Special Review Paper: Enzymes in the Leather Industry

by

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Abstract

The leather industry has been facing new challenges including the need to improve and optimize processes to achieve the required quality in their final articles and meet the requirements of environmental legislation. The enzymatic treatment of leather is a promising technology. Enzymes are organic substances, generally proteins, known as biocatalysts for multiplex che mical reactions. They have been explored commercially in the detergent, food, pharmaceutical, diagnostic, fine chemical and other industries. Commonly, the most promising enzyme sources are microorganisms. Several studies have reported enzyme use in different stages of leather production, describing a decrease in the use of chemicals for the depilation and liming steps, and some have also reported the complete replacement of chemicals with enzymes. Some authors use enzymes to increase the exhaustion of dyes in dye baths and to increase the effectiveness of tanning in the tanning step. The identification of a new enzyme is a long process, requiring screening for specific criteria, isolation and selection of new bacteria, and the investigation of the nutrients and conditions necessary for growth and enzyme production by the selected microorganism. Finally, issues such as pH, temperature and product inhibition of the enzyme during its application in the processing of skins/hides should be studied.

1. Introduction

Environmental laws are becoming more stringent, and concerns about the negative environmental impacts of industrial development are growing. As a result, many technological changes that influence the actions of public and private institutions have occurred. The leather industry is subject to these developments and seeks new, alternative processes to decrease its environmental impact and excessive use of chemicals. Leather production has increased worldwide. Brazil is the second largest producer and the fourth largest exporter of leather in the world. The world trade in leather and leather goods increased from $4 billion in 1972 to $60 billion in 2000.[1,2] The estimated world leather production for 2011 was 311 million hides (except buffalo hides).[3] Hide manufacturing involves the use of many chemical products and physical operations; consequently, this process generates huge amounts of liquid and solid waste.

According to the European Chemical Products Policy, substances with hazardous properties used in production of consumer products will be restricted beginning in 2012 and banned from any type of application in 2020. The Regulation on Registration, Evaluation, Authorization and Restriction of Chemicals (REACH), which has been in force since 2007, consists of a single system of record-keeping for new and existing substances that are manufactured or imported by the European Union. This regulation establishes new requirements for the industry and establishes a new approach for the control of chemicals.[4]

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Many alternatives have been studied for reducing the pollution load of tannery waste, including chemical product replacements, reduction of the environmental impacts of the waste and reutilization of the wastes for new products and subproducts.[5-17] Among these alternatives is the use of biotechnological enzymes.[11,18-25] Figure 1 presents the main chemical products responsible for the contamination of the liquid effluent from leather processing. The main advantages of enzyme use are the shortening of the productive process and the creation of active sites in the hides, which are likely to improve the effectiveness of chemicals such as dyes and tanning agents.

2. BIOTECHNOLOGY

Biotechnology is defined as the use of knowledge about biological processes and the properties of living things to solve problems and make useful products. The knowledge that enables the use of biological agents (organisms, cells, organelles, molecules) to obtain goods or provide services can be called biotechnology and includes basic science (molecular biology, microbiology, cell biology, genetics, genomics and embryology), applied science (immunological, chemical and biochemical techniques) and other technologies (computers, robotics and process controls). Biotechnology has impacts on diverse areas of production, offering new employment opportunities, disease-resistant plants, biodegradable plastics, biofuels, less-polluting industrial and agricultural processes and environmental bioremediation methods.[26-28]

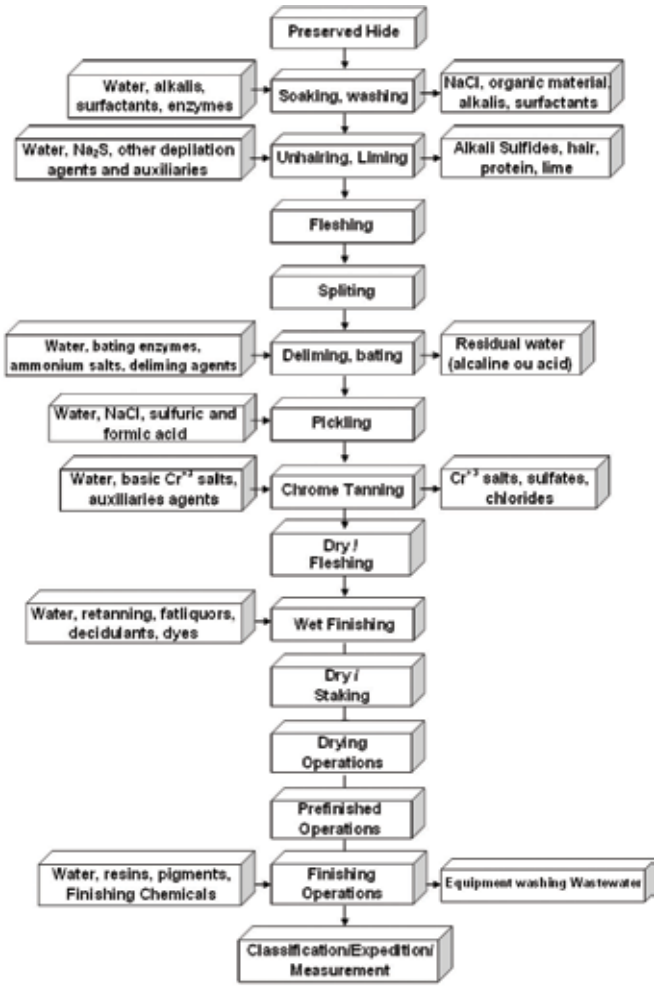

Figure 1. Leather production flowchart with chemical products and wastewater contaminants.

Recently, the industrial use of biotechnological processes (biocatalysts and biotransformations) has increased. Studies by the Organization for Economic Cooperation and Development (OECD) indicate an increased use of biotechnology in industry. Among the cases studied, fifteen of twenty-one used enzymatic processes, and for all cases, the operational costs were reduced by 9-90 % and energy and natural resources were saved.[29-32]

3. ENZYMES

Enzymes are organic substances, generally proteins, known as biocatalysts for multiple chemical reactions and have been explored commercially in the detergent, food, pharmaceutical, diagnostic, fine chemical industries, among others. They are notable molecules, especially due to their extraordinary efficiency and catalytic power, which are superior to synthetic catalysts. The high specificity of an enzyme is determined by its size and three-dimensional conformation, which creates affinity regions for substrates.[30, 33-35] Since Pasteur, research has shown that, although subject to the same laws of nature that govern the behaviors of other substances, enzymes differ from ordinary chemical catalysts in several important respects, including the following: faster reaction speeds, milder reaction conditions, greater reaction specificity and the ability to be regulated.[36]

Almost all of the reactions that characterize cellular metabolism are catalyzed by enzymes. These reactions are the metabolic basis of all living organisms and promote significant opportunities for more efficient and economical biocatalytic conversions in industry.[30]

The total number of existing enzymes is estimated to be 6000-7000; 3000 of these enzymes have described functions, but only approximately 130 have industrial uses, either as isolated enzymes or as parts of cells. Most of these enzymes are of microbial origin because microbial enzymes tend to be more stable than their counterparts in plants and animals. Of the more than 3000 different enzymes, most have been isolated from mesophilic organisms.[29] Enzymes can be classified as in Table I.

TABLE I
Enzyme classification according to the type of catalyzed reaction.

Classification	Reaction Catalyzed Type
Oxidoreductases	Redox reactions
Transferases	Functional group transfers
Hydrolases	Hydrolysis reactions in which molecules are divided into two or more molecules through the addition of water
Lyases	Breaking of covalent bonds and release of water, ammonia and carbon dioxide molecules
Isomerases	Intra-molecular rearrangement reactions
Ligases	Formation of new molecules by forming bonds between two molecules using energy (ATP)
Carbohydrases	Hydrolysis of glycosidic bonds between the monosaccharides of oligosaccharides or polysaccharides

3.1 Enzyme Applications

The applications of enzymes are obviously linked to the world market and can be divided into industrial applications and enzymes for medical, analytical and scientific uses.[37-43] The main applications, especially the industrial ones, are within the so-called biotechnology area. This term has become a catchall for activities related to science and technology in the last decade, which makes it difficult to precisely define. However, biotechnology certainly involves microbiology, biochemistry and genetic engineering and the chemical and biochemical processing of materials by biological agents, as illustrated in Figure 2. Among these agents, enzymes are often used to improve processes and to enable the use of new raw materials, improving their physical and chemical characteristics.[44]

Enzymes are used in various applications, including the following: the food, agriculture, paper, leather, and textile industries, resulting in significant cost savings. According to some authors, the use of enzymes is an important component of sustainable industrial development.[30] Bioremediation techniques use biological agents (e.g., living microbes or enzymes) for the removal, transformation or detoxification of contaminating pollutants from the environment, transforming such pollutants to less toxic forms by natural processes.[33]

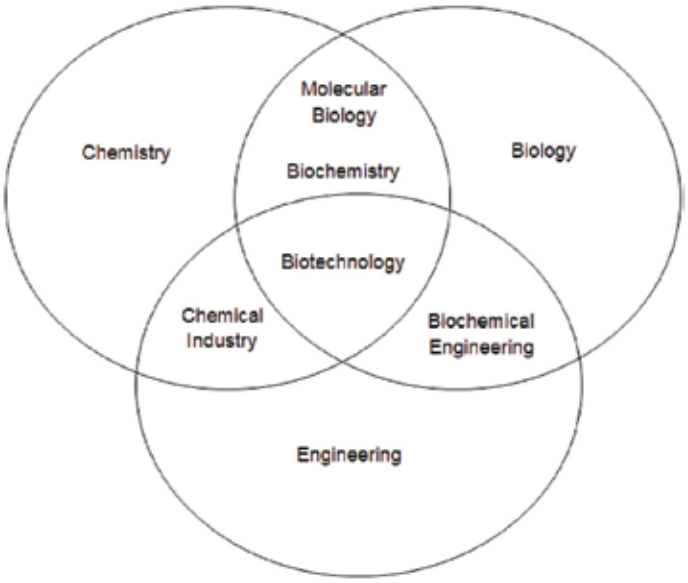

Figure 2. Knowledge areas in biotechnology.

3.1.1 Enzymes Applied to Cleaner Leather Production

Biotechnology has been used in the tanning industry for some years, but most of the enzyme preparations used in this sector do not have sufficient specificity. Currently, biological methods have been used with relative success in the processes of soaking, unhairing and bating and in the partial removal of natural fat.[30,35,45]

The environmental impacts of producing and delivering enzymes to the tannery, on the one hand, and conserving chemicals and electricity, on the other hand, have been evaluated. A small 'investment' in energy and CO_2 emissions in enzyme production resulted in considerable savings when the products replaced chemicals and electricity in the soaking and unhairing/liming processes.[46]

There are also options for the biotechnological treatment of wastewater and solid protein waste.[47-53] Mass losses in leather processing are up to 50 %. The best removal processes recover soluble proteins that may have commercial use. Enzymes can degrade the untanned[54, 55] and tanned solid leather wastes.[56]

The following are among the enzymes that can be applied in hide processing:

Proteases

Proteases are enzymes that hydrolyze proteins and peptides. Alkaline proteases are a group of physiologically and commercially important enzymes that are primarily used as additives in detergents. They have a specific role in the hydrolysis and cleavage of proteins.[34, 47, 57] The genus *Bacillus* has been a predominant and productive source of alkaline proteases used in various industrial applications. Alkaline

proteases are generally produced by submerged cultivation. Each microorganism has its own conditions for maximum enzyme production.[34, 58] Proteases act to break protein peptide bonds through catalytic hydrolysis and are used to remove non-fibrillar proteins, such as albumin and globulin.[59] An attempt has been made to design an eco-friendly vegetable tanning process combining pickle-free tanning and the application of proteolytic enzymes to improve the exhaustion of vegetable tannins. Such an approach has resulted in tannin exhaustion in excess of 95%, a 10% increase when compared to the conventional vegetable tanning process.[60] An acid protease was applied to increase the diffusion of vegetable tanning agents and thus achieve better utilization. Properties such as tensile strength and elongation were not significantly different between the products of conventional and enzymatic processes.[60]

Keratinases

Keratin is the main structural protein of hides (epidermis), hairs, wools, nails and feathers. The protein forms rigid fibers and is present in the epidermis and skeletal tissues. Feathers contain more than 90 % keratin. Keratinases has several important uses in biotechnological processes: as alternatives for the depilation of skins, as detergents and fertilizers, in animal foods and cosmetics, in the degradation of industrial waste and in the production of biodegradable films. Animals and plants are unable to efficiently hydrolyze keratin. Keratin is a very stable protein but can be hydrolyzed by intracellular and extracellular keratinases produced by certain fungi, bacteria and actinomycetes.[61-63] Specific enzymatic assays demonstrate that keratinase can act on a large variety of soluble and insoluble protein substrates.[64]

Lipases

Most lipases that are currently produced are obtained from fungi and yeasts, but the interest in bacterial lipases has increased due to the greater stability of these enzymes when exposed to high temperatures or other severe conditions compared with the enzymes obtained from other microorganisms.[65]

Lipases catalyze the hydrolysis of triglycerides into glycerol and free fatty acids, and microorganisms are vigorous producers of lipases. The production of lipases from *Aspergillus niger* has been studied, and the characteristics (optimum pH and temperature, stability and ability to remove olive oil) of the extracellular lipases obtained were determined.[66] Lipases are enzymes that are soluble in water and have an important role in digestion metabolism.[67] In leather production, lipases act on the fats and lipids of meats and greases and on the oils produced by the skin's glands.[68] Lipases are used for degreasing and unhairing. The application of lipases with surfactants promotes a more efficient degreasing. Lipases and proteases, when used together in the retanning step, remove grease, dirt and other stains; thus, it is possible obtain a more uniform and brilliant color. Lipase utilization has been shown to reduce stains caused by natural fat as well as wrinkles and other types of hide discoloration.[57]

Amylases

Amylases are responsible for the degradation of starch molecules and are widely distributed in nature. Amylases have important biotechnological applications in industries such as the textile, pulp and paper, leather, detergent, beer, bread, infant cereal, animal feed, chemical and pharmaceutical industries and for the liquefaction and saccharification of starch. These enzymes can be obtained from various sources, including plants, animals and microorganisms, but microbial enzymes generally have the largest industrial demand. Large amounts of microbial amylases are commercially available and have applications in the hydrolysis of starch.[69,70] In leather production, amylases can be used to create openings in the fibrous structure of hides.[71]

Collagenases

Collagenases are metalloproteases that can be obtained from various sources. The modes of action of collagenases are dependent upon the source from which they were obtained. Bacterial collagenases cleave preferentially at Gly-X bonds (X is often a neutral amino acid) in the sequence -Gly-Pro-X-Gly-Pro-X. Tanned collagen (cross-linked by tanning substances) is resistant to the action of collagenase; this enzyme does not hydrolyze chromium-tanned leather but does cause openings in the fibrous structure.[60]

Collagenases obtained from several microorganisms have been studied in detail but encountered difficulties in degrading and solubilizing leather, which mainly consists of collagen.[47]

Bacterial collagenase was applied to leather dyeing. The use of enzymes has also made it possible to obtain softer leathers without changes in strength properties.[60] Table II summaries some enzyme applications in leather processing.

3.1.2 Evaluation of Enzymatic Activity on Leather

In addition to knowledge of the characteristics of the enzymes to be applied to the hide for many different purposes, it is important to quantitatively assess the actions of the enzymes on the hide. The recent literature[72-75] discusses quantitative determinations of protein, interfibrillar protein (glycosaminoglycans and proteoglycans) and hydroxyproline and the evaluation of grain quality using optical and/or electron microscopes. The effects of enzymatic actions should also be evaluated based on the mechanical strength of the resulting leather. Glycosaminoglycans are also called mucopolysaccharides; these are linear carbohydrates with high molecular weights that are formed by the polymerization of uronic acid and hexamine (glucosamine or galactosamine). The most common glucosamine is hyaluronic acid. Dermatan sulfate is partially removed during the beamhouse process.[76] The dermatan sulfate removed during the beamhouse process

TABLE II
Leather process steps in which enzymes can be utilized.

Step	Technology currently used	Alternatives
Soaking	Surfactants, neutral salts, acids and bactericides	Proteolytic and lipolytic enzymes
Unhairing/Liming	Calcium hydroxide and sulfides	Enzymes with proteolytic actions (especially on collagen and keratin)
Bating	Pancreatic enzymes	
Pickling	Acids, particularly sulfuric acid and formic	Proteolytic enzymes to enhance exhaustion of vegetable tanning agents
Dyeing	Dyes and some auxiliaries, acids to fix dies	Collagenase

is associated with the opening up of the fiber structure; its efficient removal results in an open fiber structure, facilitates chemical product diffusion and produces a soft leather with an increased surface area, but the excessive removal of dermatan sulfate results in a loose and weak leather.[77] Proteoglycans are extracellular proteins bonded to glucosamines. The major proteoglycan is decorin, which consists of a single chain of dermatan sulfate attached to a polypeptide chain. The efficient removal of proteoglycans is important for the quality of the leather as this removal increases softness and flexibility.[72]

Removal of interfibrillar material, such as proteoglycans and glycosaminoglycans, is a fundamental part of the process of conventional liming and bating. For the bating step, the removal of interfibrillar material is facilitated by the use of proteolytic enzymes. The authors[73] developed a methodology to determine the removal of these proteins during the liming and bating process. These methods included the construction of standard curves for determining the concentrations of glucosamines using chondroitin sulfate and using mucin to quantify the proteoglycans.[73]

Hydroxyproline is a characteristic amino acid of collagen and is not found in other proteins. Among the amino acids in the collagen structure, the most common are hydroxyproline (11.28 %), proline (11.77 %), glycine (33.43 %), alanine (11.97 %) and arginine (5.04 %).[78] Mammalian hides include 13.45 g of hydroxyproline per 100 g of collagen, whereas fish skins include between 7 and 9 g of hydroxyproline per 100 g of collagen.[79] For this reason, the hydroxyproline content has been used as an indicator for the determination of collagen concentrations in hide or to signal undesired collagen release during processing.

In addition to the interfibrillar protein analyses, another common evaluation of enzyme applications in the various stages of hide processing is the determination of tensile strength and elongation as well as tear strength.[16,80-82]

3.2 Isolation and Screening of Enzyme-producing Microorganisms

The search for a new enzyme begins with microorganisms found in nature. Researchers have examined soil samples collected in areas with different climatic conditions ranging from tropical forests to glacial regions. Enzymes can be obtained from various sources. Among the main sources of enzymes are animals, plants and microorganisms.

Most industrial enzymes are obtained from microorganisms. The advantages of using microbial enzymes rather than enzymes obtained from plants and animals are numerous. Among these advantages, the following should be mentioned:[29,83]

a. Plants and animals grow slowly relative to microorganisms.

b. Enzymes are only a small portion of the bodies of animals or plants; therefore, large areas of land or a large number of animals would be required for large-scale production. These limitations make enzymes of plant and animal expensive. Microbial enzymes are not subject to these problems and can be produced in the desired amounts.

c. Microbial enzymes tend to be more stable than their counterparts in plants and animals.

d. The main advantage of the microbial production of enzymes is the diversity of microbial enzymes, which reflects the diversity of microbial species in nature.

e. Finally, the discovery and understanding of the genetic bases responsible for the control of physiological functions in microorganisms allows for the manipulation of microorganisms for the production of certain metabolites, including enzymes.

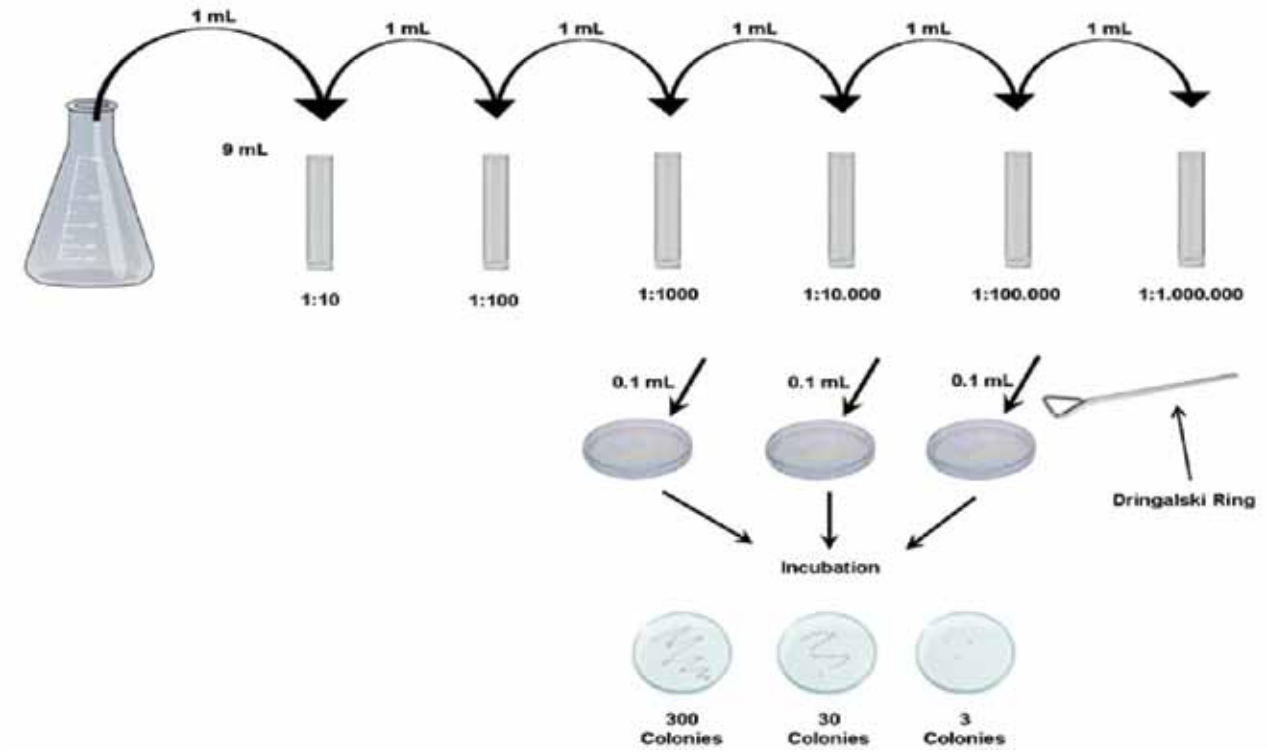

Figure 3. Methodology for microorganism isolation.

The first stage in the isolation and selection of microorganisms for the production of enzymes is the isolation of strains capable of producing the desired product, as shown in Figure 3. For this purpose, an intensive selection and testing of several different strains is necessary to identify those strains that exhibit the desired properties.[22,23,25,33,34]

Currently, several new sources of enzymes are being studied. Exotic microorganisms, such as extremophiles, have been considered important sources of enzymes. Organisms follow a distribution pattern based on pH conditions. Most well known microorganisms proliferate under conditions of near-neutral pH. When the pH is not close to neutral, the number of microorganisms is reduced.[34]

The standard practice with many microorganisms is to cultivate a large number of plates containing culture medium and agar, as shown in Figure 4. Typically, the microorganisms are isolated using subsequent plating and screening for desired characteristics. Then, the microorganisms are grown in specific media to estimate proteolytic or lipolytic activities using suitable substrates, such as skimmed milk or casein, starch, tributyrin or butter. The isolated strains that exhibit the highest activities are selected and maintained in a manner suitable for subsequent use.

Microorganisms that produce enzymes with applications in leather production, including proteases, keratinases and collagenases, have been isolated from various habitats, such as soil,[47,58,64,84] and the waste and effluents from tanneries.[85, 22,23,25]

Figure 4. Plate containing cultivation medium and colonies of an isolated bacterial strain.

Ogino et al. (2008)[47] isolated 76 species of microorganisms able to degrade leather wastes at neutral pH and 23 species of microorganisms able to degrade leather waste at basic pH. The most common methods described in the literature to obtain enzymes with proteolytic activities involve the use of culture media containing agar and proteins. The most common proteins used are casein,[58,64] skimmed milk[22, 23,25,85-87] and ground feathers.[84,88] Proteolytic activity is detected by the

formation of a translucent halo around a colony that is generated by substrate consumption, which indicates protease production.

3.3 Enzyme Production and Microbial Nutrition

Most enzymes are obtained from submerged cultures, but some are produced in semi-solid media.[83,89]

Semi Solid Medium

This type of medium is commonly used for fungal microorganisms, which are more amenable to high rates of enzyme production in conditions of low humidity and high aeration. The temperature can be maintained near 30 °C by air circulation. The production period is generally 30-40 hours but can last up to seven days. The optimum yield is determined by sampling and evaluation of enzyme production.[83]

Submerged Production

Most enzyme production is achieved via submerged cultures in bioreactors. The culture medium must contain all the nutrients necessary for growth, including adequate sources of carbon, nitrogen, metals, and trace elements. However, in some cases, the medium suitable for growth is not sufficient for the production of the desired enzyme. The pH and temperature must be adjusted for each organism. The temperature and pH for growth, production of the enzyme and enzyme stability are not necessarily the same for all enzymes. The temperature adopted for the cultivation is usually determined by considering these three factors. The need for oxygen is greater when the microorganism used in the production of the enzyme is aerobic, so aeration and agitation are employed in submerged production.

It is essential that the microorganisms are cultured under optimal growth conditions to increase the production of enzymes. The culture conditions that promote the production of proteases are significantly different from the culture conditions that promote cell growth. In the industrial production of alkaline proteases, high concentrations of complex carbohydrates, proteins and other components in the culture medium are used. To develop an economically viable technology, research has focused on methods to achieve the following: (a) increase the efficiency of alkaline proteases (b) optimize the production conditions (c) use less expensive culture media.

In most organisms, organic and inorganic nitrogen forms are metabolized to produce amino acids, nucleic acids, proteins and cellular components. Alkaline proteases include up to 15.6 % nitrogen, and their production is dependent on the provision of carbon and nitrogen in the medium. Although complex nitrogen sources are normally used for the production of alkaline proteases, the need for a particular nitrogen source varies from organism to organism. Increases in the yields of alkaline proteases have been observed by several researchers when sugars, such as lactose, maltose, sucrose, and fructose, are used in microbial cultivation. Various organic acids, such as acetic acid, methyl acetate, citric acid or sodium citrate, have produced good results regarding the production of alkaline proteases. [34] In some cases, enzyme production requires the presence of divalent metal ions (calcium, cobalt, boron, iron, magnesium, manganese). Potassium phosphate has been the source of potassium used in most studies. Phosphate is used to buffer the media, but in excess, phosphate inhibits cell growth and enzyme production.

In summary, the nutritional requirements of microorganisms are the same as those of all living beings. The following are the most important factors in microbial nutrition.[90,91] Fungi and most bacteria are chemotrophic and obtain energy via chemical reactions in which suitable substrates are oxidized. Lithotrophic microorganisms oxidize inorganic compounds, while organotrophs oxidize organic compounds. The first group includes bacteria that oxidize sulfur to produce sulfuric acid. The second group includes fungi and a significant number of bacteria.

Carbon Sources

For the autotrophic microorganisms, the sole carbon source is CO_2 or bicarbonate, from which such organisms can synthesize all required organic compounds. Most bacteria are heterotrophic and require organic sources of carbon; the most common sources of organic carbon are carbohydrates, amino acids, lipids, alcohols, and polymers, such as starch and cellulose. In fact, any natural organic or synthetic compound may be used by some microorganisms. This versatility is of great importance, allowing the use of microorganisms in a wide variety of useful transformations.

Nitrogen Sources

Microorganisms can be divided in three classes of nitrogen requirements. Some bacteria absorb nitrogen directly from the atmosphere and convert it to organic nitrogen. Numerous fungi and bacteria utilize inorganic nitrogen compounds almost exclusively, particularly ammonium salts and occasionally nitrates. Fungi and some bacteria require organic sources of nitrogen, which are represented by a variety of amino acids. In general, the addition of a protein hydrolysate or amino acids promotes the growth of most heterotrophic microorganisms.

Essential Inorganic Ions

In addition to carbon and nitrogen, microorganisms require a number of other elements in the form of inorganic compounds. Some, called macronutrients, are required in significant quantities, while others, called micronutrients, are not.

Among the macronutrients are phosphorus, in the form of phosphates, which are important in energy metabolism and in the synthesis of nucleic acids; sulfur, which is necessary for the synthesis of amino acids such as cysteine and vitamins such as

biotin and thiamin; potassium, which is an enzyme activator and osmotic pressure regulator; magnesium, which is an activator of extracellular enzymes important in sporulation; and iron, which is necessary for the synthesis of certain cytochromes and pigments. The role of micronutrients is not well known because the study of micronutrients is difficult. However, in some specific cases, a need for elements such as copper, cobalt, zinc, manganese, sodium, boron, and others has been demonstrated.

Growth factors

Growth factors are the organic compounds needed by a particular organism that cannot be synthesized by that organism. These factors must therefore be present in the medium so that the organism can grow. Many of these factors are vitamins, particularly the B complex, and sometimes these factors are amino acids or fatty acids.

Water

Water is not a nutrient, but it is absolutely essential for the growth of microorganisms because most are nourished by the passage of substances in solution through the cytoplasmic membrane. Water has a major role in regulating osmotic pressure and, due its high specific heat, in thermal regulation

Atmospheric Oxygen

Like water, atmospheric oxygen is not a nutrient. Microorganisms have different behaviors in the presence of free O_2; aerobes require the presence of free oxygen, but some aerobes are microaerophilic, requiring only a small amount, and do not tolerate normal atmospheric O_2. Conversely, anaerobic microorganisms cannot tolerate the presence of free oxygen and die quickly under these conditions, whereas facultative microorganisms can grow both in the presence and in the absence of free oxygen.

3.4 Enzyme Kinetics

The study of enzyme kinetics is an area of enzymology that focuses on enzymatic reaction rates and the factors that influence these rates. The kinetics of an enzyme are studied by evaluating the amount of product formed or the amount of substrate consumed per unit of reaction time.[33,36]

An enzymatic reaction can be expressed according to Equation 1 and comprises two elementary reactions in which the substrate forms a complex with the enzyme that subsequently decomposes into the products and enzyme:

$$E + S \underset{}{\overset{k1}{\longleftrightarrow}} ES \xrightarrow{k2} P + E \quad (1)$$

where E, S, ES and P symbolize the enzyme, the substrate, the complex enzyme/substrate and the products, respectively. According to this model, when the substrate concentration is high enough to completely convert the enzyme to its ES form, the second step of the reaction will become limiting, and the speed of the overall reaction will not vary with a further increase in the substrate concentration. The enzymatic reaction rate depends on the concentrations of the enzyme and the substrate.

In theory, all of the enzyme used to catalyze a reaction can be reused several times for the same synthesis or cleavage. In practice, however, there are some limitations. Enzymes are complicated and sensitive biomolecules, and the destruction of enzymatic activity may occur due to the presence of heat, alkalinity, acidity, trace metals or certain inhibitors.[60]Some factors that influence enzyme catalytic activities are as follows: [33,34]

pH

There is an optimum pH at which the distribution of electrical charges on the enzyme molecule, and particularly the catalytic site, is ideal for catalysis.

Temperature

Most enzymes have optimum temperatures; the optimum temperature depends on the microorganism from which the enzyme was isolated. Some microorganisms favor near-ambient temperatures; in these microorganisms, the enzymes are most active at temperatures between 30 and 40 °C. At low temperatures, the movement of molecules is slow and the reaction rate decreases as a result. At higher temperatures, molecular movement is very rapid; at these temperatures, it is difficult to maintain the conformation of the enzyme and the enzyme thus begins to undergo denaturation and loses activity. At higher temperatures, the reaction speed increases until it reaches the optimum temperature, after which the activity decreases.

Denaturation

Protein denaturation is defined as the loss of the structure that enables the protein to function. Not only high temperatures lead to denaturation. Other environmental variables that affect chemical bonds have the same effect. Thus, extreme pH values, which lead to the protonation or deprotonation of functional groups, cause the loss of enzyme activity. Detergents and nonpolar solvents can also change bonds and interactions and thus cause denaturation. In most cases, denaturation is an irreversible process.

Enzymatic Inhibition

Many substances alter the activity of an enzyme by reversibly associating with the enzyme. Substances that reduce the activity of an enzyme are known as inhibitors.

Inhibitors act through a variety of mechanisms. Some enzyme inhibitors are substances that structurally resemble the substrate of the relevant enzyme and do not react or react very slowly. These types of inhibitors can be classified into two groups:

Competitive Inhibitors

These compete directly with the substrate at the active site of the enzyme and reduce the concentration of free enzyme available for binding to the substrate. An increase in the substrate concentration leads to a decrease in the inhibitory activity by reducing the proportion of free enzyme inhibitors. This type of inhibitor is usually similar to the substrate and specifically binds to the active site of the enzyme. However, it is sufficiently different from the substrate so that it cannot react.

Noncompetitive Inhibitors

A noncompetitive inhibitor distorts the active site of the enzyme, thus making the enzyme catalytically inactive. Increasing the substrate concentration does not cause decreased inhibitory activity. The noncompetitive inhibitor does not resemble the substrate.

Denaturation

If an inhibitor binds irreversibly with the enzyme, it is classified as an inactivator/denaturant.

Enzyme Inhibitors During Hide and Leather Processing

Salts and other chemicals used in industrial baths may interfere with enzymatic activity and thus must be tested to avoid losses in processing. Table III presents the effects of various chemicals on enzyme activity, as reported by Dettmer et al. (2012b). The enzyme was partially inhibited by sodium sulfate and surfactant. EDTA, fatty alcohol, sodium carbonate and calcium hydroxide did not cause significant variations in enzymatic activity.

Studies by Dettmer et al.[22,23,25] showed that in comparisons between conventional and enzymatic unhairing processes, the enzymatic process is found to be a promising option to reduce the pollution load of the effluent generated. The enzymatic process was shown to reduce the levels of COD, BOD, nitrogen and sulfide and also resulted in a significant reduction of the processing time, from 20 hours to 6 hours; however, care was required to control the processing time and prevent damage to the leather. The authors noted that with the enzymatic unhairing process, it was possible to obtain leather with the same properties as those of leather obtained via the conventional process; similar results were obtained for both processes regarding attributes such as tensile and tear strength, percentage of chromium and shrinkage temperature. Dettmer et al.[22,23,25] also evaluated enzymatic unhairing by quantifying interfibrillar proteins, which allowed the evaluation of the enzyme efficiency for the removal of interfibrillar proteins; in addition, a possible undesired enzymatic attack on collagen was evaluated by measuring the hydroxyproline released into the wastewater. The authors also found that the residual activities of the enzymes after the unhairing process was satisfactory and allowed for the possibility of the reuse of these enzymes.

TABLE III
Effect of some inhibitors on enzymatic activity.[23]

Chemical product	Concentration	Residual Activity (%)
EDTA	0.15 % (w/v)	90.59 ± 1.31
Fatty Alcohol	0.1 % (v/v)	95.55 ± 4.92
Sodium Carbonate	0.3 % (w/v)	94.84 ± 7.69
Calcium Hydroxide	0.5 % (w/v)	98.58 ± 8.22
Calcium Hydroxide	1 % (w/v)	97.94 ± 10.31
Surfactant	0.1 % (w/v)	79.52 ± 5.10
Sodium sulfide	1 % (w/v)	61.12 ± 7.10

4. Conclusion and Future Prospects

Leather processing remains a challenging area regarding the development and application of cleaner technologies. Enzymes can be used as an alternative technology to chemical products because enzymes are natural, safe and better for the environment. However, the application costs remain high, and more research regarding the use of enzymatic technologies in the leather industry is necessary. In this review, the processes of screening, isolation, microorganism cultivation and enzyme characterization were presented, which can contribute to new research. Enzyme characterization of both commercially available and the newly isolated enzymes is important because this characterization elucidates the enzymatic properties, which can be used in future academic research and for industrial applications. These data can improve enzyme utilization in hide and leather processing via the application of specific knowledge about inhibition and the optimal pH and temperature for enzyme activities. The utilization of enzymes in the unhairing process shows promising results for reducing the pollution loads of wastes; this reduction was confirmed by analyses of the COD, BOD, nitrogen, and sulfide levels. Enzymatic processes also contribute to reductions in process time, from 20 to 6 hours, but can damage the leather if an appropriate process time is not employed. The effects of enzymatic activity on leather grain and interfibrillar proteins can be evaluated by determining the interfibrillar proteins, hydroxyproline content and leather tensile strength. In summary, the utilization of enzymes for hide and leather processing is a promising technology and can contribute substantially to the minimization of environmental impacts and the increasingly efficient use of chemical products.

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