A REVIEW OF POTENTIAL ENZYMES: PROTEASE AND KERATINASE FOR DEHAIRING PROCESS AS CLEANER AND ECO-FRIENDLY LEATHER PROCESSING

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ABSTRACT. In recent decades, there has been growing concern and negativity from the environmental impact of industrial development, which produces lots of technological changes. The significant contribution of the leather industry has a good impact on the economic sector; however, it is faced with challenges caused by pollution to the environment. Special attention is required for disposal of solid wastes because of the large amounts that are generated and legal restrictions. Environmental problems are caused by the large amount of wastes originating from chemicals during processing, especially in dehairing process. Conventional dehairing by lime sulfide process causes high chemical oxygen demand emission, biological oxygen demand, and total suspended solids. The dehairing mechanism is vaguely understood at present from the point of view of the enzyme specificity, which is needed for consistent and satisfactory hair removal without deleterious effect on the leather quality. It is known that the use of enzymes has potential as an alternative that can be used to reduce the harm caused by toxic chemicals including for waste management. Recently, there has been increasing research on application of enzymes in various leather production processes. The use of protease and keratinase enzymes in leather processing industry has the most promising potential to improve the surrounding environment. Therefore, this study aimed to review relevant literature in terms of dehairing processes without adverse effects in order to make good quality leather. Further scientific discussion is needed for understanding the gap of these critical issues in this area.

KEY WORDS: dehairing, protease, keratinase, eco-friendly, leather processing

O TRECERE ÎN REVISTĂ A POTENȚIALELOR ENZIME: PROTEAZA ȘI KERATINAZA UTILIZATE ÎN PROCESUL DE DEPĂRARE PENTRU O PRELUCRARE A PIEILOR MAI CURATĂ ȘI ECOLOGICĂ

REZUMAT. În ultimele decenii au existat preocupări și efecte adverse tot mai mari din cauza impactului asupra mediului a dezvoltării industriale, care generează o mulțime de schimbări tehnologice. Industria de pielărie contribuie favorabil în mod semnificativ la dezvoltarea sectorului economic, însă se confruntă cu provocări cauzate de poluarea mediului. O atenție deosebită este necesară pentru eliminarea deșeurilor solide din cauza cantităților mari care sunt generate și a restricțiilor legale. Problemele de mediu sunt cauzate de cantitatea mare de deșeuri provenite din substanțele chimice utilizate în timpul prelucrării, în special în procesul de îndepărtare a părului. Depărarea convențională cu soluție de var generează valori mari ale consumului chimic de oxigen, consumului biochimic de oxigen și solidelor în suspensie totale. Mecanismul de îndepărtare a părului este vag înțeles în prezent din punctul de vedere al specificității enzimatice, necesară pentru o îndepărtare uniformă și satisfăcătoare a părului, fără efecte dăunătoare asupra calității pielii. Se știe că utilizarea enzimelor poate reprezenta o alternativă în vederea reducerii daunelor cauzate de substanțele chimice toxice, cu efecte inclusiv în gestionarea deșeurilor. Recent, s-au realizat tot mai multe cercetări privind aplicarea enzimelor în diferite procese de producție a pielii. Utilizarea enzimelor protează și keratinază în industria de prelucrare a pielii are cel mai promițător potențial de a îmbunătăți mediul înconjurător. Prin urmare, acest studiu și-a propus să treacă în revistă literatura de specialitate relevantă referitoare la procesele de îndepărtare a părului pentru a obține piele de bună calitate, fără efecte adverse asupra pielii finite. Sunt necesare discuții științifice suplimentare pentru a înțelege decalajul creat de problemele critice din acest domeniu.

CUVINTE CHEIE: depărare, protează, keratinază, ecologic, prelucrarea pielii

UN EXAMEN DES ENZYMES POTENTIELLES : PROTÉASE ET KÉRATINASE POUR LE PROCESSUS D'ÉPILAGE COMME TRAITEMENT DU CUIR PLUS PROPRE ET ÉCOLOGIQUE

RÉSUMÉ. Au cours des dernières décennies, l'impact environnemental du développement industriel, qui entraîne de nombreux changements technologiques, a suscité de préoccupations croissantes et effets négatifs de plus en plus grands. La contribution significative de l'industrie du cuir a un impact positif sur le secteur économique, mais elle est confrontée aux défis causés par la pollution de l'environnement. Une attention particulière est requise pour l'élimination des déchets solides en raison des grandes quantités générées et des restrictions légales. Les problèmes environnementaux sont causés par la grande quantité de déchets provenant de produits chimiques lors du traitement, en particulier lors du processus d'épilation. L'épilation conventionnelle par le procédé au sulfure de chaux provoque des valeurs élevées pour la demande chimique en oxygène, la demande biologique en oxygène et les matières en suspension totales. Le mécanisme d'épilation est vaguement compris à l'heure actuelle du point de vue de la spécificité enzymatique, qui est nécessaire pour une épilation cohérente et satisfaisante sans effet délétère sur la qualité du cuir. Il est connu que l'utilisation d'enzymes constitue une alternative potentielle pour réduire les dommages causés par les produits chimiques toxiques, notamment pour la gestion des déchets. Récemment, de plus en plus de recherches ont été menées sur l'application d'enzymes dans divers processus de production du cuir.

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L'utilisation d'enzymes protéases et kératinases dans l'industrie du cuir est la plus prometteuse pour améliorer l'environnement. Par conséquent, cette étude fait un examen de la littérature pertinente qui fait référence au processus d'épilation pour l'obtention du cuir de bonne qualité sans effet négatif. Des discussions scientifiques plus approfondies sont nécessaires pour comprendre les lacunes de ces questions critiques dans ce domaine.
MOTS CLÉS : épilation, protéase, kératinase, éco-responsable, traitement du cuir

INTRODUCTION

Biotechnology is the utilization of biological-based materials and nature's production processes [1], which can be an alternative technology that replaced conventional technology that leads to eco-friendly production processes [2]. Among biotechnologies, the enzymatic process is an alternative that can be developed from conventional and promising processes [3]. Bacteria and fungi that grow in submerged or solid fermentation are capable of producing enzymes that can be used for industry. Fermentation process on enzyme production is further followed by filtration and cell disruption. Downstream processing of the crude enzyme collectively by precipitation followed by centrifugation and vacuum drying or lyophilization [4].

Enzymes have high specificity, are easily biodegradable, have lower toxicity than chemicals [1]. Producers can benefit by developing products that are of equal or even better quality using less raw material, chemical, water and consumption and with less problematic waste generation than traditional processes by the properties of enzymes [1, 3]. Industries use enzymes for various production processes such as leather production [5, 6], textile production [7], food production [8], and animal feed production [2].

In several articles, books, and reports during last decade, we have discussed and agree the impact of using enzymes on the environment compared to conventional processes [9-15]. However, a concrete assessment is needed that the production of enzymes and auxiliary materials for enzyme processes require more energy and raw material besides being based only based on qualitative assessments. Therefore, to assess the true environmental benefits of enzymatic processing, a qualitative environmental impact assessment is required [4].

Recently, enzyme technology has been used as a substitute for polluting chemicals in stages of leather processing. The use of enzymes in soaking, degreasing, and application of protease for dehairing in the last two decades has increased. The action of enzymes is one of the methods that the leather technologist used just to accelerate soaking and minimize the use of harmful dehairing chemicals [16, 17]. Enzymatic dehairing is part of the efforts for greener leather.

Therefore, this paper aims to update and discuss the available data on the main use of enzymes, considered as one of the most promising methods for improving environmental conditions related to leather processing. The study was conducted through a survey of scientific articles. Thus, this appears as providing an accurate database for an environmental leather guide in dehairing processes and the development of cleaner leather production.

METHODOLOGY

Mechanism of Conventional Dehairing and Enzymatic Dehairing

Good quality leather is obtained by removing the hair from the skin. In conventional leather making, the skins are treated by soaking in liquor containing lime for long duration depending on the thickness of the skin. Later, to accelerate the process, sulfides can be an alternative which results in the quick loosening of the hairs. Keratin is abundant in the main outer layer of the skin, epidermis and hair or wool. The high content of sulfur-rich amino acid and cystine causes the keratin helices to be stabilized by intra-molecular disulfide bonds. The reduction process cleaves these disulfide links by removing the keratinous substances.

Sulfide treatment affects the integrity of all layers of the skin. Sulfides are in continuous contact with the hair side of the skin so that the outer root sheath of the hair and the cortex of the hair shaft are also dissolved in the hair burning process [18]. However, the use of these sulfides leaves residual hair root or short hair in the follicle that have to removed so they are not visible in the finished leather. This short hair removal is carried out by a bating process using proteases. There is deionization of basic amino acids in collagen due to high alkaline conditions resulting in high fiber opening through an osmotic process [19]. Making the leather soft and durable depends on opening the fiber bundles [18].

The mechanism of the chemical dehairing process is much better understood, while the dehairing process using proteases lacks clear comprehension (Figure 1). The keratinolytic activity is still debated by researchers, while all researchers highly appreciate the activity of non-collagenolytic enzymes. Epidermal barrier containing keratin should be dislodged by enzymes in order to reach the hair bulb and remove the hair. In fact, proteases have been shown to have hair removal abilities even in the absence of keratinolytic activity [20, 21]. The outer root sheath which contains keratin is shed with the base membrane with an ideal protease without keratin specificity.

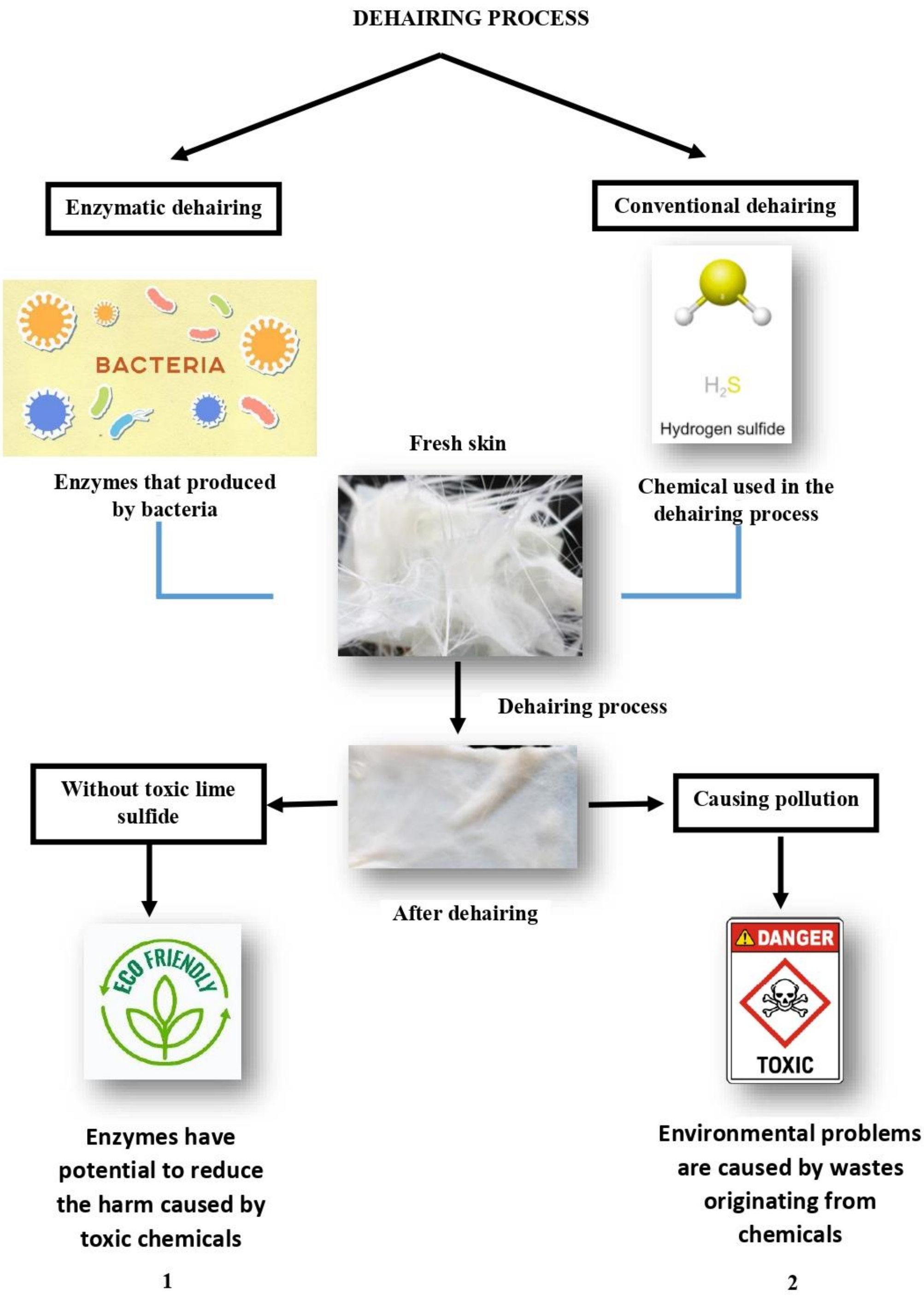

Figure 1. Schematic representation in animal hide during the dehairing process. **1** - Schematic diagram of dehairing process by enzymatic. **2** - Schematic of conventional dehairing process using chemicals

The inner root sheath can be degraded easily by protease because it contains low cysteine. The high amount of cystine due to the keratin structure affects the next layer, the cuticle is resistant to non-keratinolytic proteases. This also occurs in the cortex because some mammals share the same chemical composition. The hair bulb can be a site of attack in the process of hair removal because the hair bulb contains dermal papilla which is primarily composed of non-structural proteins. Above the hair bulb where disulfide bonds form is a zone of pre-keratinization, which has so few characteristics of keratin that it can be a point of attack in protease-mediated dehairing [22]. To loosen the hair follicle by targeting the surrounding area, a protease with the right specificity can be used.

RESULTS AND DISCUSSIONS

Enzymes

Enzymes are generally proteins, organic substances and known as biocatalysts for chemical reaction and have been used in various applications including detergent, food, pharmaceutical, diagnostic, chemical industries, agriculture, paper, and leather. According to some authors, the use of enzymes is an important component of sustainable industry [23]. Pollutants that harm the environment have to be removed, changed, or detoxified to make them less toxic through natural processes and bioremediation techniques with biological agents such as microbes that can produce enzymes [24].

The advantage of using enzymes in dehairing process, besides reducing chemicals, is also shortening the time needed, and resulting in cleaner grain layer because the enzymes attack the connection between the hair and the derma, thereby facilitating the shedding of hair without damaging it.

Proteolytic Enzymes

Protease

Protease are enzymes that hydrolyze proteins and peptides. Proteases act to break protein peptide bonds through catalytic hydrolysis and are used to remove non-fibrillar proteins [25]. Animal skin is composed of hair, unwanted protein and fat. Unnecessary fat and protein have to be removed before being processed into leather. Mechanical treatment, called soaking followed by liming process so that the hair is digested by the action of sulfides, is a conventional method to remove unnecessary parts. According to Li *et al*. [26], protein and fat can be degraded by using protease as a supplement so that it can be used in soaking and liming processes.

Pseudomonas bacteria from visceral wastes utilize protease to dehair goatskins and indicate that dehairing using enzymes generate similar or improved characteristics [5]. According to Briki *et al*. [27], protease from *Bacillus* sp. SB12 can be used for dehairing process in goat skin processing in industry. Simillar results were reported [28-30] using *Bacillus* strain for hide unhairing to replace the chemical process, which uses toxic sulfides and lime.

Conventional dehairing by lime sulfide process causes high chemical oxygen demand (COD) emissions, biological oxygen demand (BOD), and total suspended solids (TSS) [31]. One of the main contaminants of wastewater is ammonium salt which is usually added when using lime during unhairing process [32]. A reliable absorption of sulfide, nitrogen, COD, and sludge load to the environment is a good dehairing effect from the use of low sulfide and lime [33]. The Table 1 shows various enzymes that can be used for dehairing process from bacteria. From the above it can be stated that the conventional process is not friendly to the environment compared to the enzymatic dehairing process.

Table 1: Compilation of dehairing by protease from 2003-2018

Bacterial strain	Sample	Type of skin/hide	Author
Bacillus subtilis S14	Bovine hair, skins wastes and oil samples	Bovine hide	[34]
Bacillus subtilis P13	Vajreshwari hot spring (45-50°C)	Goat hide	[35]
Bacillus subtilis MTCC 6537	Tannery	Goat skin	[20]
Bacillus subtilis IH-72	Soil from tannery area	Goat skin	[36]
Bacillus subtilis P	Soil	Goat skin	[37]
Bacillus subtilis AP	Goat skin	Goat skin	[38]
Bacillus subtilis Blbc	Tannery sludge	Hide	[29]
Bacillus subtilis Strain VV, AP	Sediments from river	Goat skin	[39]

Bacterial strain	Sample	Type of skin/hide	Author
Bacillus subtilis Strain AKRS3, AP	Waste soil	Goat and sheep skin	[40]
Bacillus subtilis, P		Buffalo hide	[41]
Bacillus subtilis, AP	Soil	Goat skin	[42]
Bacillus subtilis KT004404, P		Goat skin	[43]
Bacillus subtilis AKAL7, AP	Poultry waste mixed soil	Cow hide	[44]
Bacillus subtilis R82, P		Goat skin	[45]
Bacillus pumilus UN-31-C-42	Living waste	Skin	[46]
Bacillus pumilus BA 06, AP		Pig skin	[47]
Bacillus pumilus TMS55, ASP	Marine sediment	Goat skin	[30]
Bacillus pumilus MCAS8, ASP	Lake sediment	Goat skin	[48]
Bacillus pumilus MTCC 7514, AP	Marine soil	Goat skin	[49-50]
Bacillus pumilus, AP	Soil, fish market, waste water	Goat skin	[51]
Bacillus licheniformis, AP	Soil	Bovine hide	[52]
Bacillus licheniformis ER-15, K	Soil	Bovine hide	[53]
Bacillus licheniformis RP1, AP	Water	Goat skin	[54]
Bacillus licheniformis MZK05M9, P	Laboratorium	Goat skin	[55]
Bacillus cereus MCM B-326, P	Slaughterhouse	Buffalo hide	[56-57]
Bacillus cereus IZ-06b and IZ 06r, K	Wool and skin	Sheep skin	[58]
Bacillus cereus VITSN04, AP	Soil	Goat skin	[59]
Bacillus megaterium RRM2, P		Goat skin	[60]
Bacillus megaterium DSM319, P		Cow hide	[61]
Bacillus velesensis, K	Aquatic environments	Bovine skin	[62]
Bacillus circulans, P		Goat skin	[63]
Bacillus altitudinis GVC11, ASP	Soil from slaughter	Goat skin	[64]
Bacillus halodurans JB 99, AP		Buffalo hide and goat skin	[65]
Bacillus safensis LAU 13, K	Soil	Goat skin	[66]
Bacillus sp kr10,	Feather waste	Bovine skin	[67]
Bacillus sp., AP	Lake	Cow hide	[68]
Bacillus JB 99, AP	Laboratorium	Goat skin	[69]
Bacillus sp. AMUa38, AP	Soil	Goat skin	[70]

Keratinase

Keratin can be found in hair, wools, nail, feathers and is a main structural protein of hides. The protein content of feather keratin is 91%, while water and lipids are 8% and 1%. Keratinase is included in the protease group that can be applied in cosmetics, textile, and leather industries [49]. According to Kalaikumari *et al*. [71], the use of keratinase to remove sheepskin from *Bacillus paralicheniformis* MKU3 has an efficiency up to 100%. *Bacillus cereus* and *Pseudomonas* sp. derived from poultry feathers containing keratinase showed effectiveness compared to chemicals without any damage to leather. Replacing conventional methods of dehairing with enzymatic methods will help reduce environmental pollution [57]. The use of keratinase enzyme as a substitute for sodium sulfide can be an environmentally-friendly tanning agent. The Table 2 shows various keratinase enzymes that can be used for dehairing process.

Table 2: Compilation of dehairing by keratinase from 2001-2020

Bacterial strain	Sample	Type of skin/hide	Author
Pseudomonas aeruginosa PD100, P		Cow and sheep skin	[72]
Pseudomonas aeruginosa MCM B-327, P		Hides	[73]
Pseudomonas aeruginosa MTCC 10501, AP	Slaughter soil	Goat skin	[74]
Alcaligenesfaecalis, AP	Soil	Goat skin	[75]
Lysobacter NCIMB 9497, K			[76]
Proteus vulgaris, P			[77]
Paenibacillus woosongensis TKB2, P	Poultry processing plant	Goat skin	[78]
Brevibacillus brevis US575, K	Soil	Rabbit, goat, sheep	[79]

Bacterial strain	Sample	Type of skin/hide	Author
		and bovine hides	
Vibrio metschnikovii NG155, P	Soil and water from leather and meat industry	Goat skin	[80]
Stenotrophomonas maltophilia K279	Feather	Goat skin	[81]
Brevibacterium luteolum, MTCC 5982	Laboratorium	Goat skin	[82]
Idiomarina sp. C9-1, P	Lake	Cattle hide and Goat skin	[7]

Environmental Impact of Dehairing Process

The conventional method in dehairing process causes a large amount of sulfides concentration in wastewater around 3 % w/w of skin as well as the release of high total solids in wastewater. This conventional method raises pollutants to 50–60% in which those numbers are evaluated based on several essential parameters used to evaluate the quality of wastewater, such as biochemical oxygen demand (BOD), chemical oxygen demand (COD), total dissolved solids (TDS) and total suspended solids (TSS). The imbalance of these parameters causes environmental damage. An example of using sulfide for the dehairing process is illustrated in the use of hazardous sulfide (such as H_2S resulting from the dehairing process), which impacts environmental stability. It also decreases the effectiveness of the effluent treatment plants. Even in low concentrations, sulfide has been proven to lead to a serious impact. The use of H_2S in the dehairing process is fatal, causing lethal effects even at concentrations as low as 200 ppm. The increase in concentration and toxicity causes a decrease in various human perceptions [83-84].

Experiments conducted by [19] illustrated the environmental impact of the use of enzymes in the dehairing process by comparing the performance of the conventional and enzyme-assisted methods in the dehairing process on goatskins and cowhides. According to the experiments carried out, it has been proved that the enzymatic process offered a significant contribution to the dehairing process and was more effective than employing lime and sulfide. It was evident from the reduction of total dissolved solids (TDS) and total suspended solids (TSS) by about 85% in both types of skins. Those experiments also reported that the value of pollutants in terms of biochemical oxygen demand (BOD) and chemical oxygen demand (COD) also decreased by more than 78% in both types of skins. Those achievements proved that the enzymatic method was more effective for the dehairing process in terms of environmental protection. Not only giving significant advantages, enzymatic process was also effective for reducing toxicity during the dehairing process.

However, despite the significant advantages of employing the enzymatic method, some hurdles still remain in the enzyme-assisted process. One of the important hurdles was in how much alkalinity could be generated using the enzymatic method. It could be noticed that pH during the dehairing process would be near neutral to reduce toxicity. This condition can be reached if the dehairing process can generate high alkalinity. However, the dehairing process using an enzyme cannot generate an alkaline pH as much as lime or sulfide does. Hence, it would be an advanced challenge for the next research to produce a more effective and efficient dehairing method.

Briki *et al.* [26] showed the environmental impact of using alkaline protease from *Bacillus* sp. SB12. This research illustrated that the leather tanning process produced at least 75% organic waste in which hair contributed 70% of them. Conducting chemical procedures in this process would affect the stabilization since the chemical procedure would increase the level of BOD and COD. Briki's research successfully proved that employing enzymes was one of the suitable solutions in the dehairing process. It reduced BOD and COD levels by 40% and 50%, respectively. According to the research mentioned earlier, the enzymatic method was suitable as an alternative method in the dehairing process. It significantly reduced the toxicity in order to prevent the sustainability of the environment.

Potential Use of Enzymes in Several Leather Processes

The conventional dehairing method in leather processing causes a large amount of sulfides with concentrations of around 3 % w/w of skin. Regarding to those facts, the use of sulfides which cause emissions into liquid waste can be replaced by enzymatic processes. Enzymatic processes proved to be beneficial to decrease total dissolved and suspended solids. Table 3 shows that enzymes can be used for various leather processes.

Table 3: The use of enzymes in leather processing

Stage	Traditional technology	Enzymatic alternatives
Soaking	Neutral salts, acids, bacteria	Proteolytic and lipolytic
Dehairing	Calcium hydroxide and sulfides	Enzymes with proteolytic on collagen and keratin
Bating		Alkaline active protease of alkaliphile
Pickling	Acids, particularly sulfuric acid and formic acid	Proteolytic enzymes to enhance exhaustion of vegetable tanning agents
Dyeing	Dyes and some auxiliaries, acids to fix dyes	Collagenase

CONCLUSIONS

There are three main stages (beamhouse, tanning and post-tanning) in leather processing. The dehairing process is an important stage to prepare for the next step, which is beamhouse. Beamhouse operations in leather processing which have been carried out so far cause more than 70% pollution of the total pollution generated. Sodium sulfide and lime are chemicals that play an important role during the liming and dehairing steps. Several treatments using biological or physical means have been proposed but the treatment methods have not been able to fully produce toxin-free by-products.

Social and economic sustainability are the two pillars of sustainability which are highly prioritized, but it is necessary to pay attention to environmental sustainability. In this review, various bacteria produced by protease and keratinase enzymes can be used in dehairing process. In addition, enzymatic dehairing can help reduce dependence on the use of hazardous chemicals commonly used in leather processing such as sulfide, lime and amines as well as maintain a balance between human health, wildlife, reducing water, soil and environmental pollution. Therefore, the use of enzymes can be a good alternative to conventional processes.

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