

# Role of carbohydrases in minimizing use of harmful substances: leather as a case study

Jayanthi Durga<sup>1</sup> · Ramakrishnan Ramesh<sup>3</sup> · Chellan Rose<sup>2</sup> · Chellappa Muralidharan<sup>3</sup>

Received: 10 August 2016 / Accepted: 10 December 2016 / Published online: 9 January 2017  
© Springer-Verlag Berlin Heidelberg 2017

**Abstract** Leather processing is an important industrial activity. Globally about 2.0 billion sqmt of leather is produced annually. Conventional cleansing operations carried out prior to tanning generate large amounts of waste. Among them dehairing and fibre opening process (reliming) generate large amount of effluent containing hazardous substances and alkaline sludge, resulting in high negative impact on the environment. In this study, both these pre-tanning process steps have been combined using a cocktail of carbohydrases along with optimum quantity of chemicals to minimize the environmental concerns. Carbohydrate and proteoglycan removal were chosen as the parameters of study for efficacy of unhairing and fibre opening. The morphology features of skins were analysed using scanning electron microscopy and histology. Pollution load of the enzyme aided process effluent was determined and compared with conventional process. Findings of the study indicate complete elimination of reliming process step is possible when both unhairing and fibre opening is carried out simultaneously using carbohydrases as an adjunct. Reduction in use of harmful sulphide and

lime up to 40% apart from substantial saving in time and water input is the major outcome of the present work.

**Keywords** Carbohydrases · Single-step processing · Leather making · Pollution reduction

## Introduction

Tanneries are among the oldest manufacturing industries. Tanneries are engaged in transforming the raw hides and skins into leather through several unit operations. During the last few decades, many new materials and technologies are being studied and applied in manufacturing in order to reduce the ecological impact of leather production (Jian et al. 2011). Leather manufacturing has been, very often, identified as one of the environmentally unfavourable industrial activities. The non-substantive chemicals used in the pre-tanning and tanning operations are predominantly source for large amount of the harmful substances in tanning effluents (Ludvik 1996).

Conventional pre-tanning involves use of chemicals such as lime and sulphide, aimed at the removal of non-leather making substances, which accounts for almost 80–90% of the total pollution load (Sivasubramanian et al. 2008). Besides this, sulphide in the effluent may librate hydrogen sulphide under specific conditions, a toxic gas that poses serious hazard for tannery workers. Many fatal accidents have been reported due to generation of hydrogen sulphide at high concentration, particularly at tannery effluent treatment plants (Vijayaraghavan et al. 2015). However, use of large amounts of lime ( $\text{Ca}(\text{OH})_2$ ) and sulphide ( $\text{Na}_2\text{S}$ ) for processing has remained unchanged due to non-availability of viable cost-effective alternatives. Unutilized lime contributes to significant quantity of sludge

**Electronic supplementary material** The online version of this article (doi:10.1007/s10098-016-1321-x) contains supplementary material, which is available to authorized users.

✉ Chellappa Muralidharan  
cmurali62@yahoo.com

<sup>1</sup> Academy of Scientific and Innovative Research (AcSIR), AnusandhanBhawan, 2 Rafi Marg, New Delhi, 110 001, India

<sup>2</sup> Department of Biotechnology, CSIR - Central Leather Research Institute, Adyar, Chennai 600020, India

<sup>3</sup> Leather Processing Division, CSIR - Central Leather Research Institute, Adyar, Chennai 600020, India

generation which under specific conditions can become hazardous (Schlosser et al. 1986). Several lime- and sulphide-free pre-tanning methods have been studied extensively in last few decades (Rose et al. 2007). These include unhairing methods based on proteolytic enzymes, ionic liquids and lactobacillus to replace sulphide (Seggiani et al. 2014; Sandhya et al. 2005). In leather making, many enzyme-based processes have been reported to be cost-ineffective (Ludvik 2000).

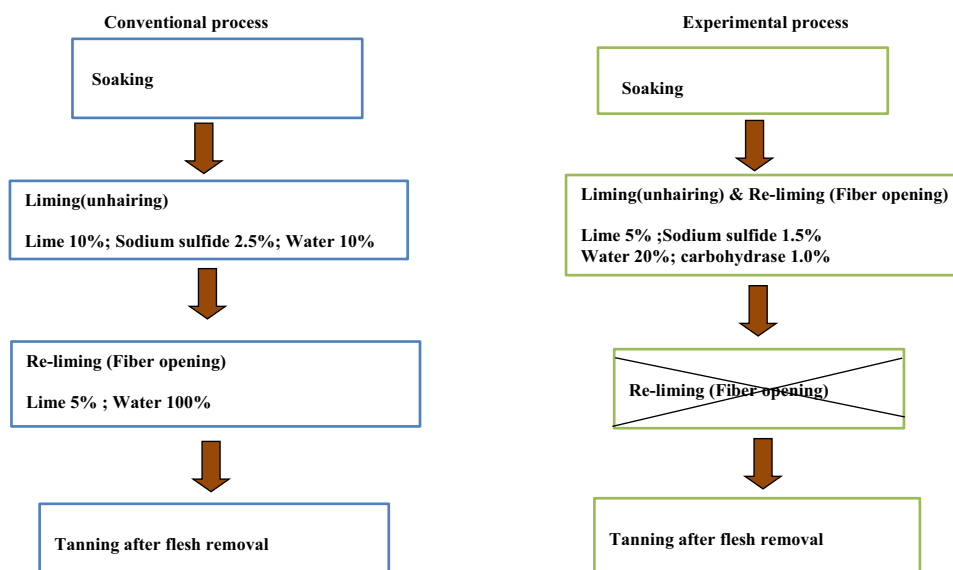
Enzymes have been widely used in leather manufacture in soaking, unhairing, bating and degreasing processes (Kandasamy et al. 2012). Currently enzyme-assisted dehairing is being used in many industries due to its better environmental performance (Senthilvelan et al. 2012). Recently, a cocktail of carbohydrases has been successfully employed to facilitate rapid fibre opening of skins in about 30 min compared to 72-h duration required in conventional process replacing lime (Durga et al. 2015). In the present study, an attempt has been made to carry out unhairing and fibre opening process in a single step with a view to optimize chemicals and time and to facilitate cleaner leather production. The results of the study indicate substantial benefits to leather making could be achieved by combining process steps through use of the carbohydrases in pre-tanning operation.

activity of carbohydrase was found to be 40,000 U/g of substrate using a method of Dey and Pridham (1972) and Miller (1972). The stability of the enzyme used in this study was in the temperature range of 25–40 °C and pH range of 6–13. All the chemicals used for leather making were of commercial grade.

## Experimental

Goat skins were used as substrate in this study. Wet salted goat skins were vertically cut into left (*L*) and right (*R*) pieces, and were marked accordingly. The left pieces were processed by conventional unhairing and fibre opening (reliming) process by pasting method using 10% lime 20% water and 2.5% sodium sulphide. The paste was applied on flesh side of the skin and left over night. Next d the skins were unhaired and relimed with 5% lime and 100% water for a d in drum.

The respective right pieces were processed by employing different concentrations of sodium sulphide and lime in the form of paste containing 20% water and 1% fibre opening enzyme (carbohydrase). Next d the skins were washed with 100% water for a period of 30 min. The pelts thus obtained from both control and experiments were assessed at this stage for fibre opening.



## Materials and methods

Cocktail of carbohydrase enzymes produced by solid-state fermentation (SSF) of *Aspergillus terreus* was used for integrated dehairing and fibre opening along with optimum quantities of sodium sulphide and lime. The enzyme

## Optimization of sulphide concentration

Experiments were carried out to optimize the quantity of sulphide for complete removal of hair in the presence of carbohydrase and reduced lime quantity. The right pieces were pasted on the flesh side with varying concentrations

of sodium sulphide, i.e. 0.5, 1.0, 1.5, 2.0 and 2.5% with 5% lime, 1% cocktail of carbohydrases and 20% water and left overnight. Skins were unhaired the next day and assessed visually. Removal of sugar and glycosaminoglycan were assayed by using standard procedures.

### Optimization of lime concentration

Second set of experiments was carried out to determine the optimum concentration of lime required for this combined process employing reduced sulphide in the presence of carbohydrase. The sulphide of optimized concentration along with 1% cocktail of carbohydrases and 20% water was used for different lime concentrations, viz. 1.0, 2.0, 3.0, 4.0, 5.0, 6.0 and 7.0 without changing other parameters. The skins were assessed visually and the carbohydrate and proteoglycan levels in the pelt samples were quantified spectrophotometrically to determine the optimum lime concentration required for maximum removal of interfibrillar materials. All the skins were converted into wet blue leathers using standard chrome tanning process (given as “Appendix 1”). Chromium content of both the leathers was analysed adopting IUC method.

Tanned leathers (wet blue) were then shaved to a uniform thickness and were converted into crust leather as per the process given in “Appendix 2”. The crust leathers were assessed and evaluated as per standard test methods and assessment procedures.

### Carbohydrate assay

Total carbohydrate content of the pelt samples, both the experimental and control, was determined by phenol–sulphuric acid method using D-glucose as standard (Dubois et al. 1956). Soaked skin sample was used as blank to compare the remaining sugar content of the both conventional and experimental samples. Sample for assay was prepared by hydrolysing 100 mg each of lyophilized sample with 0.5 N sulphuric acid solution at 100 °C in sealed tube for 4 h. Assay was carried out by using 1 ml of hydrolysed aliquot of pelt sample mixed with 5% (v/v) phenol. And then the tubes were cooled in ice for 10 min and 5 ml of concentrated sulphuric acid was added through the sides of tubes. The contents were thoroughly mixed, and the tubes were heated in a water bath at 80 °C for 20 min. After cooling the tubes to room temperature, the absorbance was noted at 490 nm using a spectrophotometer. A reagent blank was prepared in the same manner using distilled water. The amount of carbohydrate remained in the pelt samples was calculated as glucose from the standard curve drawn using glucose solution of known concentration.

### Estimation of proteoglycan

In order to estimate the amount of proteoglycan in the pelt sample, both the experimental and conventional samples were assayed by Schiff’s colorimetric method (Mantle and Allen 1978). Initially, 100 mg of sample was hydrolysed using 0.5 N sulphuric acid solution at 100 °C in sealed tube for 16–18 h and allowed to cool to room temperature. To 1 ml of hydrolysed sample, 100 µl of decolorized Schiff reagent was added and incubated at 37 °C for 2 h. Afterwards the reaction mixture was allowed to remain at room temperature for 30 min for colour development. Absorbance of the reaction product was measured at 555 nm using UV–Vis spectrophotometer and the total amount of proteoglycan present in the sample was calculated using mucin as standard.

### Scanning electron microscopic analysis

Samples from conventional and experimental pelts were cut, washed and fixed in formalin solution. Then the samples were dehydrated using a graded ethanol series and were finally freeze-dried. The dried samples were cut into approximately 5 mm thickness and examined by scanning electron microscopy. The samples were mounted both vertically and horizontally on aluminium stubs. The stubs were coated with gold using an Edwards E-306 sputter coater and introduced into the specimen chamber of a FEI-Quanta 200 scanning electron microscope. The micrographs for the cross section were obtained by operating the microscope at higher voltage.

### Histological studies

Conventional and experimental limed skins were cut and preserved in 10% formalin for 48 h. The samples of both experimental and conventional trials were fixed using formalin (10%) in phosphate-buffered saline (PBS), cassetted and blocked in paraffin wax. Sample sections of 4–5 µm thickness were cut using microtome (Leica) and mounted on glass slide. The tissue specimens thus obtained were dehydrated using series of alcohol (30, 60 and 100%) and stained using haematoxylin and eosin and visualized in bright-field microscope, to assess the extent of removal of epidermis and opening of fibre bundles of collagen and distribution in the sample.

### Analysis of chrome content

Chromium content of leathers was determined by following the official procedure (IUP 2 2000). A known quantity (~1 g) of the sample was weighed, and the percentage of chromium was estimated as per standard procedures.

Initially the samples were analysed for moisture content; chrome content was expressed on dry weight basis of leather.

### Evaluation of strength characteristics and visual assessment of leathers

Various physical properties such as tensile strength, percentage elongation at break, tear strength and grain crack strength of leather samples of experimental and conventional processes were examined as per the standard procedure (IUP 6 2000; IUP 8 2000). Samples were conditioned to the required relative humidity of  $60 \pm 4\%$  at  $20 \pm 2^\circ\text{C}$  for 48 h as per standard procedures. The crust leathers were assessed for softness, grain tightness and general appearance by hand and visual examination.

### Analysis of spent liquor

Spent liquor from both conventional and experimental processes were collected and analysed for pollution parameters such as biochemical oxygen demand (BOD), chemical oxygen demand (COD) and total dissolved solids (TDS) according to the method followed by Thangam et al. (2001) and Eaton et al. (1995). The results are expressed in parts per million (ppm).

### Results and discussion

Initially, trials were performed to optimize concentrations of sulphide and lime matching the requirements of the conventional process. Trials with different concentrations of sodium sulphide (0.5–2.5%), lime (1.0–7.0%) along with 1% carbohydrases were carried out in the study. The experimental skin was white in colour; it had cleaned grain surface compared to its control (processed by traditional method). Enzymatic fibre opening assisted the depilation of hair at its roots. On the contrary, the hairs in the control were removed by solubilization. The hair roots were still present in the deep dermis regions, leading to unclean appearance.

Breaking of *O*-glycosidic linkages of the lysyl residue of collagen enables the loosening of the collagenous fibrillar bundles which in turn facilitated the depilation of hair that has been already discussed in our earlier report (Durga et al. 2016).

### Sodium sulphide optimization

Skins subjected to combined unhairing and fibre opening process using 1% (v/w) enzyme dosage varying at varied concentrations of sodium sulphide (0.5–2.5% w/w)

**Table 1** Visual assessment of unhaird pelt

| Properties           | Conventional process<br>Sulphide 2.5% (w/w),<br>Lime 10%(w/w) | Enzyme-assisted process<br>Optimized concentration<br>Sulphide 1.5% (w/w),<br>Lime 5% (w/w) +<br>Enzyme 0.5% |
|----------------------|---------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------|
| Unhairing efficiency | 9.5 $\pm$ 0.2                                                 | 9.5 $\pm$ 0.2                                                                                                |
| Grain pattern        | 9.6 $\pm$ 0.2                                                 | 9.7 $\pm$ 0.2                                                                                                |
| substance            | 9.4 $\pm$ 0.2                                                 | 9.8 $\pm$ 0.1                                                                                                |
| Smoothness           | 9.3 $\pm$ 0.2                                                 | 9.5 $\pm$ 0.2                                                                                                |
| Pelt colour          | 9.2 $\pm$ 0.2                                                 | 9.7 $\pm$ 0.2                                                                                                |

Scale of 1–10; 1—poor; 10—best

Average value of 3 experts

**Table 2** Extent of carbohydrate and proteoglycan removal

| Sample             | Sugar removal (%) <sup>*</sup> | GAG removal (%) <sup>*</sup> |
|--------------------|--------------------------------|------------------------------|
| Control            | 67.0 $\pm$ 0.5                 | 71.6 $\pm$ 1.0               |
| 0.5% sulphide + EL | 73.5 $\pm$ 1.0                 | 77.0 $\pm$ 1.0               |
| 1.0% sulphide + EL | 74.0 $\pm$ 0.9                 | 83.0 $\pm$ 1.0               |
| 1.5% sulphide + EL | 88.0 $\pm$ 1.0                 | 86.4 $\pm$ 1.5               |
| 2.0% sulphide + EL | 78.0 $\pm$ 1.0                 | 84.0 $\pm$ 1.5               |
| 2.5% sulphide + EL | 79.5 $\pm$ 0.5                 | 85.0 $\pm$ 0.5               |

EL enzyme 1%; Lime 5.0%, GAG Glycosaminoglycan

<sup>\*</sup> Average value of 3 determinations

exhibited different degrees of unhairing. At 0.5–1.0% sodium sulphide levels, unhairing was found not satisfactory. Sulphide concentration of 1.5% along with 1% enzyme was found to be optimum requirement for complete unhairing. Visual assessment of unhaird skins indicated that experimental pelts were comparable or marginally better than the conventional pelts, and the data obtained are presented in Table 1 on a 10-point scale. The carbohydrase enzyme also was found to exhibit better functionality at this sulphide concentration (1.5%), as observed from the results of carbohydrate and proteoglycan removal given in Table 2. The removal of sugars and glycosaminoglycan at this optimum quantity is remarkably high compared to other concentrations employed. While a minimum of 1.5% sodium sulphide was found necessary for dehairing, increasing sodium sulphide concentration beyond was found not to be useful apart from adding to harmful pollution. Higher concentration of sodium sulphide beyond 1.5% also was found to adversely affect the fibre opening efficiency of carbohydrases.

**Table 3** Visual assessment of defleshed pelt

| Properties                     | Conventional process<br>Lime 5.0% (w/w) with<br>100% water (v/w) | Enzymatic process<br>Unhaired pelt with 100%<br>water (v/w) |
|--------------------------------|------------------------------------------------------------------|-------------------------------------------------------------|
| Grain pattern                  | 9.6 ± 0.1                                                        | 9.7 ± 0.1                                                   |
| substance                      | 9.4 ± 0.1                                                        | 9.8 ± 0.1                                                   |
| Smoothness                     | 9.3 ± 0.1                                                        | 9.5 ± 0.1                                                   |
| Pelt colour                    | 9.2 ± 0.1                                                        | 9.7 ± 0.1                                                   |
| Scale of 1–10; 1—poor; 10—best |                                                                  |                                                             |
| Average value of 3 experts     |                                                                  |                                                             |

**Table 4** Extent of carbohydrate and proteoglycan removal with conventional and experiment

| Sample         | Sugar removal (%)* | GAG removal (%)* |
|----------------|--------------------|------------------|
| Control        | 65.0 ± 0.5         | 73.5 ± 0.5       |
| 1.0% lime + ES | 72.4 ± 1.0         | 76.2 ± 0.5       |
| 2.0% lime + ES | 75.0 ± 1.0         | 77.0 ± 0.5       |
| 3.0% lime + ES | 81.0 ± 1.0         | 84.2 ± 1.0       |
| 4.0% lime + ES | 84.0 ± 1.0         | 86.0 ± 1.5       |
| 5.0% lime + ES | 87.0 ± 1.5         | 87.5 ± 1.5       |
| 6.0% lime + ES | 85.2 ± 1.5         | 86.3 ± 1.5       |
| 7.0% lime + ES | 84.1 ± 1.5         | 85.4 ± 1.0       |

ES enzyme 1%; sulphide 1.5%, GAG glycosaminoglycan

\* Average value of 3 determinations

### Optimization of lime

The process was carried out with different concentrations [1.0–7.0% (w/w)] of lime along with standardized concentration of 1.5% sodium sulphide and 1% carbohydrase. Compared to conventional processes employing 10% lime, lime quantity of 5% was found sufficient for combined process of unhairing and fibre opening. Visual assessment of fibre opened experimental pelts indicated comparable or marginally better features as presented in Table 3. The results of carbohydrate and proteoglycan removal are presented in Table 4. This provides clear understanding that the enzyme-assisted combined process is superior in function compared to that of the conventional chemical only process. Higher concentrations of lime beyond 7% were found to be adversely affecting the enzyme activity.

### Chromium content

The percentage of chromium oxide content in wet blue leather from conventional and experimental processes were found to be  $4.0 \pm 0.3$  and  $4.9 \pm 0.2$  (w/w), respectively. Experimental leather showed a marginal increase in chromium uptake compared to conventionally processed leather.

The increased level of chromium uptake in the enzyme-treated wet blue sample may be attributed to possible availability of chromium binding functional groups on the microfibrillar surface, due to the carbohydrase mediated deglycosylating fibre opening process, although not confirmed experimentally.

### Physical testing and visual assessment data

The strength properties such as tensile, tear and grain crack strength values were determined by following standard procedures. After dehairing, visual assessment of enzymatic pelt from goat skin revealed that there was complete and uniform removal of hair showing white clean pelt with the complete absence of hair root. The strength properties of control and experimental crust leathers are given in Table 5. It is obvious from the results that the strength properties of experimental crust leathers are comparable to that of conventionally processed crust leathers. The visual assessment and the hand evaluation of crust leathers revealed that the enzyme-assisted process led to improve the organoleptic properties such as grain pattern appearance, smoothness and fullness (Fig. 1). Both the visual and feel tests suggested that the crust leathers made out of enzyme-treated skins were fuller probably due to improved diffusion of tanning and post-tanning chemicals in to the skins because of better opening up and removal of interfibrillary materials.

### Environmental benefits

The spent liquors have been collected from conventional and experimental processes, and analysed for pollution parameters such as biochemical oxygen demand, chemical oxygen demand and total dissolved solids. The values are presented in Table 6. It is seen that the BOD, COD, TDS in the experimental process is much lower than the control process. This is mainly due to the partial replacement of lime and sulphide with the help of fibre opening enzyme. This method appreciably decreases the usage of chemicals, reduces pollution load and eliminates intermediate process, reliming. Moreover, the remarkable decrease in the BOD level indicates the decreased solubilization of hair due to judicious usage of sulphide (1.5% w/w) in the combined process as against conventional two-stage chemical process where sulphide is used to the extent of 2.5% (w/w) along with lime 5% in reliming.

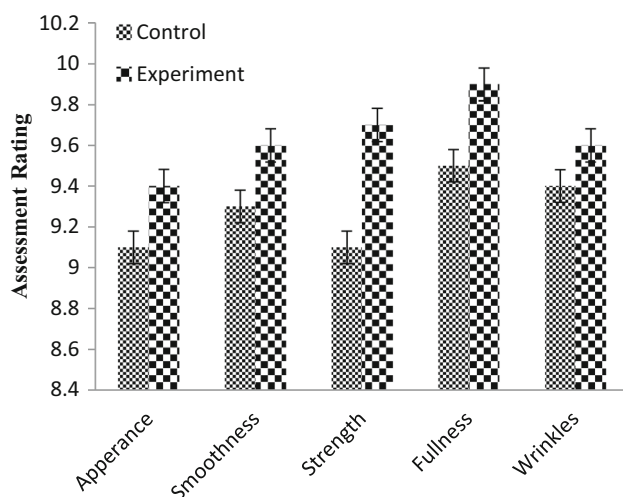
### Economic benefits of integrated process

The combination of unhairing–fibre opening by using a cocktail of carbohydrase has been developed to enhance economic benefits of leather processing. The total cost consumption of chemicals and enzyme used in

**Table 5** Physical testing results of conventional and experimental leathers

| Experiment | Tensile strength (Kg/cm <sup>2</sup> )* | % Elongation at break* | Tear strength (Kg/cm)* | Grain crack strength* |                 |
|------------|-----------------------------------------|------------------------|------------------------|-----------------------|-----------------|
|            |                                         |                        |                        | Load (Kg)             | Distension (mm) |
| C          | 242.8 ± 0.2                             | 68.8 ± 0.5             | 45.0 ± 0.2             | 41.6 ± 0.2            | 9.5 ± 0.2       |
| E          | 248.0 ± 0.2                             | 57.8 ± 0.5             | 41.9 ± 0.2             | 46.6 ± 0.2            | 8.9 ± 0.2       |

\* Average value of 3 determinations

**Fig. 1** Visual assessment of wet blue leathers**Table 6** Environmental benefits

| Process | BOD (ppm)* | COD (ppm)* | TDS (ppm)*  |
|---------|------------|------------|-------------|
| C       | 5780 ± 10  | 7560 ± 10  | 15,320 ± 10 |
| E       | 2264 ± 10  | 5040 ± 10  | 8330 ± 10   |

\* Average value of 3 determinations

conventional and experimental method are given in Table 7. The enzymatic formulation of carbohydrase has effectively used to remove the hair and interfibrillary materials such as glycosaminoglycan and proteoglycan. Reduction in usage of lime and sodium sulphide in experimental process provides cost reduction to an extent of US\$ 40/ton of skins. The experimental process would lead to saving in chemical cost due to 40–50% reduction in lime and sulphide process. In this work, a single-step process leads way to a cleaner technology. This new technology will result in increased productivity with the existing methods due to substantial time saving.

### Evaluation using scanning electron microscopy

The pelt sample processed by both conventional and experimental (enzyme-assisted) methods was examined through scanning electron microscope, and the details are

shown in Fig. 2. According to the micrographs, the cross-sectional study of the experimental sample indicated smooth and opened fibre bundles compared to that of conventionally produced pelt specimen. The micrographs confirm that the enzyme was able to bring out a conspicuous complete removal of hair and opening up of fibre structure compared to conventional process. Efficacy of enzyme-assisted and chemical-driven unhairing and fibre opening process was corroborated with the results of histological studies. Harish et al. (2015) also reported enzyme-dehaired skins exhibit better characteristics, compared to conventional process.

### Histology

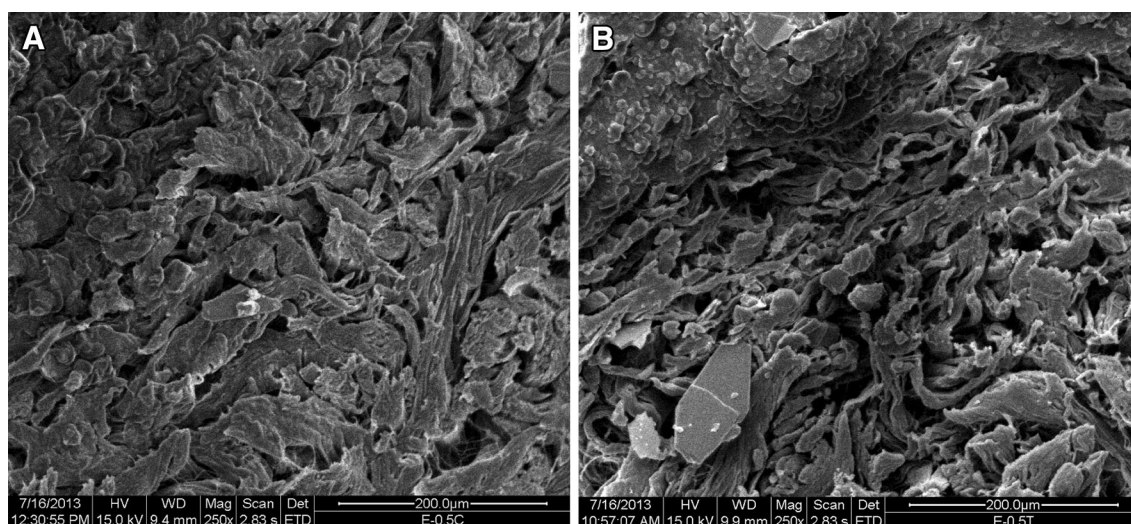
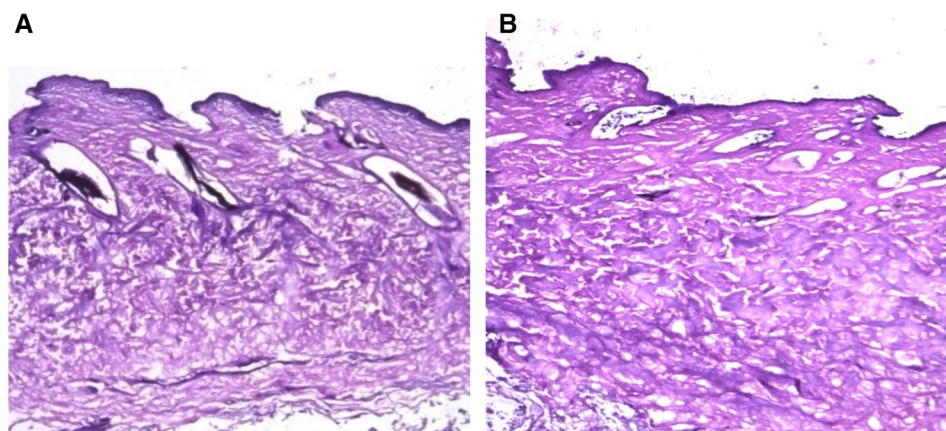
Haematoxylin- and eosin-stained sections of both the samples of chemical- and enzyme-assisted processes were analysed for extent of removal of epidermis, glandular structures and hair root. The absence of the keratinous structural features was observed in pelts obtained by enzyme-assisted single-stage treatment, whereas removal of such components was incomplete in the conventional pelts. Haematoxylin and eosin staining clearly distinguished the removal of residues of interfibrillary materials through histological studies of both conventional and experimental pelts as shown in Fig. 3. It is seen that cross section of control samples exhibited moderate opening of collagen fibre bundles, while the experimental pelts showed distinct fibres with good orientation. Complete removal of epidermal layer from skin was observed when dehairing was performed using carbohydrase.

### Conclusion

The present work deals with combining the unhairing and fibre opening by using a cocktail of enzyme in the presence of judicious concentration of lime, and sulphide has been developed to enhance economic and environmental benefits of leather making. Only 1.5% sulphide and 5% lime were found to be sufficient for unhairing when simultaneously treated with 1.0% carbohydrases for fibre opening leading to saving in 40–50% chemicals usage. Graphical representation of the process is given in Supplementary Figure. This present

**Table 7** Cost of chemicals for processing one ton of raw skins to tanned leather

| Chemicals                                    | Conventional method   |                  | Experimental method   |                  |
|----------------------------------------------|-----------------------|------------------|-----------------------|------------------|
|                                              | Quantity required (%) | Cost (US \$/ton) | Quantity required (%) | Cost (US \$/ton) |
| Lime ( $\text{Ca}(\text{OH})_2$ )            | 10                    | 15.04            | 5.0                   | 7.52             |
| Sodium sulphide ( $\text{Na}_2\text{S}$ )    | 2.5                   | 13.54            | 1.5                   | 7.9              |
| Enzyme                                       | –                     | –                | 1.0                   | 3.01             |
| Lime ( $\text{Ca}(\text{OH})_2$ )            | 10                    | 15.04            | –                     | –                |
| Ammonium chloride ( $\text{NH}_4\text{Cl}$ ) | 3.0                   | 2.71             | 2.1                   | 1.89             |
| Alkali Bate                                  | 1.0                   | 13.54            | 1.0                   | –                |
| Salt ( $\text{NaCl}$ )                       | 10                    | 6.02             | 10                    | 6.02             |
| Formic acid ( $\text{HCOOH}$ )               | 0.5                   | 0.23             | 0.5                   | 0.23             |
| Sulphuric acid ( $\text{H}_2\text{SO}_4$ )   | 1.0                   | 0.3              | 1.0                   | 0.3              |
| Basic chromium sulphate (BCS)                | 4.0                   | 42.11            | 4.0                   | 42.11            |
| Basic chromium sulphate (BCS)                | 4.0                   | 42.11            | 4.0                   | 42.11            |
| Sodium formate                               | 0.7                   | 4.74             | 0.7                   | 4.74             |
| Sodium bicarbonate                           | 1.0                   | 13.54            | 1.0                   | 13.54            |
| Total                                        | 47.7                  | 168.92           | 31.8                  | 129.37           |

**Fig. 2** Scanning electron microscopy images of control and experimental goat skins. **a** Control, **b** experiment**Fig. 3** Photomicrographs of H- and E-stained control- and experiment-treated goat skins. **a** Control, **b** experiment

invention also resulted in significant removal of interfibrillar substances without damage to collagen structure. The partial reduction in sulphide and lime lowers sulphide toxicity, hair solubilization, BOD and sludge formation. This study therefore provides an important solution to one of the long-pending problems of leather processing.

**Acknowledgements** The authors gratefully acknowledge the Council of Scientific and Industrial Research (CSIR), New Delhi, for funding this research. Authors thank “Science and Technology Revolution in Leather with a Green Touch” (STRAIT)—1190.

## Appendix 1

The pelts were washed with 200% water for 10 min. Subsequently, the pelts were delimed by adding 100% water and 1% (w/w) ammonium chloride for 45 min in a drum. Deliming was ascertained by checking the cross section of the delimed pelts for colourlessness due to phenolphthalein indicator. After deliming, bating process were carried out in the same bath for 30 min by the addition of bating enzyme. The pelts were washed with 100% water for 10 min. Pickling was carried out. 1% sulphuric acid (w/w) was added in 4 feeds at 10-min interval and tumbled in a drum for 60 min to obtain pickled skin at pH of 2.8. The pickled skins were tanned using 8% (w/w) basic chromium sulphate (BCS) in 50% pickle water for 90 min. Then 50% (w/w) water was added and the drum was run further for 30 min. To the running drum, 1% (v/v) sodium formate (mixed with 10% w/v water) was added. After 30 min, 1% (w/w) sodium bicarbonate (mixed with 10% w/v water) was added in 3 feeds at 10-min interval and continued the tumbling for 60 more min to bring the pH to 3.8.

## Appendix 2

Post-tanning operations comprise of rechroming of semi-finished wet blue leather, neutralization, dyeing, fat liquoring and finishing. The wet blue leathers obtained by the procedure under Appendix 1 to were shaved to 1.0 mm thickness. All the samples were washed in 100% (w/v) water in a drum for 10 min. After draining, the wet blue leathers were treated with 1.0% (w/w) neutralizing syntan with 100% water for 20 min. Sodium formate 0.5% (w/w) and sodium bicarbonate were then added to the drum in 3 feeds at 10-min interval, while the drum was in running mode. After ensuring the pH of the cross sections at 5.0, the leather samples were washing twice with 200% (w/v) water for 10 min. The neutralized skins were washed with water followed by treatment with resin syntan (3% w/w) and allowed to run in the drum for 20 min. After this, dyeing

(2% w/w acid dye) and fat liquoring (4% w/w synthetic fat liquor) were carried out by drumming for 30 min. Subsequently melamine- and naphthalene-based retanning syntans 4% (w/w) was added and run for 40 min followed by the addition of synthetic fat liquor 4% (w/w), polymeric fat liquor 3% (w/w) and natural fat liquor oil 4% (w/w) and further running the drum for 40 min. Finally the auxiliaries were fixed using 2% (v/v) formic acid diluted with 20% (v/v) water and added at 3 feeds at every 10-min interval and the drum was further run for 30 min and piled overnight. The leathers were set, conditioned, again set with reversible setting machine and hooked for drying. After drying, leather was staked and buffed using 400-grit emery paper.

## References

- Dey PM, Pridham JB (1972) Biochemistry of  $\alpha$ -galactosidase. *Adv Enzymol* 36:911–930
- Dubolis M, Gillis KA, Hamilton JK, Rebers PA, Smith F (1956) Colorimetric method for determination of sugars and related substances. *Anal Chem* 28:350–356
- Durga J, Ranjithkumar A, Ramesh R, Rose C, Muralidharan C (2015) Rapid fiber opening process for skins: an approach for fail-safe chemical free process. *J Am Leather Chem Assoc* 110:07–12
- Durga J, Ranjithkumar A, Ramesh R, Girivasan KTP, Rose C, Muralidharan C (2016) Replacement of lime with carbohydrases—a successful cleaner process for leather making. *J Clean Prod* 112:1122–1127
- Eaton AD, Clesceri LS, Greenberg AE (1995) Standard methods of the examination of water and waste water. The American Public Health Association (APHA), Washington
- Harish B, Khandelwal S, More V, Kalal KM, Seeta Laxman R (2015) Eco-friendly enzymatic dehairing of skins and hides by *C. breffeldianus* protease. *Clean Technol Environ Policy* 17:393–405
- IUP 2 (2000) Sampling. *J Soc Leather Technol Chem* 84:303
- IUP 6 (2000) Measurement of tensile strength and percentage elongation. *J Soc Leather Technol Chem* 84:317–321
- IUP 8 (2000) Measurement of tear load—double edge tear. *J Soc Leather Technol Chem* 84:327–329
- Jian S, Wenyi T, Wuyong C (2011) Kinetic of enzymatic unhairing by protease in leather industry. *J Clean Prod* 19:325–331
- Kandasamy N, Velmurugan P, Sundarvel A, Jonnalagadda RR, Bangaru C, Palanisamy T (2012) Eco-benign enzymatic dehairing of goat skins utilizing a protease from *Pseudomonas fluorescens* species isolated from fish visceral waste. *J Clean Prod* 25:27–33
- Ludvik J (1996) Cleaner tanning technologies, UNIDO report, pp 18–25
- Ludvik J (2000) The Scope of decrease of pollution load in leather processing (US/RAS/92/120/11-51). United Nation Industrial Development Organisation-Regional programme for pollution control in the Tanning industry in south-East Asia, 2000-08-09
- Mantle M, Allen A (1978) Periodic acid/Schiff assay for glycoproteins and Mucin. *Bio Chem Soc Trans* 6:607–609
- Miller J (1972) Experiments in molecular genetics. Cold Spring Harbor Laboratory, NY, pp 352–355
- Rose C, Suguna L, Samivelu N, Rathinasamy V, Ramalingam S, Iyappan K, Parvathaleswara T, Ramasami T (2007) Process for

- lime and sulfide free unhairing of skins or hides using animal/or plant enzymes. US Patent 7,198,647
- Sandhya C, Sumantha A, Szakacs G, Pandey A (2005) Comparative evaluation of neutral protease production by *Aspergillusoryzae* in submerged and solid-state fermentation. *ProcBiochem* 40:2689–2694
- Schlosser L, Keller W, Hein A, Heidemann E (1986) Utilisation of a *Lactobacillus* culture in the beamhouse. *J Soc Leather Technol Chem* 70:163–168
- Seggiani M, Puccini M, Vitolo S, Chiappe C, Pomeli CS, Castiello D (2014) Eco-friendly titanium tanning for the manufacture of bovine upper leathers: pilot scale studies. *Clean Technol Environ Policy* 16:1795–1803
- Senthilvelan T, Kanagaraj J, Mandal AB (2012) Application of enzymes for dehairing of skins: cleaner leather processing. *Clean Technol Environ Policy* 14:889–897
- Sivasubramanian S, Manohar BM, Rajaram A, Puvanakrishnan R (2008) Ecofriendly lime and sulfide free enzymatic dehairing of skins and hides using a bacterial alkaline protease. *Chemosphere* 70:1015–1024
- Thangam EB, Nagarajan T, Suseela RG, Chandrababu NK (2001) Application of alkaline protease isolated from *Alcaligenesfaecalis* for enzymatic unhairing in tanneries. *J Ind Leather* 37:215–222
- Vijayaraghavan R, Vedaraman N, Muralidharan C, Mandal AB, MacFarlane DR (2015) Aqueous ionic liquid solutions as alternatives for sulphide-free leather processing. *Green Chem* 17:1001–1007

Reproduced with permission of copyright owner.  
Further reproduction prohibited without permission.