Efficient Leather Dehairing by Bacterial Thermostable Protease

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Abstract

Leather processing involves many sequential steps form raw hide to the processed leather. All these steps define the quality of leather in order to perform in the prescribed conditions. Dehairing of raw hide is one of the most important steps which define removal of hair, fat and other unnecessary things form raw hide. The most convenient way to achieve perfect dehairing is use of enzyme and specifically protease which dissolve the hair protein without affecting structure of skin. In this process generally alkaline and neutral proteases have been used extensively since last two decades. Though the conventional protease are efficient for dehairing of raw hide but the stability of these protease in various temperatures and chemical environments hamper their activity. In many instances the process will run for long duration which again reduces the activity of enzymes. Thermostable proteases form microbial sources are the better option to render the problem of stability of conventional protease in the different temperatures and chemical environments. Many of bacterial strains naturally adapted their habitat in higher temperature and the enzymes of such bacterial strains are very stable. Many of thermostable proteases have been isolated, purified and implemented in various industrial processes which have been limited due to stability complication of conventional protease. Here in this article we have summarized the potential role of thermostable proteases in leather industry and more specifically in dehairing of raw hide.

Keywords: *Leather Processing, Leather Dehairing, Protease, thermostable protease*

1. Introduction

1.1 Leather and Leather Industry

Since the beginning of human civilization leather industry has been a traditional industry which uses proteolytic and lipolytic enzymes in leather processing for the conversion of raw hide into processed leather. The use of proteolytic enzymes is associated with the structure of animal skin as a raw material where enzymes are used to remove unwanted parts. A class of protease that is alkaline proteases has been sued most significantly in last few decades. [1] The use of protease improves water uptake by the dry skins, removal and degradation of protein, dirt and fats and reduces the processing time. Earlier mainly in the beginning of time pancreatic trypsin is also used for processing of leather. In the leather dehairing and dewooling protease enzymes are used to assist the alkaline chemical process. [2] The use of protease not only improves yield but also results in a more environmentally friendly process

and improves the quality of the leather (cleaner and stronger surface, softer leather, less spots). The used protease enzymes are typically alkaline bacterial proteases becoming more significant in the leather processing day by day. Another class of enzyme like lipases are used in this phase or in bating phase to specifically remove grease. [3]

The subsequent phase in leather processing is bating which aims at deliming and deswelling of collagen. In the next phase batting proteins are partly degraded to make the leather soft and easier to dye. In the beginning pancreatic trypsin were used but they are being partly replaced by bacterial and fungal enzymes. [4] As the leather industry contributes to one of the major industrial pollution problems facing the country, and the pollution causing chemical, viz. lime, sodium sulphide, salt, solvents, etc. arise mainly from the pre-tanning processes of leather processing hence the use of biological detergents protease for leather processing has becoming more significance now a day. [5] In order to overcome the hazards outcomes by the leather industry, use of biological enzymes as an alternative has been resorted to in pre-tanning operations in the leather processing such as soaking, dehairing, bating, degreasing and offal treatment [6].

1.2 Leather Processing

The leather processing involves various operations in a cascade manner form raw hide to processes leather. The complete leather manufacturing process is divided into three fundamental sub-processes: preparatory stages, tanning, and crusting which runs in cascade manner. Subsequently another sub-process, surface coating can be added into the leather process sequence, but not all leathers receive surface treatment. During the production of leather from various sources, it is difficult to create a list of operations that all leathers must undergo [7].

In the preparatory stages are when the hide/skin is prepared for tanning. Preliminary stages may include: preservation, soaking, liming, unhairing, fleshing, splitting, reliming, deliming, bating, degreasing, frizing, bleaching, pickling, and depickling. The raw hide has to undergo many of chemical treatments in cascade manner before it turns into flattering leather. [8] Which is comprised of soaking, liming, dehairing, deliming, bating, degreasing, and pickling. For most of these steps the chemicals used are quite toxic and used in large amount in these pre-tanning operations which results the leather processing industry one of the worst offenders of the environment [9].

1.3 Leather Dehairing

The raw hide which is an ultimate source of leather comprise of epidermis and dermis layers. These layers contain many proteins and other biomolecules. The principal leather making protein, collagen, exists in hides and skins in association with various globular proteins, viz. albumin, globulin, mucoids; and fibrous proteins such as elastin, keratin, and reticulum. [10] In the process of leather manufacture, the noncollagenous constituents of raw hide are removed partially or completely in the various pre-tanning operations; where leather dehairing is one main operation and the extent of removal of these constituents decides the characteristics of the final leather. Conventionally chemicals have been used for these pre-tanning operations. Since these chemicals are becoming problem for the environmental pollutions hence use of enzymatic treatments are also necessary to get optimum results without affecting environments. [11] A class of enzyme, proteases has been used successfully in last two decades. One such treatment, bating, is the only step in leather processing where enzymatic process cannot be substituted by chemical processes. The dehairing by enzyme

offers certain desired characteristics to the finished leather. Earlier, the process was carried out using dog dung or manure. The use of this was not only unhygienic but fermentation could also not be controlled. [12]

In pre-tanning operations, the hides and skins are first subjected to a water soak. For loosening the hair, the oldest method is the 'sweating' process – a natural autolysis or breakdown process which is a mild putrefaction process induced at random. [13] Since the type and quantity of the putrefying bacteria cannot be monitored, the process itself eludes control. Moreover, since the sensitivity to attack the epidermal proteins and the fibrous proteins of the corium by the proteolytic enzymes is more or less the same, the sweating may results in serious damage to the raw hide surface. [14] Dehairing is used to be followed by opening up of fiber structure in 'liming'. The dehaired hide is transferred to an alkaline solution of lime milk where swelling occurs and the non-fibrillar proteins are dissolved. After mechanical removal of the subcutaneous tissue, deliming is performed in order to remove the adsorbed lime from the hide and to eliminate the lime swell. [15] Another component of raw hide is fat in the hide skins is removed either as soluble lime soap or hydrolysis products like fatty acids. Kerosene, chlorinated hydrocarbons, and white spirit are used in the degreasing system which adds to the toxicity of the environment and effluents [16].

1.4 Global Leather Production

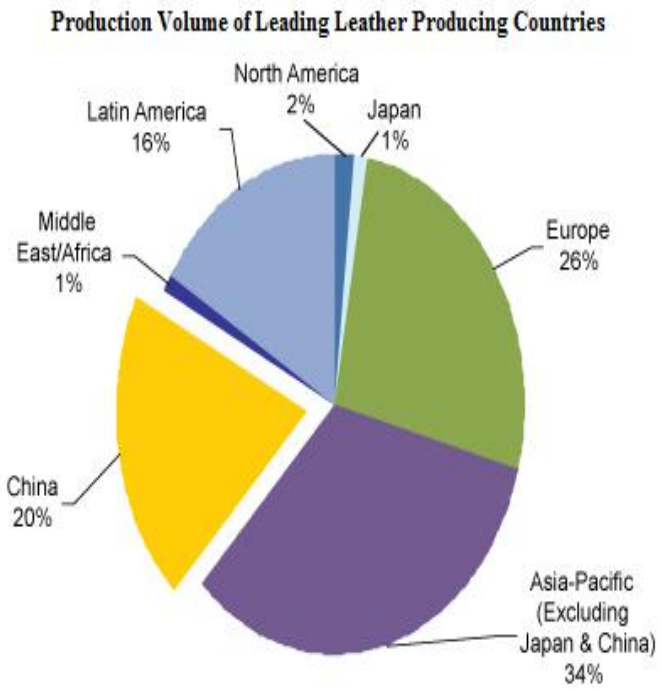

Fig. 1. Global leather production in last 20 year throughout the world. Where a major contribution from Asian continental and second form Europe. The contribution from American zone is also significant. The data have been shown is up to 2008.

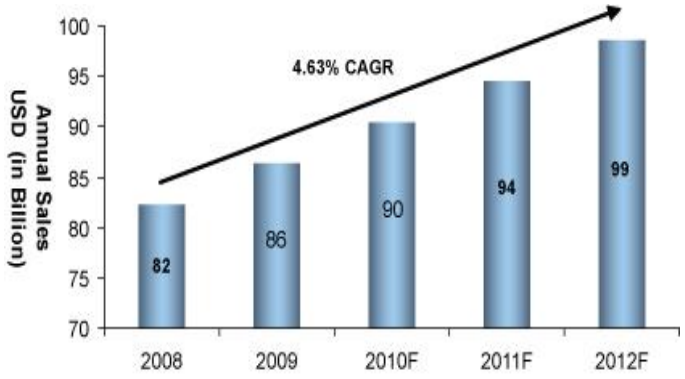

Fig. 2. The utilization of leather for various commercial applications throughout the world. The data has shown the enhanced production significantly in last 5 year which triggers the conventional leather industry for the optimization of leather production to improve yield and quality.

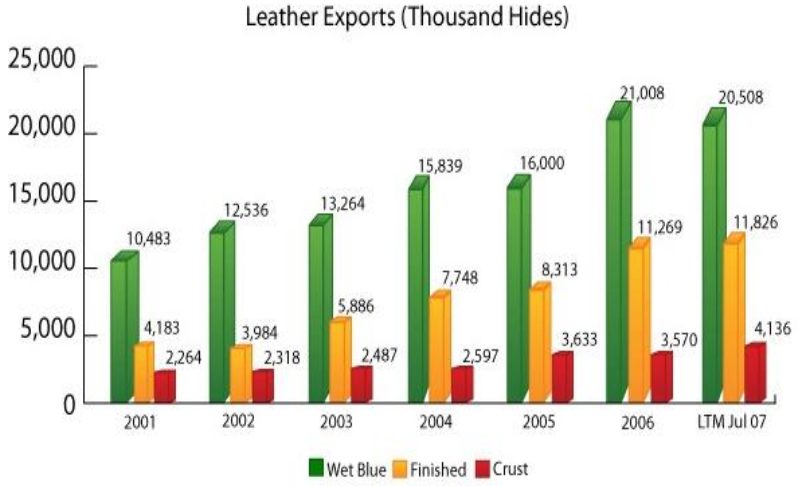

Fig. 3. The lather exports in the different forms during 2001-2007 throughout world.

2. Historical Overview

From the beginning of human civilization leather has played a key role in the development of human race. Since the prehistoric times human has used the skins of various animals to build his basic requirements. The use of hides to make clothing, shelter, carpets and even to explore intellectuals. In the Egyptian culture it was a fashion that ladies used furs as theirs jewelry. As the civilization progress man has used leather to make footwear, belts, clothing, containers for liquids, boats and many others. In other part of world Roman, Sparta and Tory during 1300 BC soldiers has custom of wearing leather shirts in the war which supposed to be a protective sheet for them not only form trauma of war but also to survive in that cold climatic conditions. [17] In ancient time in Europe, Asia and North Americans have developed skill to process the raw animal skin into leather. During the 1200 B.C. in Greek,

Roman Empire and Chinese culture leather have been used particularly for the making cloths for royal families.

Since the progression of time and development of technology human has discovered methods for the preserving and softening leather treating animal skins with such things as smoke, grease and bark extracts. [18] The art of tanning leather using the bark of trees probably originated among the Hebrews. In primitive societies, the art was a closely guarded secret passed down from father to son. As civilization developed in Europe, tanners and leather workers united in the trade guilds of the middle Ages, as did the craftsmen in other fields. Royal charters or licenses were issued permitting people to practice leather tanning.[19] In the nineteenth century, vegetable tanning, i.e., tanning using the extracts from the bark of certain kinds of trees, was supplemented by chrome tanning. This process uses chemicals and today accounts for about eighty to ninety percent of all tanning done except for the leather used in the soles of shoes and tooling leathers [20].

3. Conventional Leather Dehairing

3.1 Chemical Based Dehairing

Since the beginning of human civilization the conventional method of dehairing involves the use of lime and sodium sulphide as the lack of technology. Thus the production and quality of leather produced did not reach to optima. Though these methods have been used for centuries where the presence of these toxic chemicals in tannery waste is responsible for tremendous pollution, causing health hazards to the tannery workers and surroundings. The main ingredient of conventional leather processing lime which produces a poisonous sludge while sodium sulphide is highly toxic and has obnoxious odor. [21] In the beginning of twenty century enzyme based leather dehairing processing replaced chemical use up to certain extents as a result reduction in the pollution load to some extent, still enzyme alone, without the use of sulphide and other chemical inputs, have to optimize to avoid complete removal of use of chemicals in leather processing. [22, 23, 24]

3.2 Enzymatic Dehairing

Enzyme based leather dehairing has been considered an environmentally friendly alternative to the conventional chemical process. The ability of enzymes to digests the basal cells of the hair bulb and the cells of the malphigian layer found more efficient without disturbing the native state of skin. [25] As a result by loosening of hair with an attack on the outermost sheath and subsequent swelling and breakdown of the inner root sheath and parts of the hair that are not keratinized. Numerous significant advantages of enzymatic dehairing are [26, 27]:

(i) Minimize or complete removal of use of sodium sulphide.

(ii) Enzymatic dehairing provides complete hair removal resulting quality leather.

(iii) Creation of an ecologically conducive atmosphere for the workers.

(iv) Enzymatically dehaired leathers have shown better strength properties with surface area.

(v) Simplification of pre-tanning processes by cutting down one step, viz. bating.

(vi) A significant nature of the enzymatic dehairing process is the time factor involved.

Proteolytic enzymes have great commercial importance and contributing to more than 60% of the world's commercially produced enzymes. Approximately 50% of the enzymes used as industrial process aids are proteolytic enzymes. Proteolytic enzymes are more efficient in enzymatic dehairing than amylolytic enzymes. [28] In dehairing, the hair loosening is effected at pH 10.0 using fungal or bacterial enzymes; the treatment period being approximately 12–16 h, followed by hair removal using mechanical means [29]. The treatment period can be substantially reduced if the enzyme solution is fed in from the flesh side under pressure. Enzymatic hair loosening processes play a role wherever high-quality hair, wool or bristles are to be recovered [30].

Three methods of application are commonly used in the enzymatic dehairing process:

(i) Paint method

(ii) Dip method

(iii) Spray method

In the case of paint method, the enzyme solution is mixed with an inert material like kaolin, made into a thin paste, adjusted to the required pH, applied on the flesh side of hides and skins, piled flesh to flesh, covered with polythene sheets and kept till dehairing takes place. [31] In the dip method of enzymatic unhairing, the hides or skins are kept immersed in the enzyme solution at the required pH in a pit or tub. The disadvantage encountered in this method is the unavoidable dilution of the enzyme solution. Even though enzyme penetration is observed to be uniform, dehairing at backbone and neck is not up to the mark. A novel spraying technique has been adopted for the application of multienzyme concentrate in depilation [32].

The advantages of this method over the painting and dip methods are that

(i) Even concentrated solutions can be sprayed,

(ii) When the enzyme solution is sprayed on the flesh side with force, entry becomes easier,

(iii) Backbone and neck can be sprayed with more amount of enzyme, thereby quick process

(iv) There is no effluent arising out of this method, and

(v) After depilation, hair will be almost free from all the adhering skin tissues.

Leather dehairing by drumming is being practiced, and industrially this should be feasible. Many of other methods are in the trials with the optimized operation for the improved dehairing [33].

3.2.1 Microbial Protease

The proteases have been categorized in a very large and complex group of enzymes which differ in properties such as substrate specificity, active site and catalytic mechanism, pH and temperature activity and stability profiles. [34] In the last 30 years different classes of proteases for the commercial importance have been produced from microbial, animal and plant sources and have been implemented for enormous applications in a range of processes which take advantage of the unique physical and catalytic properties of individual proteolytic enzyme types. Subsequently with the native sources numbers of protease have been designed by genetic engineering, a wider range of enzymes has become available on a larger scale and

this has increased the scope of enzyme technology. (Nicholas et. al., 2004) [35] Microbial proteases are the most significant source in the present scenario which has been derived from a wide variety of yeasts, molds, and bacteria. [36] Among then bacterial protease have been used most as ease of isolation, purification and design at gene level.

3.2.1.1Bacterial Protease

Though the protease contributes 60% of industrial enzyme but contribution of bacterial protease is even more significant which is more than 70%. Among the all the genus of bacteria Bacillus itself contributes major content of protease for the industrial application. Proteases find applications at various steps of leather processing, e.g., neutral proteases in soaking [37] alkaline proteases in dehairing [38] and acid proteases in batting. Dehairing enzymes from *Bacillus* sp. have been reported by many researchers. [39] The concrete mixture of dehairing enzymes from *Bacillus subtilis* and *Bacillus cereus* with sodium carbonate, caustic soda and thioglycolic acid, is described in a patent. [40] The aim of the present work was to study the production, optimization and properties of extracellular proteolytic enzyme of *B. cereus* B-326 having application in dehairing of buffalo hide.

Table 1: Following are the main Bacillus strains which are in commercial use for the production of various classes of protease. Bacillus offers protease ranging from various temperature and pH. Main fact why Bacillus has been used for source of industrially importance protease ease of production and purification. Bacillus not only offers conventional protease but also unique source of thermal and chemically stable protease.

S No	Bacterial Strain	Optimal Activity at (Temp)	Optimal Activity at (pH)
1.	*Bacillus. cereus*	40-50	2-6
2.	*Bacillus subtilis*	40-60	3-8
3.	*Bacillus licheniformis*	40-80	6-9
4.	*Bacillus sp. JB-99*	50	2-10
5.	*Bacillus brevis*	55	2-8
6.	*Bacillus halodurans*	45	2-6
7.	*Bacillus sphericus*	45	6
8.	*Bacillus amyloliquefaciens*	55	3
9.	*Bacillus lentus*	40	3
10.	*Bacillus Sp KSM - K16*	50	5

3.2.1.2 Fungal Protease

Many of researchers have isolated and checked activity of various proteases for dehairing form fungal sources. The protease from Aspergilous flavus was earlier being used for dehairing, and later it was reported that simultaneous dehairing and bating is possible with the protease of *Aspergilous flavus*. Gillespie has observed that the enzyme preparation from cultures of *A. oryzae, A. parasiticus, A. fumigatus, A. effusus, A. ochraceus, A. wentii,* and *P. griseofulvum* exhibit marked depilatory activity on sheep skins. Proteolytic enzymes derived from a large number of *Bacillus* sp. and *Streptomyces* sp. have been used in dehairing of hides and skins [41, 42]. A lime and sulphide-free process of dehairing has been developed for the manufacture of suede from sheep skins using protease from *B. subtilis*. It has been reported a method of depilation in an acid medium containing *Lactobacillus* culture [43].

Central Leather Research Institute (CLRI), Chennai, India has developed Clarizyme, an alkaline serine protease, produced by *Aspergilous flavus* used for the dehairing of skins and hides. Generally *Aspergilous flavus* grows rapidly on wheat bran and produces large amounts of extracellular proteases.[44] Extensive trials have been carried out in CLRI tannery have confirmed the successful use of this enzyme as a depilatory agent. The use of this enzymatic depilation process completely eliminates the use of sulphide, a toxic pollutant. The fungal culture, *Conidiobolus* sp., isolated at National Chemical Laboratory, Pune produces high yields of extracellular alkaline protease [45]. The enzyme is active at pH 10.0 and is being tried for many industrial applications. Enzymes derived from bacteria have gained much commercial interest because of their easy production capabilities by submerged cultivation, high yield of enzyme, short duration for production, and easy recovery of the enzyme [46].

Table 2 In the fungal kingdom Aspergilous has been explored much and protease isolated have been used in the process of dehairing. Other strains like Penicillium and Conidiobolus, protease form these strain are potent too.

S No	Fungal Strain	Optimal Activity at Temp	Optimal Activity at pH
1.	*Aspergilous flavus*	40	3
2.	*Aspergilous. oryzae*	45	4
3.	*Aspergilous. parasiticus*	45	6
4.	*Aspergilous fumigatus*	45	7
5.	*Aspergilous. effusus*	55	8
6.	*Aspergilous. ochraceus*	50	8
7.	*Aspergilous. wentii*	40	8
8.	*Penicillium griseofulvum*	40	3
9.	*Conidiobolus coronatus*	45	3
10.	*Mucor mieche*	40	4

3.2.2 Animal and Plant Sources

In the primitive time the gut of various animals itself used as the source of protease, many of enzyme like trypsin have been extracted and implemented for leather processing. Some plant like papaya is rich source of protease the latex form papaya fruits has been used for the dehairing process. [47] These protease are not in industrial use in the current scenario as the isolation and purification complication. It's quite difficult to produce large scale enzyme if compare form microbial sources. More ever in case of protease form animal source that is even more complicated and as many ethical issues regarding their isolation these are not in practice [48].

4. Complications with Conventional Leather Dehairing

4.1 Stability of Conventional Protease

In the process of leather manufacturing there is extensive use of chemicals and many times process runs in various temperatures for many hours. These conditions would not be compatible with conventional protease as they are thermo and chemo sensitive hence the effect will be on the process and quality of product. [49] Many of conventional proteases form bacterial and fungal sources are having optimal activity in the temperature below 40`C and in the specific pH condition. Hence pH is another crucial factor, each class of protease are

having a definite range of pH for their activity. [50] In the process of dehairing often reaction setup is maintained for a class of protease for example neutral protease will work in the neutral pH while alkaline protease in alkaline environment [51].

4.2 Environmental Issues

Leather industry contributes to one of the major industrial pollution problems facing the worldwide. The effluent form these industry release many toxic chemicals like lime, sodium sulphide, salt, solvents, etc. which extensively used in the pre-tanning steps of leather processing. The use of conventional protease has minimized application of chemical significantly but not completely.[52] Environmental pollution has been a major irritant to industrial development. Chemical and chemical-based industries are the prime targets of the environmentalists for their crusade against pollution, and leather industry has also not been left out of the reckoning. The generation of pollution is significantly high in the pre-tanning operations compared to the post-tanning operations [53].

Of these, the most commonly practiced method of dehairing of hides and skins is the chemical process using lime and sodium sulphide. However, the use of high concentrations of lime and sodium sulphide creates an extremely alkaline environment resulting in the pulping of hair and its subsequent removal. While one cannot question the efficacy of this process, its inherent disadvantages have to be taken note of [54, 55].

Significant amongst these are:

a) It contributes in no small measure to the pollution load. Beam house processes generally account for 70–80% of the total COD of effluent from all leather making processes.

b) About 75% of the organic waste from a tannery is from the beam house and 70% of this waste is from hair which is rich in nitrogen. These figures clearly illustrate the contribution made by the lime and sulphide process towards pollution.

c) Sulphide is highly toxic with obnoxious odor. If left untreated, it can cause major problems in the sewers. The severe alkaline condition is a health hazard for the workers.

5. Thermostable Protease for Efficient Leather Dehairing

5.1 Need of Thermostable Protease

The thermostable proteases constitute a group of protease enzyme form specific microbial sources which are quite resistant to higher temperature. The optimal temperature for these proteases varies with the microbial sources. Form data which have been published in last 20 year these protease can act in the range of 40-100`C and exceptionally up to 150`C. [56] These proteases have some additional properties like chemical stability, resistant to various inhibitors and are efficient in long run processes. Often to achieve the complete dehairing protease are combined with the various chemicals at higher temperature for many hours. [57] These conditions would not favors to the conventional protease they may lose their activity resulting incomplete leather dehairing. In order to achieve successful leather dehairing thermostable proteases are the perfect ones. [58] Another benefit of use of thermostable proteases as they are potent for the complete removal of hair and other unwanted stuff form raw hide to avoid use of toxic chemicals as they are hazardous to environment [59].

5.2 Sources of Thermostable Protease

5.2.1 Bacterial Thermostable Protease

Proteases a class of proteolytic enzyme for the different industrial application constitutes more than 65% of the world enzyme market. [60] These enzymes are extensively used in the various industrial operations like food, pharmaceutical, leather and textile industries. [61] The use of these enzymes will keep increasing in the future as will the need for stable biocatalysts capable of withstanding harsh conditions of operation.

Bacilli genus alone contributes more than 70% due to ease in production and purification. In the last few decades a diverse sources of these protease has been explored which completely changed field of industrial biotechnology. [62] Till date numerous thermophilic Bacillus sp. that produce proteases have been isolated and characterized and among then the earliest isolate being Bacillus stereothermophillus [63] which is stable at 60`C. Many other Bacillus sp. also characterized with complete property suitable for industrial application runs at higher temperatures. [64] Along with this many other Bacillus stearothermophilus sp. produced an alkaline and thermostable protease which is optimally active at 85 `C. [65] Numerous unique species of Bacillus stearothermophilus TP26 that has been isolated produces an extra cellular protease having an optimum temperature of 75`C. [66] Improvement of the protease activity excreted from Bacillus stereothermophillus had also been possible using economical chemical additives in the proteolysis reactions involved in waste activated sludge which is quite complicated with conventional protease in ordinary conditions. [67] The production of unique class of proteases in a chemically defined medium, thermophilic and alkaliphilic Bacillus sp. JB-99 was also reported to produce thermostable alkaline proteases. [68] Extremely thermostable serine proteases are also produced and characterized a by the hyperthermophilic Desulfurococcus strain [69], and thermostable metallo-proteases are reported from a gram-negative thermophilic bacterium. Major area of focus in the future concerning the production of proteases is the optimization of media [70, 71] which is suitable for leather processing and other industrial applications. Hence the synthetic media have a great advantage over conventional complex media in that consistency of processes and production is enhanced protease through avoiding the variability of complex substrates [72].

5.2.2 Fungal Thermostable Protease

Another important biological source of thermostable protease is various strains of fungi. The optimal temperature for the fungal proteases are in the range from 35-55`C with few exceptions. Both the alkaline serine proteases have been isolated and used in the leather processing from *Conidiobolus* sp. were optimally active at 45°C [73] while alkaline protease from *Conidiobolus coronatus* had slightly optimal temperature and that is 40°C [74]. Extracellular protease from *Beauveria bassiana* also isolated and found optimum activity at 37°C [75]. After the complete purification protease form *Fusarium culmorum* was optimally active at 50°C for long hour operations [76].

After the purification of serine proteases from A. *fumigatus* Fresenius TKU003 [77] and *Aspergillus terreus* [78] had shown optimal temperature of 40 and 37°C respectively. The protease isolated form *Aspergillus tamarii* was active in temperature range of 30-55°C [79]. Extracellular alkaline protease isolated from *Aspergillus clavatus* was optimally active at 40°C [80]. After the complete purification Aspergillus *sydowi* protease has shown activity between 37-45`C [81]. Purified stable alkaline serine-protease from *Aspergillus clavatus* ES1 was optimally active at 50°C. After the complete purification proteases isolated from

Penicillium chrysogenum [82] and *Penicillium expansum* [83] showed maximum activities at 45 and 35℃ respectively. Crude protease extract isolated from *Penicillium* sp. had an optimum temperature of 45°C [84, 85]. Protease isolated from *Chrysosporium keratinophilum* IMI 338142 has shown optimal activity at 40°C. [86] The complete purified serine proteinase from *Paecilomyces lilacinus* had broad temperature optima in range of 30-60°C. [87] Purified Proteases isolated from *Scytalidium thermophilum* were found active at 37-45°C [88]. The purified protease form *Aureobasidium pullulans* has shown an optimum temperature of 45°C [89].

5.2.3 Recombinant Thermostable Protease

Often the habitats of thermostable microbial strains become problem for the researcher to isolate and purify the enzyme form their native wild source as maintaining those conditions in the laborites. Though if designed those conditions in lab it will not be economical as maintaining all the parameter like temperature and specific growth media. [90] The better option to overcome forms this problems to clone gene and express in the suitable host system which will be convenient to grow. The best host system used for last two decades *E. coli* which perfect for the expression for the recombinant gene. Numbers of genes coding thermostable protease form the different sources like bacterial sources and fungal sources have been successfully cloned and expressed in the E.coli system. Another benefits of having recombinant thermostable protease the expression level we can easily monitor and enhances if needed which is quite difficult in native host system [91].

6. Future Aspects

Proteases which constitute a broad class of industrially most significant enzymes are involved in diverse physiological and cellular processes. They are competent for degrading complex proteins and can also bring about synthesis of peptides especially in organic media. Protease has shown tremendous commercial value and potential applications in variety of industries including detergent, leather, pharmaceuticals and food etc. subsequently enormous additional applications are found as proteases with novel properties are being isolated and characterized from organisms in the various habitats. [92] The ubiquitous occurrence of protease in nature like plants, animals and microbes, microbial sources have several advantages. Out of these wide sources, microbial source explored most significantly due to their rapid growth, accessibility to genetic manipulation, easy scale up etc. are few of the factors which make microbes the preferred source of proteases. Advancements in technology specifically in molecular biology, protein engineering and computational biology have opened new avenues to design in new ways for desired application by site directed mutagenesis for improved and desirable properties. Continuously proteases are being isolated from novel sources with the unique properties and newer applications are being reported to existing ones, they are likely to play a very important role industry in future [93, 94].

As per concern of industrial production, the large scale processes must be economical, it is necessary to increase the enzyme yields and productivities to reduce the cost of enzyme. In order to achieve the higher yields many methods have been implemented like optimization of fermentation parameters including media formulations to produce high quality enzyme in higher amount. [95, 96] Another way is strain improvement which can be done by conventional methods like mutagenesis or asexual/parasexual recombination (Bodie et al, 1994) [97] or protoplast fusion in wild strains. (Murlidhar & Panda 2000) [98]. Recombinant DNA technology and protein engineering are classified advanced methods where

manipulation at gene level. Understanding at gene level can be done by recombinant DNA technology which describes structure function relationships of protease and provides an excellent tool for the manipulation and control of gene expression. [99] The principal objective of gene cloning is mainly for the over production of commercially important enzymes and in recent years most of the enzymes are produced by genetically engineered microorganisms. Protein engineering is another area of advanced technology which used to determine the three-dimensional structure of protein and introduce changes in amino acid sequence by site-directed mutagenesis to improve stability towards temperature and pH of the enzyme.

7. Conclusion

Leather and leather industry has been part of economy of many developed countries and many developing countries are earning foreign currency and creating employment on behalf of leather processing and production. Though it is part of economy of many countries but leather processing is one of the most important industrial pollution due to the conventional methods where extensive use of chemical results damage not only to that environment but population surrounds. Dehairing of hides is an important and unavoidable step involved in the processing of leather in the tanneries where new technology implementation is necessary. The conventional lime sulphide method employed for this purpose must be replaced by protease and further thermostable protease to avoid chemical application.

The potential for use of microbial enzymes in leather processing lies mainly in areas in which pollution-causing chemicals, such as sodium sulphide, lime and solvents are being used and conversion of waste products into potentially saleable by-products is possible. The limitation of conventional protease are render by chemically and thermostable protease. An extensive research is going on worldwide in order to hunt a common stable enzyme which will be more effective in various classes of hides. Future may witness eco labeled leather/leather products emerging as niche products, and the experience gained by the Indian leather industry in this area might greatly help India to emerge as a global leader in leather industry.

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