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Alkaliphilic Enzymes and Their Application in Novel Leather Processing Technology for Next-Generation Tanneries

Wycliffe C. Wanyonyi and Francis J. Mulaa

Contents

Abstract Leather manufacturing involves conversion of raw skin and hides into leather (stable material) through series of mechanical and chemical operations. The leather industry has attracted public outcry due to severe environmental degradation, pollution and health and safety risks. Currently the industry faces serious sustainability challenge due to extensive use of toxic chemicals and generation of hazardous waste.

W. C. Wanyonyi
Department of Physical Sciences, School of Science and Technology, University of Kabianga, Kericho, Kenya
e-mail: wwanyonyi@kabianga.ac.ke

F. J. Mulaa (✉)
Department of Biochemistry, College of Health Sciences, University of Nairobi, Nairobi, Kenya
e-mail: mulaafj@uonbi.ac.ke

This chapter describes the polluting chemicals consumed in different stages of conventional leather processing and the nature of waste generated. In order to overcome the hazards caused by toxic chemicals in tanneries and protect the environment, enzymes have been identified as a realistic alternate for chemicals used in beam house operation and waste management. Alkaline active proteases of alkaliphiles offer advantages over the use of conventional chemical catalysts for numerous reasons, for example, they exhibit high catalytic activity and high degree of substrate specificity, can be produced in large amounts and are economically viable. This is because the enzymes of these alkaliphiles are capable of catalysing reactions at the extremes of pH, temperature and salinity of leather-manufacturing processes.

The chapter describes how alkaliphilic enzyme can effectively be used in soaking, dehairing, bating and degreasing operations to prevent waste generation, help in recovery of valuable by-products, reduce cost and increase leather quality. It is worth noting that protease has the capability to replace sodium sulphide in the dehairing process. In addition, alkaline proteases have shown remarkable ability in bioremediation of waste generated during the industrial processes. Intensive efforts are being directed towards chemical-based industries to use viable clean technology in their operation to reduce their negative impact on the environment. Similarly, leather industry should adopt the use of eco-friendly reagents such as enzymes to achieve long-term sustainability and clean environment and avert health hazards. Application of enzyme technology in clean leather processing strongly depends on legislation, political will and allocation of financial resources in research, development and implementation of this potentially powerful technology.

Graphical Abstract

Keywords Alkaline active protease, Biodegradation and sustainability, Clean environment, Leather manufacturing, Pollution

Abbreviations

BOD	Biological oxygen demand
COD	Chemical oxygen demand
CTLSs	Chrome-tanned leather shavings
DHA	Docosahexaenoic acids
DPA	Docosapentaenoic acid
EC	Enzyme commission
EPA	Eicosapentaenoic
FPH	Fish protein hydrolysate
GRAS	Generally recognized as safe
PTM	Posttranslational modification
PUFAs	Polyunsaturated fatty acids
TAG	Triacylglycerols
TDS	Total dissolved solids
WE	Wax esters
WS	Wax ester synthase

1 Introduction

Leather is appreciated and treasured as one of the uttermost important ancient natural products still existing in the industrial era [1]. Leather-making operation involves conversion of raw skin and hide, a highly spoilable material from meat industry, into leather, a stable material through series of mechanical and chemical operations. The industry serves a paramount role in global economic development by providing necessities such as leather clothing, boots, balls, saddles, hunting accessories, tent coverings, containers, boat coverings, dog chews, drum heads, bookbindings, lacing, shoes, purses, gloves, luggage bags, coats, clothing accessories, automotive interiors, upholstery for boats, aircraft, furniture and other garments. In spite of the industry making important contribution to the socio-economic development of developed and developing countries, through employment, production of valuable products and export earnings, the industry has attracted public outcry due to severe environmental degradation. Chemical-based industries are the prime targets of the environmental activist for their crusade against pollution, and leather industries have not been left out of such environmental protection campaigns. Close surveillance from environmental control authorities and increased public awareness has pressurized tanneries to search for eco-friendly processing methods [2].

2 Conventional Leather Processing and Environmental Pollution

The essential protein component of leather, collagen, exists in hides and skins is association with various globular proteins specifically, globulin, albumin, mucoids and fibrous proteins such as α-keratin, elastin, and reticular fibres [3]. During leather preparation processes, the hair or scales attached to the skins/hide is often removed. In addition, the non-collagenous constituents such as fat, blood remains and flesh are also removed in pre-tanning and tanning operations. The degree of their removal and the method used determine the leather product quality. Conventional leather-manufacturing processes use huge quantities of fresh water, lime, acid, pigments, sodium sulphide, heavy oils, fungicide, salts, soap, dyes, solvents and energy. When all these chemicals are mixed at different stages of leather processing, they form a complex and highly toxic waste difficult to treat. Table 1 gives an example of overall consumption levels in a tannery [4].

Health problems, pollution and environmental degradation arising from tanneries originate from the nature of the process, raw materials and chemicals consumed in processing. The level of consumption and emission in tanneries vary significantly owing to the variability of tanneries in terms of the type (primitive to modern), kind of skins/hide processed, volumes processed, chemicals used and quality specifications of the final product. Different forms of waste in quantity and quality are generated during chemical transformation of hides and skins into leather. The use of certain procedure in production and abatement may also result in some cross-

Table 1 Main and auxiliary process chemicals for a conventional process for salted, bovine hides

Chemical consumption	Process step	Approximate weight (%)
Standard inorganic chemicals (without salt from curing, acids, bases, sulphides, chemicals containing ammonium)	Soaking, bating, liming, unhairing, deliming, fat liquoring	40
Standard organic (acids, bases, salts)	Unhairing, liming, pickling, deliming	7
Tanning chemicals (chromium, vegetable and alternative tanning agents)	Tanning and pre-tanning	23
Dyeing agents and auxiliaries	Dyeing	4
Fat-liquoring agents	Pickling	8
Finishing chemicals (pigments, special effect chemicals, binders and cross-linking agents)	Finishing, dyeing	10
Organic solvents	Degreasing	5
Surfactants	Degreasing	1
Biocides	Preservation, soaking	0.2
Enzymes	Bating, unhairing, soaking	1
Others (sequestering agents, wetting agents, complexing agents)	Stripping and bleaching	1
Total		100

media effects [5]. The quantity of chemicals utilized varies considerably depending on the quality description of the final product, the pelts treated and the procedures employed in processing. Consumption of chemicals can therefore only be given within a broad range. In addition, lack of standards in terms of quality of chemicals supplied to tanneries by different manufacturers in developing countries poses safety challenge and difficulty in finding the environmental impact of the used chemicals. Lack of material safety data sheets on most chemicals of which some are toxic to humans and environment has been a major challenge for tanneries [5].

Water used must also be taken into account when comparing consumption and emission figures. An average of 30–35 m^3 of wastewater is generated per ton of raw hide processed [6]. About 20–50% of the pelt weight is added as inorganic standard chemicals and about 3–40% as organic chemicals [5]. The inability by tanneries to treat waste and uncontrolled discharge or disposal of untreated wastes is the major course of environmental degradation facing tanneries across the globe. Huge quantities of claimable by-products are generated in the leather industry but cannot be recycled due to cross contamination with toxic chemicals. For example, when 1,000 kg raw hide is processed using traditional leather processing method, 30 m^3 of water are required and yields 150 kg hair-free leather, 30 m^3 wastewater and 700 kg solid trash [7]. In another study undertaken by Black et al. [5], processing 1,000 kg of salted raw hide using conventional leather-manufacturing method requires 22 m^3 water, 425 kg of process chemicals and 9.2 GJ of energy. The yields consist of 145 kg leather, 22 m^3 wastewater, 391 kg by-product, 906 kg solid waste, 277 kg of sludge from wastewater and 1.5 kg air emission. Disposal of toxic solid waste and huge quantities of highly polluted wastewaters is a dilemma for tanneries. Figure 1 gives an input/output overview from an advanced tannery producing upholstery leather [5].

Pre-tanning and tanning processes contribute 80–90% of the total pollution load in tanneries [8]. The waste generated by depilation/dehairing process accounts for 60% of the suspended solid, 68% of salt, 83% of BOD, 73% of COD and 76% of toxic chemicals [9]. The probable escape to the atmosphere is emissions from wet processing, finishing and effluent treatment. They include dust particles, sulphur dioxide, aerosols, hydrogen sulphide, ammonia, fume of formic acid, water vapour, carbon dioxide, chlorine, solvent vapours, volatile organic compounds (VOCs), etc. Air emissions have a negative environmental effect since some of the gases initiate photochemical reactions, increase greenhouse gases, destroy ozone layer, reduce visibility in the urban area and form acid rain or fog [10]. Traditionally, tanneries have been condemned for the discharge of noxious niff rather than any other air emissions, although the emissions of organic solvents have been a major problem. Air emissions do not only have negative health effects on tannery workforce but affect people several kilometres beyond the tannery site. Tannery ventilation requirement for health and safety of employees may limit the degree of air pollution in the buildings but not the environment outside the building [5].

The main liquid and solid outputs from leather-making process arise from decaying flesh, suspended and dissolved solids, fats, dyestuffs, organic matters, colouring pigments, dissolved lime, splitting, soluble proteins, shaving, toxic

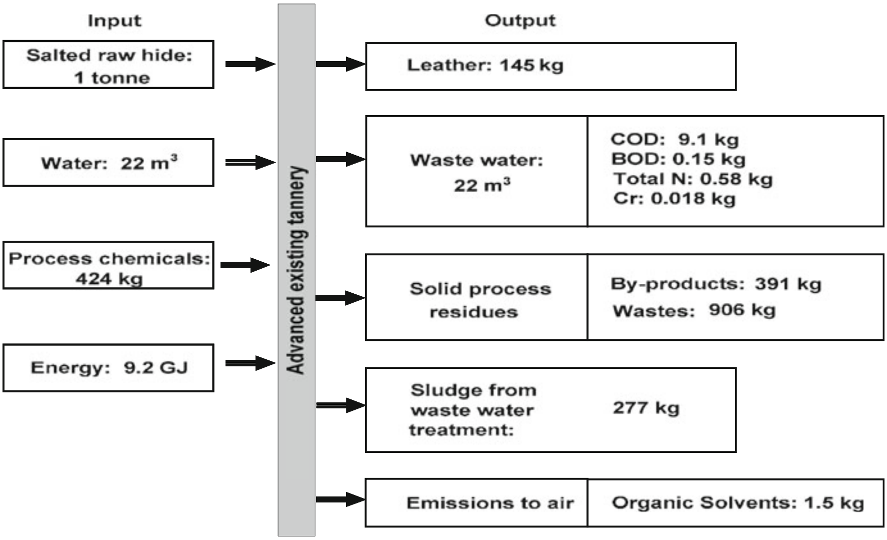
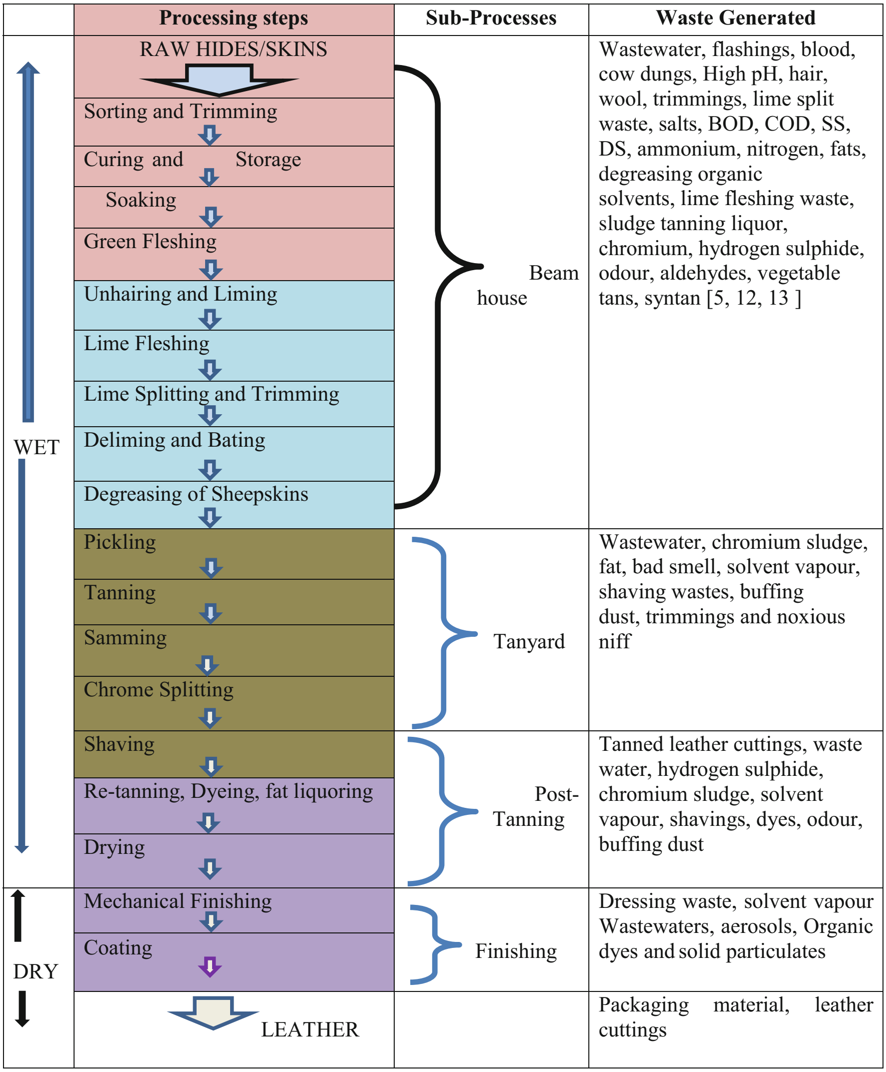


Fig. 1 Input/output overview from an advanced existing tannery for bovine salted hides per tonne of raw hide treated, producing upholstery leather (some chromium tanned) [5]

chemicals, heavy metals like chromium, etc [10]. Currently, chemical precipitation methods are normally employed for the removal of chromium from the effluent, but that leads to the formation of chrome-bearing solid wastes [11]. Leather processing can be divided into four vital subprocesses, viz. pre-tanning or beam house operations, tanning, post-tanning and finishing. Figure 2 schematically summarizes the possible steps in the production of leather through chromium tanning process and waste generated although there may be a significant variation between tanneries, depending on the kind of hide processed and quality of leather.

Legal requirements are slowly gaining momentum across the globe in forcing chemical-based industries including tanneries to apply innovative techniques in order to attain eco-friendly processing, waste reduction and waste recycling. The attention of chemical-based manufacturing industries has focused towards remodelling the processing procedures by incorporating recovery systems and improving effluent treatment methods to make processing eco-friendly and less costly and improve product quality. In order to overcome the hazards caused by excessive use of toxic chemicals in tanneries and protect the environment, enzymes have been identified as a realistic alternate for beam house operation and waste management.



	Processing steps	Sub-Processes	Waste Generated
WET	RAW HIDES/SKINS	Beam house	Wastewater, flashings, blood, cow dungs, High pH, hair, wool, trimmings, lime split waste, salts, BOD, COD, SS, DS, ammonium, nitrogen, fats, degreasing organic solvents, lime fleshing waste, sludge tanning liquor, chromium, hydrogen sulphide, odour, aldehydes, vegetable tans, syntan [5, 12, 13]
	Sorting and Trimming		
	Curing and Storage		
	Soaking		
	Green Fleshing		
	Unhairing and Liming		
	Lime Fleshing		
	Lime Splitting and Trimming		
	Deliming and Bating		
	Degreasing of Sheepskins		
	Pickling	Tanyard	Wastewater, chromium sludge, fat, bad smell, solvent vapour, shaving wastes, buffing dust, trimmings and noxious niff
	Tanning		
	Samming		
	Chrome Splitting		
	Shaving	Post-Tanning	Tanned leather cuttings, waste water, hydrogen sulphide, chromium sludge, solvent vapour, shavings, dyes, odour, buffing dust
	Re-tanning, Dyeing, fat liquoring		
	Drying		
DRY	Mechanical Finishing	Finishing	Dressing waste, solvent vapour Wastewaters, aerosols, Organic dyes and solid particulates
	Coating		
	LEATHER		Packaging material, leather cuttings

Fig. 2 Process steps in leather making (chromium tanning) and waste generated

3 Use of Enzymes in Tannery Processing

The leather industry faces serious sustainability issues due to pollution and environmental and negative health effect. New processing is a must! On this account, tanneries need to embrace cleaner production, prevent or reduce waste formation and the inevitable small amounts of waste generated disposed in an environmentally friendly way [12]. Application of neutral and alkaline-stable enzymes in tanneries could be the most effective way in reducing the use of toxic chemicals for soaking, dehairing and bating. The bio-based leather processing aims at use of enzymes instead of chemicals, thereby consuming much less energy, chemicals and water. It can significantly contribute to both economic development and cutting down environmental pollution.

Enzymes are biocatalysts that are suitable for the practice of green chemistry and can be utilized to achieve eco-friendly industrial processing. Enzymes have been utilized broadly in manufacturing of household products. Amongst the largest groups of industrial enzymes, viz. proteases, amylases and lipases, proteases account for about 59–65% of the total worldwide enzyme sale [13, 14]. Alkaline active proteases have been used in products such as cosmetics, synthesis of oligopeptides, drugs, detergents, fertilizers, cloth and in processing food and leather [15–19]. Keratinolytic protease is one of the marvellous biocatalysts able to hydrolyse and break disulphide bonds found in hair with little damage to skin/hide grain [7].

Enzymatic leather processing is a transformative and ambitious technology which simultaneously addresses major economic, social and environmental challenges affecting the tannery. The ‘greening’ of the leather processing industry, with the elimination of toxic chemicals has the potential to revive collapsed leather industries severely restricted by pollution and health concerns. Use of enzymes may drastically reduce the pollution load of the effluents, help in hair recovery and at the same time enable recycling (such as wastewater) since there is no cross contamination by toxic chemicals. Enzymes are not persistent and can be readily deactivated and biodegraded. One of the major hindrances to the uptake of enzyme technology for the leather industry just like most industries is the technical limitations around tailoring an enzymatic process to the already existing standardized industrial chemical processes. However, with the advent of technology, it has been possible to design an enzymatic process that easily fits into the already existing factory operational parameters. Successful research has been carried out in processing of cow-, goat-, sheep- and fishskins using proteases, though their use has not been fully exploited due to the following technical limitations.

1. Limited number of people with product knowledge on enzyme production, application and specificity.
2. High initial cost of equipment for enzyme production.
3. Enzymes are limited in the activity range especially with regard to pH and temperature. This requires application of more than one enzyme for complete processing.
4. If not well controlled, one can risk destroying the valuable grain surface.

4 Protease

Protease (peptidases or proteolytic enzymes) constitutes a large group of enzymes that conducts proteolysis, that is, begins protein catabolism by hydrolysis of the peptide bonds that link amino acids together in the polypeptide chain forming the protein

[20, 21]. Breakdown of peptide bonds helps in the protein degradation into their constituent amino acids, smaller peptides or it can be specific, leads to selective protein cleavage for post-translational modification (PTM) and processing [22]. Proteases are categorized as peptidases or peptide hydrolases (EC 3.4) and comprise a large family of enzymes, divided into endopeptidases (EC 3.4.21–99) and exopeptidases (EC 3.4.11–19) and grouped depending on the position of the peptide bond to be cleaved. Protease can also be classified according to the pH range where they have optimal activity: acidic (pH 2.0–6.0), neutral (pH 6.0–8.0) and alkaline (pH 8.0–13.0) [13, 23, 24].

The growing realization of a diverse array of biological, economic and technical challenges has generated renewed interest in the study of proteolytic enzymes for different industrial applications. The most common proteases of animal origin include pepsin, pancreatic, rennins, trypsin and chymotrypsin. Plant origin proteases consist of papain, bromelain and keratinases. Use of plant proteases is controlled by land availability for agriculture, labour, government policies and climate, while the recovery of animal protease in bulk is determined by the presence of livestock for slaughter, which in turn is dictated by environmental, political, religious belief and government policies. The failure of plant and animal protease to quench market demands has led to an increased interest in microbial proteases [25]. Microbial sources of protease are widely preferred for industrial application because of the following advantages:

- Require limited space for their production.
- Broad biochemical diversity.
- Rapid growth of the microorganisms and faster production.
- Flexibility in production.
- Ease of genetic manipulation to generate enzymes for different characteristics and applications.
- The enzyme can be easily recovered.
- Microorganisms can secrete large amount of enzymes for large-scale applications.
- Economical.

Microbial proteases are derived from a wide variety of microorganisms, which include bacteria, yeasts and fungi. Proteases of industrial value are mainly obtained from microorganisms, and these are *Bacillus* species from the bacterial kingdom and *Aspergillus* from the fungal kingdom because they are known to be generally recognized as safe (GRAS) [26]. Proteases have been used in laundry and detergent industries for over 50 years to facilitate release of proteinaceous materials in stains and account for about 25% of total worldwide sales of enzymes [27]. Detergent industry heavily uses thermostable alkaline proteases from thermophiles as an additive [28]. Other enzymes used in detergents include amylases, mannanase, cellulase and lipases. The use of different enzymes as detergent additives arises from the fact that proteases can hydrolyse proteinaceous stains, cellulases are effective in cleaning, colour clarification and anti-redeposition (cotton), mannanases are perfect for stain removal, amylases are effective against starch and other carbohydrate stains, while lipases are effective against oily or fat stains [29, 30]. An ideal enzyme for detergent should have broad substrate specificity, be stable at high pH and temperatures, be able to withstand oxidizing and chelating agents and be effective at low enzyme levels in detergent solutions. The leading enzyme suppliers and detergent manufacturers are actively pursuing the development of new enzyme activities that address consumer needs for improved cleaning, fabric care and antimicrobial properties [31]. As the detergent industry grow both in terms of size and complexity cleaning properties, new applications and demand of the enzyme will continue to expand.

Use of enzyme in processing of hide and skin has been practiced since ancient times. Traditionally, biocatalysts found in dog's dung were used to soak hides and skins to make them pliable by extracting protein, oil and fat constituents. Use of this method was not only unhygienic but unsustainable due to high market demand and health concern. The reasoning behind use of proteolytic enzyme from dog's dung lies in the fact that the protein is the major constituent of hair found on skins and hides. Hair is composed of α-keratin fibres and insoluble protein molecules containing a large fraction of cysteine residues and having α-helix conformation. The α-keratin is arranged in piles of fibrils. Different skin layers are composed of collagens, α-keratin and some elastin. Proteases can hydrolyse the protein fraction of dermatan sulphate, making the collagen more reachable by water and reducing the attachment of the basal layer [32]. Extremophiles can survive under extreme conditions. These include temperature (−2 to 12°C, 60 to 110°C), high pressure, radiation, salinity (2–5 M NaCl) and pH (<2, >9) [33]. Alkaline lipases from *Bacillus* strains, which grow under highly alkaline conditions in combination with other alkaline or neutral proteases, are currently being utilized in leather industry for assisted dehairing of animal hides and skin [33].

5 Enzymes from Extremophiles Microorganisms

The demand for industrial enzymes that can withstand harsh operating conditions such as high pH, temperature, salinity and pressure has greatly increased over the past decade. This has led to extensive research in exploring extremophilic microorganisms in search for novel enzymes. As a result enzymes from thermophiles and alkaliphiles have become the subject of special interest for biotechnological applications due to their efficiency and high stability at adverse operational and/or storage conditions [34]. This is because enzymes obtained from alkaliphiles are stable when added to detergents due to their inherent tolerance to high pH and can also generally function in the presence of bleaching chemicals [35]. Extreme environmental conditions require optimized interactions within the protein, at the protein–solvent boundary or with the influence of extrinsic factors such as metabolites, cofactors and compatible solutes [36]. Factors that contribute to the remarkable stability of extremozymes include an increased number of ion pairs, reduction in the size of loops and in the number of cavities, reduced ratio of surface area to volume, changes in specific amino acid residues, increased hydrophobic interaction at subunit interfaces, changes in solvent-exposed surface areas, increase in the extent of secondary structure formation and truncated amino and carboxyl termini [37].

6 Application of Protease in Cleaner Leather Processing

Pre-tanning operations in conventional leather processing generate significantly high pollution problems as compared to the post-tanning operation. Responsible chemicals for pollution in the pre-tanning processes include lime, detergents, sodium sulphide, caustic soda, sodium chloride, inorganic salt, acids and degreasing solvents. In addition, pre-tanning operations consume large amounts of energy and huge quantities of water. The sludge generated from flashings, fat waste and digested hair results in further environmental pollution. In addition, tanneries incur high costs in treatment of the waste as well in purchasing the same chemicals. Significant efforts have been made in the past decades to render pre-tanning operations in tanneries cleaner. The best strategy in reducing or eliminating pollution problems in leather industry is by targeting and replacing toxic polluting chemicals by eco-friendly chemicals and recycling some of the waste such as water.

7 Enzymatic Leather Processing

The following section summarizes the major operations performed in beam house, tanyard, post-tanning and finishing areas in leather production. All the progressive steps in the ongoing processing of hides and skins involve application of enzyme either directly or indirectly to facilitate the processing and production of desired quality. Enzymes are mainly applied in soaking, dehairing, bating, degreasing and waste treatment in effort to avert negative effect associated with use of chemicals.

7.1 Hide and Skin Sorting, Trimming and Storage

7.1.1 Sorting and Trimming

Hides and skins of good quality are sorted into different grades, types and weight. This procedure may be performed at the tannery or slaughterhouse. Unsuitable materials may be sold to other tanners or if in extremely poor conditions, discarded as waste. Selected hides/skins are then trimmed to remove spoiled parts such as edges, legs, tails, face, udders, etc. from the raw skin. This stage generates huge putrescible wastes, which require to be discarded following the set regulation on animal by-products/waste disposal. Proteolytic and lipolytic enzymes can be applied at this step to digest the spoiled parts of the hides/skins including those in poor condition and recover valuable products from the hydrolysate.

7.1.2 Curing and Storage

Curing process is performed to prevent degradation and restrains microbial attack on hides/skins before processing. For fresh hide processing immediately after skinning, this step can be omitted. Curing for long-term storage of up to 6 months includes drying, salting and brining [5]. Long-term preservation methods are used when the tannery anticipates shortage of the products in the market or when trading the hides and skins especially for intercontinental trading. Short-term curing involves preserving for 1–6 days using methods such as cooling, refrigeration and addition of biocides. These methods are used when raw materials are directly received from local sources. The raw materials are generally stored as they are received by the tannery on pallets in ventilated or air conditioned and/or cooled areas, depending on the method of curing chosen. The stored hides/skins are then taken to the beam house for processing.

7.2 Preparation for Skins and Hides for Tanning

The process in the beam house of the tannery involves removal of the skins or hides from storage and their preparation for tanning and is often carried out in processing mixer vessels or rotating drums.

7.2.1 Enzymatic Soaking

Soaking allows the hides/skins to rehydrate and helps in opening up the contracted fibre structure [15]. Soaking is necessary for solubilisation and removal of interfibrillary material and cleaning the hides and skins from dirt (salt, soil particles, dung, blood remains and soluble proteins on the surface of the skin). Soaking is mostly executed in two steps. First there is a dirt soak which removes salt and dirt. The second soak is also known as main soak which is longer and may last between 8 and 72 h. Due to the long soaking period in the main soak, in traditional leather processing method, putrefying bacteria thrive, and biocides are added to curtail their activity and growth [38].

Application of alkaline protease in the dirt soak and main soak reduces processing time and initiates the fibre opening of the hide and skin tissue. Enzymatic soaking is successfully carried out under alkaline conditions using proteolytic enzymes that are optimally active in alkaline conditions. The advantages of enzymatic soaking include:

- Loosening of the scud
- Partial dehairing during soaking process

- Solubilisation and removal of non-collagen protein
- Reduced time of soaking and faster opening of the fibre structure
- Production of leather with less wrinkled grain
- Improves softness and elasticity of the leather
- Increases the yield of hair, wool, scales and fur
- Reduces the amount of water used and wastewaters generated
- Energy efficient

7.2.2 Enzymatic Dehairing

Dehairing is the main and most polluting operations in the beam house. The aim of dehairing is to remove the hair, scales, epidermis and to some extent the interfibrillary proteins from the skin. Five methods of dehairing are generally used, i.e. (1) clipping process, (2) scalding process, (3) sweating process, (4) chemical process and (5) enzymatic process [3]. Clipping process is achieved by cuttings the wool of the sheep when the animal is either alive or dead. Scalding is the process of treating carcasses or skin with hot water or steam to loosen the hair or feather (of birds) in the follicle to aid their removal. Pork and poultry carcasses are both commonly subjected to a scalding operation during processing [39]. Sweating process is applied if the wool is of greater value than the skins and it involves hanging up soaked skins in dark humid rooms or in piles inside suitable chambers under controlled conditions of temperature and humidity for about 4–5 days where bacteria attacks keratin cells of hair and epidermis, until wool is loose. Sweating process may result in serious damage to the raw hide surface [40].

Of these, the most commonly practiced method of dehairing of hides or skins is the chemical process using high concentration of lime and sodium sulphide which creates extremely alkaline environments which result in pulping of hair and its subsequent removal. High alkalinity also helps in opening the fibre structure and hide plumping. The time span of the process may fluctuate from 18 to 168 h depending on the method used [41]. Even though this process is very efficient in hair removal, its inherent drawback has to be taken into account, noteworthy amongst these are:

- High concentration of lime blended with sodium sulphide accounts for generation of 70–80% of the total BOD and COD of effluent from all the leather-making processes. About 75% of the organic waste from a tannery is from the beam house and 70% of this waste is from dissolved hair rich in nitrogen [3].
- Sodium sulphide and hydrogen sulphide generated is highly toxic with extremely unpleasant odour. It can cause major health and pollution problems in the sewers if left untreated.
- Long processing time compromises the quality of the final leather and affects the costs.
- The process yields greenhouse and toxic gases such as carbon dioxide, hydrogen sulphide, ammonia and organic solvents.

- Workers' health is put at risk due to exposure to severe alkaline condition and toxic sulphide.
- The process consumes huge quantities of water and energy and generates a lot of wastewaters. Water polluted with these chemicals and the solubilized hair leads to an increase in alkalinity, organic nitrogen, BOD, TDS, COD and air pollution by hydrogen sulphide [15].
- Generate large quantities of solid wastes and obnoxious odour.

Enzymatic dehairing is suggested as being eco-friendly, more efficient and superior to chemical process. Dehairing, descaling and dewooling are the prominent stages where proteolytic enzymes can effectively be used in the leather processing and cut over 80% of the pollution load in the industry. Proteolytic enzymes can be used in dehairing to circumvent use of sodium sulphide and its negative environmental effect. Proteolytic enzymes are more efficient in dehairing and offer an environmentally friendly alternative to the conventional chemical process. Research by Wanyonyi et al. [15] demonstrated the technical and economic feasibility of enzymatic hair removal, in which the lime and sulphide chemicals were completely replaced by crude alkaline protease. Figure 3 shows a piece of hide successfully dehaired by alkaline proteases within 3 h as compared to a negative control which remained with hair when treated in distilled water under the same environmental conditions [42].

Skin (or hide) is usually considered to comprise of three layers. On the surface of the intact skin lies the keratinous epidermal layer comprising the epidermis and its appendages (hairs, hair root sheaths, etc.). Immediately below it, the basement membrane is to be found, attaching the epidermal layer to the underlying dermis (or corium). During leather processing, the corium is transformed into leather [43]. The proteolytic enzymes remove the hair by attacking the proteinaceous matter adjacent to the base of the hair root [44]. The ability of protease to digest the basal

Fig. 3 Photograph of hide completely dehaired by crude alkaline protease within 3 h (pH 12 and 37°C); negative control where distilled water under the same condition remained with hair

cells of the hair follicle and the cells of the Malpighian layer is found to be more efficient, and it happens without disturbing the native state of skin. The loosening of hair and its removal from the skin are accomplished after swelling and subsequent protease attack on the outermost sheath leading to breakdown of the inner root sheath (parts of the hair that are not keratinized). In enzymatic dehairing, the epidermal layer is removed from the dermis without damaging the hair and the grain layer because they are easily degraded and dissolved. Another component of raw skin (or hide) is the subcutaneous layer (connective tissue and fat) which is removed by fleshing. That layer, however, is of no relevance to dehairing except when applying the painting method [43]. Application of proteases facilitates faster dehairing process without damage to the fibrous collagen and hair or wool. It is worth noting that fully developed keratin in hair, nails and the upper part of the epidermal layer is highly resistant to chemical or biological attack, due to stable disulphide bonds (–S–S–), except from sulphide which breaks down the disulphide bonds.

Enzyme concentration, pH and temperature also play a significant role in enzymatic dehairing. pH range of 9.0–12.5 provides optimal conditions for alkaline protease activity and effectively aids in skin swelling and loosening of hair and removal. Dehairing is accomplished between 3 and 12 h when temperature range is 30–40°C. At temperature below 30°C, the duration of enzyme exposure and concentration needs to be increased for complete dehairing [15]. Further, below 15°C no appreciable dehairing within practicable time is observed. Studies have further shown that about 2% (w/w) of crude enzyme was sufficient for dehairing but 3% (w/w) of the enzyme was preferred because at this concentration even the tough hair at the neck areas was completely eliminated [44].

Enzymatic hair loosening and removal provide the necessary conditions for hair, wool, fur and scales recovery while still in good condition. Techniques commonly employed in enzymatic dehairing processes include paint, dip and spray methods. In the paint method, the enzyme solution is mixed with an inert substance like kaolin; a thin paste is made after adjusting to the prerequisite pH before applying on the flesh side of hides or skins. The skins are then piled, covered with polythene sheets and kept till dehairing takes place. In the dip method, the hides or skins are submerged in the enzyme solution at the right temperature and pH in a pit or rotating drum till all hair loosen and fall off [3]. In the spray method, a concentrated solution of enzyme is sprayed on the flesh side at a high pressure forcing its entry into the skin [3]. Difficult areas to dehair such as backbone and neck can be sprayed with more amount of enzyme, thereby accelerating the process. After dehairing, the enzyme may be reused in the second or third pile of skin or incorporated in the soaking solution till its activity diminishes. The major benefits of enzymatic dehairing include:

- Remarkable reduction or even total elimination of toxic sodium sulphide in dehairing.
- Applying enzyme can reduce the total pollution load of a tannery by over 80%.
- The process makes it possible to recover valuable hair, wool, fur and scales while still in good saleable condition.

- Enzymatic dehairing process takes short period as compared to chemical dehairing, i.e. the lime–sodium sulphide process takes about between 48 and 72 h, while the enzymatic dehairing process is accomplished within 3 and 12 h [15].
- The process eliminates the problem of waste disposal because by-products like hairs, wool, flesh, etc. can be recovered during or after dehairing.
- Enzymes are not persistent and can be readily inactivated and biodegraded.
- The process simplifies pre-tanning operations by cutting down one step, viz., bating since proteolytic enzymes are capable of removing all the hair.
- Use of enzymes greatly cuts down pungent smell and air emission.
- The process results in significant reduction in total solids, dry sludge and chemical oxygen demand in the effluent due to reduced use of chemicals.
- Treating hide/skins with enzymes produces soft, tough and pliable leather of high quality with greater surface area.
- Using the enzyme-based process creates an ecologically conducive atmosphere for the workers.

7.2.3 Fleshing

Fleshing is the mechanical scraping of the unwanted organic material from the hide and skins (connective tissue, flesh, fat, etc.). This process can be executed before soaking, after soaking, after liming or after pickling. In conventional leather processing, the process of fleshing is called green fleshing if the process is done before liming and dehairing. If fleshing is effected after liming and dehairing, it is called lime fleshing [5]. Sheepskins may be fleshed in the pickled state. Fleshing operations give rise to an effluent containing fatty and fleshy matter in suspension. If green fleshing is adopted, proteolytic and lipolytic enzymes can be utilized to hydrolyse fleshy matter to generate valuable products and recover biomolecules since the waste are not contaminated with sulphide.

7.2.4 Splitting

The uneven thickness of hides and skins is balanced out by mechanical splitting horizontally into a grain layer and flesh layer. Splitting is undertaken using splitting machines, fitted with band knife. Splitting can either be done in limed state or in the tanned condition [5]. This process generates huge quantities of flesh and fat waste, which can be solubilized and hydrolysed by use of proteolytic and lipolytic enzymes as described in fleshing to section.

7.2.5 Deliming

In enzymatic leather processing, this stage is eliminated since no lime is used. However, lowering of the pH of the hide/skin by soaking in acidified water is necessary since proteolytic dehairing is commonly done under alkaline environment. Lowering of pH is critical in preparing of the pelt for the subsequent operation since alkalinity has detrimental effect on the tanning process. The conventional deliming process is executed to eradicate the liming agent from the pelts by lowering the pH to between 8 and 9 [38]. The process involves gradual lowering of the pH using weak acidic solutions, fresh water or ammonium chloride salt. Acidification liquids which may still contain sulphide generate poisonous hydrogen sulphide gas. The process may also involve increase in temperature, removal of residual chemicals and degraded skin components. The quality of the final leather determines the extent of deliming. After deliming, the hides and skins are ready for vegetable tanning. Chrome tanning requires delimed hides and skins to be further processed through pickling and bating [5]. Once the skins and hides have been delimed, they must be taken to the next process immediately since removal of alkali provides favourable conditions for putrefying bacteria to thrive.

7.2.6 Bating

Application of protease in dehairing eliminates bating process since the hair roots are fully removed. However, the traditional chemical dehairing method leaves the skin surface with some attached hair roots and epidermal pigments which are undesirable for certain types of leather. Removal of these remnants is effected by the bating process. In the current bating process, commercially available proteolytic enzymes are used. Bating is stopped by lowering the temperature and pH and diluting the enzyme solution when the appropriate level of softening is achieved. This assessment is hard to standardize although it is based on the empirical evaluation by experienced personnel [1].

7.3 Tanyard Operations

The tanyard operations are commonly carried out in a section of the plant known as the tanyard. The operations are often carried out in the same processing vessels, with changes of float and chemicals. In chromium tanning, the vessels are usually rotating drums.

7.3.1 Degreasing

Domestic sheepskins normally contain large amount of natural grease (natural fat content is estimated to be between 10% and 20% on dry weight) which must be removed by degreasing operation. Excess fat is eliminated from fatty skins to avert the development of insoluble chrome soaps or block fat spumes formation in subsequent stage. Excess grease on the skin prevents even penetration of dye or tan, causing difficulties in the finishing processes and creating dark and greasy smudge on surface of the finished leather. Three methods are usually employed for degreasing, i.e.:

- Use of aqueous medium with organic solvent and non-ionic surfactant
- Use of aqueous medium with non-ionic surfactant
- Degreasing in solvent medium

Application of proteolytic enzyme in dehairing greatly helps in the removal and recovery of grease/fat/oil in the skin/hide by dissolving protein matter surrounding fatty tissues. Tanneries currently apply alkaline lipases at different steps of processing to eliminate natural fats from skins [44]. Lipases can remove fats and grease from skins and hides, particularly those with a moderate fat content. By combining the effects of alkaline protease, alkaline lipase and acid active lipases in dehairing, one may establish an effective method of degreasing prior to tanning and also improving the brightness and uniformity in dyeing [44]. The main advantages of using lipases are a more uniform colour and a cleaner appearance. Lipases also improve the production of hydrophobic (waterproof) leather; makers of leather for car upholstery have commented that ‘fogging’ is reduced [45].

7.3.2 Tanning

Tanning process introduces a tanning agent into the skins/hides, thereby stabilizing collagen fibres in the skin such that the hide is no longer susceptible to putrefaction or rotting. Collagen fibres are stabilized by cross-linking action of the tanning agents. The dimensional stability, resistance to mechanical action and heat resistance increase upon the tanning treatment. There are different tanning materials and methods, and one’s choice depends mainly on characteristics of the desired finished leather, cost and availability of the chemicals and the type of hides and skins. The majority of tanning agents fall into one of the following groups:

- Vegetable tannins
- Syntans
- Aldehydes
- Mineral tannages
- Oil tannage

A high percentage (80–90%) of all the leather manufactured today is tanned using basic chromium sulphate ($Cr(OH)SO_4$) because chrome tanning produces light and inexpensive leather of high thermal and bacterial resistance [44]. Although proteolytic and other enzymes are not used in the tanning process, their applications in the previous stage significantly influence the quality of tanned leather.

7.3.3 Shaving

The shaving process is carried out to reduce or standardize the thickness of hide or skin. Machines with a rapidly revolving cylinder are used to cut fine, thin fragments from the flesh side of the skin to achieve uniformity. This process can be undertaken on tanned or crusted leather. Small pieces of leather waste which are cut off are called shavings. Solubilizing of the chrome shaving using proteolytic enzyme assists in chrome recovery and solid waste management.

8 Protease in Bioremediation of Waste

Effluent discharges from tanneries create health hazards and environmental problems. When raw hides and skins are processed to leather, a number of by-products such as claws, scales, trimmings, tails, fleshings, pelt cuts, gluestock and tanned material such as cuts, buffing dust, dyed and chrome shavings are obtained [3]. As a result, the industry generates huge quantities of proteinaceous waste, chromium containing waste and wastewaters which pose serious environmental and pollution problems. Currently, the most common way practiced in the management of solid wastes is by tanneries disposing them in landfills. The disposal of untreated wastes into land and water bodies from tanneries results in air and water pollution as well as emission of greenhouse gases like methane and carbon dioxide [46]. Chromium leaching into the soil and groundwater makes it unfit for cultivation and other uses. Discharge of coloured wastewater contaminated with organic dyes from tanneries into natural streams has caused severe pollution problems, such as increased toxicity, BOD, COD and TDS of the effluent and also reduced light penetration, which has adverse effects on photosynthesis [47, 48].

Exploitation of enzymes in treatment of chrome-tanned leather shavings (CTLSs) at a commercial level is not fully developed. Studies have shown that protein is the main component of most of the CTLSs generated in tanneries [12]. However, due to non-biodegradability of CTLSs, their disposal poses a major problem for the leather industry. Enzymatic hydrolysis of CTLS is a viable method and provides a 50–60% yield of hydrolysate, which shows low ash content and a low content of chromic compounds [49]. Protein hydrolysate produced by enzymatic hydrolysis of CTLS wastes reacted with polyvinyl alcohol for producing biodegradable plastics [49].

9 Value-Added Product from Tannery Waste

Tannery wastes can be an important source of proteins and lipids, and there are reports on efforts made to recover these biomolecules [50]. The decaying of proteins and lipids is one of the major sources of offensive odours associated with tannery waste due to oxidation of disulphide bridges in proteins and unsaturated fatty acids present in these lipids. The protein-based components that can be recovered from tannery waste include protein hydrolysates, peptides and amino acids, collagen and gelatin and fish meal. The lipid-based compounds that can be recovered are oils, omega-3 fatty acids, phospholipids, squalene, vitamins, cholesterol, etc. that are required by many industries including food, agriculture, aquaculture and pharmaceuticals [51]. Proteinaceous fish meal is the main ingredient in fish feed formulation, because of its high protein content and an adequate profile of indispensable amino acids which is a requirement for the growth of fish, especially the carnivorous fish [52].

Tannery waste can also be utilized in the production of organic fertilizers and composts which have significant benefits over chemical-based products. Potential for biofuel recovery from such waste has been reported [53]. The recovery of components with potential biological activities and functionalities provides a means for value addition to the tannery processing waste and also adds to plant economy. Currently, by-products from tannery processing if not dumped are used to make low-valued products. Hides and skin from mature animals is tanned into leather at a small percent for making different products. Fish frames and heads are used for human consumption in the local community or used to make animal feeds (at small scale). Oil obtained from the by-products can be utilized for value-added products such as soap, biofuel and cosmetics.

10 Novel Biotechnological Approaches for Transforming Tannery Waste into Bioproducts Using Biocatalysts

Today tannery biowaste poses environmental risks while being an important potential feedstock resource for producing a wide range of bioproducts. The potential to exploit tannery biowastes as a raw material for bioproducts/energy requires the application of new technologies to arrive at novel and economically viable solutions. Enzymes are becoming a key element in the toolbox for the chemist. In particular, biocatalytic transformation of tannery waste is promising in production of bioproducts for use in areas such as pharmaceutical and food chemicals where target molecules are selective and complex. While new chemical catalysts are becoming available, the unique properties of proteolytic and lipolytic enzymes as biocatalysts offer green alternatives such as reduced use of organic solvents, efficient use of reagents and elimination of chemical catalysts. Enzyme technology could be the most useful technology for adding value to by-products and solving the waste problem in the tannery industry globally. A few reports are available on recovery of lipids by enzymatic hydrolysis of skin and hides and subsequent enrichment of omega-3 fatty acids using lipases [54, 55]. In the case of fishskins, omega-3 fatty acids devoid of most of the saturated fatty acids are preferred over the native fish oils since they keep the daily intake of lipids as low as possible. Omega-3 polyunsaturated fatty acids (PUFAs) are gaining recognition as important components of the human diet. They have been implicated in lowering the incidence of certain cardiovascular diseases, improving neural and retinal development in infants and slowing the growth of cancerous cells. In addition, several studies have shown fish diet containing PUFA before the period of gonad maturity affect fecundity, fertility, hatching rate and quality of eggs [56]. Two important PUFAs, eicosapentaenoic (20:5, n-3; EPA) and docosahexaenoic acids (22:6, n-3; DHA), are mainly found in sheep- and goatskins and cold water fish oils and also in substantial amounts in oil of Nile perch, a warm water fish [54]. In addition, fishskins contain docosapentaenoic acid (DPA) that also has been reported to be present in seal oil [54, 57]. Hence PUFA concentrates from fishskins would in addition to DHA and EPA present in cold water fish oil also contain DPA making them unique.

11 Bioinnovation Potential of Tannery Waste Biomass

Tannery waste is an attractive biowaste stream as it is produced in significant quantities globally. It is therefore possible to apply a cascading approach, giving priority to the transformation towards bioproducts while also permitting a possible conversion to energy, e.g. to bioparaffin and biodiesel. By adopting enzyme technology for recovery of protein hydrolysate, oil from skin and hide waste, it would therefore also be possible to recover other functional ingredients such as collagen. Since enzymes work best at low temperatures ($\leq 55^\circ C$), the process minimizes oxidation of unsaturated omega-3 fatty acids and results in fish protein hydrolysate (FPH) with higher degree of hydrolysis, and the process is more energy efficient as compared to conventional chemical treatment and cooking methods. Soluble proteins have been used in aquaculture, in preparation of fortified animal feeds and protein supplements. Due to its high protein content, sludge emanating from such a process can be a source of plant nutrients for food production or as animal feed. Use of raw tannery waste may causes problems of odour and attracting wild/domestic animals; thus it needs to be composted prior to its use.

The by-products from tannery processing are a potential source of collagen, the especially skin. Collagen is the main component found in skin, and gelatin can be obtained through partial hydrolysis of collagen. Gelatin from warm water fishskins is reported to have properties similar to that of gelatin derived from porcine [58]. Use of enzymes to extract gelatin should be targeted in enzymatic tannery processing. The proposed gelatin production method produces gelatin with very low colour and high gel strength over a very wide range of viscosities. If further improvement in gel strength is needed, transglutaminase may be used for cross-linking some smaller molecules, thereby improving the gel strength. The high purity, enzyme-extracted gelatin is produced with a dramatic reduction in gelatin production cycle due to elimination of liming step. Further, the low-temperature enzyme method of producing gelatin results in reduction of unit production costs due to increased yield, reduced chemical cost, reduced water usage, reduced utility cost and reduced emission of greenhouse gases and climate change.

Gelatin is an attractive molecule to be used in cultivation of mammalian cells. Use of bovine or porcine gelatin is nowadays restricted, and there seems to be great potential for other sources of gelatin material including fish-skin-derived gelatin. The gelatin produced should be evaluated with respect to this area of application. Furthermore, the gelatin can be modified with cross-linking functional groups for the preparation of chirally pure hydroxyalkanoic to be used as carriers of bioactive agents in various forms (hydrogels, capsules, microspheres, films, etc.). Addition of functional groups and blending with other biopolymers containing polysaccharides and lipids will confer to the gelatin buffering capacity and hydrophobicity, thus protecting the sensitive bioactive agents during gastric transit.

Tannery waste biomass is an abundant feedstock globally and has the potential to be exploited as a rich source of fatty acids for use in the biosynthesis of wax esters and lipid-based biopolymers. Wax esters are esters of long-chain aliphatic alcohols and fatty acids and are used as high-performance lubricants for engines, transmission and hydraulic systems. They are also used in cosmetics, foods and pharmaceuticals. Currently, these compounds are produced at a scale of 3 million tons per year from mineral oils. The last few years have seen a push towards the production of biodegradable lubricants from renewable sources. However, so far, the only natural sources of wax esters are whale sperm oil and jojoba oil, which are too expensive for wide range use. As an alternative, researchers have recently begun to explore lipid sources for the synthesis of bio-WEs. Synthesis of jojoba oil-like wax esters such as palmityl oleate, palmityl palmitoleate and oleyl oleate from oleate requires first the CoA activation of a fatty acid catalysed by an acyl-CoA synthetase and in a second step esterification with a fatty alcohol catalysed by a WE synthase (WS). Genes encoding these two enzyme functions have only recently been identified [59]. Both WSs not only catalyse esterification of fatty acids with long-chain alcohols but also esterification of diacylglycerol which results in the production of both triacylglycerols (TAGs) and WEs. The ratio of produced WEs (the desired product) and TAGs depends on the fatty acids used.

Despite several reports indicating more valuable products can be obtained from such by-products, there is no information on an integrated approach of recovery oil, omega-3 fatty acids, biodiesel, protein hydrolysate, amino acids, enzymes, gelatin, biofertilizer, etc. at large scale from such by-products globally. Such an approach is feasible in the tannery industry and will greatly add value to the tannery residues while addressing growing environmental concerns. This is an incentive to the tannery and other agroprocessing industries to generate more revenue and also present products of more value to the consumer.

12 Conclusions

The industry is celebrated for playing a paramount role in global economic development by providing valuable leather products, employment and foreign exchange. However, the industry faces serious sustainability issue due to pollution and environmental and negative health effect. Tanneries need to embrace cleaner production, prevent or reduce waste formation and the inevitable small amounts of waste generated be disposed of in an environmentally friendly way. Enzymes have been identified as a realistic alternate for toxic chemicals used in beam house operation.

Proteolytic and lipolytic enzymes in particular can be effectively used in soaking, dehairing, bating and degreasing operation to and help in waste reduction and recovery of valuable by-product, reduce cost and increase leather quality. We have demonstrated that proteases and lipases from extremophilic microorganisms have the capability to replace toxic sodium sulphide used in dehairing process. In addition, studies have shown that proteolytic enzyme can be used in bioremediation of generated waste. Exploitation of enzymes in treatment of chrome-tanned leather shavings (CTLSs) at a commercial level is not fully developed. Leather industry should adopt use of eco-friendly enzyme to achieve long-term sustainability and clean environment and avert health hazards. However, adoption of new technology by stakeholders in leather industry is critical but is difficult to implement due to resources involved. Implementation of enzyme-based technology in leather processing is strongly dependent on the legislation (nationwide and international), the political will and the financial resources put into research, development and implementation of this powerful technology.

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