Microbial enzyme technology as an alternative to conventional chemicals in leather industry

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Leather industry contributes to one of the major industrial pollution problems facing the country, and the pollution causing chemicals, viz. lime, sodium sulphide, salt, solvents, etc. arise mainly from the pretanning processes of leather processing. In order to overcome the hazards caused by the tannery effluents, use of enzymes as a viable alternative has been resorted to in pre-tanning operations such as soaking, dehairing, bating, degreasing and offal treatment. This review focuses on the use of microbial enzymes as an alternate technology to the conventional methods, and highlights the importance of these enzymes in minimizing the pollution load.

ENVIRONMENTAL pollution has been a major irritant to industrial development. Chemical and chemical-based industries are the prime targets of the environmentalists for their crusade against pollution, and leather industry has also not been left out of the reckoning. The generation of pollution is significantly high in the pre-tanning operations compared to the post-tanning operations¹. The chemicals mainly responsible for pollution in pre-tanning processes are lime, sodium sulphide, and caustic soda apart from common salt and degreasing chemicals. In fact, one third of the pollution caused by the leather industries results from the wastes generated during dehairing operations². The wastes from the tanneries are let out into the drains which in turn empty into the main sewerage causing hazard to those who use this water. Many tanneries have been forced to close down because of their noncompliance with the standards laid down. In a short span of time, Indian leather industry has faced serious challenges such as German ban on pentachlorophenate, certain azo dyes, formaldehyde, etc. on one hand, and court order for compliance with environmental regulations on the other³. The attention of tanners is focused towards revamping the processing methods, recovery systems, and effluent treatment techniques to make leather processing eco-friendly. Intensive efforts are being directed towards using a viable alternative technology for pre-tanning processes using enzymes^{4,5}. This could be one of the ways of solving the industrial pollution problems resulting from tannery effluents.

Conventional leather processing

The raw hide has to undergo a series of chemical treatments before it turns into a flattering leather. This includes soaking, liming, dehairing, deliming, bating, degreasing, and pickling. For all these steps, the chemicals used are quite toxic. Thus due to these pretanning operations, the leather processing industry is one of the worst offenders of the environment.

The principal leather making protein, collagen, exists in hides and skins in association with various globular proteins, viz. albumin, globulin, mucoids; and fibrous proteins such as elastin, keratin, and reticulin. During leather manufacture, the noncollagenous constituents are removed partially or completely in the various pre-tanning operations; the extent of removal of these constituents decides the characteristics of the final leather. Besides chemical treatment, certain enzymatic treatments are also necessary to get optimum results. One such treatment, bating, is the only step in leather processing where enzymatic process cannot be substituted by chemical processes. The process of bating gives certain desired characteristics to the finished leather. Earlier, the process was carried out using dog dung or manure⁶. The use of this was not only unhygienic but fermentation could also not be controlled.

In pre-tanning operations, the hides and skins are first subjected to a water soak. For loosening the hair⁷, the oldest method is the 'sweating' process – a natural autolysis or breakdown process. It is a mild putrefaction process induced at random. Since the type and quantity of the putrefying bacteria cannot be controlled, the process itself eludes control. Moreover, since the sensitivity to attack the epidermal proteins and the fibrous proteins of the corium by the proteolytic enzymes is more or less the same, the sweating may result in serious damage to the hide surface. Dehairing is used to be followed by opening up of fibre structure in 'liming'. The dehaired hide is transferred to an alkaline solution of lime milk where swelling occurs and the nonfibrillar proteins are dissolved. After mechanical removal of the subcutaneous

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tissue, deliming is performed in order to remove the adsorbed lime from the hide and to eliminate the lime swell.

The fat present in the hide skins is removed either as soluble lime soap or hydrolysis products like fatty acids. Kerosene, chlorinated hydrocarbons, and white spirit are used in the degreasing system which add to the toxicity of the environment and effluents¹. The various steps of the pre-tanning processes of leather manufacture are shown in Figure 1.

Enzymes in pre-tanning

An important enzyme used in pre-tanning processes belongs to the group of proteolytic enzymes, proteases. Obtained by microbial fermentation, the proteases are meant for use in the leather industry for dehairing, bating and soaking processes, and in the detergent industry for breaking down proteinaceous matter caused by body secretions, food stuffs, and blood⁸. The main advantages of the use of enzymes are specificity, stereospecificity, activity under mild conditions, possibility of producing 'natural' products, nonpollutants, and biodegradability⁹.

Although enzymes from plants, animals, and microbial sources have been used for decades, large-scale use of microbial enzymes received a boost only in 1960s following the introduction of fermentation technology. The enzymes or enzymatic formulations need not be pure but must be cheap compared to that of commercial chemicals used in leather industry.

Animal proteases and microbial proteases from bacteria and fungi are used in the pre-tanning processes of leather

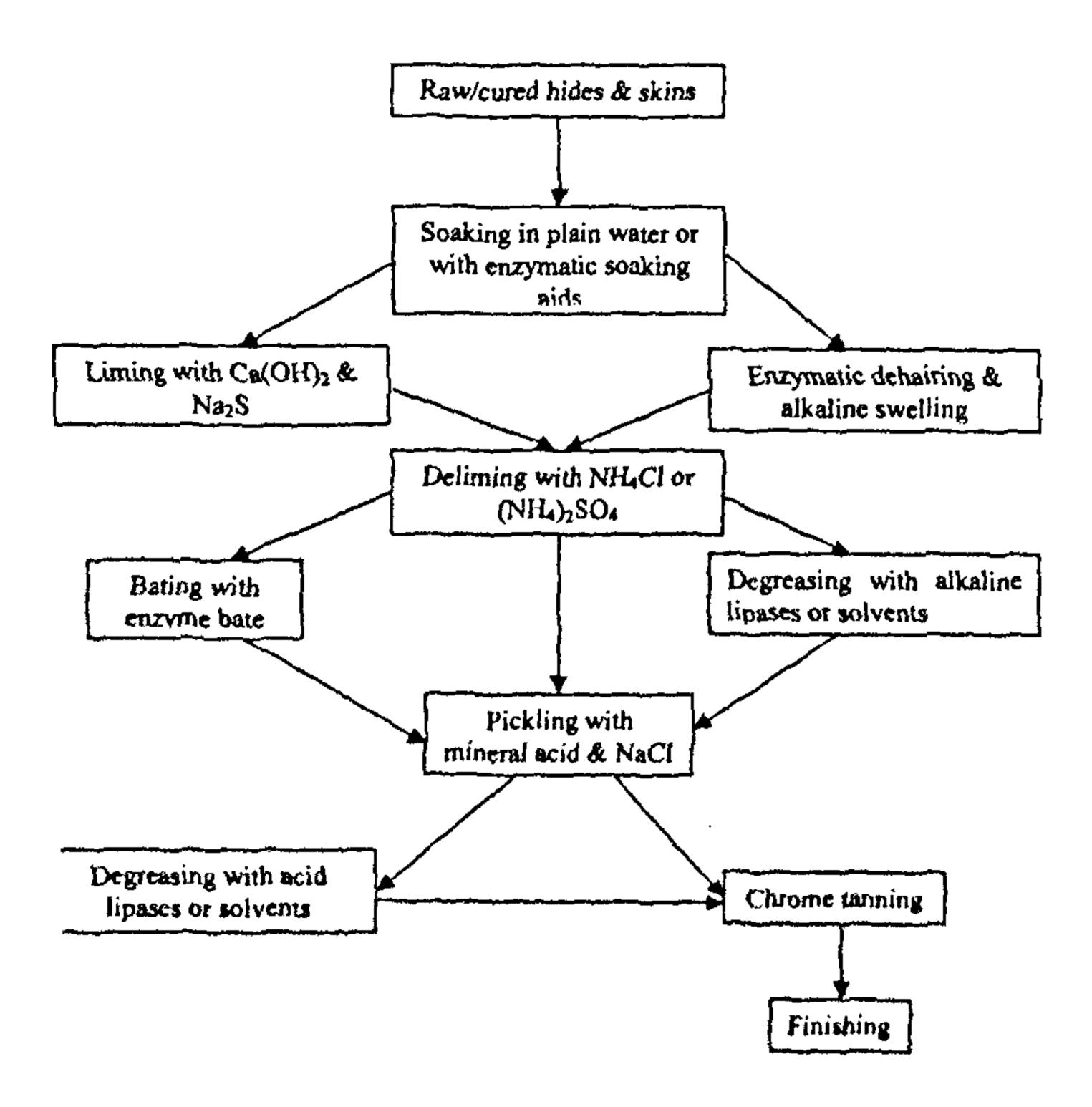


Figure 1. Flow chart of beamhouse operations.

manufacture. The most important criteria for their selection are their specificity, pH activity range as well as pH and thermal stability. If an enzyme is to act uniformly, it must be able to diffuse into the hide and this is obviously acheived with skins rather than with hides. In the latter case, an accumulation of enzyme at the surface of the grain occurs. A pronounced difference between the pH value of the solution and that of the hide is also possible ¹⁰.

The animal proteases are mixtures of trypsin, chymotrypsin, and various peptidases which may contain amylase or lipase as secondary enzymes. Mainly for economic reasons, enzymes from microorganisms have come to play a significant role in recent years and enzyme products of microbial origin are already being produced on a wide scale¹¹.

Since microorganisms can be made to propagate rapidly and profusely, they are an ideal source for enzymes¹². Mainly, neutral and alkaline proteases are obtained from bacteria, which differ in their pH activity range. Fungal proteases are also classified according to the pH activity range¹³: fungal acid proteases act between pH 2.5 and 6.0 and can be derived from A. satoi. These are used for bating prior to pickling and serve to open up the fibre structure. Fungal alkaline proteases¹⁴ belong to the same group of serine proteases as alkaline bacterial proteases. However, these are more heat sensitive and are quickly deactivated above 60°C. Fungal neutral proteases are mainly obtained from Aspergillus or Penicillium species Table 1 shows the various enzymes produced by various microorganisms used in the leather industry.

Apart from bacterial and fungal proteases, specific proteases like keratinases¹⁵ are known. Keratinases which hydrolyse keratins, are obtained from Streptomyces fradiae and can be used for dehairing¹⁰. Some of the important lipase-producing microorganisms used in degreasing are shown in Table 1. Lipases are used (i) in the oil and fat industry to modify fats for use in foods; (ii) in detergent compositions; (iii) for fatty acid production, lipid synthesis via reversal of hydrolysis and lipid modification by interesterification, and (iv) in degreasing of hides and skins¹⁶.

Enzymes in soaking

Soaking is the first operation in the tannery wherein the hides and skins are cleaned and softened with water 17. Wet-salted or freshly slaughtered hides and skins do not require any chemical agent for their proper soaking 4. Soaking is necessary for solubilization and elimination of salts and globular proteins contained within the fibrous structure of hides and skins. It is carried out under alkaline conditions at low temperature between 10°C and 20°C in water treated with antiseptics such as sodium hypochlorite, sodium pentachlorophenate, formic acid,

etc.¹. It is accelerated by some of the nonionic detergents and additives such as sodium sulphide or sodium tetrasulphide.

The advantages of enzymatic soaking include loosening of the scud, initiation of the opening of the fibre structure, and production of leather with less wrinkled grain when used at an alkaline pH of less than 10.5 (ref. 6). Use of enzyme preparation in soaking of rabbit skins improves the softness and elasticity, and increases the area yield of the fur by 3.3% while reducing the processing time by 10-20 h (ref. 18).

Grimm¹⁸ has described a soaking method using proteolytic enzymes and carbohydrases in the pH range of 5.5 to 10.0. Enzymes from Aspergillus parasiticus, A. flavus, A. oryzae, and Bacillus subtilis have been used alone or in mixtures. Rokhvarger and Zubin²⁰ suggested the use of carbohydrase from the mold culture A. awamori in soaking. Botev et al.21 have reported the use of bacterial amylase for soaking dried wool lamb skins. Alkaline proteases of bacterial and fungal origin have been used for soaking which reduces the need for the liming chemicals by 30-60% (refs 22,23). Soaking of dried furs in an aqueous bath containing 1% acid proteinase from Rhizopus rhizopodiformis and sodium bisulphite at 25°C for about 20 h has been reported by Asbeck et al.²⁴. Orlita and Beseda²⁵ have tested three commercial bacterial alkaline protease preparations for the soaking of salted cow hides. Thus, use of enzyme preparations results in a decrease in soaking time.

Soaking is usually carried out using a combination of proteolytic enzymes that are optimally active in the neutral or alkaline pH range. For enzymatic soaking, the average soaking period for salted raw stock is about 4 h and for dried raw stock is about 8-10 h (ref. 26). A water soak without auxiliary agents takes 24 h for salted hides, and 36-48 h for dried hides.

Table 1. Enzymes used in pretanning operations

Process	Enzyme	Microorganism
Soaking	Protease	Aspergillus parasiticus, A. flavus, A. oryzae, and Bacillus subtilis ¹⁹ , Rhizopus rhizopodiformis ²⁴
	Carbohydrases	Aspergillus awamori ²⁰
Dehairing	Protease	Aspergillus flavus ^{33,35} , Aspergillus sp. ³⁴ , Bacillus subtilis ⁴¹ , Lactobacillus sp. ⁴² , Conidiobolus sp. ³⁶ , B. amyloliquefaciens ⁶⁴ , Streptomyces griseus, S. fradiae ⁶⁵ , S. moderatus ⁶⁶
Bating	Protease	A. parasiticus ^{67,68} , S. rimosus and B. licheniformis ⁴⁸ , B. subtilis ⁶⁹ , Penicillium janthinellum ⁷⁰
Degreasing	Lipase	Rhizopus nodosus 49, A. oryzae and A. flavus 7)

Enzymes in dehairing

Dehairing is one of the main operations in the beamhouse. Five methods of dehairing are generally adopted, viz. (i) clipping process, (ii) scalding process, (iii) chemical process, (iv) sweating process, and (v) enzymatic process. Of these, the most commonly practiced method of dehairing of hides and skins is the chemical process using lime and sodium sulphide. However, the use of high concentrations of lime and sodium sulphide creates an extremely alkaline environment resulting in the pulping of hair and its subsequent removal. While one cannot question the efficacy of this process, its inherent disadvantages have to be taken note of. Significant amongst these are:

- (i) It contributes in no small measure to the pollution load. Beamhouse processes generally account for 70–80% of the total COD of effluent from all leather making processes. About 75% of the organic waste from a tannery is from the beamhouse and 70% of this waste is from hair which is rich in nitrogen. These figures clearly illustrate the contribution made by the lime and sulphide process towards pollution²⁷.
- (ii) Sulphide is highly toxic with obnoxious odor. If left untreated, it can cause major problems in the sewers.
- (iii) The severe alkaline condition is a health hazard for the workers.

Enzymatic dehairing is suggested as an environmentally friendly alternative to the conventional chemical process⁶. The enzyme digests the basal cells of the hair bulb and the cells of the malphigian layer. This is followed by loosening of hair with an attack on the outermost sheath and subsequent swelling and breakdown of the inner root sheath and parts of the hair that are not keratinized²⁸. Advantages of enzymatic dehairing are:

- (i) Significant reduction or even complete elimination of the use of sodium sulphide.
- (ii) Recovery of hair of good quality and strength with a good saleable value.
- (iii) Creation of an ecologically conducive atmosphere for the workers.
- (iv) Enzymatically dehaired leathers have shown better strength properties and greater surface area.
- (v) Simplification of pre-tanning processes by cutting down one step, viz. bating.
- (vi) A significant nature of the enzymatic dehairing process is the time factor involved. The lime-sulphide process takes about 16 h, whereas the enzymatic dehairing would be also completed between 12 and 20 h (ref. 29).

Proteolytic enzymes are of great commercial importance, contributing to more than 40% of the world's commercially produced enzymes³⁰. Approximately 50% of the enzymes used as industrial process aids are proteolytic enzymes³¹. Proteolytic enzymes are more efficient in enzymatic dehairing than amylolytic enzymes¹.

Microbial proteases are derived from a wide variety of yeasts, molds, and bacteria³². Yeast proteases are mainly intracellular in nature and therefore these enzymes have not gained significant commercial interest. The protease from A. flavus was earlier being used for dehairing, and later it was reported that simultaneous dehairing and bating is possible with the protease of A. flavus³³. Gillespie³⁴ has observed that the enzyme preparation from cultures of A. oryzae, A. parasiticus, A. fumigatus, A. effusus, A. ochraceus, A. wentii, and P. griseofulvum exhibit marked depilatory activity on sheep skins.

CLRI has developed Clarizyme, an alkaline serine protease, produced by A. flavus used for the dehairing of skins and hides³⁵. A. flavus grows rapidly on wheat bran and produces large amounts of extracellular proteases. Extensive trials carried out in CLRI tannery have confirmed the successful use of this enzyme as a depilatory agent. The use of this enzymatic depilation process completely eliminates the use of sulphide, a toxic pollutant.

The fungal culture, *Conidiobolus* sp., isolated at NCL, produces high yields of extracellular alkaline protease³⁶. The enzyme is active at pH 10.0 and is being tried for many industrial applications. Enzymes derived from bacteria have gained much commercial interest^{37,38} because of their easy production capabilities by submerged cultivation, high yield of enzyme, short duration for production, and easy recovery of the enzyme.

Proteolytic enzymes derived from a large number of Bacillus sp. and Streptomyces sp. have been used in dehairing of hides and skins^{39,40}. A lime and sulphide-free process of dehairing has been developed for the manufacture of suede from sheep skins using protease from B. subtilis⁴¹. Schlosser et al.⁴² have reported a method of depilation in an acid medium containing Lactobacillus culture.

In dehairing, the hair loosening is effected at pH 10.0 using fungal or bacterial enzymes; the treatment period being approximately 12–16 h, followed by hair removal using mechanical means¹⁰. The treatment period can be substantially reduced if the enzyme solution is fed in from the flesh side under pressure⁴³. Enzymatic hair loosening processes play a role wherever high-quality hair, wool or bristles are to be recovered.

Three methods of application are commonly used in the enzymatic dehairing process: (i) paint method, (ii) dip method, and (iii) spray method. In the paint method, the enzyme solution is mixed with an inert material like kaolin, made into a thin paste, adjusted to the required pH, applied on the flesh side of hides and skins, piled flesh to flesh, covered with polythene sheets and kept till dehairing takes place. In the dip method of enzymatic unhairing, the hides or skins are kept immersed in the enzyme solution at the required pH in a pit or tub. The disadvantage encountered in this method is the unavoidable dilution of the enzyme solution. Even though

enzyme penetration is observed to be uniform, dehairing at backbone and neck is not up to the mark. A novel spraying technique has been adopted for the application of multienzyme concentrate in depilation⁴⁴. The advantages of this method over the painting and dip methods are that (i) even concentrated solutions can be sprayed, (ii) when the enzyme solution is sprayed on the flesh side with force, entry becomes easier, (iii) backbone and neck can be sprayed with more amount of enzyme, thereby making the process quicker, (iv) there is no effluent arising out of this method, and (v) after depilation, hair will be almost free from all the adhering skin tissues. Of late, dehairing by drumming is being practiced, and industrially this should be feasible.

Enzymes in bating

Bating is a very important process in which enzymes have been successfully employed for centuries. The concept of softening hides by treating them in a warm infusion of animal dung has been termed as 'bating' and the product used for such process is known as a bate. The main object of bating is to remove some of the nonleather-forming proteinous materials like albumins, globulin, and mucoids from hides and skins, and to allow splitting up of collagen fibres to facilitate the penetration of tanning materials and other processing chemicals, thereby giving the finished leather the desired characteristic properties like feel, softness, pliability, etc.¹.

Deliming and bating, the subsequent steps in the processing of the pelts after liming, are really two separate operations although they are usually carried out in one step and often overlap each other. The principal materials which a bate contains are a proteolytic enzyme, a carrier for the enzyme like wood flour, and a suitable deliming agent like ammonium chloride or sulphate or both. The deliming agents are used for the removal of lime salts which are used during the dehairing process.

The comparatively richer source for the proteolytic enzyme is the pancreas from bovine and pig. The proteolytic enzymes in the pancreas are present in inactive forms; chymotrypsin as chymotrypsinogen, trypsin as trypsinogen, and carboxypeptidase as procarboxypeptidase. A process has been patented for the activation of pancreatic enzymes by the use of acid protease from A. fumigatus⁴⁵.

Underkofler and Hickey⁴⁶ have described a process for the manufacture of enzyme bate from mold source. Trabitzch⁴⁷ have reported the use of enzymes from Aspergillus species in bating and dehairing. A procedure has been developed for bating pig skins, using an enzyme preparation from B. subtilis, and bated skins exhibit good physicochemical properties¹⁸. Bacterial preparation from S. rimosus and B. licheniformis have been tested for their bating action and it is found that solubilization of collagen

has been less pronounced under the influence of microbial proteases than under the influence of pancreatic protease⁴⁸. A combination of both mold and pancreatic enzymes in suitable proportions will be an ideal bate for different types of leather.

In bating, pancreatic enzymes are used in combination with neutral and alkaline bacterial or fungal proteases. After loading the drum with the pelts, the float is fed in at 35-37°C and, then, the bating agent containing enzyme, ammonium salts and carrier material is added.

Enzymes in degreasing

Degreasing is an essential step in the production of glove and clothing leather. In this process there is removal of excess natural fats from greasy skins. The presence of natural grease in raw hides and skins, especially woolly sheep skins, results in various defects, viz. fatty spues, uneven dyeing and finishing, waxy patches in alum-tanned leathers, and pink stain on wet blues. During the degreasing operation in the pretanning process, the fat or grease is removed from the interfibrillary spaces of the skins to facilitate the even penetration of tanning materials, fat liquors, and dyes, etc. Degreasing helps to obtain soft and pliable leather for garment manufacture.

Degreasing is carried out after pickling, using aqueous emulsification with detergents, or by solvent extraction. It is well known that organic solvents like kerosene, petrol, perchloroethylene and trichloroethylene are highly unsafe and hazardous to the workers and heavily pollute the environment. The detergents, though not hazardous while handling and storing, cause serious pollution problems. These detergents and solvents add to the BOD load of the pickling effluent, and the chlorinated hydrocarbons and solvents add to the toxicity of the effluent⁴⁹.

Enzymatic degreasing is suggested as a viable alternative to combat the pollution problems caused by the use of solvents and detergents. Lipases which are projected as alternatives for solvents and detergents, catalyze the breakdown of fats and can be obtained from animal, microbial and plant sources. The advantages of using enzymes for degreasing are the elimination of solvents, reduction in surfactants, and possible recovery of valuable by-products. The disadvantages are that the lipases do not remove all types of fats in the same way that solvents do, and they add cost to the process.

In 1966, Trabitzsch⁴⁷ described the potential for lipases in degreasing skins. Baldano and Shestakova⁵⁰ compared the enzymatic and solvent degreasing of pig skin and have shown that both these methods remove approximately 50% of the grease. Yeshoda et al.⁵¹ used a fungal lipase for the degreasing of woolly sheep skins, pH range of 3.2-3.6 at 37°C for 1 h. Subsequently, Yeshoda et al.⁵² observed that degreasing and bating could be carried out simultaneously in the pH range of 7.8-8.0. An acid lipase

from Rhizopus nodosus has been noticed to be very effective in the degreasing of sheep skins⁴⁹. Zhang reported use of alkaline lipase in combination with the proteinase and pancreatin in softening pig skin to improve the degreasing effect⁵³. Pfleiderer et al.⁵⁴ carried out degreasing of hides by soaking in an acidic bath containing a proteolytic enzyme (0.01-3.0%), and a nonionic surfactant (0.2-1.5%) or its mixture with anionic emulsifiers. A combination of proteolytic enzymes and emulsifiers gives optimum results in wet degreasing of sheep skins¹.

CLRI has developed a potent fungal lipase from A. niger⁵⁵ and a potent bacterial lipase⁵⁶. Comparative studies on degreasing of sheep skins using the bacterial lipase and commercial detergent-based degreasing agent Gelon-PK have been carried out. Improved degreasing results with the bacterial lipase, with added advantages of better softness, smoothness, and improvement in other physical properties⁵⁷. Furthermore, the lipase without detergent is observed to show 70% degreasing in 2 h, with the effluent showing minimal pollution load.

Enzymatic degreasing can be carried out with acidic or alkaline lipases of fungal or bacterial origin. For degreasing, pickled pelts are kept immersed in an enzyme bath containing microbial lipase and water pH of 3.6, and left in the same bath overnight at a temperature of 28–32°C. The degreased pelts are then removed from the bath and subjected to salt wash twice with water and common salt for 40 min. The washed pelts are repickled, chrome tanned and taken for further processing⁵⁰. The use of an alkaline lipase at a pH of 9.0 to 9.3 in the degreasing of pig skin results in short degreasing time and high degreasing efficiency⁵⁸.

Enzymes for by-products utilization and effluent treatment

Enzymes could be used in the treatment of fleshings and effluent from tannery processes. A combination of hydrolytic enzymes, viz. proteases, carbohydrases, and lipases would be required. The advantages to be realised include a protein by-product suitable for animal feed as well as energy conservation and fat recovery. Again, the major disadvantage would be the cost⁶.

When raw hides are processed to leather, a number of by-products such as native hide material (claws, tails, necks, fleshings), pelt waste (trimmings, machine fleshings, gluestock, pelt cuts), and tanned material (shavings, leather cuts, buffing dust, chrome cuttings) are obtained¹⁰.

Braeumer et al.⁵⁹ have described the enzymatic conversion of glue stock and other hide offal to technically useful byproducts by hydrolysing the pulverised hide wastes with an alkaline protease, pH 9.0-

13.0, in the presence of urea, and then at pH 2.0-5.0 in the presence of a strong acid. Bronowski et al.60 have shown that treating fleshings with pancreatic enzymes instead of heat treatment for separating the fat from the proteinaceous matter requires much less energy, and the yield is increased from 60-65% to over 90%. Sauer⁶¹ has described a process for the utilization of fleshings which consists of the enzymatic hydrolysis of the proteins, conditioning of the resulting liquid, and separating the fats and solids present in the hydrolysate. The outstanding feature of the process is a recovery of 91% of the fat in the fleshings and the application of the hydrolysate directly to the soil, as a fertilizer. Iliskovic and Mersed⁶² have described the separation of fats from the fleshy wastes from cattle hide processing by treatment with enzymes.

The problem of waste treatment can be approached (i) by getting rid of the pollution by proper effluent treatment, and (ii) by controlling pollution occurring at different stages of leather manufacture². Biotechnology plays an important role in tannery effluent treatment. The secondary treatment of tannery effluents, which relies on living organisms, is normally by anaerobic lagoons and aerobic lagoons. Open waste-ponds or anaerobic lagoons are installed in few south Indian tanneries where the atmospheric temperature (20-40°C) is suitable for this operation. In these ponds, microorganisms which thrive in oxygen-less environments are allowed to digest the waste. Anaerobic lagoons can be used for cleaning wastes coming from both the vegetable tanning and chrome tanning procedures. Closed type anaerobic systems are useful for tanneries situated in cold temperatures (5-10°C). Aerobic lagoon is a shallow water-tight pond of about 2-3 m depth. The wastes are kept for about a week. Fixed or floating type surface aerators blow oxygen or air into these for helping growth of organisms. This system requires less land and is economical for larger tanneries located in urban areas.

The necessity for chromium removal in tannery waste water is another area of waste management². Microorganisms such as A. fumigatus and species of Pseudomonas when grown on chrome waste can 'leach' out chromium. Pentachlorophenol, a preservative used for raw as well as semi-processed skins, creates problems during handling and also during biological effluent treatment. P. aeruginosa could be used successfully to degrade pentachlorophenol². Other potential techniques for reduction of pollution load are recycling of immobilized enzymes to hydrolyse the solid waste, and recycling of immobilized whole cells to absorb or detoxify toxic metals in the effluent.

Indian Leather Industry Foundation (ILIFO), a non-profit association of major Indian tanners, and UNIDO's Regional Programme for Pollution Control in the Tanning Industry in South-East Asia (RePO) have recently launched a research programme to find uses for treated

tannery effluents in agriculture. At present, the experiment is on in the North Arcot district of Tamil Nadu, where several fruit, flower and vegetable plants are grown with irrigation from treated tannery effluents. Like treated effluent, tannery sludge also contains some nutrients which could be applied to agricultural fields. Disposal of sludge generated in the tannery effluent treatment process is a major bottleneck in tackling tannery pollution⁶³.

Conclusion

The tanneries in future will use a combination of chemical and enzymatic processes. The potential for use of microbial enzymes in leather processing lies mainly in areas in which pollution-causing chemicals, such as sodium sulphide, lime and solvents, are being used and conversion of waste products into potentially saleable by-products is possible. Future may witness ecolabelled leather/leather products emerging as niche products, and the experience gained by the Indian leather industry in this area might greatly help India to emerge as a global leader in leather industry.

- 1. Puvanakrishnan, R. and Dhar, S. C., in Enzyme Technology in Beamhouse Practice, NICLAI Publication, Madras, 1988, pp. 178.
- 2. Venkata Rao, P. and Puvanakrishnan, R., Sci. Rep., 1993, 30, 58-61.
- 3. Subramaniam, R. and Chandra Babu, N. K., Leather Age, 1998, 20, 37-40.
- 4. Puvanakrishnan, R. and Dhar, S. C., J. Indian Leather Technol. Assoc., 1985, 33, 19-22.
- 5. Feigel, T., World Leather, 1998, 11, 54.
- 6. Puvanakrishnan, R. and Dhar, S. C., Leather Sci., 1986, 33, 177-191.
- 7. Taylor, M. M., Bailey, D. G. and Feairheller, S. H., J. Am. Leather Chem. Assoc., 1987, 82, 153-164.
- 8. Anantharaman, A., The Hindu, 1996, Dated July 4, p. 26.
- 9. Vic, G., Biofutur, 1991, 105, 40-46.
- Pfleiderer, E. and Reiner, R., in Biotechnology (eds Rehmond, H. J. and Reed, G.), VCH Verlagsgesells Chaft mbH, Weinheim, 1986, 8, pp. 729-743.
- 11. Ruttloff, H., Huber, J., Zickler, F. and Mangold, K. H., Industrielle Enzyme., Steinkopff, Darmstadt, 1979, 1st edn., p. 107.
- 12. Rehm, H. J., Industrielle Mikrobiologie., Springer-Verlag, Berlin-Heidelberg, New York, 1980, 2nd edn.
- 13. Bergkvist, R., Swedish Patent No. 11134, 1961.
- 14. Longnecker, D. S. and Kalnitzky, G., *Iowa State J. Sci.*, 1963, 38, 31-40.
- 15. Noval, J. J. and Nickerson, W., J. Bacteriol., 1959, 77, 251-263.
- 16. Sztajer, H. and Zboinska, E., Acta Biotechnol., 1988, 8, 169-175.
- 17. Puvanakrishnan, R. and Dhar, S. C., J. Indian Leather, Technol. Assoc., 1988, 36, 197-201.
- 18. Jareckas, G., Shibakovskaya, N. M., Maceiviciene, O. and Strumskiene, A., Kozh-Obuvn. Prom-st., 1985, 6, 44-46,
- 19. Grimm, O., German Patent No. 1230169, 1966.
- 20. Rokhvarger, O. D. and Zubin, A. M., Kozh-Obuvn. Prom-st., 1971, 13, 30-32.
- 21. Botev, L. Esaulenko, L. and Toshev, T., Kozh-Obuvn. Prom-st., 1976, 17, 13-15.
- 22. Monsheimer, R. and Pfleiderer, E., East German Patent No. 2059453, 1972.

- 23. Monsheimer, R. and Pfleiderer, E., East German Patent No. 2308967, 1974.
- 24. Asbeck, A., Pfeiffer, H. F., Plapper, T. and Schimid, R., German Patent No. 2836824, 1980.
- 25. Orlita, A. and Beseda, J., Kozarstvi, 1985, 35, 293-295.
- 26. Pfleiderer, E., Leder, 1975, 26, 149-154.
- 27. Purushotham, H., Malathi, S., Rao, P. V., Rai, C. L., Immanuel, M. M. and Raghavan, K. V., J. Soc. Leather Technol. Chem., 1994, 80, 52-56.
- 28. Kuntzel, A. and Stirtz, T., J. Am. Leather Chem. Assoc., 1958, 53, 445-466.
- 29. Malathi, S. and Dhar, S. C., Leather Sci., 1987, 34, 67-76.
- 30. Ward, O. P., in *Microbial Enzymes and Biotechnology* (ed. Fogarty, W. M.), Applied Science, London, 1983, pp. 251.
- 31. Godfrey, T. and Reichelt, J., Industrial Enzymology, Nature Press, New York, 1983.
- 32. Matsubara, H. and Feder, J., in *The Enzymes* (ed. Boyer, P. D.), Academic Press, NY, 1971, vol. III, pp. 721.
- 33. Rohm, O., German Patent No. 88, 740, 1953.
- 34. Gillespie, J. M., J. Soc. Leather Trad. Chem., 1953, 37, 344-353.
- 35. Malathi, S. and Chakraborty, R., Appl. Environ. Microbiol., 1991, 57, 712-716.
- 36. Srinivasan, M. C., Vartak, H. G., Powar, V. K. and Sutar, I. I., Biotechnol. Lett., 1983, 5, 285-288.
- 37. Aunstrup, K., Annu. Rep. Ferment. Proc., 1978, 2, 125.
- 38. Lambert, P. W. and Meers, J. L., Philos. Trans. R. Soc. London, 1983, B300, 263.
- 39. Toyoda, H. and Futami, A., Bull. Japan Assoc. Leather Technol., 1962, 8, 49.
- 40. Stellmach, J. J., Leather Shoes., 1963, 145, 40-44.
- 41. Lakshmibarathi, K., Dhar, S. C. and Ghani, M. A., Leather Sci., 1978, 25, 279-281.
- 42. Schlosser, L., Keller, W., Hein, A. and Heidemann, E., J. Soc. Leather Technol. Chem., 1986, 70, 163-168.
- 43. Pauckner, W., Congress report of the Scientific Association of the Leather, Shoe and Leather Processing Industries, Budapest, 1982, p. 309.
- 44. Chandrasekaran, S. and Dhar, S. C., Leuther Sci., 1985, 32, 297-304.
- 45. Dhar, S. C. and Panneerselvam, M., Indian Patent No.153246, 1980.
- 46. Underkofler, L. A. and Hickey, R. J., in *Industrial Fermentations*, New York Chemical Publishing Co., NY, 1954, vol. II, p. 109.
- 47. Trabitzsch, H., J. Soc. Leather Technol. Chem., 1966, 50, 382-389.
- 48. Pokomy, M., Restek, A. and Petravic, J., Kozarstvi., 1983, 33, 224-226.
- 49. Muthukumaran, N. and Dhar, S. C., Leather Sci., 1982, 29, 417-424.

- 50. Baldano, Z. K. and Shestakova, I. S., Mater. Nauchn. Konf. Vost. Sib. Tekhnol. Inst. Sekts.Khem. Technol., 1972, 11th, 95; thru CA, 82, 113205g (1975).
- 51. Yeshodha, K., Dhar, S. C. and Santappa, M., Leather Sci., 1978a, 25, 77-86.
- 52. Yeshodha, K., Dhar, S. C. and Santappa, M., Leuther Sci., 1978b, 25, 267-273.
- 53. Zhang, X. and Zhang, Y., Pige Keji., 1982, 7, 16-61; Sauer, O., Leder-und Hautemarkt. Gerbereiwissenschaft und Praxis, 1984, 36, 70.
- 54. Pfleiderer, E., Taeger, T. and Wick, G., Braz. Pedido P. I., BR 8401624, 1984.
- 55. Kamini, N. R., Mala, J. G. S., Rao, D. R. and Puvanakrishnan, R., Indian Patent, 1998 (under process).
- 56. Hernachander, C., Naidu, R. B. and Puvanakrishnan, R., Indian Patent, 1998 (under process).
- 57. Senthamarai, S., Project Report M. Tech., Leather Technology, Anna University, 1998.
- 58. Hu, T., Zhou, Z., Zhong, J., Cao, Q., Huang, C. and Gu, X., Pige Keji, 1983, 3, 19-20.
- 59. Braeumer, K., Eckmayer, Z., Monsheimer, R. and Pfleiderer, E., East German Patent No. 2 705671, 1978.
- 60. Bronowski, K., Sagata, J. and Studniarski, K., Leder, 1979, 30, 8-12.
- 61. Sauer, O., Leder-und Hautemarkt. Gerbereiwissenschaft und Praxis, 1984, 36, 70; thru JALCA abstracts, 80, 190 (1985).
- 62. Iliskovic, N. and Mersed, I., Koza Obuca, 1985, 34, 130; 103, 162149C (1985).
- 63. Brief News (ed. Ashwani Gupta) Footwear and Leather, 1998, 2, 28.
- 64. George, S., Raju, V., Krishnan, M. R. V., Subramanian, T. V. and Kunthala Jayaraman., *Process Biochem.*, 1995, 5, 457-462.
- 65. Kubota, M. and Kimura, S., Hikaku Kagaku, 1967, 14, 146-154.
- 66. Chandrasekar, S. and Dhar, S. C., J. Ferment. Technol., 1983, 61, 511.
- 67. Bose, S. M., Dhar, S. C. and Das, B. M., Tanner, 1956, 11, 17-20.
- 68. Bose, S. M., Dhar, S. C. and Das, B. M., Tanner, 1956, 11, 27-31.
- 69. Jareckas, G., Proizrod. Primen. Milkrobin. Ferment. Prep., 1974, 1, 85.
- 70. Thangamani, A., Ghosh, R. K. and Goswami, P. K., Indian Patent No. 127236, 1970.
- 71. Markin, I. V., Kozh-Obvun Prom-st., 1963, 5, 30.

ACKNOWLEDGEMENTS. We thank Dr T. Ramasami, Director, Central Leather Research Institute, Madras, for his permission to publish this work. We gratefully acknowledge the financial assistance extended to Mrs N. R. Kamini and Mr C. Hemachander by the Department of Biotechnology, Government of India, and to Ms J. Geraldine Sandana Mala by Council of Scientific and Industrial Research, Government of India.