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USE OF ENZYMATIC PROCESSES IN THE TANNING OF LEATHER MATERIALS

Magdalena Lasoń-Rydel[1,2], Katarzyna Sieczyńska[1], Dorota Gendaszewska[1], Katarzyna Ławińska[1,*], Tomasz P. Olejnik[3]

1 Łukasiewicz Research Network – Lodz Institute of Technology, Marii Sklodowskiej-Curie 19/27, 90-570 Lodz, Poland
2 Interdisciplinary Doctoral School, Lodz University of Technology, Zeromskiego 116, 90-543 Lodz, Poland
3 Faculty of Biotechnology and Food Science, Lodz University of Technology, Stefanowskiego 4/10, 90-924 Lodz, Poland
*Corresponding author. E-mail: katarzyna.lawinska@lit.lukasiewicz.gov.pl

Abstract:

The policy of sustainable development, the need to save natural environmental resources, and the use of waste as raw materials in new production processes allow the use of enzymes in many industries. Enzymatic tanning and chrome tanning are two different methods used in the leather industry to transform raw hides into a durable and usable material. Enzymatic tanning uses natural enzymes that are biodegradable and environmentally friendly. Additionally, enzymatic tanning requires less water and generates less waste than chrome tanning. Moreover, enzymatic tanning can result in softer and more flexible leather with better uniformity. Enzymes selectively break down collagen fibers, resulting in a more even tanning and a consistent leather product. The use of combined enzymatic technologies with non-obvious leather finishing methodologies in tanning is forced by European Union regulations limiting the use of hazardous substances and generating significant amounts of corrosive wastewater for the environment. However, tanning with enzymes is not a perfect process; therefore, this work presents the advantages and disadvantages of tanning with enzymes and describes new technological trends in the tanning industry.

Keywords:

Enzymatic processes, tanning, leather

1. Introduction

The European Union (EU) is an important global supplier of leather to the international market. Italy accounts for around 15% of the world's leather production and around 60% of total European production. About 40–90% of the leather products fabricated in individual member states of the EU are exported. Asian regions, particularly the Far East, are developing economies and are becoming more and more important markets for EU tanners. This industry is mainly relevant in developing countries, such as Brazil, China, and India, with Brazil having the largest commercial cattle herd in the world. Other countries also have large leather goods industries. For example, there are 2,009 tanneries in India, which are gaining more and more market share, and in Brazil, finished leather is responsible for 59.6% of leather export revenue [1] (Figure 1).

Leather products remain the consumer's first choice due to their durability, flexibility, and uniqueness. Leather is still the most optimal material for footwear as it has many properties, which are important from a comfort and healthiness point of view (especially in the case of children's footwear) [3,4]. At the same time, the most effective form of recycling livestock skins is the processing and finishing of skins by tanning. However, the leather industry produces toxic waste and non-biodegradable industrial pollutants, as well as consuming large amounts of energy and water. Pollution from the leather industry poses a serious threat to the environment and human health [5], breaking the boundary of clean production [6] and the principles of the circular economy. Environmental degradation has attracted the attention of the business world, leading to the implementation of practices aimed at reducing environmental pollution and creating a circular economy [7–10]. Chemical dyes and colorants are being replaced with bio-based dyes. Chrome split formation can be reduced if splitting is performed before tanning. Water-based liquors can replace organic solvent-based liquors, reducing hydrocarbon emissions. As an alternative to traditional leather preservation, phyto-based preservation options for raw hides and skins can be used, which reduces total dissolved solids and chloride content by approximately 70%. Traditional unhairing can be replaced with oxidative unhairing, which reduces biochemical oxygen demand (BOD) and chemical oxygen demand (COD) by approximately 40%. There are high-performance tanning technologies based on copolymers that increase the absorption of tannins to the level of 95%, for example, glyoxal/cotton wool tanning, which is characterized by the production of leather with increased organoleptic properties. Modern leather dyeing systems use high-efficiency dyeing systems, e.g., a copolymer of collagen hydrolysate grafted with starch, which exhausts the dyeing bath by up to 96%. In order to reduce unpleasant odors, and often volatile organic substances and odors harmful to human health and the environment, systems based on biofilters are used to capture harmful gas emissions from the tannery environment.

Environmentally friendly leather production technologies in the tanning industry concern not only the use of new methods in the area of leather production, tanning, and finishing processes, but also in the processes of utilization of harmful tanning waste; e.g., a biodegradation system based on the laccase enzyme is being tested for resistant wastewater after tanning [11]. One of the most innovative directions in the development of new

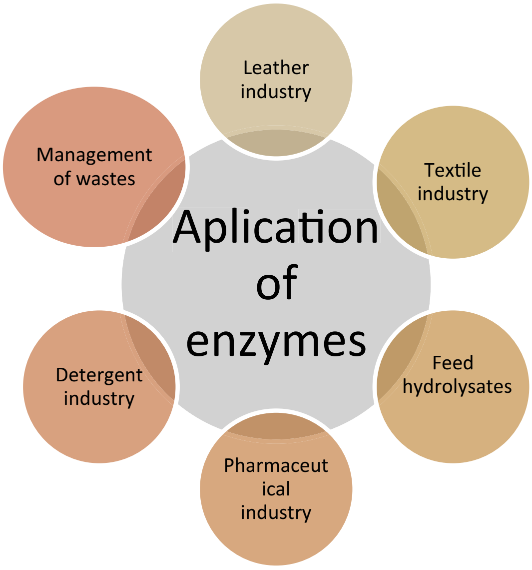

Figure 1. Use of enzymes in various industrial sectors [2].

technologies in leather production processes is the use of enzymes. The most widely used group of enzymes in the tanning industry are proteolytic enzymes. Proteases can be found in almost all living organisms at some point during their lifetimes [12]. Proteases have been broadly grouped into three primary classes based on the sources from which they are obtained: animal proteases, plant proteases, and microbial proteases. Proteolytic enzymes are bioactive macromolecules that constitute indispensable parts of life on earth, including microorganisms, animals, and plants [13]. Leather manufacture consists of mainly three steps of processing: (a) pre-tanning also known as beam house process, (b) tanning, and (c) post-tanning. Conventional chemical-based leather processing involves the use of many hazardous chemicals that generate and discharge a wide range of pollutants. The use of enzymes in tanning processes, e.g. alkaline proteases with optimal activity in a wide pH range (neutral to alkaline reaction) significantly reduce the level of pollution generated by the tanning industry [14]. These enzymes are ideal for commercial applications due to their compatibility with metal ions, compatibility with commercially available detergents, denaturants, and resistance to pH and temperature changes. Although this is not the only direction in the development of new technologies in leather-making processes, with the development of enzyme engineering, green and efficient proteases and biotechnology processes have been introduced into the leather-making process. The interdependencies of the use of environmentally friendly technologies in tanning, taking into account their impact on the natural environment and the mechanisms of the circular economy, are shown in Figure 2.

2. Transformation of raw leather into finished leather

Figure 3 illustrates the transformation of raw leather into finished leather. To transform raw hides and skins into finished hides, the skins are subjected to numerous physical, physicochemical, and chemical operations. The most important steps are divided between beamhouse operations (salt shake-off to bating), tanning operations (pickling and tanning), post-tanning operations (deacidulation to fixing), pre-finishing operations

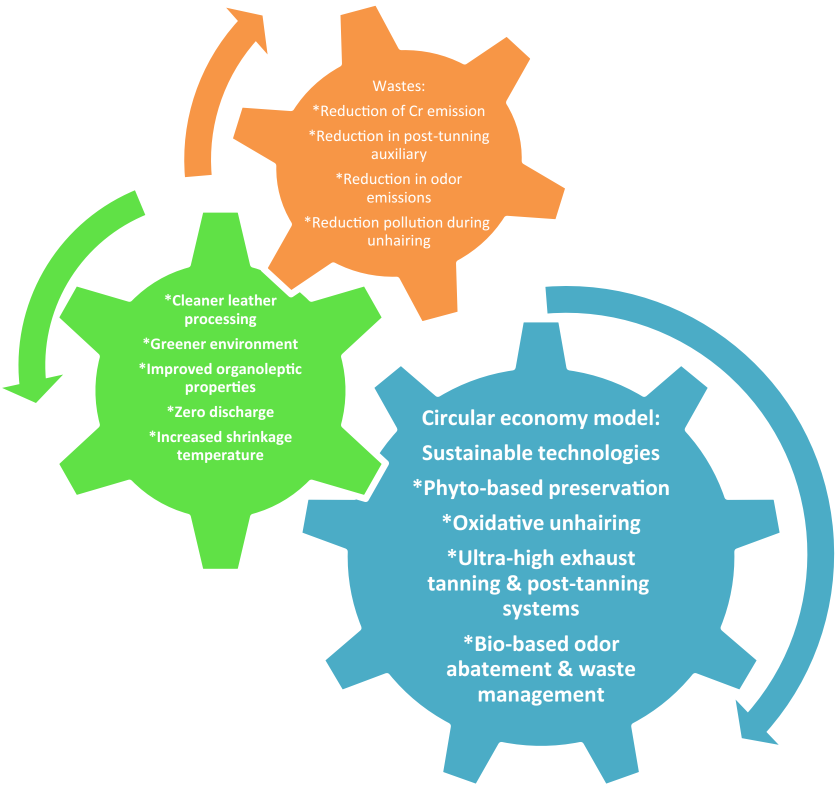

Figure 2. Leather processing showing basic raw materials and sewage [14].

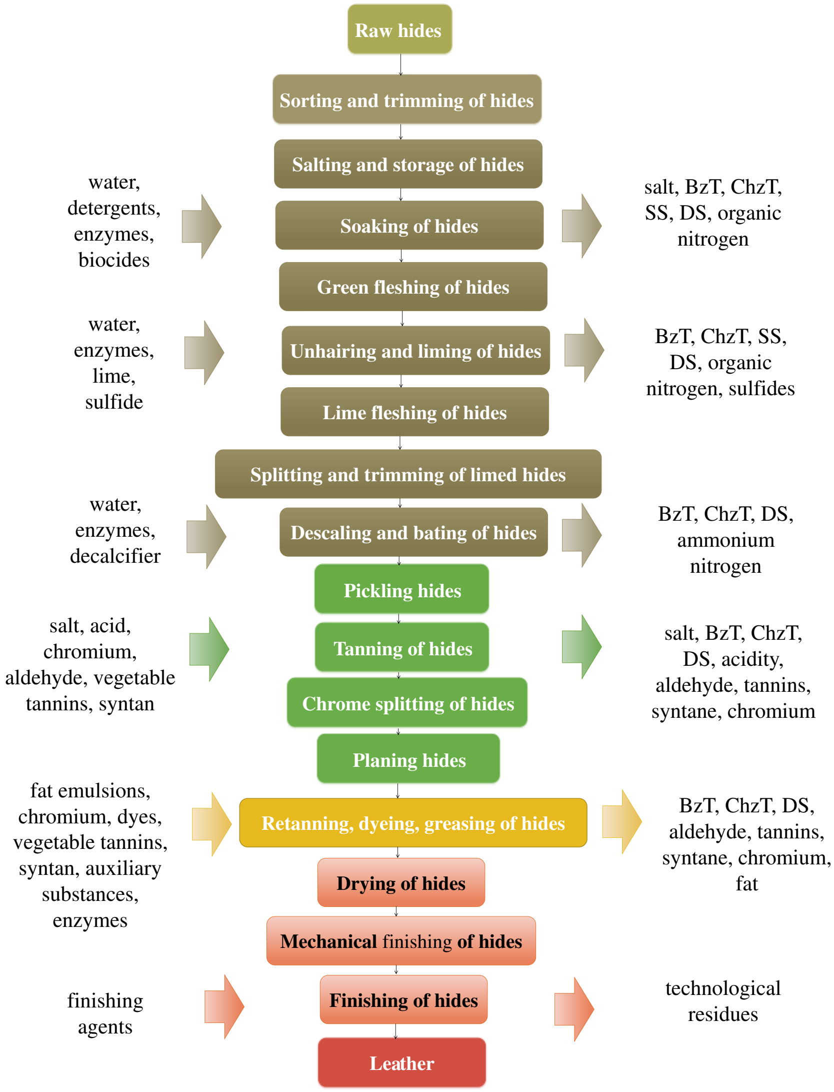

Figure 3. Leather processing showing basic raw materials and sewage [15].

(samming to sanding), and finishing operations [15]. The following list provides a brief description of the various steps involved in the tanning process.

2.1. Processes in the wet workshop

Soaking allows the skins to reabsorb any water lost after skinning, salting, and transportation. Soaking is also used to clean the hides of feces, blood, and dust and remove fibrous material. The soaking methods used depend on the condition of the skins. The hides are usually pre-soaked to remove salt and dust. The main soaking operation may last from several hours to several days [16]. Putrefying bacteria may develop during soaking. To limit their activity, biocides are added. Depending on the type of raw material, other additives may also be used, e.g., surfactants and enzyme preparations [17]. Unhairing and liming are performed to remove the hair, cuticle, and to some extent proteins fibrillary skins. The skins are thereby prepared for the removal of adhering flesh and fat in the fleshing process. The fibrous structure of the hide is opened in the liming step (by swelling) for cleaning and degreasing [18]. The keratin from the hair and epidermis is also removed from the hide by the action of reducing agents, usually sodium sulfide. After the liming process, the presence of lime or other alkali in the hides is no longer necessary and may have an adverse effect on further tanning. The descaling process involves a gradual lowering of the pH (by rinsing with fresh water or weak solutions of acids or salts, e.g., ammonium chloride, ammonium sulfate, or boric acid) and increasing the temperature. Residues of chemicals and decomposed components are removed from the hide.

2.2. Tannery process

In the tanning process, collagen fibers are stabilized with tannins, increasing their resistance to mechanical factors and high temperatures so that the hide is less prone to decay and rotting. Basic chromium sulfate is added to the pickled hide in drum II, with continuous stirring for 4–6 h to allow for absorption. To promote the final reaction of chromium to collagen, the temperature is increased to around 50°C and the pH of the bath is increased to 3.8–4.0 by the addition of basifying agents, such as sodium basic salts or magnesium oxide [14]. Unfortunately, the presence of these substances leads to high COD, BOD, suspended solids, and conductivity [19].

The tanning process modifies the rawhide by stabilizing the collagen fibers against enzymatic attack, mold, and chemical damage. Chrome tanning is still the most popular method of tanning leather, due to its ability to produce high-quality leather [20], with properties such as excellent hydrothermal stability, better dyeing properties, and softness [21]. The chromium tanning process is not an effective technology, due to the absorption rate of chromium Cr(III) by skin collagen, which ranges from 55 to 70% [22]. A large amount of chromium is discharged into the wastewater, resulting in large losses of chromium tannin. The process increases environmental pollution and wastewater treatment costs and additionally causes huge losses of chromium [23].

Untreated tannery wastewater contains up to 3,000–3,500 mg/l of chromium, which classifies tannery waste as one of the most dangerous industrial wastes [24]. Under oxidizing environmental conditions, Cr(III) is easily oxidized to Cr(VI), which is harmful to humans and causes allergies, cancer, or necrosis of the liver and kidneys. Cr(VI) causes significant damage to the environment. It reduces the rate of seed germination, leads to high teratogenic and carcinogenic rates, threatens the growth of aquatic organisms, and finally threatens the ecosphere by affecting the process of mitosis [24].

2.3. Post-tanning or bath finishing

Neutralization and rinsing followed by retanning, dyeing, and greasing are most often carried out in one technological container. Specialized activities can also be carried out at this stage of production, to ensure that the leather has certain properties, e.g., hydrophobic or waterproof properties, oleophobicity, gas permeability, flammability resistance, or antistatic properties [25].

3. Enzymatic processes

Enzymatic processes have always been part of leather processing. The first patent appeared at the beginning of the twentieth century and concerned the use of enzymes in bating [26]. In Poland, enzymatic processes were included as part of an industrial scientific description of collagen technology in the 1960s, but they were still poorly understood. Today, enzymes are used for many purposes in the industrial tanning and finishing of leather. Nonetheless, despite the obvious environmental benefits resulting from the use of enzymes in the leather industry, this technology has been developing much slower than technologies for the use of enzymes in the food, pharmaceutical, or cosmetic industries. Enzyme-based processes are used in some stages of leather production, including [27,28]:

- soaking – enzymes have been developed for loosening the scud and initiating fiber opening, producing leather with a less wrinkled grain, decreasing soaking time, improving softness and elasticity, and increasing area yield.

- unhairing – enzymes are used for (partial) elimination of toxic chemicals, reducing the pollution load, enabling milder process conditions, and providing non-toxic working conditions. The use of enzymes results in leather with better smoothness and strength properties, simplifying the pre-tanning process. Good quality hair with commercial value can be recovered.

- bating – removes non-leather foaming proteinaceous materials, for example, albumins and globulins, enhances the penetration of tanning chemicals and other technological process, smooth and supple leather. Bating is entirely dependent on proteolytic enzymes. Among others, proteases have long been used in the bating stage of leather processing.

- degreasing – eliminates chemicals (solvents, surfactants), facilitates even penetration of tanning materials, fat liquor and dyes, and results in soft leather.

- tanning and post-tanning – improves softness of leather, improves properties such as dye affinity, uniformity of color, dye penetration, and absorption [28].

Enzymes have been widely used in leather making during soaking, dehairing, bating, and degreasing steps, which are depicted in Figure 4 [22].

3.1. Soaking

Soaking is the first stage of a wet workshop in leather processing technology. Soaking can be divided into two stages [29]:

a) The first soaking is used for preliminary cleaning of the skin by removing large amounts of dirt and unwanted materials.

b) The second soaking uses water and small quantities of imbibing substances to hydrate the skin proteins and open the contracted fibers of the dried skins, solubilizing the denatured proteins and eliminating the salt used in the preservation step. Residual blood, excrement, and earth are removed from the skin [30].

To produce multifunctional enzyme preparations, multi-enzymatic systems are used. The enzyme preparations most frequently used in the soaking process are alkaline proteases. Alkaline proteases are used to ensure faster absorption of water and to reduce the soaking time [31]. The addition of a small amount of amylase and lipase may assist in the degradation and eventual removal of glycoproteins (resulting in fiber opening) and of lipoproteins in the soaking process itself, by aiding structural opening and allowing the chemicals to penetrate more rapidly [32]. Multienzyme systems containing proteases and lipases are more efficient due to the synergistic effects of the different enzymes [33]. Scientists continue to explore new and more effective enzyme preparations for use in soaking hides, and there are reports on the use of enzymes such as amidases, hyaluronidases, phospholipases, chondroitinases, and lignocellulases. However, these enzymes are not widely used due to their low specificity [34].

3.2. Unhairing

The process of hair removal is the next step after the soaking process. Leather makes up 7% of cattle weight, and only 25% of this weight is effectively used in the tanning industry. This

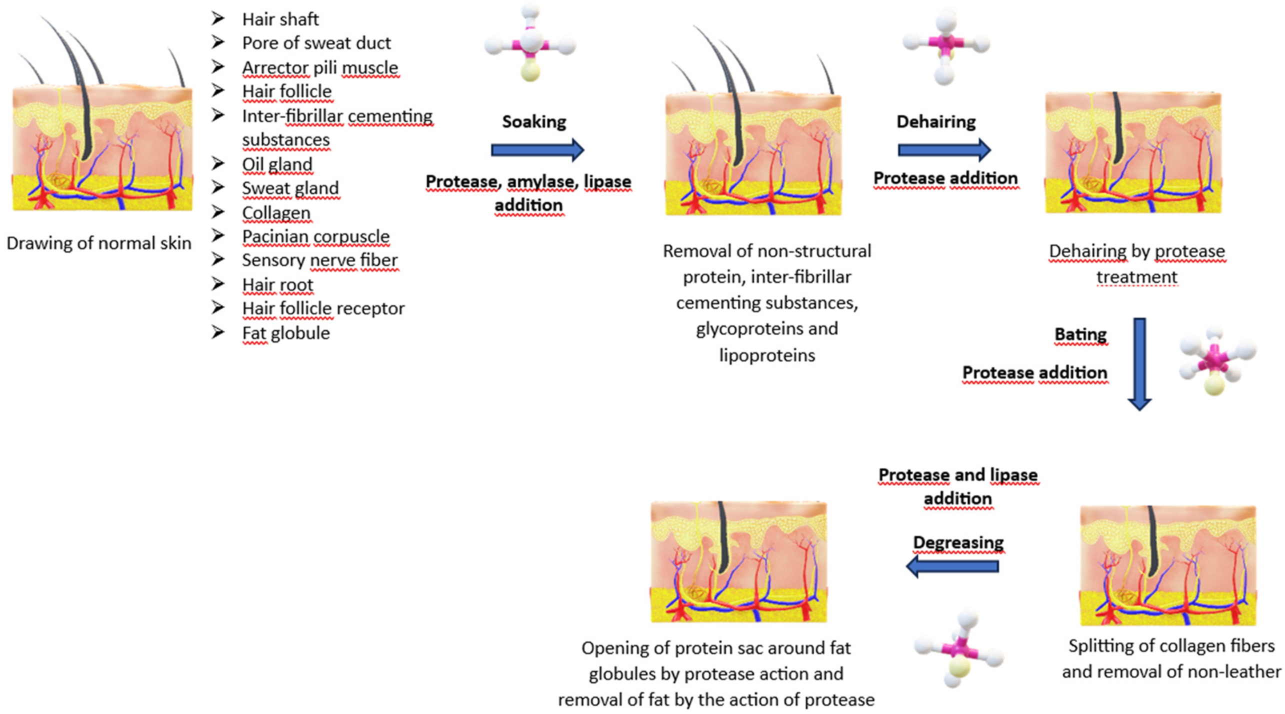

Figure 4. Mechanism of enzymatic actions they perform on skin at different leather stages [22].

results in a large amount of solid waste. Other process intermediates and by-products also have a significant impact on the environment. Hair removal is performed with the use of chemicals and usually generates about 60–70% of the total pollution from leather processing. The unhairing process produces high concentrations of environmentally toxic waste. Enzymatic processes using keratinase (the vast majority of which belong to the group of proteolytic enzymes) are now used for enzymatic environmentally friendly dehairing, replacing the traditional chemicals CaO and Na_2S [35]. Proteases have received special attention, due to their ability to hydrolyze keratin, which is an insoluble fibrous protein. Keratin is a major constituent of hair, feather, wool, and nails [36].

Proteases are increasingly being considered as a reliable alternative to conventional lime sulfide, to avoid problems caused by sulfide contamination. The main advantage of enzymatic unhairing is the complete elimination of lime and sulfide from the wastewater. Hair can be used for making many products such as brushes and carpets, and flesh is a main raw material for the glue/gelatin industry [37]. Hydrolyzed hair can also be used as an agricultural fertilizer and soil improver, in animal/poultry feed, and for the production of cosmetics, pharmaceuticals, and amino acids (e.g., cysteine) [38]. Proteases can be isolated from microorganisms (e.g., *Bacillus* sp., *Aspergillus* sp., and *Amycolatopsis* sp.). The greatest technological advantages of microbial proteases include low cost, high production, and efficient activity [39]. Enzymatic–oxidative unhairing does not contribute to the degradation of the hair and generates less organic waste with a lower pollutant load. The combined use of crude enzymatic extract and hydrogen peroxide has been shown to reduce processing time by hair saving (reducing hair destruction). The enzymatic extract was produced by cultivating the *Bacillus subtilis* strain BLBc 1 [40].

In the green unhairing cycle used in the leather industry, some keratinolytic proteases are used that exhibit high resistance to reducing agents (S^{2}-, dithiothreitol, and β-mercaptoethanol) and are quite stable under extreme conditions [41]. High-alkalinity and high-concentration detergents are needed in the chemical unhairing process. Most enzymes are not stable under these conditions, except a few keratinolytic proteases [42]. Yongquan examined the enzymatic activity of extracellular alkaline protease from *Bacillus cereus* TD5B and its potential application as a sheep skin dehairing agent [43]. The *Bacillus cereus* TD5B strain was screened for extracellular alkaline protease production on skimmed milk agar media. Its alkaline protease activity was measured at concentrations of 1, 1.5, and 2%. The results showed that 2% alkaline protease had the highest enzymatic activity. The highest tensile strength of sheep leather was obtained after dehairing with 1% alkaline protease.

In addition to its undoubted advantages, including greater process efficiency, non-toxic sewage and sludge, and a more stable product, the use of keratinase or keratinolytic protease in the unhairing of hides in the tanning industry has the following three basic disadvantages [44]:

a) High cost of enzyme production with the inability to use the biocatalysts again.

b) The keratinolytic activity/caseinolytic activity (*K/C*) ratio, which evaluates substrate specificity, is below 0.5 in the case of most keratinolytic proteases and keratinases, which decreases unhairing efficiency and leather quality substantially.

c) There is no enzymatic technology that does not damage the hides in the unhairing process [44].

The latest scientific reports indicate that a novel serine protease is produced with the bio-enzyme G3726, which is heterologously expressed by the *B. subtilis* strain SCK6. With good stability at alkaline pH, the crude enzyme could efficiently and cleanly complete the dehairing of goatskins without damaging the skin collagen, while the leather obtained shows excellent physical properties. Overall, protease G3726 is an attractive candidate for industrial applications, especially in detergent formulations and leather manufacturing [45].

3.3. Bating

Bating is the process of the removal and hydrolysis of the keratose remaining on the surface of a de-limed pelt, as well as the removal of scud consisting of epidermis, hair roots, pigments, fats, sebaceous glands, and sweat gland debris. Generally, it is the process of removing proteins other than collagen, using proteolytic enzymes such as albumin or globulin [46]. In the conventional bating process, pelt bating is carried out in a deliming solution with the help of enzyme preparations (a mixture of protease and ammonium salt), at 30–37°C and pH 7.0–8.5 [47]. Commercially produced trypsin is considered the best enzyme for pelt bating, but it is mainly extracted from bovine pancreatic tissue, which has many disadvantages, such as purity, poor batch stability, unpleasant odor, potential cross-infection, the limited availability of the raw material, and damage to mammal immunogenicity [48]. The search for new enzyme products for bating processes in the leather industry has led to research into neutral and alkaline microbial proteases, which are a class of enzymes with promising industrial applications in pelt bating. However, in alkaline environments, these enzymes have little effect on hides and do not remove plaque and the remains of hair grains satisfactorily. Therefore, it has been suggested to use enzyme preparations that are active in acid environments [49]. The acid protease from *Aspergillus usamii* is known to be an effective bating agent for sheep pelts and has been found to be more effective than neutral protease [50]. Recent studies have investigated one-bath pickling bating chrome as a method of replacing the conventional trypsin method. The innovative pickling bating method based on microbial origin acidic protease L80A can provide outstanding performance for pelt bating [51].

3.4. Degreasing

The degreasing process is another stage in tanning. The quantities of natural fats in skin are very high, especially in some types of sheepskins: 20–30% of the total weight of skins is composed of fats [52]. The degreasing process can be broken down into the following three successive stages:

a) breakdown of the protein membrane of the fat-containing;

b) removal of the fat;

c) emulsification of the fat in water or solubilization in solvent.

Three-component processes using two enzymes (proteolysis, lipolysis, and emulsification) are used in the drift-greasing process for effective degreasing. Some authors suggest using alkaline lipase combined with proteinase and pancreatin to improve the degreasing effect and soften pig skin [27]. Kanagaraj et al. claims that a combination of enzymes might be necessary not only to break down products but also to release them from the hide [53]. Hydrolysis of ovine storage lipid using lipid hydrolases was demonstrated in an attempt to understand the mechanism of degreasing. Lipases were used as agents for degreasing. Lipases are known to play a critical role in leather processing and represent an environmentally sound method of removing fat. In the case of bovine hides, tensides can be replaced completely with lipases and surfactants. However, the disadvantages are that lipase does not remove all types of fats in the same way [16].

Ejaz et al. used LIP2 lipase from the yeast *Yarrowia lipolytica* (YLLIP2) to degrease sheep skins [54]. The results showed that using only 10 mg of lipase/kg of raw hides enabled successful degreasing in only 15 min at pH 7. Comparative scanning electron microscope, attenuated total reflectance – fourier transform infrared spectroscopy, and physicochemical analyses showed that the enzymatically treated leather had better properties than the chemically treated leather. However, differences were observed in the composition of the wastewater, where the proportion of solid waste was higher for enzymatic degreasing than for conventional degreasing with chemicals.

3.5. Tanning/post-tanning

In the tanning process, collagen fibers are stabilized with tannins and with the help of tannin cross-links, so the hides are less prone to decay or rotting. The resistance of the leather to mechanical factors and to high temperatures increases. There are many different tanning methods and materials that may be chosen, depending on the required properties of the finished leather, the cost of the materials, the technology available in the plant, and type of raw material. Most tannins can be assigned to one of the following groups:

- mineral tannins,
- vegetable tannins,
- syntans,
- aldehydes,
- fatty tannins.

The most commonly used tanning agent is basic chromium sulfate ($Cr(OH)SO_4$). Currently, 80–90% of all hides are tanned with chromium salts [55]. The present study focused on the use in the tanning process of proteolytic enzymes during the wet finishing of leather and the dyeing of leather. The authors reported improvement in properties such as dye affinity, bath exhaustion, uniformity of color, dye penetration into the hide, wettability of the fiber, dye absorption, shrinkage, and tension in stretched wool fibers. In the study of Rakib et al. [56], an attempt was made to develop an eco-friendly vegetable tanning process combining pickle-free tanning and proteolytic enzymes. Leather dyeing is a post-tanning process, but it is worth noting that ecofriendly dyeing using enzymes to achieve increased uptake of dye can result in uniform dyeing with intense and bright shades. Staining with proteolytic enzymes also improves the absorption of the dye, which results in deeper shades of color. Researchers have attempted to develop an eco-friendly vegetable tanning process combining pickle-free tanning and the application of proteolytic enzymes [28]. The results showed more than 95% tannin exhaustion, an increase of 10% compared with the conventional vegetable tanning process.

3.6. New trends in enzyme treatment in leather industry

Proteases, also called proteinases, peptidases, or proteolytic catalysts, represent important enzymes commonly used in the leather industry. Proteases are a large group of enzymes belonging to the class of hydrolases, associated with the processing of long protein chains into short chains and the degradation of peptide bonds connecting amino acid residues [57].

According to historical data, proteases were classified based on source, molecular size, charge, substrate specificity, and catalytic activity. The division of proteases according to the mechanism of action, the species of microorganisms producing them, and the presence of amino acids in the active site is shown in Figure 5.

The applications of proteases isolated from different microbial sources in different industries are represented in Table 1 [58].

Proteases are widely used as catalysts for various organic transformations; meanwhile, biocatalysis mainly involves the effective use of proteases as process catalysts in specific environments. Enzymatic biocatalysis has remarkable properties under moderate conditions, such as high activity, biodegradability, and specificity. Currently, extensive research focuses on the use of techniques that increase the stability of enzymes through, for example, the process of immobilization, aggregation, or changing their chemical structure [57].

Despite the enormous applications of enzymes as potential biocatalysts and process catalysts in various organic reactions, enzymes are associated with certain limitations such as high cost, lack of effective availability, instability, low substrate specificity, and requirement for multiple cofactors [33].

In the tanning industry, acidic proteases are of great interest in processes such as unhairing, as shown in Figure 6.

Meanwhile, it should be noted that in recent years, there has been a huge increase in the use of alkaline protease as an industrial catalyst. The increasing use of alkaline proteases, not only in the tanning industry, is due to their biocatalytic activity in various organic transformations, namely,

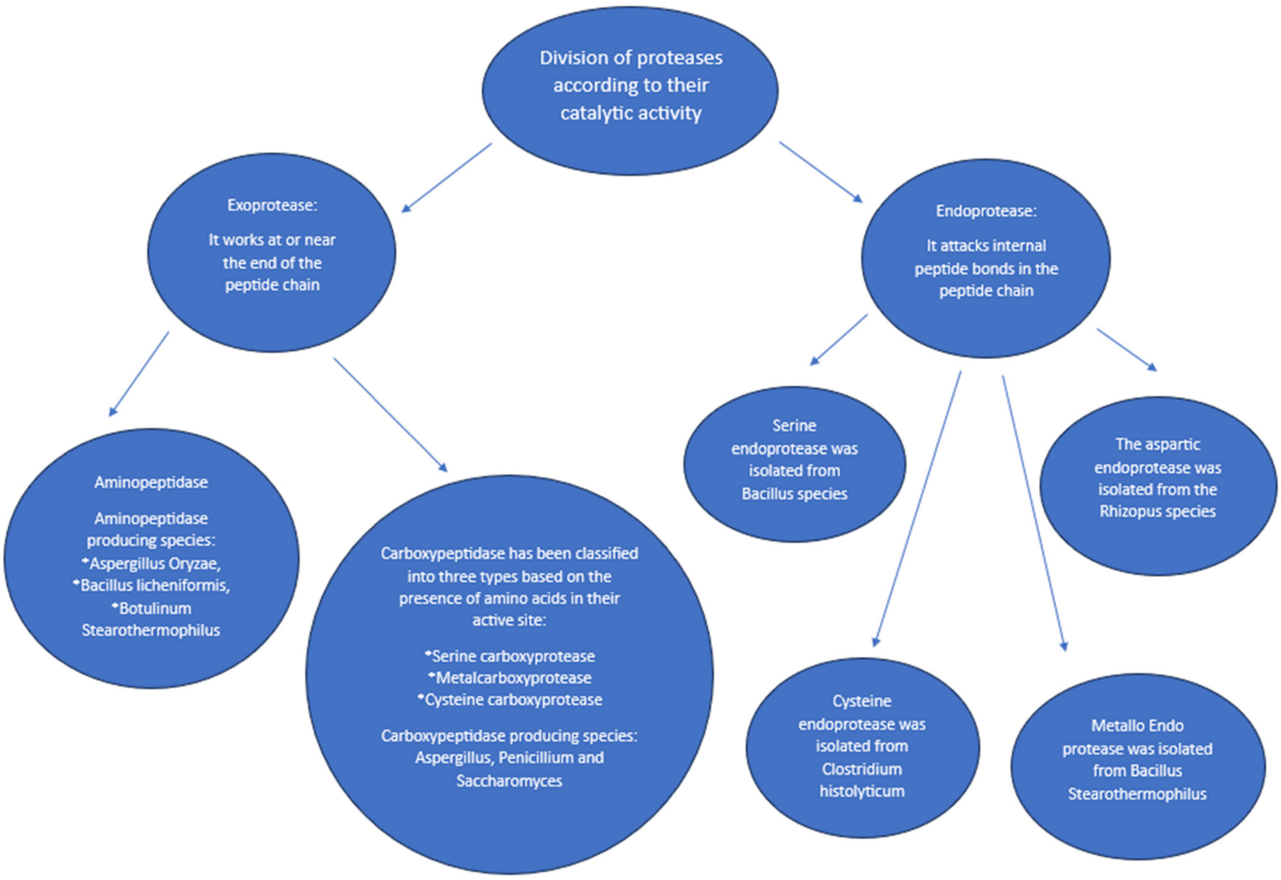

Figure 5. Division of proteases according to the mechanism of action, the species of microorganisms producing them, and the presence of amino acids in the active site [57].

Table 1. Applications of proteases isolated from different microbial sources in different industries [58]

Microbial stains	Applications
Aspergillus niger	Food, pharmaceutical, cosmetic
B. subtilis	Leather industry
A. niger	Detergents and leather industry
A. niger	Detergent
A. niger	Detergent
Aspergillus tamari	Leather processing
B. subtilis	Industrial purposes
A. niger	Peptide production
Bacillus sp. No. AH-101	Degradation of human hairs
Conidiobolus coronatus	Silver recovery
Aspergillus oryzae NRRL-447	Feather degradation
Aspergillus flavus MTCC 277	Leather treatment, detergents
Bacillus amyloliquefaciens	Feather degradation
Aspergillus sojae ATCC20235	Peptide synthesis
Bacillus sp. SSR1	Laundry detergent
Pseudomonas fluorescens	Detergent and textile industries
Aspergillus oryzae U1521	Animal feed and food processing
Aspergillus flavus	Leather processing
Bacillus cereus	Laundry detergent
B. subtilis PE-11	Detergent industry
Bacillus horikoshii	Industrial, medical applications
B. subtilis	Deproteinize crustacean wastes
B. subtilis	Industrial applications
Bacillus sp. KG5	Food industry
Engyodontium album BTMFS10	Detergent industry
Bacillus licheniformis RP1	Chitin extraction, chicken feather degradation, dehairing agent
Stenotrophomonas maltophilia	Detergent and environmental bioremediation in cold, regions
Mutant *Bacillus licheniformis* B18	Collagen replacement therapy, detergent stability, waste treatment
Streptomyces	Leather, keratin waste treatment, animal feeding industry, cosmetic industry
Chaetomium globosum	Detergent industry, feathers degradation, removal of gelatin layer from X-ray, film
Penicillium chrysogenum	Textile industry
Bacillus safensis	Detergent industry

Knoevenagel condensation, aldol reaction, transesterification reaction, separation of amino acids in organic solvents, kinetic separation of carboxylic acids, and enantioselective hydrolysis of amino acid esters or fragmentation of *p*-amidobenzyl ethers [57].

Basic proteases constitute the largest group with optimal protease activity at neutral to alkaline pH and are serine or metal proteases. Alkaline proteases owe their success in commercial applications to the following features: compatibility with metal ions, resistance to commercially available detergents, and resistance to pH and temperature changes.

In leather tanning processes, during the chemical removal of hair, lime and sulfide act as a shaving blade, leaving the hair follicles intact [59]. These intact hair roots are then removed in the next skin wiping step. This is an important pre-tanning step where not only are hair follicles and other unwanted proteins removed from the de-haired skin, but they also help split the

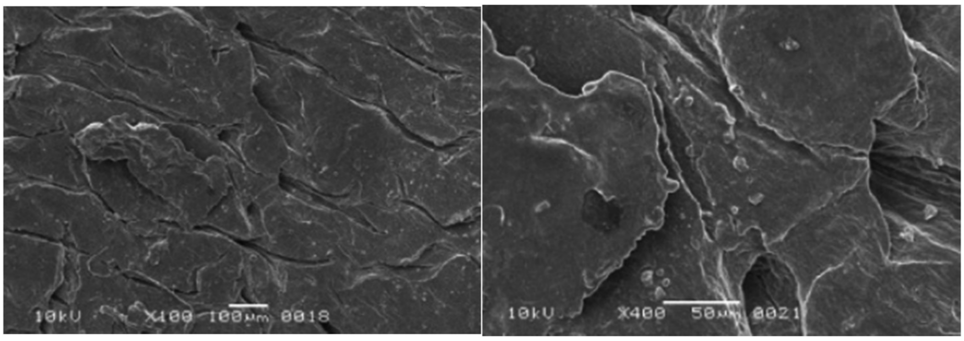

Figure 6. Mechanism of enzymatic unhairing at 100 and 400 μm [33].

fiber into fibrils. Efficient rubbing of the skin results in high porosity and thumbprints; smoothness and better elasticity of treated leather can be obtained by using alkaline serine protease from *B. subtilis* ZMS-2. The enzyme at a concentration of 2% w/w effectively removes hair bulbs and undesirable non-structural proteins (globulin, albumin, elastin) from the skin without affecting the main structural protein – collagen [60]. Moreover, not all proteolytic enzymes due to their properties, they are suitable for combing/combing collagenolytic effect. During the de-bristle/de-combing process, proteases are selectively degraded soft keratin tissues inside the hair follicles, thus causing them to be pulled out intact hairs without disturbing the tensile strength of the processed leather. This hair removal at the root level is a very important stage of leather processing, which ensures the ideal smoothness and suppleness of the processed leather, which is influenced by this enzyme.

Most tanneries in Europe today use wet blue leather for processing. This is due to the avoidance of environmentally harmful leather tanning stages in the first stages of the wet workshop. However, wet blue leather is stored in warehouses and basements for a long time before it is processed further, which may cause the leather to dry out and thus make it more difficult for chemicals and enzymes to penetrate in the further stages of leather processing [61]. Therefore, the enzymatic process (i.e., treating wet blue with acid protease) is more widely used in tanneries to improve the comprehensive properties of leather. Acid proteases can hydrolyze proteins either internally or externally cause cleavage at low pH 2–4 to produce small peptides and amino acids [61]. The wet blue bath environment typically has a pH of around 3.5. Hence, acid proteases are the choice of enzyme preparations for moderate hydrolysis of skin protein and good dispersion of skin collagen fiber optical network. However, this process also has some drawbacks: acid protease proteins slowly penetrate the collagen structure (the process takes about 2 h), which significantly reduces the effectiveness of the process. Additionally, the large size of the enzyme molecules further delays the skin penetration process. A solution to this problem was proposed by Tochetto et al. who found that the surface charge of nanoparticles (NPs) was an important factor that affects their penetration into cancer cells and the penetration effect of NP was improved when NPs were negatively charged [62]. Tamilselvi et al. developed supramolecular nanocarriers with the ability to switch surface charges and found that the reversal of surface charge promotes penetration nanocarriers for skin biofilm [63]. The results suggested a slow penetration of acid protease into wet blue mainly due to the electrostatic attraction between the acid protease (usually negatively charged) and the wet blue surface (positively charged). Moreover, optimizing the regulation of the electrostatic interaction between wet blue and acid protease is a future direction of research to increase the effectiveness of wet blue processing in the wet workshop.

Research on the use of enzymes as leather tanning agents is inextricably linked to the need to reduce the use of chromium sulfate in this process. Although the development of various improved chromium tanning methods has significantly reduced the use of chemicals and water in leather tanning, this change is not sufficient due to the production of wastewater containing harmful chromium content. Additionally, there is strong evidence that when treated leather is disposed in the environment, some of the released trivalent chromium is converted to carcinogenic hexavalent chromium. Over the years, many sustainable alternatives to chromium tanning have been developed, based on chemical and enzymatic cross-linking, various bio-based polymers, enzymes, modified zeolites, and nanostructured materials. Proteases are arguably the most extensively utilized enzymes for dehairing hides and skins. Some researchers have utilized them in soaking, rehydration, pickling, and chrome tanning. The search for new protease sources prompted researchers to focus on enzyme engineering utilizing recombinant and immobilization technology [64].

3.7. Use of enzymes in the textile industry – in comparison with the tanning industry

The tanning industry and the textile industry are two sectors that are responsible for a large amount of chemical pollutants emitted into the environment. The use of enzymatic processes in the tanning and textile industries can save both industries from collapse caused by strict restrictions on the emission of pollutants into the environment. Despite similarities in the area of using enzymes as process-efficient molecules while saving energy, both industrial sectors struggle with different types of problems and use different types of enzymes, replacing chemical processes with enzyme-based technologies. In the tanning industry, the most widely used proteases are responsible for the breakdown of peptide bonds present in proteins and animal cells. In the textile industry, the enzymatic technology has a high application potential from the fiber treatment to products' finalization [65]. In the textile preparation, the enzymes can modify the fibers' structure, degrade the gum, remove natural pigments, give the fiber a white color, dye, give shine, and soften the textiles [66]. In terms of type, the global textile enzymes market is divided into enzymes such as cellulase, amylase, catalase, pectinase, and laccase.

Catalase and amylase constitute the greatest demand on the market. The cellulase segment has a significant share due to the increase in demand for cellulase in the so-called bio-polishing. Cellulases are used in the final stage of textile production: they are used to remove starch sizing and to soften and reduce the fabric's tendency to pill. The use of enzymes in textile production significantly improves the quality of final products, including clothing.

Based on the applications in which enzymes are used quite extensively, the global market is segmented into:

- enzymes for bio-polishing fabrics (i.e., biological enzyme finishing, the purpose of which is to improve the surface finish of cotton fabric with cellulase to obtain a longer-lasting anti-adhesive effect, thereby increasing the overall smoothness and softness of the fabric);
- starch-sizing enzymes (pre-treatment of cotton);
- bleaching enzymes (including cotton enzymes, which are necessary to remove brown and yellow discoloration of cotton fibers caused by cotton flower proteins and pigments);
- bioleather/bioskin enzymes.

Increasing demand for textile enzymes in the apparel industry and increasing application to bring technological and manufacturing improvements in various end-use industries are the key factors in the textile enzyme market. Rapid industrialization and urbanization and growing environmental concerns are expected to drive the demand for textile enzymes in the near future. Similar predictions regarding the demand for enzymes are observed in the leather product market, but the sizes of both industries are significantly different, with an advantage for the textile sector. This does not change the fact that in both these industries, intensive work is underway on the use of enzymes instead of chemical technologies [67]. A representation of enzyme application is shown in Figure 7.

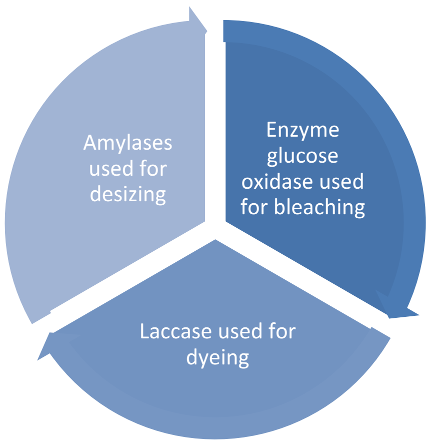

Figure 7. Diagram of textile processing steps where enzymes can be industrially used [67].

4. Innovative trends in the leather industry

The global leather trade is worth 150 billion USD annually. However, solid, liquid, and gaseous emissions from the leather sector damage the environment. Therefore, current trends in the tanning industry focus on implementing a range of more sustainable and environmentally friendly options (e.g., the use of enzymes at various stages of leather processing) in all leather manufacturing unit operations. Researchers have proposed the preservation of raw hides and hides with phyto-based methods, such as using the paste from leaves [68]. This could reduce the total amount of dissolved solids and chlorides by approximately 70%, while the leather cured by this technique showed comparable physical strength of leather with respect to conventionally cured leather. Another innovative solution is oxidative unhairing, which can reduce the BOD and COD by about 40%. This method has been suggested as an alternative to traditional unhairing, which is harmful to the environment [69]. The finished leather obtained by these processes shows good physical, mechanical, and technical properties, comparable with leather treated using the traditional process. The obtained leather was technically assessed as satisfactory and suitable for use in the production of high-quality upper leathers. Highly exhaustive tanning technologies based on copolymers can increase absorption by up to 95% [70]. New methods of extracting a cellulose derivative from further waste from the sugar sector for use in leather finishing have also been described [71]. Cellulose was found to impart fullness to the finished leather without impacting its aesthetic properties. Gandu et al. described the possibility of using an innovative odor reduction system based on biofilters, capturing harmful gas emissions from the tannery environment. The deodorization of gases emitted from tanneries, e.g., NH_3 and H_2S, using eco-friendly and cost-effective methods is necessary for the safe disposal of industrial emissions [72].

Another new trend in the tanning industry is the use of its rich protein resources for, for example, the production of bacteria producing keratinolytic proteases. Microorganisms are an excellent source of enzymes and are of increasing interest due to their wide biochemical diversity, ease of isolation and screening, susceptibility to genetic manipulation, and cost-effective and consistent production [73]. Bacteria producing keratinolytic proteases have been isolated and found in various habitats, including tannery soil [74]. Keratinolytic proteases have potential applications in various areas of industrial processes and are widely used in food and feed, medicine, detergents, silk degumming, leather, silver recovery, waste management, and pharmaceutical industry [75]. Current industry demand for keratinolytic proteases with beneficial properties continues to improve the search for new sources of enzymes. Many studies have shown that bacterial isolates can produce keratinolytic proteases from a variety of environments. Research on the optimization of biotechnological processes producing keratinolytic enzymes by bacteria led to the isolation of a new *B. subtilis ES5*, which strongly decomposes feathers, from soils originating from traditional leather tanning.

Producing keratinase from waste chicken feathers using soil bacteria from tanneries could play an important role in producing the enzyme using inexpensive substrates. The enzyme substrate obtained in this way has a promising ability to produce keratinase without additional carbon and nitrogen sources and is stable in alkaline pH, surfactants and commercially available detergents. Due to its stable properties, the product can be used in the production of environmentally friendly detergents.

Recently, trends in the production of leather products have included a completely new area of using living organisms to produce leather products. An alternative to leather of animal origin or leather substitutes in the form of plastics are products made from the leather of filamentous fungi. Basically, these alternative materials have been observed with various limitations, especially their undesirably high hydrophilicity [76]. Mycelium products still have several drawbacks that require improvement. Pure mushroom mycelium leather substitutes were found to have low tensile strength, poor performance properties, and lack of thickness uniformity [77]. Therefore, many efforts are currently being invested in circumventing these challenges; therefore, many believe that leather substitutes derived from fungal biomass are one of the most cost-effective and feasible options. There are currently 36 application-related patent worldwide materials of fungal origin, such as leather substitutes or textiles [78]. Companies point out the huge potential of leather materials made from mycelium in terms of sustainable development, low flammability potential, low production costs, fast growth rate, and relatively low carbon footprint [79]. Filamentous fungi have been identified as extremely rich and a diverse group of species and products originating from this group of organisms are now considered essential building blocks of the transition toward a more sustainable future for us and the planet because they also provide a pollution-free environment. Production of leather alternative materials from filamentous fungi obtained through environmentally benign processes has been recognized as one of these sustainable solutions. In this biogenic process, agricultural waste is recycled into cost-effective, environmentally friendly, and highly versatile leather-like products.

The process fits perfectly into the principles of closed-loop bioeconomy, the aim of which is to increase the efficiency of resource use and reduce the accumulation of waste to the necessary minimum.

However, despite the commercial progress made in the development of fungal skin substitutes, there is still a gap in scientific knowledge in this area. The production process of leather from filamentous fungi requires filling the knowledge gap regarding the fungi themselves and their species due to the huge number of occurrence of these organisms in the world. The strength properties of the produced materials also require optimization, especially in the context of the excellent strength properties of leather made from animal skins [80].

Each of these sustainable leather technologies can be applied to reduce the ecological impact of leather production.

5. Conclusions

Proteases are divided according to their source of origin into three basic classes: animal, plant, and microbial proteases [63]. In the protease database MEROPS, there are 259 different substances cataloged by amino acid sequence similarity [81]. Proteolytic enzymes differ in working pH, working temperature, substrate selectivity, and active site specificity [82]. Due to disadvantages such as uncertainty of operation in a wide range of pH and temperature, non-resistance of operation in alkaline pH, poor hair removal efficiency, and significant damage to collagen or low tolerance to chemical reagents in leather processing [83], more research is needed on the use of these enzymes in the leather processing industry.

The leather-producing industry increasingly uses proteolytic enzymes, lipases, and amylases. Almost all major leather manufacturing processes from soaking to finishing use enzymatic products to improve the efficiency and performance properties of the finished products. Thanks to the use of enzymes, more environmentally friendly leather products are being produced with fewer solvents, salts, acids, and surface-active agents. The use of biocatalysis in production processes is an essential element in creating a circular economy. The energy and mass already produced must be recycled to matter with the least possible loss and without the use of additional energy resources. The use of enzyme preparations has some problems, primarily concerning their efficiency, specificity, and high cost, but it is one of the possibilities for achieving more environmentally friendly leather production [8]. Protein engineering of keratinolytic protease opens further opportunities for leather biotechnology, either in the unhairing process or the treatment of organic-rich wastewater. Using a biocatalyst of keratinase is an environmentally friendly and sustainable way to produce high-quality leather with low pollution emissions. More work is needed to improve the performance of keratinase in the unhairing process [36]. Microorganisms are natural sources of commercial enzyme preparations (proteases from microbial sources account for around 40–60% of the total global enzyme sales) [84], due to their physiology and biochemistry, facile culturing conditions, and the ease of manipulating their cells. Some fungal strains (*Aspergillus, Penicillium*, and *Rhizopus)* are considered useful for the production of protease enzymes [85].

Another interesting avenue for the development of alternative leather production methods is the use of alkaline proteases, which have high catalytic activity and substrate specificity and can be produced in large quantities at low cost. Their use in the processes of soaking, dehairing, bathing, and degreasing hides reduces waste, recovers valuable by-products, lowers costs, and improves leather quality. These enzymes can catalyze reactions at the extremes of pH, temperature, and salinity during leather manufacturing processes [86].

The tanning industry generates nearly 2 MT of waste biomass annually, most of which goes to landfills. The biomass in landfills contaminates soil and water, contributes to global warming, and makes land unsuitable for use due to the bioaccumulation of pollutants. However, leather biomass mainly contains protein (30–35% collagen) and fat. It is a possible raw material for high-value materials, including biofilms, bioplastics, biofiber, superabsorbent material, additives for various industries, sizing agents, and surfactants. It is considered as a replacement for materials based on fossil fuels. Utilizing this biomass for value-added material production can both bring economic benefits and help prevent pollution [85]. Managing waste from the leather industry is crucial, as valuable components can be recovered (methods that produce digestate, biogas, collagen hydrolysate, fat, or fertilizers) and reused in the same sector (chrome) or in other branches of industry, such as the pharmaceutical, food, or medical industry. The greatest challenge is the introduction of zero-waste technologies, where each stream can be recycled. New sustainable technologies need to be integrated with the waste management hierarchy, ensure the maximization of material recycling, and include valorization methods for all material streams [86].

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