Journal of Cleaner Production 17 (2009) 507–515

Contents lists available at ScienceDirect

Journal of Cleaner Production

journal homepage: www.elsevier.com/locate/jclepro

Cleaner tanning practices for tannery pollution abatement: Role of enzymes in eco-friendly vegetable tanning

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ARTICLE INFO

Article history:
Received 11 July 2006
Received in revised form
18 August 2008
Accepted 26 August 2008
Available online 30 October 2008

Keywords:
Vegetable tanning
Acacia mollisima
Enzyme assisted tanning
Pickling
Cleaner technologies in tanning
Total solids
Chemical oxygen demand
Spent tan liquor

ABSTRACT

Concern about pollution related problems in the global scenario are persuading all the processing industries to adopt cleaner manufacturing practices. Thus, the leather industry is also under pressure to look for effective alternative tanning materials for chromium. Natural products like vegetable tannins are regaining importance. However, there are limitations in the use of vegetable tanning materials because of its high organic load in the effluent, which are difficult to degrade leading to high chemical oxygen demand (COD). Moreover, conventional vegetable tanning process requires partial pickling that involves the use of sodium chloride, to suppress osmotic swelling. This results in very high amount of total dissolved solid (TDS) content in wastewaters. In this investigation, an attempt has been made to design an eco-friendly vegetable tanning process combining pickle-free tanning and application of proteolytic enzymes to improve the exhaustion of vegetable tannins. Such an approach has resulted in more than 95% tannin exhaustion in the case of the experimental process, an increase of 10% compared with the conventional vegetable tanning process. The tanned leathers showed slight improvement in hydrothermal stability. Physical and tactile evaluation of experimental leathers has been better than conventionally tanned leathers. Surface colour values illustrated negligible variation in colour and shade between control and experimental leathers. The resultant leather showed opened up, split compact fibre structure that has been well coated, indicating that the enzyme assisted tanning process did not bring about any major change or destruction on the fibre structure of the leathers. The optimized system has been field tested in a commercial tannery. The results showed that the enzyme assisted tanning process is efficient in terms of improved quality of leather and also led to reduction in total solids (TS), chlorides and COD loads. The enzyme assisted tanning system presented appears to be a viable option for combating pollution arising from the conventional vegetable tanning system.

1. Introduction

Tanning process involves conversion of putrefiable skin or hides to a nonputrescible material. Leather making involves operations like soaking (rehydration), liming, deliming, pickling, tanning, post-tanning and finishing processes [1]. Conventional methods employed in leather processing subject the skin or hide to wide variations in pH. Such pH changes demand the use of acids and alkalis, which lead to the generation of salts resulting in net increase in TS, chlorides and sulfates in tannery wastewater. Pickling process contribute to high dissolved solids content in the effluent as it involves the use of sodium chloride salt along with sulfuric acid [2,3]. Globally 90% of the leathers tanned by basic chromium sulphates (BCS) result in 50–70% chromium uptake [4]. This poor uptake results in material wastage on one hand and ecological imbalances on the other. The international specification for the discharge of chromium in wastewater is in the range of 0.1–2 ppm [5]. Even a high-exhaust chrome tanning system does not provide such low concentration. Concerns have been expressed on the toxicity of chromium(III) [6,7]. Discussion exists regarding the possible conversion of chromium(III) to chromium(VI) in certain soil conditions [8]. Moreover, the disposal of chromium containing solid wastes and sludge is posing major challenge [9]. On the whole, the popular chrome tanning method has come under the close scrutiny of the environmental authorities in the industrialized as well as developing countries due to increased awareness about environment and health. Hence, there is constant search for eco benign tanning materials and methods. Tanning methods involving the use of vegetable tannins and other natural materials [10–12] are regaining importance in the recent times.

Vegetable tannins have been used for conversion of animal hides to leather for thousands of years. The invention of chrome tanning in 1858 AD replaced vegetable tanning slowly because of the versatile nature of basic chromium sulphate. The leathers manufactured in the

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0959-6526/$ – see front matter
doi:10.1016/j.jclepro.2008.08.021

world today are predominantly based on chromium salts. The vegetable tanning agents used for processing of different types of leathers are leached from wood, bark, leaves, roots etc., which are water-soluble, non-crystalline ingredients of plant matter. The vegetable tannins possess distinct astringency. Chemically, tannins are mixture of several molecular species [13]. The tannin extract is a complex mixture of polyphenols. Vegetable tannins are classified as ester derived hydrolysable and flavanoid derived condensed tannins [14,15]. Tannins have molecular weight range of 500–3000 Da [16], containing sufficient hydroxyls and other suitable groups (carboxyl) to form effectively strong complexes with protein and other macromolecules [17,18]. Even though vegetable tanning agents are natural materials, they are known for low biodegradability and the presence of phenol content and colour in the effluents. Vegetable tannins in the range of 350,000–400,000 ton are being used across the world for leather processing. The conventional vegetable tanning system exhibits an exhaustion of about 85%. This means that 60,000–80,000 ton of vegetable tannins are let out in 1,000,000–1,500,000 m^3 of spent vegetable tanning liquor in effluent. Hence, to overcome the problems associated with conventional vegetable tanning process, it is necessary to device suitable strategies for improving the exhaustion of vegetable tanning in the tanning process.

Conventional vegetable tanning process requires partial pickling of the delimed skins/hides since the vegetable tannins possess optimal particle size for penetration into the matrix at pH 4.5–5.0 [19,20]. Spent pickle liquor has high amount of dissolved solids content, as this partial pickling involves the use of 8–10% sodium chloride salt along with required quantity of sulfuric acid. Dissolved solids contributed by salts are not amenable for treatment by the conventional effluent treatment methods. The State of Tamil Nadu in India which accounts for about 5% global leather production has stringent norm of 2100 mg/l for TDS. There exist various tertiary treatment methods for the treatment of dissolved solids. They are electrodialysis, reverse osmosis, and thermal distillation. However the techno-economic feasibility of these methods is yet to be established. Hence, there is need for better strategic practices leading to improved process alternatives to decrease the TDS in the wastewater.

In the present approach, pickle-free enzyme assisted vegetable tanning using commercial vegetable tanning agent (Wattle GS powder, (*Acacia mollisima*)) at a starting pH of 4.5–5.0 has been attempted. Enzymes have found uses in various pre-tanning processes of leather manufacture such as soaking, unhairing, bating, dyeing and degreasing [21–23]. However, the information available on the use of enzymes in tanning operations is scanty. In the present study, twin objective of decreasing TDS and chlorides using pickle-less vegetable tanning system and enzyme assisted opening up of the fibre matrix with acid protease to enhance the diffusion of vegetable tannins for achieving better exhaustion of tannins has been carried out. Processes starting from soaking up to deliming are according to conventional pre-tanning system. The uptake of vegetable tannin, shrinkage temperature of the leathers, effluent loads in terms of COD, chlorides, TS and characteristics of leathers have been compared with conventional vegetable tanning process.

2. Experimental methods

Conventionally dehaired and delimed goatskins of area 5.6 sq ft have been chosen as the raw material for this study. The chemicals used for leather processing have been of commercial grade. The chemicals used for analytical techniques have been of laboratory grade.

2.1. Pickle-less enzymatic vegetable tanning

Two delimed goatskins have been taken for each experimental trial. The process up to deliming has been carried out using conventional method.

2.1.1. Optimization of enzyme concentration for pickle-less enzymatic tanning

Preliminary trials have been carried out to find out the suitable concentration of enzyme and duration of enzyme treatment in tanning. Ten delimed goatskins have been taken for the study. The skins have been treated with 0.5% oxalic acid and 0.2% organic non-swelling acid (naphthalene sulphonic acid) along with 150% water (percentages based on fleshed weight) to obtain pelts with pH of 4.5–5.0 through the cross-section. Enzyme treatment has been carried out in the same bath. Experiments have been carried out with varying concentrations of enzymes, viz., 0.1, 0.2, 0.3, 0.4 and 0.5% in the same bath. The leather processing drum has been run for 30 min with enzyme and 20% of tanning agent, spray dried powders of wattle (*Acacia mollisima*) has been added in two feeds in the same drum as described in experimental process (Section 2.1.2). Complete penetration of tanning agent has been ascertained. The pH of the pelts has been adjusted to 3.5–3.7 using 0.2% formic acid. The process liquor from the trials has been analyzed for the exhaustion of vegetable tannins. Based on the shrinkage temperature values of the tanned leathers, exhaustion of tannins and visual assessment of tanned leathers, 0.2% concentration of enzyme has been optimized.

2.1.2. Optimization of duration of enzyme treatment for pickle-less enzymatic tanning

Another set of experiments has been carried out as described in Section 2.1.1 to determine the optimum duration at 0.2% enzyme concentration. The duration of treatment experimented are 15, 30, 45 and 60 min. The leathers have been then sammed and shaved to 1.0–1.1 mm thickness and assessed by an experienced tanner. From the study, 30 min enzyme treatment duration has been found to be optimum based on the shrinkage temperature measurements, exhaustion profile and visual assessment.

2.1.3. Control vegetable tanning process

The process given below has been followed using delimed goatskins as raw material.

add	Water	100%
	NaCl	10%; run the drum for 10 min
	H_2SO_4	0.75%; 3 feeds at 15 min interval + 30 min; pH of the cut section has been 4.5–4.7; Then 50% float (solution) has been drained.
add	Basyntan P[a]	2%; run the drum for 1 hr
	Wattle	10%; run the drum for 1 hr
add	Wattle	10%; run the drum for 3 hr, then complete penetration of vegetable tannin has been ascertained.
add	Formic acid	0.1%; run for 45 min, final pH has been found to be 3.5–3.7.

[a] Commercial synthetic pre-tanning chemical – used to reduce grain harshness because of astringency of vegetable tannin liquors.

2.1.4. Optimized experimental tanning process

To the delimed goatskins:

add	Water	100%
	Non-swelling organic acid	0.2%; run the drum for ½ hr
	Oxalic acid	0.5%; 3 feeds at 15 min interval + 30 min; pH of the cut section has been 4.5.
add	Enzyme[a]	0.20%; run the drum for ½ hr
	Wattle	10%; run the drum for 1 hr
add	Wattle	10%; run the drum for 3 hr, then complete penetration of vegetable tannin has been ascertained.
add	Formic acid	0.1%; run the drum for 45 min, final pH has been found to be 3.5–3.7.

[a] Acid protease that had maximum activity at pH 4.5 at 40 °C has been used.

2.1.5. Comparison of matched pairs of control and optimized experimental leathers

Matched pair comparison of control and experimental trial at optimized enzymatic treatment has been carried out using ten delimed goatskins. The ten left halves have been used for control process and ten right halves have been processed using optimized enzymatic process. Both experimental and control tanned leathers have been post tanned using conventional post-tanning process. All the leathers after vegetable tanning have been piled for 24 hr. The tanned leathers have been converted into crust upper leathers using post-tanning and mechanical operations. The leathers have been compared for colour, fastness, strength and organoleptic properties and subjected to scanning electron microscope (SEM) analysis.

2.2. Measurement of hydrothermal stability of leathers

The shrinkage temperature, which is a measure of hydrothermal stability of leather, has been measured using Theis shrinkage meter. The temperature at which the collagenous fibre shrinks to one third of its original length is noted as the shrinkage temperature of the fibre. The shrinkage temperature measurements have been carried out for all leathers at tanned stage.

2.3. Objective assessment of softness through compressibility measurements of leathers

Softness of leathers can be numerically measured based on their compressibility [24]. Circular leather pieces (2 cm^2 area) from matched pair control and experimental leathers have been obtained as per IUP method [25] and conditioned at 80 ± 4 °F and 65 ± 2% R.H. over a period of 48 hr. The samples have been spread uniformly over the solid base of the compressibility and resilience (C & R) tester. The initial load acting on the grain surface has been 100 g. The thickness at this load has been measured 60 s after the load has been applied. Subsequent loads have been added and the change in thickness has been recorded 1 min after the addition of each load. Logarithm of leather thickness (Y axis) has been plotted against logarithm of load (X axis).

2.4. Reflectance and colour difference measurements of leathers

The matched pair control and experimental leathers made in this study have been subjected to the reflectance measurements using Milton Roy Colour mate HDS instrument. Colour measurement (L, a, b, h and C) have been recorded and the total colour difference (ΔE) and hue difference (ΔH) have been 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 = overall colour difference; ΔL = lightness difference; Δa and Δb = difference in a and b values, where 'a' represents the red and green axis and 'b' represents the yellow and blue axis; ΔH, hue difference; ΔC, chromaticity difference.

$\Delta L < 0$ sample is darker, $\Delta L > 0$ sample is lighter,
$\Delta a < 0$ sample is greener, $\Delta a > 0$ sample is redder,
$\Delta b < 0$ sample is bluer, $\Delta b > 0$ sample is yellowier,
$\Delta c < 0$ 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

2.5. Fastness to artificial light of leathers

The matched pair control and experimental samples have been cut from the official sampling position [26]. The leather specimens have been conditioned at 80 ± 4 °F and 65 ± 4% R.H. for 48 hr. The resistance of the colour of the optimized experimental and control leathers to an artificial light source, Xenon lamp has been measured using IS 6191–1971 (LF: 4) method [27]. One side of the leather has been exposed to light from Xenon arc under prescribed conditions for 20 hr, along with eight dyed Blue wool standards having increasing levels of fastness. Black panel temperature has been maintained at 63 ± 1 °C and the relative humidity has been 30 ± 5%. Fastness has been assessed by comparing the fading of crust leathers with that of the standards, from standard 1 (very low light fastness) to standard 8 (very high light fastness), where each standard being approximately twice as fast as that preceding one. Rating has been given on a scale of 1–8 points, where higher points indicate better fastness. The same methodology has been repeated for control and matched pair experimental leathers aged for 6 months.

2.6. Physical testing and hand evaluation of leathers

Samples for various physical tests from matched pair control and experimental crust leathers have been obtained as per IUP method [26]. Specimens have been conditioned at 80 ± 4 °F and 65 ± 2% R.H. over a period of 48 hr. Physical properties such as tensile strength, % elongation at break, tear strength and grain crack strength have been examined as per the standard procedures [28,29]. Matched pair experimental and control crust leathers have been assessed for softness, fullness, grain flatness, grain smoothness, grain tightness (break) and general appearance by hand and visual examination. The leathers have been rated (on a scale of 1–10 where 1 is poorest and 10 is the best) for each functional property by experienced tanners.

2.7. Analysis of spent liquors from vegetable tanning

Spent tan liquors from matched pair experimental and control tanning experiments have been collected and analyzed for COD, chlorides and TS (dried at 103–105 °C for 1 hr) as per the standard procedures [30].

2.8. Analysis of exhaustion of vegetable tanning spent liquors

Spent tan liquors from control, all pickle-less enzymatic tanning experiments and matched pair processes spent tan liquors have been analyzed for % uptake of vegetable tannins [30]. Wattle has been used for preparation of standard graph. Known concentrations of wattle have been prepared. The sample has been neutralised to pH of 7 using 0.1 N NaOH. From the known concentrations of the sample, 0.5 ml has been taken and 0.5 ml water has been added and made upto 1 ml. 5 ml of solution A (Solution A: 1 ml of 1% $CuSO_4$ and 2% Sodium Potassium Tartrate in the ratio of 1:1 + 50 ml of 1 g NaOH in 150 ml water and 5 g Na_2CO_3 added and made up to 250 ml) has been added to the samples and allowed to stand for 10 min. Then 0.5 ml of follins reagent (Folin-Ciocalteu reagent – folin:water (1:1)) has been added to the sample and allowed it to stand for 30 min and the absorbance has been measured at 660 nm

using *UV–visible spectrophotometer* (*Hitachi, Japan*). The respective absorption value for the particular concentration has been plotted. From this plot, the amount of tannins present in the waste liquor (after filtering) has been analyzed with the same procedure mentioned above and the exhaustion has been calculated as follows:

$$\%\ \text{exhaustion} = \frac{(\text{amount of tannin offered} - \text{amount of tannin present in the effluent})}{\text{amount of tannin offered}} \times 100$$

2.9. Scanning electron microscopic analysis of leather samples

Samples from matched pair experimental and control leathers have been cut from the official sampling position [26]. The samples have been first washed in water. Subsequently, the samples have been gradually dehydrated using standard procedure [31]. All specimens have been then coated with gold using an Edwards E306 sputter coater. Leica Cambridge Streoscan 440 scanning electron microscope has been used for the analysis. The micrographs for the grain surface and cross-section have been obtained by operating the SEM at an accelerating voltage of 20 kV with different magnification levels.

2.10. Field trials at commercial tannery

Enzyme assisted experiments have been carried out at a commercial tannery to adapt the enzyme assisted tanning process for better exhaustion of vegetable tannins and also to adjust their quality requirements and production schedules. Twenty goatskins have been taken and processed using optimized enzyme assisted vegetable tanning process at onsite conditions of the tannery. The leathers have been piled for 24 hr after tanning trials. Experimental and conventional vegetable tanned leathers from their conventional production process have been converted into crust upper leathers using their conventional post-tanning process. Spent tan liquors, vegetable tanned and post-tanned/crust leathers of conventional and enzyme assisted process have been analyzed for different parameters as described in Sections 2.6–2.8 to study the efficacy of the system and find its suitability at commercial scale.

3. Results and discussion

3.1. Laboratory scale studies

3.1.1. Optimization of process parameters for enzymatic pickle-free vegetable tanning

20% concentration of vegetable tannins has been chosen for obtaining good quality leather. Experiments have been conducted to optimize the important parameters of enzyme treatment, viz., the percentage enzyme concentration and the duration of enzyme treatment. The other parameters such as temperature and pH have been kept constant at 30 °C and 4.5 respectively as the protease used for the study has optimum activity under these conditions.

3.1.2. Optimization of concentration of treatment of enzymes

The exhaustion of vegetable tannins and shrinkage temperature of the tanned leather at different concentrations of acid protease treatment are given in Table 1. From the table, it is seen that the uptake of vegetable tannins by the pelt increases with increasing concentration of acid protease up to 0.2% concentration of enzyme offered. There has been no significant increase in the shrinkage temperature and fixation of the vegetable tannins above 0.2% concentration. Hence, 0.20% percentage concentration appears to be sufficient for maximum uptake of vegetable tannins and has been taken as the optimized concentration for better exhaustion of vegetable tannins. The shrinkage temperature of the leathers has been found to 86 °C and exhaustion of vegetable tannins at this concentration is found to be 97%.

3.1.3. Optimization of duration of treatment of enzymes

The fixation of vegetable tannins to the leather in terms of percentage exhaustion and shrinkage temperature for various treatment durations are given in Table 2. It is evident from the table that the uptake of vegetable tannins increases gradually with time. It requires minimum of 30 min to bring about significant exhaustion in tanning bath. At time intervals beyond 30 min, there has been slight increase in exhaustion of vegetable tannins, however longer time of exposure may result in decrease in the strength of the matrix due to high fibre splitting. Hence 30 min of enzymatic treatment has been taken as the optimum duration. The treatment of acid protease for time period of 30 min resulted in vegetable tannins uptake of 97%. Hence pH 4.5 at 0.20% concentration of acid protease for 30 min has been taken as optimized conditions for better exhaustion of vegetable tannins.

3.1.4. Mechanism of pickle-less enzyme assisted vegetable tanning of leathers

The approach of developing pickle-less enzymatic vegetable tanned leathers is based on the concept that the enzyme helps in opening up of the fibrous collagen network, there by enhancing the diffusion of vegetable tannins into the leather matrix and also the contact surface areas in the enzyme treated collagen available for interaction with vegetable tannins increases. The primary objective of this work is to open up the collagen matrix by enzyme treatment for improved exhaustion of vegetable tannins and the secondary objective is to decrease TDS and chlorides using pickle-less vegetable tanning by avoiding the use of salt in the pickling process. Vegetable tannins result in surface fixation or case hardening if used directly after deliming. Hence, the pH of the pelts has been reduced to pH 4.5–5.0 using oxalic and organic non-swelling acid. Also the pH of vegetable tannin liquor will make the pH of the residual float in the drum to be 4.5–5.0 and hence inner cross-section of the pelt cannot have pH less than 4.5 or 5.0. This will result in better penetration of vegetable tannins as the collagen matrix will also be well opened up by the enzymatic treatment. An interesting observation has also been made regarding the duration of tanning. The time taken for penetration and completion of

Table 1
Exhaustion of vegetable tannins and shrinkage temperature of the leathers tanned with varied concentration of enzymes

Process parameters	% Acid protease	% Exhaustion of vegetable tannins	Shrinkage temperature, T_s (°C)	Time taken for penetration of vegetable tannins (hr)
Control		85.2 ± 2.14	82 ± 2	6.5
Experimental concentration[a]	0.1	89.5 ± 1.68	82 ± 2	5.5
	0.2[b]	97.4 ± 1.91[b]	86 ± 1[b]	4.5[b]
	0.3	97.3 ± 0.51	86 ± 1	4.0
	0.4	98.5 ± 1.88	86 ± 1	3.8
	0.5	98.6 ± 1.41	86 ± 1	3.4

[a] Enzyme treated at pH 4.5, 30 min.
[b] Optimized experimental conditions – 0.2% enzyme, pH 4.5, 30 min.

Table 2
Exhaustion of vegetable tannins and shrinkage temperature of the leathers tanned at varied duration of treatment of enzymes

Process parameters		% Exhaustion of vegetable tannins	Shrinkage temperature, T_s (°C)	Time taken for penetration of vegetable tannins (Hrs)
Experimental duration[a] (min)	15	90.2 ± 0.88	82 ± 1	5.4
	30[b]	97.3 ± 1.84[b]	86 ± 2[b]	4.5[b]
	45	98.4 ± 1.45	86 ± 2	4.0
	60	98.3 ± 1.82	86 ± 2	3.8

[a] Enzyme treated at 0.20%, pH 4.5.
[b] Optimized experimental conditions – 0.2% enzyme, pH 4.5, 30 min.

tanning is comparatively lower than the time taken for conventional vegetable tanning as given in Tables 1 and 2. This is due to the fact that the diffusion of vegetable tannins is faster in enzyme assisted process. The mechanism of this approach is based on the fact that the vegetable tannin particulates with higher molecular weight [15] can penetrate rapidly after enzymatic treatment, as the fibre matrix in the pelt is well opened up. It has been reported from the fixation profile of vegetable tannin at various pH values that penetration is facilitated at pH 4.5–5.0 [32] while fixation is promoted at lower pH 3.5–3.7 and maximum fixation of vegetable tannins on collagen occurs at pH around 3.5 [33]. Since the pH of the aqueous solution of vegetable tannin in process solution is around 4.5–4.7, the penetration of vegetable tannins is facilitated at this pH [34]. The acid protease used in this study has optimum activity at the same pH, 4.5. The opened up matrix by enzyme treatment and the pH of leather prevents the surface fixation on the skins and aids proper penetration of vegetable tannins. Finally, the pH is adjusted to 3.5–3.7 using minimum amount of formic acid to fix maximum and the well penetrated vegetable tannins.

4. Performance of the leathers

4.1. Effect of pickle-less enzymatic treatment on surface colors

Fig. 1 shows the reflectance measurement data vs visible wavelength for both control and matched pair experimental crust leathers. The absorbance maxima has been 450 nm for both control and matched pair experimental leathers. Absorbance maximum is the wavelength at which, the reflectance is minimum as seen in Fig. 1. Since the absorbance maxima is similar for both control and matched pair experimental leathers, it can be concluded that there is no significant variation in the colour or shade between control and matched pair experimental leathers. The *L*, *a*, *b*, *C*, *H* values of control and matched pair experimental leathers and the colour differences are given in Table 3. It is observed that the matched pair experimental samples show total colour difference (ΔE) of 3.592 compared to control leather, which means the overall colour difference is negligible. The values of 'ΔL', 'ΔC', 'ΔH', 'Δa' and 'Δb' are 2.481, 3.349, 0.827, 0.441 and 3.089 respectively. The values indicate that the matched pair experimental leather possesses negligible difference in the colour measurement parameters with that of control leather. These results are similar to that observed in the visual assessment. The *L*, *a*, *b*, *C*, *H* values of the matched pair control and matched pair experimental leathers and the colour differences are given in Table 3. It is observed that the matched pair experimental samples show total colour difference (ΔE) of 3.592 compared to control leather, which means the overall colour difference is negligible. The values of 'ΔL', 'ΔC', 'ΔH', 'Δa' and 'Δb' are 2.481, 3.349, 0.827, 0.441 and 3.089 respectively. The values indicate that the experimental leathers possess negligible difference in the colour measurement parameters with that of control leather. The results are similar to that observed in the visual assessment as given in Table 6.

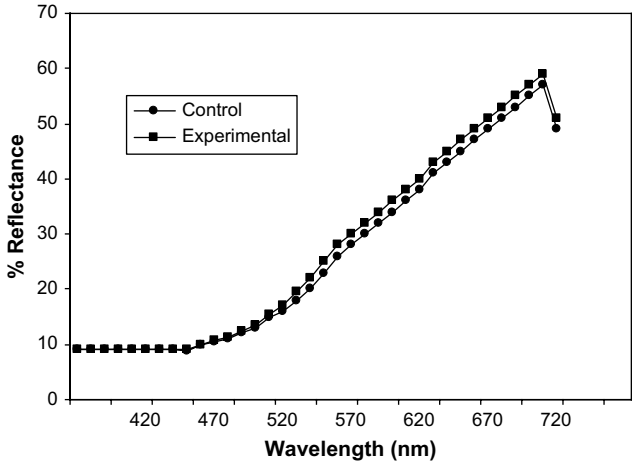

Fig. 1. Plot of percentage reflectance vs wavelength for control and matched pair experimental leathers.

4.2. Effect of pickle-less enzymatic treatment on light fastness

Vegetable tannins are well known to reduce the light fastness characteristics of leathers. Hence fastness to light and rubbing of both control and experimental crust leathers under artificial light (Xenon lamp) has been studied and is given in Table 4. The leathers from both control and matched pair experimental processes exhibit similar values that are equivalent to the Blue wool standards 4.5 (given in parenthesis in Table 4). The effect of ageing of crust leathers for six months on the fastness properties has been also studied and the values are given in Table 4. It is observed that both control and experimental samples do not show significant change in the fastness properties upon ageing.

4.3. Effect of pickle-less enzymatic treatment on strength properties

It is essential to analyze the strength characteristics upon treatment with enzymes as one may expect, opening up of fibre structure influencing the strength of the leather. Tensile, tear strength and grain crack tests have been carried out for the matched pair control and matched pair experimental crust leathers both along and across the backbone line. The mean of the values corresponding to along and across backbone has been calculated. The average values are given in Table 5. The values of various strength properties of matched pair experimental leathers are found to be comparable to that of the control leathers.

5. Assessment of opening up of fibre bundles: implicit approach

5.1. Opening up of fibre bundles through scanning electron microscope

Scanning electron micrograph analysis has been performed to investigate the grain characteristics and fibre structure of the

Table 3
Measurement of colour difference of control (C) and matched pair experimental (E) leathers

Parameters	*L*	*C*	*H*	*a*	*b*	–
C	53.718	13.612	36.103	38.141	68.112	
E	57.987	14.916	39.564	42.205	67.142	
	ΔL	ΔC	ΔH	Δa	Δb	ΔE
Total colour difference	d = 2.481	S = 3.349	I = 0.827	MR = 0.441	MY = 3.089	3.592

Table 4
Fastness to rubbing and light fastness characteristics of control (C) and matched pair experimental (E) leathers

Sample	Before ageing			After ageing		
	Wet rubbing	Dry rubbing	Light fastness[a]	Wet rubbing	Dry rubbing	Light fastness[a]
C	4.0–4.5	4.5–5.0	3.5–4.0 (4.5–5.0)	4.0–4.5	4.5–5.0	3.0–3.5 (4.0–4.5)
E	4.5–5.0	4.5–5.0	3.5–4.0 (4.5–5.0)	4.5–5.0	4.5–5.0	3.0–3.5 (4.5–5.0)

[a] Value in parenthesis indicates the corresponding blue wool standard.

tanned leathers. The scanning electron micrographs of tanned samples from control and experimental tanning processes showing the cross-section at a magnification of 600X are given in Fig. 2a and b, respectively. Both the control and experimental leathers showed compact fibre structure, which is well coated that is characteristic of vegetable tanned leather. It is clear from the photomicrograph of the experimental leather, enzyme assisted process has opened up the fibre matrix and also well coated by vegetable tannins. There is also no major change or destruction of the fibre structure in the enzyme assisted processed leather.

5.2. Softness measurements on pickle-less enzymatic treatment

The enzymatic vegetable tanning system employs vegetable tannins at an opened up fibre structure at pH of 4.0–4.5, which resulted in uptake of vegetable tannins and results in better quality leathers. Vegetable tannins are known to produce hard leathers and generally employed for producing heavy duty leathers. Hence, it is important to evaluate the extent of softness on the final leather. Quantitative assessment of softness for both control and matched pair experimental leathers has been made through compressibility measurements. The plot of logarithm of thickness vs. logarithm of load for the control and matched pair experimental leathers exhibited linear fit [35] as shown in Fig. 4a and b. The corresponding equation of the line has been obtained. Based on the equation negative slope angles (compressibility index, CI) have been calculated and the values are 8.89° and 8.47° for the control and matched pair experimental leathers. Higher values signify more softness in the leather. It is evident that the experimental leather exhibit comparable negative slope (CI) angle with that of control leather. This shows that the experimental leathers concentration comparable but slightly lower softness to that of the control leathers.

5.3. Bulk properties of leathers - hand evaluation of leathers

It is known that the results of hand and visual evaluation method are not objective but subjective, which varies from person to person. Yet it could be taken as reliable, if carried out by experienced persons and averaged. The hand and visual evaluations have been done for both tanned and crust leathers. The tanned stage assessment values are shown in Fig. 3 and Table 6. It is seen that the field trial experimental leathers have good bulk properties which are better in comparison to that of control leather. Vegetable tannin patches and case hardening have not been observed for both control and experimental leathers. Grain smoothness is slightly good for control leathers than experimental leathers. Crust leather from both control and experimental processes has been evaluated for various bulk properties by hand and visual evaluation. The average of the rating for the leathers corresponding to experiment has been calculated for each functional property and is given in Fig. 3. Higher numbers indicate better property. The experimental leathers exhibit better fullness compared to control leathers. This is primarily due to improved penetration and fixation of vegetable tannins in the experimental process, compared to control process. Other properties such as softness, grain tightness and smoothness are comparable to that of conventionally processed leathers. The overall appearance of field trial experimental leathers is better than the control leathers.

6. Environmental tolerability

The spent vegetable tan liquor contains highly contaminate matter and it contributes to exorbitantly high COD, dissolved and suspended solids. Hence, it is vital to assess the environmental impact from both conventional and pickle-free enzyme assisted vegetable tanning process. Composite liquor from the conventional process of the field trial has been prepared by mixing spent liquors from pickling and vegetable tanning processes in control. It should be noted that the field trial experimental vegetable tanning process does not have pickling process and hence spent vegetable tan liquor alone has been considered as composite, which contained enzymes in the spent vegetable tan liquor. COD, TS, chlorides and % uptake of vegetable tannin are the parameters that have been chosen for analyzing the environmental impact. The emission loads of control and enzyme assisted spent tan liquor has been calculated by multiplying COD/TS values (mg/l) with volume of effluent (l) per metric ton of raw skins processed. It is seen from Table 7 that the COD, chlorides and TS values of matched pair experimental spent tan liquor are lower than that of control. The emission loads of commercially experimented process have been almost similar to matched pair laboratory experimental results. Partial pickling and vegetable tanning processes contribute nearly 50 kg of TS for processing 1 metric ton of raw skins conventionally. It is evident that the enzymatic vegetable tanning method reduces the COD, TS and chloride loads by 25, 76 and 97%, respectively. The reduction in COD, chlorides and TS loads helps in achieving cleaner vegetable tanning. Especially the reduction of chlorides by 97% is a significant achievement in avoiding pollution due to chlorides. Implementation of pickle-less enzymatic vegetable tanning method could bring considerable change in the tanning industry making the

Table 5
Physical testing data of control (C) and matched pair experimental (E) leathers

Sample	Tensile strength	Extension at break	Tear strength	Grain crack resistance	
	(Kg/cm^2)	%	(Kg/cm)	Load (Kg)	Distension (mm)
C	201 ± 6	55 ± 3	37 ± 4	30 ± 2	11.4 ± 0.4
E	$198' \pm 12$	54 ± 5	35 ± 3	31 ± 3	11.8 ± 0.5

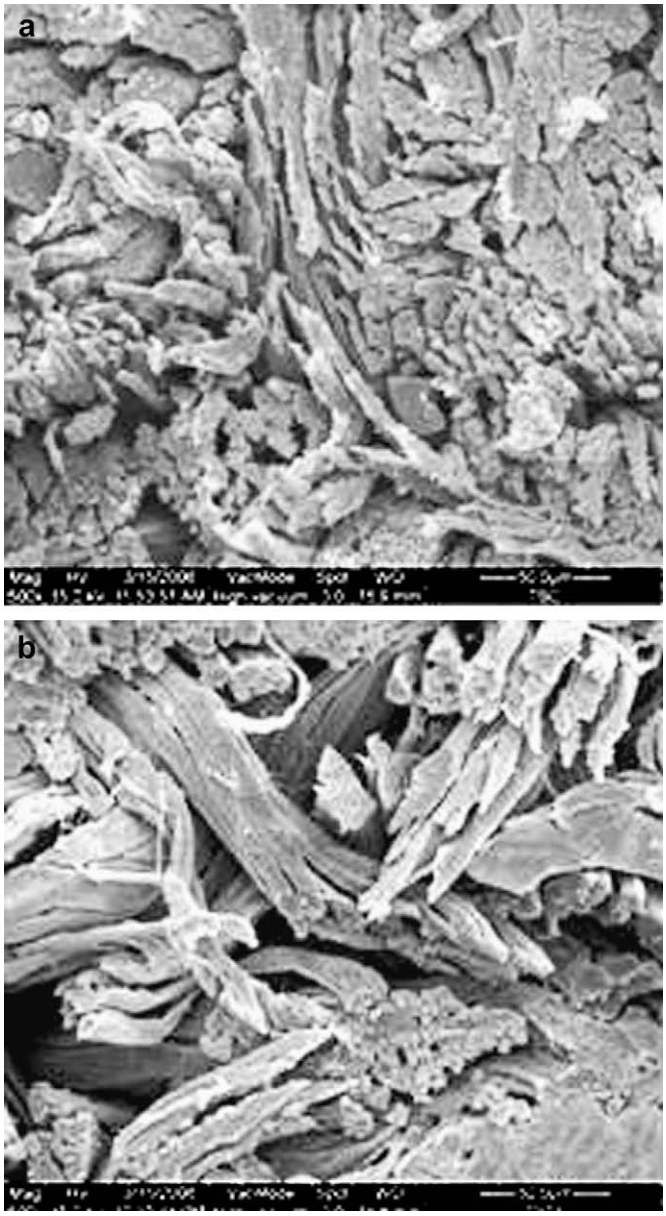

Fig. 2. Scanning electron micrograph of vegetable tanned leather sample showing the cross-section of (a) control at X600 magnification and (b) matched pair experiment at X600 magnification.

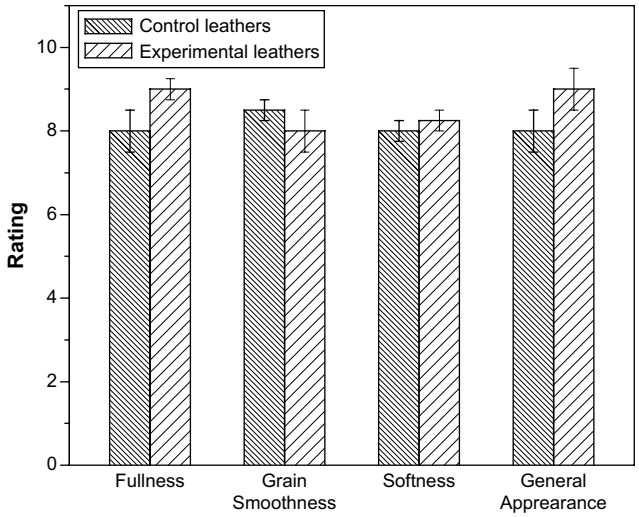

Fig. 3. Graphical representation of organoleptic properties of the control and matched pair experimental leathers.

experimental leather process to have the benefit of zero chromium emission in the composite liquor as well as harmless solid wastes.

6.1. Financial and technical practicality

Development of any new process requires commercial feasibility and cost effectiveness. The commercial field trial experimental process implemented in this work primarily involved the use of biological materials like enzymes in order to achieve lower TDS, chlorides and COD of effluent as well as better quality leather. The total chemical costs for processing 1 ton of goatskins through field trials conventional and experimental process schemes are given in Table 8. It could be seen that the experimental process exhibits higher chemical cost as compared to the control process. The total chemical cost of the conventional vegetable tanning process is about US$ 221.45, assuming partial pickling and 20% wattle GS powder, whereas experimental pickle-less enzymatic vegetable tanning process is about US$ 223.61. Hence, the possible increase in chemical cost is about US$ 2.16, for processing 1 t of raw goatskins. The reduction in discharge of effluent, BOD, COD, TDS, chloride and TS loads would provide additional benefit in effluent treatment costs [36]. Apart from this, the disposal of sludge and waste generated through control-based process causes both ecological and economic concerns. Similarly, the formation of dry sludge can also be avoided. The combination of bio-products (enzymes) in tanning with natural tanning material like vegetable tannins leads to an achievable eco-option to the conventional intricate leather processing. Such eco-option can be followed in both developed as well as developing countries as it has potential to produce natural leathers with near zero pollution load from tanning operations without affecting the leather qualities.

7. Field trials at tannery

The skins processed using the optimized enzyme assisted vegetable tanning system and conventional vegetable tanned leathers have been processed into shoe upper leathers. The leathers from the optimized enzyme assisted commercial tannery processed leathers and conventional tanned leathers from both laboratory and tannery have been compared. The shrinkage temperature, % exhaustion of vegetable tannins and time taken or penetration of tannins has been found to be comparable for enzyme assisted laboratory and commercial trials. It has been observed that the commercial experimental samples showed slight colour difference; the enzyme assisted commercial samples have been slightly darker than the laboratory samples. The fastness properties of the experimental samples did not show significant change. The field trial tested leathers have been found to be comparable to conventional processed leathers. The enzyme assisted tannery processed leathers have been found to be efficient in terms of improved quality of leather in both aesthetic and functional properties and also led to reduction in pollution load with improved cost effectiveness and technical feasibility.

8. Conclusions

Enzymes have been used in tanning as an eco-friendly approach for achieving better exhaustion of vegetable tannins. The approach is based on the concept that the enzymes act as biocatalysts in

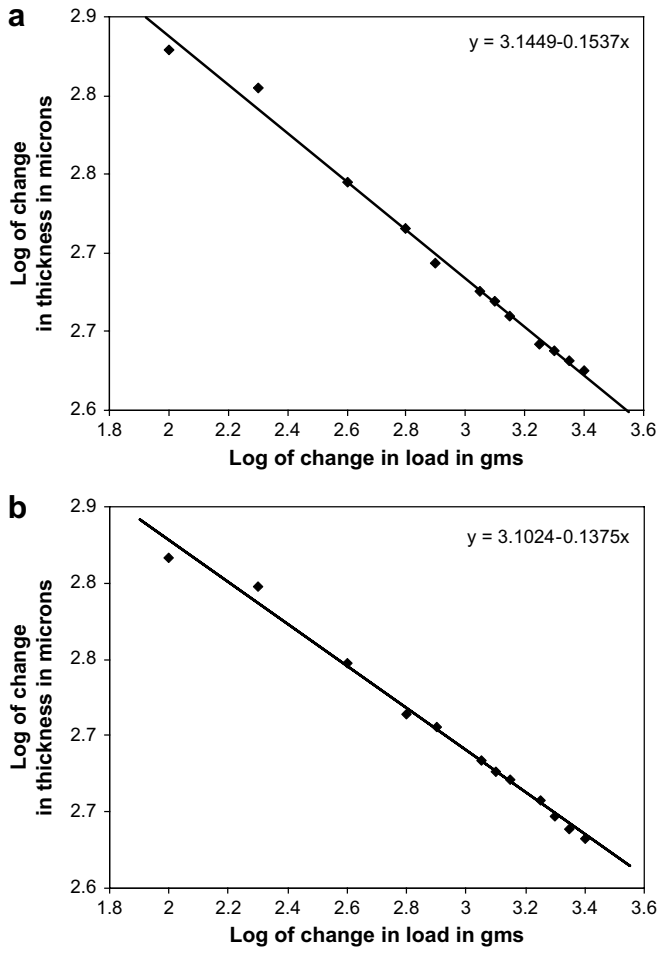

Fig. 4. Plot of log of change in load vs log of change in thickness for (a) control and (b) matched pair experimental leathers.

Table 6
Visual assessment data for control and matched pair experimental leathers after tanning

Parameters	Control	Experimental
Tannin patches	Nil	Nil
Grain smoothness	8.5	8.0
Fullness	8.0	9.0
Colour of leather	8	9.0
Case hardening	Nil	Nil
General appearance	8	8.5

Table 7
Environmental impact of control and experimental processes

Parameters	Control[a]	Experimental[b]
% Uptake of tannin	85.4 ± 2.14	97.6 ± 0.88
Volume of effluent (l/metric ton of raw skins)	605	590
COD	19989 ± 210	14925 ± 180
TS	84548 ± 620	20956 ± 250
Chlorides	24320 ± 80	728 ± 30
Emission load (Kg/metric ton of raw skins)		
COD	11.8	8.7
TS	49.9	11.8
Chlorides	14.3	0.42

[a] Composite of spent pickle and tan liquor of field trial.
[b] Spent tan liquor alone considered as composite field trial.

Table 8
Cost estimates of the conventional and experimental tanning processes

Chemicals/bio-products	(US$/t of raw skins)	
	Control	Experimental
Lime	24.98	24.98
Sodium sulfide	16.82	16.82
Ammonium chloride	1.38	1.38
Sodium chloride	6.32	
Sulfuric acid	3.21	
Wattle GS powder	168.74	168.74
Oxalic acid		4.56
Acid bate		6.13
Total	221.45	222.61

opening up the fibrous leather network, which enhance the diffusion of vegetable tannins into the leather matrix and also the contact surface area in the leather exposed for interaction with vegetable tannins increases. 0.20% concentration of enzyme, at pH of 4.5 for 30 min with 20% vegetable tannins has been found to be optimum with respect to the uptake of vegetable tannins and achieving significant hydrothermal stability. The hydrothermal stability of enzymatically processed leather is higher compared to conventionally processed leather. The enzyme treatment resulted in leathers with fullness and no major significant variation in the properties as compared to the conventional vegetable tanned leathers. The scanning electron micrographs of enzymatic vegetable tanned leathers exhibited better opening up of fibre bundles as well as separation of fibres.

One of the main advantages of the current approach is the unprecedented environmental benefits achieved. The enzymatic vegetable tanning effluent shows reduction in TS, chloride and COD loads by 76, 97 and 25% as compared to control process, apart from achieving better uptake of vegetable tannin and quality improvement in finished leathers. The experiment tanning process has the cost advantage due to net saving from the reduced effluent treatment cost. In general, this approach provides an abundant scope for decreasing the pollution load. Analyzing the various results from the experiments, an outlook at the use of enzymes in tanning process is indispensable for vegetable tanning. The adoption of pickle-less enzymatic vegetable tanning method could bring significant change in the tanning industry by making it environmentally sustainable in the context of cleaner production.

Acknowledgments

Authors thank M/S Karthick Creation, Chennai, India, manufacturers of different kinds of leathers for facilitating us to implement the outcome of this enzyme assisted vegetable tanning process. The authors also thank Ms. A. Yasothai, for her timely help in carrying out the experiments at M/S Karthick Creation.

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