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Studies on the influence of bacterial collagenase in leather dyeing

Swarna V. Kanth, R. Venba, B. Madhan, N.K. Chandrababu*, S. Sadulla

Central Leather Research Institute, Adyar, Chennai 600020, India

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Abstract

Collagenase enzymes are nontoxic and eco-friendly biocatalysts. Dyeing is an important process in the leather industry, which employs many synthetic colorants. Many good dyes suffer from incomplete exhaustion and this causes concern, as the biotreatability of the unexhausted dyes in effluent is normally difficult. Hence in the present study, an attempt has been made to improve the exhaustion of dyes by using bacterial collagenase enzymes as biocatalysts. The effect of process parameters of enzymatic treatment such as pH, temperature and duration on the exhaustion of the dye, levelness of dyeing, shade brightness, dye penetration and color intensity have been studied and the conditions are optimized. Uptake of dye as high as 99% has been observed by the treatment of collagenase. The change in shades due to enzymatic treatment has been quantified by reflectance measurements and compared with the visual assessment data. Scanning electron microscopy analysis showed a well opened-up fibre matrix for the collagenase treated leather. The strength properties are not significantly altered and the bulk properties like softness have been found to be improved by the use of collagenase.
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Keywords: Leather; Dyeing; Collagenase; Exhaustion; Colour; Intensity

1. Introduction

The living cell exclusively produces enzymes or biocatalysts and no life in any form can exist without enzymes. They are proteins in nature and catalyze certain biochemical reactions. Enzymes are remarkable because of their extraordinary specificity and catalytic power, which are greater than those of man-made catalysts. Theoretically all enzymes that are employed for catalyzing reactions can be used over and over again for performing the same specific catalytic cleavage or synthetic reactions. In practice, however, there are quite a few limitations. Since enzymes are very complicated and sensitive biomolecules, destruction of enzyme activity is liable to occur due to the presence of heat, alkalinity, acidity, traces of metal ions and certain inhibitors. On the other hand, the enzymes are activated by certain compounds and also in favorable conditions of pH, time, temperature, etc. Enzymes have found uses in various processes of leather manufacture.

Collagen, the major fibrous protein present in hide or skin is tanned to make it resistant to physical, chemical and biological effects. Such a tanned skin/hide is called leather. All the unwanted non-collagenous materials are removed during leather processing. Hence leather processing involves a number of unit processes and operations, which are broadly classified as pretanning, tanning, post-tanning and finishing operations that are well addressed [1]. Dyeing is one of the inevitable steps of imparting color to the leather carried out in post-tanning operations. The information available on the use of enzymes in dyeing process of post-tanning operations in leather manufacture is scanty.

Leather is a difficult substrate to dye to level and consistent shade due to the unique nature of the raw material that has variations within the matrix [2,3]. To achieve the objective of a level and uniform dyeing with maximum uptake of dyes, the leather dyer therefore needs to be experienced and have a thorough understanding of the dyeing properties of the dyes and auxiliaries used. Uniformly tanned leather, proper post-tanning treatment and also suitable selection of dyes are essential for an even shade and maximum uptake of dyes [4]. It had been shown that different types of dyes have

* Corresponding author. Fax: +91 44 24911589.
E-mail address: tanneryclri@yahoo.com (N.K. Chandrababu).

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different exhaustion rates and this can lead to problems in achieving levelness and shade consistency [5]. The major reason for such dyeing behavior is their varying affinity for the leather substrate and the incompatibility between the dyes [6]. The behavior of dyes is primarily determined by the charge characteristics of both the dye and the leather to be dyed [7]. However, use of appropriate auxiliaries can minimize the differences in dyeing behavior. In all the cases, a balance has to be obtained between the dyeing conditions, post-tanning chemicals and the auxiliaries used for dyeing. Retanning process and selected process parameters influence the uptake of dyes in leather processing [8–11]. Neutralization, retanning, dyeing and fatliquoring were applied in compact formulations to reduce the pollution load and increase the uptake of dyes in leather with special focus to upgrade the lower quality leathers [12]. Liquid dyes were (also) used in leather dyeing to improve exhaustion [13]. Earlier, attempts were made to achieve an eco-friendly coloring process based on a combination of iron salts with vegetable tannins [14,15]. Power ultrasound was used as a non-polluting method for enhancing the diffusion of dyes in to leather [16–19]. Novel approaches like development of databases for computer-match prediction in leather dyeing help in optimizing the amount of dyes used to get various shades [20]. Use of chitosan as an auxiliary enhanced the depth of shade and exhaustion of different types of dyes in the dye bath [21–28].

Technological advancements within the last decade have ushered in a new era of enzymatic products derived from microorganisms. These enzymes assist in the multiple aspects of leather processing such as wetting or soaking, dehairing, degreasing, opening-up of the fibre structure and softening [29]. The use of enzyme options in beam house process has been well exploited and there exists enormous scope for application of the same in post-tanning processes. This can pave way for the leather manufacturer to produce high quality leathers with reduced pollution load. Liposomes were used in leather dyeing to stabilize dye–liposome systems, which resulted in reduced pollution load [30–32]. The liposomes used as auxiliary products in sheepskin dyeing were reported to improve the levelness of dyeing and softness of leathers. A combination of a lipase and mild protease enzymes was used to clean the surface of chrome tanned stock of grease, dirt, scud, and other stains for the purpose of making more uniformly colored leather [33].

It is known that enzymes catalyze several biological reactions very efficiently. The role of enzymes in enhancing the dyeing behavior of textile has been established [34–37]. Hence it is perceived that enzymes can also be helpful in enhancing dyeing behavior of leather. Collagen is resistant to all enzymes except collagenase. Collagenases are a class of metalloproteinases, which are available from different sources. The mode of action of collagenase has been found to be dependent on the source from which it is obtained. Since leather being a material made out of collagen protein, it is thought that the metalloproteinase, collagenase, can bring in some catalytic activity onto the matrix in enhancing the dyeing characteristics of leather. Bacterial collagenase preferentially cleaves X-Gly (X is most frequently a neutral amino acid) bond of the -Gly-Pro-X-Gly-Pro-X- sequence in the non-polar regions of the collagen molecule [38]. Bacterial collagenases from *Clostridium histolyticum* cleave collagen at multiple sites [39], whereas, mammalian collagenases from human-fibroblast cleave collagen only at a single site (Leu–Ileu) breaking it into a 3/4th and 1/4th fragment [40]. Hence a treated collagen matrix exhibiting stability against the activity of bacterial collagenase can exhibit high resistance to degradation by any class of enzymes. It is well known that collagen tanned with various cross-linking agents such as chromium is made resistant against the degradation by collagenase. Hence, collagenase will not hydrolyze the chrome tanned leather, but bring about opening-up of the matrix. Hence in the present study we have used bacterial collagenase during post-tanning to enhance the dye uptake of leather.

The effect of bacterial collagenase in the dyeing of full chrome goat crust leather has been carried out using CI Acid Black 210 dye, which exhibited poor affinity to leather matrix and lower exhaustion levels. The study evaluates the use of this enzymes of to improve the penetration of the dye into the leather, the levelness of dyeing, shade brightness, color intensity, fixation of the dye for the purpose of making more uniform and darker colored leather.

2. Experimental

2.1. Reagents and chemicals

Basic chromium sulfate (BCS) and other post-tanning auxiliaries used for post-tanning are of technical grade. The type IA bacterial collagenase was procured from Sigma–Aldrich chemical company.

2.2. Experimental trials

Conventional chrome tanned leathers from a same lot of similar weight range and grade were selected for the study. The leathers were sammed and shaved to 1.0 mm thickness and cut into 15 × 15 cm size samples and four samples cut from each wet blue leather. Two samples were taken for each trial – quantity of chemicals calculated on shaved weight. The samples were processed into upper leathers as per the process described in Table 1. The effect of collagenolytic enzymatic treatment during post-tanning was studied, employing the bacterial collagenase in the process mentioned in Table 1. Various experimental trials (trial 1 to trial 4, mentioned below) were carried out at different conditions of enzymatic treatment after retanning the leathers, and subsequent to the enzyme treatment dyeing and fatliquor process were followed as mentioned in the table.

2.2.1. Effect of concentration of collagenase (trial 1)

Five sets of cut samples of wet blue goat leathers were rechromed, neutralized and retanned as per the process given in Table 1. After retanning, the leathers were treated with bacterial collagenase at six different concentrations viz., 0.02,

Table 1
Experimental post-tanning process

Material: goat skins, wet blue, Indian, shaved to 0.9–1.0 mm			
Washing	200% Water, 35 °C	20 min	pH 3.2
	0.2% Acetic acid, 85%		
Rechroming/neutralisation	150% Water		
	4% Chrome syntan	60 min	
	1% Neutralizing syntan		
	1% Sodium formate	20 min	pH 4.2
	1.0% Sodium bicarbonate	3 × 10 min, 30 min	pH 5.0
Wash twice with 200% water for 10 min			
Retanning	100% Water, 35 °C		
	2% Acrylic syntan	30 min	
	4% Phenol, naphthalene syntan		
	4% Melamine syntan	60 min	
Enzyme treatment			
Trial 1 – treatment at varying concentrations (0.02, 0.04, 0.06, 0.08, 0.1 and 0.12%)			
Trial 2 – treatment at varying pH conditions (4, 5, 6, 7, and 7.5)			
Trial 3 – treatment at varying running times (15, 30, 45, 60, and 75 min)			
Trial 4 – treatment at varying temperatures (40, 50 and 60 °C)			
Fatliquoring/dyeing	2% Acid Black dye		
	10% Water, 35 °C	60 min	
	8% Synthetic fatliquor	60 min	
	1.0% Formic acid, 85%		
	10% Water, 35 °C	3 × 10 min, 30 min	pH 3.5
The sample leathers were rinsed in water and piled overnight followed by setting, drying, staking, trimming and buffing			

0.04, 0.06, 0.08, 0.1 and 0.12% at pH 7.0 at 30 °C and the duration of treatment was 30 min.

2.2.2. Effect of pH (trial 2)

Five sets of cut samples of wet blue goat leathers were subjected to post-tanning using the process mentioned in Table 1. The enzymatic treatment was carried out at 0.1% concentration of collagenase at five different pH conditions viz., 4, 5, 6, 7 and 7.5 adjusted prior to collagenase treatment. The treatment was carried out for 30 min at 30 °C.

2.2.3. Effect of time (trial 3)

Five sets of cut samples of wet blue goat leathers were subjected to post-tanning using the process mentioned in Table 1. The enzymatic treatment was carried out using 0.1% concentration of collagenase at varied running times viz., 15, 30, 45, 60 and 75 min, keeping the pH and temperature constant at 7.0 and at 30 °C, respectively.

2.2.4. Effect of temperature (trial 4)

Three sets of cut samples of wet blue goat leathers were subjected to post-tanning using the process mentioned in Table 1. The enzymatic treatment was carried out using 0.1% concentration of collagenase at three different temperatures viz., 30, 40 and 50 °C, at pH 7.0 for 30 min.

2.2.5. Control process

One set of cut samples of wet blue leathers was subjected to post-tanning as mentioned in Table 1 without any enzymatic treatment.

The process liquors from all the experimental and control trials were analyzed for the exhaustion of dye. The leathers were washed, set, hooked to dry and stored at room temperature before color measurement and assessment of fastness properties.

2.3. Comparison of experimental and control trials

Matched pair comparison of control and experimental trial at optimized enzymatic treatment was carried out using 10 goat wet blue leathers. Ten left halves were used for control process and right halves were processed using optimized enzymatic process. The leathers were compared for color, fastness, strength and organolleptic properties and subjected to SEM analysis.

2.4. Analysis of dye exhaustion in the process liquor

Exhausted dye liquor was collected and analyzed for the unspent dye using a spectrophotometric method by measuring the absorbance value at the λ_{max} of the dye used, after suitably diluting the spent dye liquor using Hitachi *UV–vis spectrophotometer*. Then the amount of dye present in the spent liquor was calculated from the calibration graph drawn for the known concentration of the dye.

$$\%\text{Dye exhaustion} = \left[\left(C_g - C_t\right)/C_g\right] \times 100$$

Here C_g is the concentration of dye used and C_t is the concentration of dye in the spent liquor.

2.5. Determination of color difference

The control and experimental leathers made in this study were subjected to the reflectance measurements using a Milton

Roy Color Mate HDS instrument. Color measurements (L, a, b, h and C) were recorded and the total color difference (ΔE) and hue difference (ΔH) were calculated using the following equations:

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

$$\Delta H = \sqrt{\Delta E^2} - \Delta L^2 - \Delta C^2 \quad (2)$$

where ΔE represents the overall color difference; ΔL, the lightness difference; Δa and Δb, the differences in a and b values, wherein 'a' represents the red and green axes and 'b' represents the yellow and blue axes; ΔH, hue difference, and ΔC, chromaticity difference.

$\Delta L < 0$ sample is darker, $\Delta L > 0$ sample is lighter,
$\Delta a < 0$ sample is more green, $\Delta a > 0$ sample is more red,
$\Delta b < 0$ sample is more blue, $\Delta b > 0$ sample is more yellow,
$\Delta c < 0$ sample is brighter/more saturated, $\Delta L > 0$ sample is duller/less saturated;
l − lighter D − decrease MR − more red
d − darker I − increase MY − more yellow
w − weaker LR − less red G − greener
s − stronger LY − less yellow B − bluer.

2.6. Visual color assessment

The leather samples made from matched pair control and optimized experimental processes (full skin leathers) were subjected to visual assessment for uniformity of color, depth of shade, color shift from control and general appearance by standard tactile evaluation technique. Four experienced tanners rated the leathers on a scale of 0–10 points for each functional property with 0 as the lowest and 10 as the best. The average of ratings was calculated for each property and taken for comparison.

2.7. Determination of fastness to light

The leather samples made from matched pair control and optimized experimental processes were tested for light fastness after conditioning according to IS 6191 − 1971 (LF: 4) [41]. The samples were exposed to xenon arc light under prescribed conditions for 20 h along with the dyed blue wool standards. The black panel temperature was maintained at 63 ± 1 °C and the relative humidity was $30 \pm 5\%$.

2.8. Determination of fastness to wet and dry rub

Samples of appropriate size (5×14 cm) were cut from the leathers and were tested according to IS 6191 − 1971 (LF: 10) [42]. This method uses a SATRA Crock meter.

2.9. Physical testing analysis

The matched pair leather samples made from control and optimized experimental processes of full skin leathers were taken for physical testing measurements and the samples were cut from the official sampling position (IUP 2 [43] method). The leather samples were conditioned at 80 ± 4 °C and $65 \pm 4\%$ R.H. for 48 h. The tensile strength, elongation at break, tear strength and grain crack strength were measured as per IUP 6 [44], IUP 8 [45], and IUP 9 [46] methods.

2.10. Assessment of bulk properties

The leather samples were also subjected to visual assessment for bulk properties such as softness, fullness, grain smoothness, grain flatness, grain tightness and general appearance by four experienced tanners. Assessment was done on a scale of 0–10 points for each functional property and higher value indicates better property. The average of ratings was calculated for each property.

2.11. Scanning electron microscopic (SEM) studies

The leather samples (taken from the butt region) were cut into specimens of uniform thickness. A Quanta 200 series scanning electron microscope was used for the analysis. The micrographs for the grain surface and cross-section were obtained by operating the SEM at low vacuum with an accelerating voltage of 20 kV at different magnification levels.

3. Results and discussions

In this study, an attempt has been made to improve the exhaustion of dye with the use of bacterial collagenase. The approach is based on the concept that the enzymes act as biocatalysts in opening-up the fibrous leather network thereby enhancing the diffusion of dyes into the leather matrix and also the contact surface areas in the leather exposed for interaction with dye increase. Improved exhaustion of dyes and other post-tanning chemicals will not only reduce pollution but also result in quality leathers at reduced amount of chemicals used thereby reducing the cost of production.

3.1. Enzyme application for dye exhaustion: optimization of process parameters

In order to attain improved exhaustion and distribution of dyes into the leather, conditions on the usage of enzymes have to be optimized. In the process, as mentioned in Table 1, the wet blue leathers have been retanned followed by trials in optimization of parameters like concentration of enzyme, pH, temperature and time.

3.1.1. Optimization of concentration

The exhaustion of dye at different concentrations of collagenase treatment is shown in Fig. 1. From the figure it is seen that the uptake of dye by the leather increases with increasing concentration of collagenase. Above 0.1% concentration of collagenase, there is no significant increase in the fixation of dye and also higher % of collagenase can cause looseness to the leathers. Hence 0.1% concentration appears to be sufficient

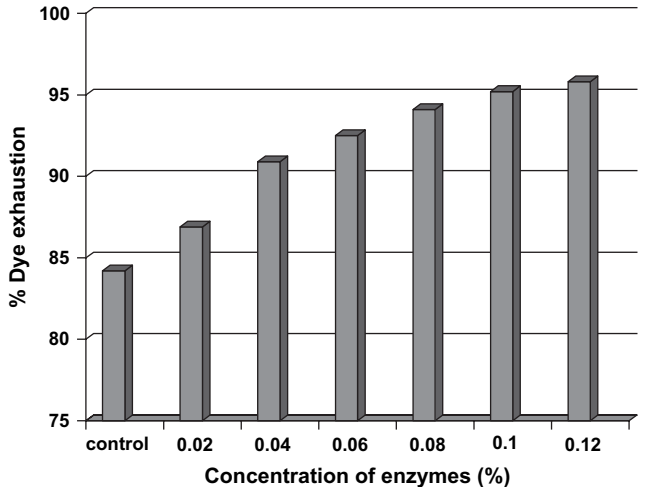

Fig. 1. Effect of enzyme concentration on the uptake of dye in the leather at room temperature for 30 min at pH of 7.0.

for maximum uptake of dye and has been taken as optimized concentration for better exhaustion of dye. The exhaustion of dye at this concentration is found to be 95%.

3.1.2. Optimization of pH

The results obtained with respect to the effect of pH on the exhaustion of dye in the process liquor are shown in Fig. 2. From the figure it can be seen that the exhaustion of dye increases gradually with increases in pH up to 7.0 and further increase in pH resulted in decrease of the exhaustion of dye. Maximum exhaustion of dye is observed when the collagenase treatment is done at pH 7.0. The bacterial collagenase used in this study has the same pH, 7.0 as the pH of maximum activity. Hence maximum dye uptake is observed at this pH. Since collagenases open up the fibre structure, the diffusion of dyes into the leather matrix is higher at this optimized pH and results in maximum uptake. Hence pH 7.0 at 0.1% concentration of collagenase has been taken as optimized concentration and pH for better exhaustion of dye.

3.1.3. Optimization of time

The fixation of dye to the leather in terms of percentage exhaustion of dye at different time intervals is shown in Fig. 3. It is evident from the figure that the uptake of dye increases gradually with time. It requires maximum of 1 h to bring about significant exhaustion in dye bath. At higher time intervals, the fixation of dye is gradual. Increased time intervals of collagenase may not be ideal for processing, as the leathers may exhibit looseness. Hence 60 min of enzymatic treatment has been taken as optimum duration. The treatment of collagenase for a time period of 60 min resulted in a dye uptake of 98%.

3.1.4. Optimization of temperature

The results obtained for the exhaustion of dye at different temperatures of collagenase treatment with constant 0.1% concentration, pH 7.0 and 30 min treatment are given in Fig. 4. It is seen that there is an increase in the exhaustion of dye with the increase in temperature of enzymatic treatment from 30 to 40 °C. The increase in exhaustion of dye could have been augmented with the distribution of dye aiding access to more reactive sites for interaction, as the bacterial collagenase enzyme used is highly active at a temperature of 37 °C. Further increase in temperatures to 50 and 60 °C resulted in decrease in the uptake of dyes as the activity of collagenase may be affected at higher temperatures.

3.2. Effect of surface color at varied condition of enzymatic treatment

Variation in color of the leather at different treatment conditions of enzyme treatment viz., concentration, pH, temperature is presented in Table 2. The color difference values of the leathers, when compared with control treated at varying enzyme concentrations viz., 0.02–0.12% are seen in the Table

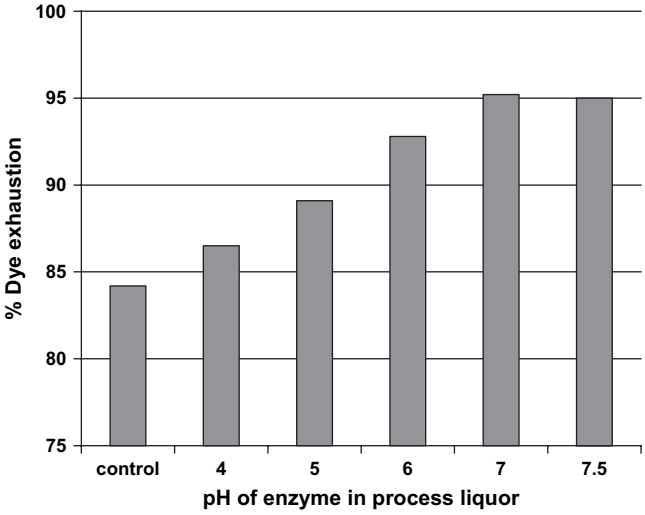

Fig. 2. Effect of pH on the uptake of dye in leather at 0.1% enzyme concentration, room temperature for 30 min.

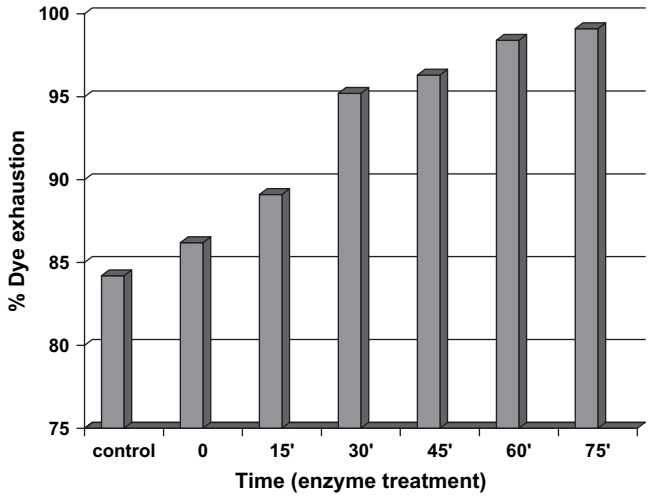

Fig. 3. Effect of time on the uptake of dye in leather at 0.1% enzyme concentration, room temperature and pH 7.0.

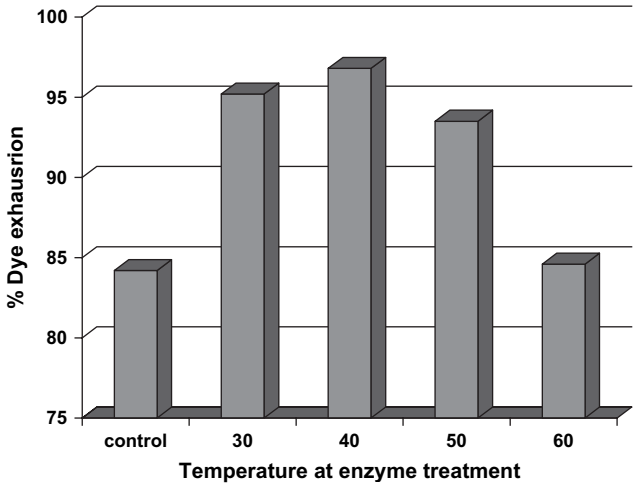

Fig. 4. Effect of temperature (°C) on the uptake of dye in leather at 0.1% enzyme concentration for 30 min and pH 7.0.

2. From the ΔL and ΔC values shown in the table, it is observed that there is an increase in the darkness and the intensity of the leathers with increase in the concentration of enzyme up to 0.1%. The leathers tend to become lighter at concentration values above 0.1%. Similar trend is observed with intensity of the color on treatment with different enzyme concentrations. The total color difference ΔE is also maximum at 0.1% concentration of enzyme. The hue difference is marginal in the enzyme treated leathers compared to control.

The study on the color differences using enzyme at different pH conditions indicated that there is a large increase in the darkness and color intensity of the leathers when enzyme treatment has been carried out at pH 7. Since collagenase activity is maximum at pH 7.0, it brings about better fibre opening at this pH resulting in the increase of contact surface areas in the leather exposed for interaction with dye. This facilitates the diffusion and exhaustion of dyes. Since the dye is added after enzyme treatment at pH 7.0, there is a possibility of the dye to react with enzyme to form dye–enzyme intermediates. Also the penetration of dye and dye intermediates will be high at higher pH of enzymatic treatment resulting in maximum uptake of dye into the leather, since the fibre structure will be well opened up. This causes an increase in surface darkness and color intensity of the dyed crust. The hue difference is (also) marginal in the enzyme treated leathers at different pH conditions.

The color difference values of leathers treated with 0.1% enzyme at pH 7.0 for 30 min at varied temperature conditions are also given in Table 2. The darkness (ΔL) is found to be highest at 40 °C. Beyond this temperature there is a decrease in darkness and the leathers have been found to be comparatively lighter. The intensity is also found to increase when the temperature of enzymatic treatment is increased.

Table 2
Color difference values of control and enzyme treated leathers

Parameters	ΔL	ΔC	ΔH	Δa	Δb	ΔE
Concentration[a] (%)						
0.02	l = 8.698	s = −0.078	D = −0.525	MR = −0.926	LY = −0.442	1.848
0.04	l = −2.589	s = −0.882	D = −0.158	MR = −0.607	MY = −0.443	2.584
0.06	l = −3.654	s = 1.423	D = −0.041	MR = 0.511	MY = 1.328	3.439
0.08	d = −2.798	s = 0.053	I = 2.047	G = −0.902	B = −1.824	5.933
0.1	d = −5.129	s = 0.119	D = −3.119	G = −1.155	B = −1.705	8.899
0.12	d = −6.387	s = −0.250	D = −0.201	MR = 0.409	MY = 0.162	10.013
pH[b]						
4	d = −2.306	w = −0.086	D = 0.439	MR = 0.409	LY = −0.161	2.840
5	d = −3.376	s = 0.102	D = −0.518	MR = 0.454	LY = −0.178	3.896
6	d = −4.240	s = −0.362	D = 0.794	MR = 0.518	LY = −0.673	4.264
7	d = −5.243	s = −0.446	D = 0.339	MR = 0.654	B = −1.058	8.689
7.5	d = −2.341	s = −0.261	D = 0.180	MR = 0.180	B = −0.858	5.352
Time[c] (min)						
15	I = 8.698	s = −0.081	D = −0.398	MR = −0.892	LY = −0.426	5.848
30	d = −5.186	s = −0.418	D = 0.421	MR = 0.598	B = −1.129	8.689
45	d = −6.425	s = −0.264	D = −0.213	MR = 0.426	MY = 0.162	9.798
60	d = −6.863	s = −0.289	D = −0.249	MR = 0.429	MY = 0.215	12.821
75	d = −6.999	s = −0.298	D = −0.292	MR = 0.488	MY = 0.315	13.018
Temperature[d] (°C)						
30	d = −5.192	s = −0.398	D = 0.310	MR = 0.632	B = −0.969	8.581
40	d = −6.215	s = −0.304	D = −0.312	LG = 0.409	MB = 0.162	10.013
50	d = −3.317	w = −0.182	I = 0.064	LG = 0.192	MB = −0.013	4.219
60	d = −0.453	w = 0.273	L = −0.015	MG = −0.268	MB = −0.048	0.552
Experimental (enzyme treatment at optimized conditions)						
0.1% pH 7, 40 °C, 60 min	d = −7.214	s = 0.558	D = −0.498	MR = 0.549	MB = −1.058	14.098

[a] pH 7, 30 °C, 30 min.
[b] Enzyme 0.1%, 30 °C, 30 min.
[c] Enzyme 0.1%, pH 7, 30 °C.
[d] Enzyme 0.1%, pH 7, 30 min.

Maximum intensity is observed at 40 °C, which subsequently decreases with further increase in temperature. The hue difference is marginal in the enzyme treated leathers, which indicates that the color of the leathers is not deviated from its original color.

The color difference values have been measured for 0.1% enzyme treatment at 7.0 pH and 40 °C at varying time intervals. The color differences for different running times, 15, 30, 45 60 and 75 min were evaluated and the values are given in Table 2. As expected the darkness (ΔL) is maximum at higher running times. Similarly the intensity is also found to increase with running time. Intensity is the highest for 60 min duration. The hue difference is marginal at different time intervals of enzymatic treatment.

3.3. Dye uptake at optimized conditions

The uptake of dye for optimized conditions viz., 0.1% concentration of collagenase, pH 7.0, 40 °C and treatment time of 60 min is found to be 98.4% whereas the exhaustion of control leather processing is found to be 84.2%.

3.4. Scanning electron microscopic (SEM) analysis of leathers

The scanning electron photomicrographs of the grain and cross-section of control and experimental leathers treated with 0.1% enzyme at pH 7.0 and 40 °C for 60 min are shown in Figs. 5 and 6, respectively. The grain structure of enzyme treated leathers (Fig. 6a) at a magnification of 250× shows clean and clearer grain pores and opened-up surface without much deposition compared to the control. The cross-sectional view of the enzyme treated leather at a magnification of 1000× in Fig. 6b shows well separated and opened-up fibres. Hence, the enzymatic treatment to leathers had favored an increase in the contact surface areas in the fibre network exposing more reaction sites for the interaction with dye, which had resulted in the increase in the exhaustion of dyes.

3.5. Visual assessment of leathers

Visual assessment for shift in color from control, uniformity of color, depth of shade and general appearance for control and experimental leathers (optimized enzymatic conditions) is carried out by standard tactile evaluation technique, and the values are detailed in Table 3. The depth of shade is uniform for enzyme treated leathers, which is in agreement with the reflectance measurement values. The intensity of the enzyme treated leather is comparatively higher than the control. There is no appreciable change or shift in color for the experimental leathers compared to control. These results are in agreement with the reflectance measurement values. The uniformity of color, dye penetration and shade is better for the enzyme treated samples. There is an overall improvement in the general appearance of the leathers treated with enzyme (optimized conditions) in the post-tanning operation.

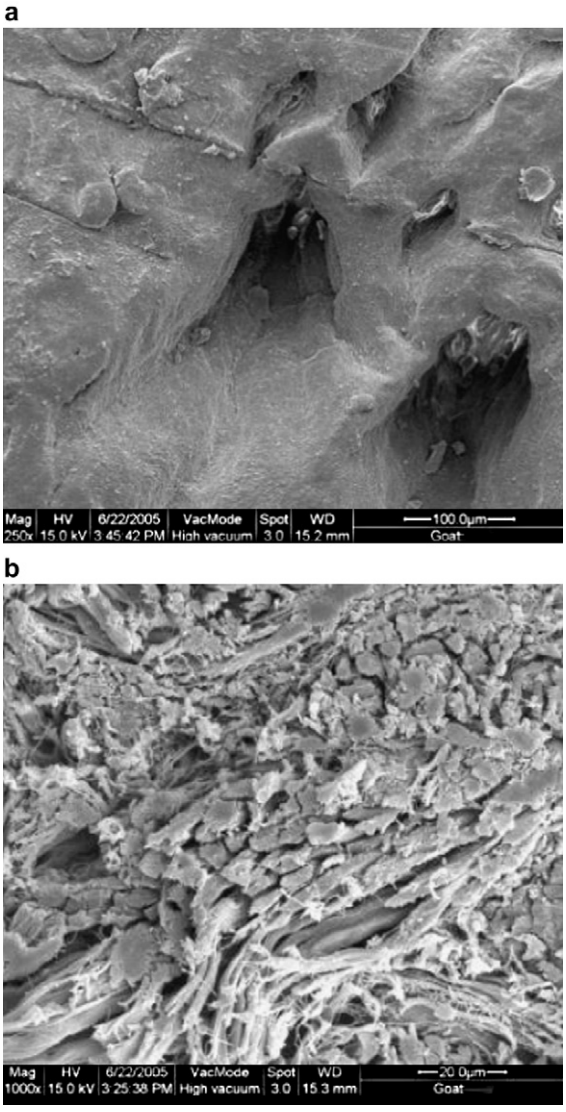

Fig. 5. Scanning electron micrographs of control leather: (a) grain structure (×250); (b) cross-section (×1000).

3.6. Light and rub fastness characteristics of leathers

The fastness of enzyme treated leathers at different conditions to rubbing and light is given in Table 4. From the table, it is evident that the fastness to wet and dry rubbing of the enzyme treated leather is better than control leathers. Enzyme treatment leathers in general exhibited moderate light fastness (rating 3 on grey scale), equivalent to the blue wool standards. The effect of ageing (6 months) on the fastness of leather has been studied. The effect of ageing on the fastness properties measured after ageing has been found to be similar to the values before ageing.

3.7. Evaluation of strength characteristics

It is imperative to analyze the strength characteristics upon treatment with enzymes. The various strength characteristics of the experimental crust leathers treated at optimized

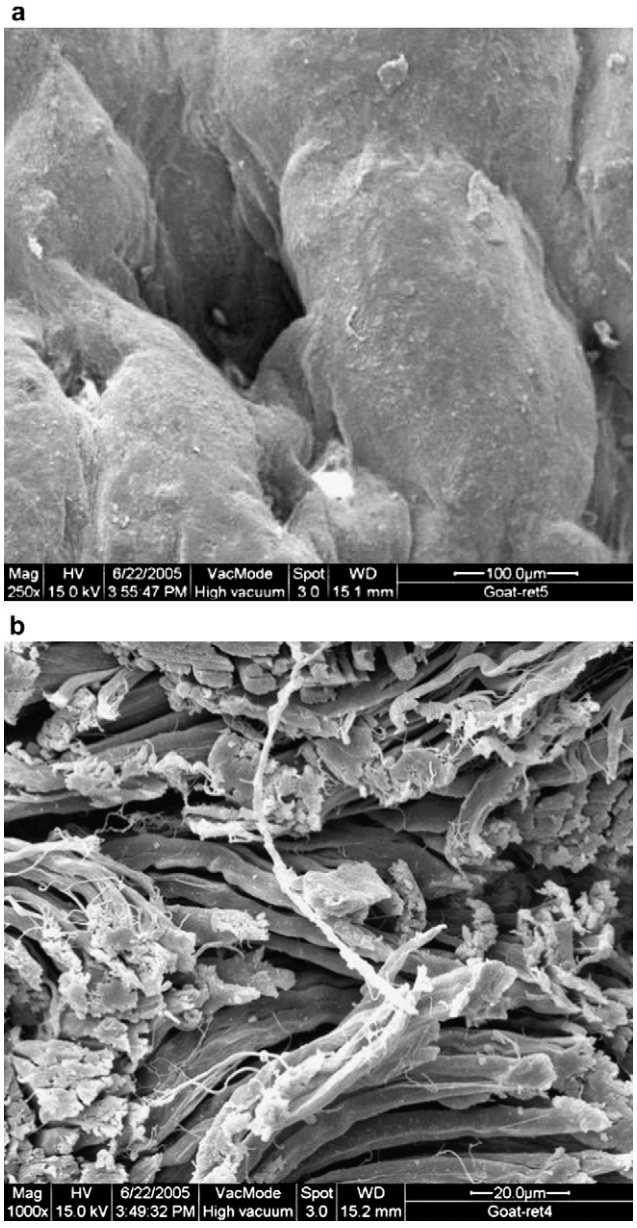

Fig. 6. Scanning electron micrographs of experimental enzyme treated leather: (a) grain structure (×250); (b) cross-section (×1000).

Table 3
Dye exhaustion and visual assessment data of control and 0.1% enzyme treated leathers at pH 7.0 for 60 min at 40 °C (optimized conditions)

Sample	% Dye exhaustion	Color shift as compared to control	Uniformity	Depth of shade	Intensity of dyeing
Control	84.5 ± 1.3	8	7.5	7	7
Enzyme treated	98.4 ± 1.5	9	9	9.5	9

Values reported are average of four samples ± standard deviation. Standard deviation of the organolleptic properties viz., color shift as compared to control, uniformity, depth of shade, intensity of dyeing are found to be less than ±0.5.

conditions of enzyme along with control leathers are given in Table 5. It is observed that the strength characteristics of the experimental leathers are not affected drastically due to enzymatic treatment. The values of various strength properties of experimental leather are found to be comparable to that of the control leathers as per BIS norms [47].

3.8. Assessment of bulk properties

The ratings of the bulk properties such as softness, fullness, feel, grain smoothness and general appearance of the leathers treated with enzymes and control are presented in Fig. 7. It is seen that all the organoleptic properties are comparable or better than the control leathers. Especially the grain smoothness and softness of the enzyme treated leathers have been found to be better than the control leathers.

3.9. Plausible mechanism of the process

Leather being a material made out of collagen protein, it is thought that the metalloproteinase, collagenase, can bring in some catalytic activity onto the matrix in enhancing the

Table 4
Fatness to wet rub, dry rub and light fastness of control and enzyme treated leathers

Sample	Before ageing			After ageing		
	Wet rubbing	Dry rubbing	Light fastness[e]	Wet rubbing	Dry rubbing	Light fastness
Control	4/5	5	3(4)	4/5	5	3/5
Concentration[a] (%)						
0.02	4/5	5	3(4)	4/5	5	3/5
0.04	4/5	5	3(4)	4/5	5	3/5
0.06	4/5	5	3(4)	4/5	5	3/5
0.08	4/5	5	3(4)	4/5	5	3/5
0.1	5	5	4(4)	5	5	4/5
0.12	5	5	4(4)	5	5	4/5
pH[b]						
4.0	4/5	5	3(4)	4/5	5	4/5
5.0	4/5	5	3(4)	4/5	5	4/5
6.0	4/5	5	3(4)	5	5	4/5
7.0	5	5	4(4)	5	5	5
7.5	5	5	4(4)	5	5	5
Time[c] (min)						
15	4/5	5	3(4)	4/5	5	4/5
30	4/5	5	3(4)	4/5	5	4/5
45	5	5	4(5)	5	5	4/5
60	5	5	4(5)	5	5	5
75	5	5	4(5)	5	5	5
Temperature[d] (°C)						
40	5	5	4(5)	4/5	5	4/5
50	5	5	4(5)	5	5	5/5
60	5	5	4(5)	5	5	5/5
Experimental (enzyme treatment at optimized conditions)	4/5	5	4(5)	4/5	5	4/5

[a] pH 7, 30 °C, 60 min.
[b] Enzyme 0.1%, 30 °C, 60 min.
[c] Enzyme 0.1%, pH 7, 30 °C.
[d] Enzyme 0.1%, pH 7, 60 min.
[e] Value in parenthesis indicates the corresponding blue wool standard.

Table 5
Strength properties of control and enzyme treated leathers (0.1% enzyme, pH 7.0, 60 min, 40 °C)

Sample	Tensile strength (kg/cm^2)	Extension at break (%)	Tear strength (kg/cm)	Grain crack resistance	
				Load (kg)	Distension (mm)
Control	248 ± 10	61 ± 3	60 ± 4	41 ± 2	9.8 ± 0.7
Enzyme treated	239 ± 10	71 ± 5	69 ± 3	43 ± 3	11.9 ± 1.1

dyeing characteristics of leather. It is well known that collagen tanned with various cross-linking agents such as chromium is made resistant against the degradation by collagenase. If collagenase is used before tanning it can cause significant damage to the hide substance due to degradation of collagen. Whereas the same collagenase when reacted with chrome tanned matrix it may not attack the matrix with the intensity it does for untanned collagen. Enzyme binding is highly specific for the substrate. Since the substrate collagen matrix is altered due to the effect of cross-linking of the tanning agent the binding of collagenase to the tanned collagen becomes unproductive. Therefore the use of collagenase on a chrome tanned matrix will not hydrolyze collagen but results in opening-up of the fibrous leather network thereby enhancing the diffusion of dyes into the leather matrix and also the contact surface areas in the leather exposed for interaction with dye increase. However, there may be very few sites in the chrome tanned leathers especially attacked by bacterial collagenase, which can exhibit some binding. Such binding can catalyze in exposing more number of functional sites for the binding of dyes and other chemicals in post-tanning operation of leather manufacture.

4. Conclusions

Enzymes were used as an eco-friendly approach for achieving better exhaustion of dye. The exhaustion of dyes in the effluent by the enzymatic treatment is well documented. A 0.1% concentration of enzyme, at pH 7.0 for 60 min and 40 °C were found to be optimum with respect to the uptake of dye, dye penetration and intensity of the color. The enzyme treated leather at the optimized conditions resulted in leathers with uniform dyeing, intense and bright shade. There is no significant change in color due to enzymatic treatment as noticed by reflectance measurements and visual assessment data. The overall fastness of the leathers treated with enzyme is comparable with those obtained by control leathers. Ageing of the colored leathers for six months does not alter the fastness significantly. The strength characteristics of the control leathers are not significantly altered. The bulk properties like softness, fullness, grain smoothness, feel and general appearance have been improved by the introduction of enzymes in the process bath. The present approach of reducing pollution load in post-tanning process with the use of enzymes at optimized conditions provides a new avenue for eco-benign dyeing process.

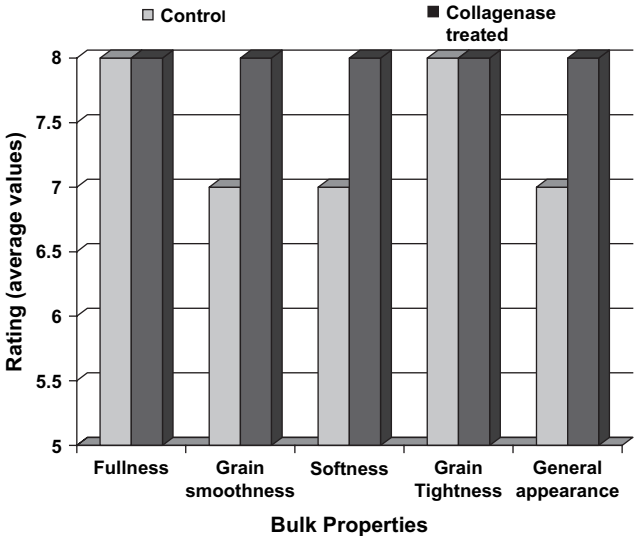

Fig. 7. Graphical representation of organoleptic properties of control and enzyme treated leather (optimized conditions).

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