].

Among the main features of this fabric is that the warp yarns are dyed while the weft yarns are not. Therefore, the texture structure of the fabric is variegated on the outside and white on the reverse side [7, 8].

Traditionally, denim textiles are dyed with various natural blue indigo dyes that create a unique blue color on the fabric [9]. Today, in addition to indigo dyes, the wrap yarn is usually dyed with other vat dyes and Sulfur dyes. Dyeing of denim textiles with these dyes is done by the mechanism that the dye becomes

insoluble in the fibers [10]. In denim textiles, the dye remains on the surface of the fibers due to the ring dyeing method. In fact, during the dyeing process, the dye molecules penetrate to some extent inside the fibers, so that the dye is practically confined to the surface and the inner layers in the fiber body remain colorless [11,12]. To give these garments an aged appearance, they can be abraded or surface treated so that the dye is removed from the outer surfaces of the fabric and the correct appearance is achieved with the desired visual effect [13-15].

Due to the nature of the texture and dyeing of the jeans' textiles, the garments they produce would not be comfortable without finishing treatments. Therefore, some finishing treatments are required to make them more flexible, grippy, and comfortable. This type of finishing of denim garments is one of the most important finishing treatments for jeans, and pumice stones with mechanical treatments were originally used for the purpose, which produced the desired appearance. This method not only noticeably reduced the resilience and strength of the fabric, but also damaged the equipment and facilities [16, 17].

Discoloration has also been studied by plasma irradiation by controlling the operational variables such as gas type, time, and frequency, an aged appearance [18, 19]. The use of laser is another method for jeans discoloration, as the creation of desired patterns and designs on these textiles is very easy and convenient [6, 16, 20]. Advanced Oxidation Process (AOP) systems, which involve the use of the combination of irradiation and active materials such as oxidizing agents, have also attracted the attention of many researchers [21]. Other materials used to discolorize and produce the desired appearance of these clothes are enzymes. The use of enzymes has particular advantages, including the fact that their treatment takes place on the surface of the textile and that they do not produce undesirable features on the textile. The discoloration treatment by the enzymes is very controllable. Cellulase and laccase are typical enzymes used for this purpose [10, 22-26].

It should be noted that each of the physical and chemical methods discussed, remove the molecules of indigo dye (Figure 1) from the surface of the fabrics by different mechanisms[27].

The most serious type of chemical degradation of the dye is achieved by the use of UV/H₂O₂ compounds [21]. When ozone is used as an oxidant, the dye molecule is cleaved to isatin and isatoic anhydride [29]. In discoloration with laccase enzyme, due to its function based on electron exchange, isatin is formed in the middle of the reaction by splitting of indigo dye, and anthranilic acid is finally formed (Figure 2) [28].

Enzymes can be used with current equipments and facilities in this industry without the need for re-tooling, and this is an important point that demonstrates the effectiveness of using enzymes in the industry to discolorize denim [30-35]. Moreover, enzymes are among the materials that are well compatible with the environment, so their use causes fewer environmental problems and leads to higher immunity and safety [36, 37].

By degrading the surface piles, this enzyme produces a fabric with a smoother surface, and by degrading the surface of the fabric, it improves hanging [33, 38-40]. The cellulase enzyme, by breaking the polymer

chain of the cellulose, reduce the tensile strength and firmness of fabric and to some extent it improves flexibility [41-43].

The second enzyme that is typical in the process of jeans discoloration is laccase. The use of laccase enzyme, which is able to degrade indigo dye, can not only increase the efficiency of discoloration but also provide a better effluent at the end of the process [30]. In fact, it can be said that cellulase produces an aged appearance by abrasion, while laccase produces a lighter shade by degrading the molecules of indigo dye and also helps to reduce the back staining caused by the treatments of the cellulose [30, 44, 45]. Laccases (benzenediol: oxygen oxidoreductases, EC1.10.3.2) are polyphenol oxidizers with multi cuppers that catalyze the oxidation of a wide range of organic and inorganic substrates by an oxygen that acts as an electron acceptor [46-48].

Because of its low redox potential, the laccase enzyme can not work on nonphenolic parts of the aromatic compound. To this end, the researchers have developed small organic compounds called mediators that have high redox potential and can be oxidized and activated by laccase. Therefore, they degrade both the surfaces with large molecules and the non-phenolic surfaces. To date, several mediators have been introduced to degrade polymers and synthetic dyes by laccase [49-51]. Mediators are low molecular weight compounds that are easily oxidized by laccase. The products of this reaction are unstable cationic radical compounds that can oxidize more complex substrates before returning to the stable state. An ideal characteristic for a mediator is that it should be a desirable substrate for laccase [40, 52].

In the present study, the cellulase and laccase enzymes are used in both processes of washing and printing to decolorize denim. The effect of sodium hydrosulphite as a mediator on the discoloration efficiency of denim dyed with indigo was investigated in both methods.

Experimental

Materials

Jeans fabrics were tufted by twill with 2/1 weave construction, warp yarn count 603 Denier (25cm^{-1}), weft yarn count 612 Denier (17cm^{-1}) and with 358 g/cm^2 weight. The used materials included Laccase enzyme (Setenzim Eco-L 30), from Setas Company and has maximum activity at 65 °C and pH=5.0, Neutral cellulase enzyme (Dsn 205-G), from Setas Company and has maximum activity at 35 °C and pH=7.0, Sodium hydrosulphite from AZ Chemicals Company, Acrylic thickener (Setaprint RST) from Setas Company, di-sodium hydrogen phosphate from Sigma-Aldrich Company, citric acid (99%) from Sigma-Aldrich Company, and finally nonionic detergent from Diadavin UN from Resin Saveh.

A buffer solution (pH=5) was made for laccase according to suggested method by T. C. McIlvaine [53]:

To produce 20ml buffer solution (pH=5), 10.30ml di-sodium hydrogen phosphate solution (0.2M) was completely mixed with 9.70ml citric acid (0.1M).

A buffer solution (pH=6) was made for mixture of laccase and cellulase according to the suggested method by T. C. McIlvaine [53]:

To produce 20ml buffer solution with pH=6, 12.63 ml di-sodium hydrogen phosphate solution (0.2M) was completely mixed with 7.37 ml citric acid (0.1M).

Methods

Jean's fabrics were decolorized with two methods included discharge printing and washing. At discharge printing method the effect of laccase enzyme (L) and mixture of laccase and neutral cellulase (NC) on discoloration of jeans fabrics were studied by the use of sodium hydrosulphite (SH) as mediator (s). Printing pastes compositions can be seen in Table 1.

By changing pH and treatment temperature, no significant difference was seen in the sample with SH discharge printed codes. In the present study, jeans fabrics were discharged printed, and then, the samples were put at a chamber for 12 hours with a constant temperature which was 65°C for laccase enzyme and 50°C for the mixture of laccase and neutral cellulase. Finally treated jeans fabrics were put in boiling deionized water to deactivate enzymes and washed with non-ionic detergent.

At washing method, the effect of laccase enzyme and mixture of laccase and neutral cellulase were studied by the use of sodium hydrosulphite and/or silver nitrate as mediator(s). Washing solution compositions are seen in Table 2.

By changing pH and treatment temperature, no significant difference was seen in the sample with SH washed codes. In the present study, jeans fabrics were washed in a bath, and then, the samples were put at a chamber for 1 hour and the duration of treatment bath was stirred with a magnet stirrer. The bath temperatures were constant which was 65 °C for laccase enzyme and 50 °C for the mixture of laccase and neutral cellulase. Finally, treated jeans fabrics were put in boiling deionized water to deactivate enzymes and washed with non-ionic detergent. Schematic diagram of the used methods to discoloration of jeans fabrics is shown in Figure 3.

Characterization

Color shift measurements were performed using a reflective spectrophotometer from Texflash Data Color for Switzerland (technical characterizations: Geometry: 0/d, diaphragm: 18 mm). Equation 1 was used to quantify the color changes in the CIELAB color space between untreated and treated fabrics.

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

The ΔL^* , Δa^* and Δb^* are parameters to show the difference between treated and untreated fabrics in Equation 2.

$$\Delta L^* = L_{treated}^* - L_{untreated}^* \quad \text{Equation 2}$$

$$\Delta a^* = a_{treated}^* - a_{untreated}^*$$

$$\Delta b^* = b_{treated}^* - b_{untreated}^*$$

The surface morphology of the specimens was evaluated through the use of scanning electron microscopy (EM3200 KYKY, China). The magnification was selected at 2500x for all of the specimens. Gold sputter coater (SBC-12, KYKY, China) was used to coat a layer of gold before the SEM experiments.

Due to the lack of interaction after the discoloration treatment between the cellulosic fibers and sodium hydrosulfide as well as laccase enzyme, there would be no change in the surface of the fibers with these materials. Consequently, just the SEM images of the samples that include cellulase enzyme chemical compound are provided.

Moisture content was performed according to ASTM D2654-1976 method. First, the fabrics were prepared according to ASTM D1776-04 at the conditioning room to provide 25 and 65% RH for 24 h and then the fabrics were weighted. The samples were put at the oven (Dry air Sterilizer FN120 for Turkey) and were weighted every 2 hours (the used balance was TE124S for Sartorius Company from Germany) until the weight had been approximately unchanged. Finally, moisture content was calculated from Equation 3.

$$\text{Moisture Content (\%)} = (M_2 - M_1) / M_1 \times 100 \quad \text{Equation 3}$$

While M_2 is the fabrics' weight after presence at the conditioning room and M_1 is the fabrics weight after existence of the conditioning room.

Crease recovery is one of the most important parameters to evaluate the fabrics' comfort. AATCC 66-2003 method and Crease Recovery Angle device (model Tester model 150) from James H. Heal & Co. Ltd (British company) were used to measure crease recovery angle. As presented in the used method, sample dimensions were $4 \times 1.5 \text{ cm}^2$ and loading on the folded sample was 500 g and remaining time of the load on the sample was 5 minutes.

Air permeability was performed according to ASTM D737-96 method by the use of SDL-Mo 21S instrument from Shirley British Company. The permeability of the air was defined as the transformed air

volume (ml/s) from 1cm² fabric under 120 Pa. Permeability was achieved by dividing air volume / surface area air transition (0.0785cm²).

Abrasion fastness was performed according to ASTM D 4158–01 test method by the use of Nu-Martindale Abrasion and Pilling Tester instrument model 404 from James H. Heal & Co. Ltd (England). The sample was round with 38 mm diameter which was put into a holder. Load was 9kPa and RPM was 60 (total round was 3500). After testing the samples were weighted and the differences in (%w/w) were reported as abrasion fastness.

Result And Discussion

Appearance and color changings of the treated specimens

Reflectance curves were plotted to investigate the reflectance properties of the specimens. The involvement of the laccase enzyme in the discoloration process leads to a significant improvement in the reflectance of the specimens, as shown in Figure 4 (Reflectance curves for printed specimens). This phenomenon was enhanced for the specimens treated with both enzymes and sodium hydrosulfite. This result shows that the use of laccase enzymes and sodium hydrosulfite in combination with cellulase enzymes has a synergistic effect on the reflectance of the specimens. The washed specimens gave the same result (Figure 5). Comparing Figure 4 and Figure 5, it is clear that the printing method gives better results in terms of reflectance of the samples.

To gain a better understanding of the color changes of the treated specimens, color coordinates in CIELAB color space were obtained (Table 3). The results presented in Figure 6 and Table 3 show that the improvement in the lightness of the specimens ranged from 0.40 to 27.42. As shown in Figure 6, the lightness of the specimen treated with laccase enzyme was greater than that of the specimen treated with sodium hydrosulfite for both methods. The simultaneous use of laccase enzyme and sodium hydrosulfite resulted in a significant synergistic increase in the lightness of the specimens treated by both methods. The addition of cellulase enzyme enhanced the previously described synergistic effect, as shown in Table 3.

It can be concluded that the combination of laccase enzyme and sodium hydrosulfite is successful in discoloration, and the addition of cellulase enzyme increases the lightness (about 1 for the washed specimen and about 3 for the printed specimen). It should be noted that in previous experiments, the application of cellulase enzyme to denim without other chemicals resulted in a decrease in lightness [54]. The addition of the cellulase enzyme to the chemicals used to discolor the jeans had no effect in the present study. It is believed that the mechanism of action of the chemicals is the probable cause of the synergistic effect of the combination of the two enzymes and sodium hydrosulfite. The indigo dye molecules were released by surface degradation of cellulose fibers by the cellulase enzyme. The laccase enzyme, on the other hand, destroyed the indigo dye molecules, and sodium hydrosulfite reduced the

soluble dye molecules. It can be assumed that sodium hydrosulfite has a synergistic effect in the presence of the two enzymes (Figure 7).

As mentioned earlier, the printing process results in better discoloration performance, which could be due to the printing process. The chemicals act on the outer surface of the fabrics in this process, and it is likely that the printing process has a higher quality of enzyme reaction than the washing process.

In this study, the increase in lightness is achieved by preparing the specimens once. However, in previous studies, the increase in lightness was 21 after treating specimens four times [40]. Therefore, this is one of the most remarkable achievements of the research.

Figure 8 shows optical images of the specimens. The accuracy of the above improvement in lightness is evident in this figure. In addition, the discoloration process changed the hues of the specimens (Table 3), as shown in the a^*b^* diagram (Figure 9).

As shown, the transition from redness to greenness was important, and the greenness of the treated specimens has increased. The blueness also improved in all treated specimens. In fact, the improvement in blueness appears to have been noticeable in the treated specimens (Figure 8). Finally, color differences (ΔE^*) in CIELAB color-space were determined to investigate the color changes of the treated specimens compared to the untreated specimens (Figure 10).

As can be seen from Figure 10, the color differences were greater than three ($\Delta E^* > 3$) in all specimens, which means that the color differences were evident in all specimens. The printed specimen with two enzymes and sodium hydrosulfite has the largest color difference. It should be noted that the aforementioned specimen showed the greatest improvement in terms of lightness. Moreover, the greatest influence on the color differences was associated with the improved lightness and then with the blueness of the treated specimens. As already mentioned, the printed specimens gave better results. It may be mentioned that in the printing process, a form of immobilization occurred, and therefore the action of the enzymes was much better than in the washing method. Since enzyme treatment is usually limited to the surface due to the size of the molecules, the printing method provided better conditions for enzymatic treatment of the specimens, resulting in highly efficient discoloration.

Surface Morphology Evaluation

The SEM images of the untreated and treated specimens are shown in Figure 11. Since there was no chemical degradation by laccase enzyme and sodium hydrosulfite on the surface of cellulose fibers during the process, the images of SEM were made only from the specimens that had been decolorized with the cellulase enzyme. As can be seen, the application of the cellulase enzyme to the cellulose fibers of the denim obviously results in degradation. It can be concluded that the anchor fibers were formed due to the surface destruction of the fibers by the cellulase enzyme [44]. The surface destruction of the cellulose fibers resulted in a kind of fibrillation on the fiber surface, which can be clearly seen in the images of the specimens treated by both printing and washing methods. It appears that the degradation

is more severe in the specimens treated with cellulase enzyme in the printing method than in the washing method, which can be seen when comparing Figure 11-b and Figure 11-d with Figure 11-c and Figure 11-e. It should be noted that some of the light spots on the longitudinal surface of the fibers in the printed specimens (Figure 11-b and Figure 11-d) may be the thickening agent remaining after the final treatment. Enzymes are large molecules whose treatment is mainly limited to the surface, and in the printing method, due to the placement of the chemicals in the presence of the printing paste, better conditions are provided to achieve higher efficiency in treating the surface of the specimens. It can be said that a kind of immobilization takes place in the printing process. Previous researches have shown that immobilization of enzymes, including their mutual presence, increases their efficiency [27, 55, 56]. When comparing the degree of degradation obtained by the two methods, it should be noted that according to the studies of Montazer and Sadighi, the presence of sodium hydrosulfite reduces oxidation by cellulase enzyme due to its reducing effect [57]. Therefore, as expected, the degradation of the specimens treated with a combination of the two enzymes with sodium hydrosulfite was lower than the specimens treated with a combination of laccase and cellulase enzymes. This is evident when comparing Figure 11-b and Figure 11-c with Figure 11-d and Figure 11-e.

Moisture Content Evaluation

In order to investigate the effects of material type and the method of discoloration on the moisture content of the denim fabrics, their moisture content was determined and compared with that of the untreated specimen. As Table 4 shows, due to the lack of chemical interaction of cellulosic fibers with sodium hydrosulfite and laccase enzyme, there are no significant changes in the moisture content of the specimens treated either individually or in combination with these materials. There was a significant increase in moisture content of the samples treated with the combination of the two enzymes and the two enzymes with sodium hydrosulfite. This is due to the degradation of cellulose fibers by the cellulase enzyme. It can be said that hydroxyl groups were formed at both ends of the chain due to the destruction of the polymer chain of the fibers. Hydroxyl groups are hydrophilic functional groups and can help in moisture absorption, increasing the moisture content. The increased moisture absorption due to cellulase treatment of cellulose fabric has been observed in previous studies [41, 42]. Compared to a study by Ebrahimi et al., the present research revealed that the application of advanced oxidation process (AOP) did not result in any significant change in the water absorption capacity and water retention properties of the denim fabric [21]. The specimens treated with the combination of cellulase-laccase enzymes and also the combination of the two enzymes with sodium hydrosulfite by washing method shows a slight increase in moisture content as compared to the printing method as compared to the untreated specimen. This may be due to the nature of the method and it can be said that when the samples are treated by the printing method, the enzymes reach the surface of the fabrics more easily and the surface of the fibers is degraded more by the cellulase enzyme. The specimens treated by both printing and washing methods with the combination of laccase-cellulase enzymes and also with the combination of the two enzymes with sodium hydrosulfite in both printing and washing methods, it is also observed that the specimens treated with the combination of enzymes without sodium hydrosulfite absorb more moisture than the other similar specimens. Due to its reducing effect, sodium hydrosulfite can prevent the oxidation of

cellulose, which was also mentioned in the study conducted by Montazer and Siddiqui [57]. Therefore, it can be concluded that the combination of sodium hydrosulfite and cellulase enzyme reduces the destructive effect of the enzyme as shown in Figure 13-d and Figure 13-e.

Crease Recovery Angle Evaluation

To investigate the effects of the materials and treatments on the wrinkling of the treated fabrics, the crease recovery angle of the specimens was analyzed and the results can be seen in Table 4. According to the results, sodium hydrosulfite increased the wrinkle resistance of the fabric. This problem was observed more in the printed specimens than in the washed specimens. No significant change in this parameter was observed in the Laccase-treated specimens, which is due to the fact that there is no interaction with cellulose fibers. This problem was also found in the research carried out by Maryan and Montazer [44]. Specimens treated with cellulase enzymes were found to have a significant decrease in the crease recovery angle, which is due to the surface degradation of the cellulosic fibers, which in return reduces the resistance of the fabric to external forces to compress the fabric. In other methods, such as using oxidizing agents by increasing the treatment variables such as concentration, time, and other parameters, the wrinkling and bending stiffness of the fabrics continuously decreased, indicating further destruction of the fiber structure [21, 58]. The wrinkle recovery of the printed specimens (reduced by 14 and 20 degrees for the specimens printed using the two enzymes and sodium hydrosulfite and the specimens printed with laccase enzyme and sodium hydrosulfite, respectively) was greater than that of the washed specimens (reduced by 7 and 9 degrees in the crease recovery angle for the washed specimens using the two enzymes and sodium hydrosulfite and washed with laccase enzyme and sodium hydrosulfite, respectively), which can be attributed to the greater degradation of the fibers due to the nature of the method.

This result can be clearly seen in the SEM images in Figure 11. More fibrillation occurred in the specimens treated by the printing method. Also, the higher wrinkle resistance of the specimens treated with two enzymes and sodium hydrosulfite compared to the specimens treated with two enzymes confirms the fact that sodium hydrosulfite reduces the destructive effect of the cellulase enzyme due to its reducing function. In a study by Montazer and Marian in which cellulase and laccase enzymes were used to decolorize indigo-dyed denim, the numerical values of the crease recovery angle increased for cellulase treated, two enzymes treated, laccase treated, and untreated specimens, respectively. 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 surface area, so with the addition of laccase, the available surface area of the cellulase enzyme was reduced and the degradation carried out by the enzyme decreased accordingly [59].

Air Permeability Evaluation

As shown in Table 4, in the specimens treated with the two enzymes as well as the two enzymes with sodium hydrosulfite, the air permeability was increased by 16 and 14 percent in the printed specimens and 19 to 16 percent in the washed specimens. It can be said that the pills can be removed by the action

of cellulase enzyme on cellulose fabrics, therefore the air permeability is higher in the specimens treated by the washing method. It also seems that the reason for the higher air permeability of the washed specimens is due to the mechanical treatment during the process of discoloration as well as the removal of more fibers from the fabric surface [37], which may contribute to the improvement and completion of the cellulase enzyme function. In a study conducted by Nasrin Hagh et. al, after treating the denim fabric with calcium hypochlorite, it was observed that the air permeability decreased with time[58]. The probable reason for this is the excessive damage, which led to the formation of too many anchor fibers on the surface of the fabric. Also, when a combination of ultraviolet irradiation and oxidizing agents was used with increasing treatment time, the air permeability remained constant, which may be due to relative destruction [21]. No decrease in air permeability in the present study indicates controlled degradation (Figure 11). In conclusion, the lower air permeability in the specimens treated with the two enzymes without sodium hydrosulfite may be due to the greater degradation at fabric surface (Figure 11-b and Figure 11-d). Therefore, the air permeability and moisture content improve the wearing comfort of the garment made from the fabric, both of which improved after the discoloration process in this study.

Abrasion Fastness Evaluation

Table 5 shows the results of the abrasion fastness test results of the specimens. Firstly, it can be seen that for the printed specimens treated with sodium hydrosulphite and / or the laccase enzyme, compared to the same washed specimens, the washed specimens have a higher abrasion fastness. It can be said that the washing method produced anchor fibers and fuzzy fibers and pills [39] due to surface degradation by the cellulase enzyme [44]. They were removed from the fabric surface by the mechanical treatment, while this was not the case in the printing method. The fact that the mechanical treatments increase the areas on the fabric surface available for destructive treatment by the cellulase enzyme was mentioned in the studies of Kahn et al. [37]. Moreover, the specimens treated with two enzymes and sodium hydrosulfite showed lower abrasion fastness with both methods. The reason may be the destructive effect of the cellulase enzyme on the cellulose fibers, but this destructive effect is higher in the printing method and resulted in lower fastness compared to the washing method (Figure 11). It can be said that due to the limited effect of the enzyme on the fabric surface in the printing method, this opportunity was given to the cellulase enzyme as much as possible. Also, the abrasion fastness is higher for samples treated with two enzymes and sodium hydrosulfite compared to the specimens treated with the two enzymes. It can be said that the reason for this mechanism is the decrease of the destructive effect of the cellulase enzyme with sodium hydrosulfite by preventing the oxidation of cellulose by the enzyme [57].

Conclusion

Researchers have proposed several physical and chemical methods for decolorizing denim dyed with indigo. One of these methods is the use of enzymes, which is a desirable method in this field. This is because enzymes have limited surface treatment and high controllability. Laccase and cellulase are two enzymes used for this purpose. The laccase enzyme breaks down the dye molecules, and the cellulose

enzyme decolorizes the fabric by degrading the fabric surface and releasing the dye molecules. Researchers have developed a group of chemical compounds called mediators to increase the efficiency of discolorization using the laccase enzyme. In the present study, in addition to the cellulase enzyme, sodium hydrosulfite (used as a reducing agent in the dyeing process of this fabric) was used to increase the efficiency of discoloration of indigo dyed denim fabric using laccase enzyme in both printing and washing processes and significant results were obtained. The increase in lightness by 24.56 units in the specimens treated by the printing method including the combination of laccase enzyme and sodium hydrosulfite, and the increase in the lightness by 27.42 units in the specimens treated by the printing method containing the combination of cellulase and laccase enzyme with sodium hydrosulfite is an outstanding result compared to previous studies. A significant increase in lightness was obtained by using oxidizing agents such as potassium permanganate or by a high number of treatments. It should be noted that oxidizing agents have a high destructive effect on the cellulose fabrics. The significant increase in the blueness of the treated specimens and the improvement in the greenness show the better color properties after the discoloration process. Also, the increase in moisture content and air permeability indicates the improvement in comfort of the treated specimens, which is very important for the garment industry. In conclusion, sodium hydrosulfite can be used as an effective and powerful mediator to increase the efficiency of decolorization via laccase enzyme and decolorize denim fabrics dyed with indigo.

Declarations

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Availability of data and material: 'Not applicable'

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Authors' contributions: 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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Tables

Table 1 *Printing pastes compositions for discharge printed samples*

Sample code	SH	L	NC	Buffer solution	Acrylic thickener	Balance
	(g/kg printing paste)					
SH discharge printed (SDP)	75	-	-	600	175	X
L discharge printed (LDP)	-	20	-	600	175	X
L and SH discharge printed (LSDP)	75	20	-	600	175	X
L and NC discharge printed (LNDP)	-	10	10	600	175	X
L, NC and SH discharge printed (LNSDP)	75	10	10	600	175	X

Table 2 *Washing solutions compositions for washed samples*

Sample code	SH (g/l)	L (g/l)	NC (g/l)
SH washed (SW)	75	-	-
L washed (LW)	-	20	-
L and SH washed (LSW)	75	20	-
L and NC washed (LNW)	-	10	10
L, NC and SH washed (LNSW)	75	10	10

Table 3 *Colour indices of the untreated and the treated specimens*

	L*	a*	b*	C*	h°	ΔL^*	Δa^*	Δb^*	ΔE
Untreated	27.1	1.36	-12.2	12.28	-83.64	0.0	0.0	0	0
SDP	29.53	0.094	-16.85	15.46	-89.68	2.43	-1.27	-4.65	5.3982
LDP	34.45	-0.92	-17.44	18.34	86.98	7.35	-2.28	-5.24	9.3101
LSDP	51.66	-0.39	-14.06	14.07	88.411	24.56	-1.75	-1.86	24.6924
LNDP	35.47	-0.84	-14.78	14.8	86.747	8.37	-2.2	-2.58	9.031
LNSDP	54.52	0.14	-12.8	12.8	-89.37	27.42	-1.5	-0.6	27.4676
SW	27.14	0.48	-15.72	15.73	-88.25	0.04	-0.88	-3.52	3.6286
LW	31.28	0.66	-12.55	12.57	-86.99	4.18	-0.7	-0.35	4.2526
LSW	39.04	0.32	-18.28	18.28	-89	11.94	-1.04	-6.08	13.4392
LNW	32.04	-0.7	-18.38	17.46	87.819	4.94	-2.06	-6.18	8.1755
LNSW	42.22	-0.5	-18.34	18.35	88.45	15.12	-1.86	-6.14	16.4248

Table 4 *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
SDP	4.1	85	6.42
LDP	4.28	60	6.43
LSDP	4.33	80	6.45
LNDP	4.79	42	7.40
LNSDP	4.74	48	7.29
SW	4.08	78	6.62
LW	4.11	61	6.68
LSW	4.26	74	6.72
LNW	4.54	53	7.62
LNSW	4.43	55	7.43

Table 5 *Abrasion fastness of the untreated and the treated specimens*

Specimen code	Before	After	Weight Loss (%)
Untreated	0.4088	0.4054	0.8317
SDP	0.4178	0.4143	0.8380
LDP	0.4127	0.4092	0.8481
LSDP	0.4132	0.4097	0.8470
LNDP	0.4057	0.4012	1.1091
LNSDP	0.4028	0.3987	1.0180
SW	0.4103	0.4074	0.7068
LW	0.4064	0.4035	0.7136
LSW	0.4117	0.4087	0.7287
LNW	0.3984	0.3947	0.9290
LNSW	0.408	0.4045	0.8580

Figures

Figure 1

The Chemical Structure of Indigo Dye [28]

Figure 2

Indigo dye degradation mechanism using laccase enzyme [28]

Printing method

Washing method

Figure 3

Schematic diagram of the used methods to discoloration of jeans fabrics: (printing method-upper): a) printing paste preparation, b) printing paste, c) placing the printed fabric in the insulated compartment, d) discoloration process, e) enzyme deactivation, f) washing. (washing method-lower): a) discoloration bath preparation, b) discoloration process, c) enzyme deactivation, d) washing.

Figure 4

Reflectance curves of the untreated and the printed specimens

Figure 5

Reflectance curves of the untreated and the washed specimens

Figure 6

ΔL* for Untreated and treated specimens

Figure 7

Schematic diagram of possible decolorization process mechanism: a) cellulose indigo dyed degradation fiber with NC and release of indigo dye molecules (1) – degradation of released indigo dye molecules with L (2), b) reduction of indigo dye molecules with SH (1) – degradation of reduced indigo dye molecules with L (2), c) combination of cellulose fiber indigo dyed degradation with NC and reduction of indigo dye molecules with SH (1) – mix degradation of released indigo dye molecules and reduced indigo dye molecules with L (2)

Figure 8

Images of the untreated and the treated specimens: a) Untreated, b) SDP, c) LDP, d) LSDP, e) LNDP, f) LNSDP, g) SW, h) LW, i) LSW, j) LNW, k) LNSW.

Figure 9

Block diagram of the untreated and the treated specimens

Figure 10

ΔE^*_{ab} diagram of the untreated and the treated specimens

Figure 11

SEM micrographs: a) Untreated specimen, b) LNDP, c) LNW, d) LNSDP, e) LNSW