Application of Laccases with Cellulases on Denim for Clean Effluent and Repeatable Biowashing

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ABSTRACT: In this study, we try to use laccases with cellulases to investigate their effects on the garments and possible reusing of biowashing effluent to save water, enzyme, and energy. To achieve these goals, denim garments treated with different percentages of laccases and cellulases and the remaining baths (effluent) used three times with three different methods. Color indices of fabric samples were measured by colorimeter on the front and back of garment and on the white pocket. The effluents in the remaining baths were also monitored by spectrophotometer after each processing. The XRD spectrums were used to calculate the crystalline degrees of the chosen samples. The fiber surfaces of some treated samples were observed by SEM. The results revealed that using optimum concentration of laccases and cellulases produces the same biowashing effect on the garment after three-repeated biowashing. By using this method, laccases helps to discolor the effluent and enzyme residuals of both cellulases and laccases are useful for repeated processing. Overall, this method is recommending a biowashing process with considerable reduce in consumption of laccases, cellulases, water, time, and energy. © 2008 Wiley Periodicals, Inc. J Appl Polym Sci 110: 3121–3129, 2008

Key words: denim; stone washing; effluent; reusing; laccases; cellulases; back-staining; biowashing

INTRODUCTION

Dyeing effluent is the main source of water pollution by textile industries and some limitations for effluent discharge were currently performed by environmental regulations. Many researchers worked on decolorization of dyestuff in effluent by various physical, chemical, and biological methods. The effluent of stone washing usually contains indigo dyes, which removed from the garment surface and colors the effluent.[1]

In stone washing with cellulases, one of the major factors causing high indigo back-staining on denim fabrics (re-deposition of indigo on white weft yarns) is the high ability of cellulases protein to bind cellulose and binding to indigo. The structures and properties of indigo molecule are caused nonpolar counteract between the indigo and cellulases molecules. Indigo dyes' molecules contain aromatic rings involved in hydrophobic interactions and the heterocycle ring contain —NH and =O groups. This may form hydrogen bonds with other molecules (water molecules).[2–4]

During denim stone washing, the indigo removed from garments and adds into the effluent. This causes significant problems to the environment, water, and human. Indigo cannot be easily degraded or removed with physical and chemical processes. In one method, effluent can be partially discolored by treatment with chlorites and potassium permanganate. However, this causes a substantial environmental pollution and damages to the human health. Indigo is known as environmentally harmful chemical because of toxic aromatic amines groups formation in wastewater.

The ability of microorganisms to discolor dyes has received many attentions. Microbial depolarization and degradation of dyes was seen as a cost-effective method to remove these pollutants from the environment, and may open up possibilities for reusing dyeing effluent and reducing water consumption.[5]

Dyes can be eliminated by a wide variety of aerobic or anaerobic organisms that are preferably employed as mixed cultures because of their relative robustness and versatility against xenobiotic compounds. Recently, a combination of an anaerobic/aerobic pilot plant for the treatment of colored textile effluents was described.[6]

The crucial step in dye degradation is the cleavage of the chromophore rendering dye fragments more susceptible to biodegradation by less specialized organisms. The pretreated effluent can be treated in common municipal wastewater treatment plants or the decolorized effluents can be reused in textile

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processing. Oxidative enzymes involved in the lignolytic systems of white-rot fungi are the most important classes of enzymes suited for enzymatic dye degradation. Besides, peroxidases, laccases seem to be the most promising enzymes.[6]

Laccases (benzenediol: oxygen oxidoreductase, EC 1.10.3.2) are copper-containing oxidases that accept a wide range of aromatic alcohols and amines as substrates. They are not dependant on any cofactors. Only molecular oxygen (air) is needed as a cosubstrate. They catalyze the removal of a hydrogen atom from the hydroxyl group of ortho- and para-substituted mono- and polyphenolic substrates and from aromatic amines by one electron abstraction. This form free radicals capable of undergoing further depolymerization, repolymerization, demethylation, or quinone formation. This process is industrially used to achieve the stone washed effect of indigo dyed denim fabric by means of milder enzymatic decolorization.[4–6] Laccases have been reported to bleach indigo fabrics and laccases-based systems are able to degrade indigo both in solution and on denim, leading to various bleaching effects on the fabric. Another laccases application is the oxidative transformation or polymerization of dye precursors to improve dyeing efficiency.[6–9]

The biodegradability of indigo and its industrial effluents by fungi have been studied with *Phellinus gilvus*, Phone Rochaete Chrysporiwn, *Pvcnoporus sanguineus*, and *Pleurotus sajor-caju*. Tests of immediate biodegradability have proved that indigo can be classified easily as biodegradable by fungi, and toxicity is greatly reduced.[9,10] The indigo degradation product is isatin (indole-2, 3-dione), which further decomposes to anthranilic acid (2-aminohenzoic acid). Oxidation of substrates by laccases was believed to involve the reduction of molecular oxygen. The kinetics of indigo degradation with laccases shows that laccases from *Polyporus sp*. are more effective than others. This is due to the faster conversion of indigo into isatin, which further decomposes to anthranilic acid as a final reaction product. Laccases activity in fungal cultures can be increased by the addition of different aromatic compounds, such as gallic acid, ferulic acid, xylidine, guaiacol, syringaldazine, and veratryl alcohol, which have been widely used to stimulate laccases production.[8,11,12]

In this work, it was tried to optimize washing conditions to make possible reusing of washing bath contains water, cellulases, and laccases. This helps to reduce water, energy, and enzymes consumptions. To achieve these goals, different mixtures of cellulases and laccases are examined for biowashing. The effluent of stone washing was also reused for three times by adding a portion of cellulases and laccases.

MATERIALS AND METHODS

Material

Denim fabric (Blue jean) used, was 100% cotton, twill 2/1 weave construction, weft and warp yarns count of 15 Nm, weft density of 20 per cm, warp density of 26 per cm, and fabric weight of 265 g/m^2. To prepare garment samples, the fabric with 30 $\times$ 50 cm^2 were selected and sewed in the leg form with two pockets one on face (jean material) and other on back side (white cotton material). The backside pocket was plain weave white cotton fabric with 30 Nm open-end spinning yarns on weft and warp, weft density of 24 per cm, warp density of 30 per cm, and weight of 106 g/m^2 (Fig. 1).

Auxiliaries used were as follows: industrial acetic acid 70%, dispersing agent (Verlane N60) to prevent back-staining composed of polyacrylates and alkyl phosphonate with anionic structure from Rudolf Chemie. Co., anticreasing (Rucolin JES) composed of polyacrylamide with nonionic structure from Rudolf Chemie. Co (Tehran, Iran).

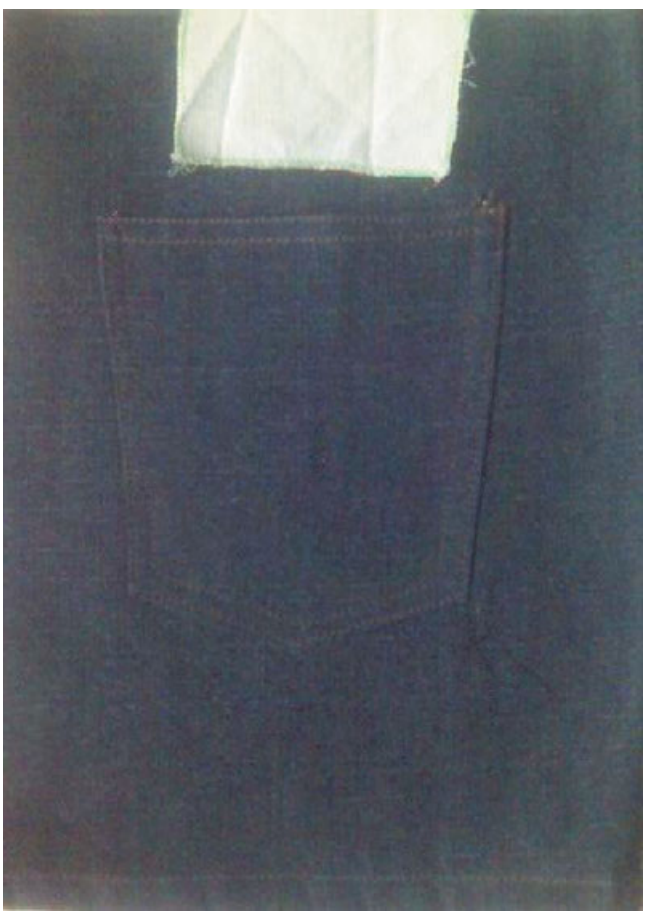

Figure 1 Garment sample with two pocket one the face and other (white pocket) on the back which turn to the face in the picture. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

Enzymes used were as follows: neutral cellulases (Roglyr Ultra 97655) from Rotta. Co. which is soluble at 40–50°C and has maximum activity at 55°C and pH = 7. This is a new enzyme with higher activity comparing with acidic cellulases. Lacasses (Denilite IT), from Novo Chemie. Co., are soluble at 55–65°C and has maximum activity at 60°C and pH = 4.5; however, it is active at pH = 7 with 80% activity. Amylases (Rotta-amylase 189) from Rotta. Co has range of activity at 60–100°C.

A rotary drum washer with 5 kg capacity (steel, r.p.m. = 25) was used for desizing, stone washing, and biowashing.

Methods

Desizing

Samples were desized for 15 min at 70°C, pH = 7, 1 mL/L amylases, and then washed thoroughly. In all of the experiments, weight of samples 450 g, Liquor to Goods Ratio (L : G) 50 : 1, dispersing agent 2 g/L and anticreasing 3 g/L were adjusted. Denim samples were treated with three different methods for indicating the best possible method of effluent reusing. To compare the samples treated with stone washing effluent and mixture of laccases and cellulases with cellulases treated sample, a control sample (cellulases treated sample) was prepared. To do this, a desized sample was treated with 9% neutral cellulases on weight of garment (9 Nc) to achieve a reasonable decolorization on the garment for 1 h at 55°C and pH = 7.

Reusing of effluent by first method

Step 1: A desized sample was treated with 9% neutral cellulases and 2% laccases on weight of garment for 60 min at 60°C and pH = 7. The sample produced in this step named as 9Nc2L-1 (9 Nc = 9% neutral cellulases, 2 L = 2% laccases, 1 = Step 1).

Step 2: The remaining bath of Step 1 used as a new liquor bath and the temperature raised to 60°C and 20% of cellulases used in Step 1 (1.8%) was added to this bath and then biowashing was carried out similar to Step 1. This sample is coded as 1.8Nc0L-2 (1.8 Nc = 1.8% neutral cellulases, 0 L = 0% laccases, 2 = Step 2).

Step 3: This step was carried out similar to Step 2 by using the remaining bath of step two with a new sample. This sample is coded as 1.8Nc0L-3 (1.8 Nc = 1.8% neutral cellulases, 0 L = 0% laccases, 3 = Step 3).

Step 4: This step was carried out similar to Step 3 by using the remaining bath of Step 3 with a new sample. This sample is coded 1.8Nc0L-4 (1.8 Nc = 1.8% neutral cellulases, 0 L = 0% laccases, 4 = Step 4).

Reusing of effluent by second method

Step 1: A desized sample was treated with 9% neutral cellulases and 2% laccases on weight of garment for 60 min at 60°C and pH = 7. The sample produced in this step was named as 9Nc2L-1 (9 Nc = 9% neutral cellulases, 2 L = 2% laccases, 1 = Step 1).

Step 2: The remaining liquor bath of Step 1 used as a new liquor bath and the temperature raised to 60°C and 4.5% cellulases (50% cellulases used in Step 1) along with 0.4% laccases (20% of laccases used in Step 1) was added to the bath and then biowashing carried out was similar to Step 1. This sample is coded as 4.5Nc0.4L-2 (4.5 Nc = 4.5% neutral cellulases, 0.4 L = 0.4% laccases, and 2 = Step 2).

Step 3: This step was carried out similar to Step 2 by using the remaining liquor bath of Step 2 with a new sample. This sample is coded as 4.5Nc0.4L-3 (4.5 Nc = 4.5% neutral cellulases, 0.4 L = 0.4% laccases, and 3 = Step 3).

Step 4: This step was carried out similar to Step 3 by using the remaining liquor bath of Step 3 with a new sample. This sample is coded as 4.5Nc0.4L-4 (4.5 Nc = 4.5% neutral cellulases, 0.4 L = 0.4% laccases, and 4 = Step 4).

Reusing of effluent by third method

Step 1: A desized sample was treated with 9% neutral cellulases and 2% laccases on weight of garment for 60 min at 60°C and pH = 7. The sample produced was named as 9Nc2L-1 (9 Nc = 9% neutral cellulases, 2 L = 2% laccases, 1 = Step 1).

Step 2: The remaining liquor bath of Step 1 used as a new liquor bath and the temperature raised to 60°C and 6.3% cellulases (70% cellulases used in step one) and 1% laccases (50% of laccases used in step one) was added to this bath and then biowashing carried out similar to Step 1. This sample is coded as 6.3Nc1L-2 (6.3 Nc = 6.3% neutral cellulases, 1 L = 1% laccases, and 2 = Step 2).

Step 3: This step was carried out similar to Step 2 by using the remaining liquor bath of Step 3 with a new sample. This sample is coded as 6.3Nc1L-3 (6.3 Nc = 6.3% neutral cellulases, 1 L = 1% laccases, and 3 = Step 3).

Step 4: This step was carried out similar to Step 3 by using the effluent of Step 3 with a new sample. This sample is coded as 6.3Nc1L-4 (6.3 Nc = 6.3% neutral cellulases, 1 L = 1% laccases, and 4 = Step 4).

Testing methods

Each denim swatches cut to 0.5 × 0.5 cm^2 and coated with gold by Sputter Coater device from BAL-TEC.CO, Switzerland. Microscopic pictures of samples were produced with double zoom (25,200 μ)

TABLE I
The Colorimetric Properties of Face of Treated Samples with Effluent

Samples	L^*	a*	b^*	C^*	h	ΔE
Untreated F	23.5	0.42	−4.93	10.30	267.39	3.75
Desized F	22.51	0.77	−8.38	8.42	275.22	0.00
9NC F	28.77	−0.65	−10.27	10.29	266.47	6.64
9NC2L-1F	28.78	−1.15	−8.29	8.37	262.12	6.55
1.8NC 0L-2 F	26.49	−0.77	−9.53	9.57	265.37	4.41
1.8NC 0L-3 F	25.61	−0.76	−9.63	9.46	265.37	3.67
1.8NC 0L-4 F	25.99	−0.63	−9.92	9.94	266.35	4.05
9NC2L-1 F	28.78	−1.15	−8.29	8.37	262.12	6.55
4.5NC 0.4L-2 F	26.51	−0.80	−9.45	10.35	268.91	4.42
4.5NC 0.4L-3 F	26.33	−0.78	−9.56	10.76	268.85	4.28
4.5NC 0.4L-4 F	26.03	−0.69	−9.65	10.55	268.95	4.01
9NC2L-1 F	28.78	−1.15	−8.29	8.37	262.12	6.55
6.3NC 1L-2 F	27.16	−1.07	−8.19	10.30	267.39	5.00
6.3NC 1L-3 F	27.08	−0.96	−8.59	10.51	268.02	4.85
6.3NC 1L-4 F	26.30	−0.80	−9.10	10.00	268.88	4.16

F, Face of sample; L^*, lightness; a^*, redness–greenness; b^* yellowness–blueness; ΔE, color difference with desized sample; C^*, chroma; h, hue.

by electron microscope device from Phillips Holland.

Three selected samples were exposed to X-ray by XRD device from Siemens. Co D5000 with lamp Cuk_a. The degree of crystalline was calculated by using eq. (1).

$$X = \frac{I_C}{I_C + I_A} \times 100 \quad (1)$$

In this equation, X is degree of crystalline, I_A is area of amorphous, and I_C is area of crystalline.

The colorimetric properties of samples were obtained by Data color device model microflash 200d with angle 10° and D_{65} lamp (standard light). Each denim swatches composed of three parts (face, backs, and white pocket). The average values L^* (lightness), a^* (redness–greenness), b^* (yellowness–blueness), and ΔE (color difference with desized sample) reported. In addition, the whiteness index (W) for white pocket was recorded.

To measure the absorption spectrum of biowashing effluent spectrophotometer device M-350 from England was used. The effluent from biowashing treatments were diluted by water and evaluated with control sample (water).

RESULTS AND DISCUSSIONS

Color and whiteness measurements

The results of chromaticity indices, color changes, and whiteness of the samples treated with biowashing effluent are illustrated in Table I. The results of the first method (Step 1) showed that sample treated with mixture of cellulases and laccases have higher L^* than treated samples with cellulases alone. It can be proposed that the treated samples with mixture of enzymes are brighter than treated sample with neutral cellulases (9 Nc) alone. The results of samples treated with effluent subsequently showed that using of effluent in subsequent processing by the first method leads to the production of a lower lightness or in other words, the effectiveness of laccases decline in subsequent processing. However, the values of L^* are not much differed from treated sample in the first step and with a control sample (treated sample with neutral cellulases). It means that the decolorization occurred but with the lower degree.

In the second and third method, more cellulases and laccases used in the remaining liquor bath and then greater lightness values obtained. Reusing of effluent for several times lead to produce a lower lightness. However, the changes of lightness are not remarkable when comparing the sample produced in Step 3 with the samples treated with mixture of cellulases and laccases and control sample. In fact, the values of L^* illustrated that in the third method, it is possible to reuse the effluent for four times.

The values of b^* for samples back are shown in Table II. Generally, by increasing the times of reusing of effluent, a lower concentration of laccases remains in the effluent and thereby samples back are bluer. This is explaining why staining on back of sample increases. In the second and the third methods, the concentration of cellulases and laccases increases by reusing the effluent, the back staining decreases more than in the first method. It can be also seen that, the treated samples with stone washing effluent have smaller back staining than the treated sample with neutral cellulases alone.

TABLE II
The Colorimetric Properties of Back of Treated Samples with Effluent

Samples	L^*	a^*	b^*	C^*	h	ΔE
Untreated B	39.48	−0.81	−3.25	3.35	255.99	3.08
Desized B	41.07	−0.38	−4.86	4.88	265.58	0.00
9NC B	43.71	−1.18	−7.28	7.38	260.81	2.62
9NC2L-1B	43.90	−1.95	−3.00	3.15	262.50	3.73
1.8NC 0L-2 B	41.91	−1.29	−4.21	4.35	255.43	1.39
1.8NC 0L-3 B	41.70	−1.27	−4.50	4.20	252.39	1.14
1.8NC 0L-4 B	41.52	−1.25	−4.86	5.02	255.52	0.97
9NC2L-1 B	43.90	−1.95	−3.00	3.15	262.50	3.73
4.5NC 0.4L-2 B	43.52	−1.64	−4.13	6.36	265.10	2.17
4.5NC 0.4L-3 B	43.52	−1.55	−4.18	5.78	267.57	1.02
4.5NC 0.4L-4 B	43.46	−1.53	−4.36	6.38	265.21	2.11
9NC2L-1 B	43.90	−1.95	−3.00	3.15	262.50	3.73
6.3NC 1L-2 B	43.77	−1.75	−4.10	5.31	265.15	1.47
6.3NC 1L-3 B	43.67	−1.65	−4.22	5.43	266.34	2.08
6.3NC 1L-4 B	43.55	−1.61	−4.32	5.13	266.50	1.30

B, Back of sample; P, white pocket; L^*, lightness; a^*, redness–greenness; b^*, yellowness–blueness; ΔE, color difference with desized sample; C*, chroma; h, hue.

By comparing the amounts of **ΔE** for different samples in Tables I–III, it can be seen that an increase or a decrease in enzyme concentration during biowashing causes a change in color. However, this change is varying and cannot be related to the concentration of enzymes.

The values of b^* for white pocket samples are shown in Table III. It can be observed that increasing times of reusing the effluent causes an increase in the blueness values of pocket material. The whiteness values of pockets also decrease with increasing times of using effluent. With increasing concentration of cellulases and laccases in the second and third method, the whiteness of samples pocket increase more than in the first method. It can be resulted that the back staining on the samples decreases. The values of whiteness on white pocket recognized that increasing of laccases concentration leads to lower staining in comparing with treated samples with neutral cellulases alone. The results of these experiments in terms of chromaticity indices, color changes, and whiteness indicated that method two and three by using more cellulases and laccases are recommend applying these methods as the valuable methods in repeated biowashing process by effluent reusing. However, the method three is more reasonable for its reproducibility.

Crystallinity

Desized, neutral cellulases and mixture of laccases and cellulases samples were selected to measure the

TABLE III
The Colorimetric Properties of White Pocket of Treated Samples with Effluent

Samples	L^*	a^*	b^*	C^*	h	ΔE	W
Untreated P	89.88	−0.55	7.34	7.37	94.30	20.59	76.1
Desized P	73.02	−2.50	−4.31	4.99	239.89	0.00	45.2
9NC P	72.20	−2.92	−5.05	5.69	242.63	1.10	44.0
9NC2L-1P	78.39	−0.66	−0.55	0.86	219.66	6.81	53.9
1.8NC 0L-2 P	74.07	−2.38	−0.99	2.98	143.00	3.48	46.8
1.8NC 0L-3 P	71.80	−2.68	−1.74	2.78	164.61	2.84	43.4
1.8NC 0L-4 P	71.39	−3.03	−1.97	3.18	197.71	2.89	42.8
9NC2L-1 P	78.39	−0.66	−0.55	0.86	219.66	6.81	53.9
4.5NC 0.4L-2 P	77.88	−1.11	−0.68	2.27	240.72	6.22	53.0
4.5NC 0.4L-3 P	77.70	−1.95	−1.03	2.06	242.41	5.74	52.7
4.5NC 0.4L-4 P	75.49	−2.31	−1.87	3.16	245.43	3.47	49.1
9NC2L-1 P	78.39	−0.66	−0.55	0.86	219.66	6.81	53.9
6.3NC 1L-2 P	78.23	−1.41	−0.60	1.49	199.23	6.55	53.6
6.3NC 1L-3 P	78.14	−1.43	−0.69	0.60	225.26	6.98	54.0
6.3NC 1L-4 P	76.45	−1.55	−0.92	1.07	239.19	5.21	50.6

P, White pocket; L^*, lightness; a^*, redness–greenness; b^*, yellowness–blueness; ΔE, color difference with desized sample; W, whiteness; C^*, chroma; h, hue.

TABLE IV
Crystallinity Percentages of Different Samples

Samples	Crystallinity (%)
Desized sample	65
Treated sample with neutral cellulases (9 Nc)	69
Treated sample with laccases (2 L)	61
Treated sample with mixture of laccases and cellulases (9Nc2L)	63

degree of crystallinity. The percentage of crystallinity for different samples is calculated and reported in Table IV.

As a result of cellulases treatment on the cellulose, the percentage of crystallinity increases. This can be due to penetration of enzyme into amorphous region that may reduce the amorphous region. The results show that the crystallinity percentage of samples treated with laccases decreases. It can be proposed that laccases has a negative effect on the crystallinity. This can be explained by possible acidic hydrolysis of cellulose in acidic media used for laccases treatment or by the oxidation of cellulose by laccases itself or oxidation compounds possibly exist in the commercial laccases. When mixture of laccases and cellulases are applied on the cellulose fabric, a limited decrease occurred in the crystallinity percentage. In this case, laccases react with lower activity because of application of laccases in the neutral media and cause a lower decrease in crystallinity. In other word, decreasing of crystallinity by laccases in acidic media may be reduced by using a neutral pH treatment. For the samples treated with mixture of enzyme, the neutral medium was used and a small reduction of the crystallinity can be related to the action of laccases.

The color evaluation of effluent

The effluents of five different samples were selected to study the absorption by spectrophotometer. The selected samples of effluents from the second method were as follows:

1. Biowashing effluent of neutral cellulases (control sample = Sample 1),
2. Biowashing effluent of neutral cellulases and laccases (Sample 2),
3. Biowashing effluent of Sample 2 (Sample 3),
4. Biowashing effluent of Sample 3 (Sample 4),
5. Biowashing effluent of the first method treated with 3 mL/L H_2O_2 at 60°C for 15 min (finished stone washing effluent).

Absorption spectrums of selected effluent were shown in Figure 2.

Figure 2 shows that the curve belongs to Sample 1 (control sample) has an absorption at 550 nm and 650 nm. This demonstrates the existence of indigo in colored effluent. Absorption spectrum of effluent including laccases (Sample-2) shows no absorption at 400–800 nm. It can be proposed that this effluent is free from indigo and the removal indigo during stone washing by cellulases in effluent degraded by laccases in the processing bath. It can be observed from absorption spectrums of effluents of Samples 3 and 4 in Figure 2 that effluents of reused baths for stone washing (Steps 3 and 4) have no absorption at

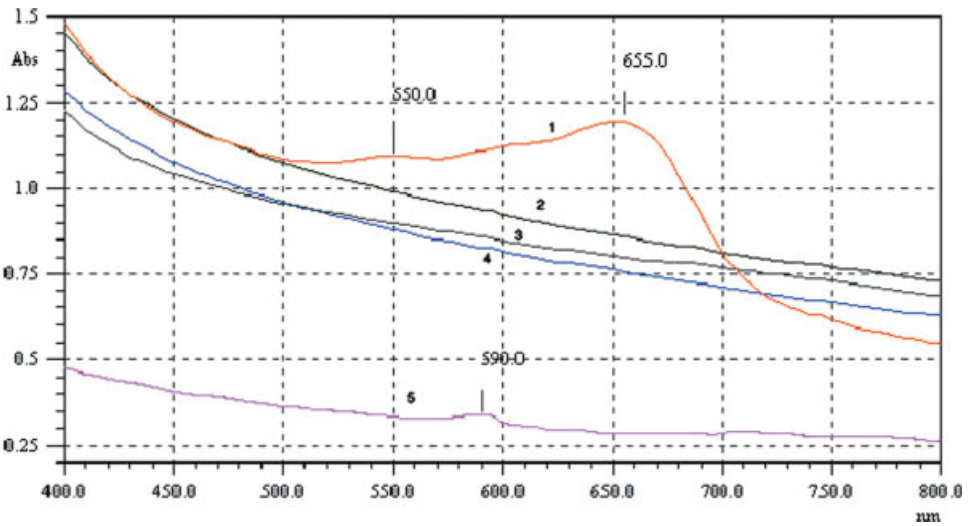

Figure 2 Absorption spectrums of different selected effluent. 1, Control effluent; 2, first step effluent; 3, second step effluent; 4, third step effluent; 5, finished effluent. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

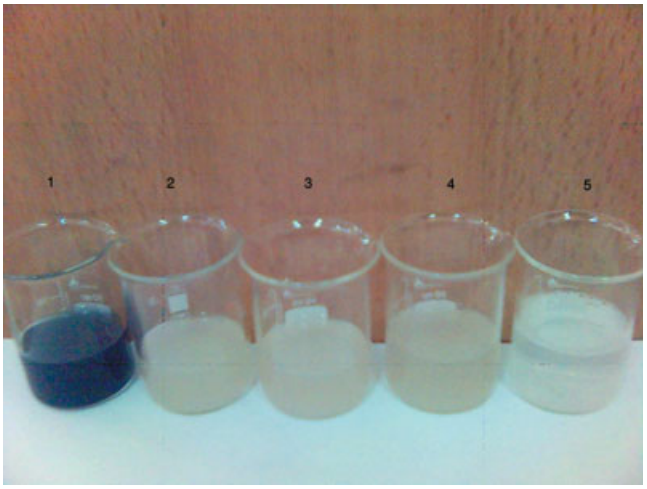

Figure 3 Selected samples of effluent. 1, Control effluent; 2, first step effluent; 3, second step effluent; 4, third step effluent; 5, finished effluent. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

400–800 nm. Therefore, due to the presence of laccases in effluent, the separated indigo from previous steps were degraded and no indigo was remained in the effluent. Considering these findings, the effluent is not colored and cellulases and laccases remain in the effluent as they are biocatalysts and are not consuming during the stonewashing processes. Therefore, it can be possibly considered reusing effluent including water and enzymes and reduce the consumption of water, energy, and enzymes. In absorption spectrum of effluent Sample 5 in Figure 2 a small absorption at 590 nm can be seen that can be a proof for very small amount of indigo remaining in effluent. Overall, absorption spectrum of treated effluent with H_2O_2 is similar to absorption spectrum of water alone and only a small pick appears at 590 nm.

As Figure 3 recognized while effluent of control sample (Sample 1) is colored illustrating indigo in the effluent, the Effluents 2 and 3 (Step 2 and 3) are not colored as they include laccases, which acts on indigo, and can degrade it. Effluent 5 is clear water similar to tap water that is recognized the effect of hydrogen peroxide on effluent including laccases.

SEM's pictures

Study of fabric surface

Microscopic pictures of sample surfaces are shown in Figure 4. Figure 4(a,b) demonstrated that surfaces of desized sample covered by the anchor fiber which are likely removed by the neutral cellulases treatment because of hydrolyzing of cellulose and free fiber by cellulases.

The picture of treated sample with mixture of laccases and cellulases [Fig. 4(c)] showed that laccases is not able to remove the anchor fibers and just caused a change in color. Therefore, clearly decreased the effect of cellulases caused a reduction in removal of anchor fibers.

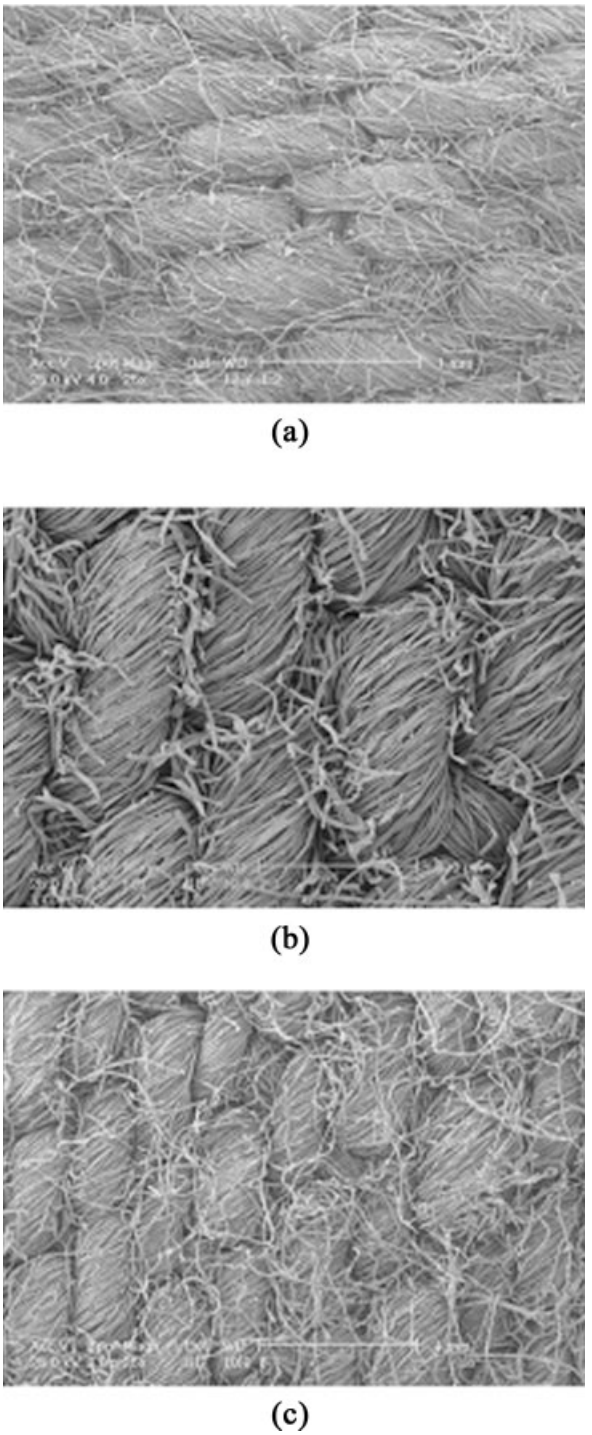

Figure 4 SEM pictures of different treated samples, (a) desized sample, (b) treated sample with neutral cellulases, and (c) treated sample with mixture of cellulases and laccases (magnification: ×25).

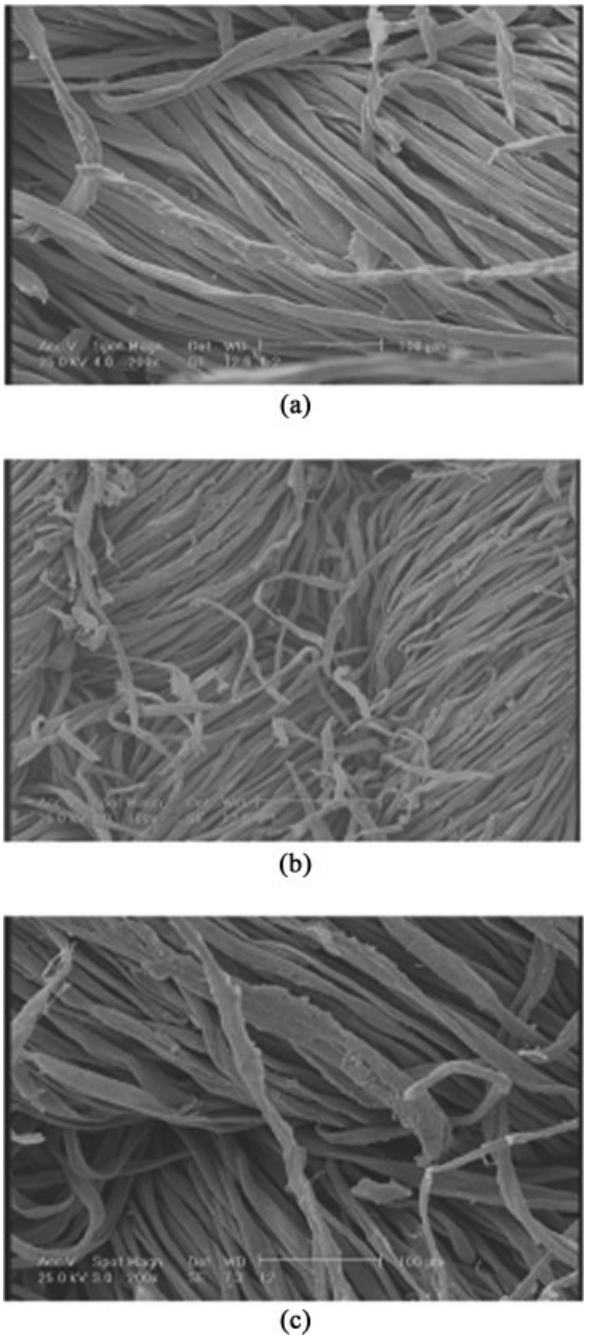

Figure 5 SEM pictures of different treated samples, (a) desized sample, (b) treated sample with neutral cellulases, and (c) treated sample with mixture of cellulases and laccases (magnification: ×200).

Study of fiber surface

Microscopic pictures of surfaces of samples were shown in Figure 5. The fiber entanglements can be clearly observed. The picture of desized sample [Fig. 5(a)] showed the fiber in the surface unchanged. This means that the fibers have not been damaged by desizing. It can be seen in pictures of treated samples with neutral cellulases [Fig. 5(b)] that the surface of outer fibers was damaged and some fibers were broken. However, inner fibers seem to remain unchanged without damage. In other words, during stone washing only surface fibers of outer fibers are damaging and inner fibers have not been affected. This means that the inner fibers are less accessible for the cellulases.

It can be observed from Figure 5(c) that entanglements of fiber surfaces of treated sample with mixture of laccases and cellulases are damaged less than treated samples with neutral cellulases. This can be due to reducing of cellulases activity when mixed with laccases. Overall, it can be considered that laccases along with cellulases reduce the fiber surface damage.

CONCLUSION

Energy and water play an important role in human life. However, dyeing and stone washing treatments need a lot of water and generating effluent contains dyes and unsafe materials for environment. On the other hand, denim washing is known as one of the finishing treatment with wide usage because of creating special appearance and updating clothes. In this research work, we try to study the possibility of reusing stone washing effluent and indicating the color differences produced with several times of reusing effluent.

The results of samples reflection illustrated that increasing numbers of reusing stone washing effluent causes a decrease in the lightness but this is very small and is not significant in comparing with the control sample and sample produced by step one. By increasing the cellulases and laccases concentration in the second and third methods, the lightness increases and in fact, the second and third methods are more useful. The whiteness of white pocket for treated samples has been gained when the percentage of cellulases and laccases increases in the latter methods. The whiteness index of these samples show a little difference with samples treated in first step (in all methods) and control sample.

The degree of crystallinity showed that samples treated with cellulases have a higher crystallinity and the samples treated with laccases produce a lower crystallinity and for those treated sample with mixture of cellulases and laccases, a low degree of crystallinity obtained.

By comparing of absorption spectrum of selected effluents, it can be recognized that the effluent of sample treated with laccases has no absorption in visible region. It means that the laccases decompose the removed indigo during stone washing. In this way, laccases cause discoloring of effluent that recommending reuse of stone washing effluent for several times.

The SEM pictures showed that fibers of treated sample with neutral cellulases damaged significantly

but fibers of treated sample with laccases and cellulases are little affected. In other word, laccases along with cellulases reduce the activity of neutral cellulases and the fiber damages are limited to the outer fibers and inner fibers remain unaffected.

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