

## Enzyme technology applications in leather processing

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The emphasis on the use of enzymes has come about because of the unique properties of the enzymes. The most important properties are the catalysis of chemical reactions at high rate under mild environmental conditions of pH, temperature and pressure, specificity of reactions, minimal side reactions, simple operations, non toxic nature and non polluting effluent generations. The leather industry world over is coming under pressure from environmental regulations to comply with the pollution and discharge legislation. The current activity in the area of leather processing is shifting towards the design and utilization of cleaner and softer technology like enzymatically enhanced processes. The enzymes are successfully employed for the better quality leather production with less pollution impact and also for the treatment of waste discharged from the industry. The leather processing from the raw skins to the finished products required the various steps like curing, soaking, liming, dehairing, bating, pickling, degreasing and tanning. The various processing principles have been discussed in brief along with application of suitable enzymes, their properties and sources. It showed that leather industries have enormous potential for the wide range of applications of several industrial enzymes.

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Interest in enzyme system is a result of a desire to utilize their vast catalytic potential, high specificity and high catalytic activity under mild environmental conditions of pH, temperature and pressure. The use of enzymes, although only recently understood, has been going on for centuries. Since micro-organisms are responsible for the fermentation of beer, wine, bread, cheese and various vegetables, all these processes are examples of cell-mediated conversions or application of enzymes. Current technology makes it possible to isolate, purify, even to immobilize (bind to fixed support) the specific enzymes needed for a desired function. Enzymes claim potential applications in agriculture, leather, food, textile and in pharmaceutical industries<sup>1-6</sup>. Enzymes play significant role in industrial effluent treatment, water treatment, petroleum sludge degradation, crude oil spill treatment, fly ash dump reclamation, eco-restoration of mine dumps and degraded eco-system<sup>7</sup>. Enzymes are also used for many more typical applications like fuel oil additive to improve dispersion and flame temperature, mould release agent in building and construction industry, removal of dead tissue and dissolution of blood clots, reverse

hydrolysis in the aspartame synthesis, surfactant for bitumen in the surfaced roads, modification of protein rich materials, and removal of turbidity in the beverage.

Animal skin goes through a series of operations prior to the making of various leather goods. The enzymatic action in leather production was reported to have started at research level in the early of the 20<sup>th</sup> century and first patent was taken by Rohm<sup>8</sup> in 1910 for the use of enzyme in bating. It took more than seventy years to apply them on industrial scale for dehairing process. Later on enzymes were successfully employed for the better quality leather production and also for the waste treatment in the leather industry. The prime stages in leather processing are – curing, soaking, liming, dehairing, bating, pickling, degreasing and tanning. The discharges and refuges disposed from all these processing stages in the leather production, causes severe health hazards and environmental problems to the entire eco-system. The huge amount of industrial effluents contain relatively higher amount of sulphide and chromium for improving the quality of tanning in the leather production. The leather industry world over is therefore, coming under high pressure from environmental regulation to comply with the pollution and discharge legislation. Because of the restriction of

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the environmental protection agency (EPA), dealing with dissolved solid levels in plant effluents, processing of brine cured hide is sometimes uneconomical. This is the reason, US exports a large volume of the hides to other countries, where environmental restrictions are less stringent, and it buys finished leather from abroad, at a value added price. As a result, the leather industry is looking for cleaner option for the dehairing process. The current activity in this area indicates that the trend is shifting towards design and utilization of cleaner and safer technology like enzymatically enhanced processes<sup>6,9-10</sup>. A number of different enzymes (proteases, lipases, amylases) have been used in leather processing in these directions.

Traditionally, enzymes found in dog were used to treat leather to make it pliable by removing some protein components. The reason behind the use of protease lies in the fact that the protein is the major constituent of hair and skins. Hair is composed of  $\alpha$ -keratin fibres, insoluble protein molecules containing a large fraction of cysteine residues and having an  $\alpha$ -helix conformation. The  $\alpha$ -keratin is arranged in piles of fibrils. Different skin layers are composed of collagens,  $\alpha$ -keratin, and some elastin. Collagen contains a large fraction of glycine, alanine, proline, and hydroxy proline. These are arranged in a triple helix conformation<sup>11</sup>. The use of enzymes with different specific constituents in leather makes it possible, selective hydrolysis of the noncollagenous constituents of the skin.

Dehairing is the single largest process in leather production, which requires huge amount of industrial enzymes like proteases, amylases and lipases. Works on enzymatic option for the dehairing process, were carried out by Raju *et al.*<sup>12</sup> using a strain belonging to *Bacillus* isolated and evaluated for its efficacy. The activity and the performance of the enzymes satisfied the condition necessary for its application in

dehairing. Enzymes have good potential to be exploited as an environmentally friendly option in dehairing as the trend is shifting towards cleaner technology. Therefore, the potential for the industrial use of enzymes in leather processing is very high because of their marked properties as highly efficient and selective catalysis. The resulting saving in process time increases efficiency and allows increased leather output as well.

### Enzyme applications

Bull hides, buffalo hides, steer hides, heifer hides, calf hides, bovine skins, goat skins, sheep skins and many more precious hides and skins have been the concern of high interest for leather professionals all around the world. Table 1 shows some typical events in relation to the chronological development of the leather technology and the enzymatic involvement. From the data of chronological development, it is observed that leather technology in the early of the twenty-century rarely used any enzymatic treatment, although the demand for quality leather has been the matter of concern throughout the ages.

The various important processing methods involved in the leather manufacture are curing, soaking, liming, dehairing, bating, pickling, degreasing and tanning. All these successive steps in the leather production involve enzymatic action directly or indirectly for facilitating the procedures and enhancing the leather output of desired quality. Table 2 showed the extent to which the enzymatic action have been involved at different stages of leather processing. Enzymes are mainly used in soaking, unhairing, bating, degreasing and waste processing of leather industries.

### Curing

Curing is the process of preserving the hides from getting spoiled well before the exercise of various

Table1—Major land marks in the leather technology development in 20<sup>th</sup> century

Year	Researchers	Some typical events in leather technology
1913	Rohm & Haas	Attempts for enzymatic dehairing process were made
1914	Loveland F A	Concept of white weight and comparative were studied
1922	Mc Laughlin	Brine-cured hides were found much better white weight
1931	Theis E R	Effect of salt in lime liquor with Na <sub>2</sub> S <sub>n</sub> were studied
1958	Strandine & Connick	Effect of storage on brine-cured hides were studied
1960	Mycek & Clarke	Research report on transglutaminase were made public
1970	Folk, Cole & Chung	Basic research on mechanism of enzyme action started
1972	Chung	Isolation of transglutaminase from hair follicle done
1985	Blair & Sirolime	Blair - Sirolime methods of dehairing process developed

Table 2—Enzymatic function and its involvement at different leather processing stages

Stages	Enzymes involved	Function of enzymes
Curing	Enzyme are directly not involved	To preserve hides and skins
Soaking	Alkaline & pancreatic proteases	To remove non fibrillar proteins
Dehairing	Alkaline & neutral proteases	To improve the waste water quality
Degreasing	Lipases & proteases	To remove fats
Bating	Trypsin & alkaline proteases	To make soft, supple and pliable
Tanning	Enzyme are directly not involved	To influence the quality of tanning
Waste processing	Trypsin & proteolytic enzymes	Chrome- tanned-waste processing

processing and to use them purposefully later on. If the hides and skins are not cured just after flaying, they get putrefied within two-three days. Therefore, hides are required to immediate thorough curing to stop them from deterioration. Hides are steeped in a brine bath and dried in the sun and salt is added to the flesh side. For curing, dry, airy and clean places are preferred. Curing is done at controlled temperature, pH, moisture and by using toxic materials. In curing, use of biocides has been preferred although these are inimical to the environment. Radiation curing is one among several curing methods that is theoretically an attractive and alternate method but practically this is not feasible. Therefore, salt curing is still prevalent in many countries. Some times the presence of organisms on cured hides reduces the value of the hides as a raw material for leather manufacture. The quality of leather manufactured from the brine cured cattle hides are known to deteriorate on prolonged storage, particularly at elevated temperatures. The deterioration is probably due to the presence of proteolytic enzymes produced by micro-organisms growing on the hide. Curing the hide with the salt to overcome the degenerative process has been used for long. If the hide is properly salt cured, the activity of the organism is readily controlled<sup>13</sup>.

#### Soaking

Soaking is the first tanning operation for treatment of hides and skins with water. Hides are first soaked for rehydration before further processing. The better the rehydration, superior the leather. In this stage hides and skins are washed and soaked in surfactants and anti-microbial compounds. This process is preferred to facilitate the further processing of leather. Green hides and skins are soft enough and therefore do not require any soaking<sup>14</sup>. A salt-cured hide requires a soaking process that raises the moisture content from 45 percent to greater than 52 percent. This is achieved through a carefully designed and monitored soaking process. The longer the soak, the

more significant the bacterial threat is to the crude material. The method of soaking for a pack of hides and skins depends mainly upon its conditions and to a less extent upon the type of leather which is going to be produced. In developed countries like Europe and America, proteolytic and amylolytic enzymes are widely used for soaking. Use of enzymes in soaking have been tried successfully since 1966 because surfactants in excess amounts cause pollution<sup>15</sup>. Several works on swelling for brine-cured hides with enzyme soak has also been reported providing important information about swelling versus immature collagen<sup>16,17</sup>. In a study of various types of soaks on the rehydration of brine cured hides, Tancous *et al.*<sup>18</sup> exercised six different soaking methods (three enzymatic methods and three conventional methods) and compared for their effectiveness in rehydrating the center portion. The ultimate goal of the study was to reduce the incidence of hard spots. A 45 percent reduction in the soaking time over conventional methods and a 40 percent reduction in the amount of sulphide used for dehairing was found. The enzymatic method, which employed the use of, protease + surfactant, was the best of the six methods attempted. The type of soaks used and the soaking times are listed below:

- Protease + surfactants → 5 hours
- Lipases + surfactants → 5 hours
- Proteases + lipase → 5 hours
- Soda ash + surfactants → 9 hours
- Regular process (sodium tetrasulphide + surfactant) → 9 hours
- Modified process (sodium tetrasulphide + surfactant) → 9 hours

For the two set of experimental values determined, the typical values for sulphide (mg/L) used in the regular processing were 2490 and 2340 and in the protease + surfactants were 1330 and 1490, a 41 percent reduction. Values for the protease + lipase

process were 1450 and 1340, a 42 percent reduction over the regular process. Values for soda ash + surfactant; regular process modified, and lipase + surfactant were 2090, 2180; 2332, 2530; and 2410 and 2360. With regard to the effect of soak types, the centre layers of the soaked hides varied considerably depending upon the type of soak used. The protease + surfactant appeared as the best rehydration of the center layers at 59.9 percent, the other process were less effective. The other soak type showed lower values at 55.3, 56.6, 56.2 and 55.6 percent for the protease + lipase, soda ash, regular processing and regular processing modified. Zung's<sup>19</sup> work has recently shown that protease has positive impact in the soaking of salt-cured hides. By using a special historical techniques, Zung found that enzymes definitely penetrated both sides of the hide to the depth of one-twentieth to one-tenth of the thickness and that some enzymes penetrated into the centre of hide by means of the blood vessels. When leather was produced, only a short, four-hour soaking time was cleaner than that of the control leather. Protein between the fibres of hides are removed by proteinases. The removal of the proteins disturbs the molecular structure and enables faster rehydration or soaking.

#### Liming

Hides and skins are taken for the liming operation after soaking. It is learned that most of the skins are not sufficiently swollen and they need a liming treatment for desired swelling. In this process, the soaked hides and skins are treated with milk of lime. It gives the desired swelling of the collagen structure which helps to open up the fibre bundles. The objective of this process is to remove the hairs, nails, hooves and other keratinous matters and also to remove the interfibrillary soluble proteins like mucins. The quality of the finished leather is largely controlled in the liming process. High abrasive resistance of sole leather, high tensile strength of picking band or belting leathers are largely dependent on the process of liming. The liming method used on sheep skins is the painting of the flesh side with a lime paint, a mixture of slaked lime and sodium sulphide solution or slaked lime and red arsenic.

#### Dehairing

Dehairing is the process of removing the hairs and furs from the hides without any damage to them. The process of dehairing largely depends upon the

phenomenon of hair loosening. Loosening of the hair is due to the chemical reaction of lime liquor on the hair root or base of the hair shaft. This weakening of the hair is dependent on the breakdown of the disulphide link of the amino acid, cystine, which is characteristic of the keratin class of proteins e.g. wool and hair. The process of dehairing achieved using a variety of techniques was reviewed by Germann<sup>20</sup>. The most commonly employed methods for dehairing rely upon the use of sulphide during liming to destroy keratin, the principal components of hair. This produces an effluent with a chemical oxygen demand of about 60,000 mg/L and constitutes the polluting aspect of leather manufacture. The use of sulphide in dehairing can be circumvented by the use of proteolytic enzymes<sup>21,22</sup>. These are used as supplement in the chemical dehairing processes. The enzymes remove the hair by disturbing the proteinaceous matter present at the base of the hair. Proteolytic enzymes can also attack the dermal collagen, damaging fine fibres in the grain enamel<sup>23-24</sup>. Therefore, there is a definite need to identify specific proteases that can remove hair without damaging the fibrous collagen. A number of enzymes have been proposed and include proteases from bacterial, fungal and vegetable origin<sup>23-25</sup>.

Dehairing is the process where enzymatic involvement is the most important factor to expedite the process. Enzymatic dehairing, either in the alkaline range or in the acid range, has been widely exercised<sup>26</sup>. The primary studies on dehairing by Raju *et al.*<sup>12</sup> with the extracellular protease secreted by the *Bacillus* isolate, showed that it has a dual pH—maxima at pH 7.5 and 9.0 and the temperature maxima at 37°C. It requires the presence of complex protein substrate in the medium for optimal enzymatic action. In enzymatic dehairing, apart from pH, temperatures also play significant role. At temperatures ranging from 32-37°C unhairing could be accomplished between 18-24 h. At temperature below 32°C, the duration of enzyme application needs to be increased for complete dehairing. Further, below 25°C no appreciable enzymatic dehairing within a reasonable period is envisaged. Studies conducted on the temperature stability of the enzyme indicate that the enzyme is stable between temperature ranging from 20 to 50°C. Their studies also showed that although even 2% (w/w) of crude enzyme was sufficient for dehairing, 3% (w/w) of the enzyme was preferred because at this concentration even the tough

hair at the neck region was removed completely. Pal *et al.*<sup>27</sup> have experimentally carried out the investigation for dehairing goat and sheep skin using the enzyme secreted by *Rhizopus oryzae*. They studied the effect of units of activity of enzymes, effect of pH, and effect of hydration on dehairing process. Subsequently, they evaluated the physical properties of wet blue leather for both goat skin and sheep skin in the prevailing condition of lime-sulphide dehairing and enzymatic dehairing. An enzyme powder was obtained after the drying of enzyme extract and this was used to prepare a paste that was painted on the flesh side of both the goat and sheep skins. The skins were piled flesh to flesh and left at room temperatures (33-35°C) overnight. The hair was removed using a blunt knife. After dehairing, the skins were further processed to the wet blue stage. Their physical evaluation also included the general appearance, feel, fullness, gloss and grain smoothness. They finally concluded that an economic viable enzymatic dehairing process can be favourably compete with the chemical dehairing process. They also observed that enzymatic dehairing could be accomplished within 11-12 h provided the temperature was maintained in the range of 30-37°C. The enzyme used by them was stable from pH 3-11 and temperature up to 80°C. They optimized the prevailing condition for dehairing hide and skin with 46% hydration, 83% humidity, pH 8.0 and 2 h of incubation period.

Thangam *et al.*<sup>28</sup> made an investigation of alkaline protease isolated from *Alcaligenes faecalis* for enzymatic dehairing in tanneries. The enzyme used by them was relatively stable in the pH range of 8-11 and at temperature up to 30°C for 24 h. Their results indicated that the protease produced by *Alcaligenes faecalis* was best suitable for dehairing and could be exploited as an eco-friendly dehairing agent in leather processing. Their results envisaged to reduce the pollution load to the environment and help in bating operations for improved yield and soft leather. In their work goat-skins were washed and cut into two halves. The paint method of the dehairing was followed in all the studies. The enzyme powder obtained after lyophilization was used to prepare a paste that was painted on the flesh side of the goat skin. The dehairing trials were conducted by following sulphide free and less sulphide methods using various concentrations of enzymes and sulphide. The right halves of the skin were applied with the enzyme

preparations and treated as experimental skins. The left halves were the control and dehaired by following a conventional lime-sulphide method of using 2.5% sodium sulphide and 10% lime. The efficacy of the dehairing process in relation to different temperature, pH, enzyme concentration and less sulphide methods were measured by applying a scale such as difficult dehairing, slightly difficult dehairing and moderate dehairing. The complete removal of hair was achieved even with 0.5% enzyme concentration. At 0.25% enzymes concentration, only moderate dehairing was noticed after 18 h of applications. Their findings indicated that the use of 0.5% of crude powder was sufficient to reduce sulphide from 2.5 to 1%.

A recent report communicated by Paul *et al.*<sup>29</sup> involved the proteolytic enzymes for providing a suitable alternative to destructive sulphide dehairing which included the use of a diverse array of enzymes many of which were rather non-specific. The enzymes caused loosening of the hair, without damaging the fibrous collagen of dermis. The enzymes included were proteases from bacterial, fungal and vegetable origin. Another enzyme for use in depilation was dispase, a neutral proteinase of bacterial origin. The enzymes have been used successfully for a number of years in biomedical field to achieve separation of epidermal and epithelial cells from the underlying matrix through the destruction of the basement membrane. In their study, incubation of bovine skin with dispase caused detachment of the epidermis of the level of the epidermal/dermal junction leaving a clean grain surface. Sloughing of the epidermis was observed initially at the surface but with prolonged treatment extended into the hair follicles to bring about in hair loosening. This is likely to be a result of enzymatic cleavage of the collagenous components of the basement membrane upon which these structures reside. However, the microscopic examination did not show any evidence of grain damage, indicating that the fibrous collagen of the dermis has not been degraded by the enzyme.

Ever since the onset of the industrial enzymatic dehairing process in 1990, considerable amount of work has been carried out and some researchers presented the review report elaborately on the phenomenon of enzymatic dehairing<sup>30</sup>. But most of the enzymatic process development is propriety in nature. Many people rationalize enzymatic dehairing as a sound alternative to the lime-sulphide process owing to the severe problem created by sulphide and

chromium in tanneries. The reason being, these two components are the major pollutants of the leather processing<sup>9</sup>. The advantage of enzymatic dehairing is the reduction of sulphide content in the effluent, and elimination of the bate in the delimiting, reduction in the COD and BOD of the effluent of toxic chemicals and easy handling<sup>15,31</sup>. Therefore, economically viable enzymatic dehairing process can compete well with the chemical dehairing process. Disadvantage of enzymatic dehairing are that enzymes are more expensive than the conventional process chemicals, require careful control, and because of the effect on the structure of the hides and skin, may require subsequent process changes<sup>32</sup>. Ultrafiltration of the enzymatic dehairing using proteolytic enzyme was carried out by Cassano *et al.*<sup>33</sup> during the unhairing operation thus creating an enzymatic reactor for the production of dehaired skins. Ultrafiltration permits to control the enzymatic action on skin because the enzyme was rejected by the membrane and is accumulated in the feed tank, while the sulphide component (used in low concentration) is rejected with the permeate. The advantages of the coupled enzymatic/UF system were: control of enzyme action; reduction of sulphide requirement; shortening of unhairing-liming time; then possibility to recover hair and to reduce pollution of waste water and cost of cleaning up processes. Disadvantage for adopting enzymatic dehairing is sometimes ruled out when a cost effective enzyme is available.

#### **Bating**

Bating is the process of beating the leather cruelly with heavy and sudden stroke using metal rods or wooden logs in prevailing condition. The purpose of bating is loosening and peptization of the non-collagenous skin structure through the removal of the residues of the interfibrillary proteins, epidermis and scuds. This makes them soft and supple and to prepare them for tanning. Strong bating is required to achieve a soft and pliable leather such as purses and gloves, whereas slight bating is required for the soles of shoes. Bating is in fact an offensive stage in the preparation of quality leather. Bating brings about the following effects in the pelts: removal of lime, produce silky grain, remove swelling and plumbing, increase the degree of stretch possessed by the finished leather. Failure to remove the non-collagenous proteins causes a cementing together of the fibres when the leather is dried and results in firmness and lack of flexibility. It is well known that

the classical bating process in the alkaline condition makes use of proteolytic enzymes, which are of pancreatic or bacterial origin, and the efficiency of the process depends on the enzyme concentration as well as temperature, pH and time during the bating process. The bating in alkaline conditions is today universally recognized by the entire leather industry, but to be effective it should be conducted at 95-100°F and at pH 7.5-8.5, otherwise the enzyme efficiency drops drastically<sup>34</sup>. The effect of bating enzymes occur through the diffusion of the enzymes into the hide but the greatest concentration is found on the outer layers. On the unsplit pelts, penetration of the bating enzymes to the inside of the hide are insufficient to digest unwanted proteins, this is particularly true in the neck and butt area. It is worth to note that whether the enzymes are free or bound to the particles. If the enzymes are free then the particulate can be removed before adding the substrate. If the enzymes are particle bound, clarification may decrease the activity. In fact, particulate interferes with the exposure of the substrate, which is insoluble to the enzymes.

#### **Degreasing**

Hides and skins, specially domestic sheep skins, contain large amount of natural grease which is generally removed in the tannery by liming operation. But sometimes it so happens that the hides and skins contain appreciable amount of grease even after liming. This residual grease is responsible for fatty acid spues, uneven dyeing and finishing, waxy patches in alum tanned leathers and pink stains in chrome blues, etc. Tanners were therefore trying for a long time to find a suitable means to get rid of this residual grease in hides and skins. Treatment of tanned leathers with a suitable solvent of fat makes the former hard and horny and therefore, for pliability, extra fat liquoring was necessary. On the other hand, it was practically impossible to remove grease from pelts by similar treatment due to the presence of large percentage of water in the pelts. Moreover, in hides and skins, grease always remains inside fat cells made up of reticulin or other type of tissues. Unless these cells are ruptured, grease cannot be removed by any degreasing method. Sun drying, ruptures the fat cells almost completely, and therefore it is easy to remove grease from flints by any suitable degreasing methods. But wet salted and fresh hides and skins require special treatment, before degreasing, to rupture the fat cells. With one percent sulphuric

acid, ten percent common salt and storage for few weeks after pickling rupture almost all the fat cells and therefore, degreasing is done mostly after pickling. Three methods used for the removal of the grease are aqueous emulsification, solvent extraction and pressure degreasing.

During the last five years enormous progress has been made in the field of degreasing process in an aqueous medium, both as regards to the use of contaminating chemicals and in the application of technology of the products. The best time for carrying out the aqueous degreasing process is on pickled skin, as the deposits are more accessible to the surfactants. The application of the enzymes mainly of the lipase type, in different stages of the leather process has been studied<sup>35-38</sup>. When enzymes are applied in aqueous medium (pickling phase), there was combined action involving rupture of the membranes surrounding the fat cells and triglyceride splitting, all of which helps to improve the degreasing process.

Palop *et al.*<sup>39</sup> studied the effectiveness of the degreasing with lipase enzyme. They started with five English domestic lamb skins which underwent a conventional beam house operation (soaking, unhairing, deliming and bating). Then these were treated with a reference pickle, standard degreasing with 8% ethoxylated fatty alcohol (EPA) and enzyme treatments as follows:

Skin 1: Reference pickle—standard degreasing

Skin 2: Reference pickle + 1.5% acid protease—standard degreasing

Skin 3: Reference pickle + 1.5% acid lipase—standard degreasing

Skin 4: Reference pickle + acid protease + acid lipase—standard degreasing

Skin 5: Reference pickle + acid protease + acid lipase—standard degreasing + 1.5% neutral lipase

Samples were taken from both pickled and degreased skins for initial and residual fat determination. Percent degreasing effectiveness was expressed as  $[(\% \text{ initial fat} - \% \text{ residual fat})/\% \text{ initial fat}] \times 100$ . It was observed that the reference degreasing process (skin no.1) had an effectiveness of 58%. The addition of an acid protease (skin no.2) to reference pickle has no influence on the degreasing effectiveness, since it remains at 58%. Treatment with acid lipase increased the degreasing effectiveness to 78% (skin no.3). The combine effect of protease and lipase gave effectiveness 78.5% and further increase

in effectiveness up to 88% was produced by adding lipase to the degreasing process in a neutral medium (skin no. 5). Waters and Price<sup>40</sup> observed that treatment with acid lipase did not rupture the fat cells, but it broke down the triglycerides of the natural fat released from the cells due to reaction of the acid of the pickle process. The natural fat was composed of fatty acids 10, triglycerides 56, waxes 23, phospholipids 6 and cholesterol, 5%. So with the breakdown of the triglyceride with lipase the fatty acid composition increased from 10 to 40% which became of enormous assistance to emulsify the diglycerides and monoglycerides, fatty acid and glycerol which were formed in the rupture of the triglycerides<sup>39</sup>.

Mitchell and Ouellette<sup>41</sup> evaluated the combination of lipase and protease to clear the surface of the chrome tanned stock of grease, dirt, scud and other stains for the purpose of making more uniformly coloured leather. Lipase and protease were tried since the undesired material on the surface of chrome tanned blue stock are proteins, fats and oils. A significant reduction in grease stain, neck wrinkle discolouration was observed. Also improvement was observed in brightness and uniformity in dyeing. This was accomplished using extremely low amount of a combination of two enzymes selected to remain particularly active in the condition of retanning with respect to pH, temperatures, running times and presence of other chemicals. The low pH conditions found in the retanning of blue stock required to select enzymes active in low pH range. 0.015% acid lipase and 0.3% acid protease was best combination.

#### Tanning

Tanning is the last stage in leather manufacturing. It is the process of converting unstable raw hides into leather, with adequate strength properties and resistance to biological and physical attacking agents. In fact, it is the process of introducing a tanning agent into the hides. This is accompanied by the introduction of additional cross links into collagen, which bind the active group of the tanning agents to the functional group of the protein. Tanning makes collagen more resistant to the hydrolysis by acids and enzymes, but not by alkalis. Thus, the tanned collagen as a rule binds less water than the native one. Tanning with chrome compounds or with tannins does not prevent alkaline collagen hydrolysis<sup>14</sup>. Chrome tanning is the most important tanning method to obtain light, inexpensive leather of high thermal and

bacterial resistance. It improves the colour, appearance and look of the finished leather as well. Almost all skins and hides are coloured, using chrome tanning after these are treated with acid solution for delimiting purposes. Although, enzymes are not directly involved at this stage, however, the enzymatic treatment in the previous stage significantly influences the quality of tanning.

#### Physical evaluation

The details which correlate the enzymatic processing with the physical properties of the leather is scanty in literature. The evaluation of physical properties of the leather includes the measurements of tensile strength, tearing strength, stitch tear strength, and percent elongation. Raju *et al.*<sup>12</sup> reported the determination of elongation of break tongue tear strength, brushing strength and tensile strength of the dyed crust leather samples, cut from the identical portion in the butt area. They conducted the experiments of dehairing by the enzyme extra-cellular protease secreted by the *Bacillus spp.* The enzyme concentrations applied were 1 to 3 percent in the pH optimum 7.5 to 9.0 and temperature optimum 32 to 37°C. Their specimens compared favorably after enzymatic dehairing process with regard to tensile strength, elongation of break and tongue tear resistance. Pal *et al.*<sup>27</sup> also proceeded for the physical evaluation of the leather which included the measurements of tensile strength, tearing strength, stitch tear strength and percent elongation with the application of enzymes as depilant. Their measurement data are shown in Table 3. However, they did not make any conclusion based upon their enzymatic study. In the study conducted by Thangam *et al.*<sup>28</sup>, they also measured the strength properties of the leathers treated by the enzyme secreted from *Alcaligenes faecalis* at pH optimum 9.0 and temperature optimum 55°C. The properties of the enzyme treated leathers were comparable to those of

control leather. The tear strengths of the experimental leathers were considerably better when compared to the corresponding control leather. These indicated that the enzymes treatment did not have an adverse effect on the strength of the leather. However, there is not any scientific details in the literature which can correlate the physical properties of leather and the enzymatic action. This invites the attention of researchers to make a dent in this direction for improving the leather quality and facilitating the process in leather production.

#### Waste processing

Effluent discharges from leather processing industries create health hazards and environmental problems unless these wastes are properly treated. Fleshings, the major solid waste generated at the pretanning operations of leather processing, were hydrolyzed using pancreatic enzymes with a view to evolve a simple method for solid waste management by Kumarguru *et al.*<sup>42</sup>. The proteolytic activity of the pancreatic homogenate with casein was found to be 80 units/mL. Fleshings treated with pancreatic enzyme preparation showed a six fold increase in proteolysis against the control at the end of 7 days. The protein content, collagen and the free fatty acids in the hydrolysate supernatant were 80.0, 10.64 and 72.86 mg/mL respectively. The optimum pH for the enzyme preparation was 8.5. The hydrolysis was observed by almost total liquefaction of the fleshing. Bajza and Marcovic<sup>43</sup> studied the effect of alkaline protease on untanned leather (hide) waste. Trimmings obtained after liming had the alkalinity that corresponds to pH 10. The enzyme which was active in this pH was used. The process was conducted at constant temperature 55°C favourable for the enzyme. The enzyme was a commercial preparation of alkaline protease Protoderm 100T, produced from submerged cultivation of *Bacillus* genus. It was observed that leather solubility increased by increasing the enzyme

Table 3—Physical evaluation of wet blue leather

Experimental details	Tensile strength (Kg/cm <sup>2</sup> )		Elongation (percent)		Tearing strength (Kg/cm)	Stitch tear strength (Kg/cm)
	1st	2nd	1st	2nd		
Goat skins						
I. Lime-sulphide dehairing	120	212	90	60	34	38
II. Enzymatic dehairing	131	269	60	60	56	89
Sheep skin						
I. Lime-sulphide dehairing	140	176	80	66	48	55
II. Enzymatic dehairing	175	200	66	70	48	71



concentration from 500 to 15,000 units per gram of leather. Leather waste breakdown yielded a water soluble hydrolysate, which could be concentrated on a vacuum evaporator and then dried to fine flour for various purposes.

The major pollutants in leather industry are sulphide and chromium. Chromium in the effluent is a serious threat to the water resources. In many countries it is not allowed to exceed 5 ppm. But the chrome letout in the effluent is about 40 percent of that input offered. Therefore, reduction have been made to the offered chrome from 2.5 percent Cr<sub>2</sub>O<sub>3</sub> to 2 percent Cr<sub>2</sub>O<sub>3</sub>. But mere reduction does not do well. Nowadays conversion of tannery by-products into industrially useful products has been given prime attention as an alternate solution. Enzymes have been applied by Cabeza *et al.*<sup>44</sup> for gelatine isolation from chrome shavings. Chrome shavings were pretreated for 6 to 24 h with enzyme solutions at the optimum pH for the enzyme. Then gelatin was extracted at 70°C, pH 8.0. To evaluate the effectiveness of the process, the protein yield i.e. the amount of initial protein recovered as gelatin was determined. Pepsin rose to a maximum of 6.10% using 0.01% of enzyme, higher than the control (without enzyme) value (4.33%). Trypsin had a maximum of 14.70 % using 0.25% of enzyme in the extraction. This value was much higher than the control and the values obtained with pepsin. Kolomaznik *et al.*<sup>45</sup> has reported on enzymatic dechromation of chrome-tanned wastes by using proteolytic enzyme (ALCALASE of Novo Nordisk, Denmark). The mechanism of enzymatic reaction was as follows.

#### **Enzymatic hydrolysis**

Chrome-tanned-wastes → Gelatin/Protein hydrolyzate + Chrome sludge

They sought for the potential application of the reaction products in different industrial sectors. The chrome sludges were found suited for pigment in glassmaking, heat-resistant bricks and alkaline chromate. The use of hydrolysates was best suited for concrete admixture, grinding of cement hydration of lime, protective coating and plaster binder. The chrome sludges were further processed and recycled tanned salt were produced. The typical composition of chrome cake thus produced was Na<sub>2</sub>O-0.42%, MgO-6.40%, Al<sub>2</sub>O<sub>3</sub>-0.40%, SiO<sub>2</sub>-0.89%, P<sub>2</sub>O<sub>5</sub>-0.12%, SO<sub>3</sub>-1.50%, CaO-2.50%, Cl-0.33%, Cr<sub>2</sub>O<sub>3</sub>-15.90%, MnO-0.13%, Fe<sub>2</sub>O<sub>3</sub>-2% and the Ignition loss - 68.81%. Cabeza *et al.*<sup>46</sup> studied the pilot plant trials of a

process to treat chrome shavings to isolate protein products and purified chromium. The process used two enzymes, pepsin and alkaline protease, in two consecutive extractions with isolation of high quality gelatin and a hydrolysate. Chrome shavings were pretreated with 0.1% pepsin at pH 3 -3.5 and at room temperature for 8 h and gelatin is then extracted at pH 8 and 70°C. Gelatin removed by using filter press and hydrolysed protein was then isolated by second extraction from sludge using 0.005% alkaline protease at pH 8 and 70°C for 3 h. The remaining solid after filter press, called chrome cake, was chemically treated to prepare it for recycling in the tannery industry.

#### **Industrial enzymes**

The commercial exploitation of enzymes is not new. The use of yeast as bio-catalyst dates back to about 6000 B C. However, the production of enzymes at large scale cannot boast of so ancient a history. The first large scale production of enzymes came about only in 1874, with the first industrial batch of chymosin. From 1913 onwards the detergent industry became a prime concern of enzymes. The leather industry in 1917 and the starch industry in 1950 set a similar trend. The last three decades have been marked by an explosive advancement in the field of commercial production of enzymes. The increasing demand of quality leathers in the country as well as in the world is increasing fast. This invites new process-efficient and clean technology in the leather production. Industrial enzymes have appeared to develop the so called clean technology and economic process in this direction. Industrial enzymes related to leather processing are usually mixture of different enzymes and these are standardized with diluents. Based upon the type of bio-chemical reaction, enzymes are assigned to large groups.

Many industrial enzymes are being manufactured from animal and plant system. However, major share is obtained by cultivation of microbes. About twelve categories of enzymes are used for industrial purposes. Table 4 showed the global production of industrial enzymes. Most of these are hydrolytic enzymes used for the depolymerisation of natural substrates to low molecular mass. The largest group being proteolytic enzymes from bacteria (59%) followed by carbohydrases (20%) in terms of the relative sales value<sup>1</sup>. It is evident that the global production of industrial enzymes is increasing fast

Table 4—Global production of industrial enzymes

Enzymes	Amount (Ton )
Bacillus protease	550
Aspergillus amyloglucosidase	350
Bacillus amylase	350
Glucose isomerase	60
Microbial rennet	25
Fungal amylase	20
Pectinase	20
Fungal protease	15

Table 5—Country wise production of commercial protease enzymes

Country	Amount (Ton)
Denmark	249
Netherlands	100
USA	64
Japan	42
Germany	32
France	16
UK	11
Switzerland	11
Others	5

owing to the multiplying demand of typical enzymes in different industrial sector. Among the industrial enzymes producing countries, Denmark top in protease production with its annual production of 249 tones, which is approximately 47 percent of the global production (Table 5). The worldwide market of industrial bulk protease (trypsin) of the animal pancreas origin are approximately  $10^7$  \$ for their comprehensive application in the leather production. However, there is much more likelihood of increasing its sales market in near future.

Proteases used by the detergent industry are also suitable for soaking purpose since they are relatively resistant to the increased pH of about 10 used in the soaking bath. Among different industrial enzymes pepsin, trypsin, rennin and transglutaminase are the most frequently used enzymes in leather manufacturing process. These are the typical enzymes with their specific utility in soaking, bating and dehairing process. The details for scores of industrial enzymes and their applications are widely available throughout the literature. Properties of some of the important industrial enzymes are given in Tables 6-8.

Trypsin is formed in the intestine from trypsinogen which in turn is formed in the pancreas. Trypsinogen becomes trypsin when a splitting of Val-(ASP)<sub>4</sub>-Lys

Table 6—Properties of proteases<sup>1</sup>

Organisms	pH Range for haemoglobin	pH stability
<i>Aspergillus saitoi</i>	3.5 - 4.5	2.0 - 5.0
<i>Aspergillus oryzae</i>	3.0 - 4.0	5.0
<i>Panceilomyces varioti</i>	3.5 - 5.5	3.0 - 5.0
<i>Mucor pusillus</i>	3.5 - 4.5	3.0 - 6.0

Table 7—Properties of lipases<sup>1</sup>

Source	Optimum pH	Temperature stability °C
Porcine pancrease	6.5 - 9.5	40 - 45
<i>Rhizopus</i> species	6.0 - 7.5	35 - 40
<i>Mucor javanicas</i>	5.5 - 8.0	40 - 45
<i>Candida cylindracea</i>	5.0 - 7.5	40 - 45

Table 8—Properties of amylases<sup>1</sup>

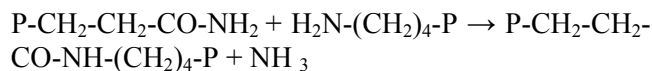
Enzymes	Optimum pH	Optimum temp °C
Bacterial $\alpha$ -amylase	6.5 - 7.5	95.0
Fungal $\alpha$ -amylase	5.0 - 6.0	80.0
Malt $\alpha$ -amylase	4.5 - 7.0	85.0
Pancreatic amylase	6.0 - 7.0	75.0

hexapeptide occurs. Many trypsin like enzymes are also known. Their way of action and composition are close to trypsin. They attack the denatured proteins. According to the research report communicated by Cabeza *et al.*<sup>44</sup> and Taylor *et al.*<sup>47</sup> trypsin proved to be an effective enzyme for the isolation of protein product during the treatment of chrome shavings. They developed the commercial trypsin preparation that proved to be not only efficient in solubilizing the shavings but also cost effective.

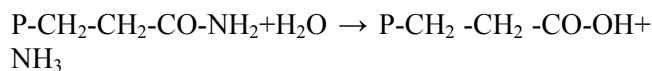
Pepsin, has been reported being applied during pickling and on chrome tanned hides and skins. This acid enzyme has been extensively used on pigs and sheep skins, which by nature are very greasy. The proteolytic action of this acid enzyme hydrolyzes the cell membranes and a good part of grease is eliminated. Hence this enzyme also acts as a degreasing agent. Pepsin is an enzyme characteristic of the mammalian stomach structure, with molecular weight of 35, 000 and a large amount of dicarboxylic, aliphatic and aromatic amino acids. This enzyme has an optimum activity at pH around 2.0, but is still active up to pH 6.0, and becomes active in the presence of HCl. The enzyme product has the

advantage of a lower cost for the leather industry. The hydrolyzed protein components of this enzyme product assure a better stabilization of the pepsin activity and do not require the addition of other agents. According to a report communicated by Deselnicu *et al.*<sup>34</sup> pepsin enzymes work more gently than the alkaline proteolytic enzyme and can be used at lower temperature such as 70°F. The diffusion and the penetration of this acid pepsin enzyme through the inner layer is much faster and more effective than during the alkaline bating. This acid enzymatic process also has tremendous advantages for those tanneries which process wet blue hides from different sources. This new acid pepsin enzymatic process is covered by a US patent application<sup>48</sup>.

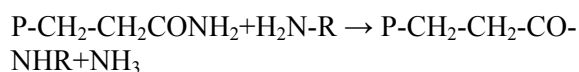
Another class of important industrial enzymes is transglutaminases. Transglutaminase is an enzyme capable of forming inter or intra-molecular cross-links in many proteins and has the utility in the modification of gelatin byproducts from the leather industry. The enzymes catalyze an acyl transfer reaction between the  $\gamma$ -carboxamide group of peptide bound glutamine residues as acyl donors and primary amines as acceptors. When the  $\epsilon$ -amino group of peptide bound lysine acts as acyl acceptor, an  $\epsilon$ -( $\gamma$ -glutamyl) lysine cross-link is formed;



This enzyme produced by fermentation is commercially available, relatively inexpensive and environmentally safe. In the absence of amine substrate, transglutaminase can catalyze the deamidation of glutamine residue during which water molecules are the acyl acceptors.



Transglutaminase can also catalyze an acyl transfer reaction between the  $\gamma$ -carboxamide group of peptide bound glutamine residue and various primary amines.



The earliest report on transglutaminase appeared in 1959-1960 when Mycek *et al.*<sup>49,50</sup> and Clarke *et al.*<sup>51</sup> described their experiments in the incorporation of primary amines into protein and in deamidations of

protein using this enzyme. During 1960 and 1970, Folk *et al.*<sup>52-56</sup> exercised basic research that resulted in understanding the structural requirements of specific substrate for this enzyme as well as the mechanism of enzyme action. In 1980s Motoki and Nio<sup>57</sup>, Motoki *et al.*<sup>58</sup>, Nio *et al.*<sup>59,60</sup> and Ikura *et al.*<sup>61-64</sup> published several research papers describing practical application of transglutaminase isolated from guinea pig liver particularly with food proteins. The description included cross-linking between different proteins, studies on the mechanism of gelation of protein solutions as effected by transglutaminase, and incorporation of amino acids. Because of the isolation problem, it was not commercially feasible to use this enzyme preparation. In 1989, Ando *et al.*<sup>65</sup> isolated the enzymes from a *Streptovorticillium* species and, based on this discovery two companies were jointly granted a patent<sup>48</sup> and shortly after this began producing the enzyme mainly for modification of food products. They demonstrated the feasibility of using microbial transglutaminase to alter the physical properties of gelatin by-products from the leather industry and thus increasing the potential markets for new value-added products. The characteristics of microbial transglutaminase isozyme, as reported by Taylor *et al.*<sup>9</sup> that it is calcium independent and has broad specificity. The enzyme is a single polypeptide chain with a molecular weight of about 38000 and this is about one half the molecular weight of the transglutaminase derived from pig liver. The -SH group of single cysteine is involved in the catalytic reaction.

The most important feature of these industrial enzymes is their activity. For all sorts of industrial and research purposes, enzymatic activity is one of the major determinants in their application. Various complexing agents may decrease the activity of metal dependent enzymes. However, in a report communicated by Didato and Bryant<sup>66</sup>, they did not observe any inhibition of enzymatic activity in tannery soaking procedures when normal dosages of microbicides up to 600 ppm Busan 1630 (2 bromo- 4' - hydroxy acetophenone) were applied. Microbicides are also essential along with the enzymes in the processing of leather. Six commercial proteases were assayed in the presence of four selected industrial microbicides. None exhibited any significant inhibition of the protease studied. The comparison of enzymes based on specific activity must be conducted. Except for proteases,  $\alpha$ -amylases and

lipases, many more enzymes are still being developed. However, their commercialization subjects to both the technical feasibility and the economics of utilization.

### Discussion

The emphasis on the use of enzymes in the industry has come about because enzymes have unique properties such as catalysis of chemical reactions at high rate, operations at ambient conditions, selectivity of substrate, minimal side reactions, simple operations, availability in large amount from microbial sources, reusability if immobilized, nontoxic nature and non polluting effluent generations. During the early days of enzyme technology, development was slow. This was due to impediments in the fields of enzyme stabilization, production on large scale, cofactor regeneration and lack of enzyme immobilization facilities. Advancements in the various areas of biotechnology have overcome most of the hurdles and currently, the pace of development of enzyme technology is quite rapid. The advancements in the techniques of genetic engineering which permit the manipulation of cellular DNA, have led to the opening up of a new field called protein engineering. The structure of a protein can now be altered by offering specific and precise changes in the DNA molecule, which ultimately will be reflected in the protein formed. The structurally altered enzyme thus obtained has different physico chemical properties which distinguishes it from its normal cellular component. The physico chemical differences engineered into the enzyme would, of course, depend on the requirements of the relevant industry. It has also been possible to increase manifold production of microbial enzyme by inserting extra copies of the gene responsible for producing the enzymes. Capability has now been developed to make use of microbes to express important enzymes of animal and plant origin. Recently, many novel industrial enzymes that prove to be efficient in the process of dehairing, soaking, bating and solubilizing the chrome shaving have been developed.

Leather industries have enormous potential for the wide range of applications of several industrial enzymes such as proteases (alkaline, neutral and acidic), lipases, amylases, pepsin, trypsin, renin and around glutaminase etc.<sup>67-69</sup>. Dehairing and dewooling are the prominent stages where enzymatic application can effectively govern the leather processing with least environmental pollution. Enzymes also have vital application in the effluent treatment from the

tanneries. The parameters involved in the enzyme applications are the parameters like enzyme and substrate concentration, pH and temperature. The cost factor in the enzyme operation which are required to be solved are enzyme production costs (or purchase price), enzyme life, turn over number, equilibrium constant for reaction, conversion efficiency, temperature stability, operation concentration of substrate, and microbial contamination or maintenance of low bacterial counts. The future is still waiting for the involvement of enzymes for their successful utilization in the quality leather production.

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