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ScienceDirect
Chemosphere 70 (2008) 1015–1024
CHEMOSPHERE
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Ecofriendly lime and sulfide free enzymatic dehairing of skins and hides using a bacterial alkaline protease

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Received 30 April 2007; received in revised form 11 September 2007; accepted 12 September 2007
Available online 5 November 2007

Abstract

The ever-increasing attention to the environmental impact of leather industry has necessitated the development of enzyme-based processes as potent alternatives to pollution causing chemicals. In this study, a hair saving process is developed for dehairing of skins and hides using a bacterial alkaline protease preparation, completely eliminating the use of lime and sulfide. To evaluate the efficacy of the enzymatic process, comparative studies have been carried out with two controls; a conventional lime–sulfide process and enzyme-assisted process using commercial dehairing enzyme with reduced quantities of lime and sulfide. The developed process requires a shorter duration of 6 h for complete dehairing of skins and hides than control groups and also, it avoids the use of silicate carriers since the enzymatic dehairing is carried out by dip method. Histological and scanning electron microscopic analyses of the dehaired pelts obtained from enzymatic process reveal complete removal of hair and epidermis with moderate opening up of fiber structure in both dermis and corium. Moreover, the collagen is not damaged and resulting in a leather of good quality. The developed process has resulted in a remarkable reduction of effluent load in terms of biochemical oxygen demand, chemical oxygen demand, total dissolved solids and total suspended solids. Physicochemical studies conclusively show that the leathers produced by enzymatic process are equivalent to or better than that obtained by control systems. Thus, the developed enzymatic process offers immense potential for greener mode of dehairing of skins and hides in leather industry coupled with environmental excellence.

Keywords: Protease; Leather; Pollution; Collagen; Liming; Proteoglycan

1. Introduction

Among the pretanning processes in leather manufacture, an environmentally constrained operation is dehairing wherein the sludge forming saturated lime liquor in conjunction with toxic sharpeners such as sodium sulfide are employed in high concentrations. Conventional dehairing processes employing lime and sulfide contribute to 50–60% of total pollution load in terms of biochemical oxygen demand (BOD), chemical oxygen demand (COD), total dissolved solids (TDS) and total suspended solids (TSS) besides a high alkaline effluent of 100% toxicity (Taylor et al., 1987; Marsal et al., 1999). Hydrogen sulfide, emanating from the dehairing process is proven to be fatal even in concentrations as low as 200 ppm (Hannah and Roth, 1991; Roth et al., 1995). The extensive use of hazardous sulfide not only leads to unfavorable consequences on the environment but also undermines the efficacy of the effluent treatment plants (Davies, 1997). Hence, rationalization of the dehairing process by systemic use of proteases in place of lime and sulfide becomes an issue of primary importance in leather processing (Puvanakrishnan and Dhar, 1988). This ultimately leads to a substantial reduction of effluent load and toxicity in addition to improvement in leather quality (Puvanakrishnan and Dhar, 1986).

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0045-6535/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.chemosphere.2007.09.036

Currently, many commercial enzyme preparations are being used for enzyme-assisted dehairing processes; but the use of lime and sulfide is not completely eliminated (Frendrup, 2000). Proteolytic enzymes attack the collagen of the grain layer to a certain degree, thereby imparting damage to the grain structure (Cantera et al., 1996). Although many reports are available for dehairing, either free of lime or sulfide and or both, none of these methods have found commercial application in tanneries (Taylor et al., 1987; Kamini et al., 1999; Macedo et al., 2005; Thanikaivelan et al., 2005). Factors impeding the implementation of enzymatic dehairing include the cost of production of enzymes, scale up and bioprocessing, suitability and efficacy of enzyme preparation to process different raw materials such as skins and hides without damaging the collagen by hydrolysis and the inability of the enzyme to remove fine hair. Some of the reported lime and sulfide free enzymatic dehairing processes adopt either an enzyme carrier such as sparingly soluble kaolin in significant quantities (Dayanandan et al., 2003) or soluble silicates (Saravanabhavan et al., 2005). This may contribute to a significant rise in COD and TDS and TSS of effluent. Among the numerous studies undertaken to develop an alternative to dehairing processes, dehairing by proteases from microbial sources exhibits advantages in terms of scalability, efficiency and pollution control.

Short duration enzymatic dehairing using a protease preparation avoids the problem of collagen damage and thereby eliminates the constraint of high degree of control over the process. Hitherto, there is no detailed study on short duration enzymatic dehairing process for skins and hides avoiding the use of above chemicals. This study describes a process for hair saving enzymatic dehairing of skins and hides by dip method in a short duration using an effective bacterial alkaline protease preparation that completely obviates the use of lime, sulfide and other silicates reported earlier. The efficacy of the developed process is substantiated by histological studies, SEM analysis of dehaired pelts and chrome crusts, physicochemical studies on dyed chrome crusts and analysis of effluent from dehairing process. Also, studies have been taken up to find out whether the process results in a significant reduction of effluent load when compared to conventional and enzyme-assisted dehairing processes.

2. Materials and methods

2.1. Materials and dehairing experiments

Freshly flayed wet-salted goatskins and cowhides were used for dehairing experiments. SPIC Biodart (a bacterial protease based commercial dehairing enzyme) from Southern Petrochemical Industries Corporation Ltd., Chennai, India was used in the enzyme-assisted dehairing process. Chemicals used in leather processing and biochemical analysis were of commercial and analytical grade, respectively. Protease used for enzymatic dehairing was obtained from *Bacillus subtilis* MTCC 6537, as identified by Microbial Type Culture Collection (MTCC), Institute of Microbial Technology, Chandigarh, India and it was deposited in MTCC under Budapest Treaty. This strain was isolated from the effluent obtained from KAR Tannery, Dindigul, India. Medium components used for the production of protease as well as salting out were of commercial grade. Pilot scale protease production was carried out by employing a 300 l fermentor (Bioengineering AG, Switzerland) with a working volume of 200 l. The medium (pH 6.7 ± 0.1) constituents were glucose – 0.5%, casein – 0.5%, soyabean meal – 1.5%, NaCl – 0.1%, $MgSO_4$ – 0.025%, KH_2PO_4 – 0.04% and $CaCl_2 \cdot 2H_2O$ – 0.02% and a suitable antifoam agent. 5% of a 24 h bacterial culture, which was developed in a 20 l fermentor containing the above-mentioned medium, was used as seed inoculum. Briefly, fermentation was carried out at 30 °C by maintaining aeration around 0.5–0.7 vvm and agitation at 150 rpm. At the end of 30 h, the culture medium was harvested and centrifuged at 10000*g* using continuous centrifuge to remove the biomass. This cell free broth was subjected to ultrafiltration using 10-kDa membrane (Millipore) to obtain about 10-fold concentrate. Further, this concentrate was subjected to salting out employing 60% ammonium sulphate and the resulted precipitate was lyophilized. This lyophilized protease preparation having an activity of 750 U g^{-1} was used in this study. One unit of protease activity was defined as the liberation of one mg tyrosine equivalent of casein substrate in 10 min using tyrosine standard.

Three different groups of large-scale dehairing experiments viz., conventional lime–sulfide process, enzyme-assisted process using a commercial enzyme and enzymatic process free of lime and sulfide were carried out. These processes were represented as negative control, positive control and experimental groups, respectively (Table 1).

Ten wet-salted goatskins and 10 cowhides, each weighing approximately 2.5 and 12 kg, respectively, were initially soaked with water for 6 h employing a float of 300% water. Prior to dehairing step, all the soaked skins and hides were cut along the backbone line into two halves namely right and left, and numbered. The soaked weight of all right and left halves was noted.

All the left halves of skins and hides were used for experimental dehairing process using protease obtained from *B. subtilis*. Among the right halves of skins and hides, half of them were used for conventional dehairing and the rest were used for enzyme-assisted dehairing. Thus, comparison of experimental left halves with corresponding control right halves was made to evaluate the efficacy of the developed experimental enzymatic dehairing against control systems.

For the conventional process, 10% lime and 2% sodium sulfide were added along with 10% water and the paste thus prepared, was applied on the flesh side, left for 18 h at ambient temperature (28–32 °C) and dehaired using conventional beam and knife method. Similarly, 1.5% enzyme, 5% lime, 0.5% sodium sulfide and 10% water were used to

Table 1
Details of the enzymatic process in comparison with conventional and enzyme-assisted systems

Process	Goatskins			Cowhides		
	Conventional	EA	Experimental	Conventional	EA	Experimental
Soaking						
Wet salted skins/hides	1000	1000	1000	1000	1000	1000
Water	3000	3000	3000	3000	3000	3000
Soaked skins/hides	1160	1160	1160	1180	1180	1180
Green fleshed hides	–	–	–	1040	1040	1040
Dehairing						
Mode of dehairing	Paste and pile	Paste and pile	Dip method	Dip method	Dip method	Dip method
Water	116	116	1160	1040	1040	1040
Lime	116	58	–	104	62.4	–
Sodium sulfide	23.2	5.8	–	31.2	20.8	–
Sodium carbonate	–	–	2.3	–	–	4.2
Enzyme	–	17.4	11.6	–	20.8	20.8
Effluent	750[a]	750[a]	830	730	770	795
Dehaired pelts	880	865	860	920	890	875
Dry hair	30	33	38	–	–	22
Reliming						
Water	880	865	860	920	890	875
Lime	88	86.5	86	92	89	87.5
Effluent	610	575	550	605	560	530
Relimed pelts	1045	1020	1025	1090	1075	1060
Fleshed weight	820	825	825	940	930	940

Weight of the above components was presented in kg.
EA – Enzyme Assisted.
[a] Water used for washing.

make a paste for enzyme-assisted process. For experimental group, optimized dip method was adopted for goatskins, wherein the soaked skins were suspended in dehairing float of pH 7.5–9.0 comprising 1% enzyme, 100% water and 0.2% sodium carbonate for pH adjustment. The float was left for 6 h and dehaired. All the percentages were based on soaked weight (Table 1).

Dehairing of control and experimental hides was performed using dip method. Soaked halves of control and experimental hides were subjected to green fleshing wherein the flesh tissue underlying the *corium stratum* was removed to some extent without causing any damage to corium matrix. For conventional process, the hides were dipped in 100% water float comprising 10% lime and 3% sulfide for 18 h and dehaired the next day. Similarly, 2% enzyme, 6% lime, 2% sulfide and 100% water were employed for positive control. For experimental group, hides were suspended in a 100% water float of pH 7.5–9.0, containing 2% enzyme and 0.4% sodium carbonate. The float was left for 6 h and dehaired. All the percentages were based on defleshed weight (Table 1).

Subsequently, both controls and experimental groups of dehaired pelts were relimed using 100% water with 10% lime (based on the weight of dehaired pelts) for 2 d with occasional handling. The relimed control pelts were then delimed, bated and pickled in a drum whereas the relimed experimental pelts were pickled without bating. The pickled skins and hides were finished as dyed crusts as per conventional procedures.

2.2. Histological analysis

Samples of 1 cm^2 were cut from identical official sampling portions (Official Methods of Analysis, 1965) of the corresponding dehaired pelts of skins and hides, washed thoroughly and were fixed in formal saline (prepared by adding 0.9 g sodium chloride in 100 ml of 10% formaldehyde solution). Samples were then dehydrated with ethanol series. After embedding in paraffin block, sections of 6 μm were obtained using microtome and they were stained using hematoxylin and eosin to examine the histological features.

2.3. Scanning electron microscopic (SEM) studies

Samples were cut from experimental and control dehaired pelts and dyed crusts as mentioned earlier. Samples of dehaired pelts were washed, fixed in buffered formalin, dehydrated using a graded methanol series and finally with acetone. Subsequently, acetone was completely replaced by flushing with Freon Mafron R-22 gas and then samples were freeze-dried. The dried samples were cut into 3 mm thickness, mounted vertically and horizontally on copper stubs in order to view cross and surface details, coated with 20 nm of gold by direct current sputtering and examined in a FEI Quanta 200 Environmental SEM unit operated at an accelerating voltage of 12 kV. The dyed crust samples were directly taken for gold coating and subsequent SEM analysis.

2.4. Physicochemical, objective and softness assessment of leathers

For physical tests, the finished leathers of experimental and control groups were conditioned for 3 d at 26 ± 2 °C and 65 ± 2% relative humidity. Then, samples were cut from identical positions of the two counterparts as per IULTCS method (IUP 2, 2000). Physical testing (IS 2961, 1964; SLP 9, 1996; IUP 6, 2000; IUP 8, 2000) as well as chemical analysis of dyed crusts were carried out for control and experimental groups after taking samples from the official sampling position. The Cr_2O_3 content was determined as per the standard procedure (IUC 8, 1998). Samples were analyzed for their moisture content and all the chemical constituents were expressed on dry weight basis. All the dyed crusts were assessed for general appearance, grain tightness, fullness, softness, grain flatness and dyeing characteristics. Softness of leathers was numerically measured based on compressibility (Lokanadam et al., 1989).

2.5. Analysis of effluent from dehairing process

The effluent from the dehairing process for control and experimental groups of skins and hides were analyzed for various parameters such as BOD, COD, TDS, TSS, calcium and sulfide (APHA, 1989). Sulfide and calcium content in the effluent of experimental group were not estimated since the developed process was free of sulfide and lime.

3. Results and discussion

3.1. Dehairing experiments and material balance

Preliminary trials were taken up to standardize the optimal conditions such as pH, enzyme concentration and duration for complete dehairing of skins and hides using the bacterial protease preparation by dip method (data not shown). The optimal conditions for the production of dehaired pelts from goatskins and cowhides by dip method for 6 h were 1% enzyme, 0.2% sodium carbonate and 100% float at pH 7.5–9.0 and 2% enzyme, 0.4% sodium carbonate and 100% float at pH 7.5–9.0, respectively. Visual observation studies on the experimental dehaired pelts from goatskins and cowhides apparently revealed complete absence of fine hairs and epidermis and the experimental pelts were whiter than the controls due to elimination of sulfide in the process. It was reported earlier that the removal of residual fine hairs remained the greatest obstacle to the development of hair saving enzymatic process (Paul et al., 2001).

Process details were presented for processing 1000 kg of salted skins and hides (Table 1). Paste method was adopted for conventional and enzyme-assisted dehairing of goat skins. However, dip method was employed for experimental dehairing since the enzyme did not have action upon hair components. In addition, the bath did not contain sulfide and recovery of intact hair was possible after dehairing. Microscopic analysis confirmed that the hair recovered from experimental dehairing was intact in both skins and hides and the hair was not damaged. Presence of hair root was noticeable in the dislodged hair of the experimental system whereas it was absent in the control dehairing systems (Fig. 1a–e). This could possibly explain that control systems might leave residual hair on the grain surface since the hair recovered from these systems had damaged ends. The structure of hair from the conventional dehairing of cow hide was not shown as it was completely destroyed in the bath. The effluent derived from experimental dehairing of skins and hides consisted of a complex mixture of degraded noncollagenous proteins, proteoglycans, insignificant amount of enzyme, dislodged intact hair and epidermal keratinous substances. The effluent from controls contained the above constituents besides reacted and unreacted products of lime and sulfide and, in addition, hair was present in pulped form due to degradation in alkaline bath containing hair destructive sulfide.

On the other hand, experimental process for both skins and hides yielded significant quantity of intact hair of good quality that could be a value added saleable byproduct (Table 1). Recovered intact hair from skins and hides is used in the manufacture of felt, organic fertilizer and poultry feed stuff. Also, the recovered hair when subjected to hydrolysis by thermal, biological and chemical means has an array of applications ranging from biogas generation, regeneration of keratins, recovery of melanin for the preparation of suntan lotions and cosmetics, hair conditioner, pharmaceutics, retanning agent as well as chrome exhaustion in leather manufacture and in the manufacture of synthetic products such as nylon (Cantera and Buljan, 1997).

It was observed that the weight of experimental dehaired pelts from skins and hides was less when compared to controls (Table 1). This could be explained by the fact that enzymatic treatment removed higher amounts of non-leather forming substances. Also, absence of swelling in enzyme dehaired pelts could be due to the elimination of lime in the process. With the result, the pelts absorbed low quantity of water during the process while controls absorbed more water as well as lime. However, the weight of enzyme dehaired pelts, when subjected to alkaline swelling in subsequent reliming process, was increased by 20% and it was equivalent to that of control relimed pelts. It was also evident from the data that water absorption was relatively more in experimental relimed pelts than controls indicating adequate swelling of relimed pelts. More water absorption by pelts in the presence of lime led to build up of hydrostatic pressure, which in turn could enhance the splitting of fiber bundles (Herfeld and Schubert, 1969; Ramasami et al., 1999).

3.2. Assessment of enzymatic dehairing by histology

Efficacy of this process was corroborated with histological studies on the sections of dehaired pelts. Hematoxylin

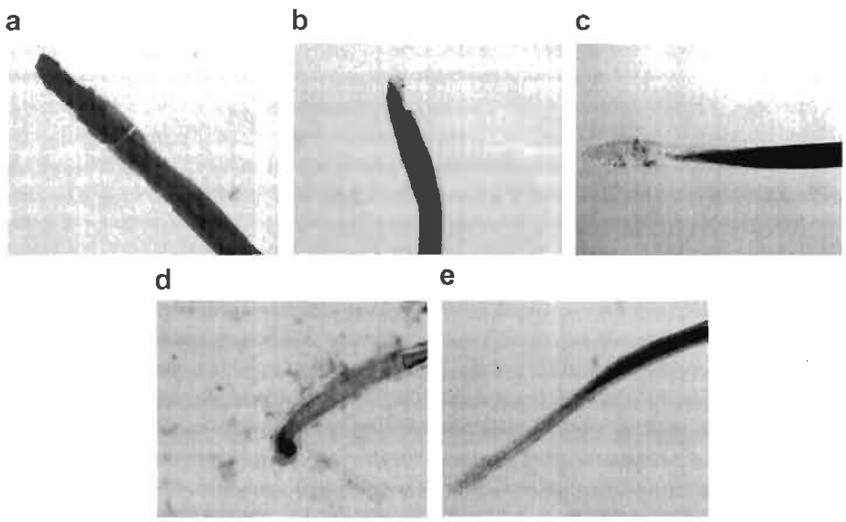

Fig. 1. Microscopic structure of the hair from goat skins (a–c) and cow hides (d, e) from conventional (a), enzyme-assisted (b, d) and enzymatic dehairing (c, e). (×150).

and eosin staining clearly distinguished the features in terms of the extent of removal of epidermis, glandular structures, hair shafts and follicles after different treatments (Fig. 2a–f). Complete absence of the above structural features was evident in sections of dehaired pelts of skins and hides obtained from experimental system (Fig. 2c and f) whereas incomplete (Fig. 2a and d) and moderate removal (Fig. 2b and e) was observed in conventional and enzyme-assisted systems, respectively. Remnants of epidermis were seen in the focal area of control systems of skin and hide. Moreover, opening up of collagen fiber structure was complete, regular and even in dermis and corium of experimental dehaired pelts than in controls, while the fiber structure appeared to be compact and moderately opened up in the conventional and enzyme-assisted systems, respectively. It was also observed that there was no apparent damage to the collagen fibers in enzyme dehaired pelts. Earlier reports on enzymatic and enzyme-assisted dehairing preparations required careful controls as they were shown to attack the dermal collagen, leaving residual hairs, damaging fine fibers in the grain enamel and thereby reducing the quality of leather (Cantera et al., 1996; Paul et al., 2001). One of the advantages of the present enzymatic process was that it required only 6 h for complete dehairing of skins and hides. Besides, this short duration obviated the problem of collagen damage by protease and hence high degree of control was not required.

3.3. SEM analysis of pelts and dyed crusts

Further information pertaining to grain and cut surface of dehaired pelt and dyed crust samples from cowhides was obtained by SEM analysis. Cowhides were taken for SEM studies because the thickness of hides was more than that of the skins and in addition, dehairing of hides was reasonably difficult than that of skins. SEM analysis showed that the grain structure of dehaired pelt was clean without any damage in both control and experimental systems (Fig. 3a–c). The grain surface appeared to be more even and smoother in experimental system than controls. In addition, hair pores were free of any hair residues in the experimental system. Cut surface features revealed moderate opening up of collagen fiber bundles in conventional system when compared to well opened up fiber bundles in enzyme-assisted and experimental systems (Fig. 3d–f). The architecture of collagen fibrous structure of skins constituted fiber bundles with numerous collagen fibrils. Proteoglycans, cementing substances present abundantly in the various layers of skin, interacted with collagen molecules at a fibril level (Scott, 1980). Protease of this study was capable of opening the fibrous structure by degrading these interfibrillar substances and proteolytic cleavage of the core protein backbone of proteoglycan could release fragments consisting of a peptide portion with varying numbers of covalently attached polysaccharide chains. Extent of removal of these substances from fibers of dermis and corium determined the degree of opening up of collagen fiber bundles (Alexander et al., 1986). This could further accelerate the penetration of protease through collagen matrix to act upon anchoring proteins around the hair follicles eventually facilitating the removal of hair.

Though dehaired pelts of the experimental system exhibited better opening up of collagen fiber structure in skins and hides, they lacked adequate plumpness due to lower degree of fiber splitting and fiber swelling. Swelling of fibers in pelts was related to osmotic phenomenon and it was generally achieved by employing a high concentration of lime in both dehairing and reliming operations leading to complete elimination of interfibrillary substances from collagen matrix. During liming, collagen fibers swelled osmotically

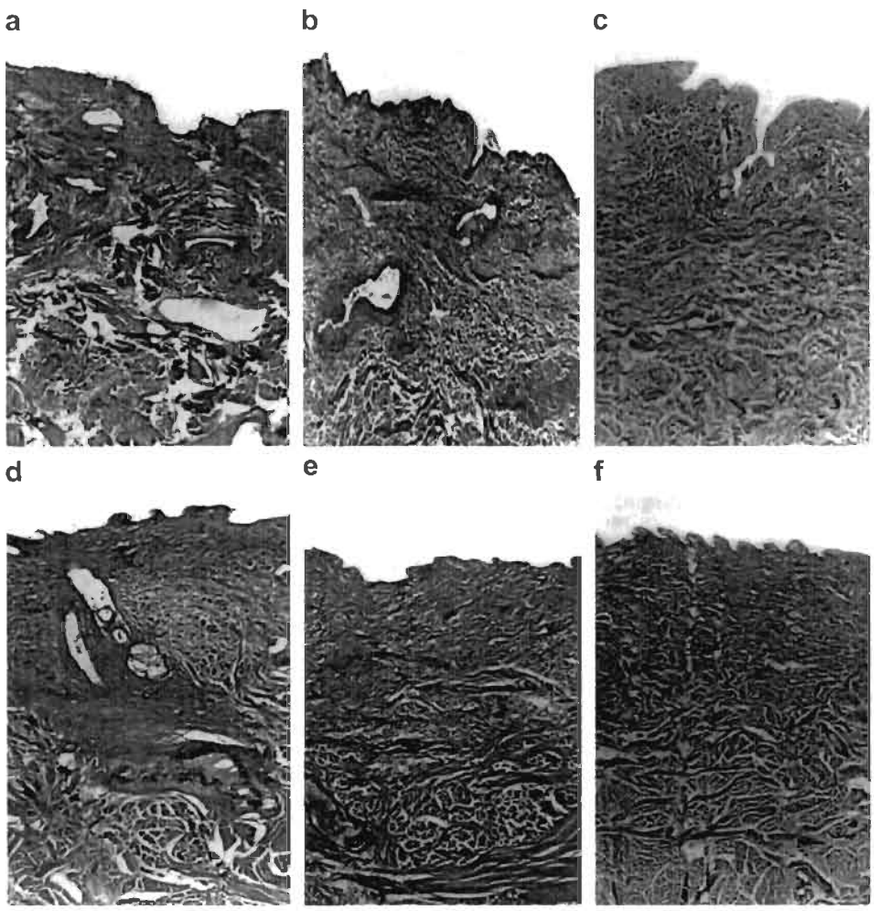

Fig. 2. (a–c) showing staining of the sections of pelts from goat skins and (d–f) showing the sections of pelts from cow hides from conventional (a, d), enzyme-assisted (b, e) and enzymatic dehairing (c, f). (H&E ×175).

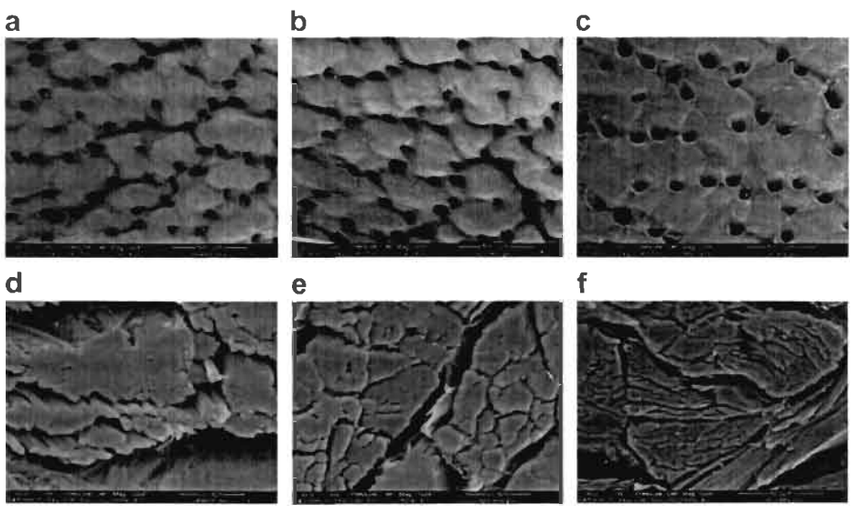

Fig. 3. SEM showing grain surface of dehaired pelts from (a) conventional, (b) enzyme-assisted and (c) enzymatic dehairing of cow hides (80×); cut surface of dehaired pelts from (d) conventional, (e) enzyme-assisted and (f) enzymatic dehairing of cow hides (1500×).

by taking up water from the lime solution and this decreased the cohesive forces between the fibers by breaking hydrogen bonds, causing the fiber structure to become looser (Herfeld and Schubert, 1969; Menderes et al., 1999).

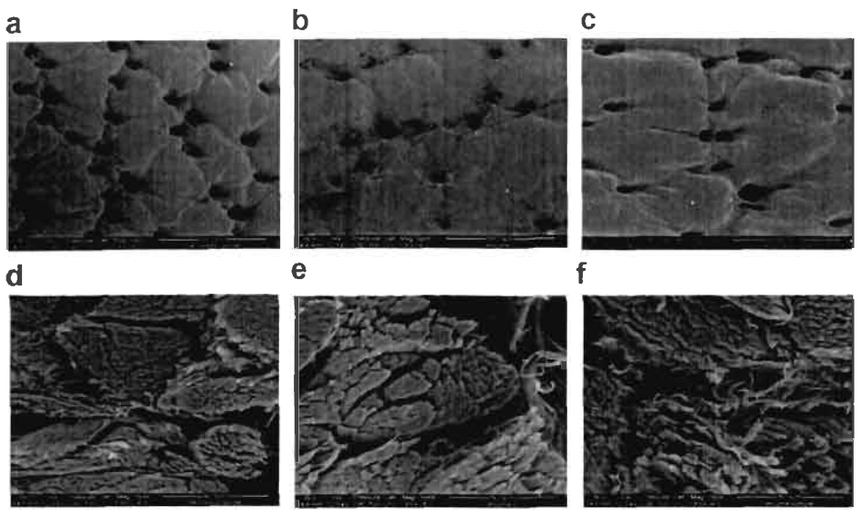

Fig. 4. SEM showing grain surface of dyed crusts from (a) conventional, (b) enzyme-assisted and (c) enzymatic dehairing of cow hides (100×); cut surface of dyed crusts from (d) f conventional, (e) enzyme-assisted and (f) enzymatic dehairing of cow hides (1000×).

However, enzyme of this study avoided the use of lime in dehairing operation. To obtain well-split fiber bundles with more relaxed fibrils and high angle of fiber weave, reliming was carried out for all experimental and control skins and hides using 10% lime.

SEM analysis of the grain surface of dyed crusts from experimental and control systems revealed no grain damage with clean hair pores (Fig. 4a–c). The cut surface of experimental system in particular, showed longitudinal fine splitting of the larger fiber bundles into elementary fibers (Fig. 4f).

3.4. Physicochemical, objective and softness assessment of dyed crusts

Physical characteristics of the control and experimental dyed crusts, on an average, were shown to be in agreement with the standards and they showed no significant difference between them. However, the tensile strength and the degree of elongation at break were found to be slightly higher in experimental leathers when compared to controls (Table 2). High water vapor permeability values of experimental dyed crusts were comparable to controls and this validated the observation of higher porosity and well opened up fiber structure for the experimental crusts. Semi quantitative assessment of leather quality as a measure of softness was carried out for control and experimental dyed crusts. Compression index values deduced from compression measurement data of experimental dyed crusts from skins and hides were found to be higher than that of controls and this indicated an increased softness for experimental crusts (Table 2). Softness of leathers was indirectly related to opening up of fiber bundles (Thanikaivelan et al., 2002). Crusts of well-opened fiber structure had more softness than the ones, which were moderately or incompletely opened up. This could clearly show that this enzymatic process produced soft leathers of well-opened fiber structure and of more even colour when the leathers were dyed. Chemical analysis of the dyed crusts obtained from control and experimental dehairing systems exhibited a rather similar pattern (Table 3). Increase in the content of hide substance from experimental dyed crusts indicated that the collagen content was not affected by enzyme treatment. High chrome content in experimental leathers could be attributed to the well split fiber bundles wherein more number of collagen cross-linking sites were available for fixation with tanning agents.

All the finished leathers produced by control and experimental systems were assessed for their quality with regard to properties such as grain flatness, fullness, softness, grain tightness, dyeing characteristics and general appearance and they were rated. The results showed that the properties of the experimental leathers were comparable to that of control leathers though experimental leathers displayed better properties in terms of softness and grain flatness (data not shown). In contrast, it was shown earlier that enzymatic dehairing resulted in grain irregularities such as loss in fullness and smoothness of the grain, and also, an irregular 'buffing effect' resulting in uneven dyeing later (Cantera et al., 1996).

3.5. Environmental impact

Ecofriendly nature of the enzymatic dehairing process of skins and hides was assessed in terms of pollution control parameters such as TDS, TSS, BOD, COD, calcium and sulfide. Although the paste method of dehairing of skins did not generate effluent in the form of liquor, disposal of their contents into liquid stream would significantly contribute to pollution problems besides toxicity as they contained high content of sulfide and lime. In order to analyze the pollution impact of the process, prior to hair

Table 2
Physical characteristics of dyed crusts produced from goatskins and cowhides

Skins/hides	Method	Tensile Strength (N mm^{-2})		Elongation at break (%)		Tear Strength (N mm^{-1})		Distension at crack (mm)	Load at grain crack (kg)	Water vapor permeability (mg cm^{-2} h^{-1})	Compression Index
		Parallel	Perpendicular	Parallel	Perpendicular	Parallel	Perpendicular				
Skins	BIS norms	20–25	15–20	40–50	60–80	40–50	30–40	>7	>20	–	–
	Conventional	23.25 ± 0.96	17.80 ± 0.52	47.96 ± 2.64	67.80 ± 4.04	43.06 ± 4.16	36.62 ± 3.27	9.5 ± 0.40	20 ± 2	12.30 ± 0.56	6.22 ± 0.32
	Enzyme assisted	22.34 ± 0.86	18.34 ± 0.64	48.80 ± 2.16	73.06 ± 3.22	42.40 ± 1.48	39.57 ± 1.53	9.4 ± 1.20	18 ± 4	11.76 ± 0.78	5.68 ± 0.21
	Experimental	23.79 ± 1.08	18.96 ± 0.38	48.43 ± 3.28	73.52 ± 3.84	42.90 ± 2.48	37.28 ± 1.78	10 ± 0.60	22 ± 2	11.88 ± 0.46	6.93 ± 0.46
Hides	BIS norms	25–30	20–25	40–60	60–80	40–50	30–40	>8	>40	–	–
	Conventional	26.98 ± 1.12	22.26 ± 0.98	47.86 ± 2.36	59.74 ± 4.23	45.13 ± 3.76	39.73 ± 2.21	10.35 ± 0.90	44 ± 2	8.11 ± 1.13	4.70 ± 0.11
	Enzyme assisted	27.63 ± 1.34	23.37 ± 1.18	49.08 ± 3.42	56.40 ± 3.89	42.15 ± 2.56	38.54 ± 1.67	10.30 ± 1.10	46 ± 4	10.23 ± 1.28	5.24 ± 0.23
	Experimental	27.15 ± 1.56	24.20 ± 1.03	48.74 ± 2.78	61.32 ± 3.70	44.87 ± 2.12	39.28 ± 1.98	10.55 ± 1.05	46 ± 2	9.98 ± 1.04	7.21 ± 0.28

Values were means ± standard deviation for four samples ($n = 4$) from four different sets comprising each of ten skins. Values for hide samples were also presented in the similar manner.

removal, the control skins were washed with known amount of water to suspend the adhering contents of depilatory agents and other skin components that were removed during the process. The effluent, thus collected, was subjected to analysis of environmental parameters. Effluent liquors from enzymatic dehairing of skins and hides as well as control dehairing of hides were directly taken for analysis as they employed dip method. Enzymatic dehairing process had certain advantages over the lime–sulfide system with regard to the effluent disposal problems. In the liming process, the effluent problems were principally due to the large quantity of total solids arising from lime and sodium sulfide. Table 4 showed that TDS and TSS of enzymatic dehairing effluent were greatly reduced by about 85% for both skins and hides compared to dehairing with lime and sulfide. This could be mainly due to the elimination of sludge forming, sparingly soluble lime in the process. Pulped hair and keratinous substances contributed to high BOD and COD in the effluent from lime–sulfide process. The extent of reduction in terms of BOD and COD in the enzymatic dehairing effluent was observed as 78% and 84% for skins and 85% and 90% for hides, respectively. These figures indicated that the proportional reduction of BOD and COD was rather more in the enzymatic dehairing effluent from hides than that of skins. This could be because of pulped hair present in effluent of control hides.

To assess the impact of the enzymatic process upon environment, analysis of effluent load was carried out by multiplying concentration (mg l^{-1}) with the volume of dehairing effluent (L) per ton of wet-salted hides and skins (Table 4). Reduction of TDS and TSS load in the effluent from the enzymatic dehairing of skins and hides was found around 85% against the conventional process. BOD and COD load from the effluent of enzymatic dehairing of skins were reduced by 76% and 82%, respectively when compared to conventional dehairing. Similarly, reduction of BOD and COD for experimental dehairing of cow hides was 84% and 89%, respectively. Moderate reduction, i.e., 40–60% in the load of the above parameters was obtained in enzyme-assisted system against conventional process. Pretanning processes generally accounted for 70–80% of the total COD of effluent from all leather making processes (Marsal et al., 1999). About 75% of the organic waste from a tannery was from pretanning processes and 70% of this waste was from hair rich in nitrogen (Kamini et al., 1999). Elimination of hair destructive sulfide in the process led to the complete recovery of undamaged intact hair thereby resulting in huge reduction in COD. Despite moderate reduction in all the above parameters, enzyme-assisted dehairing with commercial enzyme still resulted in significant pollution problems due to the use of lime and sulfide. However, analysis of effluent from enzymatic dehairing of skins and hides devoid of lime and sulfide showed that TSS, TDS, BOD and COD were not only reduced to a greater magnitude but also toxicity due to sulfide was completely eliminated (Davies, 1997). Contrary to control dehairing, the enzymatic process did not generate

Table 3
Chemical analyses of dyed crusts produced from (a) goatskins and (b) cowhides using conventional, enzyme-assisted and experimental dehairing systems (On 0% moisture basis)

Characteristics	Goatskins			Cowhides		
	Conventional	Enzyme-assisted	Experimental	Conventional	Enzyme-assisted	Experimental
Oils and fats	13.7 ± 0.7	15.4 ± 0.6	14.6 ± 0.7	12.3 ± 0.6	11.5 ± 0.4	11.3 ± 0.3
Water solubles	14.5 ± 0.6	13.2 ± 0.4	14.0 ± 0.5	7.5 ± 0.4	6.3 ± 0.3	5.9 ± 0.2
Hide substance	61.5 ± 2.8	64.8 ± 2.3	64.2 ± 2.5	66.1 ± 3.2	67.0 ± 2.9	67.2 ± 3.0
Insoluble ash	1.9 ± 0.2	1.6 ± 0.1	1.6 ± 0.1	2.5 ± 0.3	2.5 ± 0.1	2.3 ± 0.2
Fixed organic Matter	8.5 ± 0.6	5.0 ± 0.5	5.6 ± 0.3	11.7 ± 0.6	12.7 ± 0.7	13.3 ± 0.4
Cr_2O_3	3.4 ± 0.3	3.4 ± 0.2	3.5 ± 0.1	3.5 ± 0.3	3.1 ± 0.1	3.5 ± 0.2

Values were means ± standard deviation for four samples ($n = 4$) from four different sets of experiments.

Table 4
Analyses of pollution parameters and load from the effluent of dehairing processes of hides and skins

Parameters	Goatskins			Cowhides		
	Conventional	EA	Enzymatic	Conventional	EA	Enzymatic
(a) Effluent parameters (mg l^{-1})						
TDS	22930	13920	3490	41760	23390	7020
TSS	10770	3680	1430	19860	5540	2920
BOD	5200	2810	1120	12300	6800	1800
COD	14900	9400	2420	36300	21700	3680
Ca^{2+}	2860	1740	–	3080	2260	–
Sulfide	4120	1700	–	5750	4250	–
pH	11.6	11.2	7.4	12.4	12.1	7.6
(b) Effluent load (kg t^{-1})						
TDS	17.20	10.44	2.90	30.48	18.01	5.58
TSS	8.08	2.76	1.19	14.50	4.27	2.32
BOD	3.90	2.11	0.93	8.99	5.24	1.43
COD	11.18	7.05	2.01	26.50	16.71	2.93
Ca^{2+}	2.15	1.31	–	2.25	1.74	–
Sulfide	3.09	1.28	–	4.20	3.27	–

Results were presented as an average of three samples ($n = 3$) from three different sets of experiments.
EA – Enzyme Assisted.

high alkaline effluent as lime was eliminated in the process. It could be noticed that the pH of the effluent from the experimental system for skins and hides was near neutral, indicating that it was less toxic. In addition, treatment of an effluent with neutral pH would be less expensive than that having a high alkaline pH.

4. Conclusions

In this study, an ecofriendly dip method of enzymatic dehairing of skins and hides was developed using a bacterial protease avoiding the use of lime and sulfide. Comparative studies were carried out with controls such as conventional lime–sulfide and enzyme-assisted process using a commercial enzyme. The process resulted in complete removal of hair and epidermis with even and well opened collagen fiber bundles in a shorter duration as substantiated by histochemical and SEM studies. Analysis of dyed crusts confirmed that the leathers produced by enzymatic dehairing displayed better physicochemical properties. The developed process resulted in a significant reduction of pollution parameters as well as toxicity. As such, no systematic study was carried out on the mechanism of enzymatic dehairing using a bacterial protease and this will be taken up elsewhere.

Acknowledgements

The authors thank Dr. A.B.Mandal, Director, CLRI, Chennai for his kind permission to publish this work. The financial assistance by CSIR, New Delhi to one of the authors, Mr.S.Sivasubramanian, is gratefully acknowledged.

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